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A protective effect of adiponectin against oxidative stress in Japanese Americans: the association between adiponectin or leptin and urinary isoprostane

Shuhei Nakanishi*, Kiminori Yamane, Nozomu Kamei, Hideki Nojima, Masamichi Okubo, Nobuoki Kohno

Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8551, Japan

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Abstract

Adiponectin, which is produced by adipose tissue, is thought to play an important role in inflammation. On the other hand, adiposity, or the hypertrophy of adipose tissue, has been reported to increase oxidative stress. Accordingly, the possibility exists that adiponectin, as well as leptin, influences oxidative stress, resulting in a proinflammatory state. However, the relationship between adiponectin and oxidative stress is unclear. We examined 259 Japanese Americans living in Hawaii who were diagnosed as having normal glucose tolerance (NGT), impaired glucose tolerance, or diabetes by a 75-g oral glucose tolerance test. First, we measured their serum adiponectin, leptin, and high-sensitivity C-reactive protein levels as markers of inflammation, and urinary 8-iso-protaglandin $F_{2\alpha}$ (isoprostane) as a relevant marker of oxidative stress. We investigated the relationship between adiponectin or leptin and isoprostane among these subjects. In the diabetic subjects, the adiponectin and leptin levels were significantly lower and higher, respectively, than among the NGT subjects. Urinary isoprostane levels tended to decrease significantly after a rise in adiponectin levels ($P = .014$) among the NGT subjects. Next, we investigated the association between the 2 adipocytokines and isoprostane by regression models. Adiponectin was negatively but significantly associated with urinary isoprostane levels adjusted for age, gender, and smoking status, whereas leptin was positively and significantly correlated with urinary isoprostane levels ($P = .014$ and .004, respectively). With respect to adiponectin, this association was attenuated but still significant when further adjustments were made for waist-to-hip ratio, body mass index, percent body fat, C-reactive protein levels, glucose tolerance status, or homeostasis model assessment. In conclusion, this study suggests that adiponectin and leptin might be associated with oxidative stress levels. These results also suggest the possibility that adiponectin might modulate oxidative stress, leading to antidiabetic and anti-arteriosclerotic effects.

1. Introduction

Obesity is one of the major risk factors for cardiovascular disease [1] and type 2 diabetes [2]. In addition, obesity has been reported to be associated with oxidative stress [3]. Oxidative stress is increased under a hyperglycemic state [4] or in the presence of atherosclerotic lesions [5]. At the same time, adipose tissue is known to produce and secrete a variety of bioactive substances known as adipocytokines. Adiponectin and leptin are considered to be among the most important adipocytokines. However, the relationship between adipocytokines, especially adiponectin, and oxidative stress remains unclear.

In recent years, these 2 adipocytokines have been thought to possess various physiological activities. For example, hypoadiponectinemia was an independent risk factor for progression to type 2 diabetes [6], coronary artery disease [7], and so forth. The pathophysiology of these diseases is believed to be related to chronic inflammation [8,9]. Moreover, adiponectin is negatively correlated with C-reactive protein (CRP) levels in the plasma and adipose tissue [10], and therefore, might possess anti-inflammatory activity [10,11].

In contrast, leptin was reported to regulate body fat mass through actions on food intake and energy expenditure [12] and correlated with the waist-to-hip ratio (WHR) [13], CRP levels in vivo [14], and oxidative stress leading

* Corresponding author. Radiation Effects Research Foundation, Hiroshima 732-0815, Japan. Tel.: +81 82 261 3131; fax: +81 82 263 7279.
E-mail address: nshuhei@rerf.or.jp (S. Nakanishi).

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to atherosclerosis in vitro [15,16]. These results suggest that leptin might be related to a proinflammatory status, which might provide a common pathogenic mechanism that contributes to several complications observed in obese and diabetic subjects [16,17].

If adiponectin protects against oxidative stress and/or leptin enhances oxidative stress, both adipocytokines might be exceedingly important in the regulation of inflammation related to atherosclerosis or type 2 diabetes.

Isoprostanes, also known as 8-iso-protaglandin $F_{2\alpha}$, are prostaglandin-like compounds produced by the free radical–mediated peroxidation of lipoproteins. Isoprostanes are a reliable and clinically relevant marker of oxidative stress and can be accurately measured in both plasma and urine [3,5,18].

