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CLINICAL TRIAL REPORT

Intramyocellular Lipids, Insulin Resistance, and Functional Performance in Patients with Severe Obstructive Sleep Apnea

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Purpose: An increasing number of studies have linked the severity of obstructive sleep apnea (OSA) with metabolic dysfunction. However, little is known about the lipid compartments (intramyocellular [IMCL] and extramyocellular [EMCL] lipids) inside the musculature in these patients. The present study was designed to investigate the IMCL and EMCL, biochemical data, and functional performance in patients with severe OSA, and to examine the correlations between intramuscular lipid contents and test variables.

Participants and Methods: Twenty patients with severe OSA (apnea-hypopnea index [AHI]: ≥30/h; body mass index [BMI]: 26.05±2.92) and 20 age- and BMI-matched controls (AHI <5/h) were enrolled. Proton magnetic resonance spectroscopy was used to measure the IMCL and EMCL of the right vastus lateralis muscle. Biochemical data, including levels of fasting plasma glucose, insulin, lipid profiles, and high-sensitivity C-reactive protein (hsCRP), were measured. Insulin resistance index (IR) was calculated using the homeostasis model assessment method. Performance tests included a cardiopulmonary exercise test and knee extension strength and endurance measurements.

Results: Patients with severe OSA had significantly (P<0.05) lower values of IMCL (14.1 ±5.4 AU) and EMCL (10.3±5.8 AU) compared to the control group (25.2±17.6 AU and 14.3 ±11.1 AU, respectively). Patients with severe OSA had significantly higher hsCRP, IR, and dyslipidemia compared with controls (all P<0.05). Furthermore, IMCL was negatively correlated with AHI, cumulative time with nocturnal pulse oximetric saturation lower than 90% ($TSpO_2$<90%) (ρ=−0.35, P<0.05), IR (ρ=−0.40, P<0.05), glucose (ρ=−0.33, P<0.05), and insulin (ρ=−0.36, P<0.05), and positively correlated with lowest oximetric saturation (ρ=0.33, P<0.01).

Conclusion: Skeletal muscle dysfunction and metabolic abnormalities were observed in patients with OSA that did not have obesity. IMCL was positively correlated with aerobic capacity and muscular performance, but negatively correlated with AHI and IR. Large-scale clinical trials are required to explore the complicated mechanism among OSA, intramuscular metabolism, and insulin action.

Clinical Trial Registration: ClinicalTrials.gov Identifier: NCT00813852.

Keywords: ^{1}H magnetic resonance spectroscopy, insulin resistance, obstructive sleep apnea, skeletal muscle

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Introduction

Obstructive sleep apnoea syndrome (OSA) is characterized by repeated occlusion of the upper airway during sleep, resulting in periods of intermittent hypoxemia.[1] An increasing number of studies have linked the severity of OSA with metabolic

dysfunction.[2,3] Particularly, both glucose intolerance and insulin resistance are closely correlated with the level of nocturnal oxygen desaturation in patients with OSA.[2] As skeletal muscles have the largest tissue mass for the uptake of circulating glucose, it is speculated that altered intramuscular energy metabolism may implicate the development of insulin resistance.[4,5] Previous studies have reported that oxidative metabolism in the skeletal muscle is compromised in patients with intermittent hypoxia[6] and in patients with OSA.[7,8] The increased reliance on anaerobic energy sources and decreased oxidative capacity may thus influence insulin sensitivity.[4,5] However, little is known about the lipid compartments inside the musculature in patients with OSA.

Besides the subcutaneous fat layer, there are two more lipid compartments located inside the musculature: the extramyocellular (EMCL) and intramyocellular lipids (IMCL). The EMCL nestles in layers along the muscles fiber bundles, whereas the IMCL consists of microscopic, nearly spherical fat droplets within the myocytes, and acts as a fuel source for the myocytes.[9] Type I fibers contain more IMCL than type II (glycolytic) fibers.[10] Several studies reported a significant association of increased IMCL with insulin resistance.[11] It is believed that in Caucasian people, the deposition of IMCLs represents an early abnormality in the pathogenesis of insulin resistance. However, investigations on IMCL in Indians and South Asian populations failed to show any relationship between IMCL and insulin sensitivity.[12,13] Additionally, both OSA and insulin resistance are pro-inflammatory conditions. However, to our knowledge, the relationships between IMCL and pro-inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP), have not been extensively investigated.

