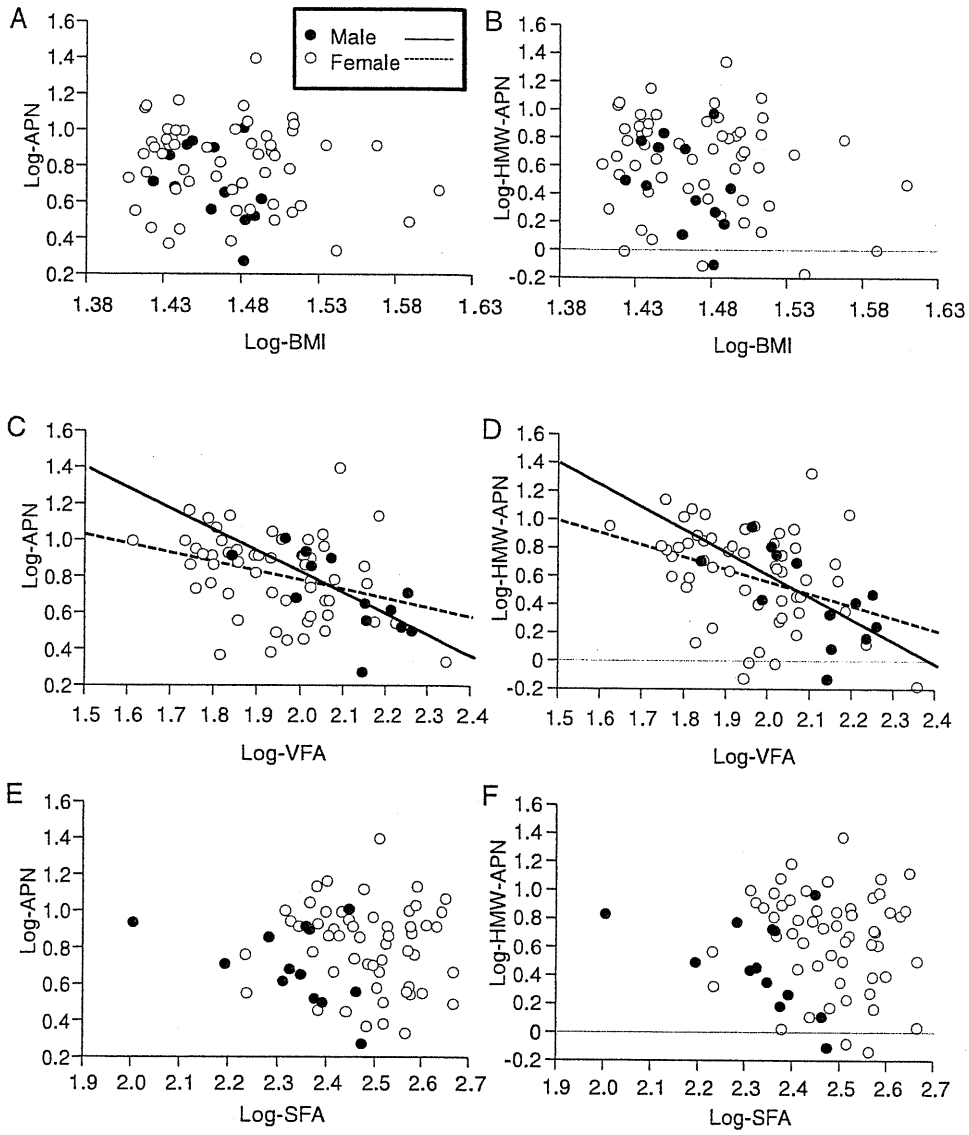

Fig. 1. Relationships between circulating total adiponectin levels (APN), circulating high molecular weight adiponectin levels (HMW-APN), and fat distribution, i.e. body mass index (BMI) (A, APN; B, HMW-APN), visceral fat area (VFA) (C, APN; D, HMW-APN) and subcutaneous fat area (SFA) (E, APN; F, HMW-APN) in males (closed circle) and females (open circle). Pearson's correlation coefficient was used to examine the relationships between APN, HMW-APN and fat distribution in males (solid line) and females (dotted line). In all cases, $p < 0.05$ was considered significant.

thermore, we and another author have shown that APN is negatively correlated with VFA and SFA as well as BMI in the general population[6] and young men[7]; however, there is a considerable variation in circulating adiponectin levels among subjects with a similar BMI, even in obesity[1]. The present study demonstrated that APN and HMW-APN correlated with VFA, but not BMI and SFA, in obese subjects, which is the first report to our knowledge. Taken together with substantial evidence showing a relationship between visceral adiposity and cardiometabolic disorders, the present results suggest the hypoadiponectinemia may represent the dysfunction of adipose tissue in obesity and is associated with atherosclerosis.

Table 2. Results of uni- and multivariate analyses of correlation between log-APN, log-HMW-APN and various parameters

	Log-APN			Log-HMW-APN		
	Males	Females		Males	Females	
	Univariate p value	Univariate p value	Multivariate F value	Univariate p value	Univariate p value	Multivariate F value
Log-BMI	0.109	0.064	–	0.064	0.055	–
Log-VFA	0.009	0.002	4.14	0.016	0.005	6.33
Log-SFA	0.051	0.070	–	0.068	0.133	–
Systolic blood pressure	0.877	0.779	–	0.496	0.868	–
Diastolic blood pressure	0.708	0.254	–	0.570	0.199	–
Glucose	0.158	0.409	–	0.338	0.331	–
Total cholesterol	0.517	0.596	–	0.710	0.477	–
Triglyceride	0.098	0.217	–	0.119	0.225	–
HDL-cholesterol	0.168	<0.001	10.75	0.109	<0.001	9.57

Univariate: Pearson's correlation analysis, Multivariate: Stepwise multiple regression analysis. Parameters with F value >4.0 were subsequently entered into the regression analysis as independent variables.

Reducing visceral fat accumulation to improve hypoadiponectinemia is a potential strategy to prevent atherosclerotic cardiovascular disease in obesity.

Acknowledgments

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Original Article

Nocturnal Falls of Adiponectin Levels in Sleep Apnea with Abdominal Obesity and Impact of Hypoxia-Induced Dysregulated Adiponectin Production in Obese Murine Mesenteric Adipose Tissue

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Aim: Obstructive sleep apnea-hypopnea syndrome (OSAS) is associated with atherosclerotic cardiovascular disease. We reported recently daytime hypoadiponectinemia and nocturnal falls in circulating adiponectin concentrations (Δadiponectin) in OSAS patients, in part due to hypoxic stress. The present study investigated the association between Δadiponectin and fat distribution in OSAS males, and the effect of hypoxic stress on adiponectin production in obese yellow-KKAy mice.
Methods: The participants in this study were 43 Japanese males who visited the clinic and were newly diagnosed with OSAS. Venous blood samples were collected before sleep and after wakingup. We investigated the effect of hypoxia on adiponectin expression in mesenteric and subcutaneous fat tissues of obese yellow-KKAy mice. We measured adiponectin secretion into media under hypoxic conditions in an *ex-vivo* model of yellow-KKAy mice.
Results: In OSAS males with a relatively higher body mass index (BMI), Δadiponectin correlated inversely with the waist-hip ratio, but not with BMI, waist circumference or hip circumference. In obese yellow-KKAy mice, exposure to hypoxia for 2 days suppressed plasma adiponectin levels, with no apparent change in mesenteric and subcutaneous fat tissue adiponectin mRNA expression. In an *ex-vivo* study of obese yellow-KKAy mice, hypoxic stress reduced adiponectin in the supernatant of mesenteric fat tissues, but not subcutaneous fat tissues.
Conclusions: These findings suggest that abdominal obesity, representing abundant mesenteric fat tissue susceptible to hypoxic stress, partly explains Δadiponectin in OSAS patients, and that reduction of visceral fat accumulation may combat OSAS-related atherosclerotic cardiovascular diseases in abdominal obesity.

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Key words; Nocturnal falls of adiponectin, Body fat distribution, Waist-hip ratio, Mesenteric fat tissues, Obstructive sleep apnea-hypopnea syndrome

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAS) is associated in some patients with insulin resistance and hypertension, leading to atherosclerotic cardiovascular disease[1]. Cardiovascular diseases are the most serious complications in OSAS[2]. Previous studies indicated that people who died suddenly in the early hours of the morning from cardiac causes had significant sleep apnea compared to those who died suddenly from cardiac causes during other intervals[3], and from angina attacks during night sleep (i.e. nocturnal cardiac events)[4, 5].

Increasing evidence suggests that body fat accumulation, especially visceral fat accumulation, plays a role in the development of atherosclerosis. Recent

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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, which are involved in energy metabolism, inflammatory response, and cardiovascular functions[6]. Adiponectin, as an adipocytokine, was identified by our group from the human adipose tissue cDNA library[7]. We demonstrated previously the association between visceral obesity and OSAS[8]. We recently reported daytime hypoadiponectinemia and nocturnal falls in circulating adiponectin concentrations (Δadiponectin) in severe OSAS patients, presumably due to hypoxic stress[9], possibly that might be associated with nocturnal cardiac events. The association between fat distribution and Δadiponectin in OSAS patients remains unclear.

Aim

The aim of the present study was to clarify the possible association between fat distribution and nocturnal falls in circulating adiponectin concentrations in male patients with OSAS, and the effect of hypoxic stress, seen partly in OSAS, on adiponectin production in the subcutaneous and mesenteric fat tissues of obese mice.

