Effects of apolipoprotein E genotype on serum lipids in obstructive sleep apnoea

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ABSTRACT There is increasing evidence that intermittent hypoxia resulting from obstructive sleep apnoea (OSA) is independently associated with dyslipidaemia. Currently, no data exist on potential links between OSA-related dyslipidaemia and susceptibility genes for dyslipidaemia in such patients. Our aim was to study the effects of the apolipoprotein E (*APOE*) genotype and sleep apnoea severity on atherogenic dyslipidaemia in patients with OSA.

519 clinically stable subjects prospectively recruited at a tertiary referral teaching hospital underwent full polysomnography. *APOE* gene polymorphisms were assessed using real-time PCR.

In all *APOE* genotype groups, serum triglycerides increased while high-density lipoprotein (HDL) cholesterol was reduced with increasing severity of OSA in each *APOE* genotype group, whereas the deleterious effects of OSA on serum apolipoprotein (Apo)B levels were observed in ε2 carriers and the ε3/ε3 genotype only. Nevertheless, the ε4 allele carriers had ApoB levels within the risk range, irrespective of nocturnal hypoxia. In addition, among patients with the high-risk ε4 genotype, those with the most severe nocturnal hypoxia had significantly higher triglyceride and lower HDL cholesterol levels compared with nonhypoxic ε4 subjects. *APOE* genotype and the oxygen desaturation index were both independent predictors of serum triglyceride levels ($p=0.009$ and $p<0.001$, respectively; $R^2=0.148$) and ApoB levels ($p=0.001$ and $p=0.003$, respectively; $R^2=0.104$).

Our findings suggest that OSA has adverse effects on several lipid parameters over and above the effects carried by *APOE* genotype. Further st1udies are needed to analyse the effects of high-risk genotypes on metabolic and cardiovascular outcomes in patients with OSA.

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OSA has adverse effects on several lipid parameters in addition to the effects carried by the *APOE* genotype http://ow.ly/sKOUW

Received: June 11 2013 | Accepted after revision: Oct 24 2013 | First published online: Nov 14 2013

Support statement: This work was supported by the Slovak Research and Development Agency under contract No. APVV-0134-11, VEGA 1/0112/11 and VEGA 1/0111/12 of the Ministry of Education, Slovakia.

Conflict of interest: None declared.

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Introduction

Atherosclerosis is one of the main causes of morbidity and mortality in patients with obstructive sleep apnoea (OSA) [1–3]. Atherogenic dyslipidaemia, characterised by an increase in plasma triglycerides, reduction in high-density lipoprotein (HDL) cholesterol and a predominance of small, dense, low-density lipoprotein (LDL) particles, is common in patients with OSA [4, 5]. Evidence from a murine model of OSA has demonstrated that chronic intermittent hypoxia induces upregulation of genes involved in lipid biosynthesis in the liver [6] and leads to decreases in plasma triglyceride clearance [7]. Therefore, the increased cardiovascular risk carried by OSA has been attributed, at least in part, to lipid abnormalities induced by intermittent hypoxia.

Genetic and environmental factors play an important role in the aetiology of atherosclerosis [8]. Although significant evidence links OSA to proatherogenic factors, such as the metabolic syndrome [9–12], systemic inflammation [13–15] and oxidative stress [16, 17], little is known about the role of genetic factors for increasing atherosclerotic risk in OSA. In particular, no data are available on potential links between OSA-related dyslipidaemia and susceptibility genes for dyslipidaemia in such patients.

Apolipoprotein E (*APOE*) is probably the most studied susceptibility gene for atherosclerosis and dyslipidaemia [18, 19]. There are three common alleles of the *APOE* gene (ε2, ε3 and ε4). Individuals carrying the ε4 allele have higher total and LDL cholesterol levels than individuals with the commonest ε3ε3 genotype and those carrying the ε2 allele have lower levels of total and LDL cholesterol [19–21]. In addition, associations between *APOE* genotype and triglycerides [18] and circulating apolipoprotein (Apo)E and ApoB concentrations have been reported in the general population [18, 22, 23]. Taken together, ε4 allele carriers represent the risky *APOE* genotype [19–23]. Nevertheless, the role of *APOE* gene polymorphisms in lipid metabolism disturbances in OSA, a condition known to be associated with increased cardiovascular risk, is unclear. *APOE* genotype is related to circulating ApoE levels in humans, with the lowest ApoE levels observed in ε4 allele carriers [18]. This finding, coupled with observations of increased serum lipids and profound atherosclerotic plaque growth in ApoE-deficient mice exposed to intermittent hypoxia [24], suggests that *APOE* genotype and hypoxia may both play a distinct role in OSA-related increased cardiovascular risk. Therefore, the purpose of our study was to investigate the effects of *APOE* genotype on atherogenic dyslipidaemia in patients with OSA.