Invasive technique (eg, skeletal muscle biopsy) and noninvasive methods (eg, computed tomography, proton magnetic resonance spectroscopy [^{1}H-MRS], and magnetic resonance imaging) can be used to assess IMCL.[14] The invasive nature of muscle biopsies and the safety issues concerning ionizing radiation from computed tomography limit the determination of the lipid stores. ^{1}H-MRS is a noninvasive tool allowing the quantification of skeletal muscle lipids, especially for differentiating between IMCL and EMCL.[15] With skeletal muscle lipids and energy production playing such an influential role in glucose control and lipid oxidation,[16] ^{1}H-MRS provides data that will supply information gained from circulating substrates and signaling hormones. Although ^{1}H-MRS has been extensively used in the investigation of metabolism in other diseases,[16] only few studies utilized this technique to investigate skeletal muscles' metabolism in patients with OSA.[17,18]

Therefore, the purposes of this study were to (1) investigate the IMCL and EMCL, biochemical data, and functional performance in patients with severe OSA versus controls, and (2) examine the correlations between intramuscular lipid contents and biochemical (insulin, glucose, hsCRP, and lipid profiles) and performance variables (peak oxygen consumption, and strength and endurance of knee extensors).

Methods

Participants

Male patients (ranging from 40 to 65 years of age) who were referred to the Center of Sleep Disorder of a teaching hospital for evaluation of sleep apnea were prospectively recruited in this study since September 2007. We have previously reported the details of the recruitment procedures and methods.[19,20] Briefly, the consecutive participants who were newly diagnosed with severe OSA with whole-night polysomnography (PSG) (apnea-hypopnea index [AHI] of $\geq$30 h^{-1}) were recruited. The control group included age- ($\pm$3 years), weight- ($\pm$3 kg), and height-matched ($\pm$5 cm) subjects without OSA (AHI <5 h^{-1}). Control subjects were patients who were referred to the sleep laboratory for snoring, sleep disturbance, or excessive daytime sleepiness and were confirmed to not have OSA. Exclusion criteria were coronary heart diseases, nervous system diseases, abnormal pulmonary function, morbid obesity, diabetes under insulin management, alcoholism ($\geq$50 g per day), and recent infection. Additionally, participants were excluded if they had any missing value(s) for our primary measures. All participants had never been treated for OSA. Biochemistry, MRS, CPET and other tests were all performed by personnel blinded to the OSA status of the patients.

This trial was conducted in accordance with the Declaration of Helsinki. This study was approved by the Institutional Ethics Committee of the National Taiwan University Hospital (www.ClinicalTrials.gov; NCT00813852) and all subjects provided their written informed consent prior to enrolment.

Outcome Measures

Diagnosis of OSA

Whole-night PSG (Embla N7000, Medicare Flaga, Reykjavik, Iceland) was performed in the sleep lab following the protocol described in the previous studies.[19,20] The

sleep stages and respiratory events were scored according to the American Academy of Sleep Medicine standard.[1] In short, apnea was defined by the absence of airflow ≥10 seconds and hypopnea was a ≥50% decrease in airflow ≥10 seconds associated with reduced arterial oxygen saturation of ≥3% or an arousal. The oxygen desaturation index (ODI) was defined by the number of ≥4% arterial oxygen saturation per hour. All of the sleep studies were analyzed by the same investigator to maximize inter- and intra-scorer reliability. The level of daytime sleepiness was assessed using the Epworth sleepiness scale in the morning after nocturnal PSG. Normal values range from 2 to 10. Scores over 10 indicate daytime sleepiness.[21]

Biochemistry Investigations

Fasting (overnight) venous blood samples were drawn from the antecubital vein on the next morning of PSG to estimate hsCRP levels, plasma glucose, insulin level, total cholesterol, triglycerides, and high-density lipoprotein cholesterol level. All tests were performed by personnel blinded to the clinical outcomes. hsCRP levels were measured using CRP-Latex (II) immunoturbidimetric assay kit (Denka Seiken, Tokyo, Japan) on a Hitachi 911 immunoannalyzer (Roche Diagnostics, Indianapolis, IN, USA).[22] The concentrations of glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol were assayed according to the previously described methods.[23] The value of low-density lipoprotein cholesterol was further calculated using the Friedewald formula.[24] Serum insulin concentration was estimated using the ^{125}I-insulin radioimmunoassay kit (Medicorp Inc., Montreal, Canada). The homeostasis model assessment method was applied to calculate insulin resistance by using the following formula: fasting serum insulin (mu/l (micro-units)) × fasting blood glucose (mmol/l)/22.5.[25]