Materials and Methods

Human Studies

Participants

We studied 43 Japanese males (apnea hypopnea index (AHI) ≥5, age; 41.4±1.6 years, mean±SEM) between February 2006 and March 2007 who visited the clinic and were newly diagnosed with OSAS[8]. OSAS was diagnosed according to the guidelines of the American Academy of Sleep Medicine Task Force[10], as reported previously[8]. All recordings were scored manually by an experienced polysomnographic technologist, as described in detail previously[11]. Sleep duration was estimated using the self-reported sleep time and recorded data. The Medical Ethics Committee of Osaka University approved this study. All subjects enrolled in the study were Japanese and each gave written informed consent. This study (called The Osaka University Visceral Fat Study (O-VFStudy)) is registered under number UMIN 000002997 (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000003633&language=E).

Anthropometry and Laboratory Tests

Height, weight, waist circumference and hip circumference were measured in a standing position. Body mass index (BMI) was calculated using the formula [weight (kg)/height (m)^2]. Waist circumference (WC) at the umbilical level was measured with a non-stretchable tape in late expiration while standing (in cm). Hip circumference(HC) was measured horizontally at the level of the greater trochanter of the femur, also with the subjects standing[12]. The waist-to-hip ratio (WHR) was defined as WC divided by HC.

Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subjects had rested in a sitting position for at least 10 minutes. Venous blood samples were collected to measure blood glucose, hemoglobin A1c, immunoreactive insulin (IRI), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) after wakingup while the subject was in the supine position. Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula[13]. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or treatment for hypertension. Diabetes mellitus was defined according to the World Health Organization criteria, and/or treatment for diabetes mellitus. Dyslipidemia was defined as an LDL-C concentration of >140 mg/dL, TG concentration >150 mg/dL, HDL-C concentration <40 mg/dL, and/or treatment for dyslipidemia. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR (mU/L mg/dL)=[(fasting IRI×fasting glucose)/405]. In each sleep study that included adiponectin monitoring, venous blood samples were obtained before sleep and after wakingup while the subject was in the supine position. For the purpose of the present study, serum samples obtained at baseline from each study participant and stored at −20℃ were thawed and assayed for serum adiponectin concentrations using the sandwich enzyme-linked immunosorbent assay (ELISA) (Otsuka Pharmaceutical Co., Tokushima, Japan). No patients were on medications known to increase serum adiponectin levels, such as pioglitazone[14].

Animal and Cell Culture Studies

Animals and Exposure to Hypoxia

Male control KK and yellow-KKAy mice (each group; $n=6$) were obtained from Clea Japan (Tokyo, Japan) and housed under a 12-h dark-light cycle (lights on 8:00 A.M. to 8:00 P.M.) and constant temperature (22℃) with free access to food (Oriental Yeast, Osaka, Japan) and water. Male mice were housed in cages exposed to room air (ambient atmosphere) or sustained hypoxic chambers (Teijin Pharma, Osaka,

Japan, ~10% O_2), as we reported previously[9]. Both in control KK and obese yellow-KKAy mice, there were no significant differences in body weight, plasma adiponectin levels, and blood glucose levels at baseline between control (room air) and hypoxic (10% O_2 concentration) groups.

Measurement of Plasma Adiponectin Concentrations and Adipose Adiponectin mRNA Expression in Control KK and Obese Yellow-KKAy Mice

All experiments were conducted in mice at age 14 weeks. Mice were sacrificed under pentobarbital sodium anesthesia (50 mg/kg body weight) at the indicated times under each condition, and then the mesenteric and subcutaneous fat tissues and blood samples were collected. Each sample was subjected to measurement of plasma concentrations and mRNA expression levels (using real-time quantitative polymerase chain reaction; rt-PCR), as described previously[9]. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

Measurement of Adiponectin Secretion into Media Under Hypoxic Conditions in an ex-vivo Model of Obese Yellow-KKAy Mice

Each pair of mesenteric fat tissue and subcutaneous fat tissue was obtained from 14-week-old male obese KKAy mice housed under room air. Each tissue was weighed and 250 to 300 mg were placed into a ϕ 3 cm-dish filled with 1 mL fetal calf serum (FCS)-free complete Dulbecco's modified Eagle's medium (DMEM), similar to the method described previously[15]. Next, the tissues were minced into small pieces, and the medium was removed and washed with 1 mL calcium- and magnesium-free PBS, incubated with 1 mL DMEM-free FCS for one hour under 1% O_2 hypoxia or control conditions (18-21% O_2 – 5% CO_2) (each group, $n=6$). An aliquot of the culture media was subjected to measurement of adiponectin in media using ELISA. We could not investigate non-obese KK mice because a sufficient amount of mesenteric fat tissue could not be obtained for the experiment.

Statistical Analysis

All values are expressed as the mean ± SEM. Relationships between two continuous variables were analyzed using scatter plots and Pearson's correlation coefficients. Differences among groups were compared by the unpaired Student's t-test for experiments involving only two groups. In all cases, p values < 0.05 were considered significant. All statistical analyses were performed with StatView-J 5.0 (Statistical Analysis System Inc., Cary, NC).

Table 1. Clinical characteristics of male subjects with OSAS (AHI ≥ 5)

Number	43	
Age (years)	41.4 ± 1.6	(25-77)
Body weight (kg)	87.3 ± 2.7	(55.4-133.3)
Body mass index (BMI) (kg/m^2)	29.7 ± 0.7	(23.2-38.2)
Waist circumstance (cm)	98.9 ± 1.7	(82.0-126.0)
Hip circumstance (cm)	105.7 ± 1.3	(89.0-126.0)
Waist-hip ratio	0.93 ± 0.01	(0.85-1.03)
AHI (events/hour)	42.6 ± 4.1	(5.3-104.0)
ODI < 4.0% (events/hour)	363.1 ± 35.6	(3.7-89.3)
SpO_2 < 90% time rate (%)	22.1 ± 3.3	(0.1-67.0)
Baseline SpO_2 (%)	94.6 ± 0.3	(92-97)
Lowest SpO_2 (%)	71.9 ± 1.5	(54-87)
Systolic blood pressure (mmHg)	131 ± 2	(104-170)
Diastolic blood pressure (mmHg)	86 ± 2	(66-118)
Pulse rate (/min)	84 ± 2	(60-114)
Fasting glucose (mg/dL)	106 ± 2	(88-144)
Hemoglobin A1c (%)	5.1 ± 0.1	(4.5-7.3)
Immunoreactive insulin (mU/L)	16.5 ± 1.6	(3.0-44.0)
HOMA-IR (unit)	4.5 ± 0.5	(0.7-12.9)
Low density lipoprotein-cholesterol (mg/dL)	125 ± 4	(76.2-176.8)
Triglyceride (mg/dL)	158 ± 12	(70-444)
High density lipoprotein-choresterol (mg/dL)	44 ± 2	(27-88)
Diabetes mellitus (%)	14.0	
Dyslipidemia (%)	76.7	
Hypertension (%)	39.5	
Serum adiponectin before sleep (μg/mL)	6.0 ± 0.4	(1.7-17.2)
Serum adiponectin after wake-up (μg/mL)	5.2 ± 0.4	(1.2-13.5)
Δadiponectin (%)	−13.1 ± 2.1	(−36.5-16.6)

Data are presented as the mean ± SEM (range). OSAS: obstructive sleep apnea hypopnea syndrome, AHI: apnea hypopnea index, SpO_2: percentage of arterial O_2 saturation from pulse oximetry, ODI: oxygen desaturation index, 90% time: time at desaturation below 90% in minutes of total bedtime, HOMA-IR: homeostasis model assessment of insulin resistance, Δadiponetin = (serum adiponectin concentration after waking-up − before sleep)/after waking-up.

Results

Human Studies

The characteristics of the subjects enrolled in this study are presented in **Table 1**. The mean BMI was

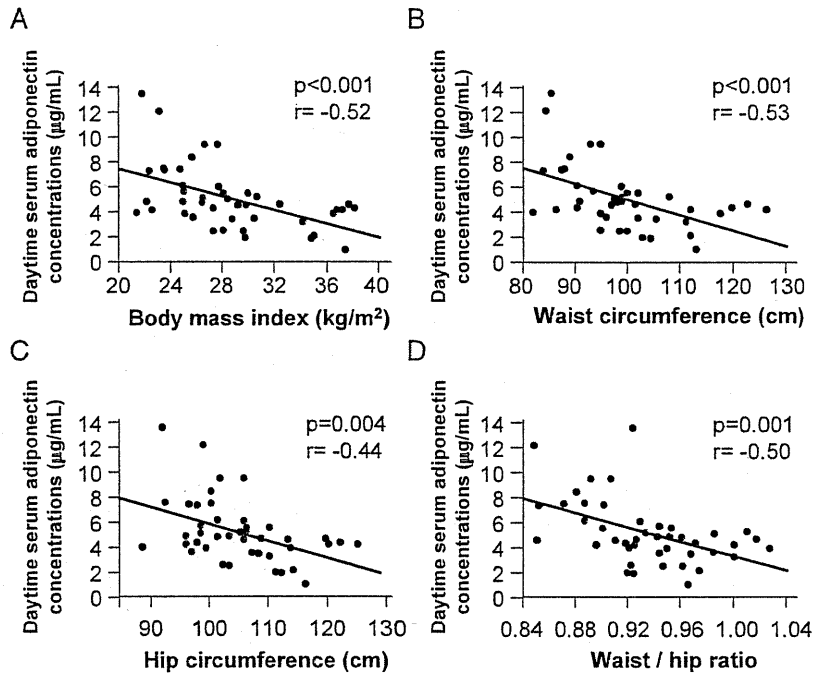

Fig. 1. Human studies.

Relationships between daytime serum adiponectin concentrations (in the morning) and body mass index (A), waist circumstance (B), hip circumstance (C), and waist-hip ratio (D) in patients with obstructive sleep apnea-hypopnea syndrome (OSAS).