Subjects and methods

Subjects

Clinically stable subjects without OSA (fewer than five obstructive apnoeas or hypopnoeas per hour of sleep), and patients with OSA (five or more obstructive apnoeas or hypopnoeas per hour of sleep and excessive daytime sleepiness) referred to the sleep unit at a tertiary referral teaching hospital (Dept of Respiratory Medicine, Faculty of Medicine, P.J. Šafárik University and L. Pasteur University Hospital, Košice, Slovakia) for a diagnostic sleep study were prospectively recruited between 2006 and 2012. Exclusion criteria were as follows. 1) Chronic respiratory diseases other than OSA, such as bronchial asthma, chronic obstructive pulmonary disease, restrictive pulmonary disorders or hypoventilation syndrome; 2) known hereditary metabolic disorders; 3) type 1 diabetes; 4) hypothyroidism; or 5) regular use of sedatives, antidepressant or antipsychotic medication or alcohol. The study was approved by the L. Pasteur University Hospital ethics committee, and all subjects provided written informed consent.

Sleep assessment

All participants underwent full attended diagnostic overnight polysomnography (Alice 4; Respironics Inc., Murrysville, PA, USA), comprising continuous recording of electroencephalography (EEG), electro-oculography, electromyography, ECG, thoracic and abdominal impedance belts, thermistor for nasal and oral airflow, pulse oximetry and microphone for snoring. All records were scored manually following the American Academy for Sleep Medicine (AASM) 2007 guidelines [25]. Apnoea was identified as a drop in airflow of >90% from the baseline excursion for ⩾10 s; hypopnoea was defined as a reduction in airflow of ⩾50% of baseline for ⩾10 s accompanied either by a decrease in haemoglobin saturation for ⩾3%, an EEG-recorded arousal, or both. The apnoea/hypopnoea index (AHI) was defined as the number of apnoea and hypopnoea events per hour of sleep. Oxygen desaturation index (ODI) was defined as the number of oxygen desaturations of haemoglobin of ⩾3% per hour of sleep. In addition, the length of time with an arterial oxygen saturation measured by pulse oximetry (Sp_{O_2}) <90% was used to assess the degree of nocturnal hypoxia. The classification of OSA severity was based on the AASM guidelines as follows [25]. Mild: AHI ⩾5 and <15 episodes·h^{-1}; moderate: AHI ⩾15 and <30 episodes·h^{-1}; and severe: AHI ⩾30 episodes·h^{-1}.

DOI: 10.1183/09031936.00098513

Biochemical measurements

A peripheral venous blood sample was drawn into EDTA tubes between 06:00 h and 07:00 h, after polysomnography and after 12–14 h of overnight fasting, and stored at -20°C until analysis was undertaken. Fasting cholesterol, triglycerides, HDL cholesterol, fibrinogen, ApoA-1 and ApoB were measured by routine enzymatic methods. LDL cholesterol was calculated using the Friedewald equation.

Genetic analyses

DNA was extracted from peripheral blood leukocytes and amplified using real-time PCR using an air thermocycler (LightScanner32; Biofire Diagnostics Inc., Salt Lake City, UT, USA). To determine *APOE* polymorphisms, the Light-Mix Kit *ApoE C112R R158C* (TIB Molbiol, Berlin, Germany) was used. *APOE* genotypes were pooled in agreement with the method described in the recent meta-analysis [19] into three groups: ε3 homozygous group (ε3ε3 reference group, n=328), ε2 allele carriers (consisting of ε2ε2, ε2ε3 and ε2ε4 genotypes, n=62) and ε4 allele carriers (consisting of ε3ε4 and ε4ε4 genotypes, n=129).

Statistical analysis

Power calculations were performed based on published data on the effects of *APOE* genotype on serum triglyceride levels in the general population [16] and on the expected variability of triglycerides in OSA as observed by our group [12]. For a standard deviation of 0.72, a power calculation indicated that we would need a total cohort of $\geqslant$247 patients to detect a difference of 0.34 mmol·L^{-1} with a power of 80% at a 0.05 significance level.