Vital Signs and Body Composition Measurements

Upon arrival to the laboratory, subjects rested in a chair for at least 5 min before the heart rate and blood pressure were measured. Next, weight and height were measured, and the body mass index (BMI) was calculated. Body composition was then measured using a bioelectrical impedance analyzer (Maltron BioScan 920, Esgender, UK), with 800 μA of current at a frequency of 50 kHz. The measurement procedure was carried out according to the user's manual. Once the measurements had stabilized, the analyzer calculated the percentage of body fat directly from the equation and displayed the value. Previous studies have demonstrated an excellent test–retest reliability and validity for the method comparable to hydrostatic weighing and dual-energy X-ray absorptiometry.[26,27]

Functional Performance of Knee Extensors

A Cybex 6000 (Cybex, Division of Lumex Inc., Ronkonkoma, NY, USA) was utilized to measure the strength and the endurance of right knee extensors. Each subject was instructed to perform five maximal isometric knee extension contractions at a 60° knee flexion angle. Each contraction lasted for 5 seconds, with a resting period of at least 15 seconds between trials.[28] The mean of five maximal isometric contraction peak torques (Newton-meter, N-m) for each subject was calculated for data analyses. The endurance test consisted of 30 cycles of alternative knee extension/flexion isokinetic contractions with the speed of 180°/second. The muscular endurance was evaluated by total works (N-m) generated in 30 cycles of contractions as the total area under the torque curve for knee extension movement which would be automatically provided by the Cybex system.[28]

Cardiopulmonary Exercise Test

Each subject performed a symptom-limited maximal exercise test with continuous electrocardiographic monitoring on a cycle ergometer. All participants were encouraged to exercise until exhaustion. The criteria for termination included at least two of the following conditions: volitional fatigue, heart rate at or near 90% of the age-predicted maximum, or a respiratory exchange ratio >1.15.[29] The graded exercise protocol consisted of three minute stages, starting at 25 watts with a 25-watt increment at each successive stage, while maintaining a pedalling rate of 50—60 rpm. Respiratory gas exchange measurements, including peak ventilation (V_{Epeak}) and oxygen consumption (VO_{2peak}), were obtained during exercise using a computer-controlled, breath-by-breath metabolic measurement system (Vmax29 Metabolic Measurement System; SensorMedics, Anaheim, CA, USA). The tester was blinded to the results of the biochemical analyses.

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

Localized ^{1}H MRS spectrum of the vastus lateralis were acquired using a 1.5 Tesla whole-body imager (Sonata, Siemens, Erlangen, Germany). During the measurement acquisition procedure, the subjects remained supine, with the vastus lateralis complex of the right thigh positioned within the homogeneous volume of the magnet. Before performing ^{1}H MRS, multi-slice magnetic resonance images in three orthogonal planes of the right thigh

(above knee) were acquired using a standard spin-echo pulse sequence [echo time (TE) = 15 ms, repetition time (TR) = 520 ms, 5 mm slice thickness; 256 × 256 matrix]. Then, these scout images were acquired to position the volume of interest. Volumes of interest (typically 15 × 15 × 25 mm^3) centered within the vastus lateralis muscle were placed to avoid vascular structures and gross adipose tissue deposits. For volume selection, a single voxel PRESS (Point Resolved Spectroscopy) technique was applied. Chemical shifts were reported using water as the internal standard at 4.7 ppm. The IMCL and EMCL peaks were well identified in the longer echo time sequence (TR=3000 ms, TE=20 ms). The spectra were analyzed, including amplitude and area under curve. The spectra obtained several chemical components: TMA= trimethylamines (Choline), at 3.2 ppm; TCr= total creatine methyl, at 3.0 ppm; EMCL(-CH2), 1.5 ppm; IMCL(-CH2), 1.3 ppm; EMCL(-CH3), 1.1 ppm; IMCL(-CH3), 0.9 ppm. Contributions of IMCL and EMCL contents were estimated by de-convolution of the lipid resonance peak (0.8–1.6 ppm). The percentages of IMCL and EMCL, respective to water, were determined by calculating the area under each peak using system software (Java-based MR user interface (jMRUI)).[30]

Data Analyses

Statistical analyses were performed using the Statistical Software for Social Sciences v.19 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as numbers and percentages. Between-group comparisons of baseline measurements were examined using the independent Student's *t*-test, Mann–Whitney *U*-test, or a chi-squared test. The differences of exercise test parameters between the two groups were tested using the Student's *t*-test. The associations among the variables were investigated using the Spearman correlation coefficient (ρ). The α value was set at 0.05.