In this report, we hypothesized that adiponectin has a protective effect against oxidative stress, whereas leptin has a permissive effect for oxidative stress. Therefore, we measured serum adiponectin, leptin, and urinary isoprostane levels among Japanese Americans living in Hawaii.

2. Subjects and methods

The study subjects were Japanese Americans enrolled in a medical survey in the US state of Hawaii in 2002. This survey is part of a long-term epidemiological study of the risk factors for diabetes and cardiovascular disease, initiated in 1970, in which the subjects were limited to a population genetically identical to the Japanese population. This epidemiological study was previously described in detail elsewhere [19,20]. The study population consisted of 106 men and 153 women, including those who were under current therapy for hyperlipidemia and/or hypertension. All participants had not been diagnosed with diabetes before and were free of infectious symptoms, autoimmune diseases, or any other acute conditions as assessed by a medical interview. The smoking status (current, past, or none) was also assessed using standard interviewing procedures. Beforehand, subjects with serum creatinine levels higher than 2 mg/dL were excluded. All subjects underwent physical measurements and provided blood and urine samples after an overnight fast. The WHR was defined as the minimum abdominal circumference among measurements of the waist, the xiphoid process and the iliac crests, divided by the hip circumference as measured over the femoral heads. The percent body fat (% fat) was assessed by measuring the bioelectric impedance from foot to foot [21]. The participants underwent a 75-g oral glucose tolerance test (75gGTT), and glucose intolerance was diagnosed according to the 1997 American Diabetes Association criteria [22]. The collected blood was centrifuged, and the obtained serum and urine samples were immediately frozen at -80°C. The samples were subsequently brought back to Japan. Insulin was measured by a double-antibody radioimmunoassay. C-reactive protein levels were measured using a highly sensitive, latex-enhanced immunonephelometric assay [20]. Serum adiponectin, leptin, and urinary isoprostane concentrations were determined by enzyme-linked immunoassay (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan) [10], radioimmunoassay (Linco Research, Inc, St Charles, MO) [14], and enzyme immunoassay (Oxford Biomedical Research, Inc, Oxford, MI) [3], respectively. The urine data were expressed as the ratio to the urinary creatinine levels measured in the same samples. Insulin resistance was evaluated with a homeostasis model assessment (HOMA) [23]. This study was approved by the Ethics Committee of the Hiroshima University and the Council of the Hiroshima Kenjin-Kai Association in Hawaii.

3. Statistical analysis

The results were expressed as means $\pm$ SE. Because the triglyceride, adiponectin, leptin, CRP, isoprostane, and WHR data did not show normal distributions, the data were analyzed after logarithmic transformation. First, continuous variables were compared by analysis of covariance, and if they were found to be statistically significant, the Tukey-Kramer method was used to assess the relationship between categories. Next, all subjects were divided into tertiles according to their adiponectin or leptin concentrations, based on the population for each group undergoing a 75gGTT. Regarding adiponectin, we divided the normal glucose tolerance group (NGT) into categories comprised of less than 7.55, 7.55–1.5, and 11.5 μg/mL or more; for the impaired glucose tolerance group (IGT), categories of less than 7.0, 7.0–10.1, and 10.1 μg/mL or more; and for the diabetes group (DM), categories of less than 5.6, 5.6-7.7, and 7.7 μg/mL or more. With respect to leptin, we divided the NGT into categories of less than 4.0, 4.0–7.1, and 7.1 ng/mL or more; for IGT, categories of less than 5.2, 5.2–9.2, and 9.2 ng/mL or more; and for DM, less than 3.8, 3.8-9, and 9 ng/mL or more. We compared these concentrations with the isoprostane levels of the 3 categories in each group. The number of subjects for adiponectin were 56, 55, and 56, respectively, for NGT; 24, 23, and 24, respectively, for IGT; and 8, 8, and 7, respectively, for DM. With respect to leptin, the subject number was 56, 56, and 55, respectively, for NGT; 23, 23, and 24, respectively, for IGT; and 7, 7, and 8, respectively, for DM. Lastly, multiple regression analyses were performed to investigate the associations between urinary isoprostane as a dependent variable, and either adiponectin or leptin as independent variables, after adjusting for age, gender, smoking status, WHR, body mass index (BMI), % fat, CRP, HOMA, and glucose tolerance status (NGT, IGT, or DM). For all data analysis, SAS package version 8.2 (SAS Institute, Cary, NC) was used.

