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ORIGINAL CONTRIBUTIONS

Obstructive Sleep Apnea Is Associated with Fatty Liver and Abnormal Liver Enzymes: a Meta-analysis

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Abstract
Background Obstructive sleep apnea (OSA) is associated with the cluster of clinical conditions that comprise the metabolic syndrome, including nonalcoholic fatty liver disease (NAFLD). Our primary purpose was to estimate the effect of OSA on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Our secondary purpose was to investigate the potential influence of OSA on histological severity of NAFLD to explore whether chronic intermittent hypoxia is associated with inflammation and fibrosis.
Methods Our literature search identified 11 studies, from which we extracted information about numbers of control subjects and OSA patients, and ALT, AST, and NAFLD.
Results From a total of 668 OSA patients and 404 controls, we found that the standardized difference in mean values of ALT and AST levels in patients with OSA was significantly different from that in the controls. Meta-regression showed that the association was independent of body mass index and type 2 diabetes. Fatty liver was associated with OSA in five studies with 400 subjects. OSA was significantly associated with liver fibrosis in 208 subjects, but not with lobular inflammation.
Conclusions Routine assessment of liver enzymes and liver damage should be implemented in OSA patients because they have an increase of 13.3 % of ALT and 4.4 % of AST levels, and a 2.6-fold higher risk of liver fibrosis when they have NAFLD, which is 2.6 times more frequent in OSA patients.

Keywords Alanine aminotransferase · Aspartate aminotransferase · Fatty liver · Hypoxia · Metabolic syndrome · Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis · Obstructive sleep apnea

Authors' contributions CJP and SS designed the study, analyzed and interpreted the data, and prepared and wrote the manuscript. CJP performed the statistical analysis. Both authors read and approved the final manuscript

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Introduction

The metabolic syndrome (MS) comprises a combination of risk factors for cardiovascular disease (CVD). To the classical components initially regarded as syndrome X (insulin resistance, dyslipidemia, central obesity, and arterial hypertension), some other clinical phenotypes have recently been added, such as nonalcoholic fatty liver disease (NAFLD), which is not only the hepatic manifestation of MS [1, 2] but is also independently associated with an increased risk of CVD [3, 4] and a proatherogenic profile [3, 5–7]. In addition, a serious and potentially life-threatening respiratory condition characterized by repetitive apnea and hypopnea, known as obstructive sleep apnea (OSA), has been associated in several epidemiological studies with MS components such as obesity, insulin resistance, and hypertension [8] but also with increased risk of CVD [9–11]. Hence, it has been suggested that OSA is part of

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the cluster of vascular risk factors, and the name of syndrome Z has been suggested to define the interaction between OSA and CVD [12].

OSA is not only associated with repeated cessation of breathing while sleeping, it is also accompanied by hypoxia in target tissues and hemodynamic changes associated with dysregulation of the sympathetic nervous system [12]. Moreover, multiple cycles of hypoxia/reoxygenation, such as those occurring during OSA, are also consistently associated with chronic inflammation [13]. Interestingly, evidence from experimental studies has shown that chronic intermittent hypoxia might be involved in the pathogenesis of NAFLD [14]. Observational human studies have suggested that OSA is associated with the histological severity of NAFLD, particularly among patients with morbid obesity [14]. Nevertheless, the evidence remains controversial because most of the studies included patients with all the features of MS and did not adjust for potential confounding variables, such as body mass index (BMI) or type 2 diabetes (T2D). Some observational cross-sectional studies have reported that OSA is a risk factor for developing nonalcoholic steatohepatitis (NASH) [15–20]. In contrast, two other studies have reported evidence suggesting that the association between OSA and NASH is coincidental [21, 22]. Another important clinical observation is the putative association between OSA and elevated liver enzymes. In this regard, some [23–25] but not all [15, 16, 18, 20–22] studies have reported that OSA is associated with elevated serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST), and the reason for the discrepancy among these studies might be their small sample size. This association, however, sounds biologically plausible because chronic intermittent hypoxia might modulate the shift towards a necroinflammatory response in the liver tissue and could even explain the severity of liver damage. In fact, induction of hypoxia-induced factor 1α seems to be an important mediator of NAFLD development in animal models [14, 26]. Nevertheless, as the evidence is still inconclusive, at the present time, it is not possible to establish the putative association of OSA with NAFLD or elevated liver enzymes.

Meta-analysis is a reliable way to address discrepancies between studies; therefore, we decided to evaluate the potential association between OSA and liver enzymes and NAFLD-related phenotypes using a systematic review. In view of the evidence mentioned earlier, our primary purposes were to estimate from the available literature the effect of OSA on the level of ALT and AST in different populations, and evaluate systematically the study characteristics that could be responsible for the association; for instance, potential confounders such as BMI, sex, and T2D. Our secondary purpose was to review the potential influence of OSA on the histological disease severity of NAFLD to address the question of whether chronic intermittent hypoxia is associated with higher levels of liver inflammation and fibrosis.

