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Impact of Obstructive Sleep Apnea on Liver Fat Accumulation According to Sex and Visceral Obesity

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Abstract

Rationale

Associations between obstructive sleep apnea (OSA) and liver fat accumulation have been frequently investigated because both morbidities are common. Visceral fat was reported to be closely related to OSA and liver fat accumulation. Recently, sex differences in the association between OSA and mortality have gained much attention.

Objectives

To investigate the associations among OSA, liver fat accumulation as determined by computed tomography, and visceral fat area and their sex differences.

Methods

Studied were 188 males and 62 females who consecutively underwent polysomnography and computed tomography.

Results

Although the apnea-hypopnea index was positively correlated with liver fat accumulation in the total males, none of the OSA-related factors was independently associated with liver fat accumulation in either the total male or female participants in the multivariate analyses. When performing subanalyses using a specific definition for Japanese of obesity or visceral obesity (body mass index (BMI) $\geq$25 kg/m^2 or visceral fat area $\geq$100 cm^2), in only males without visceral obesity, percent sleep time with oxygen saturation <90%, in addition to BMI, insulin resistance, and serum triglyceride values, was independently correlated with

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liver fat accumulation (R^2 = 15.1%, P<0.001). In males, percent sleep time of oxygen saturation <90% was also a determining factor for alanine aminotransferase values regardless of visceral fat area. In contrast, OSA was not associated with liver fat accumulation or alanine aminotransferase values in females whether or not visceral obesity was absent.

Conclusions

Sex differences in the visceral fat-dependent impact of OSA on liver fat accumulation existed. Although the mechanisms are not known and ethnic differences may exist in addition to the specific criteria of visceral obesity in Japan, the treatment of male patients with OSA might be favorable from the viewpoint of preventing liver fat accumulation and liver dysfunction even in patients without obvious visceral fat accumulation.

Introduction

Obstructive sleep apnea (OSA) is characterized by nocturnal intermittent hypoxemia represented as the number of episodes of oxygen desaturation per hour over total sleep time (oxygen desaturation index: ODI) and sleep-associated hypoxia represented as the percentage of time spent with oxygen saturation (SpO_2) below 90% to total sleep time (%T<90). Fatty liver disease is the most common chronic liver disease worldwide [1]. Fatty liver disease is a progressive disease from simple steatosis to steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. Thus, both OSA and fatty liver disease are highly prevalent and each has an impact on patients' prognosis.

During the decade following the first study [2], OSA and fatty liver disease have been attracting interest as an important target of study, and many studies have shown that OSA is associated with the progression of fatty liver disease by histology, radiology, and biomarkers [3–12]. Among studies investigating the association histologically, Mishra et al., Polotsky et al., and Aron-Wisnewsky et al., respectively, reported the lowest SpO_2 during sleep, a mean fall in SpO_2 caused by OSA, and the ODI to be associated with inflammation and fibrosis in fatty liver [6, 8, 10]. These findings showed that hypoxia related to OSA should play an important role in the progression of fatty liver disease. However, it is still unclear whether OSA is associated with the onset or early stage of fatty liver disease observed as liver fat accumulation (LFA). In addition, although recent human studies have mainly investigated the association between OSA and fatty liver disease in morbidly obese participants [5–11], in animal models of OSA, exposure to intermittent hypoxia for 5 days increased liver triglyceride content in lean mice without fatty liver but not in obese mice with fatty liver already at baseline, and it took 12 weeks to identify a similar and significant increase in obese mice [13, 14]. In these studies, sterol regulatory element binding protein 1 (SREBP-1), a key transcription factor of lipid biosynthesis, and stearoyl-coenzyme A desaturase 1 (SCD-1), an SREBP-1-induced enzyme of lipid biosynthesis, were upregulated in the liver in parallel to the increase in liver triglyceride content. It took 5 days in the lean mice and 12 weeks in the obese mice until the activation of SREBP-1 became significant. However, the mechanisms of SREBP-1 and SCD-1 activation or the rationales for the differences in the duration of intermittent hypoxia exposure were not clearly reported [13, 14]. Thus, an association among the hypoxemia induced by OSA, LFA, and obesity (especially the visceral fat accumulation (VFA), which is more influential in LFA than the body mass index (BMI) [1, 15]) has not been established. In addition, the thresholds

of the degree of obesity or the VFA required for a significant association between OSA and LFA are not well known.

On the other hand, sex differences have been recognized in the epidemiology of fatty liver disease, such as its prevalence, severity, and prognosis [1, 16], and sex hormones and differences in patterns of body fat accumulation, including VFA, are considered to be main etiologies of sex differences [1, 17]. Thus, sex must be considered an important factor in fatty liver disease. Nevertheless, a direct comparison of OSA and fatty liver disease between males and females has not been made by radiological studies.

From this background, it has been suggested that it is important to understand the association among sex, VFA, OSA, and OSA-induced hypoxemia. We hypothesized that there is a sex and visceral fat-dependent impact of OSA on LFA. To test that hypothesis, we measured OSA, LFA, and VFA simultaneously and quantitatively.

Methods

Participants

We retrospectively surveyed 449 consecutive adults (≥20 years old) who were admitted to Kyoto University Hospital for polysomnography on suspicion of OSA between October 2008 and August 2010. Excluded were 97 individuals who met the following exclusion criteria: being administered medicines known to cause LFA; being serum HBs antigen and/or HCV antibody positive; having blood brain natriuretic peptide levels >100 pg/ml [18]; the presence of known or CT-diagnosed steatohepatitis, liver cirrhosis, or liver disease other than fatty liver disease; or the presence of another clinically serious disease. We clinically recommend an unenhanced abdominal CT examination for those suspected to have OSA to check the VFA [19–21]. Those who agreed to our recommendation underwent abdominal CT between the day when participants were referred to our clinic with suspicion of OSA and the day when OSA therapy was started. The study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (IRB approval number E-1307) and written informed consent was obtained from all participants.

CT Scanning and measurements

Unenhanced transverse CT was performed with an Aquilion 64 CT system (Toshiba Medical Systems Corporation, Tochigi, Japan) running on 135 kVp, 440 mA, and a 0.5-s scan time. The abdomen from the top of the liver to the lower region of the umbilicus was scanned. In this study, LFA was represented as the CT values for liver (CT_{LFA}) in continuous variables because of the negative correlation between LFA and CT_{LFA}. It has been reported that the CT_{LFA} is best for prediction of LFA among several CT parameters [22]. According to the measurement method used in this report, the CT_{LFA} was determined by averaging the CT values of 12 regions of interest placed on the CT liver images reconstructed at 7 mm intervals. Each region of interest was determined as a circular area of 100 ± 5 mm^2, and was placed to avoid apparent vessels in each liver parenchyma of the 12 sections defined by the modified Couinaud segmentation system (S1 Fig). Furthermore, evaluating liver fat content is frequently performed by adjusting for CT values of the spleen. The CT values for spleen parenchyma were determined by averaging the CT values of 3 regions of interest placed in the cross sections dividing the spleen into 4 equal parts. The interclass correlation coefficient for inter-reader comparisons was 0.99 for CT_{LFA} in a randomly selected sample of 30 participants. To confirm the results, we also evaluated LFA by the liver/spleen ratio [23–25]. VFA and subcutaneous fat accumulation (SFA) were each measured as areas in a CT image of the umbilical level [26] using an image analysis program (AZE Virtual Place 99, AZE of America, Ltd., Irvine, CA, USA) [27]. Visceral obesity

(VO) was defined by VFA $\geq$ 100 cm^2 according to criterion for both Japanese males and females (VO_{100}) proposed by the Japan Society for the Study of Obesity [28], which has been adopted by the Ministry of Health, Labor, and Welfare of Japan [29].

