Obstructive sleep apnea and effects of continuous positive airway pressure on triglyceride-rich lipoprotein metabolism

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Abstract **This study aimed to explore lipoprotein metabolism in obstructive sleep apnea (OSA) and the effects of continuous positive airway pressure (CPAP). We studied 15 men with severe OSA [apnea-hypopnea index (AHI) ⩾30 events/hour] and 12 age-, BMI-, and waist circumference-matched volunteers without OSA (AHI <5 events/hour). Carotid intima-media thickness (CIMT) was determined by a blind examiner. After 12 h fasting, a triglyceride-rich chylomicron-like emulsion, labeled with [^{14}C]cholesteryl oleate and [^{3}H]triolein, was injected intravenously followed by blood sample collection at preestablished times. Fractional clearance rate (FCR) of the radiolabeled lipids was estimated by compartmental analysis of radioisotope decay curves. Compared with controls, patients with OSA showed a significant delay in both cholesteryl ester FCR (0.0126 ± 0.0187 vs. 0.0015 ± 0.0025 min^{-1}; P = 0.0313) and triglycerides FCR (0.0334 ± 0.0390 vs. 0.0051 ± 0.0074 min^{-1}; P = 0.0001). CIMT was higher in the OSA group: 620 ± 17 vs. 725 ± 29 μm; P = 0.004. Cholesteryl ester FCRs were inversely related to total sleep time <90% (r = −0.463; P = 0.029) and CIMT (r = −0.601; P = 0.022). The triglyceride FCR was inversely correlated with AHI (r = −0.537; P = 0.04). In a subgroup of patients treated with CPAP for 3 months (n = 7), triglyceride FCR increased 5-fold (P = 0.025), but the cholesteryl ester FCR was unchanged. In conclusion, severe OSA decreased lipolysis of triglyceride-rich lipoproteins and delayed removal of remnants. CPAP treatment may be effective to restore the lipolysis rates.**—Drager, L. F., T. M. Tavoni, V. M. Silva, R. D. Santos, R. P. Pedrosa, L. A. Bortolotto, C. G. Vinagre, V. Y. Polotsky, G. Lorenzi-Filho, and R. C. Maranhao. **Obstructive sleep apnea and effects of continuous positive airway pressure on triglyceride-rich lipoprotein metabolism.** ***J. Lipid Res.*** **2018.** 59: **1027–1033.**

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Obstructive sleep apnea (OSA) is a common clinical condition characterized by recurrent episodes of complete (apneas) or partial (hypopneas) obstructions of the upper airway, leading to increased negative intrathoracic pressure, sleep fragmentation, and chronic intermittent hypoxia (CIH) during sleep (1). There is growing evidence that OSA is independently associated with cardiovascular events, including myocardial infarction and stroke (2–5). Mechanisms by which OSA leads to cardiovascular complications are not completely understood. Metabolic dysfunction may be involved in the increased risk of CVD in patients with OSA (6). It is known that OSA increases insulin resistance and worsens the control of type 2 diabetes (7–9), but the effects of OSA on lipid metabolism are not well established (10, 11). We have previously utilized a mouse model and have shown that CIH, the hallmark of OSA, delayed the clearance of triglyceride-rich lipoproteins (TRLPs) by inhibiting lipoprotein lipase (LpL) in the

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Abbreviations: AHI, apnea-hypopnea index; CE, cholesteryl ester; CIH, chronic intermittent hypoxia; CIMT: carotid intima-media thickness; FCR, fractional clearance rate; LpL, lipoprotein lipase; OSA, obstructive sleep apnea; TRLP, triglyceride-rich lipoprotein.

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adipose tissue (12). LpL catalyzes the lipolysis of TRLP, liver-synthesized VLDLs, and chylomicrons produced in the intestine from dietary fats. In this model, LpL inactivation was induced by upregulation of a powerful LpL inhibitor, angiopoietin-like protein 4 (Angptl4) (13), via hypoxia inducible factor 1 (HIF-1). The HIF-1–mediated increase in adipose Angptl4 and the resulting inhibition of LpL contribute to progression of atherosclerosis in apoE KO mice exposed to CIH (13). The implications of these findings to TRLP metabolism in patients with OSA are unknown.