The data are presented as mean±SD for all variables that were normally distributed, and as median (interquartile range) for variables that were not normally distributed. Differences between the groups were analysed using ANOVA for normally distributed variables and ANOVA on ranks for nonparametric variables. Discrete data were analysed using the Chi-squared test. Because the distributions of HDL cholesterol and triglycerides were skewed we log-transformed the variables for use in regression models. Least-squares linear regression analysis was used to assess the unadjusted relationships between parameters of OSA severity and serum lipid levels. For those lipid parameters that concurrently correlated with OSA severity and *APOE* genotype in univariate analyses we further assessed lipid levels in subgroups of subjects

TABLE 1 Basic demographic characteristics and polysomnographic findings of the study subjects

	No OSA	Mild OSA	Moderate OSA	Severe OSA	p-value
Subjects n	128	126	66	199	
Sex					
Male	68 (53)	89 (71)	45 (68)	167 (84)	<0.001
Female	60 (47)	37 (29)	21 (32)	32 (16)	
Age years	47.8±12.1	49.5±12.0	51.6±11.0	51.2±10.6	0.035
BMI kg·m^{-2}	28.2±4.3	29.6±4.6	31.1±4.9	33.9±6.0	<0.001
Current smoker	44 (34)	44 (35)	20 (30)	91 (46)	0.051
Arterial hypertension	58 (45)	53 (42)	37 (56)	124 (62)	0.001
Type 2 diabetes	4 (3)	8 (6)	7 (11)	35 (18)	<0.001
Statin use	12 (9)	16 (13)	16 (24)	40 (20)	0.011
Fibrate use	3 (2)	3 (2)	1 (2)	14 (7)	0.056
NREM min	349.4±41.7	365.1±62.5	361.0±47.7	377.1±66.3	0.001
S1 NREM min	51.3±47.6	53.6±39.4	45.2±37.0	62.4±69.1	0.089
S2 NREM min	244.1±69.8	255.6±60.1	259.0±54.8	281.1±86.5	<0.001
SWS min	54.1±34.0	55.8±34.9	56.7±34.4	33.6±30.4	<0.001
REM min	66.5±37.2	68.4±32.0	71.7±36.7	57.2±38.8	0.007
AHI events·h^{-1}	2.3±1.4	9.4±2.9	20.8±4.1	60.4±21.5	<0.001
ODI events·h^{-1}	2.4±4.4	6.1±5.2	14.1±10.3	47.0±25.9	<0.001
Arousal index events·h^{-1}	18.4±14.3	22.3±11.5	26.1±12.0	54.6±21.7	<0.001
S_{pO_2} <90% min	5.5±18.1	9.9±38.8	27.9±76.1	92.8±105.1	<0.001
Lowest S_{pO_2} %	89.3±5.1	85.9±5.9	80.0±10.5	68.2±17.9	<0.001
***APOE* ε2ε2 + ε2ε3 + ε2ε4 genotype**	19 (15)	17 (14)	7 (11)	19 (10)	0.256
***APOE* ε3ε3 genotype**	71 (55)	80 (63)	46 (69)	131 (66)	0.169
***APOE* ε3ε4 + ε4ε4 genotype**	38 (30)	29 (23)	13 (20)	49 (24)	0.328

Data are presented as n (%) or mean±SD, unless otherwise stated. OSA: obstructive sleep apnoea; BMI: body mass index; NREM: non-rapid eye movement (REM); S1: stage 1; S2: stage 2; SWS: slow wave sleep; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; S_{pO_2}: arterial oxygen saturation measured by pulse oximetry; *APOE*: apolipoprotein E.

divided according to quartiles (Q) of ODI or S_{pO_2} <90% within each genotype group. In multivariate analysis, multiple linear regression models were used, with lipid levels as dependent variables and age, sex, ODI or AHI, *APOE* genotype, body mass index (BMI), smoking, diabetes and statin use as independent variables. Analyses were conducted using SPSS for Windows software (version 14.0; IBM, Chicago, IL, USA). $p<0.05$ was considered statistically significant.

Results

Characteristics of the subjects

519 subjects participated in the study; 128 had no OSA, 126 suffered from mild OSA, 66 from moderate OSA and 199 from severe OSA. Basic demographic characteristics and polysomnographic findings in the study group are summarised in table 1. Male sex, higher age, higher BMI, arterial hypertension, type 2 diabetes and statin use were all associated with greater severity of OSA. Importantly, no differences in *APOE* genotype distribution were observed between patients grouped by OSA severity.