Polysomnography

Polysomnography was performed in the standard manner from 22:00 until 6:00 the following morning according to recommendations in the American Academy of Sleep Medicine's (AASM's) manual (SomnoStar pro, Cardinal Health, Dublin, OH, USA) [30]. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin, and 12-lead electroencephalograph. Ventilation was monitored by inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer and supplemented by an oronasal thermal sensor. Arterial oxygen saturation (SpO_2) was monitored continuously with a pulse oximeter, and 4% ODI [31], %T<90, and the lowest SpO_2 during sleep were determined as indices of hypoxia (intermittent hypoxia, burden of hypoxia, and degree of desaturation). Apnea was defined as a cessation of airflow for at least 10 seconds and hypopnea was defined as an abnormal respiratory event lasting at least 10 seconds with a reduction > 50% in airflow by nasal pressure as compared to baseline or with an oxygen desaturation of > 3% or an arousal, which was proposed as an alternative scoring rule for hypopnea by AASM [30] and is usually used in clinical practice in Japan [32–34]. The AHI and 4%ODI were defined as the average number of apnea and hypopnea or 4% oxygen desaturation episodes per hour over the total sleep time, respectively. AASM recommended another hypopnea rule defined by an abnormal respiratory event lasting at least 10 seconds with a reduction >30% in airflow or with an oxygen desaturation of >4%. Therefore, we also scored AHI throughout sleep time, during REM, and in the supine position in all participants by using hypopnea as defined by this rule (AHI_{AASM}).

Data collection

Fasting venous blood samples were taken in the morning after overnight polysomnography. Insulin resistance was evaluated based on the homeostasis model assessment index (HOMA-IR) calculated from fasting plasma glucose and fasting serum insulin levels. Basic clinical parameters such as clinical history, medications, drinking habit, and smoking status were obtained from questionnaires. Daily alcohol intake was calculated from kinds and quantity of liquor and frequency of drinking and was used as a continuous variable. These questionnaires and anthropometric measurements were conducted on the day of polysomnography. Obesity was defined by BMI $\geq$25 kg/m^2 according to criterion for Japanese proposed by the Japan Society for the Study of Obesity [28], which has been adopted by Ministry of Health, Labor, and Welfare of Japan [35].

Statistical analysis

Results are expressed as mean ± standard deviation or number of participants. Following testing for normality and equality of variance, continuous variables were compared by unpaired *t* test, Welch's tests, or Mann-Whitney U tests, and categorical variables for male and female participants were compared by Fisher's exact test. Prevalence of fatty liver among Japanese males and females was 40% and 22%, respectively [36], and the ratio of prevalence of OSA among males to that among females was about 2.3 [37]. When calculated from these data, the sample size to achieve 80% power at a 5% significance level in detecting the sex difference in fatty liver of OSA patients was 248 (173 males and 75 females). In addition, clinic-based studies reported that the prevalence of OSA (AHI $\geq$ 5 h^{-1}) was 67% [4] and that 84% of those with

OSA and 64% of those without OSA had fatty liver [10]. From these data, the sample size for detecting the influences of OSA on fatty liver was estimated to be 162.

The correlations between CT_{LFA}, serum aspartate aminotransferase (AST) values, or serum alanine aminotransferase (ALT) values and other independent variables were analyzed by Pearson's or Spearman's correlation coefficients and then stepwise multiple regression analyses were performed using variables yielding a P-value < 0.10 by univariate analysis. Independent variables included into these analyses were age, BMI, neck circumference, waist circumference, systolic blood pressure, diastolic blood pressure, daily alcohol intake, use of lipid-lowering agents, current smoking, AHI, 4%ODI, %T<90, lowest SpO_2 during sleep, arousal index, REM sleep, AHI during REM, supine sleep time, AHI in supine position, Epworth Sleepiness Scale score, VFA, SFA, serum C-reactive protein (CRP), triglycerides, HDL-cholesterol and LDL-cholesterol levels, fasting plasma glucose levels, and the homeostasis model assessment of insulin resistance. When two independent variables had very strong collinearity ($\gamma > 0.80$), each was entered into the multivariate analysis separately and the best-fit model was adopted. P-values < 0.05 were considered statistically significant. All analyses were performed using JMP 9.0.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Inclusion criteria and characteristics of study participants

From the 449 adults who underwent diagnostic polysomnography during the study period, 97 participants were excluded due to the exclusion criteria and 102 declined the CT examination, leaving 250 to undergo abdominal CT (Fig 1). Between those who did and did not undergo CT examination, there were no significant differences in age, BMI, waist circumference, AHI, AST, and ALT. Characteristics of the 250 study participants (188 males, 62 females) are shown in Table 1. In this study population, age, BMI, and CT_{LFA} did not differ between males and females. Of the participants, 89 (34.8%) were taking lipid-lowering agents [38]. Of the 62 females, 14 were premenopausal, 40 were postmenopausal (1 was receiving hormone-replacement therapy), and the menopausal condition in 8 was unknown.

Association between OSA and CT_{LFA} by univariate regression analyses in male and female participants

AHI was negatively correlated with CT_{LFA} in all participants (Table 2). Although the number of females might not be sufficient for separate analyses, in a sex-specific analysis AHI was negatively correlated with CT_{LFA} in males ($\gamma = -0.288$, $P < 0.001$) but not in females ($\gamma = -0.011$, $P = 0.933$). In females, even when limited to the postmenopausal females without hormone-replacement therapy ($n = 39$), AHI was not correlated with CT_{LFA}.

As the Japanese criterion for visceral obesity is VFA greater than 100 cm^2 (VO_{100}), we firstly conducted regression analyses of the participants stratified according to the presence or absence of VO_{100}. In the males, AHI was not significantly correlated with CT_{LFA} in males with VO_{100} ($n = 113$) ($\gamma = -0.126$, $P = 0.182$) but was significantly correlated with CT_{LFA} in males without VO_{100} ($n = 75$) ($\gamma = -0.376$, $P < 0.001$). In the females, none of the OSA-related indices was correlated with CT_{LFA} whether VFA was over or under 100 cm^2 (S1 Table).

Multivariate regression analyses for CT_{LFA} in males with and without VO_{100}

When multivariate analyses were conducted in male participants, none of the OSA-related factors was independently associated with CT_{LFA} in the total male participants (Table 3, Fig 2A),

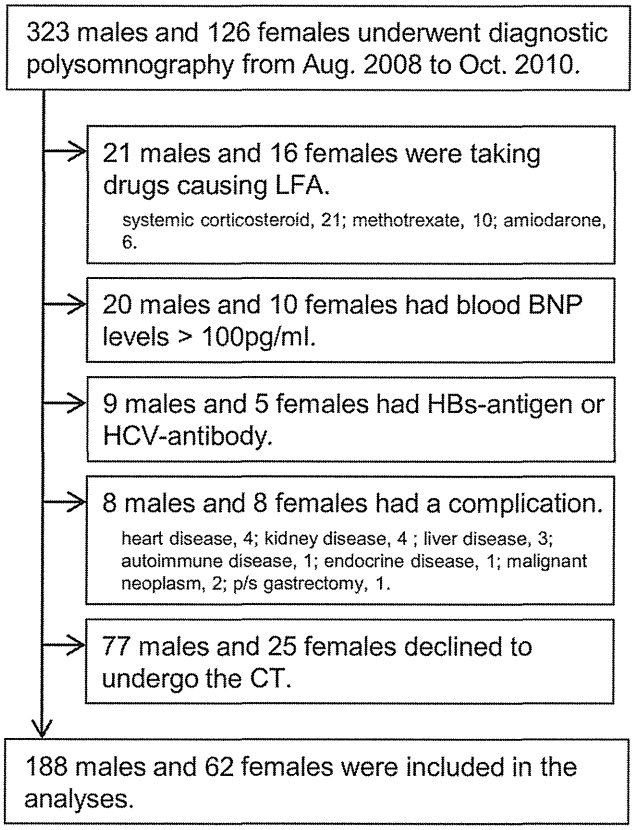

Fig 1. Flow chart of enrollment of study participants.

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male OSA participants (AHI $\geq$ 10 h^{-1}, n = 164), or in male control participants (AHI < 10 h^{-1}, n = 24). In similar analyses, %T<90 was an independent determinant of CT_{LFA} not in males with VO_{100} but in males without VO_{100} (R^2 = 15.1%, P < 0.001) (Table 4, Fig 2B and 2C). When 4%ODI was separately entered into the stepwise analysis for males without VO_{100} because of its strong collinearity (γ = 0.830 with %T<90), it remained in the model as an independent determinant of CT_{LFA} (R^2 = 8.8%, P = 0.013; Table 4 model B). Moreover, we used AHI_{AASM} instead of the AHI that we had adopted. In this case, AHI_{AASM} was entered into the analysis separately from 4%ODI and comprised the stepwise model for CT_{LFA} in males without VO_{100} in almost the same pattern as produced by 4%ODI (R^2 of AHI_{AASM} = 7.6%, cumulative R^2 of the model = 50.9%). When the liver/spleen ratio was adopted as a parameter representing LFA, both of these indices of OSA-related hypoxemia were independent determinants of LFA (%T<90, R^2 = 10.5%, P = 0.003; or 4%ODI, R^2 = 6.3%, P = 0.019). Since there was a significant correlation not between alcohol intake and CT_{LFA} but between the use of lipid-lowering agents and CT_{LFA}, we excluded users of lipid-lowering agents (n = 20) or non-OSA participants (AHI < 10 h^{-1}, n = 11) from the males without VO_{100} and performed a similar analysis. As a result, the independent contributions of %T<90 to CT_{LFA} did not change even in these subanalyses.