Materials and Methods

Data Sources and Study Selection

We searched for published studies on PubMed, Medline, and Google Scholar using the keywords and terms ("obstructive sleep apnea" or "sleep apnea, obstructive") or ("sleep" or "chronic intermittent hypoxemia" and "apnea" and "obstructive") or ("obstructive sleep apnea" or "obstructive" and "sleep" and "apnea") and ("liver" or "liver") and ("enzymology" or "enzymology" or "enzymes"). In addition, the references of the retrieved articles were checked and the PubMed link "related articles" was used to identify additional papers; the search was also performed in The Cochrane Library. This yielded a total of 138 hits. The literature search was done on studies up to November 2012 and availability of an English language abstract or paper for review. There were no country restrictions. The authors reviewed all abstracts independently to determine the eligibility criteria or to establish the appropriateness of the research issue. Details about inclusion and exclusion criteria and data collection can be seen in the Appendix.

We followed the appropriate methods for conducting a meta-analysis as stipulated in the Guidelines for Meta-Analyses of Observational Studies in Epidemiology group [27].

Inclusion and Exclusion Criteria

Inclusion criteria include observational or cohort (population-based or hospital-based) case–control studies that provided raw data dealing with chronic intermittent hypoxemia or OSA and liver enzymes, in which information about the number of subjects in each category (control subjects and OSA patients evaluated by a reliable method, such as overnight polysomnography) and data about serum ALT and AST levels could be extracted. Exclusion criteria include studies that referred to subjects with active alcohol consumption, duplicate publications, unpublished papers, and papers that included only data about patients with OSA without valid controls, and studies that included therapeutic intervention before measuring outcomes of interest.

Data Collection

The primary outcomes to evaluate were ALT and AST levels in patients with OSA compared with controls without OSA (no-OSA or controls). Secondary outcomes were fatty liver and histological outcomes such as presence/absence of NASH, liver inflammation, and fibrosis, according to OSA/no-OSA status.

For each study, information was collected concerning the demographic information of the subjects (age, sex, country of origin as a proxy of ethnicity), study design, liver enzymes, BMI, and T2D. Assessment of fatty liver (evaluated by liver ultrasound or biopsy) was also included in the analysis. The

Table 1 Characteristics of the studies of the relation of obstructive sleep apnea (OSA) with liver enzymes and liver-related phenotypes

First author, year	References	Country	Age (range years)	Sex, male proportion	Number of subjects, OSA/no-OSA	ALT mean±SD, OSA/no-OSA	AST mean±SD, OSA/no-OSA	Clinical condition of patients	BMI mean±SD OSA/no-OSA	Type 2 diabetes *N* total (*n* OSA/ *n* no-OSA)	Assessment of OSA	Outcome of histological evaluation NASH total (*n* OSA/*n* no-OSA)
Acarturk G, 2007	15	Turkey	Adult (41–60)	F, 0	20/25	21.1±5.3/22.8±11	19.7±3.9/20±5.9	Obese	39.9±8/39±6.2	NA	NP	NA
Aron-Wisnewsky J, 2012	16	France	Adult (41–60)	M/F, 0.1	34/33	33.6±16.3/30±19.3	23.3±6.6/21.7±7.8	Morbid obese/ bariatric surgery program	48.3±7/45.7±5.7	15 (13/2)	ODI	NASH *n*=8 (7/1)
Daltro C, 2010	21	Brasil	Adult (21–40)	M/F, 0.35	16/23	40.9±18/32.1±17.7	25.4±10.7/22±7.3	Morbid obese/ bariatric surgery program	41.9±5.1/41.4±4.5	2 (NA)	NP	NASH *n*=32 (18/14)
Jouet P, 2007	22	France	Adult (21–40)	M/F, 0.13	48/9	40.9±17.3/41.1±14.5	23.6±11.2/22±8.6	Morbid obese/bariatric surgery program	54.6±9.8/45.5±5.5	6 (5/1)	NP	NASH *n*=21 (17/4)
Turkay C, 2012	31	Turkey	Adult (41–60)	M/F, 0.75	62/44	NA	NA	Clinical suspicion of OSA	NA	NA	NP	NA
Kheirandish-Gozal L, 2008	25	USA	Children (4–17)	M/F, 0.5	343/175	18.9±3.7/17.6±3.5	15.7±3.3/14.8±3.7	Habitual snoring	NA	NA	NP	NA
Polotsky V, 2009	18	USA	Adult (21–60)	M/F, 0.25	45/45	12.3±6.2/11.1±6.1	17.8±5.9/17.5±6.6	Morbid obese/bariatric surgery program	49.9±7.9/48.1±7.9	NA	NP	LB only available in a minority of subjects with abnormal ALT (*n*=20)
Mishra P, 2008	17	USA	Adult (41–60)	M/F, 0.3	82/19	NA	NA	NAFLD	NA	33 (NA)	NP	NASH *n*=79 (NA)
Tanne F, 2005	19	USA	Adult (41–60)	M/F, 0.6	44/35	28±16/19±8	14±7/11±4	Clinical suspicion of OSA	30.37±7/27±6.4	6 (5/1)	NP	LB only available in a minority of subjects with abnormal ALT (*n*=18)
Byrne T, 2012	23	USA	Adult +60 (range NA)	M/F, 0.5	18/53	30.9±14.8/22.1±9.1	28.1±12.9/25.7±8.9	Clinical suspicion of OSA	NA	34 (25/9)	NP	NA
Tatsumi K, 2005	20	Japan	Adult (41–60)	M/F, 0.9	83/41	28±22.8/28.2±22.4	21.8±12.8/23.3±9.6	Non obese	25.8±2.7/25.1±4.5	NA	NP	NA