29.7 kg/m^2 and mean WC was 98.9 cm. Our previous report showed daytime hypoadiponectinemia and Δadiponectin in severe OSAS patients, in part due to hypoxic stress[9]. First, we investigated the relationship between daytime circulating adiponectin concentrations and each of BMI and fat distribution in males with OSAS. Anthropometric measurements such as WHR, as an indicator of body fat distribution in obesity, are usually assessed using standard methods. In males with OSAS, serum adiponectin concentrations measured in the early morning correlated significantly with BMI, WC, HC, and WHR (**Fig. 1**), similar to a previous report[16]. Next, we analyzed the correlation between Δadiponectin and each of BMI and fat distribution in males with OSAS. Interestingly, Δadiponectin correlated inversely and significantly with WHR only ($p=0.026$, $r=-0.34$), but not with BMI, WC, or HC (**Fig. 2**), suggesting that fat distribution, such as visceral and subcutaneous fat tissue, is an important factor in Δadiponectin in OSAS.

AHI correlated significantly with BMI ($r=0.37$, $p=0.016$), WC ($r=0.36$, $p=0.019$), HC ($r=0.32$, $p=0.035$), and tended to correlate with WHR ($p=0.054$); however, there was no correlation between AHI and daytime serum adiponectin levels ($p=0.148$), Δadiponectin ($p=0.401$) (data not shown).

Animal Studies

Next, we studied the effect of sustained hypoxia on adiponectin expression in mesenteric and subcutaneous fat tissues of obese yellow-KKAy mice, as reported previously in lean mice[9]. Exposure to sustained hypoxia (10% O_2 concentration) for 2 days suppressed plasma adiponectin levels in obese yellow-KKAy mice (**Fig. 3A, left**), with no apparent change in mesenteric and subcutaneous fat tissue adiponectin mRNA expression in obese yellow-KKAy mice (**Fig. 3A, middle and right**). Exposure to sustained hypoxia for 2 days suppressed plasma adiponectin levels in control KK mice, with no apparent change in mesenteric and subcutaneous fat tissue adiponectin mRNA expression in obese yellow-KKAy mice (data not shown), compared with the control (room air), similar to our previous report on epididymal fat tissues of lean mice[9]. Exposure to sustained hypoxia for 4 days resulted in significant falls in plasma adiponectin concentrations of control KK and obese yellow-KKAy mice, and significant decreases in mesenteric

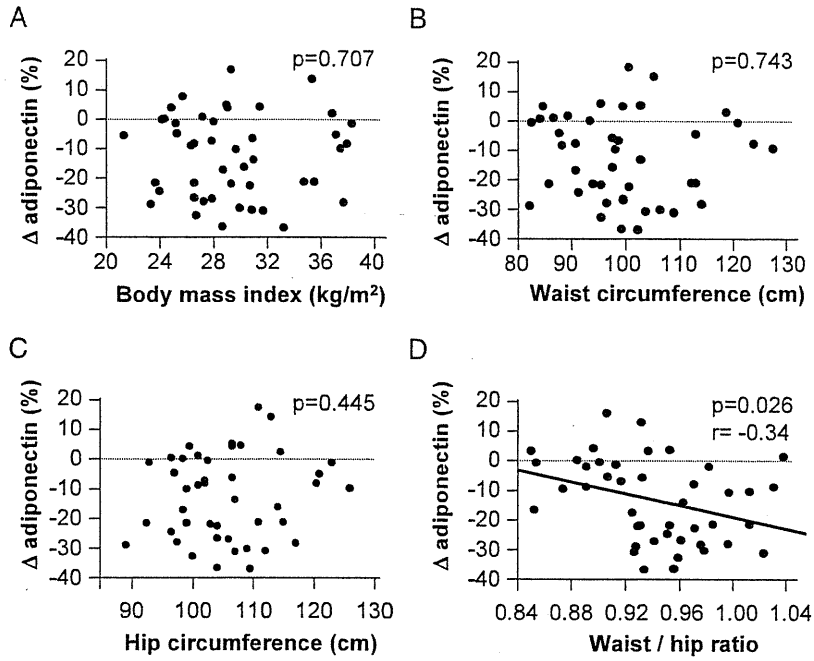

Fig. 2. Human studies.

Relationships between percent change in serum adiponectin level (Δadiponectin) and body mass index (A), waist circumstance (B), hip circumstance (C), and waist-hip ratio (D) in patients with obstructive sleep apnea-hypopnea syndrome (OSAS). Δadiponectin was calculated by the formula: [(serum adiponectin concentration after wake-up − before sleep) × 100/serum adiponectin concentration after wake-up].

and subcutaneous fat tissue adiponectin mRNA expression levels in obese yellow-KKAy mice (data not shown), compared with the control (room air), similar to our previous report on epididymal fat tissues of lean mice[9].

Animal *Ex-Vivo* Studies

Next, we performed an *ex-vivo* experiment to examine the effects of exposure to 1% O_2 on adiponectin concentration in media containing mesenteric and subcutaneous fat tissues of 14-week-old male yellow-KKAy mice. Adiponectin concentrations in these media increased linearly during the 2-hour hypoxia (data not shown); therefore, we conducted *ex-vivo* studies under each condition for 1 hour. Adiponectin concentrations in media cultures of mesenteric fat tissues were lower following 1-hour exposure to 1% O_2 (**Fig. 3B, left**) than the control condition, while those from subcutaneous fat tissues were not different (**Fig. 3B, right**) between control and hypoxic conditions. We also examined the adiponectin mRNA expression level in each *ex-vivo* fat tissue under control conditions and 1-hour exposure to 1% O_2. There were no significant changes in adiponectin mRNA expression levels in mesenteric and subcutaneous fat *ex-vivo* tissues between control and hypoxic conditions (date not shown). In addition, we examined the cytotoxicity of 1-hour exposure to 1% O_2 to mesenteric and subcutaneous *ex-vivo* fat tissues by measuring lactic dehydrogenase. No evidence of cytotoxicity was noted in both mesenteric and subcutaneous fat (data not shown). These results indicate that mesenteric fat tissue is susceptible to hypoxic stress with regard to adiponectin production, compared with subcutaneous fat.

Discussion

Two major findings of the present study were: 1) nocturnal falls in circulating adiponectin concentrations correlated negatively with WHR in male OSAS subjects with abdominal obesity; 2) in *ex-vivo* studies of obese mice, exposure of mesenteric fat tissue, but not subcutaneous fat tissue, to hypoxia resulted in the suppression of adiponectin level in the culture media.

Previous studies demonstrated the possible association between sleep apena and visceral obesity[8, 17-19]

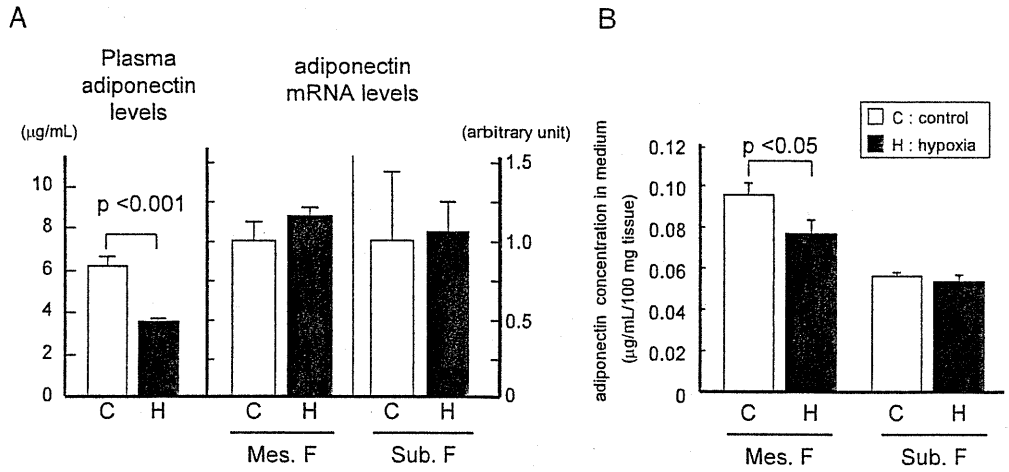

Fig. 3. Animal studies.

Effect of hypoxia on adiponectin in obese yellow-KKAy mice ($n=6$, each). A. plasma adiponectin concentrations in obese yellow-KKAy mice under control (20% O_2) and hypoxic exposure (10% O_2) for 2 days were measured by ELISA, as described in Materials and Methods. Expression levels of adiponectin mRNA in mesenteric fat tissue (mes. F), and subcutaneous fat tissue (sub. F) of obese yellow-KKAy mice under control (20% O_2) and hypoxic exposure (10% O_2) for 2 days. Data were normalized against 18s mRNA. Data are the mean ± SEM. Similar results were observed in other series of experiments. B. Fifteen-week-old yellow-KKAy mice were used in these ex-vivo experiments ($n=7$, each), as described in Materials and Methods. Mes. F: mesenteric fat ex-vivo tissues. Sub.F: subcutaneous fat ex-vivo tissues. C: control (18-21% O_2 − 5% CO_2), H: hypoxic stress (1% O_2) conditions. Data are the mean ± SEM. Similar results were observed in other series of experiments.

and metabolic syndrome[20], and recent studies reported that obese subjects with sleep apnea also suffer from daytime hypoadiponectinemia[21, 22]. Our recent study showed daytime hypoadiponectinemia and nocturnal falls in circulating adiponectin concentrations in patients with severe OSAS, in part due to hypoxic stress[9]. Previous studies also demonstrated that daytime circulating adiponectin levels correlated negatively with BMI, WC, HC and WHR in male patients with OSAS, although daytime circulating levels of leptin correlated positively with BMI, WC, or WHR[18, 23-26]. To our knowledge, the present study is the first to assess the relationship between nocturnal falls in circulating adiponectin concentrations and fat distribution in male subjects with OSAS. These results may partly relate to the characteristics of this study population, i.e. male OSAS subjects with obesity. It is known that fat distribution, such as WHR or the visceral fat area/subcutaneous fat area ratio obtained from CT cross-sectional images in the umbilical region[27], is useful in obese subjects, although WC is known to be a simple parameter of visceral fat accumulation in the general population. The current study showed that the nocturnal fall in circulating adiponectin correlated inversely with fat distribution, i.e.WHR, in male OSAS subjects with abdominal obesity (**Fig. 2**), suggesting that fat distribution matters.