The effects of OSA on serum lipid levels

Serum HDL cholesterol levels were decreased, while triglyceride and ApoB levels were increased in those patients with mild, moderate and severe OSA compared with subjects without OSA ($p=0.020$, $p<0.001$ and $p=0.030$, respectively) (table 2). There was no observable effect of OSA severity on total or LDL cholesterol or ApoA-1. Importantly, adjustments for BMI or full adjustments (*i.e.* for sex, age, BMI, smoking, diabetes and statin use) had no effect on the observed differences in serum lipids between patients grouped by OSA severity.

HDL cholesterol levels were inversely correlated with AHI, ODI and arousal index ($p<0.001$ for all). In contrast, AHI, ODI and arousal index were directly correlated with triglyceride ($p<0.001$ for all) and ApoB levels ($p<0.001$, $p=0.001$ and $p=0.002$, respectively) (table 3).

The effects of APOE genotype on serum lipid levels

The effects of *APOE* genotype on serum lipids are displayed in table 4. There were significant differences between the ε2 allele carriers and the ε3ε3 homozygous group, as well as the ε4 allele carriers with respect to

TABLE 2 Plasma lipid levels in subjects grouped by severity of obstructive sleep apnoea (OSA)

	No OSA	Mild OSA	Moderate OSA	Severe OSA	p-value
Subjects n	128	126	66	199	
Cholesterol mmol·L^{-1}					
Unadjusted	4.97±0.88	4.82±0.86	5.05±1.25	5.06±1.00	0.183
Adjusted for BMI	4.94±0.84	4.84±0.76	5.00±1.14	5.09±0.95	0.183
Fully adjusted#	4.96±0.88	4.84±0.83	5.01±1.22	5.07±0.98	0.233
LDL cholesterol mmol·L^{-1}					
Unadjusted	2.99±0.77	2.90±0.75	2.98±0.98	2.98±0.90	0.853
Adjusted for BMI	2.99±0.75	2.90±0.65	2.97±0.89	3.00±0.85	0.713
Fully adjusted#	2.99±0.80	2.91±0.71	2.96±0.95	2.97±0.89	0.898
HDL cholesterol mmol·L^{-1}					
Unadjusted	1.11 (0.95–1.40)	1.10 (0.95–1.35)	1.13 (0.97–1.39)	1.04 (0.89–1.21)	0.004
Adjusted for BMI	1.13 (1.00–1.40)	1.10 (0.96–1.34)	1.13 (0.99–1.36)	1.05 (0.94–1.24)*	0.007
Fully adjusted#	1.14 (0.95–1.40)	1.09 (0.94–1.35)	1.12 (1.00–1.36)	1.04 (0.92–1.23)*	0.009
Triglycerides mmol·L^{-1}					
Unadjusted	1.42 (10.5–1.95)	1.42 (1.05–2.02)	1.50 (1.11–2.31)	1.90 (1.35–2.67)	<0.001
Adjusted for BMI	1.43 (1.07–1.91)	1.56 (1.14–2.15)	1.62 (1.32–2.30)	1.98 (1.44–2.67)*	<0.001
Fully adjusted#	1.43 (1.04–1.94)	1.44 (1.01–2.17)	1.64 (1.05–2.35)	1.94 (1.40–2.73)*	<0.001
ApoA-1 g·L^{-1}					
Unadjusted	1.36±0.27	1.34±0.25	1.36±0.22	1.33±0.27	0.785
Adjusted for BMI	1.36±0.26	1.34±0.24	1.36±0.22	1.34±0.25	0.875
Fully adjusted#	1.37±0.27	1.34±0.26	1.34±0.22	1.33±0.27	0.682
ApoB g·L^{-1}					
Unadjusted	0.88±0.21	0.83±0.20	0.86±0.26	0.93±0.22	0.030
Adjusted for BMI	0.86±0.20	0.85±0.17	0.88±0.23	0.94±0.21*	0.003
Fully adjusted#	0.88±0.22	0.85±0.18	0.89±0.25	0.94±0.22*	0.012

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein. #: adjusted for sex, age, BMI, smoking, diabetes and statin use. *: $p<0.05$ compared to no OSA.