NP nocturnal polysomnography, *ODI* oxygen desaturation index, *NA* not available, *F* female, *M* male, *LB* liver biopsy, *NASH* nonalcoholic steatohepatitis

evaluation of histological disease severity was based on data from liver biopsy from NAFLD patients, including the presence of lobular necroinflammation (grade ≥1) and fibrosis (stage ≥1). NASH was not included as a variable in the analysis because its histological definition was not uniform across the studies.

All quantitative variables had to be expressed as mean± standard deviation (SD); standard error (SE) was converted to SD. Odds ratios (ORs) were obtained or calculated for OSA patients against healthy control subjects.

Statistical Analysis

For liver enzymes ALT and AST, effect size measure stands for Cohen's standardized mean difference (D), which was the mean difference (between cases and controls) divided by the common within-group SD.

For the dichotomous variables fatty liver, lobular inflammation, and liver fibrosis, effect stands for OR. Fixed effect model using the Mantel–Haenszel method was used to summarize the results, which yielded the corresponding pooled OR and corresponding 95 % confidence interval (CI).

The DerSimonian and Laird method was used to combine the OR and D for the outcomes of interest using a random effects meta-analytical technique [28].

Heterogeneity was evaluated with the Q statistic and I^2 statistic, a transformation of Q that estimates the percentage of the variation in effect sizes that is due to heterogeneity. An I^2 value of 0 % indicated no observed heterogeneity, and larger values showed increasing heterogeneity.

Meta-regression analysis (random effects model, within-study variance estimated with the unrestricted maximum-likelihood method) was also performed to assess the influence of study-related factors on outcomes and analysis of heterogeneity.

To check for publication bias, we used a visual inspection of funnel plots and the Begg and Mazumdar's rank correlation test (this test reports the rank correlation—also known as rank correlation coefficient or simply Kendall's τ—between the standardized effect size and the variances, or standard errors, of these effects) [29]. A p value ≤0.05 was considered to be statistically significant.

All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

Results

We evaluated 11 studies that met the selection criteria as described in the Appendix, and the study characteristics are shown in Table 1. Data from three studies that fulfilled the eligibility criteria were included after personal contact with the investigators [21–23]. Data on one further study [24] were unavailable because the authors did not disclose the raw data and our efforts to contact them were unsuccessful; one study was excluded because OSA was evaluated by a sleep apnea questionnaire [30], and other studies were excluded because the authors included patients with OSA managed with noninvasive ventilation and variables of interest were analyzed after medical intervention with continuous positive airway pressure [31–35]. Absence of controls was another reason for exclusion [36]. All the studies scored well in terms of adequate descriptions of selection criteria and reference test, blind assessment of the reference test, and availability of clinical data.

Study Characteristics

All studies included male and female subjects, except one that was based only on women [15]. The histological disease severity of NAFLD was evaluated in the included studies by needle biopsy obtained during bariatric surgery. Lobular inflammation and fibrosis were analyzed as dichotomous variables (presence or absence) according to OSA, and effect stands for Mantel–Haenszel OR.

Histological assessment of liver specimens was performed uniformly across the studies as follows: liver steatosis was graded 0–3 [16, 17, 22] and 0–4 [21], and lobular inflammation and fibrosis were scored 0–3 and 0–4, respectively. The degree of fatty liver infiltration was assessed in all the studies by the Brunt criteria [37]. NASH evaluation was based on descriptive histological features and no information about any specific scoring system was provided by the authors. Moreover, no information about adequacy of the liver biopsies for histological assessment was provided. Nevertheless, it is important to note that most of the specimens were obtained during bariatric surgery and sample size should not have been an issue.

OSA was assessed by standard overnight polysomnography in 10 studies [15, 17–23, 25, 38], and the number of apnea and hypopnea episodes per hour of monitoring was calculated (apnea–hypopnea index, AHI); absence of OSA was defined as AHI <10/h [15, 18, 19, 21–23] or AHI <5 [20, 25]. One study evaluated the severity of OSA by measurement of oxygen saturation index [16].