The hydrolysis of the TRLP triglycerides on the endothelial surface of capillaries is a crucial process for energy storage by the organism. After lipolysis, the triglyceride-depleted remnants are removed by the liver. Defects in lipolysis of TRLP and in remnant removal predispose to development of atherosclerotic CVD (14–17). Therefore, the aim of this study was to investigate whether the metabolism of TRLP is impaired in patients with OSA and whether disturbances in TRLP metabolism could be reversed by OSA treatment. Chylomicron-like emulsion labeled with radioactive triglycerides and cholesteryl esters (CEs) was used to assess the intravascular metabolism of TRLP in OSA patients and controls. The OSA indexes and the plasma clearance of the radioactive labels obtained by compartmental analysis were correlated with carotid intima/media thickness values, recognized markers of subclinical vascular disease and predictors of cardiovascular events (18). The following prespecified hypotheses are: *1*) OSA elicits defects in the major steps of TRLP metabolism, namely, decreased lipolysis and removal of remnants from the circulation; *2*) The lipolysis and removal of remnants from the circulation are inversely correlated with the hypoxemia burden during sleep; *3*) OSA treatment with continuous positive airway pressure (CPAP) is effective in restoring the lipolysis rates and improves the accumulation of remnants.

MATERIALS AND METHODS

During a 1-year period, we selected patients with severe OSA free of overt CVDs (including hypertension, diabetes mellitus, cerebrovascular, aortic, cardiac disease, or smoking habit as well as chronic use of medications) from the Sleep Laboratory, Heart Institute (InCor). Simultaneously, we invited healthy individuals from hospital staff after they completed a Berlin Questionnaire, indicating a low risk of OSA. Particular attention was paid to anthropometric characteristics to obtain two groups with a comparable age (±5 years), BMI (±3 kg/m^2), and waist circumference (±5 cm). Due to the lack of previous human data but based on previous experimental study (12), we speculated the required sample size based on the assumption that patients with OSA will present 50% of the observed fractional clearance rate (FCR) of CE and triglycerides in the matched controls. Using a significance level (α) of 5% and a power (1-β) of 80%, we therefore estimated that at least 11 participants per group needed to be enrolled. Hypertension was carefully excluded based on the average of two or more properly measured, seated blood pressure readings on at least two office visits, according to current guidelines, by experienced physicians not involved in the study. We also considered out-of-office measurements showing normal values of blood pressure. In addition, venous blood was collected for the measurement of fasting glucose and plasma lipids levels by standard laboratory methods. FFAs were measured by enzymatic methods, and plasma noradrenalin levels were determined by HPLC. The local ethics committee approved the protocol, and all participants gave written, informed consent.

Sleep study

All participants underwent a standard overnight polysomnography (EMBLA; Flaga hf. Medical Devices, Reykjavik, Iceland), including electroencephalography, electrooculography, electromyography, oximetry, thermistor, and pressure cannula measurements of airflow, and measurements of ribcage and abdominal movements during breathing. Apnea was defined as complete cessation of airflow for at least 10 s. Hypopnea was defined as a 30% reduction in respiratory signals for at least 10 s associated with oxygen desaturation of 3%. The apnea–hypopnea index (AHI) was calculated as the total number of respiratory events (apneas plus hypopneas) per hour of sleep. The AHI cutoff for control subjects and patients with severe OSA was less than 5 and more than 30 events per hour of sleep, respectively. Patients with OSA had been recently diagnosed and were naive to treatment.