The present study focused on the effect of hypoxia on adiponectin expression in mesenteric and subcutaneous fat tissues of obese yellow-KKAy mice. The results demonstrated that hypoxic stress resulted in the suppression of plasma adiponectin levels in obese yellow-KKAy mice, with no apparent change in mesenteric and subcutaneous fat tissue adiponectin mRNA expression in obese yellow-KKAy mice (**Fig. 3A**). For clarify the mechanism of their posttranscriptional dysregulation, we also investigated the effect of hypoxia on adiponectin production from mesenteric and subcutaneous fat *ex-vivo* tissues of obese mice. Exposure to hypoxia suppressed adiponectin secretion from mesenteric *ex-vivo* fat tissue from obese mice, but not from subcutaneous fat from the same mice (**Fig. 3B**). Based on these findings, we propose that visceral fat accumulation in obese subjects with OSAS might be associated with nocturnal falls in circulating adiponectin concentrations. The hypoxia-induced decrease of adiponectin production in mesenteric fat may be partly susceptible to decreased circulating adiponectin levels because subcutaneous fat is the largest proportion of total body fat content. Our previous works demonstrated that accumulated visceral fat associated strongly with the production of

reactive oxygen species (ROS), locally[28] and systemically[29]. Although not evaluated here, hypoxic stress might also occur more overtly in visceral fat tissue, related to hypoxia-induced ROS production, as reported previously in pulmonary vessels[30, 31] and cardiomyocytes[32]. Further studies are necessary to elucidate the precise mechanism in OSAS patients.

Conclusion

In conclusion, we demonstrated for the first time that exposure to hypoxia resulted in the dysregulation of adiponectin production from mesenteric fat tissues of obese mice. This association may explain the nocturnal falls in circulating adiponectin concentrations seen in OSAS patients with visceral obesity. The results suggest that reduction of visceral fat accumulation might be therapeutically beneficial in OSAS-related disease, especially OSAS-related nocturnal cardiac events in abdominal obesity.

Limitations of the Study

Our study had several limitations. First, the cross-sectional design makes it difficult to establish a cause-effect relationship. Second, the results may not be valid in non-Japanese populations. Third, it was difficult to recruit a sufficient number of subjects, and thus further multicenter studies of larger samples or controlled samples (non-OSAS versus OSAS, or non-obese versus obese) should be conducted in the future. Fourth, the present study used anthropometric measures, such as WC, HC, and WHR, to account for the effects of body fat distribution, because we could not measure visceral fat and subcutaneous fat areas using a computed tomography scan, as this clinic was unequipped. Further research on both visceral and subcutaneous fat areas measured by computed tomography scanning or magnetic resonance imaging is needed to delineate the effects of visceral and subcutaneous adiposity. Fifth, the present study lacks the clear advantage of diabetes and hypertension. Finally, although hypoxia (intermittent and sustained), reoxygenation, neuro-hormonal abnormality, abnormal metabolism, low sleep quality and other factors in OSAS during sleep could explain the nocturnal fall in circulating adiponectin levels, the current study used a sustained hypoxic model, as we reported previously[9].

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Disclosure Statement

The authors declare no conflict of interest.

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Impact of Sleep-Disordered Breathing, Visceral Fat Accumulation and Adiponectin Levels in Patients with Night-Time Onset of Acute Coronary Syndrome

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Acute coronary syndrome (ACS) during sleep occurs at a relatively low frequency and the pathogenic background remains uncertain. The aim of the present study was to determine the significance of sleep-disordered breathing (SDB) and excess visceral fat with nocturnal dysregulation of adipocytokines in night-time onset of ACS. SDB, visceral fat area (VFA), and changes in circulating adipocytokine levels were assessed in 109 consecutive patients with ACS. SDB and VFA were assessed by cardiorespiratory monitoring and computed tomographic scan, respectively. Visceral fat accumulation was more common in patients with (12 to 7 A.M.) than without (7 to 12 A.M.) night-time onset of ACS ($p < 0.05$). In patients with night-time onset of ACS, those with excess visceral fat were significantly more likely to have SDB and nocturnal dysregulation of adiponectin than those without such accumulation ($p < 0.05$), but there was no difference between those with and without excess visceral fat (VFA cutoff 100 cm^2) in patients with non–night-time onset of ACS. In conclusion, night-time onset of ACS is associated with excess visceral fat and SDB (referred as to "syndrome Z"). SDB and excess visceral fat are treatable risk factors. Decrease of excess visceral fat and treatment of SDB could be beneficial in in preventing nocturnal cardiac events. (Am J Cardiol 2011;108:1266–1271)

Acute coronary syndrome (ACS) generally occurs with a diurnal periodicity that peaks during morning to daytime activity.[1–3] Fewer patients have onset of ACS during sleep and factors that influence the likelihood of ACS occurring during sleep are unknown but are potentially affected by several factors that differ between patients including the presence of sleep apnea. Many but not all patients with visceral obesity have sleep-disordered breathing (SDB), especially obstructive sleep apnea (OSA).[4–7] Patients with OSA have a high frequency of sudden cardiac death from night to early morning.[8–10] The cluster of visceral obesity, multiple risk factors, and OSA[11–13] is referred to as "syndrome Z."[14] Recently we found dysregulation of adipocytokines during sleep in patients with obesity and OSA.[15,16] A combination of SDB and excess visceral fat with dysregulation of adipocytokines may participate in increasing ACS risk during sleep. The aim of the present study was to clarify this point.

Methods

Of patients with clear-onset ACS who were admitted and underwent revascularization within the first 24 hours after admission to Kokura Memorial Hospital from April through September 2009, 109 consecutive Japanese patients who underwent overnight cardiorespiratory monitoring (Somté, Compumedics, Melbourne, Australia) before discharge from the hospital (about 1 week after onset of ACS) to assess the presence of SDB with informed consent were included in this study. Table 1 presents characteristics of study participants. Shift workers and patients with coronary spasm and nonsignificant organic stenosis were excluded from the study. All patients with ACS underwent coronary angiography and successful revascularization with percutaneous coronary intervention procedures within the first 24 hours after admission. ACS included ST-segment elevation acute myocardial infarction (n = 66, average peak creatinine kinase 3,137 ± 2144 IU/L mean ± SD), non–ST-segment elevation acute myocardial infarction (n = 27, average peak creatinine kinase 691 ± 192 IU/L), and high-risk unstable angina (n = 16, average peak creatinine kinase 182 ± 16 IU/L).[17] ST-segment elevation acute myocardial infarction was diagnosed when new or presumed new ST-segment elevation 1 mm was seen in any location or new left bundle branch block was found on the index or qualifying electrocardiogram with 1 positive cardiac biochemical marker of necrosis. Non–ST-segment elevation acute myocardial infarction was diagnosed in the presence of 1 positive cardiac biochemical marker of necrosis without new ST-segment elevation seen on the index or qualifying elec-

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Table 1
Baseline characteristics of patients with acute coronary syndrome (n = 109)

Age (years)	66 ± 12 (40–91)
Men/women	93/16
Body mass index (kg/m^2)	23.8 ± 3.4 (17.8–37.4)
Waist circumference (cm)	87 ± 10 (64–112)
Hip circumference (cm)	92 ± 6 (80–112)
Total fat area (cm^2)	239 ± 95 (69–523)
Visceral fat area (cm^2)	127 ± 63 (22–361)
Subcutaneous fat area (cm^2)	112 ± 55 (18–284)
Polysomnographic findings	
Apnea–hypopnea index (events/hour)	11 ± 12 (0–61)
Baseline arterial oxygen saturation (%)	95 ± 2 (91–98)
Lowest arterial oxygen saturation (%)	83 ± 4 (72–94)
4% oxygen desaturation index (events/hour)	14 ± 12 (1–69)
Percent time at arterial oxygen saturation <90%	2 ± 4 (0–25)
Apnea–hypopnea index	
<5	46 (42%)
≥5–<15	37 (34%)
≥15–<30	19 (18%)
≥30	7 (6%)
Obstructive sleep apnea index/central sleep apnea index/mixed apnea index/hypopnea index	3.5 ± 5.7/1.5 ± 3.1/1.8 ± 3.7/4.3 ± 4.2
Serum adiponectin (μg/ml)	9.2 ± 6.6 (2.3–38.3)
Plasma total plasminogen activator inhibitor-1 (ng/ml)	24.5 ± 11.6 (5.0–61.0)
Serum soluble CD40 ligands (pg/ml)	2.4 ± 1.9 (0–8.3)

Data are presented as mean ± SD (range) or number of subjects (percentage).

Table 2
Correlations among visceral fat area, subcutaneous fat area, and various clinicobiochemical parameters

	VFA		SFA	
	r	p Value	r	p Value
Apnea–hypopnea index	0.15	0.008	0.18	0.062
Peak creatinine kinase	0.17	0.077	0.11	0.264
Adiponectin after waking	−0.41	0.0001	−0.19	0.052
Total plasminogen activator inhibitor-1 after waking	0.28	0.004	0.25	0.008
Soluble CD40 ligands after waking	0.21	0.034	0.08	0.441

The p values by Pearson correlation analysis and $p < 0.05$ were considered statistically significant.
SFA = subcutaneous fat area.

trocardiogram. High-risk unstable angina was the absence of ST-segment elevation on electrocardiogram and serum biochemical markers indicative of myocardial necrosis within each hospital laboratory's normal range. With a discharge diagnosis of ACS the affected coronary artery was the left anterior descending coronary artery in 59 patients, left circumflex coronary artery in 11 patients, and right coronary artery in 39 patients. Date and time of onset of ACS (i.e., angina or chest pain) were determined through an interview and retrospective analysis of clinical charts. This strategy for assessing the onset time of ACS has been validated previously.[18,19] Onset of ACS was defined as the time when chest pain occurred and was divided into 2 periods (night-time 12 to 7 A.M., non–night-time 7 to 12 A.M.) according to the time of venous sample collection in the morning as reported previously.[15,16] Numbers of patients with a history of myocardial infarction or angina and a family history of coronary artery disease were 16 (15%) or 11 (10%) and 11 (10%), respectively. The medical ethics committees of Osaka University and Kokura Memorial Hospital approved this study. All participants were Japanese and each gave a written informed consent. This study (Osaka University Visceral Fat [O-VF] Study) is registered under number UMIN 000002997.