DOI: 10.1183/09031936.00098513

TABLE 3 Correlations between plasma lipid levels and markers of obstructive sleep apnoea severity

	AHI events·h^{-1}		ODI events·h^{-1}		S_{pO_2} <90% min		Arousal index events·h^{-1}	
	R	p-value	R	p-value	R	p-value	R	p-value
Cholesterol mmol·L^{-1}	0.074	0.097	0.041	0.356	0.030	0.499	0.055	0.216
LDL cholesterol mmol·L^{-1}	0.002	0.966	0.023	0.607	0.005	0.909	0.038	0.400
HDL cholesterol mmol·L^{-1}	-0.222	<0.001	-0.224	<0.001	0.163	<0.001	-0.190	<0.001
Triglycerides mmol·L^{-1}	0.292	<0.001	0.270	<0.001	0.158	<0.001	0.277	<0.001
ApoA-1 g·L^{-1}	0.072	0.131	0.096	0.045	0.062	0.195	0.028	0.560
ApoB g·L^{-1}	0.180	<0.001	0.160	0.001	0.117	0.014	0.145	0.002

AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; S_{pO_2}: arterial oxygen saturation measured by pulse oximetry; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein.

LDL cholesterol, triglycerides, ApoA-1 and ApoB levels, and these differences remained significant after adjustments for BMI or after adjustments for all covariates. *Post hoc* analyses revealed that compared with the ε3ε3 (reference) group, ε4 allele carriers had significantly higher triglyceride and ApoB levels ($p<0.05$ for both). *APOE* genotype had no effect on total or HDL cholesterol levels. The exclusion of subjects on hypolipidaemic therapy (n=99) or ε2ε4 heterozygous subjects (n=6) from the analyses made no difference to any of these findings (data not shown). Importantly, sex distribution, age, BMI, AHI and ODI were similar in the ε2 carriers, ε3ε3 homozygous group and as the ε4 allele carriers ($p=0.944$, $p=0.733$, $p=0.352$, $p=0.299$ and $p=0.457$, respectively).

Relationships between sleep apnoea severity and serum lipid levels across APOE genotype groups

Across all *APOE* genotype groups, there were significant increases in serum triglyceride levels with increased severity of nocturnal hypoxia, in association with reductions in serum HDL cholesterol (table 5). In contrast, serum ApoB levels increased from Q1 to Q4 ODI in ε2 carriers and the ε3ε3 homozygous group, but not in ε4 carriers. *Post hoc* analyses revealed that among the risky ε4 allele carriers those patients with Q4 ODI had significantly higher triglyceride levels and significantly lower HDL cholesterol levels compared with subjects with Q1 ODI. Similarly to the entire cohort, severity of OSA was not related to total or LDL cholesterol or to ApoA-1 levels in patients grouped by *APOE* genotype. To strengthen these analyses further, we performed analogical tests after dividing patients grouped by quartiles of AHI or time of S_{pO_2} <90% within the respective genotype groups. Importantly, discrimination of sleep apnoea severity by AHI made no material difference to the observed effects of OSA on serum lipids within the respective genotype groups. Relationships between serum HDL cholesterol, triglycerides or ApoB and S_{pO_2} <90% in univariate analyses were weaker compared with those with AHI or ODI (table 3); consequently, dividing the cohort by S_{pO_2} <90% weakened the observed differences between OSA severity and serum lipids (data not shown).

Both *APOE* genotype and ODI, in addition to sex and BMI, independently predicted serum triglyceride levels in multivariate stepwise regression analysis ($p=0.009$, $p<0.001$, $p=0.001$ and $p=0.010$, respectively; $R^2=0.148$). In addition, *APOE* genotype and ODI were both independent predictors of serum ApoB levels ($p=0.001$ and $p=0.003$, respectively; $R^2=0.104$). To strengthen these analyses we further analysed independent effects of *APOE* genotype and OSA severity on lipid parameters in analogical models with AHI as an independent variable. Indeed, both *APOE* genotype and AHI independently predicted serum triglycerides ($p=0.011$ and $p<0.001$, respectively; $R^2=0.120$). ApoB was independently predicted by AHI, while *APOE* genotype approached but did not reach statistical significance in this model ($p=0.001$ and $p=0.061$, respectively; $R^2=0.031$). We also analysed an independent effect of an interaction term (*APOE* genotype*ODI) on serum triglyceride and ApoB levels. Nevertheless, this interaction term did not reach statistical significance in either of these models. Serum HDL cholesterol was independently predicted by ODI ($p=0.008$), but not by *APOE* genotype. No significant predictor of LDL cholesterol was identified in multivariate models.