Plasma kinetics of chylomicron-like emulsions

The chylomicron-like emulsion kinetic study was performed as previously described (19). Chylomicron-like emulsion mixture had triolein 76.5 ± 4.1%, free cholesterol 1.9±.3%, cholesteryloleate 11.2 ± 3.0%, and phosphatidylcholine 10.4 ± 1.3%; and size range from 80 to 110 nm. Lipid mixtures were emulsified by ultrasonic irradiation and purified by ultracentrifugation in density gradients. The ^{14}C-cholesteryl oleate (CE) and ^{3}H-triolein (TO; Amersham, UK) were added to the lipid mixtures for determination of plasma kinetics. The emulsion was sterilized by passage through a 0.2 μm filter. Study subjects reported to the laboratory by 8:00 AM after a 12-h overnight fast, and blood was collected for lipid analysis. The radiolabeled emulsion was then injected in a bolus (volume, 200–300 μl) containing 74 kBq (2 μCi) of the ^{14}C and 148 kBq (4 μCi) of the ^{3}H label into the antecubital vein. In order to obtain blood samples for radioactivity measurement, the antecubital vein of the contralateral arm was cannulated, and a slow infusion of heparin-free saline was started to maintain catheter patency. The total saline volume infused did not exceed 100 ml. Blood samples were collected at preestablished intervals of 2, 4, 6, 10, 15, 20, 30, 45, and 60 min after injection of the emulsion. After blood collection, plasma was separated by centrifugation, and an aliquot of 1 ml was transferred to counting vials containing 7 ml of the scintillation solution 2,5 difeniloxazole:1,4 bis(-5 phenyl-2-oxazoly)benzene:Triton X-100/toluene (5 g:0.5 g:333 ml/667 ml). Radioactivity in the samples was determined using a Packard 1660 TR spectrometer (Packard, Meridien). The estimation of the in vivo time course of the catabolism of the chylomicron-like emulsion was evaluated by compartmental analysis. The clearance from plasma of CEs reflects tissue uptake from plasma of remnant particles in various degrees of delipidation. The FCR in min^{-1} were calculated using the ANACOMP software as previously described (19). Conformity of the radioactivity dose administered to patients with the international regulations was demonstrated in our previous study (20).

Carotid-intima media thickness

All participants had their vascular properties evaluated within 2 weeks after polysomnography. Carotid-intima media thickness (CIMT) was made by an experienced observer (L.A.B.) blinded to the clinical condition of each participant. All measurements were performed with the patient in a recumbent position while awake. CIMTs (in micrometers) were evaluated with a high-resolution echo-tracking system (Wall Track System, Medical Systems

Arnhem, Oosterbeck, The Netherlands) coupled with a conventional two-dimensional vascular echograph (Sigma 44, Kontrom Instruments, Watford, UK) equipped with a 7.5 MHz probe. As previously described (21), measurements were performed on the right common carotid arteries 1 cm below the bifurcation at the site of the distal wall. CIMT was measured at the thickest point, not including plaques, on the near and far walls with a specially designed computer program. A high rate of CIMT reproduction has been previously demonstrated (22). Plaque was defined as a localized thickening greater than 1.2 mm that did not uniformly involve the whole artery.

Statistical analysis

Data were analyzed with SPSS 10.0 statistical software (SPSS, Inc., Chicago, IL). Quantitative variables were expressed as the mean ± SD. After checking normality with the Kolmogorov–Smirnov test, unpaired Student *t*-test was used to compare means. Pearson correlation coefficients between polysomnographic, lipid, and CIMT data were obtained. Multiple regression analysis was used to identify variables that were independently associated with the vascular parameters and to adjust for possible confounding factors, including fasting triglycerides (that may influence TRLP metabolism) (23). Categorical variables were expressed by the frequency distribution, and their association was tested with likelihood ratio tests. A value of $P < 0.05$ was considered significant.

RESULTS

We screened 120 consecutive patients who were diagnosed with severe OSA in our Sleep Laboratory over a 1 year period. Twenty-five of them were found to be eligible for the study after excluding patients with comorbid diabetes, hypertension, heart failure, chronic kidney, and liver disease, as well as cigarette smokers and patients chronically using any medications. Fifteen patients agreed to participate and were enrolled in the study. A control group of 12 subjects without OSA (confirmed by overnight polysomnography) was selected out of 20 healthy volunteers matched by age (±5 years), BMI (±3 kg/m^2), and waist circumference (±5 cm) with low risk for OSA by the Berlin Questionnaire (24).