For overnight cardiorespiratory monitoring, the recorded signals (airflow, arterial oxygen saturation, thoracic and abdominal wall movements) were analyzed for number of apneas and hypopneas during sleep. The oxygen desaturation index, lowest arterial oxygen saturation, baseline arterial oxygen saturation, and time at desaturation <90% in minutes of total bedtime were measured for the entire night. Apnea was defined as cessation of airflow >10 seconds. Hypopnea was defined as a decrease in airflow signal to <70% of the preceding level associated with >4% desaturation. Sleep apnea was categorized as OSA, central sleep apnea, and mixed apnea as reported previously.[15] Apnea–hypopnea index was defined as the total number of apneas/hypopneas per hour of recording time according to the guideline.[20] An apnea–hypopnea index ≥5 established the diagnosis of SDB. All recordings were scored manually by an experienced polysomnographer and duration of sleep was estimated using self-reported sleep time and recorded data as reported previously.[15]

Height and weight were measured in a standing position. Body mass index was calculated as weight (kilograms) divided by height (meters) squared. Waist circumference (centimeters) at the umbilical level was measured with a nonstretchable tape in late expiration while standing. Hip circumference was measured horizontally at the level of the greater trochanter of the femur with the patient standing. Waist-to-hip ratio was defined as waist circumference divided by hip circumference. Visceral fat area (VFA) and subcutaneous fat area were computed or measured manually on computed tomographic scan at the umbilical level. Visceral fat accumulation was measured by computed tomographic scan and defined as VFA ≥100 cm^2.[21]

Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the patient had rested in a supine position for ≥10 minutes. Venous blood samples were collected for laboratory measurements after the subject awoke and was in a supine position. In each sleep study that included adiponectin monitoring, venous blood samples were obtained before sleep onset (~10:00 P.M.) and after waking (~7:00 A.M.) while the subject was in a supine position. For the present study serum samples that

Table 3
Comparison between patients with and without visceral fat accumulation (visceral fat area cutoff 100 cm^2)

	VFA <100 cm^2	VFA ≥100 cm^2	p Value*
Subjects (men/women)	45 (36/9)	64 (57/7)	0.19
Age (years)	67 ± 11	65 ± 13	0.29
Body mass index (kg/m^2)	21.7 ± 2.2	25.2 ± 3.4	<0.0001
Visceral fat area (cm^2)	63 ± 22	146 ± 43	<0.0001
Subcutaneous fat area (cm^2)	101 ± 49	146 ± 65	<0.001
Culprit coronary lesion (left anterior descending coronary artery/left circumflex coronary artery/right coronary artery)	27/4/14	32/7/25	0.74
Peak creatinine kinase (IU/L)	1,806 ± 2,277	2,269 ± 1,998	0.27
Smoke (never/ex/current)	20/5/20	19/12/33	0.27
Diabetes mellitus	26 (58%)	36 (56%)	0.87
Hypertension	18 (40%)	44 (69%)	<0.01
Dyslipidemia	15 (33%)	40 (62%)	<0.01
Apnea–hypopnea index (events/hour)	8 ± 8	12 ± 14	<0.05
Adiponectin after waking (μg/ml)	12.9 ± 8.2	6.5 ± 3.1	<0.0001
Adiponectin before sleep onset (μg/ml)	12.5 ± 8.1	6.6 ± 3.0	<0.0001
Total plasminogen activator inhibitor-1 after waking (ng/ml)	22.5 ± 10.0	27.3 ± 11.4	<0.05
Total plasminogen activator inhibitor-1 before sleep onset (ng/ml)	23.1 ± 13.2	19.5 ± 7.0	0.10
Soluble CD40 ligand after waking (pg/ml)	2.0 ± 2.0	2.7 ± 1.8	0.07
Soluble CD40 ligand before sleep onset (pg/ml)	1.5 ± 1.3	1.8 ± 1.7	0.44

Data are presented as mean ± SD or number of patients (percentage).
* By Pearson correlation or chi-square analysis.

were obtained at baseline from each participant and stored at −20°C were thawed and assayed for circulating adiponectin (Otsuka Pharmaceutical, Co., Tokushima, Japan), total plasminogen activator inhibitor-1 (PAI-1; (LPIA•tPAI test; Mitsubishi Kagaku Iatron, Tokyo, Japan), and soluble CD40 ligand levels (Quantikine Human Soluble CD40 Ligand Immunoassay, R&D Systems, Inc., Minneapolis, Minnesota).

Hypertension (n = 62, 57%) was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or current treatment for hypertension (calcium channel antagonists in 21, angiotensin-converting enzyme inhibitors in 53, angiotensin receptor blockers in 46, β blockers in 71, diuretics in 9). Diabetes mellitus (n = 62, 57%) was defined according to World Health Organization criteria or as nonfasting plasma glucose concentration ≥6.1 mmol/L, and/or current treatment for diabetes mellitus (sulfonyl ureas in 9, biguanides in 1, α-glucosidase inhibitors in 18, insulin in 2). Dyslipidemia (n = 65, 60%) was defined as low-density lipoprotein cholesterol concentration >3.6 mmol/L, triglyceride concentration ≥1.69 mmol/L, high-density lipoprotein cholesterol concentration <1.04 mmol/L, and/or treatment for dyslipidemia (statins in 82, fibrates in 2). We included subjects receiving medications with antiplatelet drugs (aspirin in 107, ticlopidine in 41, clopidogrel in 61). With regard to smoking status 49% (n = 53) of subjects were current smokers, 15% (n = 17) were former smokers, and 36% (n = 39) were nonsmokers.

All values were expressed as mean ± SD (range, minimum to maximum) and p values <0.05 were considered statistically significant. Relations between 2 continuous variables were analyzed using scatter plots and Pearson correlation coefficients. Differences among groups were compared by 1- or 2-way analysis of variance with Fisher's protected least significant difference test for multiple-group analysis or unpaired Student's *t* test for experiments involving only 2 groups. Frequencies of each group were compared by chi-square test. All statistical analyses were performed with STATVIEW-J 5.0 (SAS Institute, Cary, North Carolina).

Results

Frequency of SDB diagnosed by overnight cardiorespiratory monitoring was 58% (n = 63) in patients with ACS, although mean body mass index was 23.8 kg/m^2 (Table 1). In patients with ACS VFA correlated positively with circulating total PAI-1 levels and negatively with circulating adiponectin levels (Table 2) as reported previously in the general population.[22] Subcutaneous fat area correlated positively only with total PAI-1. The present study found a positive correlation between VFA and soluble CD40 ligand levels in patients with ACS as in a previous study.

As presented in Table 3 patients with visceral fat accumulation (VFA ≥100 cm^2) had a higher body mass index, larger VFA and subcutaneous fat areas, higher total PAI-1, and lower adiponectin than patients with VFA <100 cm^2. Prevalence of hypertension and dyslipidemia was higher in patients with VFA ≥100 cm^2 compared to patients with VFA <100 cm^2. These results suggest that excess visceral fat in patients with ACS correlates with metabolic disorders and dysregulation of adipocytokines.

To clarify the significance of visceral fat accumulation on night- and non–night-time onset of ACS, we divided patients into 4 groups according to time of onset of ACS and visceral fat accumulation (Table 4). Prevalences of VFA ≥100 cm^2 was 68% (n = 17 or 25) in patients with night-time onset of ACS and 56% (n = 47 of 84) in those with non–night-time onset of ACS. Furthermore, prevalence of SDB and apnea–hypopnea index was significantly higher in those with VFA ≥100 cm^2 than in those with VFA <100 cm^2 in patients with night-time onset of ACS (Figure 1,

Table 4
Comparison of patients with and without visceral fat accumulation (cut-off visceral fat area (100 cm^2) stratified by time of onset of acute coronary syndrome (night-time and non–night-time)