Results

Demographic Characteristics and Sleep Examination Results

Table 1 shows the basic demographic characteristics and sleep examination results of the patients with severe OSA and of the control subjects. The mean age of the study participants was 50.3±6.1 years; a majority of these were in the "overweight" category, with a mean BMI of 25.93 ±2.81 kg/m^2. Age and anthropometric characteristics were matched in the two groups. Compared with the control group, patients with severe OSA demonstrated significantly higher values of Epworth sleepiness scale measurements, AHI, ODI, and the cumulative time with nocturnal pulse oximetric saturation lower than 90% ($TSpO_2$< 90%) (P<0.05 for all); the lowest oximetric saturation (lowest SpO_2), arousal index, sleep efficiency, and blood pressure were observed in the severe OSA group (P<0.05 for all). However, both systolic and diastolic blood pressure were considered "normal" based on the most recent American Heart Association recommendations.[31] Additionally, the numbers of participants with hypertension and diabetes were not significantly different between the two groups (P>0.05). Daily energy expenditure was not significantly different between the two groups (P>0.05).

Table 1 Patient Characteristics and Sleep Measurements

	OSA Group (n=20)	Control Group (n=20)	*P*-value
Age (years)	50.2±5.6	50.4±6.7	0.919
Body weight (kg)	73.8±7.8	72.4±7.8	0.581
Body height (cm)	168.4±5.3	167.5±4.2	0.546
Body mass index (kg/m^2)	26.05±2.92	25.82±2.76	0.799
Heart rate (bpm)	77.9±4.4	76.7±4.6	0.401
Systolic blood pressure (mmHg)	128.6±6.9	120.0±6.5	0.001
Diastolic blood pressure (mmHg)	83.5±7.5	76.7±6.4	0.003
Epworth sleepiness scale	11.5±3.6	8.4±3.2	0.007
Apnea-hypopnea index (/hr)	48.0±18.9	2.4±1.2	<0.001
Oxygen desaturation index (/hr)	42.5±21.5	2.2±1.0	<0.001
Lowest SpO_2 (%)	72.2±8.2	89.5±2.4	<0.001
$TSpO_2$ < 90% (%)	18.4±23.5	0.1±0.1	0.001
Arousal index (/hr)	11.1±9.2	4.6±2.5	0.013
Sleep efficiency (%)	82.9±9.6	92.6±3.8	<0.001
Energy expenditure (kcal/kg/day)	35.20±2.71	37.31±5.99	0.159
Hypertension (%)	4 (20%)	3 (15%)	0.500
Diabetes (%)	4 (20%)	3 (15%)	0.500

Notes: Values are as group mean ± SD or case numbers (percentage). $TSpO_2$ <90% is the cumulative time with nocturnal pulse oximetric saturation lower than 90%. A *P*-value less than 0.05 is statistically significant.

Outcome Measurement Results

Table 2 presents the IMCL and EMCL data for the two groups. Significantly lower values for both IMCL and EMCL were observed in the OSA group (10.3±5.8 AU (arbitrary unit) and 14.3±11.1 AU, respectively) compared with the control group (14.1±5.4 AU and 25.2±17.6AU, respectively) (P<0.05). However, although the OSA group had a higher ratio of IMCL to EMCL than the control group (95.0±64.8% and 77.6±36.8%), the difference was not

Table 2 Results of Proton Magnetic Resonance Spectroscopy and Biochemistrical Analyses

