anti-atherogenic effect in Watanabe Heritable Hyperlipidemic rabbits. From the observation that the baseline lipid profile was not different between the two groups of exposure and non-exposure in secondary prevention, the drug might exhibit greates effectiveness in post-cardiovascular disease patients, in possibly advanced lipid accumulation and inflammation, which are associated with the circulation of oxidized LDL[28].

In primary prevention, we observed an almost significant increase of events in the exposed group (**Table 3**), and an apparently increased risk (HR 1.5), although not statistically significant after adjustment (**Table 4**). We suppose, however, that the ideal effects of probucol might be concealed by the following factors noted in primary prevention. The exposed group had a worse lipid profile (TC, LDL-C and HDL-C levels), higher HbA_{1c}, and thus definitely a higher risk than the unexposed group. Furthermore, 8 (nearly 30%) of the 27 patients experiencing cardiovascular events in the exposed group discontinued probucol when they had events. This was consistent with the different finding between primary and secondary preventions in the exposed group: less than half of the patients (113 of 233) in primary prevention continued on probucol, while 53 (72%) of 74 patients continued in secondary prevention. This estimation might be conservative.

The controversial and paradoxical action of probucol– lowering HDL-C– level was not associated with the risk of CV events in the cohort, therefore, the association between low levels of HDL-C and an increased risk for CV events or death indicated by the early Framingham Heart Study[29] may not be extrapolated to probucol-treated patients. This proposition is consistent with recent findings that a lowered HDL-C level is not always atherogenic, but that the quality or function of HDL-C is more important than the HDL-C levels[30]. In fact, increased levels of HDL-C with torcetrapib, a CETP inhibitor, were not associated with a significant clinical benefit in patients with coronary disease[31], FH[32] or mixed dyslipidemia[33].

We speculate that enhanced reverse cholesterol transport by CETP activation as a result of probucol treatment also contributed to the detected risk reduction in the cohort. The observed positive outcome of probucol, a CETP activator, might be a mirror image of the negative clinical trial results for the CETP inhibitor[34]. Reports[35, 36] of increased coronary heart disease in CETP deficiency despite increased HDL-C levels, and the molecular approach to review CETP deficiency[37] support our hypothesis, at least in Japanese genealogy. Interestingly, a recent basic research reports that human CETP expression enhances the mouse survival rate in an experimental systemic inflammation model[38], indicating for the first time a role for CETP in the defense against the exacerbated production of proinflammatory mediators.

For the safety evaluation, we found no cardiotoxic adverse drug reaction including QT/QTc prolongation or torsade de pointes, in this study, although probucol can cause them[16, 39, 40].

We obtained these results from an observational study with no control for inaccuracy, unexpected bias or confounding factors. We could not assure the precision of the baseline measurements due to unrecorded data. The participant centers were major hospitals for FH, but not all hospitals in Japan, because the study was conducted as part of a post-marketing study by a pharmaceutical manufacturer within the framework of the Japanese government regulations. Some restrictions on collecting data might have resulted in unexpected small numbers in the unexposed group in secondary prevention, although we think that the study cohort represents nearly a nationwide population of heterozygous FH in Japan. The results derived from patient data in Japan can not necessarily be generalized to patients in western countries.

Despite these limitations of the study, however, we could evaluate the outcome of long-term probucol treatment in the medical practice setting for FH, a high-risk population, for as long as 20 years in Japan. The significant risk reduction of CV events observed in the secondary prevention group holds clinical significance and suggests some beneficial therapeutic actions of this drug in arteriosclerotic diseases. The hypothesis from the findings warrants a randomized controlled trial for verification of the secondary prevention, and needs further research into the molecular mechanisms or roles of CETP in pathogenesis.

Author Contributions

Dr. Yamashita had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Matsuzawa, Kita, Saito, Fukushima, Matsui. Acquisition of data: Yamashita, Bujo, Arai, Harada-Shiba, Saito, Kita, Matsuzawa. Analysis and interpretation of data: Yamashita, Bujo, Arai, Harada-Shiba, Matsui, Saito, Fukushima, Kita, Matsuzawa.

Drafting of the manuscript: Yamashita, Bujo, Arai, Harada-Shiba, Matsui, and Fukushima. Critical revision of the manuscript for important intellectual content: Yamashita, Matsui, Fukushima, Kita, Saito,

and Matsuzawa. Statistical analysis: Matsui and Fukushima. Administrative, technical, or material support: Fukushima, Matsui, Kita, Saito, and Matsuzawa. Study supervision: Yamashita, Fukushima, Matsui, Kita, Saito, and Matsuzawa.

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From the formerly Daiichi and Otsuka, Dr. Matsui, Dr. Fukushima, Dr. Matsuzawa, and Dr. Kita received fees and expenses for meetings related to protocol design, statistical and clinical interpretation of the data; Dr. Bujo, Dr. Arai, Dr. Harada-Shiba received honoraria and travel expenses for lectures, Dr.Yamashita, Dr. Bujo, Dr. Arai received fees and travel expenses for a meeting related to clinical interpretation of the data. Dr. Yamashita received consultancy fees from Otsuka. Dr. Matsuzawa is contracted as a short-term adviser to Otsuka in medical science. Dr. Saito received travel expenses only.

POSITIVE Investigators

Osaka University Hospital, Suita (S. Yamashita, T. Maruyama); National Cardiovascular Center, Suita (M. Harada-Shiba); Sumitomo Hospital, Osaka (Y. Minami); Chiba University Hospital, Chiba (H. Bujo); Asahi General Hospital, Asahi (N. Hashimoto); Kawatetsu Chiba Hospital, Chiba (M. Takahashi); Nishifuna Naika, Funabashi (M. Shinomiya); Kashiwado Hospital, Chiba (K. Kosuge); Numazu City Hospital, Numazu (Y. Hayashi); Toho University Sakura Medical Center, Sakura (K. Shirai, Y. Miyashita); Matsudo City Hospital, Matsudo (T. Oeda); Kyoto University Hospital, Kyoto (M.Yokode, H. Arai); Hiroshima General Hospital of West Japan Railway Company, Hiroshima (K. Takata); Maizuru Kyosai Hospital, Maizuru (R. Tatami); Kido Hospital, Niigata (T. Miida)

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Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome

Yasuhiko Nakagawa,[1] Ken Kishida,[1] Shinji Kihara,[1] Mina Sonoda,[1] Ayumu Hirata,[1] Atsutaka Yasui,[1] Hitoshi Nishizawa,[1] Tadashi Nakamura,[1] Ryoko Yoshida,[2] Iichiro Shimomura,[1] and Tohru Funahashi[1]

[1]*Department of Metabolic Medicine, Graduate School of Medicine, Osaka University; and* [2]*Yoshida Suimin-kokyu Clinic, Osaka, Japan*

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Nakagawa Y, Kishida K, Kihara S, Sonoda M, Hirata A, Yasui A, Nishizawa H, Nakamura T, Yoshida R, Shimomura I, Funahashi T. Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome. *Am J Physiol Endocrinol Metab* 294: E778–E784, 2008. First published January 15, 2008; doi:10.1152/ajpendo.00709.2007.—Previous reports demonstrated that adiponectin has antiatherosclerotic properties. Obstructive sleep apnea-hypopnea syndrome (OSAHS) is reported to exacerbate atherosclerotic diseases. We investigated nocturnal alternation of serum adiponectin levels before sleep and after wake-up in OSAHS patients and the effect of sustained hypoxia on adiponectin in vivo and in vitro. We measured serum adiponectin concentrations in 75 OSAHS patients and 18 control subjects before sleep and after wake-up and examined the effect of one-night nasal continuous positive airway pressure (nCPAP) on adiponectin in 24 severe OSAHS patients. We investigated the effects of hypoxia on adiponectin in mice and cultured adipocytes with a sustained hypoxia model. Circulating adiponectin levels before sleep and after wake-up were lower in severe OSAHS patients than in control subjects [before sleep: 5.9 ± 2.9 vs. 8.8 ± 5.6 μg/ml ($P < 0.05$); after wake-up: 5.2 ± 2.6 vs. 8.5 ± 5.5 μg/ml ($P < 0.01$), respectively; means ± SD]. Serum adiponectin levels diminished significantly during sleep in severe OSAHS patients ($P < 0.0001$), but one-night nCPAP improved the drop in serum adiponectin levels [−18.4 ± 13.4% vs. −10.4 ± 12.4% ($P < 0.05$)]. In C57BL/6J mice and 3T3-L1 adipocytes, hypoxic exposure decreased adiponectin concentrations by inhibiting adiponectin regulatory mechanisms at secretion and transcriptional levels. The present study demonstrates nocturnal reduction in circulating adiponectin levels in severe OSAHS. Our experimental studies showed that hypoxic stress induced adiponectin dysregulation at transcriptional and posttranscriptional levels. Hypoxic stress is, at least partly, responsible for the reduction of serum adiponectin in severe OSAHS. Nocturnal reduction in adiponectin in severe OSAHS may be an important risk for cardiovascular events or other OSAHS-related diseases during sleep.

nasal continuous positive airway pressure

RECENT STUDIES HAVE DEMONSTRATED that adipose tissue is not only a passive reservoir for energy storage but also produces and secretes a variety of bioactive molecules called adipocytokines, including adiponectin (1a, 20), tumor necrosis factor-α, leptin, and plasminogen activator inhibitor type 1 (PAI-1) (36). Dysregulated production of adipocytokines is associated with the pathophysiology of obesity-related diseases (1a, 9, 27). The biological functions of adiponectin, which we identified as an adipocytokine in the human adipose cDNA library (20), include improvement of glucose (21) and lipid metabolism (26), prevention of inflammation (31) and atherosclerosis (24), and cardiovascular protection (14, 30, 38). Serum adiponectin levels are low in visceral obesity (1a), insulin resistance (10), type 2 diabetes (9), and cardiovascular diseases (29). Previous studies demonstrated the possible association between visceral obesity and obstructive sleep apnea-hypopnea syndrome (OSAHS) (39, 40). More recent studies reported that obese subjects with OSAHS had hypoadiponectinemia (36, 46).