VFA threshold level of the association between OSA and CT_{LFA}

To determine the threshold of VFA levels at which %T<90% was a significant determinant of CT_{LFA} in males, stepwise models were examined for VFAs of 115 cm^2, 130 cm^2, and 145 cm^2.

Table 1. Characteristics of study participants.

Characteristics	All participants	Males	Females	P-value*
Number (%)	250	188 (75.2)	62 (24.8)	...
Age, y	57.3 ± 13.1	56.5 ± 13.7	59.7 ± 11.1	0.095
BMI, kg/m^2	26.6 ± 4.9	26.5 ± 4.6	27.0 ± 5.8	0.480
Neck circumference, cm	39.8 ± 4.6	40.7 ± 4.2	37.2 ± 5.4	<0.001
Waist circumference, cm	96.7 ± 10.2	96.1 ± 9.3	98.6 ± 12.4	0.149
Systolic blood pressure, mmHg	125.8 ± 13.7	125.7 ± 14.0	126.0 ± 12.8	0.870
Diastolic blood pressure, mmHg	77.8 ± 10.1	78.7 ± 10.3	75.0 ± 9.1	0.012
Alcohol intake, g/d	13.0 ± 23.0	16.8 ± 25.3	1.6 ± 4.5	<0.001
User of lipid-lowering agents, n(%)	89 (35.6)	63 (33.5)	26 (41.9)	0.284
Current smoker, n (%)	36 (14.5)	32 (17.1)	4 (6.5)	0.039
CT parameters				
Time from PSG to CT, m	-0.3 ± 1.2	-0.2 ± 1.0	-0.5 ± 1.6	0.173
CT_{LFA}, HU	54.1 ± 12.8	53.8 ± 12.0	55.1 ± 15.0	0.523
VFA, cm^2	113.6 ± 60.6	118.1 ± 61.7	100.2 ± 55.8	0.044
SFA, cm^2	160.4 ± 95.7	145.6 ± 82.9	205.2 ± 116.5	<0.001
Sleep parameters				
AHI, h^{-1}	31.6 ± 20.1	34.0 ± 20.1	24.4 ± 20.0	0.001
4%ODI, h^{-1}	23.8 ± 20.5	26.2 ± 20.6	16.7 ± 18.7	0.002
%T<90, %	13.3 ± 20.1	14.9 ± 20.6	8.7 ± 17.8	0.001
Lowest SpO_2 during sleep, %	79.4 ± 10.1	79.0 ± 10.1	80.8 ± 9.9	0.119
Arousal Index, h^{-1}	31.0 ± 16.1	33.3 ± 16.0	23.9 ± 14.2	<0.001
REM sleep, %	14.9 ± 6.2	14.8 ± 6.2	15.1 ± 6.1	0.760
AHI during REM, h^{-1}	35.3 ± 23.7	34.4 ± 23.6	37.9 ± 24.1	0.318
Supine sleep time, %	69.0 ± 26.2	69.0 ± 26.7	69.0 ± 24.4	0.987
AHI in supine position, h^{-1}	39.8 ± 24.1	43.3 ± 24.5	29.3 ± 23.1	<0.001
Epworth sleepiness scale score	9.8 ± 5.3	10.0 ± 5.3	9.0 ± 5.1	0.180
Blood parameters				
AST, IU/L	24.5 ± 12.0	24.8 ± 11.9	23.6 ± 12.3	0.204
ALT, IU/L	28.3 ± 20.3	29.7 ± 20.8	24.3 ± 18.3	0.011
CRP, mg/dL	0.15 ± 0.24	0.14 ± 0.22	0.20 ± 0.28	0.284
Triglycerides, mg/dL	141.6 ± 89.6	150.1 ± 94.3	115.7 ± 67.7	0.002
HDL-cholesterol, mg/dL	48.8 ± 12.3	47.1 ± 11.6	54.3 ± 12.8	<0.001
LDL-cholesterol, mg/dL	112.4 ± 30.7	113.3 ± 29.9	109.8 ± 33.3	0.448
Fasting plasma glucose, mg/dL	104.5 ± 26.6	104.4 ± 24.0	104.6 ± 33.5	0.968
HOMA-IR	2.82 ± 3.17	2.71 ± 2.88	3.17 ± 3.92	0.772

All values are number (percentage) or mean ± standard deviation.

*Males vs. females.

A lower CT_{LFA} means higher liver fat accumulation. Among users of lipid-lowering agents [38], 7 were taking pioglitazone, 76 statins, 4 eicosapentaenoic acid, 5 ezetimibe, 6 tocopherol, and 11 telmisartan; among them, 20 were taking 2 of these medicines.

Abbreviations: BMI, body mass index; CT, computed tomography; PSG, polysomnography; CT_{LFA}, CT values for liver; VFA, visceral fat accumulation; SFA, subcutaneous fat accumulation; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; %T<90, percentage of time spent with SpO_2 below 90% to total sleep time; SpO_2, oxygen saturation measured by pulse oximetry; REM, rapid eye movement; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

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From these analyses, %T<90 was a significant determinant of CT_{LFA} until VFA reached 130 cm^2 (VO_{130}).

Table 2. Correlation coefficients of CT_{LFA} in all participants and separately in males, and females.

	All participants	Males	Females
Age, y	0.26‡	0.25‡	0.27†
BMI, kg/m^2	-0.61‡	-0.63‡	-0.58‡
Neck circumference, cm	-0.41‡	-0.35‡	-0.55‡
Waist circumference, cm	-0.55‡	-0.54‡	-0.56‡
Systolic blood pressure, mm Hg	-0.17†	-0.17†	-0.20
Diastolic blood pressure, mm Hg	-0.22‡	-0.21†	-0.27†
Alcohol intake, g/d	-0.02	-0.02	0.16
§Use of lipid-lowering agents	-0.05	-0.13*	0.12
§Current smoking	-0.12*	-0.12	-0.08
VFA, cm^2	-0.49‡	-0.49‡	-0.53‡
SFA, cm^2	-0.49‡	-0.59‡	-0.43‡
AHI, h^{-1}	-0.21‡	-0.29‡	-0.01
4%ODI, h^{-1}	-0.24‡	-0.29‡	-0.09
%T90, %	-0.26‡	-0.31‡	-0.11
Lowest SpO_2 during sleep, %	0.13†	0.20†	-0.05
Arousal Index, h^{-1}	-0.03	-0.04	0.03
REM sleep, %	-0.03	0.04	-0.18
AHI during REM, h^{-1}	-0.22	-0.33‡	0.04
Supine sleep time, %	0.12*	0.06	0.28†
AHI in supine position, h^{-1}	-0.21‡	-0.27‡	-0.05
Epworth sleepiness scale score	-0.17†	-0.16†	-0.16
CRP, mg/dL	-0.30‡	-0.28‡	-0.34†
Triglycerides, mg/dL	-0.41‡	-0.42‡	-0.44‡
HDL-cholesterol, mg/dL	0.36‡	0.38‡	0.33†
LDL-cholesterol, mg/dL	-0.13†	-0.16†	-0.06
Fasting plasma glucose, mg/dL	-0.22‡	-0.22†	-0.23*
HOMA-IR	-0.40‡	-0.44‡	-0.35†

*P <0.10

†P <0.05

‡P <0.001, which indicate variables entered into the multivariate regression analyses.

§Correlation coefficients of these variables are indicated by Spearman's ρ, and the others are indicated by Pearson's γ. CT_{LFA} is negatively correlated with liver fat accumulation.