Discussion

This observational study has demonstrated that serum triglycerides increased while HDL cholesterol was reduced with increasing severity of OSA in each *APOE* genotype group, whereas the deleterious effects of OSA on serum ApoB levels were observed in ε2 allele carriers and the ε3ε3 genotype only. Nevertheless, the ε4 allele carriers had ApoB levels within the high risk range irrespective of nocturnal hypoxia. In addition, among patients with the high-risk ε4 genotype those with the most severe nocturnal hypoxia had

TABLE 4 Plasma lipid levels in subjects grouped by apolipoprotein E (*APOE*) genotype

	APOE genotype			p-value
	ε2ε2 + ε2ε3 + ε2ε4	ε3ε3	ε3ε4 + ε4ε4	
Subjects n	62	328	129	
Cholesterol mmol·L^{-1}				
Unadjusted	4.73±0.91	4.98±0.97	5.07±1.02	0.164
Adjusted for BMI	4.78±0.98	4.98±0.99	5.08±0.98	0.126
Fully adjusted#	4.75±0.97	4.97±0.98	5.10±0.97	0.097
LDL cholesterol mmol·L^{-1}				
Unadjusted	2.67±0.75*	2.98±0.82	3.05±0.92	0.024
Adjusted for BMI	2.69±0.84*	2.98±0.84	3.06±0.84¶	0.025
Fully adjusted#	2.65±0.83*	2.98±0.82	3.06±0.82¶	0.017
HDL cholesterol mmol·L^{-1}				
Unadjusted	1.08 (0.97–1.35)	1.10 (0.95–1.32)	1.05 (0.87–1.30)	0.159
Adjusted for BMI	1.05 (0.99–1.40)	1.10 (0.94–1.32)	1.05 (0.88–1.29)	0.180
Fully adjusted#	1.06 (0.97–1.39)	1.10 (0.94–1.33)	1.07 (0.89–1.33)	0.489
Triglycerides mmol·L^{-1}				
Unadjusted	1.88 (1.29–2.66)	1.50 (1.12–2.20)	1.73 (1.21–2.58)*	0.023
Adjusted for BMI	1.88 (1.41–2.66)	1.60 (1.21–2.26)	1.71 (1.24–2.60)	0.239
Fully adjusted#	1.88 (1.37–2.65)	1.58 (1.13–2.31)	1.73 (1.24–2.59)*	0.045
ApoA-1 g·L^{-1}				
Unadjusted	1.37±0.27	1.36±0.26	1.29±0.24*,¶	0.028
Adjusted for BMI	1.38±0.26	1.36±0.25	1.29±0.26*	0.054
Fully adjusted#	1.39±0.25	1.36±0.25	1.29±0.25*,¶	0.039
ApoB g·L^{-1}				
Unadjusted	0.81±0.19¶	0.88±0.22	0.94±0.23*,¶	0.003
Adjusted for BMI	0.81±0.21¶	0.89±0.22	0.94±0.22¶	0.001
Fully adjusted#	0.81±0.21¶	0.89±0.21	0.95±0.21*,¶	<0.001

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein. #: adjusted for sex, age, BMI, smoking, diabetes and statin use. *: $p<0.05$ compared with ε3 carriers; ¶: $p<0.05$ compared with ε2 carriers.

significantly higher triglyceride and lower HDL cholesterol levels compared with nonhypoxic ε4 subjects. Previously, potential links have been observed between *APOE* genotype and the risk of OSA in some, but not all, studies [26, 27]. In addition, some observations suggested a significant effect of *APOE* genotype on clinical outcomes in terms of cognitive impairment [28] and memory performance in OSA [29], without examining the effects on serum lipids. To our knowledge, our data are the first to examine lipid status within the various *APOE* genotype groups in patients with OSA, and to demonstrate that sleep apnoea has adverse effects on several lipid parameters over and above the effects carried by the *APOE* genotype.