In patients with OSAHS, repetitive nocturnal episodes of apneas elicit hypoxemia, hypercapnia, increased sympathetic activities, surges in blood pressure, increases in cardiac wall stress, and cardiac arrhythmias (19, 35). OSAHS is also associated with hypercoagulability, vascular oxidative stress (44), systemic inflammation, and endothelial dysfunction (18) during sleep. Patients with OSAHS have severe perturbations of autonomic, hemodynamic, humoral, and vascular regulation probably due to hypoxemia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors during sleep that contrast with the physiology for normal sleep (32). In the present study, we measured serum adiponectin levels before sleep and after wake-up in OSAHS patients and control subjects and also examined the alteration in serum adiponectin levels during one-night sleep. We further examined the effect of one-night nasal continuous positive airway pressure (nCPAP) on the alteration of serum adiponectin levels.

Hypoxia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors in OSAHS during sleep could explain the nocturnal fall in circulating adiponectin levels (19, 35). The present study focused on hypoxic stress, although other factors could be involved. On the other hand, previous studies reported that adiponectin is regulated by several factors at both transcriptional (22) and posttranscriptional (28) levels. Previous studies demonstrated that exposure to 1% O_2 hypoxia results in transcriptional suppression in vitro (3, 8, 41, 45). We therefore focused attention on dysregulation of posttranscriptional levels of adiponectin by exposure to hypoxia, similar to the previous report on the regulation of adiponectin by testosterone (28). We investigated the effect of hypoxia on adiponectin in mice and cultured cells, using the sustained hypoxia stress method.

Address for reprint requests and other correspondence: K. Kishida, Dept. of Metabolic Medicine, Graduate School of Medicine, Osaka Univ., 2-2 B-5, Yamada-oka, Suita, Osaka, 565-0871, Japan (e-mail: kkishida@imed2.med.osaka-u.ac.jp).

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MATERIALS AND METHODS

Human Studies

Patients. We studied 93 Japanese patients with OSAHS, including 78 men (45.5 ± 15.0 yr, mean ± SD) and 15 women (51.5 ± 13.0 yr) between February 2006 and March 2007, who were newly diagnosed as having OSAHS. The control group consisted of 18 Japanese control subjects who were free of OSAHS, including 15 men (40.4 ± 12.3 yr) and 3 women (45.2 ± 12.4 yr). All participants underwent overnight cardiorespiratory monitoring (Osaka University: Somté, Compumedics, Melbourne, Australia; Yoshida Suimin-kokyu Clinic: Alice 4 Diagnostics Sleep System, Respironics). Each polysomnographic recording was analyzed for the number of apneas and hypopneas during sleep. The oxygen desaturation index (ODI), the lowest oxygen saturation, the desaturation index, and the time at desaturation below 90% in minutes of total bed time for the entire night were measured. Apnea was defined as arrest of airflow >10 s. Hypopnea, or partial closure of the airway during sleep, was defined as ≥30% reduction in airflow associated with ≥4% desaturation. An obstructive apnea was defined as the absence of airflow in the presence of rib cage and/or abdominal excursions. The apnea-hypopnea index (AHI) was defined as the total number of apneas and hypopneas per hour of sleep. The diagnosis of OSAHS was based on AHI of ≥5 [control = 18 (13 men and 5 women)] and classified as mild AHI ≥5 to <15 [n = 24 (21 men and 3 women)], moderate AHI ≥15 to <30 [n = 12 (8 men and 4 women)], or severe AHI ≥30 [n = 39 (36 men and 3 women)], according to the guidelines of the American Academy of Sleep Medicine Task Force (1).

Twenty-four of 39 patients who had AHI >30 were titrated with nCPAP during polysomnography (Fuji Respironics) by experienced technicians. The critical pressure was determined by an automatic CPAP device (REM star Auto M series with C-Flex, Respironics). The recording methods were described in detail previously (13, 34).

All subjects were engaged in little or no physical activity, but all had a regular annual health check. Each subject was asked to complete a questionnaire on sleep symptoms, family history, medical history, and medications. Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subject had been resting in the supine position for at least 10 min after wake-up. Mean values were determined from two independent measurements taken at 5-min intervals.

Diabetes mellitus was defined according to World Health Organization criteria and/or treatment for diabetes mellitus. Dyslipidemia was defined as a total cholesterol concentration of >220 mg/dl, triglyceride concentration >150 mg/dl, HDL-cholesterol concentration <40 mg/dl, and/or treatment for dyslipidemia. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or treatment for hypertension. Patients with a previous diagnosis of dyslipidemia, hypertension, or diabetes mellitus and receiving drugs for any of these conditions were also included in this study. The numbers of patients on medications known to increase serum adiponectin levels, such as pioglitazone (7), angiotensin receptor blockers (16), and/or fibrates (22), were two (control), two (mild OSAHS), one (moderate OSAHS), and three (severe OSAHS). These subjects were not excluded from the study. We also included subjects receiving medical treatment for diabetes mellitus, dyslipidemia, or hypertension. The numbers of patients with diabetes mellitus were 7 (control), 7 (mild OSAHS), 3 (moderate OSAHS), and 10 (severe OSAHS). The numbers of patients with dyslipidemia were 6 (control), 11 (mild OSAHS), 6 (moderate OSAHS), and 18 (severe OSAHS). The numbers of patients with hypertension were 6 (control), 6 (mild OSAHS), 5 (moderate OSAHS) and 19 (severe OSAHS). Patients with recent myocardial infarction or stroke, upper airway surgery, class III/IV heart failure, pregnancy, or chronic renal failure, or who were on systemic steroid treatment or hormonal replacement therapy, were excluded from this study.

Measurement of serum adiponectin concentrations. In each sleep study, venous blood samples were obtained before sleep and after wake-up while the subject was in the supine position. For the purpose of the present study, serum samples that were obtained at baseline from each study participant and stored at −20°C were thawed and assayed for adiponectin levels by sandwich enzyme-linked immunosorbent assay (ELISA) (Otsuka, Japan) (1a, 7, 11, 15, 28). The Medical Ethics Committee of Osaka University approved this study. All subjects enrolled in this study were Japanese, and each gave written informed consent.

Animal and Cell Culture Studies

Animals and exposure to hypoxia. Male C57BL/6J mice (each group n = 5 or 6) were obtained from Clea Japan (Tokyo, Japan) and kept under a 12:12-h light-dark cycle (lights on 8:00 AM to 8:00 PM) and constant temperature (22°C) with free access to food (Oriental Yeast, Osaka, Japan) and water. Male mice were housed in cages exposed to room air (ambient atmosphere) or in hypoxia chambers (Teijin Pharma, Osaka, Japan) at ~10% O_2. This O_2 concentration is often used for hypoxia stress study in vivo (25).

Measurement of serum adiponectin concentrations and adipose adiponectin mRNA expression in mice. Mice were used at 10–13 wk of age in this study, because serum adiponectin levels decrease gradually in younger mice and can be influenced by body weight gain in older mice. Mice were killed under pentobarbital sodium anesthesia (50 mg/kg body wt) at the indicated times under each condition, and then various tissues and blood samples were collected. Each sample was subjected to measurement of serum and mRNA [with real-time quantitative polymerase chain reaction (rt-PCR)] as described previously (1a, 7). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

Measurement of adiponectin secretion into medium and mRNA expression in cell cultures. 3T3-L1 cells were maintained and differentiated as described previously (7, 28). On *day 7*, the cells were cultured for 12 h under 10% or 15% O_2 hypoxia or control conditions (18–21% O_2-5% CO_2; each group n = 6). An aliquot of the culture medium was subjected to measurement of adiponectin (by ELISA), and the cells were harvested for mRNA (rt-PCR) as described previously (7, 28). Briefly, total RNAs were extracted by using RNA STAT-60 (Tel-Test, Friendswood, TX). First-strand cDNA was synthesized from 320 ng of total RNA with the Thermoscript reverse transcription-polymerase chain reaction system (Invitrogen, Carlsbad, CA). rt-PCR amplification was conducted with the ABI PRISM 7900HT Sequence Detection system and the SDS Enterprise Database (Applied Biosystems, Foster City, CA) using SYBR Green polymerase chain reaction Master Mix (Applied Biosystems). The final result for each sample was normalized to the respective 36B4 in consideration for its stability, as reported previously (8). We also investigated 18S ribosomal RNA and cyclophilin as other internal standards in this study. The sequences of the primers used for rt-PCR were as follows: adiponectin, 5′-GATGGCAGAGATGGCACTCC-3′ and 5′-CTTGCCAGTGCTGCCGTCAT-3′; 36B4, 5′-AAGCGCGTCCTGGCATTGTCT-3′ and 5′-CCGCAGGGGAGCAGTGGT-3′.