Time of Onset of ACS	12–7 A.M.		7–12 A.M.	
	VFA <100	VFA ≥100	VFA <100	VFA ≥100
Subjects (men/women)	8 (6/2)	17 (17/0)†	37 (30/7)	47 (40.7)
Age (years)	64 ± 14	62 ± 12	68 ± 10	66 ± 13
Body mass index (kg/m^2)	21.0 ± 2.2	25.8 ± 3.8†	21.9 ± 2.3	24.9 ± 3.3§
Waist–hip ratio	0.9 ± 0.1	1.0 ± 0.1†	0.9 ± 0.0	1.0 ± 0.1§
Visceral fat area (cm^2)	56 ± 29	151 ± 50†	64 ± 21	145 ± 41§
Subcutaneous fat area (cm^2)	81 ± 34	147 ± 60†	105 ± 41	145 ± 68§
Smoke (never/ex/current)	4/0/4	2/1/14	16/16/5	22/7/18
Diabetes mellitus	4 (50%)	7 (41%)	22 (35%)	29 (47%)
Hypertension	5 (63%)	13 (76%)	12 (35%)	31 (66%)§
Dyslipidemia	5 (63%)	7 (41%)	20 (54%)	33 (70%)
Apnea–hypopnea index (events/hour)	3 ± 3	14 ± 15‡	9 ± 8	12 ± 14
Adiponectin before sleep onset (μg/ml)	16.9 ± 12.7	6.3 ± 2.1‡	11.6 ± 6.8	6.8 ± 3.2§
Adiponectin after waking (μg/ml)	18.0 ± 12.7	6.2 ± 2.0†	11.7 ± 6.7	6.7 ± 3.4§
Change in adiponectin (%)*	5.6 ± 4.7	−0.4 ± 6.4‡	0.6 ± 12.1	−2.3 ± 11.0
Total plasminogen activator inhibitor-1 before sleep onset (ng/ml)	16.0 ± 5.6	19.6 ± 6.7	24.1 ± 13.7	19.4 ± 7.2
Total plasminogen activator inhibitor-1 after waking (ng/ml)	17.6 ± 8.0	28.6 ± 13.2‡	21.7 ± 10.8	26.8 ± 10.8‖
Change in total plasminogen activator inhibitor-1 (%)*	37 ± 19	33 ± 34	12 ± 71	25 ± 28
Soluble CD40 ligand before sleep onset (pg/ml)	1.5 ± 1.2	1.4 ± 1.7	1.5 ± 1.3	1.9 ± 1.6
Soluble CD40 ligand after waking (pg/ml)	1.4 ± 1.5	2.3 ± 1.8	2.2 ± 2.0	2.8 ± 1.8
Change in soluble CD40 ligand (%)*	−90 ± 227	117 ± 437	−29 ± 256	−15 ± 280

Data are presented as mean ± SD or number of patients (percentage).
* This parameter was calculated by the formula (circulating parameter concentration after waking minus before sleep onset × 100/circulating parameter concentration after waking).
† $p < 0.01$; ‡ $p < 0.05$ compared to without visceral fat accumulation in same group.
§ $p < 0.01$; ‖ $p < 0.05$ compared to without visceral fat accumulation in same group.

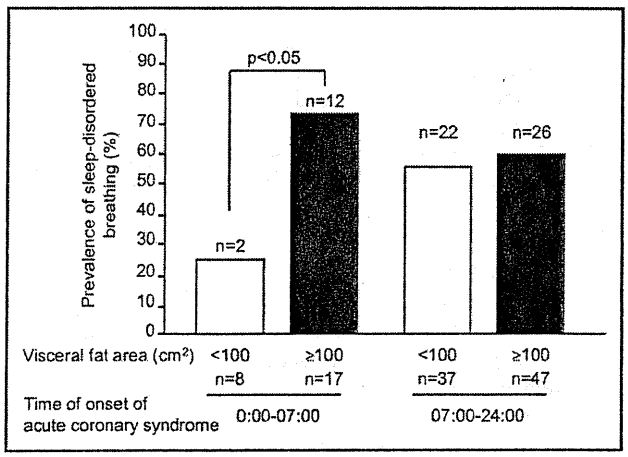

Figure 1. Prevalence of sleep-disordered breathing in patients with acute coronary syndrome with and without visceral fat accumulation (visceral fat area cut-off value 100 cm^2) stratified by time of onset of acute coronary syndrome. Differences among groups were compared by analysis of variance with Fisher's protected least significant difference test for multiple-group analysis or unpaired Student's *t* test for experiments involving only 2 groups. Frequencies of each group were compared by chi-square test. A p value <0.05 was considered statistically significant.

Table 4). In contrast, prevalence of SDB and apnea–hypopnea index was not different between those without and those with excess visceral fat in patients with non–night-time onset of ACS. Of patients with night-time onset of ACS, those with VFA ≥100 cm^2 had significantly lower circulating adiponectin levels and higher circulating total PAI-1 levels and tended to have higher circulating soluble CD40 ligand levels ($p = 0.051$) than those with VFA <100 cm^2 (Table 4). Further analysis of patients with night-time onset of ACS showed that change in adiponectin in those with VFA ≥100 cm^2 was −0.4 ± 6.4%, whereas the change in those with VFA <100 cm^2 was 5.6 ± 4.7%; the difference between the 2 groups was significant (Table 4).

Discussion

The major finding of the present study was that night-time onset of ACS was associated with SDB in patients with visceral fat accumulation, who also had hypoadiponectinemia and nocturnal dysregulation of circulating adiponectin concentrations, compared to those without visceral fat accumulation. In contrast, prevalence of SDB and change in adiponectin were not different between those with and those without visceral fat accumulation in patients with non–night-time onset of ACS. These results suggest that an association of SDB and excess visceral fat with nocturnal dysregulation of adiponectin may play some role in the development of night-time onset of ACS.

Although obese East and South Asians including Japanese have a relatively milder degree of adiposity compared

to European and American subjects,[23] fat distribution, specifically visceral fat, still correlates well with various diabetogenic, atherogenic, prothrombotic, and proinflammatory metabolic abnormalities (referred to as "metabolic syndrome") that increase the risk of cardiovascular diseases. The present study identified a high prevalence of classic coronary risk factors, e.g., dyslipidemia and hypertension (at 60% and 57%, respectively). There was also a large proportion of diabetics despite a relatively low body mass index (average 23.8 kg/m^2; Table 1), which could reflect these ethnic and racial differences.[24]

Association of OSA with "syndrome X"[25] is currently referred to as syndrome Z.[14] There is tight linkage between visceral obesity and OSA but not all patients with excess visceral fat have OSA. In the present study there was no difference in prevalence of SDB with or without visceral fat accumulation in patients with non–night-time onset of ACS. There are differences in craniofacial structure that may contribute to the risk of OSA in the Japanese population.[26] We have demonstrated that coexistence of excess visceral fat and SDB was associated with night-time onset of ACS (Table 4). Although visceral fat is a known risk factor for ACS onset during the daytime, we have shown that the combination of visceral fat distribution in combination with OSA significantly increases the likelihood of ACS onset during sleep. This finding supports the concept of an adverse synergistic combination of the metabolic syndrome and OSA (syndrome Z).

Repetitive hypoxia, sympathetic activation, and hypercoagulation in SDB may contribute to night-time ACS, although the precise mechanism remains unclear. Dysregulation of adipocytokines in excess visceral fat may also play some role in the development of ACS at night (night coronary plaque erosion and rupture). Adiponectin increases the expression of tissue inhibitor of metalloproteinase-1 in macrophages[27] and thus may inhibit matrix degradation in atheromatous plaques. Although PAI-1 is synthesized in endothelial cells and liver, we previously reported that it is synthesized in adipose tissue and that circulating levels of PAI-1 correlated positively with VFA.[28] The present study demonstrated that circulating PAI-1 levels positively and adiponectin levels negatively correlated with VFA in patients with ACS (Table 2). Importantly, the association of SDB in subjects with excess visceral fat further caused nocturnal dysregulation of adiponectin (Table 4). Hypoxia in adipocytes regulates positively the expression of the PAI-1 gene and negatively the expression and secretion of adiponectin.[15,16]

The present study provided new findings of the positive relation between serum soluble CD40 ligand levels and VFA (Table 2) in patients with ACS. An increase in soluble CD40 ligand level denotes increased risk of cardiovascular events in unstable angina and probably represents platelet activation.[29] There was also a negative relation between circulating soluble CD40 ligand and adiponectin levels (data not shown), suggesting adiponectin may inhibit thrombus formation and platelet aggregation.[30]

In conclusion, the present study indicates that subjects with excess visceral fat who develop ACS at night-time are more likely to have SDB and dysregulated adiponectin production during sleep compared to those without excess visceral fat. In patients with excess visceral fat assessment of SDB and decrease of visceral fat seem important for the prevention of ACS, especially with regard to night-time coronary plaque rupture and erosion.

There are several limitations to this study. First, factors that underlie the onset of ACS during sleep in patients without visceral fat accumulation or SDB remain uncertain. Further analysis will be needed to clarify this point. Second, we evaluated and measured various laboratory parameters, fat distribution, and SDB at about 1 week after onset of ACS; therefore, medical therapy for ACS may have affected these parameters. Third, there is bias in single-center trials. Fourth, the present study did not include control patients without ACS. Fifth, the strategy for assessing time of onset of ACS has been validated previously,[18,19] although there may be a difference in time from plaque rupture and the erosion event to a patient's ACS-related symptoms. To clarify the exact time of onset of ACS further study is needed on the time-of-day question including whether the patients were awakened from sleep by chest pain. Sixth, we used conventional overnight cardiorespiratory monitoring to assess SDB without electroencephalography. Duration of sleep by self-reported sleep time and recorded data might overestimate real sleeping time based on electroencephalogram and thus underestimate the apnea–hypopnea index. Although polysomnography is required to accurately assess sleep duration and structure and is currently considered the "gold standard" method to diagnose OSA attended or not, time spent on analysis and interpretation of data obtained by technical and medical staff means it may not be the most cost-effective or convenient method of assessing SDB in the large number of patients who present with ACSs and require secondary prevention of adverse outcomes in this urgent setting.

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Impact of occupational stress on stroke across occupational classes and genders

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ABSTRACT

The aims of the present study were to analyze the association between incident stroke, occupational class and stress and to examine whether the association is found in both men and women in a prospective study of Japanese male and female workers. A total of 3190 male and 3363 female Japanese community-dwelling workers aged 65 or under with no history of cardiovascular disease were followed. Occupational stress was evaluated using a demand-control questionnaire. The impact on stroke was examined in stratified analyses of occupational classes. We identified 147 incident strokes (91 in men and 56 in women) during the 11-year follow-up period. Men with high strain jobs (combination of high job demand and low job control) were nearly three times more likely to suffer from a stroke than men with low strain jobs (combination of low job demand and high job control). Among male workers in low occupational classes (blue-collar and non-managerial work), job strain was associated with a higher risk of stroke. In contrast, there was no association between job strain and incident stroke among male workers in high occupational classes (white-collar and managerial work). No statistically significant differences were found for stroke incidence among the job characteristic categories in all the female participants. However, significant, over five-fold excess risks were found among white-collar and managerial female workers exposed to high job strain, compared with their counterparts with low strain jobs. Our study of Japanese workers provided supportive evidence for vulnerability to occupational stress among lower occupational class workers in males but not in females.