4. Results

First, we compared the clinical characteristics of subjects after adjusting for age, gender, and fsmoking status (Table 1). Among the DM subjects, WHR, BMI, fasting glucose,

Table 1
Clinical characteristics of subjects

	NGT	IGT	DM
N (men/women)	167 (70/97)	70 (23/47)	22 (13/9)
Age (y)	65.7 ± 1.2	69.0 ± 1.8	70.4 ± 3.3†
SBP (mm Hg)	134 ± 3	137 ± 3	137 ± 5
DBP (mm Hg)	73 ± 2	75 ± 2	77 ± 3
% Fat	26.6 ± 1.5	28.4 ± 1.7	29.8 ± 2.4
WHR	0.85 ± 0.03	0.86 ± 0.04	0.89 ± 0.05†
BMI	23.7 ± 0.6	24.7 ± 0.7	26.0 ± 1.0†
Fasting glucose (mmol/L)	4.76 ± 0.11	5.12 ± 0.13	6.22 ± 0.18†,‡
2-h Glucose (mmol/L)	5.39 ± 0.22	8.39 ± 0.25†	12.20 ± 0.35†,‡
Fasting insulin (pmol/L)	48.6 ± 7.9	54.5 ± 9.2	82.3 ± 12.5†,‡
2-h Insulin (pmol/L)	307.2 ± 50.8	543.1 ± 58.6†	500.0 ± 79.8†
T-CHO (mmol/L)	5.45 ± 0.15	5.30 ± 0.17	5.33 ± 0.24
TG (mmol/L)	1.64 ± 0.22	1.88 ± 0.25	2.63 ± 0.34†
Adiponectin (μg/mL)	11.82 ± 0.97	9.11 ± 1.12	7.44 ± 1.53†
Leptin (ng/mL)	6.65 ± 0.92	7.85 ± 1.05†	8.55 ± 1.44†
CRP (mg/L)	0.15 ± 0.03	0.12 ± 0.04	0.24 ± 0.05†
Urinary isoprostane (ng/g Cr)	0.62 ± 0.19	0.76 ± 0.22	1.32 ± 0.30†

Data are expressed as means ± SE. All data are adjusted for age, gender, and smoking status except age.
SBP indicates systolic blood pressure; DBP, diastolic blood pressure; T-CHO, total cholesterol; TG, triglycerides.
† $P < .05$ toward NGT.
‡ $P < .05$ toward IGT by Tukey-Kramer method.

insulin, 2-hour glucose, triglycerides, CRP, and urinary isoprostane were all significantly higher than in the NGT subjects. Adiponectin was significantly lower in the DM and IGT subjects than in the NGT subjects. Leptin was significantly higher in the DM subjects than in the NGT subjects. In addition, in the IGT subjects, fasting glucose, 2-hour glucose, and insulin were all significantly higher than in the NGT subjects.

Next, we compared the urinary isoprostane levels divided into tertiles of adiponectin levels in the NGT, IGT, and DM subjects (Fig. 1). Regarding the adiponectin tertiles, among the NGT subjects, urinary isoprostane levels by increasing adiponectin tertiles were 0.72 ± 0.09, 0.60 ± 0.09, and 0.55 ± 0.09 ng/g Cr, respectively ($P = .014$ for trend), with the high category of adiponectin having significantly lower isoprostane levels than the low category ($P = .021$). Among the IGT subjects, these levels were 0.54 ± 0.30, 0.90 ± 0.30, and 0.92 ± 0.30 ng/g Cr, respectively (no trend). Among the DM subjects, the levels were 2.02 ± 0.82, 0.80 ± 0.89, and 1.21 ± 0.90 ng/g Cr, respectively (no trend), with the low category of adiponectin having higher isoprostane levels than the middle category, but the difference was not statistically significant ($P = .196$). Regarding the leptin tertiles, among the NGT subjects, the urinary isoprostane levels were 0.54 ± 0.09, 0.59 ± 0.09, and 0.74 ± 0.09 ng/g Cr, respectively (no trend), with the high category of leptin having higher isoprostane levels than the low category, but the difference was not statistically significant ($P = .270$). Among the IGT subjects, these levels were 0.62 ± 0.30, 0.95 ± 0.30, and 0.81 ± 0.30 ng/g Cr, respectively. Among the DM subjects, the levels were 0.78 ± 0.84, 0.54 ± 0.84, and 2.42 ± 0.78 ng/g Cr, respectively (no trend), with the high category of leptin having higher isoprostane levels than the