Pulse-chase studies. Pulse-chase studies were performed to analyze the secretion steps of the newly synthesized adiponectin proteins, according to the procedure described previously (11, 28). 3T3-L1 adipocytes (*day 7*) were plated onto six-well plates and were incubated with fetal calf serum (FCS)-free complete Dulbecco's modified Eagle's medium (DMEM) for 12 h. For metabolic labeling, cells were washed with PBS and incubated with methionine- and cysteine-free DMEM without serum for 30 min to deplete the intracellular pools. The depletion medium was removed, and the cells were incubated in 1 ml of methionine- and cysteine-free DMEM containing 100 μCi/ml of L-[^{35}S]methionine and L-[^{35}S]cysteine (Pro-mix L-[^{35}S] in vitro labeling mix; GE Healthcare). For immunoprecipitation of radiola-

beled adiponectin in medium and cell lysates, metabolic labeling was performed for 2 h. The labeling medium was then replaced with 1 ml of FCS-free DMEM and set into an incubator under control condition or hypoxia (15% O_2-5% CO_2 atmosphere) for 1, 2, 4, 8, or 12 h. The medium and cell lysates were collected at indicated times for immunoprecipitation assays. At each time point, the medium was collected, and the cells were washed with PBS without calcium and magnesium ions, suspended in 300 μl of disruption buffer [mmol/l: 10 Tris·HCl (pH 7.5), 150 NaCl, 5 EDTA, 10 benzamidine, and 1 PMSF, with 1% Nonidet P-40], and lysed with three repetitive freeze-thaw cycles. The cell lysates were adjusted to equal protein concentration with disruption buffer and subjected to immunoprecipitation. A total of 500 μl of cell lysates (100 μg of total protein) was mixed with an equal volume of disruption buffer lacking EDTA and Nonidet P-40 and immunoprecipitated with 5 μl of rabbit polyclonal antibody against mouse adiponectin (OCT12202) overnight at 4°C, followed by incubation with 40 μl of protein G beads for 2 h at 4°C. For the medium, aliquots (300 μl) of metabolically labeled culture medium were added to 200 μl of 2.5× immunoprecipitation buffer [IPB; 1× IPB = (mmol/l) 10 Tris·HCl (pH 7.5), 150 NaCl, 1 EDTA, 10 benzamidine, and 1 PMSF, with 1% Nonidet P-40] and immunoprecipitated as described above. The immunoprecipitates were then washed three times with 1× IPB, followed by solubilization with a sample buffer, and subjected to SDS-PAGE. After electrophoresis the gel was dried, and radiolabeled proteins were analyzed by autoradiography. The band intensities were quantified by densitometry.

Statistical Analysis

Continuous variables are presented as means ± SD and were compared by one-way or two-way analysis of variance (ANOVA) with Fisher's protected least significant difference test for multiple-group analysis or unpaired Student's *t*-test for experiments with only two groups. In all cases, P values <0.05 were considered statistically significant. All analyses were performed with the STATVIEW 5.0 system (HULINKS, Tokyo, Japan). Animal and cell experiments were performed at least three times. We used power analysis to set the minimum requirement of case numbers required to obtain "statistically significant" results for validation of our hypothesis.

RESULTS

Human Studies

Serum adiponectin levels in patients with OSAHS and control subjects. The characteristics of the subjects enrolled in this study are presented in Table 1. AHI, ODI 4%, and the percentage of arterial O_2 saturation from pulse oximetry (Sp_{O_2}) <90% were significantly higher and the lowest Sp_{O_2} were significantly lower in OSAHS patients than in the control subjects (Table 1). Serum adiponectin concentrations at 7:00 AM in patients with severe OSAHS (5.2 ± 2.6 μg/ml, mean ± SD) were significantly lower than in control subjects (8.5 ± 5.5 μg/ml; Fig. 1) ($P < 0.01$).

Next, we focused on nocturnal alternation in serum adiponectin levels. The mean serum adiponectin concentrations before sleep (at 8:00 PM) in patients with severe OSAHS (5.9 ± 2.9 μg/ml) were significantly lower than in control subjects (8.8 ± 5.6 μg/ml, $P < 0.05$; Fig. 1). Furthermore, in patients with severe OSAHS, adiponectin levels were significantly lower after wake-up (5.2 ± 2.6 μg/ml) than before sleep (5.9 ± 2.9 μg/ml, $P < 0.0001$; Fig. 1). However, there were no significant differences in circulating adiponectin levels between the two samples obtained at 8:00 PM and 7:00 AM in moderate OSAHS, mild OSAHS, and control groups. There was no significant difference in the form of adiponectin multimers between before sleep and after wake-up in patients with severe OSAHS (data not shown).

Effects of one-night nCPAP treatment on serum adiponectin levels. nCPAP is the gold standard treatment for OSAHS (23). We investigated the effect of one-night nCPAP treatment

Table 1. *Clinical and biochemical characteristics of subjects*

		OSAHS		
	Control (AHI < 5)	Mild (5 ≦ AHI < 15)	Moderate (15 ≦ AHI < 30)	Severe (30 ≦ AHI)
Number	18	24	12	39
Sex, male/female	13/5	21/3	8/4	36/3
Age, yr	43.8±12.2	45.9±12.6	52.1±14.1	46.4±14.3
BW, kg	66.9±14.8	77.1±19.9	77.5±17.3	87.5±19.0[a,d]
BMI, kg/m²	23.8±4.0	26.7±5.8	28.9±4.0[f]	30.9±6.4[a,c]
Waist circumstance, cm	86.7±10.9	92.2±13.7	98.9±10.5[f]	100.7±12.8[a,d]
AHI, events/h	2.4±1.5	8.9±2.7[j]	19.4±4.3[f,h]	57.8±18.6[a,c,e]
ODI 4%, events/h time in bed	2.0±2.0	6.7±4.6[i]	16.6±6.9[f,h]	52.5±22.0[a,e]
Baseline Sp_{O_2}, %	96.8±1.4	95.6±1.9[i]	95.2±2.1[f]	94.4±2.0[a,d]
Lowest Sp_{O_2}, %	88.3±3.9	84.1±5.6[i]	78.8±3.3[f,h]	67.6±10.7[a,c,e]
Sp_{O_2} <90%, % time in bed	0.1±0.1	1.6±2.9[i]	6.7±7.6[f,h]	30.3±21.9[a,c,e]
Fasting glucose, mg/dl	112±26	103±14	117±43	113±19[d]
IRI, μU/ml	9.1±5.7	12.5±7.4	14.1±7.5	19.1±13.8[a]
HOMA-IR	2.4±2.1	3.2±2.4	3.7±2.7	5.3±4.1[a,c]
Total cholesterol, mg/dl	208±40	202±29	207±58	202±25
Triglyceride, mg/dl	187±146	155±79	167±92	156±80[a]
HDL cholesterol, mg/dl	56±17	44±8[j]	41±12[g]	45±11[a]
Systolic blood pressure, mmHg	113±18	125±15[j]	125±11[g]	129±15[a]
Diastolic blood pressure, mmHg	72±11	75±11	79±11	85±14[c]
Serum adiponectin before sleep, μg/ml	8.8±5.6	7.9±5.4	6.0±3.0	5.9±2.9[b]
Serum adiponectin after wake-up, μg/ml	8.5±5.5	7.9±5.6	5.8±3.0	5.2±2.6[a,d]

Data are means ± SD. OSAHS, obstructive sleep apnea-hypopnea syndrome; BW, body weight; BMI, body mass index; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; Sp_{O_2}, arterial O_2 saturation from pulse oximetry; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment-insulin resistance. [a]$P < 0.01$ (control vs. severe); [b]$P < 0.05$ (control vs. severe); [c]$P < 0.01$ (mild vs. severe); [d]$P < 0.05$ (mild vs. severe); [e]$P < 0.01$ (moderate vs. severe); [f]$P < 0.01$ (control vs. moderate); [g]$P < 0.05$ (control vs. moderate); [h]$P < 0.01$ (mild vs. moderate); [i]$P < 0.01$ (control vs. mild); [j]$P < 0.05$ (control vs. mild).

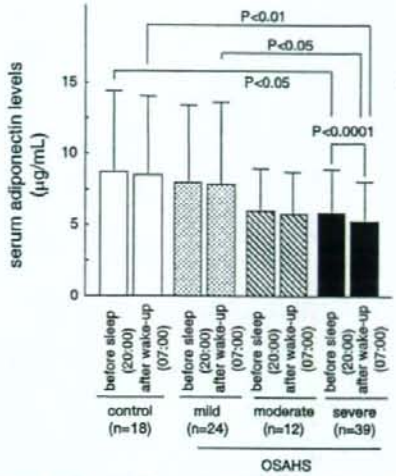

Fig. 1. Human studies. Circulating adiponectin levels measured by ELISA (1a) before sleep and after wake-up in control subjects (n = 18) [apnea-hypopnea index (AHI) <5/h] and patients with obstructive sleep apnea-hypopnea syndrome (OSAHS; n = 75) [mild: AHI 5–15/h (n = 24); moderate: AHI 15–30/h (n = 12); severe AHI ≥30/h (n = 39)]. Similar results were obtained on other independent days. Data are means ± SD.

on serum adiponectin levels in 24 patients with severe OSAHS (AHI ≥ 30). One-night nCPAP treatment significantly decreased AHI, ODI 4%, and the time spent at Sp_{O_2} <90% (data not shown). Individual data are shown in Fig. 2*A*. The percent change in serum adiponectin level [Δadiponectin: (serum adiponectin concentrations after wake-up − before sleep)/before sleep (%)] before one-night nCPAP treatment was −19.1 ± 13.1%, whereas that after nCPAP treatment significantly improved to −10.9 ± 11.8% ($P < 0.05$; Fig. 2*B*).

Animal and Cell Culture Studies

The present study focused on the effect of hypoxia on adiponectin, which is at least partly a pathophysiological factor in severe OSAHS, although other OSAHS-related factors could be involved. We investigated the effect of exposure to sustained hypoxia on adiponectin in C57BL/6J mice and cultured 3T3-L1 adipocytes, using the sustained hypoxia stress method. Exposure to hypoxia for 4 days resulted in significant suppression of serum adiponectin concentrations and significant change in adipose adiponectin mRNA expression compared with control ($P < 0.01$). Furthermore, exposure to hypoxia for 2 days suppressed serum adiponectin levels, with no apparent change in adipose mRNA expressions (Fig. 3*A*). Exposure to hypoxia inhibited adiponectin secretion from cultured 3T3-L1 adipocytes at both transcriptional (10% O_2 hypoxia) and posttranscriptional (15% O_2 hypoxia) levels, compared with control (Fig. 3*B*). For further analysis of the posttranscriptional dysregulation of adiponectin, we performed pulse-chase experiments to examine the inhibitory effects of 15% O_2 hypoxia on the secretion of newly synthesized adiponectin protein in 3T3-L1 adipocytes. The secretion of radiolabeled adiponectin was continuously inhibited with exposure to 15% O_2 hypoxia and from 1 to 12 h of chasing period (Fig. 4*A*). However, adipocytes exposed to hypoxia showed retention of the labeled adiponectin intracellularly (Fig. 4*B*).