Abbreviations: CT_{LFA}, CT values for liver; BMI, body mass index; VFA, visceral fat accumulation; SFA, subcutaneous fat accumulation; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; %T<90, percentage of time spent with SpO_2 below 90% to total sleep time; SpO_2, oxygen saturation measured by pulse oximetry; REM, rapid eye movement; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

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Interaction of obesity with the association between OSA and CT_{LFA}

When participants were stratified by the presence or absence of obesity defined by Japanese criterion (BMI $\geq$ 25 kg/m^2) [28] instead of VO, independent determinants of CT_{LFA} were age, BMI, VFA, and triglycerides in males with obesity (n = 110); BMI, HDL-cholesterol, and HOMA-IR in males without obesity (n = 78); BMI, triglycerides and HOMA-IR in females with obesity (n = 35); and neck circumference in females without obesity (n = 27). However, none of the OSA-related indices was detected as a determinant of CT_{LFA} in these stratified groups (S2 and S3 Tables).

Table 3. Stepwise multiple regression models for CT_{LFA}.

	All participants (n = 250)			Males (n = 188)			Females (n = 62)		
Variables	**β**	**R^2, %**	***P* value**	**β**	**R^2, %**	***P* value**	**β**	**R^2, %**	***P* value**
Age	0.11	2.7	0.031	0.17	4.3	0.004	–	–	–
BMI	-0.44	27.0	<0.001	-0.39	24.5	<0.001	-0.39	22.3	<0.001
Neck circumference	–	–	–	–	–	–	-0.32	17.6	0.002
CRP	-0.11	3.3	0.023	-0.18	8.6	0.010	–	–	–
Triglycerides	-0.21	8.7	<0.001	-0.14	5.9	0.020	-0.33	14.5	<0.001
HDL-cholesterol	0.12	4.2	0.026	0.14	5.5	0.015	–	–	–
Fasting plasma glucose	-0.11	2.4	0.022	-0.11	2.5	0.041	–	–	–
Cumulative R^2	...	48.5	...	...	51.2	...	...	54.4	...

Variables entered into the stepwise regression analyses were indicated in Table 2 by variables yielding P-value <0.10; only variables left in one or more of the models are shown in this table. Minus sign means the variable was not selected through univariate or stepwise selection procedures. CT_{LFA} is negatively correlated with liver fat accumulation.

Abbreviations: CT_{LFA}, CT values for liver; β = standard regression coefficient; R^2 = coefficient of determination; BMI, body mass index; CRP, C-reactive protein.

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Association between OSA and serum transaminase values in univariate and multivariate regression analyses

In the males, while AHI was significantly correlated with serum AST and ALT values, multiple regression analyses showed that %T<90 was an independent determinant of not AST but ALT values (Fig 3A and 3D). However, in males without VO_{100}, %T<90 was an independent determinant of AST and ALT values (Fig 3C and 3F). This independent contribution of %T<90 to transaminase values did not change even after non-OSA participants, excessive drinkers, or users of lipid-lowering agents were excluded from the males without VO_{100}.

In the females, OSA was not correlated with serum AST or ALT values in the total female group, in postmenopausal females without hormone-replacement therapy, in females with VO_{100}, or in females without VO_{100}.

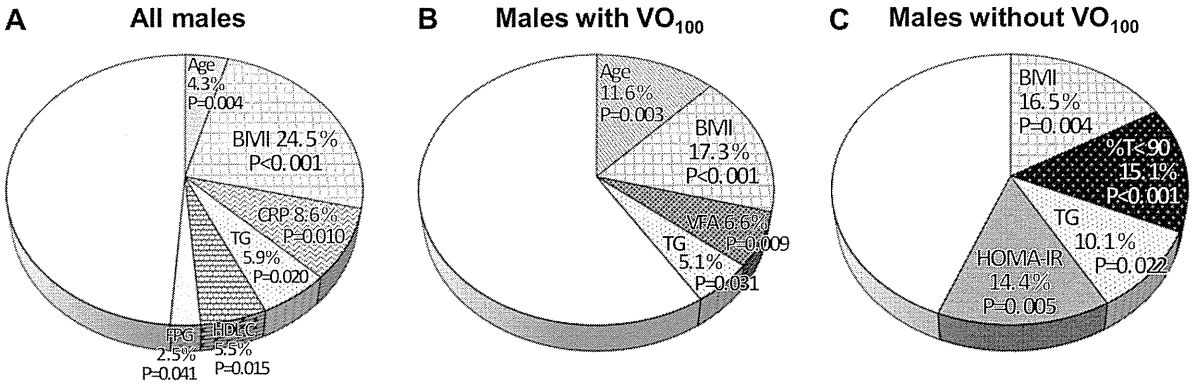

Fig 2. Coefficients of determination (R^2) in stepwise multiple regression models for CT_{LFA} in all males and males stratified according to VO_{100}. (A) All males, (B) males with VO_{100}, and (C) males without VO_{100}. Variables entered into the stepwise regression analyses were selected from age, BMI, neck circumference, waist circumference, systolic and diastolic blood pressures, alcohol intake, use of lipid-lowering agents, current smoking, VFA, SFA, AHI, 4% ODI, %T<90, lowest SpO_2, arousal index, REM sleep, AHI during REM, supine sleep time, AHI in supine position, Epworth sleepiness scale score, CRP, triglycerides (TG), HDL-cholesterol (HDL-C), LDL-cholesterol, fasting plasma glucose (FPG), and HOMA-IR when yielding a P-value <0.10 by univariate analysis.

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Table 4. Stepwise multiple regression models for CT_{LFA} in males with and without VO_{100}.

	Males with VO_{100} (n = 113)			Males without VO_{100} (n = 75)					
				Model A			Model B		
Variables	β	R^2, %	*P* value	β	R^2, %	*P* value	β	R^2, %	*P* value
Age	0.26	11.6	0.003	–	–	–	–	–	–
BMI	-0.33	17.3	<0.001	-0.29	16.5	0.004	-0.31	17.9	0.003
%T<90	–	–	–	-0.31	15.1	<0.001	...	...	...
4%ODI	–	–	–	...	...	...	-0.22	8.8	0.013
VFA	-0.21	6.4	0.009	–	–	–	–	–	–
Triglycerides	-0.17	5.1	0.031	-0.21	10.1	0.022	-0.23	11.0	0.018
HOMA-IR	–	–	–	-0.26	14.4	0.005	-0.26	13.9	0.011
Cumulative R^2	...	40.4	...	...	56.0	...	...	51.5	...

Variables entered into the stepwise regression analyses were selected from age, BMI, neck circumference, waist circumference, systolic and diastolic blood pressures, alcohol intake, use of lipid-lowering agents, current smoking, VFA, SFA, AHI, 4%ODI, %T<90, lowest SpO_2, arousal index, REM sleep, AHI during REM, supine sleep time, AHI in supine position, Epworth sleepiness scale score, CRP, triglycerides, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, and HOMA-IR when yielding a P-value <0.10 by univariate analysis; %T<90 (Model A) and 4%ODI (Model B) were entered into the analyses separately for their strong collinearity; only variables left in one or more of the models are shown in this table. Minus sign means the variable was not selected through univariate or stepwise selection procedures. CT_{LFA} is negatively correlated with liver fat accumulation.

Abbreviations: VO_{100}, visceral obesity (VFA ≥100 cm^2); β = standard regression coefficient; R^2 = coefficient of determination; BMI, body mass index; % T<90, percentage of time spent with SpO_2 below 90% to total sleep time; ODI, oxygen desaturation index; VFA, visceral fat accumulation; HOMA-IR, homeostasis model assessment index of insulin resistance.

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Discussion

In this study, we demonstrated significant associations among OSA, LFA, sex, and VFA. Such associations have not been fully investigated in previous studies. That is, the %T<90 (rather than 4%ODI), namely sleep-associated hypoxia, was a major independent risk factor for LFA evaluated by CT_{LFA} but only in males with VFA < 130 cm^2. Moreover, %T<90 was associated with serum ALT values regardless of VO criteria. However, such associations between OSA and LFA or serum transaminase values were not observed in females at all.