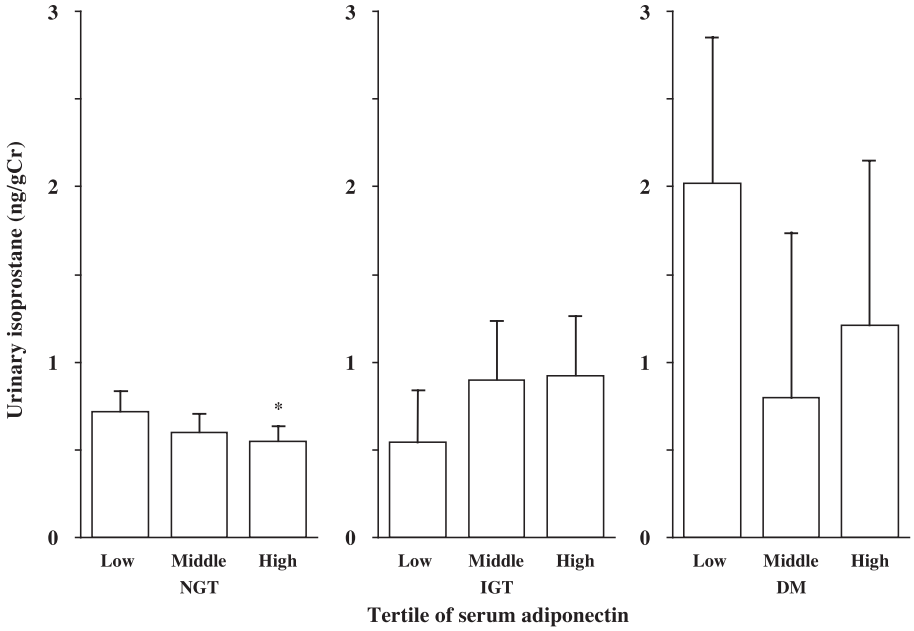

Fig. 1. Urinary isoprostane levels were divided into adiponectin tertiles according to the 75gGTT groups. By increasing adiponectin tertiles among the NGT subjects, the mean adiponectin levels were 5.32 ± 0.60, 9.47 ± 0.61, and 18.87 ± 0.60 μg/mL, respectively. Among the IGT subjects, these levels were 4.49 ± 0.51, 8.58 ± 0.52, and 14.68 ± 0.52 μg/mL, respectively. Among the DM subjects, the levels were 4.39 ± 0.56, 6.83 ± 0.60, and 9.76 ± 0.60 μg/mL, respectively. The error bars indicate SEs. The trend analysis was $P = .014$, .969, and .875 among the NGT, IGT, and DM subjects, respectively. *$P < .05$ compared with the lowest tertile by the Tukey-Kramer method.

Table 2
The relationship of serum adiponectin by regression analysis with urinary isoprostane as the dependent variable

Adjustment	β	SE	P
Adiponectin only	−.360	.138	.010
Adjusted for age, gender, and smoking status	−.385	.157	.014
Adjusted for age, gender, smoking status, and WHR	−.388	.159	.015
Adjusted for age, gender, smoking status, and BMI	−.307	.160	.047
Adjusted for age, gender, smoking status, and % fat	−.340	.163	.039
Adjusted for age, gender, smoking status, and CRP	−.368	.161	.023
Adjusted for age, gender, smoking status, and glucose tolerance status	−.345	.164	.037
Adjusted for age, gender, smoking status, and HOMA	−.373	.175	.034
Adjusted for age, gender, smoking status, WHR, BMI, % fat, CRP, HOMA, and glucose tolerance status	−.301	.184	.104

middle category, but the difference was not statistically significant (P = .920, data not shown).