ALT and AST Levels

Data regarding ALT were extracted from nine studies [15, 16, 18–23, 25] that included 1,072 individuals (668 patients with OSA and 404 control subjects), and the analysis showed that the standardized difference in mean values of ALT levels was significantly different in OSA patients from controls either in the fixed ($p<5\times10^{-6}$) or the random model ($p<0.002$) (Fig. 1). Although we did not observe heterogeneity among

Model	Study name	Popul	Outcome	Statistics for each study				Sample size		
				Std diff in means	Lower limit	Upper limit	p-Value	OSA	Control	Total
	ACARTÜRK G et al. 2007	MO	ALT	-0.190	-0.779	0.399	0.5272	20	25	45
	Aron-Wisnewsky J et al. 2012	MO	ALT	0.202	-0.278	0.682	0.4101	34	33	67
	Daltro C et al. 2010	MO	ALT	0.494	-0.154	1.141	0.1350	16	23	39
	Jouët P et al. 2007	MO	ALT	-0.012	-0.724	0.700	0.9740	48	9	57
	Kheirandish-Gozal L et al. 2008	GP	ALT	0.358	0.174	0.541	0.0001	343	175	518
	Polotsky VY et al. 2009	MO	ALT	0.195	-0.219	0.609	0.3558	45	45	90
	Tanne F et al. 2005	HB	ALT	0.688	0.231	1.144	0.0032	44	35	79
	Byrne TJ et al. 2012	HB	ALT	0.668	0.085	1.250	0.0246	35	18	53
	Tatsumi K et al. 2005	HB	ALT	-0.009	-0.383	0.365	0.9631	83	41	124
Fixed				0.298	0.171	0.425	0.0000	668	404	1072
Random				0.279	0.105	0.453	0.0017	668	404	1072

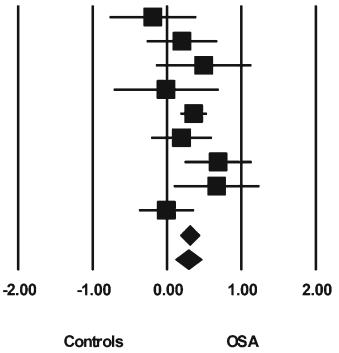

Fig. 1 Summary estimates for standardized difference (*D*) (effect); the corresponding 95 % CI (lower and upper) and significance (*p* value) were estimated by fixed and random effects analysis for serum ALT level (as continuous variable) between OSA patients and controls without OSA. The first author of the study and the year of publication are shown after study name; *Popul* indicates design features, *GP* general population, *HB* hospital-based, and *MO* morbid obesity. In the graph, *numbers* indicate *D* values, *filled squares* stand for the effect of individual studies, and *filled diamonds* express combined fixed and random effects

studies as assessed by the *Q* statistic ($p<0.19$), the I^2 (30.1 %) value was high. We removed one study at a time and observed that after removing the study of Tatsumi and Saibara [20], a more robust effect was obtained (fixed *D*, 0.338; 95 % CI, 0.203–0.472; $p<1\times10^{-6}$; random *D*, 0.329; 95 % CI, 0.160–0.499; $p<0.0002$) with lower $I^2=18$ %.

From the Begg and Mazumdar's rank correlation test (two-tailed, $p<0.93$), it seems that there was no publication bias. Information about AST levels was extracted from the same previously mentioned studies, and the analysis showed that the standardized difference in mean values of AST levels was also significantly different in OSA patients from controls either in the fixed or the random model ($p<0.0023$) (Fig. 2), without evidence of heterogeneity (*Q* statistic $p<0.57$; I^2, 0.0) and without publication bias (Begg and Mazumdar's rank correlation test, two-tailed, $p<0.92$). One study was excluded from the analysis of liver enzymes because the authors did not disclose data as mean± SD/SE, and our repeated efforts to contact them were unsuccessful [38].

Model	Study name	Popul	Outcome	Statistics for each study				Sample size		
				Std diff in means	Lower limit	Upper limit	p-Value	OSA	Control	Total
	ACARTÜRK G et al. 2007	MO	AST	-0.059	-0.647	0.529	0.8450	20	25	45
	Aron-Wisnewsky J et al. 2012	MO	AST	0.222	-0.259	0.702	0.3657	34	33	67
	Daltro C et al. 2010	MO	AST	0.385	-0.259	1.028	0.2415	16	23	39
	Jouët P et al. 2007	MO	AST	0.147	-0.565	0.860	0.6853	48	9	57
	Kheirandish-Gozal L et al. 2008	GP	AST	0.262	0.079	0.444	0.0050	343	175	518
	Polotsky VY et al. 2009	MO	AST	0.048	-0.365	0.461	0.8202	45	45	90
	Tanne F et al. 2005	HB	AST	0.511	0.060	0.962	0.0263	44	35	79
	Byrne TJ et al. 2012	HB	AST	0.205	-0.365	0.775	0.4812	35	18	53
	Tatsumi K et al. 2005	HB	AST	-0.127	-0.501	0.248	0.5075	83	41	124
Fixed				0.198	0.071	0.324	0.0022	668	404	1072
Random				0.198	0.071	0.324	0.0022	668	404	1072