Impact of OSA on LFA and sex difference

There are two main putative mechanisms by which OSA increases LFA, one of which is the activation of triglyceride biosynthesis in liver through a pathway from hypoxia-inducible factor-1α (HIF-1α) [13, 14, 39], and the other is the increase in serum free fatty acid values observed in OSA patients [40, 41]. Ryan et al. reported that HIF-1α activity was induced by exposure to sustained hypoxia in a dose-dependent manner but not by exposure to intermittent hypoxemia [42]. In our study of humans, 4%ODI representing intermittent hypoxemia was also associated with LFA, but its effect on the determinants was smaller than %T<90 representing sleep-associated hypoxia (Table 4, Models A and B). Thus, sleep-associated hypoxia rather than intermittent hypoxemia seems to have the greater influence on LFA, as was also reported recently [12]. In a mouse model exposed to intermittent hypoxemia (12 times/h or 60 times/h), intermittent-hypoxemia exposure caused not only intermittent-hypoxemic effects but also sustained-hypoxic effects on the liver and fat tissues, and the higher frequencies of intermittent hypoxemia produced more severe effects [43].

One of the important findings of this study was a sex difference in the association between OSA and LFA. Few studies have investigated sex differences in the association between OSA

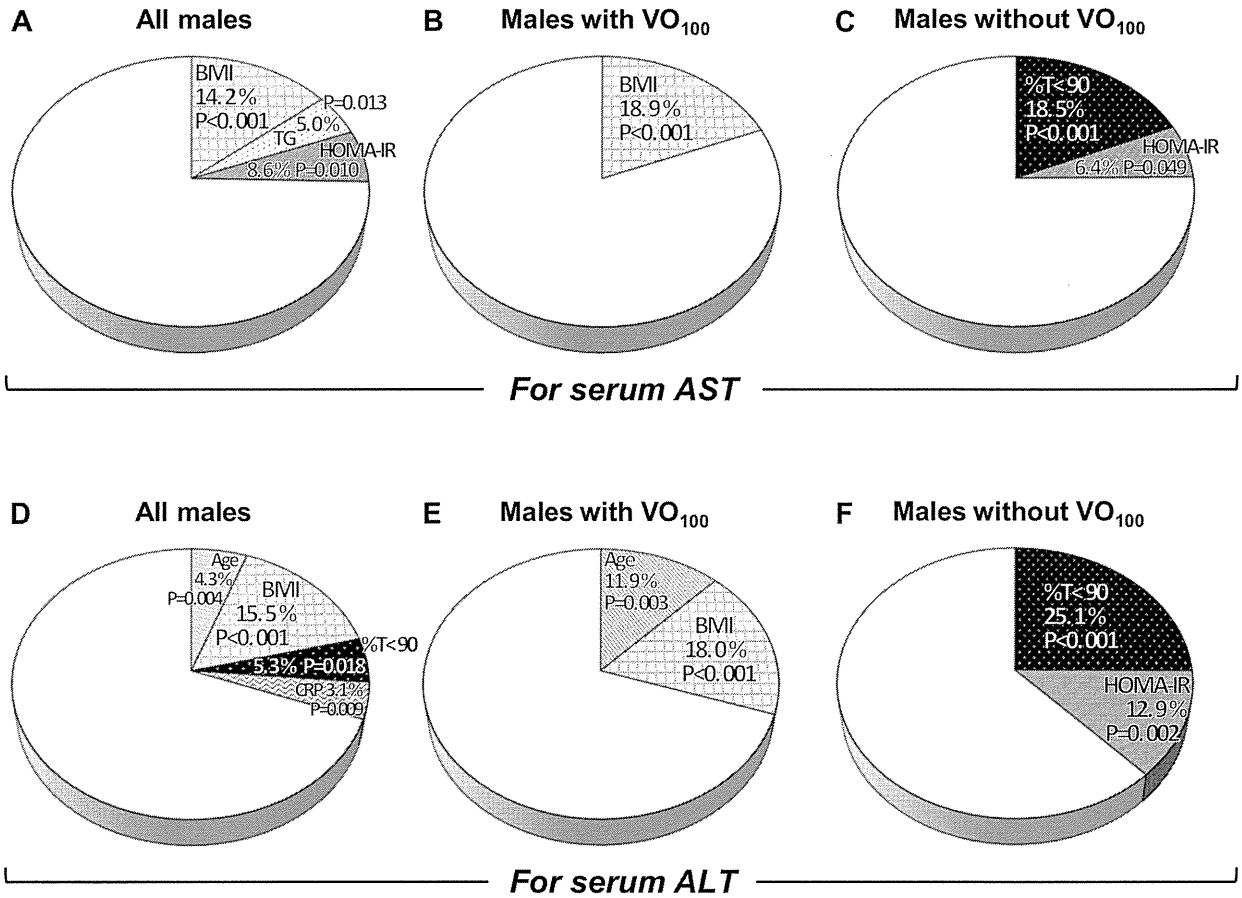

Fig 3. Coefficients of determination (R^2) in stepwise multiple regression models for serum transaminase values in all males and males stratified according to VO_{100}. Serum AST and ALT values in all males (A, D), in males with VO_{100} (B, E), and in males without VO_{100} (C, F). Variables entered into the stepwise regression analyses were selected from age, BMI, neck circumference, waist circumference, systolic and diastolic blood pressures, alcohol intake, use of lipid-lowering agents, current smoking, VFA, SFA, AHI, 4%ODI, %T<90, lowest SpO_2, arousal index, REM sleep, AHI during REM, supine sleep time, AHI in supine position, Epworth sleepiness scale score, CRP, triglycerides (TG), HDL-cholesterol (HDL-C), LDL-cholesterol, fasting plasma glucose (FPG), and HOMA-IR when yielding a P-value <0.10 by univariate analysis.

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and LFA. In this study, although there was no significant difference in age, BMI, and CT_{LFA} between males and females, an independent association between OSA and LFA was observed only in males but not in females. One reason for this may be the influence of estrogen, which was reported to inhibit HIF-1α and key enzymes for triglyceride biosynthesis [44, 45]. However, even when excluding premenopausal females in this study, the result did not change. In addition to the low prevalence of severe OSA in females (Table 1), the age of onset of OSA is later in females than in males, with the prevalence of OSA increasing in females after menopause. Thus, females may have less cumulative exposure to OSA than men with similar severities of OSA [46]. In addition, sex differences in unmeasured health behaviors such as diet or exercise as well as changes over time in risk factors such as alcohol intake and obesity cannot be excluded as causes of the sex difference in OSA-associated LFA. Since the number of females was small in this study, the cumulative effects of OSA and the effects of estrogen on OSA-related diseases including LFA should be studied further in large-scale studies.

Visceral fat-dependent impact of OSA on LFA

The second important finding of this study is that an association between OSA and LFA was observed only when VFA was less than 130 cm^2. Similarly, the improvement by continuous positive airway pressure therapy in insulin sensitivity of OSA patients was reported to be much greater in non-obese than in obese patients [47]. In addition, it was the VFA rather than the BMI that impacted the association between OSA and LFA in our study. This may be explained by the dose-dependent impact of VFA, which is the main source of free fatty acids and is more greatly associated with LFA than SFA or BMI [1, 15, 48]. Tatsumi et al. reported that OSA was not a risk factor for fatty liver in Japanese, who had an average VFA of 156 cm^2 [4]. These results, including ours, suggest that a large VFA, which would be greater than 130 cm^2 in Asians, overwhelms OSA in terms of its impact on LFA. Similar findings were also observed in animal examinations. LFA at baseline was much greater in obese mice than in lean mice, and it took a much longer time to identify a significant increase in LFA by intermittent hypoxia in the existing fatty liver of obese mice [13, 14]. Recently, Minville et al. reported that %T<90, HOMA-IR, and serum triglyceride values were independently associated with fatty liver, similar to our results [12]. However, contrary to our findings, they found an association between OSA and LFA only in morbidly obese patients. Although the SteatoTest, which they used to determine fatty liver, is convenient and well-validated, it is a scoring system consisting of several blood markers such as triglycerides and glucose and, therefore, potentially strongly correlates with HOMA-IR and triglycerides and tends to be influenced by obesity [49]. CT evaluation of fatty liver is more sensitive, specific, and quantitative [22, 23]; therefore, we believe our findings would also be accurate and that CT would be more suitable for evaluation of fatty liver than biomarkers.