Table 1 shows the anthropometric, clinical characteristics, and **Table 2** shows laboratorial data of the participants. The two groups were not different in respect to age, BMI, blood pressure, glucose, and fasting total cholesterol, LDL, and HDL cholesterol levels. Fasting triglycerides and FFA levels in the plasma were higher in the OSA group. OSA patients also showed a trend for higher plasma noradrenaline levels. As expected from the design, sleep apnea differed between the groups. In addition, OSA was associated with higher CIMT values.

Figure 1A and B show the plasma decay curves of radioactively labeled CEs and triglycerides in OSA patients and control subjects. Apparently, the decay curves did not markedly differ from one another, but, as calculated from the parameters of the compartmental analysis, the FCR of the emulsion CEs was indeed markedly diminished in the OSA compared with the control group (0.0015 ± 0.0025 and 0.0126 ± 0.0187 min^{-1}, respectively, $P = 0.031$). The FCR of the emulsion triglycerides was also greatly diminished in the OSA group (0.0051 ± 0.0074 and 0.0334 ± 0.0390 min^{-1}, $P = 0.0001$).

Seven out of 15 OSA participants treated with CPAP for 3 months agreed to repeat the experiments. The mean CPAP usage was 5.6 ± 0.8 h/night. No significant differences in glucose, triglycerides, and cholesterol levels were observed in these patients after the treatment period (data not shown).

CPAP treatment increased 5-fold the triglyceride FCR, from 0.0065 ± 0.0088 to 0.0302 ± 0.0245 min^{-1} (Fig. 1F). The increase in the CE FCR was not statistically significant (Fig. 1E).

Table 3 shows the Spearman correlations between demographic, sleep, vascular, and metabolic parameters with CE FCR, triglycerides FCR emulsion labels, and CIMT. The CE FCR values negatively correlated with the total sleep time with O_2 saturation <90%. It was found that the triglycerides FCR values positively correlated with minimal O_2 saturation during sleep and inversely correlated with AHI, total sleep time with O_2 saturation <90%, and CIMT values. CIMT values positively correlated with AHI and total sleep time with O_2 saturation <90%, but inversely correlated with baseline and minimum O_2 saturation during sleep.

Multivariate analysis (**Table 4**) showed that BMI and the presence of OSA were independently associated with CE FCR. White race, BMI, waist circumference, and the presence of OSA were independently associated with triglycerides FCR.

TABLE 1. General characteristics and sleep data of controls and patients with OSA

	Control (n = 12)	OSA (n = 15)	*P*
Age (y)	41 ± 6	41 ± 8	0.7358
BMI (kg/m^2)	27.9 ± 2.0	28.4 ± 2.9	0.6495
Waist circumference (cm)	94 ± 7	97 ± 8	0.2895
Systolic blood pressure (mmHg)	115 ± 5	119 ± 10	0.1815
Diastolic blood pressure (mmHg)	73 ± 8	74 ± 8	0.7300
Heart rate (bpm)	72 ± 5	72 ± 13	0.9797
CIMT (μm)	620 ± 17	725 ± 29	0.004
Sleep parameters			
AHI (events/hour)	2.5 ± 1.8	45.1 ± 18.6	<0.0001
Baseline O_2 saturation (%)	96 ± 1	96 ± 1	0.457
Minimal O_2 saturation (%)	91 ± 2	77 ± 7	<0.0001
Total sleep time <90% (%)	0	19 ± 23	<0.0001

Data are expressed as mean ± SD.

TABLE 2. Serum biochemistry of controls and patients with OSA

	Control (n = 12)	OSA (n = 15)	*P*
Glucose (mg/dl)	92 ± 6	89 ± 8	0.2609
Cholesterol (mg/dl)			
Total	206 ± 27	193 ± 39	0.3624
LDL	131 ± 26	119 ± 36	0.3215
HDL	50 ± 15	45 ± 14	0.4154
Non-HDL	156 ± 25	145 ± 36	0.3779
Triglycerides (mg/dl)	104 ± 47	147 ± 56	0.0452
FFAs (mEq/L)	0.43 ± 0.12	0.85 ± 0.18	<0.0001
Noradrenaline (pg/ml)	204 ± 83	269 ± 107	0.0975

Data are expressed as mean ± SD.