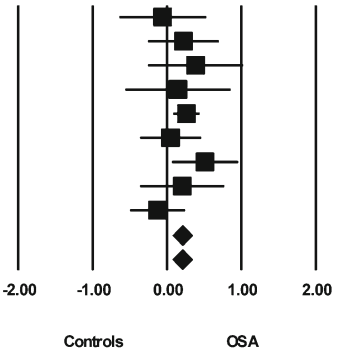

Fig. 2 Summary estimates for standardized difference (*D*) (effect); the corresponding 95 % CI (lower and upper) and significance (*p* value) were estimated by fixed and random effects analysis for serum AST level (as continuous variable) between OSA patients and controls without OSA. The first author of the study and the year of publication are shown after study name; *Popul* indicates design features, *GP* general population, *HB* hospital-based, and *MO* morbid obesity. In the graph, *numbers* indicate *D* values, *filled squares* stand for the effect of individual studies, and *filled diamonds* express combined fixed and random effects

Model	Study name	Popul	Outcome	Statistics for each study				
				MH odds ratio	Lower limit	Upper limit	p-Value	Total
	ACARTÜRK G et al. 2007	MO	Fatty liver	0.409	0.085	1.975	0.2659	45
	Aron-Wisnewsky J et al. 2012	MO	Fatty liver	3.314	1.014	10.838	0.0475	67
	Daltro C et al. 2010	MO	Fatty liver	1.842	0.311	10.915	0.5010	40
	Kheirandish-Gozal L et al. 2008	GP	Fatty liver	4.109	1.343	12.567	0.0132	142
	Turkay C et al. 2012	HB	Fatty liver	4.563	1.923	10.830	0.0006	106
Fixed				2.848	0.239	33.975	0.4081	400
Random				2.556	1.184	5.515	0.0168	400

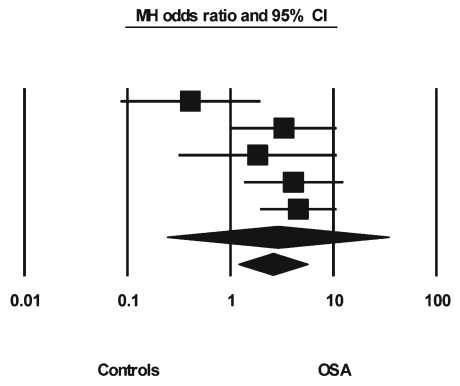

Fig. 3 Summary estimates for OR (effect); the corresponding 95 % CI (lower and upper) and significance (*p* value) were calculated by fixed and random effects meta-analysis for fatty liver (as dichotomous variable) between OSA patients and controls without OSA. The first author of the study and the year of publication are shown after citation name; *Popul* indicates design features, *GP* general population, *HB* hospital-based, and *MO* morbid obesity. In the graph, *numbers* indicate OR, *filled squares* stand for the effect of individual studies, and *filled diamonds* express combined fixed and random effects. The symbol size is proportional to the number of individuals involved in each study

Is OSA Associated with Liver Enzymes Regardless of Male Sex, Obesity, and T2D?

The epidemiological evidence has shown that male sex and obesity are strongly associated with the presence of sleep-disordered breathing [39]. Hence, we evaluated by meta-regression analysis whether the association between OSA and liver enzymes was influenced by male sex, and initially this analysis seemed to demonstrate that sex did not have a significant impact on the level of either ALT (slope=1.11, $p<0.70$) or AST (slope=−0.08, $p<0.79$) as *D* of both enzymes did not correlate with male sex. However, considering that the removal of the study of Tatsumi and Saibara [20] gave a more homogeneous and robust estimation, we repeated the analysis excluding this study, and we found that the OSA effect on ALT was dependent on male sex in each study (slope=0.95, $p<0.02$); regarding AST, the correlation with male sex was still not significant (slope=0.54, $p<0.18$). Thus, our results showed that male sex modulated the OSA effect on ALT levels, although more research is needed to address this question.

We next wondered whether BMI might influence the association between OSA and liver enzymes, and surprisingly meta-regression analysis showed that this association was independent of obesity because *D* of both enzymes did not correlate with BMI (ALT slope=−0.006, $p<0.54$) and AST (slope=0.001, $p<0.92$).

OSA is associated with T2D [40], and NAFLD and T2D are strongly associated; therefore, we performed a meta-regression analysis and observed that *D* for ALT (slope=0.64, $p<0.50$) and AST (slope=−0.44, $p<0.64$) did not correlate

Model	Study name	Popul	Outcome	Statistics for each study				Sample size		
				MH odds ratio	Lower limit	Upper limit	p-Value	OSA	Control	Total
	Aron-Wisnewsky J et al. 2012	MO	inflammation	3.286	1.198	9.010	0.0208	34	33	67
	Daltro C et al. 2010	MO	inflammation	1.140	0.231	5.624	0.8718	16	24	40
	Jouët P et al. 2007	MO	inflammation	0.556	0.095	3.246	0.5140	13	9	22
	Mshra P et el. 2008	MO	inflammation	1.904	0.627	5.782	0.2559	82	19	101
Fixed				1.721	0.108	27.520	0.7009	145	85	230
Random				1.800	0.905	3.579	0.0939	145	85	230