Lastly, we investigated the associations between adiponectin or leptin and urinary isoprostane levels by regression models (Tables 2 and 3). Adiponectin was significantly and negatively correlated with isoprostane (P = .010) by the simple regression model. After adjustments for age, gender, and smoking status, the significant negative association with isoprostane remained (P = .014). After even more adjustments for WHR, BMI, % fat, CRP, glucose tolerance status, or HOMA, the significant negative association remained (P = .015, .047, .039, .023, .037, and .034, respectively). However, after adjusting for all factors as stated above, the negative association disappeared (P = .104). Regarding leptin, it was significantly positively correlated with isoprostane (P = .036) by the simple regression model. After adjustments for age, gender, and smoking status, the significant positive association remained (P = .004). However, in contrast to adiponectin, after more adjusting for BMI, the association disappeared (P = .103).

5. Discussion

In this study, we measured 2 serum adipocytokines, adiponectin, and leptin, as well as urinary isoprostane, and investigated possible relationships between these markers. As a result, adiponectin and leptin were significantly lower and higher, respectively, among diabetic subjects than among NGT subjects. Furthermore, adiponectin had a negative, whereas leptin had a positive, relationship to oxidative stress levels independent of CRP, glucose tolerance status, WHR, % fat, or HOMA. These results suggest that these 2 adipocytokines may be important in the regulation of inflammation related to atherosclerosis and type 2 diabetes.

Adipocytes synthesize and secrete several cytokines. In other words, adipose tissue is hypothesized to be a factor directly modulating proinflammatory vs anti-inflammatory cytokine levels. Adiponectin levels are decreased in type 2 diabetes and coronary artery diseases [7,24]. In addition, adiponectin deficiency elevates CRP mRNA levels in the adipose tissue of mice [10]. Moreover, lower adiponectin levels are thought to contribute to the development of atherosclerosis through the modulation of inflammatory cascades [11]. Accordingly, adiponectin is thought to play an important role as an anti-inflammatory cytokine. On the other hand, adiponectin was a negatively significant variable for predicting urinary isoprostane concentrations (Table 2). Isoprostane has been shown to be increased in type 2 diabetes and cardiovascular diseases [5]. Such diseases are pathophysiologically associated with oxidative stress. In brief, these results suggest the possibility that adiponectin might have anti-atherogenic and antidiabetogenic effects by modulating oxidative stress. In addition, the significance of the relationship between adiponectin and urinary isoprostane levels remained even after adjusting for age, gender, and smoking status. After further adjustment for WHR, BMI, % fat, CRP, HOMA, or glucose tolerance status, the negative association remained, but it was significantly attenuated after further adjusting was made for BMI or % fat among these 6 factors (Table 2). Therefore, adiponectin may be negatively associated with oxidative stress, at least in part, through adiposity. Further study is needed, however, because other unknown factors, such as tumor necrosis factor α (TNF-α) [25], might exist.

The mechanisms responsible for the modulation of oxidative stress by adiponectin are unclear. Because this

Table 3
The relationship of serum leptin by regression analysis with urinary isoprostane as the dependent variable

Adjustment	β	SE	P
Leptin only	.241	.114	.036
Adjusted for age, gender, and smoking status	.411	.143	.004
Adjusted for age, gender, smoking status, and WHR	.411	.145	.005
Adjusted for age, gender, smoking status, and BMI	.319	.195	.103
Adjusted for age, gender, smoking status, and % fat	.357	.167	.034
Adjusted for age, gender, smoking status, and CRP	.445	.162	.007
Adjusted for age, gender, smoking status, and glucose tolerance status	.385	.146	.009
Adjusted for age, gender, smoking status, and HOMA	.486	.182	.008
Adjusted for age, gender, smoking status, WHR, BMI, % fat, CRP, HOMA, and glucose tolerance status	.410	.232	.078