DISCUSSION

We found significantly lower levels of serum adiponectin in patients with severe OSAHS, similar to previous reports in OSAHS patients (36, 46). The alternation of serum adiponectin levels during one-night sleep in severe OSAHS has not been reported. In the present study, we found nocturnal reduction in serum adiponectin levels in patients with severe OSAHS. In addition, such reductions were ameliorated by one-night nCPAP treatment. These results indicate that one-night nCPAP treatment attenuates the nocturnal reduction of serum adiponectin levels. Although high-molecular-weight adiponectin is significantly low in patients with coronary artery disease or obesity (11, 12), in the present study there was no significant difference in the form of adiponectin multimers between before sleep and after wake-up in patients with severe OSAHS (data not shown). In mice and cultured 3T3-L1 adipocytes, exposure to hypoxia decreased adiponectin concentrations by inhibiting adiponectin regulatory mechanisms at both secretion and transcriptional levels.

nCPAP reduces the risk of fatal and nonfatal cardiovascular outcomes (23). In the present study, one-night nCPAP treatment reduced the nocturnal reduction in serum adiponectin,

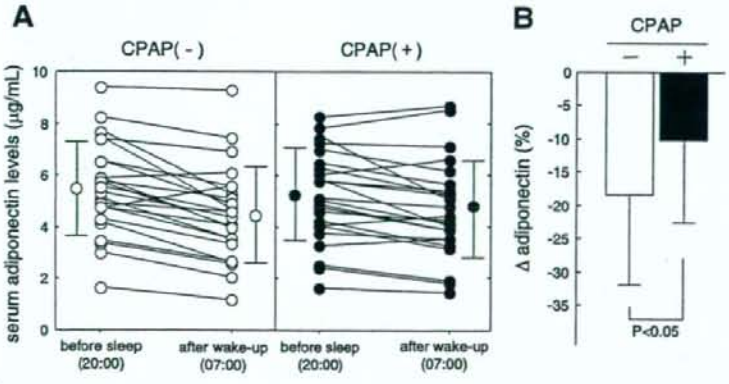

Fig. 2. Human studies. Serum adiponectin levels in patients with severe OSAHS (n = 24) before sleep and after wake-up, with or without whole 1-night nasal continuous positive airway pressure (nCPAP) treatment. *A*: individual serum adiponectin levels quantified by ELISA. *B*: Δadiponectin with or without whole 1-night nCPAP treatment. Data are means ± SD. Similar results were obtained on other independent days.

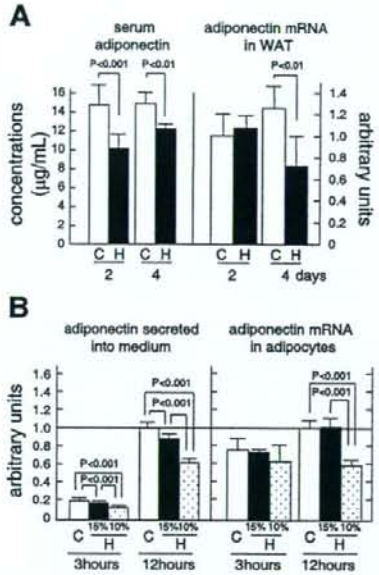

Fig. 3. *A*: mouse studies. Dysregulation of adiponectin in hypoxic mice. Mice were housed in chambers under control (C; $n = 5$) or hypoxic (H; $n = 6$) conditions for the indicated time periods. Levels of serum adiponectin were measured by ELISA (1a). Total mRNA was extracted from the tissue of individual mice and subjected to real-time quantitative PCR analysis to determine the mRNA levels of adiponectin in white adipose tissue (WAT; epididymal fat tissues). Data were normalized against 36B4 mRNA. The values of mice at 2 days were arbitrarily set as 1.0. Data are means ± SD. Similar results were obtained in 2 other independent experiments. *B*: cultured cell studies on secretion and adiponectin protein and mRNA levels in 3T3-L1 adipocytes. 3T3-L1 cells were cultured on *day 7* under control ($n = 6$) or hypoxic (15% or 10% O_2, $n = 6$) conditions for the indicated time periods. Levels of adiponectin secreted into the culture medium for the indicated time intervals were analyzed by ELISA. Adiponectin mRNA expression levels in adipose tissues were measured as described in MATERIALS AND METHODS. The values of the 12-h control group were arbitrarily set as 1.0. Data are means ± SD. This experiment was performed 3 times with similar results.

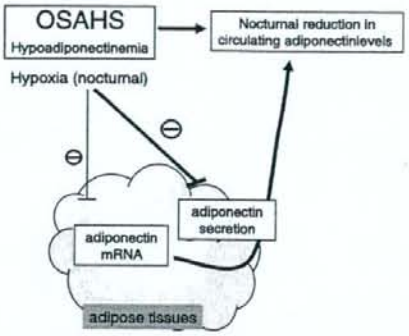

Fig. 5. Schematic presentation of the nocturnal reduction of adiponectin in patients with severe OSAHS. Profound hypoxemia suppresses adiponectin mRNA level in adipose tissue, as reported previously (3, 8, 41, 45). In addition, hypoxia inhibits the secretion of adiponectin. Hypoxic stress of local adipose tissue during sleep seems to play, at least in part, a role in dysregulation of adiponectin production, although other OSAHS-related factors could be involved. Nocturnal reduction of adiponectin may be an important risk for OSAHS-related diseases in patients with severe OSAHS.

suggesting that cyclical hypoxemia can have a short-term effect. The effects of CPAP treatment may be related to improvement of sleep quality, metabolism, and other factors; therefore, improvement of the drop in adiponectin levels with one-night nCPAP may be due not only to removing hypoxia partly but also to alteration of other OSAHS-related factors. Although we did not investigate the long-term effect of nCPAP treatment in the present study, several reports found no significant changes in serum levels of adiponectin after 3 mo of long-term nCPAP treatment (6, 42), suggesting that the lack of a long-lasting change in adiponectin can be explained by the influence of body mass on adiponectin secretion, which was unchanged during nCPAP treatment. Considered together, the results of one-night nCPAP seem different from those of long-term nCPAP treatment. Longitudinal and interventional studies are required to compare the long- and short-term effects of nCPAP.

Hypoxia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors in OSAHS during sleep could explain the nocturnal fall in circulating adiponectin levels (19, 35). The present study focused on hypoxic stress (intermittent and sustained), although other factors could be involved. We examined the effect of intermittent hypoxia on adiponectin in cultured cells, using the intermittent hypoxia model to investigate the effect of desaturation-reoxygenation or other complex mechanisms (33). Preliminary results showed that the fall in adiponectin levels was dependent on the total time of

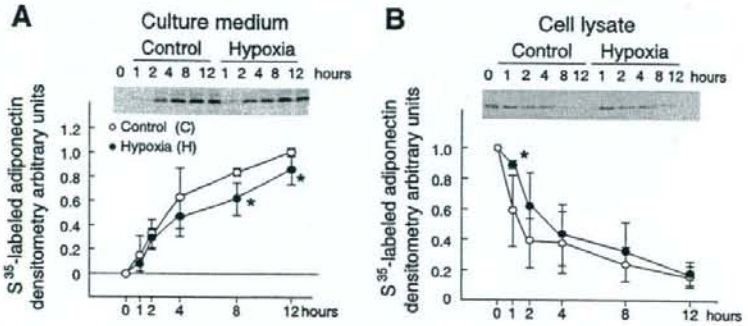

Fig. 4. Pulse-chase experiments of ^{35}S-labeled adiponectin under control or hypoxic (15% O_2 hypoxia) conditions in 3T3-L1 adipocytes. Representative autoradiographic data of adiponectin secretion (*A*) and adipose adiponectin proteins (*B*) are shown. Band intensities were quantified by densitometry in the media and cell lysates at the indicated time intervals ($n = 5$). Data are means ± SD. $^*P < 0.01$ vs. control. Similar results were obtained in 3 other independent experiments.

hypoxic exposure, regardless of whether the hypoxic stress was sustained or intermittent (data not shown). We therefore investigated the regulation of adiponectin under sustained hypoxia both in vivo and in vitro. We tried several hypoxic concentrations of O_2 in these experiments, including 3%, 5%, 8%, 9%, 10%, and 15% in vitro. The present study demonstrated that exposure to 3%, 5%, 8%, 9%, or 10% hypoxia resulted in suppression of adiponectin mRNA expressions, similar to 1% O_2 hypoxia in previous studies (3, 8, 41, 45), and 15% O_2 hypoxia suppressed adiponectin production posttranscriptionally, in agreement with a previous report on the regulation of adiponectin by testosterone (28). The results of these in vivo and in vitro studies suggest dysregulated adiponectin production at transcriptional and posttranscriptional levels by hypoxic stress.

Figure 5 provides a summary of a working model based on the results of our previous (8) and present studies. In OSAHS, while multiple pathophysiological mechanisms influence adiponectin production, hypoxic stress of local adipose tissue during sleep seems to play, at least in part, a role in dysregulation of adiponectin production. Further studies should examine the regulation of adiponectin by other OSAHS-related factors.