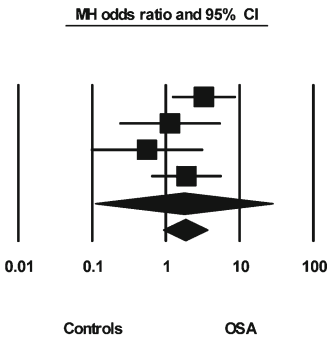

Fig. 4 Summary estimates for OR (effect); the corresponding 95 % CI (lower and upper) and significance (*p* value) were calculated by fixed and random effects meta-analysis for liver lobular necroinflammation between OSA patients and controls without OSA. The first author of the study and the year of publication are shown after citation name; *Popul* indicates design features, *MO* morbid obesity. In the graph, *numbers* indicate OR, *filled squares* stand for the effect of individual studies, and *filled diamonds* express combined fixed and random effects. The symbol size is proportional to the number of individuals involved in each study

with T2D. Thus, we suggest that the association between OSA and liver enzymes was also independent of T2D.

Fatty Liver and Histological Severity of NAFLD

Data regarding fatty liver disease as a disease trait were extracted from five homogeneous studies ($p<0.11$; I^2, 47.9 %) with no evidence of publication bias (Begg and Mazumdar's rank correlation test two-tailed, $p<0.10$) [15, 16, 21, 25, 38]. These included 400 individuals (252 patients with OSA and 148 controls) and the analysis showed a significant association between OSA and fatty liver in the random model (OR, 2.556; 95 % CI, 1.184–5.155; $p<0.017$) (Fig. 3). As I^2 was high, after removing one study at a time, a more robust estimation of the OR was found (3.778; 95 % CI, 2.154–6.626; $p<0.00004$) after removing the study of Acarturk et al. [15]. Interestingly, a trend toward male sex dependency was also observed for this outcome by meta-regression (slope=1.81, $p<0.054$).

We observed that the interval in the fixed model was higher compared with the random one; therefore, we used a different approach to examine the association between OSA and fatty liver. The weighted OR method, which gives a relative weight to each study that is proportional to the inverse of total variance, showed that fatty liver was significantly associated with OSA in both fixed and random models (OR, 2.939; 95 % CI, 1.731–4.989; $p<0.00007$ and OR, 2.556; 95 % CI, 1.185–5.513; $p<0.017$, respectively).

In addition, we found four homogeneous reports ($p<0.33$; I^2, 12.8 %) [16, 17, 21, 22] without evidence of publication bias (Begg and Mazumdar's rank correlation test, two-tailed, $p<0.9$), including 230 subjects (145 patients with OSA and 85 controls) that reported retrieval data about liver histological scores for lobular inflammation according to OSA, and no significant association was observed in the fixed or random model (Fig. 4).

Nevertheless, the comparison between cases and controls in three studies [16, 17, 21], including 208 subjects (132 patients with OSA and 76 controls), showed that OSA was significantly associated with liver fibrosis in the random model ($p<0.008$) (Fig. 5), without evidence of either heterogeneity (Q statistic $p=0.0$; I^2, 39.3 %) or publication bias (two-tailed $p<0.34$); weighted OR showed significant results in fixed or random models (OR, 2.586; 95 % CI, 1.288–5.188; $p<0.0074$).

NASH as a dichotomous trait was evaluated in a small sample of 162 subjects, and no significant association was observed with OSA (fixed model OR, 1.873; 95 % CI, 0.739–4.74; $p=0.18$ and random model OR, 2.185; 95 % CI, 0.669–7.133; $p=0.19$).

One study was excluded from the analysis of histological scores as liver biopsy was restricted to 18 patients with abnormal liver enzymes [19].

Discussion

The epidemiological evidence suggests that OSA is strongly linked not only to the cluster of MS-associated phenotypes but also to increased cardiovascular risk. As a consequence, physicians are aware that patients with OSA may also show comorbidity, particularly obesity, arterial hypertension, and insulin resistance, and because of that, they routinely perform screening tests for these conditions in subjects with sleep-disordered breathing.

Recent studies have shown that fatty liver and abnormal liver enzymes are also associated with OSA, and it has been speculated that OSA might worsen progression of NAFLD. Unfortunately, the current evidence is inconclusive and even controversial because a positive association, particularly with liver enzymes, was observed in some but not all of the studies. To provide a more objective basis for clinical recommendations and to determine the impact of OSA on liver-related phenotypes, we conducted a meta-analysis. Interestingly, we observed that

Model	Study name	Popul	Outcome	Statistics for each study				Sample size		
				MH odds ratio	Lower limit	Upper limit	p-Value	OSA	Control	Total
	Aron-Wisnewsky J et al. 2012	MO	Fibrosis	4.833	1.499	15.582	0.0083	34	33	67
	Daltro C et al. 2010	MO	Fibrosis	2.167	0.476	9.860	0.3173	16	24	40
	Mshra P et el. 2008	MO	Fibrosis	1.694	0.589	4.871	0.3276	82	19	101
Fixed				2.898	0.118	71.142	0.5147	132	76	208
Random				2.586	1.289	5.189	0.0075	132	76	208