Methods in CT measurements

In this study, the CT_{LFA} was measured in all participants by a single well-calibrated method that allowed the evaluation of the whole burden of LFA by setting regions of interest in each anatomical section of the liver [22]. As mentioned in Methods, CT_{LFA} was reported to be best for prediction of LFA among several CT parameters. In addition, we confirmed the results by the liver/spleen ratio, a method that is also frequently used to measure LFA [23–25]. Results using the two different methods were almost the same. Therefore, it can be considered that the results of this study are accurate.

Study limitations

Limitations of this study should be mentioned. Firstly, we cannot know causality or the mechanisms of our findings because this was a cross-sectional observational study. This study was done at the time of diagnosis of OSA, so the duration of OSA was unclear. Secondly, we measured LFA using CT images instead of biopsy specimens. CT measurement can be used for evaluation of LFA, particularly in those who should avoid an invasive biopsy [1], and also can evaluate the whole burden of LFA regardless of heterogeneously spreading fat. Thirdly, our study also included fewer females than the calculated sample size and OSA was less severe in the females than in the males. These factors might have caused β errors in analyses concerning female participants. Therefore, the results could have been different with a greater number of postmenopausal female participants. However, significant differences in the associations among VFA, LFA, and OSA between males and females in this study may support previous knowledge that the prevalence of LFA and the pattern of body fat distribution differ by sex [1, 28]. Fourthly, although we adopted obesity criteria for Japanese proposed by Japan Society for the Study of Obesity (VFA $\geq$100 cm^2 or BMI $\geq$25 kg/m^2, irrespective of sex) [34], some studies implied that the cutoff value of VFA for Japanese females should be less than 100 cm^2 [50, 51].

Larger-scale and more recent studies supported the Japan Society's criteria [52, 53], but there would still be room to examine the cutoff values for VFA in fatty liver especially for Japanese females. Fifthly, self-reported sleep duration and quality, which were recently reported to be associated with fatty liver independently of OSA [54], were not considered. In future investigations of fatty liver, sleep duration and quality should be evaluated objectively in a home setting. Finally, ethnic differences as shown between BMI and the prevalence of OSA should be further studied.

Conclusions

OSA-related hypoxemia (%T<90) was a significant risk factor for LFA in patients with a VFA of 130 cm^2 or less and for an elevation of ALT regardless of VO criteria. However, these associations were observed only in males, which might be the same phenomenon as the sex difference observed in the cardiovascular prognosis of OSA patients. These results suggested that treatment for male OSA with hypoxemia during sleep might be warranted from the viewpoint of preventing LFA in males without overt VFA and liver dysfunction.

Supporting Information

S1 Fig. Modification of Couinaud segmentation system. Each ROI for measurement of attenuation was placed in the liver parenchyma of each section. (A) Level of right hepatic vein; (B) level of umbilical portion of left portal vein; (C) level of posterior branch of right portal vein. Abbreviations: HV, hepatic vein; IVC, inferior vena cava, UP, umbilical portion; Post.PV, posterior branch of right portal vein.
(TIF)

S1 File. Study protocol.
(DOC)

S2 File. Study protocol in the original language.
(DOC)

S1 Table. Stepwise multiple regression models for CT_{LFA} in females with and without VO_{100}.
(DOC)

S2 Table. Stepwise multiple regression models for CT_{LFA} in males with and without obesity (BMI $\geq$ 25 kg/m^2).
(DOC)

S3 Table. Stepwise multiple regression models for CT_{LFA} in females with and without obesity (BMI $\geq$ 25 kg/m^2).
(DOC)

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Author Contributions

Conceived and designed the experiments: YT KT TK TC MM KC. Performed the experiments: YT YC YH KM T. Hitomi TO. Analyzed the data: YT KT KM MA SH T. Handa TO KC.

Contributed reagents/materials/analysis tools: KT YH YC TK T. Handa T. Hitomi MM. Wrote the paper: YT CK.

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Obstructive sleep apnea and abdominal aortic calcification: Is there an association independent of comorbid risk factors?

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ABSTRACT

Background: No studies have addressed the relationship between obstructive sleep apnea (OSA) and abdominal aortic calcification (AAC), a marker for subclinical atherosclerosis and future cardiovascular events.
Objectives: To investigate 1) the association between OSA severity and AAC, and 2) whether OSA can impact the extent of AAC independent of comorbid atherogenic risk factors.
Methods: 390 participants aged 40–70 years underwent polysomnography and abdominal computed tomography. AAC was separately quantified in the upper and lower abdominal aorta using the modified Agatston scoring method, and the total AAC score was calculated as a sum of the two scores. OSA was defined as none/mild (apnea-hypopnea index [AHI] <15, n = 87), moderate (AHI 15–30, n = 129), and severe (AHI ≥30, n = 174).
Results: Log-transformed total AAC score adjusted for age and body mass index (BMI) was greater in participants with an elevated AHI (3.4 for none/mild OSA, 3.7 for moderate OSA, and 4.2 for severe OSA, p = 0.04). Multivariate linear regression analysis including age and BMI as covariates showed that severe OSA was associated with higher scores for the lower and total AAC (β = 0.15 and 0.14, p = 0.01 and 0.01, respectively). The association did not persist after additionally adjusting for traditional atherogenic risk factors including visceral fat, smoking, hypertension, dyslipidemia, and diabetes.
Conclusions: Severe OSA was associated with a greater extent of AAC, which was dependent on coexisting atherogenic risk factors. Comorbid cardiometabolic disorders may largely mediate the association of OSA with subclinical atherosclerosis.

1. Introduction

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder characterized by recurrent episodes of partial or complete obstruction of the upper airway, leading to intermittent hypoxia and frequent arousals during sleep. Although accumulating evidence indicates that untreated OSA accelerates atherosclerosis, which may mediate the increased cardiovascular disease burden in patients with OSA, frequently-occurring coexisting cardiometabolic disorders make it difficult to assess the independent effect of OSA on the progression of atherosclerosis [1,2]. In this context, exploring the relationship between OSA and a marker for subclinical atherosclerosis could provide indirect evidence of whether OSA per se should be included as a risk factor for atherosclerosis and subsequent adverse outcomes [3].

Arterial calcification occurs as part of the atherosclerotic process thus potentially serving as a surrogate marker for subclinical atherosclerosis. Clinically, coronary artery calcification (CAC) has been extensively studied as a predictor of cardiovascular morbidity and mortality [4,5], and abdominal aortic calcification (AAC) also has been shown to be an independent predictor of future cardiovascular events [6]. To date, the association of OSA with CAC has

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been investigated in a few studies with mixed results while no studies have addressed the relationship between OSA and AAC [7–10].

The objective of this study was to investigate whether the severity of OSA is associated with the extent of AAC, and, if so, to determine whether OSA per se is causal for AAC independent of comorbid atherogenic risk factors.

2. Methods

2.1. Study participants

A total of 684 patients aged 40–70 years underwent polysomnography at our institute from November 2008 to December 2012, and were routinely asked to have abdominal computed tomography (CT) for the evaluation of visceral fat accumulation. Of these, 263 patients declined to have CT, and an additional 31 were excluded from the present analysis due to sleep-disordered breathing other than OSA (n = 18), hemodialysis (n = 5), previous treatment for OSA (n = 3), previous treatment for abdominal aortic aneurysm (n = 3), or insufficient data (n = 2). Finally, data on the remaining 390 patients were retrospectively analyzed for this study (Fig. 1). The Kyoto University Graduate School and Faculty of Medicine Ethics Committee approved this study (E2123).

2.2. Measurement of abdominal aortic calcification

All participants underwent an unenhanced abdominal CT scan covering the level of the aortoiliac bifurcation using a 64-slice multidetector CT scanner (Aquilion 64, Toshiba Medical Systems, Otawara, Japan). Images were reconstructed in the transaxial plane with a 7-mm slice thickness, and were analyzed by one examiner using a dedicated workstation (Ziostation, Ziosoft, Inc., Tokyo, Japan). The AAC score was separately measured in two segments: the lower segment from the aortoiliac bifurcation to the renal arteries (lower abdominal aorta), and the upper segment cranial to the renal arteries with the same length as the lower abdominal aorta (upper abdominal aorta). Total AAC score was calculated as the sum of the upper and lower AAC scores.