Cardiovascular disturbances are the most serious complications in OSAHS (17, 35, 43). Gami et al. (5) reported that people with sudden death from cardiac causes during nocturnal sleep had a significantly higher AHI than those with sudden death from cardiac causes during other intervals, and that AHI correlated directly with the relative risk of sudden death from cardiac causes (5) and angina attack (2, 4) during night sleep. However, the exact mechanism of death remains unclear. Nocturnal reduction in adiponectin in patients with severe OSAHS may be an important risk for cardiovascular events or other OSAHS-related diseases during sleep. Further investigation is required.

In conclusion, the present study demonstrated nocturnal reduction in circulating adiponectin levels in severe OSAHS. Our in vivo and in vitro studies showed that hypoxic stress induced adiponectin dysregulation at both transcriptional and posttranscriptional levels. Hypoxic stress is, at least in part, responsible for nocturnal reduction of serum adiponectin levels in severe OSAHS. Evaluation of changes in circulating adiponectin levels during sleep may be conducted in OSAHS-related diseases.

Limitation of the Study

We included patients on medications known to increase serum adiponectin levels, such as pioglitazone (7), angiotensin receptor blockers (16), and/or fibrates (22); however, the results were similar in adiponectin levels when patients who were using medication that could influence adiponectin levels were excluded (data not shown).

Serum adiponectin levels are low in obesity (1a) and insulin resistance (10). The present study lacks the clear advantage of body mass index- and insulin resistance-matched studies, because we could not find a sufficient number of control subjects matched for weight and insulin sensitivity to patients with OSAHS or nonobese patients with OSAHS. Further studies of larger samples of heterogeneous OSAHS patients should be conducted in the future.

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Prognostic Value of Adiponectin for Cardiovascular Disease and Mortality

Jacqueline M. Dekker, Tohru Funahashi, Giel Nijpels, Stefan Pilz, Coen D. A. Stehouwer, Marieke B. Snijder, Lex M. Bouter, Yuji Matsuzawa, Iichiro Shimomura, and Robert J. Heine

EMGO Institute (J.M.D, G.N., M.B.S., L.M.B., R.J.H.), Departments of General Practice (G.N.) and Endocrinology (R.J.H.), VU University Medical Center, 1081 BT Amsterdam, The Netherlands; Department of Internal Medicine and Molecular Science (T.F., Y.M., I.S.), Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan; Department of Internal Medicine (S.P.), Division of Endocrinology and Nuclear Medicine, Medical University of Graz, 8010 Graz, Austria; Department of Public Health, Social and Preventive Medicine (S.P.), Mannheim Medical Faculty, University of Heidelberg, 68135 Mannheim, Germany; Department of Internal Medicine (C.D.A.S.), Academic Hospital Maastricht, 6229 HX Maastricht, The Netherlands; and Institute of Health Sciences, Faculty of Earth and Life Sciences (M.B.S.), Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands

Context: Low adiponectin concentrations are associated with the presence of an adverse cardiovascular disease (CVD) risk profile.

Objective: We studied the predictive value of adiponectin levels for all-cause and CVD mortality and CVD morbidity.

Design, Setting, and Participants: This was a population-based cohort study in Hoorn, The Netherlands, which started in 1989 and included 2484 participants, aged 50–75 yr.

Main Outcome Measures: Hazard ratios (HRs) with 95% confidence interval per SD change in log-adiponectin for all-cause and CVD mortality and CVD morbidity were calculated.

Results: Adiponectin was determined for 1077 men and 1248 women. Higher adiponectin reduced the risk of nonfatal CVD in women [HR with 95% confidence interval 0.72 (0.61–0.90) in women and 0.92 (0.79–1.06) in men], but not the risk of all-cause or CVD mortality. In contrast, after adjustment for cardiovascular risk factors, higher adiponectin was a significant predictor of all-cause and CVD mortality [HR for CVD mortality 1.45 (1.10–1.92) in women and 1.30 (1.04–1.63) in men]. Higher adiponectin was associated with an increased risk of CVD mortality in people with prevalent CVD [HR 1.27 (0.98–1.63)] and with reduced risk in people without [HR 0.90 (0.73–1.11)]. After adjustment for cardiovascular risk factors, the HRs for CVD mortality were 1.60 (1.14–2.23) for patients with and 1.38 (1.06–1.80) for patients without prevalent CVD.

Conclusions: High levels of adiponectin predict mortality, in particular in patients with prevalent CVD. We hypothesize that adiponectin protects against metabolic and vascular diseases, but in patients already afflicted with CVD, adiponectin is compensatory up-regulated and, therefore, indicates a high mortality risk. ***J Clin Endocrinol Metab* 93: 1489–1496, 2008)**

Adiponectin is an adipocytokine, which is mainly produced by the adipose tissue (1). Although it is the most abundantly produced protein of the fat cell, plasma levels are reduced in obese patients. There is growing evidence that reduced adiponectin concentrations indicate an increased cardiovascular risk because hypoadiponectinemia is associated with the components of the metabolic syndrome, in particular with insulin resistance, elevated triglycerides, and low high-density lipoprotein (HDL) (1, 2). Apart from this, adiponectin possesses anti-inflammatory properties and exerts direct antiatherosclerotic and cardioprotective effects (2). Clinical studies have shown that low adiponectin concentrations are associated with endothelial

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Abbreviations: ALT, Alanine aminotransferase; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio.

dysfunction, increased carotid intima-media thickness, and coronary artery disease (2–4). High adiponectin concentrations are independent of other cardiovascular risk factors associated with a lower prevalence of acute coronary syndromes (4). Therefore, it was suggested that low adiponectin concentrations are a cardiovascular risk factor and that therapeutic strategies that enhance the secretion or action of this adipocytokine might reduce the incidence of cardiovascular diseases (CVDs) (1, 2).

However, several recently published studies on the prospective association between adiponectin and CVD events/mortality showed inconsistent results. Five studies reported that adiponectin was not independently associated with future CVD (5–9). Low adiponectin concentrations turned out as a risk factor for future CVD in some studies (10–16), whereas others showed that high adiponectin levels were associated with an increased risk of CVD and/or mortality (17–23). The underlying mechanisms for these contradictory results are still unclear but may be due to differences in the study populations. Toward this, it was speculated that low adiponectin predicts cardiovascular events in low-risk populations for CVD, whereas in high-risk populations, a counter-regulatory increase of adiponectin occurs that is responsible for the elevated cardiovascular risk associated with high adiponectin levels (24, 25). To test this hypothesis, we studied the association of adiponectin with 15-yr all-cause and CVD mortality and 10-yr nonfatal CVD in the Hoorn Study, a large population-based study, distinguishing subjects with and without a history of CVD.

Subjects and Methods

Study population

The Hoorn Study is a Dutch cohort study of diabetes and diabetes complications in the general population, which started in 1989. The cohort and the baseline measurements have been described in detail previously (26). Briefly, a random selection of 3553 men and women of 50–75 yr old was taken from the population register. A total of 2540 (71.5%) agreed to participate, and after exclusion of 56 non-Caucasian participants, the Hoorn Study population consisted of 2484 men and women. For the present study, we excluded 159 subjects with missing adiponectin data, leaving 1077 men and 1248 women for the analyses. This number of study probands was not determined by power calculations for specific hypotheses. All participants gave their written informed consent. The study was approved by the Ethics Committee of the Vrije Universiteit Medical Center.

Baseline examination and measurements

At the baseline medical examination, a blood sample was taken from all participants after overnight fasting. Adiponectin was determined in 2004 in spare baseline plasma samples that had been stored at −80 C and had never been thawed before. Adiponectin was determined with a latex turbidometric immunoassay. The interassay and intraassay coefficients of variation were less than 2.0% and less than 3.1%, respectively. A standard 75-g oral glucose tolerance test was performed in all subjects, except those using glucose-lowering medication. Plasma glucose was determined with a glucose dehydrogenase method (Merck, Darmstadt, Germany). Diabetes and impaired glucose metabolism were defined according to World Health Organization criteria of 1999 (27). Fasting insulin was determined with an insulin-specific double-antibody RIA (antibody: LINCO SP21; LINCO Research, Inc., St. Louis, MO). Fasting triglycerides, and total and HDL-cholesterol were determined by enzymatic techniques (Roche Molecular Biochemicals, Mannheim, Germany). Serum alanine aminotransferase (ALT) enzyme activity was measured according to the method of the International Federation of Clinical Chemistry from 1985, and expressed as U/liter. Serum creatinine level was determined in μmol/liter, and renal function [glomerular filtration rate (GFR)] was estimated by the Cockcroft-Gault formula in ml/min·1.73 m^2.

Waist and hip circumference, weight, and height were measured. Body mass index (BMI) was calculated as the ratio of weight and height squared. Blood pressure was measured twice on the right arm with a random-zero sphygmomanometer (Hawksley-Gelman Ltd., Lancing, UK), and the mean was used for computations. Information about use of medication, including antihypertensive medication, smoking status (nonsmokers, ex-smokers, and current smokers), and history of CVD at baseline (assessed by Rose Questionnaire) were determined by a self-administered questionnaire. Cigarette-years were calculated as the product of years smoked and mean number of cigarettes per day.

Follow-up of morbidity and mortality

The cohort was followed with respect to morbidity and mortality. Vital status was obtained from the population register of the city of Hoorn. Causes of death were coded by reviewing death certificates, and nonfatal CVD events were classified using medical records of general practitioners and the local hospital. Causes of death were coded according to the International Classification of Diseases, Injuries and Causes of Death, ninth revision.

CVD mortality was defined with International Classification of Diseases, Injuries and Causes of Death codes 390–459 (diseases of the circulatory system) or 798 (sudden death, cause unknown) because sudden death in general is of CVD origin. Vital status until January 2004 was known for all subjects. Cause of death could not be obtained for 35 men and 28 women.