Introduction

Previous studies have indicated that workers in lower occupational classes are more vulnerable to occupational stress than workers in higher occupational classes (Hallqvist, Diderichsen, Theorell, Reuterwall, & Ahlbom, 1998; Kivimäki et al., 2002; Lynch, Krause, Kaplan, Tuomilehto, & Salonen, 1997; Theorell et al., 1998; Wege et al., 2008). Only a few studies have prospectively investigated the association between occupational class and occupational stress using stroke as the outcome (Kivimaki et al., 2009; Kuper, Adami, Theorell, & Weiderpass, 2007; Toivanen, 2008; Toivanen & Hemström, 2008; Virtanen & Notkola, 2002). Of those, one study showed that female lower occupational class non-manual workers with low job control had a significantly higher risk of stroke mortality (Toivanen & Hemström, 2008). Other prospective studies did not test whether lower occupational class workers were more susceptible when exposed to occupational stress (Kivimaki et al., 2009; Kuper et al., 2007). Thus, there is no prospective study that has addressed different vulnerabilities to stress across occupational classes using incident stroke as an outcome. In addition, there may be gender differences in the health impact of socioeconomic factors as well as occupational stress (Kopp, Skrabski, Szekely, Stauder, & Williams, 2007). Nevertheless, few studies have addressed the potential interaction between gender and occupational or employment status and gender differences in work hazard exposures and the health impact. Furthermore, the prospective associations between occupational class/occupational stress and health outcomes have not been examined extensively outside Western societies. Data from the Jichi Medical School Cohort Study of a Japanese working population, a large-scale prospective cohort study, allowed an approach to this important issue. The aims of the present study were to analyze the association between incident stroke, occupational class and stress and examine whether the association is found in both men and women in a prospective study of Japanese male and female workers.

Materials and methods

Study populations

Data were acquired from routine mass screening examinations for cardiovascular diseases in the aged, which were carried out in

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Japan in accordance with relevant legal regulations. The regulations required municipal governments to manage the program efficiently and to make it available to all residents who wished to participate. The local government office invited all potential participants in each community to attend screenings by sending letters or using public information channels. The invitation explained that people who were visiting hospitals or clinics in relation to cardiovascular diseases were exempt from the examination. Ultimately, 12,490 Japanese individuals from 12 communities across Japan participated between 1992 and 1995. The overall response rate was 65.4% (Ishikawa, Gotoh, Nago, Kayaba, & Jichi Medical School (JMS) Cohort Study Group, 2002). Because the aim of this study was to analyze the association between incident stroke, occupational class and occupational stress, the study population was limited to 3190 male and 3363 female workers with baseline ages ≤65 years who were free from stroke or myocardial infarction and completed information regarding occupations. Compared with the general working population, the study population included larger proportions of older workers and workers engaged in pre-industrial occupations (farming/forestry/fisheries) (Tsutsumi, Kayaba, Kario, & Ishikawa, 2009). Over 99% of the participants were employed by companies with fewer than 300 employees. Japanese companies are required to conduct an annual health check-up of their employees. However, for those not offered health examinations at their workplace, such as workers with pre-industrial occupations or those who were self-employed, the mass screening examination program provided an opportunity to have their health status checked. Furthermore, many small companies (local industries) or local government offices used the opportunity to get health checkups for their employees in rural districts, such as those included in our study areas. We inferred from the analysis of repeated surveys that changes in occupation or job position were infrequent in the settings studied (Kayaba, Tsutsumi, Gotoh, Ishikawa, & Miura, 2005). Some part-time employees may have been included in the study population, but this was not ascertained.

Surveillance of stroke

The established follow-up system ensured that participants were contacted annually by direct interview or telephone/letter to determine their health status. Participants were asked if they had suffered a stroke or cardiovascular disease after enrolling. They were asked which hospital they attended and when, to ascertain the incidence of these diseases. When an incident case was suspected, all the medical records were reviewed and duplicate computer tomography films or magnetic resonance imaging films for these patients were obtained. The diagnosis of stroke was based on the presence of a focal and nonconvulsive neurological deficit lasting ≥24 h, with a clear onset. Patients who had a transient ischemic attack were excluded. The diagnosis was determined independently by a diagnostic committee composed of a radiologist, a neurologist and two cardiologists. Specific causes of mortality were determined for all participants who died between the date of their health examination and the end of 2005, using the Cause-of-Death Register found at the public health center located in each community. This was done with the permission of the Agency of General Affairs and the Ministry of Health, Labor and Welfare.

Assessment of occupational class

Two categories of occupation (white-collar and blue-collar) and two categories of position (manager and non-manager) were used to reflect the occupational classes related to socioeconomic status. Participants' occupations at baseline were classified according to the National Statistics guidelines (Ministry of Internal Affairs and Communications, 1998). The following occupations were included: professional or technician (n = 196 men, 196 women); clerk (198, 342); sales worker (249, 355); service worker (250, 550); farming, forestry, or fisheries worker (1200, 1134); security worker (18, 1); transportation or communications worker (87, 4); construction worker (609, 83); craft worker or laborer (330, 657) and unclassified (53, 41). Regarding occupation, the first four job categories (from professional/technician to service worker) were classed as white-collar jobs; the remainder was classed as blue-collar jobs. If participants reported themselves to be a manager, they were classed as white-collar, regardless of their chosen job category. Positions were classed as either manager or non-manager. Subjects were categorized as managers if they reported themselves to be employers or managers at their companies. The managerial positions included relatively large numbers of employers or administrative personnel, reflecting the rural setting of our study. The majority was considered to be self-employed. In accordance with several preceding studies, we classed self-employed workers as managerial, even though they may not necessarily have had any subordinates (Fukuda, Nakamura, & Takano, 2005; Kawakami et al., 2004; Rosengren et al., 1990; Wege et al., 2008). Although our occupational classification was based on simple questions, previous analyses of this cohort found that the occupational categories showed a reasonable association with psychosocial job characteristics and lifestyle factors (Hirokawa, Tsutsumi, & Kayaba, 2006; Tsutsumi, Kayaba, Tsutsumi, & Igarashi, 2001).

Assessment of occupational stress

Occupational stress was evaluated at baseline using a Japanese version of the Demand-Control Questionnaire from the WHO-MONICA Psychosocial Study Questionnaire. The questionnaire is based on Karasek's demand-control model (Karasek & Theorell, 1990). The model states that workers who face high psychological demands and have little control over their work (i.e., job strain) are at greater risk of becoming ill. This questionnaire has the following subscales: job demands related to quantitative and qualitative workloads (5 items) and job control related to decision-making authority and skill discretion (6 items). All questions are scored on a Likert scale of 1–4. Cronbach's alpha coefficients for the job demand index and job control index were 0.69 and 0.65, respectively. The job conditions in this cohort during the follow-up period demonstrated a moderate degree of stability with 5-year-interval intraclass correlation coefficients of 0.55 (n = 378) for job demands and 0.63 (n = 377) for job control (Kayaba et al., 2005). Cross-classification of the job demand and job control scales according to their sex-specific median values produced a quadrant scheme with four psychosocial job characteristics: low job demand and high job control, representing a low strain job (reference category); high job demand and high job control, representing an active job; low job demand and low job control, representing a passive job; and high job demand and low job control, representing a high strain job.

Assessment of confounding factors

We measured the following demographic characteristics and conventional risk factors at baseline: age (18–39, 40–49, 50–59, 60–65 years); educational attainment (≤15 years: age at completion of compulsory education; 16–18 years: age at finishing senior high school; ≥19 years: age at entering college or further education); smoking status (lifetime non-smoker, ex-smoker, current smoker); alcohol consumption (non-drinker, <1 go daily (go, a traditional Japanese alcohol unit; 1 go = 28.9 g of alcohol), ≥1 go

daily); and physical activity index (<29, 29–36, ≥37) (Kannel & Sorlie, 1979).

Data analysis

Analysis was based on the incident stroke rate during the 11 years of follow-up. For each participant, person-years of follow-up were allocated according to the dates of health examinations until death, date of a move away from the study community, an endpoint (stroke) or December 31, 2005, whichever occurred first. Data regarding the movements of the study population were obtained every year from the participants' municipal governments, which recorded movements of residents in and out of the particular community. A total of 193 participants (2.9% of the analyzed cohort) moved out of their communities during the study period and were treated as censored cases. The total number of observed person-years was 71,385. The association between occupational stress and stroke were examined by Cox proportional hazard regression analysis. To investigate whether there were different susceptibilities to incident stroke across occupational classes, stratified analyses by the two occupational categories were conducted. Tests for trends across psychosocial job characteristics categories were conducted by assigning equally spaced values (e.g., 0, 1, 2, or 3) to the categories and treating the variables as continuous in the Cox proportional hazards model. The hazard ratios were estimated after adjusting for age, educational attainment, smoking status, alcohol consumption, physical activity index and study area (community). There was no adjustment for other biomedical risk factors, such as hypertension. However, as research has strongly suggested that biomedical risk factors are in the causal pathway between job strain and stroke, adjusting for these would represent an underestimation of risk (Tsutsumi et al., 2009). Ordinal variables including a small amount of missing data were represented by dummy variables. All probability values were two-tailed, and values of $P < 0.05$ were considered statistically significant. All analyses were conducted using SPSS for Windows, release 18.

The study design and procedures were reviewed and approved by each municipal government and by the Ethics Committee for Epidemiological Research at Jichi Medical School. Written informed consent was obtained from all prospective participants.