DISCUSSION

This study examined the effects of OSA on the metabolism of TRLP in subjects. We found that OSA delayed both the removal of TRLP remnants from the circulation, as indicated by the smaller clearance of the emulsion ^{14}C-CEs, and the lipolysis of TRLP, as indicated by the diminished clearance of the emulsion ^{3}H-triglycerides. Furthermore, both remnant removal and lipolysis were correlated with the severity of nocturnal hypoxemia. Finally, lipolysis process estimated by the ^{3}H-triglyceride clearance was improved by the CPAP treatment. Taken together, these findings point to a potential disruptive effect of OSA on the metabolism of TRLP that may lead to acceleration of the atherogenic process.

The present results are consistent with our previous experimental data in the animal model of intermittent hypoxia (12, 13). Previous reports on the lipid metabolism

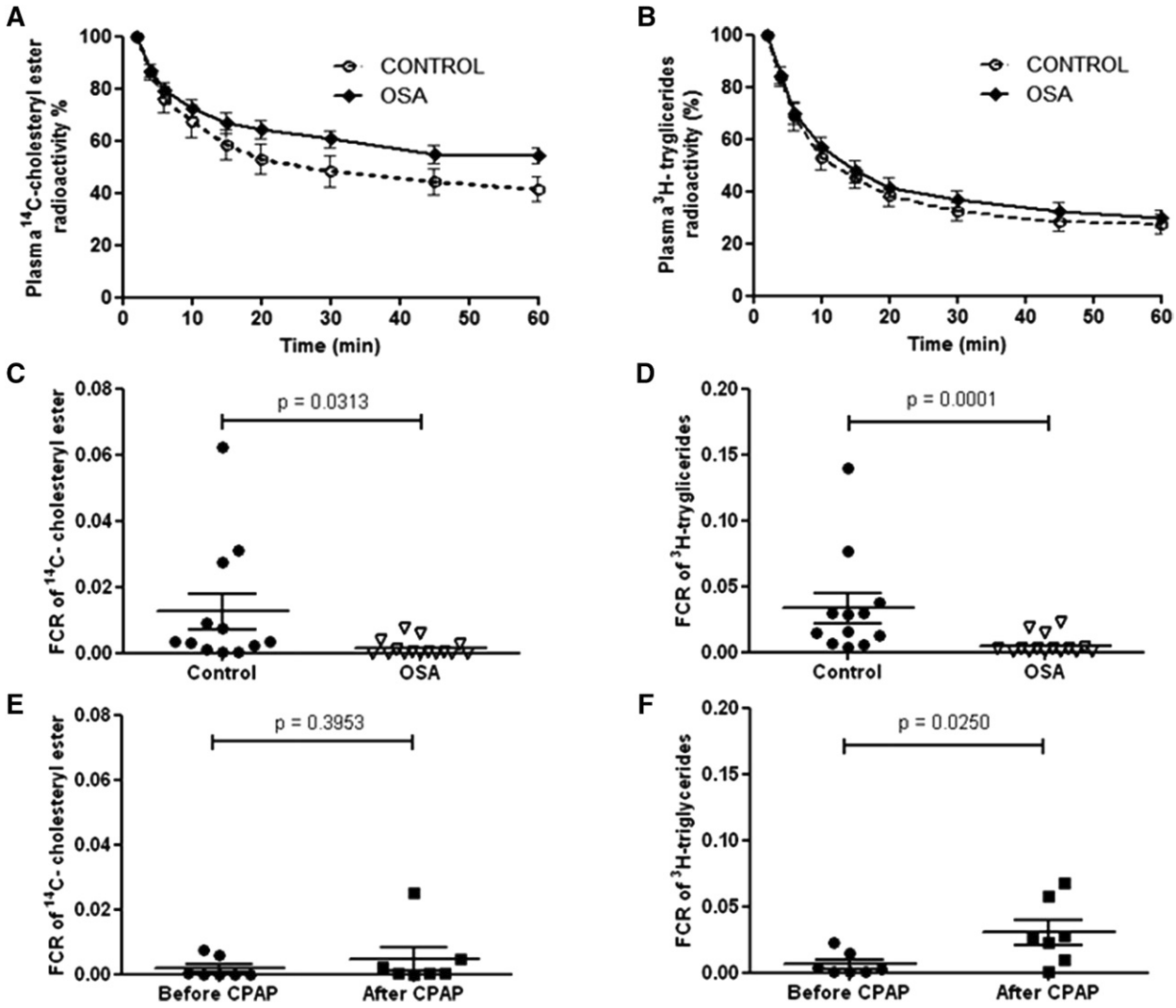

Fig. 1. Plasma decay curves of the emulsion labeled CE (A) and ^{3}H-triglycerides (TG) (B) and FCR of CE (C) and TG (D) in controls and OSA patients. Effects of CPAP treatment on FCR-CE (E) and FCR-TG (F) in OSA patients are shown.

TABLE 3. Spearman correlations between demographic, sleep, vascular, and metabolic parameters with CE and triglyceride FCR of the emulsion radiolabels and CIMT

	CE FCR	Triglyceride FCR	CIMT
Demographic data			
Age	−0.100	0.074	−0.254
BMI	0.220	0.088	−0.100
Waist circumference	−0.027	−0.083	0.241
Sleep data			
AHI	−0.327	−0.537*	0.438*
Baseline O_2 saturation	0.266	0.247	−0.387*
Minimal O_2 saturation	0.401	0.478*	−0.492*
Total sleep time <90% (%)	−0.463*	−0.480*	0.476*
Vascular parameter			
CIMT	−0.601	−0.510	1.0
Metabolic parameters			
Glucose	0.064	0.01	0.100
Cholesterol			
Total	0.088	0.086	−0.252
LDL	0.100	0.100	−0.333
HDL	0.070	0.255	−0.100
Noradrenaline	−0.245	−0.100	0.100

O_2, oxygen. * $P < 0.05$.