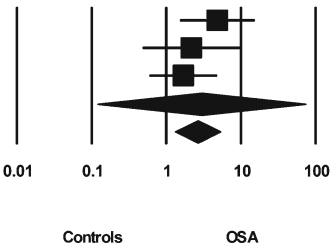

Fig. 5 Summary estimates for OR (effect); the corresponding 95 % CI (lower and upper) and significance (p value) were calculated by fixed and random effects meta-analysis for liver fibrosis between OSA patients and controls without OSA. The first author of the study and the year of publication are shown after citation name; *Popul* indicates design features, *MO* morbid obesity. In the graph, *numbers* indicate OR, *filled squares* stand for the effect of individual studies, and *filled diamonds* express combined fixed and random effects. The symbol size is proportional to the number of individuals involved in each study

OSA was strongly associated with ALT and AST levels, showing that patients with OSA had an increase of 13.3 and 4.4 %, respectively, in comparison with individuals without OSA. This conclusion results from a total of 1,072 individuals recruited from nine homogeneous studies from different ethnic groups that included patients with well-characterized OSA. Meta-regression analysis showed that this association seems to be independent of obesity and T2D.

Surprisingly, this meta-analysis showed that OSA was associated with fatty liver but not with lobular inflammation. Nevertheless, these data should be interpreted with caution because several factors may explain this observation; the most important could be related to a lack of statistical power in the sample evaluated. Conversely, patients with OSA had a 2.6-fold greater risk of liver fibrosis when they had fatty liver, and in fact they had a similar risk for having fatty liver.

Unfortunately, the lack of prospective studies about NAFLD severity and OSA in patients other than those with morbid obesity precludes generalization of these findings and emphasizes the need to explore in more detail the progression of histopathological features in patients with and without OSA.

An interesting and perhaps surprising result is that our meta-analysis was unable to demonstrate a link between obesity and elevated liver enzymes in subjects with OSA. Nevertheless, the lack of association with BMI could have been due to bias because the majority of the subjects were obese and most of them had undergone bariatric surgery.

Whether the effect of OSA on the level of liver enzymes is subject to sexual dimorphism remains an open question, although our results suggest that men are more prone to suffer from it.

The strength of this study was that it is believed to be the first to provide evidence-based data that could result in the formulation of management guidelines for OSA and NAFLD in relation to screening strategies and medical advice. In fact, OSA is associated with a 2.5-fold increased risk of having fatty liver. Furthermore, the current meta-analysis is useful for clearly understanding the magnitude of the effect of the association between OSA and liver enzymes. An additional and attractive conclusion is the putative role of OSA on NAFLD severity because patients with OSA showed more aggressive disease with higher liver fibrosis scores.

Nevertheless, it should be noted that the number of patients with available histology was significantly smaller than the whole sample, but this represents a more homogeneous sample because the pediatric population was not included. Hence, definitive conclusions need further, larger studies, with further characterization to control for potential confounding factors in a controlled fashion.

One criticism concerns the observation that few studies presented data about fatty liver or disease severity evaluated by liver biopsy. Nevertheless, limited data about these issues is reasonable because liver biopsy is an invasive and costly procedure, and patients with OSA do not have a formal recommendation for evaluation of liver disease. Thus, less invasive procedures are urgently needed to evaluate NASH in OSA patients. Moreover, the inclusion in this meta-analysis of a study that was carried out in a large pediatric population could be regarded as a potential limitation. Nevertheless, it is worth mentioning that the Q and I^2 statistics did not show evidence of heterogeneity.

Chronic intermittent hypoxia has been implicated in the pathogenesis of NAFLD, but most of the experimental evidence is based on experimental studies and in vitro models [14]. Data from human and animal studies have shown that hypoxia-related pathways have an important role in the molecular events activated by liver steatosis [7, 26]. The question of whether elevated liver enzymes in patients with OSA is a surrogate marker of liver damage or an indicator of an altered hepatic metabolism remains unanswered, and translational studies are warranted to identify the causal mechanisms.

One may speculate that repetitive cycles of hepatic hypoxia/reoxygenation and catecholamine-mediated systemic metabolic changes switch the metabolic profile of the liver towards, for instance, hypoxic mitochondrial respiration, and this may explain the mitochondrial alterations observed in liver biopsies from NAFLD patients [41]. Likewise, it is also suggested that OSA is a trigger for inflammation that might explain the increase in liver enzymes [42].

In addition, the transcriptional program triggered in response to hypoxia may also explain the increased fibrosis scores observed in OSA patients. In fact, in previous studies, it has been shown that hypoxia acts as a major stimulus of angiogenesis and fibrogenesis, particularly by the activation of hypoxia-inducible factor-1α and vascular endothelial growth factor signaling pathways [43], and by inducing migration of activated hepatic stellate cells [44]. In this regard, some other plausible molecular candidates might be the profibrogenic agents angiotensin-I-converting enzyme and transforming growth factor β1, which have recently been shown to be overexpressed in NASH patients [7].