ApoE is a multifunctional protein that is a component of several plasma lipoprotein–lipid particles and, as such, plays a key role in the metabolism of cholesterol and triglycerides [30]. In the population at large, the ε2 and the ε4 alleles have been linked to high triglyceride levels [19–21, 31]. The LDL receptor recognises ApoE and ApoB-100 and plays a crucial role in cholesterol homeostasis [32], whereas the structurally related very LDL receptor recognises ApoE, but not ApoB-100, and plays an important role in triglyceride metabolism [33]. In the present study, serum triglycerides were affected by both the severity of sleep disordered breathing and *APOE* genotype and, importantly, these differences remained significant after adjustments for BMI and other covariates. Similarly to reports in large population-based cohorts [19], the association of *APOE* genotype with triglycerides was nonlinear, with the highest levels among ε2 and ε4 allele carriers and the lowest levels in the common ε3ε3 reference group. The observation of significant independent effects of both OSA and *APOE* gene polymorphisms on triglycerides in the entire cohort led to an intriguing question: does hypoxia relate to lipid parameters irrespective of the underlying *APOE* genotype? Indeed, triglyceride levels increased with a rise in OSA severity in each *APOE* genotype group. Of interest, among the risky ε4 allele carriers, the difference in median triglyceride levels between the nonhypoxic and the most severely hypoxic subjects (Q1 *versus* Q4 ODI) was 1.02 mmol·L^{-1}. Such an increase is clinically significant, while at the triglyceride level of 1.2 mmol·L^{-1} the vast majority of LDLs are

DOI: 10.1183/09031936.00098513

TABLE 5 Plasma lipid levels across quartiles of oxygen desaturation index (ODI) in ε2 carriers, the ε3ε3 homozygous group and ε4 carriers

	ODI quartile				p-value
	Q1	Q2	Q3	Q4	
Cholesterol mmol·L^{-1}					
ε2 carriers	4.42±0.81	5.23±0.91	4.79±0.77	4.66±0.50	0.078
ε3ε3 genotype	4.94±0.91	4.91±0.97	5.03±0.96	5.03±1.05	0.800
ε4 carriers	5.04±0.92	5.06±0.79	4.83±1.15	5.43±1.07	0.142
LDL cholesterol mmol·L^{-1}					
ε2 carriers	2.31±0.70	3.00±0.77	2.84±0.70	2.53±0.38	0.118
ε3ε3 genotype	2.98±0.69	2.94±0.82	3.01±0.83	2.96±0.94	0.957
ε4 carriers	3.13±0.91	3.08±0.67	2.87±0.94	3.19±1.04	0.542
HDL cholesterol mmol·L^{-1}					
ε2 carriers	1.39 (1.07–1.75)	1.01 (0.91–1.09)	1.023 (0.90–1.20)**	1.10 (0.98–1.17)**	0.017
ε3ε3 genotype	1.19 (1.05–1.45)	1.15 (0.97–1.34)	1.10 (0.95–1.30)	1.03 (0.90–1.20)**	0.002
ε4 carriers	1.16 (1.03–1.41)	0.98 (0.88–1.33)	1.06 (0.78–1.25)	0.96 (0.84–1.10)**	0.021
Triglycerides mmol·L^{-1}					
ε2 carriers	1.05 (0.73–1.62)	2.18 (1.87–2.94)**	1.60 (1.33–2.45)	2.24 (1.88–2.72)**	0.001
ε3ε3 genotype	1.26 (0.85–1.79)	1.43 (1.04–1.98)**	1.65 (1.30–2.26)**	1.75 (1.31–2.60)**	<0.001
ε4 carriers	1.25 (1.03–1.89)	1.61 (1.23–2.53)**	1.69 (1.32–2.47)**	2.27 (1.63–4.09)**	0.002
ApoA-1 g·L^{-1}					
ε2 carriers	1.56±0.34	1.32±0.30	1.26±0.14	1.37±0.23	0.046
ε3ε3 genotype	1.36±0.26	1.40±0.27	1.34±0.22	1.36±0.24	0.587
ε4 carriers	1.30±0.22	1.32±0.27	1.24±0.23	1.28±0.22	0.618
ApoB g·L^{-1}					
ε2 carriers	0.68±0.19	0.93±0.13**	0.81±0.17	0.84±0.17**	0.011
ε3ε3 genotype	0.85±0.20	0.85±0.23	0.90±0.19	0.94±0.05**	0.027
ε4 carriers	0.91±0.22	0.99±0.19	0.90±0.21	1.03±0.26	0.121

Data are presented as mean± SD or median (interquartile range), unless otherwise stated. LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein.**: p<0.01 compared to Q1 ODI.

less atherogenic large buoyant particles, at a triglyceride level >1.9 mmol·L^{-1} most of the LDL particles are small, dense proatherogenic LDLs [34].