	OSA Group (n=20)	Control Group (n=20)	*P*-value
IMCL (AU)	10.3±5.8	14.1±5.4	0.037
EMCL (AU)	14.3±11.1	25.2±17.6	0.023
IMCL/EMCL (%)	95.0±64.8	77.6±36.8	0.304
Body fat (%)	22.30±5.26	21.17±5.02	0.492
Fat free mass (kg)	57.85±5.31	56.02±4.51	0.249
hsCRP (mg/dL)	0.23±0.14	0.12±0.06	0.039
Insulin resistance index	2.69±1.88	1.38±0.75	0.017
Insulin (μU/mL)	9.34±6.01	5.74±2.42	0.041
Fasting glucose (mg/dL)	116.2±50.5	94.0±14.6	0.081
Lipid Profiles			
Total cholesterol (mg/dL)	205.0±42.5	186.3±37.1	0.113
Triglyceride (mg/dL)	149.9±89.7	105.6±51.4	0.132
High-density lipoprotein (mg/dL)	42.1±7.0	41.6±7.1	0.730
Low-density lipoprotein (mg/dL)	138.7±37.0	125.1±29.8	0.277
VO_{2peak} (mL/kg/min)	25.68±3.57	27.84±2.74	0.038
HR_{peak} (bpm)	151.0±15.6	169.6±13.9	<0.001
Knee extensors peak torque (N-m)	135.2±19.2	158.2±13.0	<0.001
Knee extensors endurance (N-m)	44.03±15.3	60.1±20.5	0.005

Notes: Values are as group mean ± SD. A *P*-value less than 0.05 is statistically significant.

Abbreviations: IMCL, intramyocellular lipid contents; EMCL, extramyocellular lipid contents; AU, arbitrary unit; hsCRP, high-sensitivity C-reactive protein.

significant ($P>0.05$). It is important to note that patients in the OSA group demonstrated a much wider distribution of values for all lipid parameters versus the control group. Figures illustrate quantification of lipid profiles by an ^{1}H-MRS spectrum from the vastus lateralis muscle of a patient with severe OSA (Figure 1) and a control (Figure 2).

Table 2 also demonstrates the results of hsCRP levels, lipid profiles, biochemical analyses, and functional performance measurements for the two groups. Significantly higher hsCRP levels were observed in the severe OSA group (0.23 ±0.14 mg/dL) versus the control group (0.12±0.06 mg/dL) ($P<0.05$). Patients with OSA also had significantly higher levels of insulin (9.34±6.01 μU/mL) and insulin resistance index (IR) (2.69±1.88) compared with the controls ($P<0.05$), but no significant differences were observed between the two groups in fasting glucose levels and lipid profiles. Additionally, patients with OSA demonstrated a significantly lower VO_{2peak} ($P=0.038$), and strength ($P<0.001$) and endurance ($P=0.005$) of knee extensors versus the control group.

The Spearman correlation coefficients of IMCL and EMCL, ratio of IMCL to EMCL, and various parameters for all participants are listed in Table 3. IMCL was negatively correlated with PSG, ODI, TspO2<90% ($\rho=-0.35$, $P<0.05$), arousal index, hsCRP, IR ($\rho=-0.40$, $P<0.05$), glucose ($\rho=-0.33$, $P<0.05$), and insulin ($\rho=-0.36$, $P<0.05$), and positively correlated with lowest SpO_2 ($\rho=0.33$, $P<0.01$), VO_{2peak}, strength, endurance, and daily energy expenditure.

Discussion

This study demonstrates that patients with OSA had significantly lower IMCL and EMCL values, as measured by ^{1}H-MRS, compared with their age-, and BMI-matched controls without OSA. Compared with controls, patients with OSA also had significantly reduced functional performance and exhibited abnormal biochemical data, such as hsCRP, fasting insulin, and IR. It is suggested that both altered skeletal muscle and systemic metabolism are associated with OSA. Previous studies on skeletal muscle IMCL content in OSA populations are scarce and did not observe the correlation between measures of IMCL and IR in OSA.[17,18] Our results enhance the understanding of the role of skeletal muscle metabolism in severe OSA.

The physiological function of fat in the muscle is to serve as a readily available intracellular source of energy during exercise.[32] This capacity to store fat inside the muscle may have conferred an evolutionary advantage to permit physical activity during cycles of feast and famine.[32,33] The IMCL content is increased in the physically trained state to optimally match the fat oxidative capacity. However, nowadays the lower levels of physical activity combined with continuous availability of food in the population would negate the need for high IMCL content. In these conditions in which IMCLs are not being used for oxidation, IMCLs and their intermediates negatively affect insulin signaling and induce insulin resistance.[33] Therefore, the preserved capacity to store fat in the muscle may have detrimental effects on insulin sensitivity. In this respect, the capacity to use IMCLs may be more influential than the magnitude of IMCL levels per se in determining the negative effects on insulin sensitivity.