status in OSA patients are scanty. Phillips et al. (11) determined the 24 h plasma lipid profile in patients with OSA under standardized Western diet, including snacks. Meals were ingested five times with blood sample collection over the 24 h. They observed that the triglycerides and total cholesterol plasma concentrations were diminished after a 2 month treatment with CPAP. Our study confirms previous hypotheses suggesting that CIH of OSA inhibits TRLP clearance. Triglycerides are transported in plasma by chylomicrons and VLDL. Delayed triglyceride clearance was reversed by CPAP treatment, which suggests causality. In contrast, CE clearance was not changed by CPAP. Thus, our data suggest that CIH affects clearance of TRLP. LpL is a key enzyme of TRLP clearance. Consistent with this aforementioned and our findings, Iesato et al. (25) measured the serum concentration of preheparin LpL in OSA patients and non-OSA controls and observed a progressive decrease in LpL concentration in parallel to the increasing OSA severity. After 3 month treatment with CPAP, LpL concentration increased. Despite the fact that the concentration of free LpL is only residual and that the bulk of TRLP lipolysis occurs by the action of the endothelium-bound enzyme, their data are supportive to our main findings underscoring the role of OSA in delayed lipoprotein clearance.

Impaired clearance of TRLP leads to nonfasting or postprandial hyperlipidemia. Chylomicronemia elicits atherosclerosis in mice (26). Chylomicron remnants induce macrophage foam cell formation and the development of the atherosclerotic plaque (27). Postprandial hypertriglyceridemia confers risk of myocardial infarction, ischemic heart disease, stroke, and death (17, 28, 29). A body of evidence suggests that non-HDL cholesterol, the sum of the LDL, lipoprotein(a), TRLP, and TRLP remnants, is superior to LDL cholesterol as an indicator of CVD risk, particularly in patients with hypertriglyceridemia. TRLPs may contribute to the progression of atherosclerosis and CVD via multiple mechanisms (30). TRLPs enhance intimal cholesterol deposition and lead to activation of proinflammatory pathways (31). Consistent with our previous reports

TABLE 4. Multivariate analysis to determine the independent predictors of FCR of the CE and triglycerides emulsion labels