In the present study, total cholesterol and LDL cholesterol were not increased in patients with OSA, in agreement with several previous reports [11, 35, 36]. In contrast, HDL cholesterol levels were inversely related to indices of OSA severity, and these relationships remained significant after adjustments for all relevant covariates. Our observations are in line with previous studies in cohorts with large sample sizes that have shown that OSA is associated with a decrease in HDL cholesterol [37, 38]. Importantly, adverse effects of sleep disordered breathing on HDL cholesterol were observed in each *APOE* genotype group, whereas *APOE* genotype was not linked to HDL cholesterol *per se*. The difference in median HDL cholesterol between the nonhypoxic and the most severely hypoxic subjects was ⩾0.2 mmol·L^{-1}, irrespective of *APOE* genotype, and patients in the Q3 and Q4 ODI groups had HDL cholesterol levels <1.1 mmol·L^{-1} in each genotype group. Of note, such levels are associated with increased cardiovascular risk, as suggested by the recent Prospective Cardiovascular Muenster (PROCAM) study, in which HDL cholesterol <1.15 mmol·L^{-1} was associated with a 2.6-fold increased risk of myocardial infarction [39].

Similarly to triglycerides, serum ApoB levels were independently predicted by both OSA severity and *APOE* genotype. ApoB reflects the number of all atherogenic triglyceride-rich and LDL particles, including the most atherogenic fraction of small, dense LDL particles, and thus represents an additional marker of increased cardiovascular risk [40]. Serum levels of ApoB >0.9 g·L^{-1} have previously been associated with an increased cardiovascular risk [34, 41]. Importantly, in nonhypoxic subjects within the ε2 and the ε3 genotypes the average ApoB was below this risky level, whereas patients with the most severe hypoxia had significantly higher mean ApoB levels, thus suggesting an adverse effect of OSA on ApoB in these genotype groups. Previous studies have demonstrated that in the general population, plasma ApoB concentrations are the highest in ε4 allele carriers [23], and our results parallel these findings. Of note, the lack of an additional deleterious effect of sleep disordered breathing on ApoB in the ε4 group may potentially represent a ceiling

effect of *APOE* genotype on this lipid parameter, as among the ε4 allele carriers the ApoB levels were above the risky 0.9 $g \cdot L^{-1}$ level in all OSA severity groups.

Studying a well-defined cohort of subjects who all underwent full polysomnography represents one of the main strengths of this study. In addition, the proportion of ε2, ε3 and ε4 allele carriers in the present cohort of patients with OSA (12%, 63% and 25%, respectively) is similar to that observed in large epidemiological cohorts in the general population [19, 42], thus reinforcing the value of our observations on the effects of *APOE* gene polymorphisms on lipid phenotype in OSA. There are also some limitations to this study. First, the cross-sectional nature of the study design does not prove causation for the relationships between OSA, *APOE* genotype and serum lipids. Secondly, individuals with suspected OSA referred to the sleep laboratory are a discrete group and results obtained may potentially not be generalisable to the general population. Thirdly, the primary incentive of our study was to analyse, in patients with OSA, differences in serum lipids between the respective *APOE* genotype groups, and the study was powered as such. Therefore, the lack of a significant effect of the interaction term *APOE* genotype*hypoxia on serum lipids should be considered inconclusive and hypothesis generating. Further investigations in large cohorts are needed to approach this question.

In conclusion, we have demonstrated that OSA had a negative impact on serum triglycerides and HDL cholesterol irrespective of *APOE* genotype, thus suggesting deleterious metabolic consequences of sleep disordered breathing over and above the genetic effects. Increases in ApoB levels with increasing severity of OSA were confined to ε2 allele carriers and the ε3ε3 genotype only. Nevertheless, the ε4 allele carriers had ApoB in the risk range irrespective of the presence of OSA. Further studies are needed to analyse the effects of risky genotypes on metabolic and cardiovascular outcomes in patients with OSA.

Acknowledgements

The authors wish to express their gratitude to A. Schejbalova and Z. Lazarova (Sleep Laboratory, Dept of Respiratory Medicine, Faculty of Medicine, P.J. Šafárik University, Košice, Slovakia and L. Pasteur University Hospital, Košice).

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