study is cross-sectional, this relationship could be the other way around. The mechanisms of adiponectin's oxidative stress modulation are unclear. However, several explanations are plausible. One is that adiponectin affects the activation of peroxisome proliferator-activated receptor α (PPARα). Yamauchi et al [26] described an increase in PPARα ligand activity in the skeletal muscle of adiponectin transgenic mice crossed with *ob*/*ob* mice. The activation of PPARα has been implicated in the protection against oxidative damage afforded by a reduction in nuclear factor κB (NF-κB) activity in mice [27]. Another explanation is that adiponectin inhibits NF-κB signaling through an inhibition of TNF-α activation in human aortic endothelial cells [28], leading to the suppression of oxidative stress. In brief, the link between adiponectin and oxidative stress might represent a relationship between TNF-α and oxidative stress, because adiponectin expression is thought to be associated with lower TNF-α expression [25].

Among the NGT subjects, the high-adiponectin category had significantly lower urinary isoprostane levels than the low category in our study. In addition, a negative dose trend as a tertile of the adiponectin levels was observed among these subjects (Fig. 1). Among the IGT and DM subjects, no relationship was found between adiponectin and urinary isoprostane. Adiponectin, thus, may play an important role in modulating oxidative stress, even in NGT subjects. One reason for the apparent lack of association among the IGT and DM subjects is that compared with NGT subjects, hyperglycemic states are thought to elevate oxidative stress for various reasons [4], leading to elevated isoprostane levels. As a result, the impact of adiponectin on oxidative stress might have been somewhat attenuated. Another reason is that isoprostane levels might be predominantly related to obesity, rather than to glycemic status. The other reason may have been an issue of statistical power, and further study is required to clarify.

Leptin has already been discussed in terms of its relationship to oxidative stress [15-17]. The proinflammatory effects of leptin were thought to be associated with some key steps, such as an activation of the Jun N-terminal kinase/stress-activated protein kinase–dependent pathway and with NF-κB [15], the activation of protein kinase A [16], or a suppression of paraoxinase 1 activity [17]. In this study, we described a similar conclusion in vivo. In addition, as discussed in the Results section, the low category of leptin had low urinary isoprostane levels when compared with the other categories, although no significant tendency was found among the tertiles of the NGT and IGT groups. This was consistent with the result that oxidative stress stimulated by leptin might be independent of WHR, % fat, CRP, glucose tolerance status, or HOMA (Table 3). Among these factors, similar to adiponectin, the significance of the relationship between leptin and urinary isoprostane was attenuated and disappeared after further adjusting was made for BMI. Therefore, leptin may be positively associated with oxidative stress through obesity. Accordingly, although there was a very attenuated but significant relationship between adiponectin and oxidative stress adjusted for BMI, the mechanism responsible for the actions of adiponectin and leptin through oxidative stress might have fundamentally the same origin (ie, obesity).

Several limitations of this study remain. First, anti-inflammatory medications were not considered completely in this study. However, there were no differences in adiponectin, leptin, or urinary isoprostane levels between subjects who were not under therapy for hyperlipidemia or hypertension and those subjects who were being treated (data not shown). Second, renal function might influence urinary isoprostane levels, leading to the possibility of misinterpreting the relationship between adipocytokines and oxidative stress in our study. However, patients with severe renal dysfunction were excluded, because all subjects' serum creatinine levels in this study were under 2 mg/dL. Moreover, no statistical differences were observed regarding the mean serum creatinine levels; 0.78 ± 0.02 mg/dL for NGT, 0.77 ± 0.03 mg/dL for IGT, 0.73 ± 0.04 mg/dL for DM adjusted for age, gender, and smoking status. Third, as previously described, this study is cross-sectional. Accordingly, we might have overestimated the results regarding the relationships between the 2 adipocytokines and oxidative stress, because we were unable to distinguish cause from effect. Thus, the possibility exists that oxidative stress might suppress adiponectin and induce leptin. Further study on this point is needed.

In summary, we reported that adiponectin, in contrast to leptin, had a negative correlation with urinary isoprostane levels. This trend remained even after adjusting for WHR, BMI, % fat, CRP, HOMA, or glucose tolerance status. These results suggest the possibility that adiponectin might modulate oxidative stress, leading to antidiabetic and antiarteriosclerotic effects.

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