Data on nonfatal outcomes were complete until 2000, for 845 men and 909 women who gave permission to access their hospital files and/or contact their general practitioners. Nonfatal CVD was defined as documented angina pectoris (chest pain, followed by coronary artery bypass surgery or angioplasty, or in the presence of more than 50% stenosis or electrocardiographic changes or positive exercise test), myocardial infarction (in the presence of at least two of the following: typical pain, elevated enzymes, and/or electrocardiogram changes), congestive heart failure (in the presence of at least two of the following: shortness of breath, cardiomegaly, dilated neck veins, or one of the former in the presence of edema or tachycardia), stroke or transient ischemic attack (sudden onset of symptoms, neurological symptoms, or change of consciousness), or peripheral disease (by procedure or typical pain accompanied by stenosis or ankle arm blood pressure ratio < 0.90 or positive vascular stress test).

Statistical analyses

Population characteristics were compared between sex-specific quartiles of baseline plasma adiponectin levels. Analyses were performed separately for men and women because of the different fat distribution in women and because women have higher levels of adiponectin than men. For proportions, trends over the quartiles were tested with the χ^2 test with P for linear-by-linear test. For continuous variables the Pearson and age-adjusted partial correlations with the log of adiponectin were determined. Survival curves of 15-yr all-cause mortality and CVD mortality and 10-yr nonfatal CVD in quartiles of adiponectin were plotted. Age-adjusted hazard ratios (HRs) for all-cause mortality, CVD mortality, and nonfatal CVD for the second through the fourth quartile relative to the lowest quartile of adiponectin were estimated with Cox proportional hazards analyses. We first adjusted for all possible mediating or confounding variables one by one, and then combined all these variables into one model. To study the possible modifying effect of the presence of diabetes or prevalent CVD, stratified analyses were performed. A P value less than 0.05 was considered statistically significant. Statistical analyses

were performed with SAS for windows, version 8.0 (SAS Institute Inc., Cary, NC) and with SPSS, version 15.0 (SPSS, Inc., Chicago, IL).

Results

Mean adiponectin was higher in women than men, and was increasing with age and lower GFR in both genders (Tables 1 and 2). High adiponectin was strongly associated with a more favorable CVD risk profile, with lower weight, smaller waist, higher HDL cholesterol and lower triglycerides, lower insulin and glucose levels, and lower ALT. Associations were not significant for total cholesterol. In women there was a trend of lesser smoking and higher participation in sports activities with higher levels of adiponectin. In men the reverse was observed for smoking, and there was no significant association with sports activities. Adiponectin was not associated with prevalent CVD at baseline. The baseline characteristics according to adiponectin quartiles for study participants with and without prevalent CVD are shown in supplemental Tables A and B (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Until January 2004, after 15-yr follow-up, 286 men and 219 women had died, among whom 121 men and 83 women due to CVD. Until January 2000, after 10-yr follow-up, 195 men and 128 women had a nonfatal CVD event. As shown in Fig. 1, all-cause mortality was highest in both men and women with the highest adiponectin levels. The higher risk of mortality could not be attributed to any particular causes, and the association was also observed for cancer mortality, and noncancer non-CVD mortality (data not shown).

As shown in Table 3, adjustment for age did not explain the positive association between adiponectin and all-cause mortality in men. In women, but not in men, there was a U-shaped association with all-cause and CVD mortality, and a significant negative association with nonfatal CVD was observed in women and a nonsignificant trend in men. Adjustment for possible mediating or confounding variables resulted in a strong and statistically significant increased risk of CVD mortality with higher adiponectin level for both men and women (Table 4). The same pattern was observed for all-cause mortality and for nonfatal CVD (data not shown).

When we stratified for the presence of type 2 diabetes, no differences were observed in the relationships for all-cause and CVD mortality, or for nonfatal CVD (data not shown). For all-cause and CVD mortality analyses of the entire study population, we observed P values for interaction terms of prevalent CVD with dummy variables of adiponectin quartiles all less than 0.15. Therefore, we performed subgroup analyses and found that high adiponectin was associated with increased mortality risk in both sexes with prevalent CVD at baseline (Table 5). In women without prevalent CVD, the mortality risk was reduced in higher adiponectin quartiles, and in men there was a U-shaped associ-

TABLE 1. Baseline characteristics for women according to quartiles of adiponectin

	Quartiles of adiponectin				Partial correlation[a]	P value[b]
	1 (n = 310)	2 (n = 314)	3 (n = 311)	4 (n = 313)		
Adiponectin (μg/liter)	8.10 ± 1.59	12.10 ± 1.07	16.01 ± 1.28	24.61 ± 5.87		
Age (yr)	61.2 ± 7.3	60.9 ± 7.4	61.7 ± 7.3	63.6 ± 7.5	0.154	<0.001
BMI (kg/m²)	27.9 ± 4.0	27.5 ± 4.1	26.4 ± 4.1	25.7 ± 3.5	−0.235	<0.001
WHR	0.88 ± 0.07	0.85 ± 0.07	0.84 ± 0.07	0.82 ± 0.07	−0.360	<0.001
Waist (cm)	91.1 ± 10.3	88.6 ± 10.8	86.3 ± 10.1	83.3 ± 10.2	−0.307	<0.001
Hip (cm)	103.4 ± 7.5	103.8 ± 7.9	102.6 ± 7.7	101.9 ± 7.4	−0.090	0.004
Use of antihypertensives (%)	31.3	25.5	14.2	19.5		<0.001
Diastolic bp (mm Hg)	82.6 ± 10.7	80.9 ± 10.2	80.0 ± 10.4	79.7 ± 10.8	−0.121	<0.001
Systolic bp (mm Hg)	138.5 ± 21.5	134.4 ± 20.9	134.2 ± 20.6	133.4 ± 20.7	−0.143	<0.001
Cholesterol (mmol/liter)	7.02 ± 1.35	6.78 ± 1.18	6.88 ± 1.17	6.82 ± 1.15	−0.038	0.232
HDL cholesterol (mmol/liter)	1.23 ± 0.90	1.40 ± 0.33	1.50 ± 0.31	1.65 ± 0.39	0.449	<0.001
Triglycerides (mmol/liter)	2.09 ± 1.22	1.56 ± 1.02	1.33 ± 0.53	1.21 ± 0.54	−0.417	<0.001
Insulin (pmol/liter)	104.9 ± 52.0	88.8 ± 51.4	77.5 ± 33.2	76.3 ± 41.5	−0.260	<0.001
Glucose (mmol/liter)	6.23 ± 2.20	5.72 ± 1.67	5.53 ± 1.21	5.41 ± 1.40	−0.221	<0.001
2-h glucose (mmol/liter)	7.24 ± 3.84	6.34 ± 3.10	5.85 ± 2.92	5.50 ± 1.93	−0.275	<0.001
HbA_{1c} (%)	5.77 ± 1.16	5.49 ± 0.86	5.40 ± 0.70	5.35 ± 0.69	−0.190	<0.001
GFR (ml/min·1.73 m²)	75.9 ± 18.2	73.7 ± 15.7	70.5 ± 14.6	67.6 ± 14.2	−0.155	<0.001
ALT (IU/liter)	12.7 ± 12.6	11.0 ± 5.8	11.2 ± 6.1	10.0 ± 4.8	−0.137	<0.001
Current cig smoker (%)	37.3	28.6	27.7	19.4		<0.001
Cig years[c]	270 ± 325	219 ± 319	212 ± 310	163 ± 264	−0.112	<0.001
Alcohol (g/d)	5.3 ± 8.5	5.8 ± 9.7	6.2 ± 9.9	5.0 ± 7.6	0.004	0.090
Sport activity (%)	25.7	26.3	30.3	33.9		0.013
CVD (%)	19.4	16.9	11.9	17.4		0.235

Continuous data are presented as means ± SD and categorical data as percentages. bp, Blood pressure; Cig, cigarette; HbA_{1c}, glycosylated hemoglobin; WHR, waist to hip ratio.

[a] Partial (Pearson) correlations are age adjusted, except for age.

[b] P values are for partial correlation coefficients for continuous variables, or for the χ^2 test with P for linear-by-linear test for proportions.

[c] Product of number of cigarettes per day and years smoked.