AAC was quantified using the modified Agatston scoring method [11]: First, aortic calcification was identified as plaque $\geq$1 mm^2 with a density of $\geq$130 Hounsfield Units in the wall of the aorta. Then, a calcium score in Agatston units was calculated for each calcified lesion by multiplying the number of pixels with a weighted density score based on the maximum attenuation within the lesion. Finally, the total Agatston score was determined by summing the Agatston scores across all lesions within each segment. The intra-class correlation coefficients for the AAC score of the upper and lower abdominal aorta from 40 random subset samples were 0.999 and 0.997, respectively.

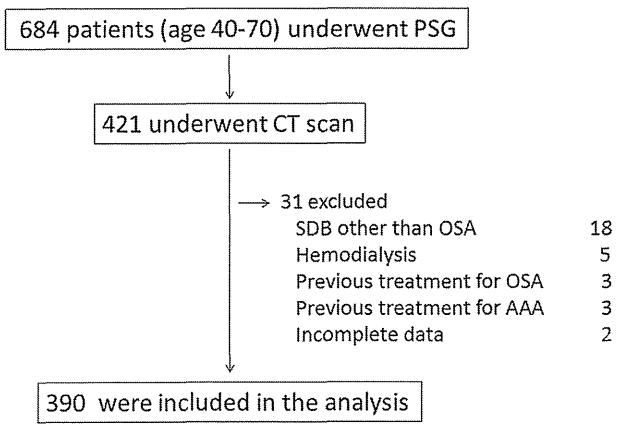

Fig. 1. Flowchart of the study. AAA, abdominal aortic aneurysm; CT, computed tomography; OSA, obstructive sleep apnea; SDB, sleep-disordered breathing.

2.3. Polysomnography

The diagnosis of OSA was confirmed by overnight polysomnography (SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Inc., Murrysville, PA, USA) as previously described [12]. Apnea was defined as the continuous cessation of airflow for more than 10 s, and hypopnea was defined as a reduction in airflow of 50% or more lasting for 10 s or more accompanied by a decrease in SpO_2 of at least 3% or arousal [13]. Apnea-hypopnea index (AHI) values were calculated as the number of episodes of apnea and hypopnea per hour over the total sleep time. OSA severity was defined by AHI as follows: none/mild OSA (AHI <15), moderate OSA (AHI 15–30), and severe OSA (30 $\leq$AHI).

2.4. Cardiovascular risk factors

To measure the abdominal visceral fat area, we used a single CT scan obtained at the level of the umbilicus (approximately at the level of L4 and L5). Visceral fat area was quantified using a specialized image analysis program (AZE Virtual Place 99, AZE of America, Ltd., Irvine, CA, USA) as previously described [14].

Hypertension was defined as 1) the current use of antihypertensive drugs with a previous diagnosis of hypertension or as 2) systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg. Blood pressure was decided based on the average of the last 2 of 5 measurements after the patients rested a few minutes in a sitting position. Dyslipidemia was defined as 1) the current use of lipid-lowering drugs with a previous diagnosis of dyslipidemia, or 2) LDL-C $\geq$140 mg/dl or HDL-C <40 mg/dl or TG $\geq$ 150 mg/dl [15]. Diabetes was defined as 1) a previous diagnosis of diabetes, or as 2) fasting plasma glucose $\geq$126 mg/dl and HbA1c $\geq$ 6.5%. Cardiovascular disease was defined based on a history of coronary artery disease, stroke, and peripheral artery disease. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

2.5. Statistical analysis

All values are expressed as mean $\pm$ SD unless otherwise stated. AAC scores were transformed by natural logarithm after adding a constant of one to each score. Characteristics of participants were compared according to the severity of OSA using one-way ANOVA or the Kruskal–Wallis test for the continuous variables, and the chi-squared test for the categorical variables. Post-hoc pairwise comparisons were performed by the Tukey–Kramer method or the Wilcoxon rank-sum test (using Bonferroni correction), as appropriate. Adjusted mean (SE) values of log-transformed AAC scores across OSA severity groups were estimated by analysis of covariance. Pearson correlation analyses or Spearman rank test were used to evaluate the bivariate relationship.

Multivariable linear regression analyses were conducted in stages to estimate the association between OSA severity and AAC: model 1 was adjusted for the covariates of age and BMI; model 2 was adjusted for the covariates of age and visceral fat area, which may act as a cause of other atherogenic risk factors; and model 3 was adjusted for model 2 covariates plus all other potential confounders including sex, smoking, hypertension, dyslipidemia, and diabetes mellitus [16]. A two-sided *P*-value <0.05 was considered to be statistically significant for all tests. All statistical analyses were

performed using JMP 7.0.2 software (SAS Institute Inc., Cary, NC, USA).

3. Results

Baseline characteristics of study participants are summarized in Table 1. Participants with an elevated AHI were more likely to be male, to have a higher BMI, a larger visceral fat area, greater number of pack years smoked, and to have hypertension or cardiovascular diseases. The lower and total AAC score was higher in participants with severe OSA compared to those with none/mild OSA. Even after adjusting for age and BMI, the log-transformed total AAC score was greater in participants with an elevated AHI (3.4 for none/mild OSA, 3.7 for moderate OSA, and 4.2 for severe OSA, p = 0.04), and was significantly higher in severe OSA compared to none/mild OSA (Fig. 2).

The relationships between log-transformed AAC scores and other variables examined are shown in Table 2. All log-transformed AAC scores were positively correlated with age, visceral fat area, hypertension, dyslipidemia, and diabetes. Positive correlations were also noted for the lower and total AAC score with male sex, smoking, AHI, and 3% oxygen desaturation index.

In multivariate linear regression analysis adjusted for age and BMI, severe OSA was associated with greater log-transformed scores for the lower and total AAC (β = 0.15 and 0.14, P = 0.01 and 0.01, respectively) (Table 3). However, the association was attenuated in the age- and visceral fat area-adjusted model, and visceral fat area was an independent predictor for greater log-transformed AAC scores across the two aortic segments and the total abdominal aorta. After additionally adjusting for traditional atherogenic risk factors, age, hypertension, and diabetes were all independently associated with three log-transformed AAC scores, and smoking was an independent predictor for greater log-transformed scores for the lower and total AAC. Neither OSA nor visceral fat area was an independent predictor for the AAC score.

4. Discussion

In this cross-sectional CT-based study, we found that severe OSA was associated with a greater extent of AAC compared to none/mild OSA even after adjustment for age and BMI. This association, however, did not persist after additionally adjusting for other atherogenic risk factors such as hypertension and diabetes, suggesting that these risk factors may mediate the association between OSA and AAC. To our knowledge, this is the first study that investigated the relationship between OSA and AAC.

Current evidence has indicated that OSA can promote arterial calcification via multiple pathways: OSA may directly (i.e. via OSA-induced physiologic cascade leading to endothelial dysfunction) or indirectly (i.e. by exacerbating atherogenic cardiometabolic disorders) accelerate atherosclerosis and subsequent vascular calcification [2]. Furthermore, the current understanding of the pathophysiology of cardiovascular calcification recognizes this condition as an actively regulated inflammatory process, thus

Table 1
Baseline characteristics according to the severity of obstructive sleep apnea.