OSA is associated with increased levels of liver enzymes and seems to be a risk factor for progressive liver disease, at least in patients with morbid obesity, who were overrepresented in the present study. The assessment of fatty liver disease by noninvasive methods in patients with OSA is advised.

The impact of OSA on NAFLD progression and disease severity remains to be addressed in long-term follow-up studies. These studies should be sufficiently powered and include both sexes, and should specifically define histological features of NASH, such as hepatocellular ballooning, as a primary endpoint. In the end, clinical intervention remains to be proven in high quality studies exploring patients other than those with morbid obesity. Finally, randomized, case–control, interventional studies are needed to implement therapeutic recommendations because the effect of continuous positive airway pressure on liver enzymes and liver-related outcomes is still controversial [34, 35, 45].

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Conflict of Interest The authors declare no conflicts of interest.

Appendix

Search methods and process of study selection.

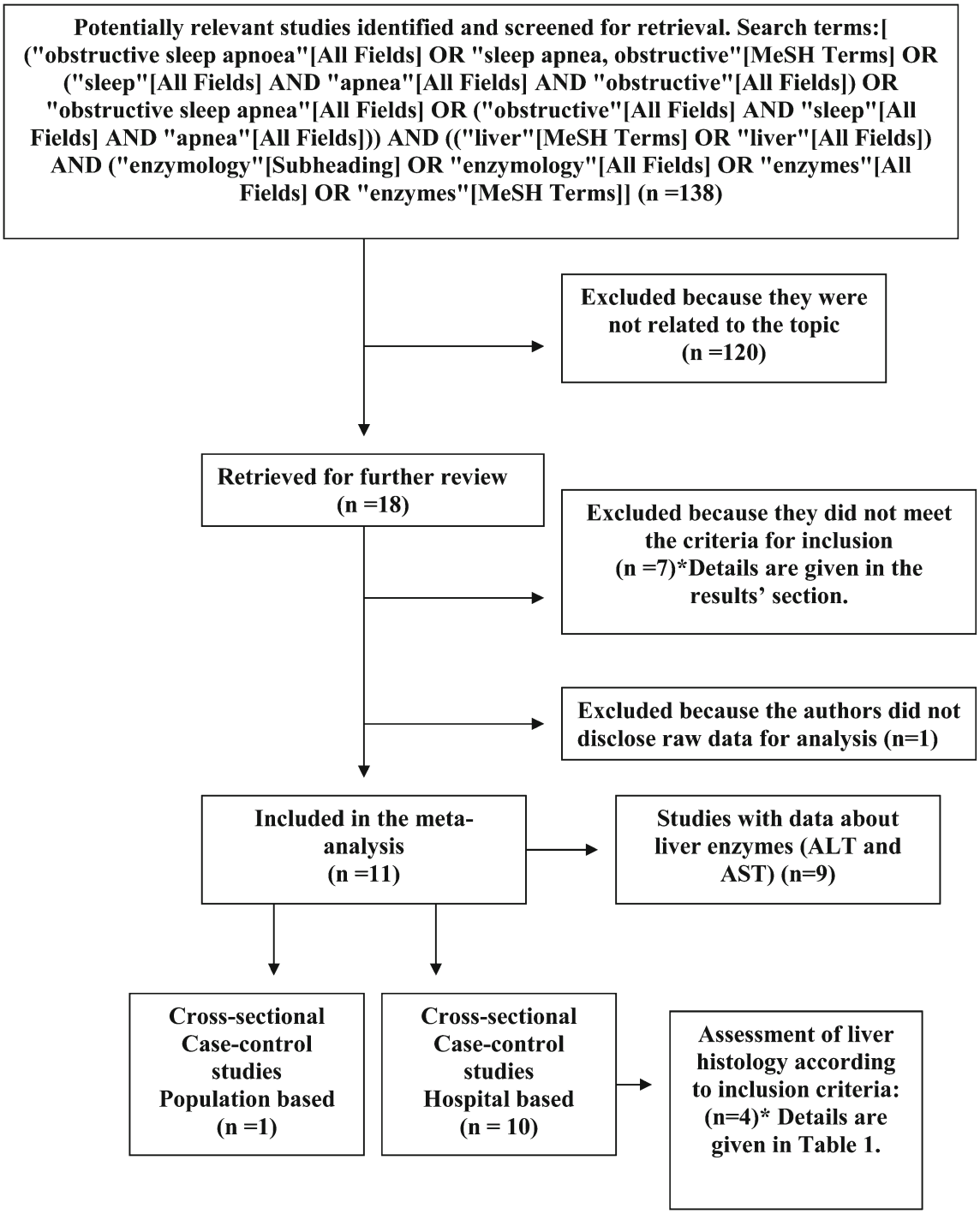

Fig. 6 MOOSE guidelines for meta-analyses and systematic reviews of observational studies

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