Coefficient	Estimate	Standard error	*t*	*P*
CE FCR				
(Intercept)	−7.629	2.754	−2.770	0.012
Age	−0.062	0.036	−1.695	0.106
White race	−0.219	0.513	−0.428	0.673
BMI	0.295	0.115	2.572	0.018
Waist circumference	−0.047	0.037	−1.269	0.219
Triglycerides	0.003	0.004	0.661	0.516
OSA	1.856	0.429	4.328	<0.001
Triglycerides FCR				
(Intercept)	−7.493	2.765	−2.710	0.013
Age	0.008	0.037	0.225	0.824
White race	1.337	0.515	2.597	0.017
BMI	0.334	0.115	2.902	0.009
Waist circumference	−0.086	0.038	−2.288	0.033
Triglycerides	−0.005	0.004	−1.215	0.239
OSA	1.983	0.431	4.604	0.000

(21, 32, 33), our current data show a strong relationship between AHI and nocturnal hypoxemia and a marker of subclinical vascular disease and predictor of CVD, namely, CIMT (18). The significant reduction of CIMT after effective CPAP treatment in severe OSA patients (34) supports the potential role of OSA in atherosclerosis. Thus, OSA-induced impairment of TRLP clearance may contribute to the progression of atherosclerosis and increased CVD risk.

Our study has some strengths and limitations. The main strengths include: *1*) we performed full polysomnography, considered the gold standard method for diagnosing OSA; *2*) in order to understand the relative role of OSA on lipid clearance in humans, we carefully selected patients and appropriate matched controls with no substantial confounding factor such as hypertension, smoking, diabetes, and regular use of any medication; and *3*) the robust evaluation of TRLP clearance. In the emulsion method used in this study to evaluate the TRLP intravascular metabolism, potential confounders such as differences in intestinal motility, microbiota, and other factors affecting fat absorption are bypassed. The chylomicron-like emulsions that are devoid of proteins acquire apolipoproteins from the native plasma lipoproteins. Acquisition of apoC II allows lipolysis of the emulsion particles by LpL and of apoE allows remnant removal by hepatic receptors that recognize this apolipoprotein (35). After injection of the doubly-labeled chylomicron-like emulsions into the plasma compartment, the plasma decay curves of radioactive CEs that are not independently removed from the emulsion particles stand for the removal of remnants. On the other hand, the triglyceride curves mirror the lipolysis process chylomicrons undergo in the plasma compartment. In this approach, artificially made emulsions with composition similar to that of lymph chylomicrons and doubly labeled with radioactive CEs and triglycerides are iv injected. Therefore, the chylomicron-like emulsion is a straightforward and precise method to evaluate TRLP, allowing the analysis of plasma kinetics and simultaneous discrimination of the two major processes occurring in TRLP metabolism, namely, lipolysis by LpL and the removal of the remnants by the liver. The following limitations should be acknowledged: first, results from this study were limited to male patients with severe OSA and no significant comorbidities. Therefore, this finding may not be true for mild or moderate OSA. While interesting to figure out the relative role of OSA, the exclusion of females, smokers, and patients with diabetes, hypertension, and coronary artery disease may limit generalizability. Second, we did not perform measurement of LpL, a key enzyme involved in the lipid metabolism. There are some concerns and pitfalls in getting peripheral blood as a reliable and representative marker of LpL activity at tissue level. Third, liver fat content may play role in triglyceride clearance (36) and was not addressed in the current study. Finally, our results are derived from a cross-sectional study, and the associations must be interpreted with caution. The sample size was calculated based on the comparisons of OSA and matched controls. However, it is reassuring that in a subgroup in whom OSA was properly treated with CPAP improved 5-fold triglyceride FCR. The lack of significance increase in the CE FCR may be related to the small sample size, rather than a lack of effect.

In conclusion, our results establish that OSA leads to proatherogenic disturbances of the TRLP metabolism. In this regard, severe OSA patients showed delayed removal of remnants and decreased lipolysis of TRLP. As shown in previous studies, those defects of TRLP metabolism are associated not only with the presence of coronary artery disease, but also with the severity and incidence of the complications of this disease.

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