TABLE 2. Baseline characteristics for men according to quartiles of adiponectin

	Quartiles of adiponectin				Partial correlation[a]	*P* value[b]
	1 (n = 267)	2 (n = 261)	3 (n = 277)	4 (n = 272)		
Adiponectin (μg/liter)	5.54 ± 0.95	7.85 ± 0.60	10.27 ± 0.83	15.58 ± 3.71		
Age (yr)	58.3 ± 6.3	60.5 ± 6.8	61.7 ± 7.4	63.7 ± 7.2	0.208	<0.001
BMI (kg/m²)	27.0 ± 2.9	26.7 ± 2.9	25.9 ± 2.9	25.1 ± 2.7	−0.264	<0.001
WHR	0.96 ± 0.06	0.96 ± 0.06	0.94 ± 0.07	0.93 ± 0.06	−0.250	<0.001
Waist (cm)	97.1 ± 8.5	96.8 ± 8.7	94.6 ± 9.4	92.4 ± 8.7	−0.255	<0.001
Hip (cm)	101.0 ± 5.5	100.8 ± 5.3	100.1 ± 5.3	99.0 ± 5.3	−0.151	<0.001
Use of antihypertensives (%)	21.0	19.5	20.6	8.8		0.001
Diastolic bp (mm Hg)	84.6 ± 9.2	84.0 ± 9.2	83.0 ± 9.9	82.7 ± 11.0	−0.094	0.006
Systolic bp (mm Hg)	135.1 ± 18.1	134.8 ± 18.3	133.7 ± 17.7	136.9 ± 21.4	−0.084	0.015
Cholesterol (mmol/liter)	6.37 ± 1.12	6.47 ± 1.14	6.34 ± 1.08	6.48 ± 1.13	0.042	0.221
HDL cholesterol (mmol/liter)	1.07 ± 0.29	1.13 ± 0.24	1.18 ± 0.29	1.32 ± 0.34	0.304	<0.001
Triglycerides (mmol/liter)	2.06 ± 1.41	1.75 ± 0.87	1.53 ± 0.71	1.38 ± 0.70	−0.247	<0.001
Insulin (pmol/liter)	97.5 ± 53.2	95.3 ± 61.5	88.1 ± 59.6	74.2 ± 49.7	−0.190	<0.001
Glucose (mmol/liter)	5.95 ± 1.44	5.95 ± 1.57	5.71 ± 1.18	5.55 ± 1.15	−0.199	<0.001
2-h glucose (mmol/liter)	6.61 ± 3.40	6.07 ± 3.20	5.53 ± 2.45	5.27 ± 1.87	−0.220	<0.001
HbA_{1c} (%)	5.54 ± 0.84	5.51 ± 0.91	5.42 ± 0.66	5.47 ± 0.69	−0.096	0.006
GFR (ml/min·1.73 m²)	86.4 ± 16.9	80.8 ± 14.4	78.8 ± 16.5	74.5 ± 17.7	−0.133	<0.001
ALT (IU/liter)	17.8 ± 13.3	14.8 ± 8.9	13.6 ± 6.7	12.3 ± 7.8	−0.157	<0.001
Current cig smoker (%)	33.8	37.6	38.9	36.7		0.456
Cig years[c]	433 ± 405	505 ± 444	507 ± 422	563 ± 558	0.087	0.012
Alcohol (g/d)	14.4 ± 15.0	14.0 ± 16.2	11.1 ± 12.8	11.7 ± 13.2	−0.046	0.184
Sport activity (%)	28.6	23.9	25.8	21.8		0.117
CVD (%)	19.1	22.4	24.9	18.8		0.895

Continuous data are presented as means ± SD and categorical data as percentages. bp, Blood pressure; Cig, cigarette; HbA_{1c}, glycosylated hemoglobin; WHR, waist to hip ratio.

[a] Partial (Pearson) correlations are age adjusted, except for age.

[b] *P* values are for partial correlation coefficients for continuous variables, or for the χ^2 test with *P* for linear-by-linear test for proportions.

[c] Product of number of cigarettes per day and years smoked.

ation with reduced risk in the second and increased risk in the third and fourth adiponectin quartile. After adjustments for cardiovascular risk factors (according to the fully adjusted model in Table 4), the HR [with 95% confidence interval (CI)] for cardiovascular mortality including both sexes was 1.38 (1.06–1.80) for patients without and 1.60 (1.14–2.23) for patients with CVD per SD change in log adiponectin. Accordingly, the HR for all-cause mortality was 1.33 (1.15–1.55) for patients without and 1.69 (1.33–2.16) for patients with prevalent CVD, with similar results for sex-stratified analyses (data not shown).

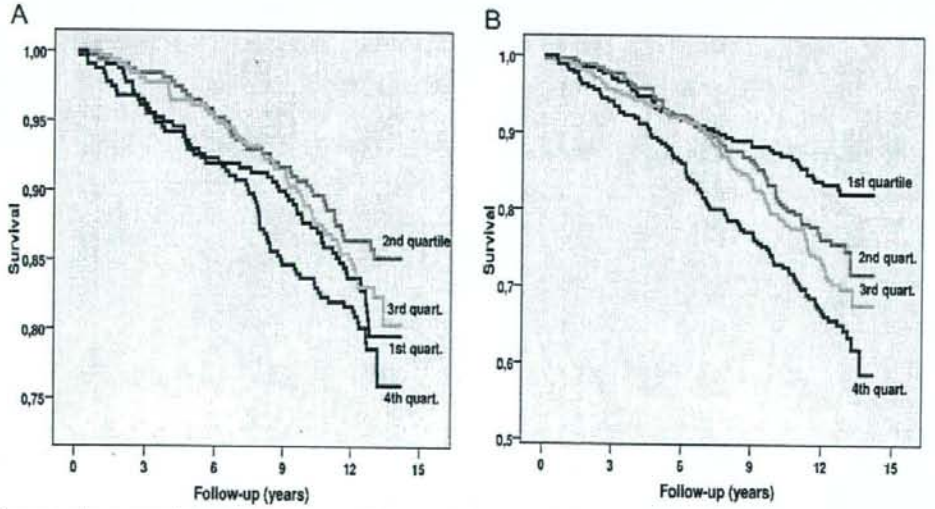

FIG. 1. A, Fifteen-year all-cause mortality according to quartiles (quart) of adiponectin in women. B, Fifteen-year all-cause mortality according to quartiles of adiponectin in men.

TABLE 3. Age-adjusted HRs (with 95% CI) for 15-yr all-cause mortality, 15-yr CVD mortality, and 10-yr nonfatal CVD in sex-specific quartiles of adiponectin

	15-yr all-cause mortality	15-yr CVD mortality	10-yr nonfatal CVD
Women			
Participants at risk	1248	1220	909
Events	219	83	128
First quartile	1.00 Reference	1.00 Reference	1.00 Reference
Second quartile	0.74 (0.50–1.10)	0.74 (0.40–1.39)	0.66 (0.42–1.06)
Third quartile	0.84 (0.58–1.23)	0.60 (0.31–1.15)	0.56 (0.36–0.91)
Fourth quartile	0.92 (0.64–1.31)	0.88 (0.51–1.53)	0.44 (0.27–0.72)
Continuous[a]	0.98 (0.86–1.12)	0.93 (0.75–1.15)	0.72 (0.61–0.90)
Men			
Participants at risk	1077	1042	845
Events	286	121	195
First quartile	1.00 Reference	1.00 Reference	1.00 Reference
Second quartile	1.17 (0.80–1.73)	1.14 (0.64–2.03)	0.86 (0.57–1.30)
Third quartile	1.26 (0.87–1.82)	1.34 (0.77–2.33)	0.99 (0.67–1.46)
Fourth quartile	1.41 (0.98–2.03)	1.13 (0.64–2.00)	0.73 (0.47–1.11)
Continuous[a]	1.13 (1.00–1.28)	1.07 (0.89–1.29)	0.92 (0.79–1.06)

[a] HR per SD change in log-transformed adiponectin.

The results for the subgroup with available data for nonfatal CVD events were similar to those from the entire study population. In this subgroup, the age-adjusted HRs for all-cause mortality were 0.98 (0.79–1.23) for women and 1.17 (0.92–1.49) for men with no history of CVD at baseline, and 1.35 (0.98–1.86) for women and 1.16 (0.86–1.57) for men with prevalent CVD, and the age-adjusted HRs for CVD mortality were 0.81 (0.58–1.13) for women and 0.93 (0.64–1.34) for men without CVD, and 1.26 (0.80–1.99) for women and 1.10 (0.75–1.62) for men with prevalent CVD. For nonfatal events the age-adjusted HRs per SD change in log adiponectin were 0.74 (0.59–0.93) for women and 0.91 (0.73–1.14) for men without CVD at baseline, and 0.73 (0.54–0.99) in women and 0.86 (0.65–1.14) in men with prevalent CVD. Baseline characteristics for the subgroup with available data for nonfatal CVD events were approximately identical to those of the entire study population (data not shown).

Discussion

High adiponectin was associated with a higher age and a beneficial CVD risk profile at baseline in the general Dutch population. Risk of nonfatal CVD was significantly reduced in women with higher adiponectin, and there was a nonsignificant trend for such an association in men. In contrast, high adiponectin was not associated with lower CVD mortality and all-cause mortality, but after adjustment for cardiovascular risk factors, high adiponectin was significantly associated with increased all-cause and cardiovascular mortality. This association of high adiponectin and increased mortality risk was more pronounced in patients with prevalent CVD than in those without. Adiponectin is considered to protect against CVD, mediated by direct effects on the cardiovascular system and by its association with a favorable cardiovascular risk profile (2). Adiponectin inhibits pro-atherogenic processes in endothelial cells, suppresses macrophage tofoam cell formation, and exerts antiinflammatory effects, *e.g.* through inhibition of nuclear factor-κB. Stimulation of AMP-activated kinase by adiponectin reduces insulin resistance, and recent data from the Hoorn Study show that high adiponectin levels are associated with a reduced risk of impaired glucose tolerance and type 2 diabetes (1, 28). Furthermore, several cross-sectional and genetic studies support the notion that low adiponectin levels are associated with all components of the metabolic syndrome and serve as a risk factor for coronary artery disease (1, 2).