Results

Table 1 shows the relationships between occupational classes and the examined variables at baseline. Male white-collar workers were younger, had higher educational attainment and were less physically active than male blue-collar workers. The two occupational categories were significantly associated. Specifically, three-quarters of white-collar workers and a third of blue-collar workers filled supervisory or managerial positions. Regarding psychosocial job characteristics, active and, to a lesser extent, low strain jobs were more prevalent among white-collar workers, while passive and, to a lesser extent, high strain jobs were more prevalent among blue-collar workers. Male managers were most likely to be in their 40s or 50s. Male managers had higher educational attainment and were less physically active than non-managerial male workers. Regarding psychosocial job characteristics, active and, to a lesser extent, low strain jobs were more prevalent among managers, while passive and, to a lesser extent, high strain jobs were more prevalent among non-managerial workers.

Female white-collar workers were younger, had higher educational attainment, were less physically active and consumed more cigarettes and alcoholic beverages than female blue-collar workers. Women managers were fewer than men. Two-fifths of white-collar workers and one-fifth of blue-collar workers filled supervisory or managerial positions. High strain jobs were prevalent among female blue-collar workers but low strain, active and passive jobs were more prevalent among female white-collar workers. Female managers were also most likely to be in their 40s and 50s. Female managers had higher educational attainment, were less physically active, and consumed more alcoholic beverages than non-managerial workers. Active and, to a lesser extent, low strain jobs were more prevalent among managers and passive and, to a lesser extent, high strain jobs were more prevalent among non-managerial workers.

A total of 147 first-ever strokes occurred during a mean follow-up of 11 years. Table 2 shows incidence rate of stroke for each psychosocial job characteristic and the associations of job characteristics with incident stroke by gender and occupational class. Multivariate analysis revealed a nearly 3-fold increased risk of incident stroke among men with high job strain, compared with their counterparts with low strain jobs (hazard ratio 2.8, *P* for trend 0.022). Among male workers in low occupational classes, high strain was associated with a higher risk of stroke, with both the effect estimates and trend being statistically significant (hazard ratio 3.9; *P* for trend 0.016 in blue-collar workers, hazard ratio 8.9; *P* for trend 0.019 in non-managerial workers). In contrast, there was no association between high strain and incident stroke among male workers in high occupational classes. No statistically significant differences were found for stroke incidence among the job characteristic categories in all the female participants. However, significant, over 5-fold excess risks were found among white-collar and managerial workers exposed to high strain (hazard ratio 5.6; *P* for trend 0.093 in white-collar workers, hazard ratio 5.3; *P* for trend 0.046 in managerial workers), whereas there was no association between high strain and incident stroke among female blue-collar and managerial workers.

Discussion

In a cohort of Japanese workers, we found a significantly higher risk of incident stroke in men with high job strain among blue-collar workers and those in non-managerial jobs, but not among white-collar workers or those in managerial positions. The opposite trends were observed in women, i.e., significant elevated risks among white-collar and managerial workers, but not among blue-collar workers or those in non-managerial positions. To the best of our knowledge, this is the first study to prospectively demonstrate a significantly increased susceptibility to incident stroke among lower occupational class male workers when exposed to occupational stress but not among higher occupational class workers. Occupational stress is known to be associated with cardiovascular diseases in men based on previous studies (Belkić, Landsbergis, Schnall, & Baker, 2004), including evidence from Japanese working populations (Kayaba et al., 1990; Uchiyama, Kurasawa, Sekizawa, & Nakatsuka, 2005; Yoshimasu & The Fukuoka Heart Study Group, 2001). Evidence showed a weaker association between job strain and cardiovascular diseases among women than among men (Belkić et al., 2004), but our findings suggest that job strain predicts cardiovascular diseases also in women of some occupations. The present study provides new insight to the literature by demonstrating a specific association with incident stroke other than western population, strengthening the evidence with a broader socioeconomic scope.

Increased susceptibility to illness among workers in low occupational classes might be the result of prolonged or more intense stress reactions and delayed recovery, compared with workers in higher occupational classes (Hemingway et al., 2005; Shishehbor, Litaker, Pothier, & Lauer, 2006; Steptoe, 2006). This could be because the former are less likely to have the resources to cope with

Table 1
Baseline characteristics according to occupational classes.

	Men					
	White-collar (n = 1290)	Blue-collar (n = 1900)	χ^2 P value	Manager (n = 1601)	Non-manager (n = 1589)	χ^2 P value
Age, years						
18–39	223 (17.3)	224 (11.8)	<0.001	191 (11.9)	256 (16.1)	<0.001
40–49	470 (36.4)	462 (24.3)		511 (31.9)	421 (26.5)	
50–59	365 (28.3)	616 (32.4)		538 (33.6)	443 (27.9)	
60–65	232 (18.0)	598 (31.5)		361 (22.5)	469 (29.5)	
Education, years						
≤15	351 (27.3)	898 (47.7)	<0.001	573 (36.0)	676 (42.9)	<0.001
16–18	644 (50.0)	855 (45.5)		779 (49.0)	720 (45.7)	
≥19	292 (22.7)	128 (6.8)		239 (15.0)	181 (11.5)	
Smoking						
Non-smoker	263 (20.4)	425 (22.5)	0.361	356 (22.3)	332 (21.0)	0.506
Ex-smoker	324 (25.2)	454 (24.0)		396 (24.8)	382 (24.1)	
Current smoker	701 (54.4)	1011 (53.5)		844 (52.9)	868 (54.9)	
Alcohol drinking						
Non-drinker	257 (20.4)	399 (21.8)	0.165	332 (21.4)	324 (21.0)	0.734
<28.9 g/day	393 (31.1)	514 (28.0)		445 (28.7)	462 (29.9)	
≥28.9 g/day	612 (48.5)	921 (50.2)		776 (50.0)	757 (49.1)	
Physical activity index						
<29 MET-h	408 (31.9)	181 (9.6)	<0.001	325 (20.5)	264 (16.8)	0.007
29–36 MET-h	513 (40.1)	673 (35.8)		603 (38.0)	583 (37.1)	
≥37 MET-h	359 (28.0)	1026 (54.6)		659 (41.5)	726 (46.2)	
Occupational position						
Manager	969 (75.1)	632 (33.3)	<0.001			
Non-manager	321 (24.9)	1268 (66.7)				
Job characteristics						
Low strain	223 (17.3)	276 (14.5)	<0.001	282 (17.6)	217 (13.7)	<0.001
Active	480 (37.2)	475 (25.0)		583 (36.4)	372 (23.4)	
Passive	279 (21.6)	644 (33.9)		352 (22.0)	571 (35.9)	
High strain	308 (23.9)	505 (26.6)		384 (24.0)	429 (27.0)	
	Women					
	White (n = 1496)	Blue (n = 1867)	χ^2 P value	Manager (n = 949)	Non-manager (n = 2414)	χ^2 P value
Age, years						
18–39	251 (16.8)	118 (6.3)	<0.001	70 (7.4)	299 (12.4)	<0.001
40–49	582 (38.9)	484 (25.9)		317 (33.4)	749 (31.0)	
50–59	484 (32.4)	761 (40.8)		390 (41.1)	855 (35.4)	
60–65	179 (12.0)	504 (27.0)		172 (18.1)	511 (21.2)	
Education, years						
≤15	457 (30.7)	976 (53.5)	<0.001	366 (38.7)	1067 (44.4)	0.012
16–18	746 (50.1)	764 (41.1)		457 (48.4)	1053 (43.8)	
≥19	287 (19.3)	119 (6.4)		122 (12.9)	284 (11.8)	
Smoking						
Non-smoker	1298 (88.1)	1720 (93.9)	<0.001	846 (91.0)	2172 (91.5)	0.905
Ex-smoker	47 (3.2)	32 (1.7)		23 (2.5)	56 (2.4)	
Current smoker	129 (8.8)	79 (4.3)		61 (6.6)	147 (6.2)	
Alcohol drinking						
Non-drinker	950 (65.1)	1302 (73.5)	<0.001	603 (65.8)	1649 (71.2)	<0.001
<28.9 g/day	409 (28.0)	400 (22.6)		244 (26.6)	565 (24.4)	
≥28.9 g/day	101 (6.9)	70 (4.0)		70 (7.6)	101 (4.4)	
Physical activity index						
<29 MET-h	518 (35.2)	332 (18.0)	<0.001	250 (26.7)	600 (25.3)	0.009
29–36 MET-h	820 (55.7)	852 (46.3)		498 (53.1)	1174 (49.5)	
≥37 MET-h	134 (9.1)	656 (35.7)		190 (29.3)	600 (25.3)	
Occupational position						
Manager	602 (40.2)	347 (18.6)	<0.001			
Non-manager	894 (59.8)	1520 (81.4)				
Job characteristics						
Low strain	318 (21.3)	358 (19.2)	<0.001	193 (20.3)	483 (20.0)	<0.001
Active	488 (32.6)	459 (24.6)		330 (34.8)	617 (25.6)	
Passive	423 (28.3)	492 (26.4)		224 (23.6)	691 (28.6)	
High strain	267 (17.8)	558 (29.9)		202 (21.3)	623 (25.8)	

MET-h, metabolic equivalent task per hour.

the burden of stressful work (Lachman & Weaver, 1998; Wheaton, 1980), or believe they have lower control and poorer skill discretion (Bosma, 2006) than the latter workers. These contexts would be in line with the current harsh situation of Japanese workers. In Japan, the long economic recession since the 1990s, international competition and the accompanying rise in competitive working climate and job insecurity has affected men (Takeda et al., 2006). In particular, the economic recession appeared to have a strong effect on the increase in suicides, especially in lower occupational class middle-aged men (Lester, Motohashi, & Yang, 1992; Ueda & Matsumoto, 2003).

Our results for male workers were in agreement with previous studies (Hallqvist et al., 1998; Kivimäki et al., 2002; Lynch et al., 1997; Theorell et al., 1998; Wege et al., 2008), but an opposite