Variable	Obstructive sleep apnea			
	None/Mild (n = 87)	Moderate (n = 129)	Severe (n = 174)	*P* value
Age (years)	57.1 ± 9.3	58.0 ± 8.5	57.7 ± 8.2	N.S.
Men	44 (51%)	88 (68%)	145 (83%)	<0.0001
Body mass index (kg/m^2)	24.9 ± 4.5	26.4 ± 4.8	28.4 ± 5.1[*]	<0.0001
Visceral fat area (cm^2)	91.9 ± 54.1	104.6 ± 54.1	148.9 ± 71.0[†]	<0.0001
Smoker				
Current	11 (13%)	14 (11%)	34 (20%)	N.S.
Past	32 (37%)	55 (43%)	79 (45%)	
Never	44 (51%)	60 (46%)	61 (35%)	
Smoking (pack-years)[a]	15.9 ± 24.9	20.0 ± 31.1	24.5 ± 30.5[‡]	0.01
Hypertension	37 (43%)	69 (53%)	110 (63%)	0.006
Systolic blood pressure (mmHg)	123 ± 16	126 ± 17	126 ± 17	N.S.
Diastolic blood pressure (mmHg)	75 ± 11	76 ± 10	78 ± 12	N.S.
Use of antihypertensive drugs	28 (32%)	59 (46%)	97 (56%)	0.001
Dyslipidemia	59 (68%)	86 (67%)	141 (70%)	N.S.
Use of statins	25 (29%)	42 (33%)	54 (31%)	N.S.
Diabetes mellitus	17 (20%)	39 (23%)	52 (30%)	N.S.
Cardiovascular diseases[b]	8 (9%)	26 (20%)	37 (21%)	0.046
Epworth sleepiness scale	8.4 ± 5.1	9.2 ± 4.9	9.3 ± 5.7	N.S.
AHI (events/h)[a]	8.5 ± 4.2	22.9 ± 3.9	50.4 ± 17.3	<0.0001[§]
Lowest SpO_2 (%)[a]	87.2 ± 5.2	80.2 ± 11.0	75.5 ± 10.0	<0.0001[§]
Mean SpO_2 (%)[a]	95.6 ± 1.8	94.7 ± 1.7[‖]	91.8 ± 3.7[†]	<0.0001
Time of SpO_2 <90% (%)[a]	1.8 ± 4.8	5.8 ± 8.0	31.0 ± 28.5	<0.0001[§]
Arousal index (events/h)[a]	17.1 ± 7.6	22.8 ± 9.6	37.0 ± 16.2	<0.0001[§]
AAC score				
Upper[c]	0 (0–24)	0 (0–44)	0 (0–56)	N.S.
Lower[c]	29 (0–235)	82 (0–507)	133 (0–661)[¶]	0.013
Total[c]	54 (0–296)	115 (0–589)	147 (0–837)[¶]	0.014

[*] P < 0.0001 vs. none/mild OSA; P < 0.01 vs. moderate OSA.
[†] P < 0.0001 vs. none/mild OSA, moderate OSA.
[‡] P < 0.05 vs. none/mild OSA.
[§] P < 0.0001 among all comparisons.
[‖] P < 0.001 vs. none/mild OSA.
[¶] P < 0.005 vs. none/mild OSA.
AHI: apnea-hypopnea index, AAC: abdominal aortic score.
[a] Kruskal–Wallis test was used to determine overall differences among groups.
[b] Including coronary artery diseases, stroke, peripheral artery diseases.
[c] Values are expressed as median (interquartile range).

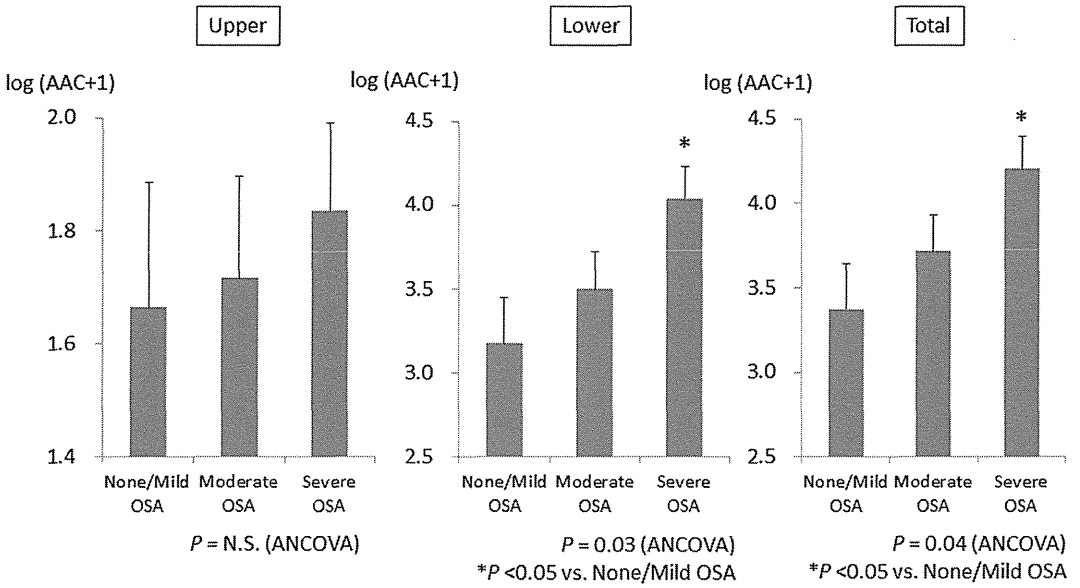

Fig. 2. Abdominal aortic calcification scores according to the severity of obstructive sleep apnea. All values are adjusted by age and body mass index, and are presented as mean ± SE. AAC: abdominal aortic calcification, ANCOVA: analysis of covariance, OSA: obstructive sleep apnea.

Table 2
Correlations between abdominal aortic calcification score and possible determinants.

Variable	Log (AAC + 1)		
	Upper	Lower	Total
Age[a] (years)	0.51*	0.49*	0.53*
Men	0.04	0.18†	0.15†
Body mass index[a] (kg/m^2)	−0.07	−0.08	−0.07
Visceral fat area[a] (cm^2)	0.14‡	0.18†	0.18†
Smoker	0.09	0.31*	0.28*
Hypertension	0.30*	0.27*	0.29*
Dyslipidemia	0.16‡	0.18†	0.18†
Diabetes mellitus	0.26*	0.21*	0.22*
AHI[a,b]	0.09	0.15‡	0.15‡
3% ODI[a,b]	0.08	0.14‡	0.14‡

*P < 0.0001.
†P < 0.001.
‡P < 0.01.
AAC: Abdominal aortic calcification, AHI: Apnea-hypopnea index, ODI: oxygen desaturation index.
[a] Correlations between these variables and AAC scores are presented by Pearson correlation coefficients (r), and the others are presented by Spearman rank correlation coefficient (ρ).
[b] Values are log-transformed.

implying that OSA-induced oxidative stress and systemic inflammation might be involved in the progression of arterial calcification [17].

To date, inconsistent results have been reported as to whether OSA is an independent risk factor for arterial calcification as assessed by CAC [7–10], while no data are currently available regarding the association between OSA and AAC. Although less frequently studied than CAC, it should be noted that AAC often precedes CAC [18], and is the most prevalent arterial calcification among peripheral arteries that predicts atherosclerosis in other vascular sites and future cardiovascular events [6,19,20]. Therefore, exploring the relationship between OSA and AAC would provide additional insights into the pathogenic significance of OSA in the progression of systemic atherosclerosis and subsequent adverse outcomes.

From these perspectives, the present study demonstrated an association between OSA severity and the extent of AAC, highlighting an increased atherosclerotic burden in patients with OSA that may lead to cardiovascular diseases in these patients. However, this association was no longer significant after accounting for other atherogenic risk factors including visceral fat area, smoking, hypertension, dyslipidemia and diabetes. Accordingly, in the presence of other atherogenic risk factors, the impact of these comorbidities probably outweighs that of OSA per se on the progression of subclinical atherosclerosis. In keeping with our results, previous studies showed that the relationship between OSA and CAC was attenuated by coexisting morbidities [8,10], and we extended these findings by evaluating OSA and AAC. Not surprisingly, the relative associations between OSA or abdominal visceral fat and AAC bear similarities to findings of a previous study documenting that coexisting morbidities largely mediate the relationship between abdominal visceral fat and CAC/AAC [21]. Viewed from another side, these findings underscore the importance of managing comorbid risk factors for atherosclerosis to prevent cardiovascular diseases in patients with OSA, as well as an effective treatment for OSA which might precipitate traditional atherogenic risk factors.

We separately measured the AAC score in the upper and the lower aorta, because the hemodynamic conditions and severity of atherosclerosis of the abdominal aorta differ substantially above and below the renal arteries [22]. In keeping with a previous study [23], abdominal aortic calcification predominantly occurred in the lower abdominal aorta, and therefore risk factors for the total AAC score were mainly defined by those included in the lower AAC score. We also found that risk factors for AAC slightly differed between the upper and lower aorta: hypertension is the most significant risk factor other than age for calcification in the upper abdominal aorta whereas smoking emerged as a dominant risk factor in the lower abdominal aorta. This may be partially explained by the previous finding that the impact of blood pressure as a cause of aortic calcification wanes with passage from the proximal to the distal aorta, probably due to a decreased impact of direct blood pressure on the aortic wall [19].

We must acknowledge several limitations. First, the cross-sectional nature of the study precluded definitive conclusions regarding the causal relationship between the potential risk factors