TABLE 4. Multivariate-adjusted HRs (with 95% CI) for 15-yr CVD mortality in sex-specific quartiles of adiponectin

Adjusted for	Age, BMI, WHR	Age, HDL, triglycerides	Age, glucose, 2 h-glucose	Age, insulin	Age, ALT	Age, GFR, smoking[a]	All previous
Women (1220 study participants at risk with 83 deaths due to CVD)							
First quartile	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference
Second quartile	0.79 (0.42–1.51)	0.94 (0.49–1.79)	0.88 (0.42–1.86)	0.74 (0.39–1.41)	0.75 (0.40–1.41)	0.86 (0.45–1.69)	1.07 (0.49–2.34)
Third quartile	0.69 (0.36–1.34)	0.91 (0.45–1.82)	0.95 (0.46–1.93)	0.67 (0.34–1.29)	0.61 (0.32–1.17)	0.64 (0.33–1.24)	1.30 (0.60–2.81)
Fourth quartile	1.13 (0.63–2.02)	1.52 (0.81–2.84)	1.36 (0.72–2.65)	0.98 (0.56–1.72)	0.90 (0.52–1.57)	1.01 (0.57–1.78)	2.36 (1.13–4.95)
Continuous[b]	1.04 (0.88–1.31)	1.15 (0.90–1.46)	1.11 (0.87–1.41)	0.99 (0.80–1.23)	0.94 (0.76–1.16)	0.97 (0.79–1.20)	1.45 (1.10–1.92)
Men (1042 study participants at risk with 121 deaths due to CVD)							
First quartile	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference
Second quartile	1.13 (0.63–2.03)	1.31 (0.72–2.56)	1.20 (0.66–2.18)	1.12 (0.62–2.01)	1.13 (0.63–2.03)	1.10 (0.61–1.97)	1.21 (0.65–1.97)
Third quartile	1.54 (0.88–2.69)	1.61 (0.91–2.86)	1.44 (0.81–2.57)	1.30 (0.74–2.27)	1.33 (0.76–2.03)	1.30 (0.74–2.26)	1.59 (0.88–2.89)
Fourth quartile	1.43 (0.81–2.57)	1.51 (0.82–2.77)	1.24 (0.68–2.56)	1.17 (0.60–2.08)	1.13 (0.63–2.01)	1.12 (0.63–2.01)	1.71 (0.90–3.26)
Continuous[b]	1.19 (0.98–1.45)	1.20 (0.98–1.47)	1.10 (0.91–1.34)	1.09 (0.90–1.33)	1.07 (0.88–1.29)	1.08 (0.89–1.31)	1.30 (1.04–1.63)

WHR, Waist to hip ratio.

[a] Adjustment for current smokers (yes/no) and product of number of cigarettes per day and years smoked.

[b] HR per 1 SD change in log-transformed adiponectin.

TABLE 5. Age- and sex-adjusted HRs (with 95% CI) for 15-yr all-cause mortality and 15-yr CVD mortality in subjects with and without CVD at baseline in sex-specific quartiles of adiponectin

	All		Women		Men	
	15-yr all-cause mortality	15-yr CVD mortality	15-yr all-cause mortality	15-yr CVD mortality	15-yr all-cause mortality	15-yr CVD mortality
No history of CVD						
Participants at risk	1886	1839	1040	1015	846	824
Deaths	340	115	160	51	180	64
First quartile	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference
Second quartile	0.73 (0.52–1.01)	0.64 (0.37–1.12)	0.58 (0.37–0.93)	0.64 (0.30–1.37)	0.96 (0.59–1.55)	0.73 (0.33–1.64)
Third quartile	0.89 (0.65–1.21)	0.72 (0.43–1.21)	0.70 (0.46–1.07)	0.42 (0.19–0.95)	1.19 (0.76–1.87)	1.18 (0.57–2.41)
Fourth quartile	0.96 (0.71–1.29)	0.77 (0.47–1.27)	0.73 (0.48–1.10)	0.64 (0.32–1.31)	1.32 (0.85–2.04)	1.03 (0.51–2.12)
Continuous[a]	1.00 (0.89–1.13)	0.90 (0.73–1.11)	0.90 (0.76–1.06)	0.79 (0.32–1.31)	1.14 (0.96–1.36)	1.07 (0.80–1.48)
History of CVD						
Participants at risk	433	417	204	201	229	216
Deaths	164	88	59	32	105	56
First quartile	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference	1.0 Reference
Second quartile	1.52 (0.92–2.50)	1.39 (0.71–2.74)	1.38 (0.64–2.99)	1.08 (0.36–3.22)	1.57 (0.80–3.05)	1.64 (0.67–4.04)
Third quartile	1.36 (0.82–2.26)	1.35 (0.68–2.66)	1.75 (0.77–3.99)	1.70 (0.59–5.32)	1.23 (0.63–2.39)	1.32 (0.54–3.27)
Fourth quartile	1.89 (1.16–3.06)	1.64 (0.85–3.17)	1.85 (0.91–3.76)	1.70 (0.67–4.29)	1.87 (0.95–3.67)	1.60 (0.63–4.11)
Continuous[a]	1.29 (1.07–1.55)	1.27 (0.98–1.63)	1.31 (1.00–1.72)	1.32 (0.91–1.91)	1.27 (0.99–1.63)	1.22 (0.87–1.73)

[a] HR per 1 SD change in log-transformed adiponectin.

However, several recent studies on the prospective association between adiponectin and CVD events/mortality showed inconsistent results (5–23). Unexpectedly, several of these studies, in particular those including patients already afflicted with or at high risk for CVD, found that high adiponectin was associated with an increased risk of mortality (17, 18, 20–23). As an explanation for these results, it was hypothesized that in CVD, a counter-regulatory increase of adiponectin occurs that represents a defense mechanism of the body against the cardiovascular alterations and the pro-inflammatory state associated with CVD (24, 25). This is in line with our results because we show that high adiponectin is significantly associated with an increased mortality risk, in particular, in patients with prevalent CVD. These findings fit well with observations that adiponectin exerts protective functions in early atherosclerosis by reducing a pro-atherogenic endothelial activation, but in patients with vascular diseases, adiponectin was positively correlated with serum concentrations of markers of endothelial activation/injury like CD146 or vascular cell adhesion molecule-1, suggesting a possible up-regulation of adiponectin in response to endothelial damage (2, 29, 30). It was also implicated that inflammation, lipotoxicity, and oxidative stress increased adiponectin expression, whereas adiponectin counteracts these pro-atherogenic influences by its antiinflammatory and antioxidative properties (1, 2, 31–33). Furthermore, it was shown that adiponectin increases in patients with myocardial dysfunction, although adiponectin is suggested to protect against heart failure because it attenuates cardiac hypertrophy (2, 18, 22, 23). All these data suggest that adiponectin protects against CVD, but it may be compensatory up-regulated in response to cardiovascular damage, or due to the wasting process in heart failure with elevation of adiponectin as a consequence of weight loss (22). Weight loss and malnutrition, both associated with high adiponectin, predict mortality in patients with CVD, and could, therefore, partially account for the relationship between high adiponectin and mortality (1, 34, 35).

Our results of an association between adiponectin and mortality in patients with CVD fit well with the concept of "reverse epidemiology," a term that is used to describe the inverse association between traditional cardiovascular risk factors (*e.g.* BMI) and mortality in patients with heart and renal failure (34, 35). However, in the present study, adjustment for BMI did not explain the associations between adiponectin and mortality. Alternatively, "adiponectin resistance," possibly due to down-regulation of adiponectin receptors as reported in obesity and insulin resistance, could also trigger a counter-regulatory increase of adiponectin in high-risk patients with prevalent CVD (1).

It should also be noted that adiponectin serum concentrations are inversely correlated with GFR and are increased in patients with albuminuria (18, 21, 29, 30). Underlying mechanisms for the elevation of adiponectin in chronic kidney disease remain unclear but may at least in part be due to reduced renal clearance (36). However, GFR in the present study could not explain the association between high adiponectin and high mortality risk. Apart from this, adiponectin correlates with age and might, therefore, also be compensatory up-regulated in the aging process of the body.

Finally, it cannot be excluded that adiponectin also exerts harmful effects that contribute to the increased mortality risk associated with high adiponectin. Toward this, it could be speculated that beneficial effects of adiponectin might become deleterious in advanced disease stages of CVD, in particular when the compensatory increase of adiponectin is overwhelming. In this context the ability of adiponectin to decrease body weight, mediated by increases in energy expenditure and by central effects in the brain, might be harmful in advanced stages of CVD, when a decline in body weight is associated with an adverse cardiovascular outcome (1, 34, 35, 37). Considering that adiponectin-mimetic therapies have been suggested to be introduced in the treatment of metabolic and CVD (1, 2), it will be important to elucidate the underlying mechanisms for the asso-

ciation between adiponectin and mortality, and to monitor carefully therapeutic interventions that aim to enhance the secretion or action of adiponectin.

Apart from low concentrations of a globular form that is cleaved from the full-length protein, adiponectin forms multimers that circulate in plasma in a low-, middle-, and high-molecular weight form (1, 2). These isoforms have different binding affinities to the adiponectin receptors (AdipoR1 and AdipoR2) and may, therefore, exert different bioactivities. Recent data indicate that the high-molecular weight form is the most active and clinically relevant form, at least for the metabolic and vascular-protective effects of adiponectin (1, 2, 38, 39). However, it was shown that globular adiponectin may contribute to myocardial hypertrophy, suggesting that not all isoforms of adiponectin protect against CVD (40).

A limitation of the present study may be that the different isoforms of adiponectin were not determined because at the time of our measurements, the recently developed ELISA systems were not available (39). Furthermore, information on nonfatal CVD was available for a subpopulation, and for a shorter follow-up period. To exclude the possibility that the differences in population size and follow-up duration explain the differences in the associations between adiponectin and nonfatal CVD and mortality, we also studied 10-yr all-cause and CVD mortality in the same subcohort. The associations did not differ from those observed in the total cohort, and for a longer follow-up duration.

During the revision process of our manuscript, other data from older men were published that confirm a positive association between adiponectin and mortality, with the most significant results for patients with heart failure (41).

In conclusion, high plasma adiponectin level is associated with a favorable lipid and glucose metabolism, and with a reduced risk of CVD events in women. However, higher adiponectin was associated with an increased risk of mortality, in particular in people with a history of CVD. We hypothesize that adiponectin protects against metabolic and vascular diseases, but in patients already afflicted with CVD, adiponectin is compensatory up-regulated in advanced disease stages and, therefore, indicates a high mortality risk in these patients. It remains a challenge for the near future to elucidate further the underlying mechanisms for our results.

Acknowledgments

Address all correspondence and requests for reprints to: Jacqueline M. Dekker, Institute for Research in Extramural Medicine, Vrije Universiteit Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. E-mail: jm.dekker@vumc.nl.

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