Several studies have investigated the relationships between IMCL and insulin sensitivity. Our results were inconsistent with previous studies, indicating that increased content of IMCL is associated with a decreased whole-body and skeletal muscle insulin sensitivity in both patients with and without diabetes.[11,34,35] Tamura et al examined the effects of diet and exercise on IMCL in patients with type II diabetes using ^{1}H-MRS.[36] They demonstrated that IMCL decreased in parallel with improved peripheral insulin sensitivity. However, some studies did not demonstrate the relationship between IMCL and insulin resistance in non-obese

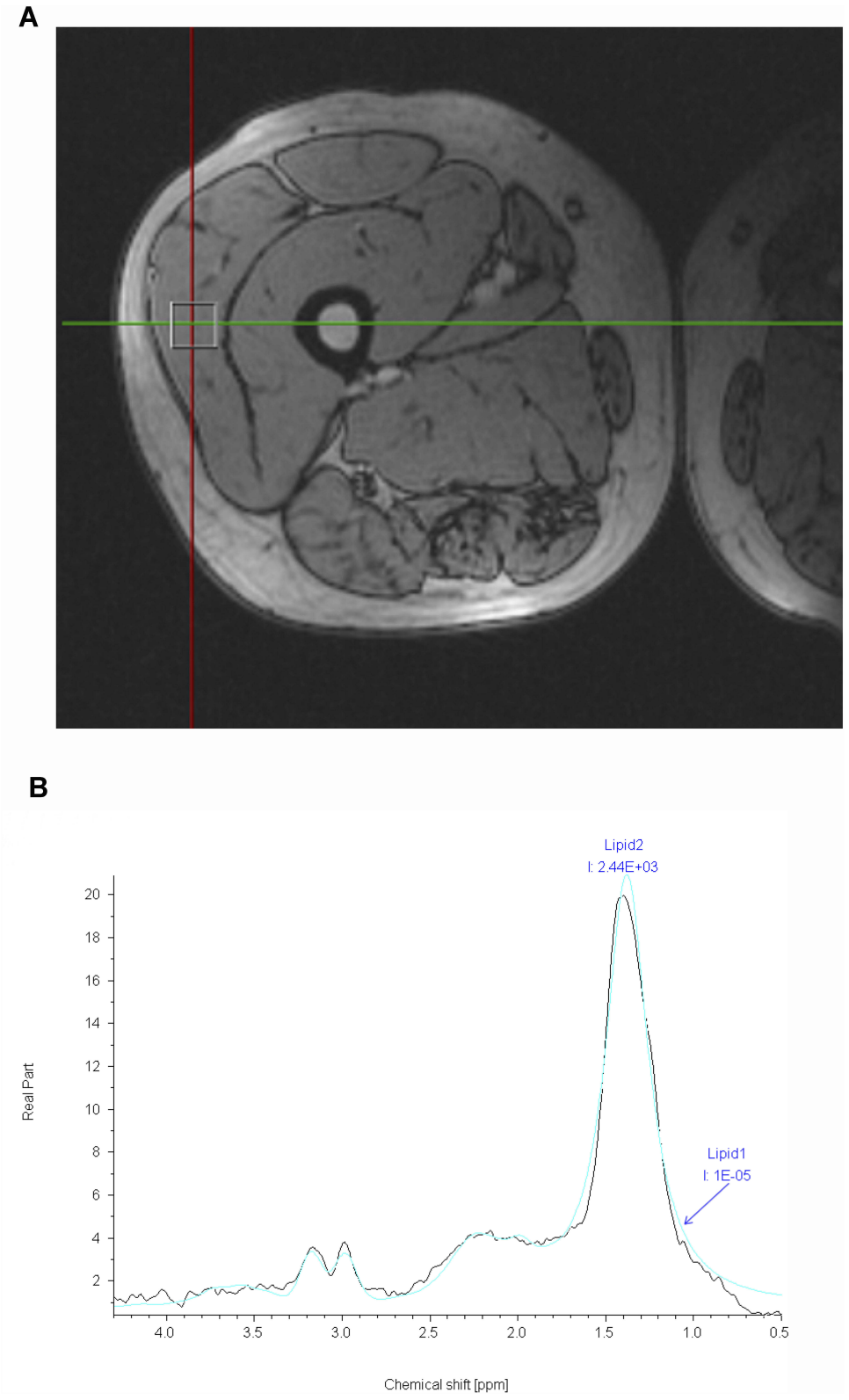

Figure 1 (**A**) ^{1}H-MRS voxel placement from the vastus lateralis muscle of a patient with severe OSA. (**B**) The peak originating from the methylene protons of IMCLs of a patient with severe OSA by an ^{1}H-MRS spectrum.

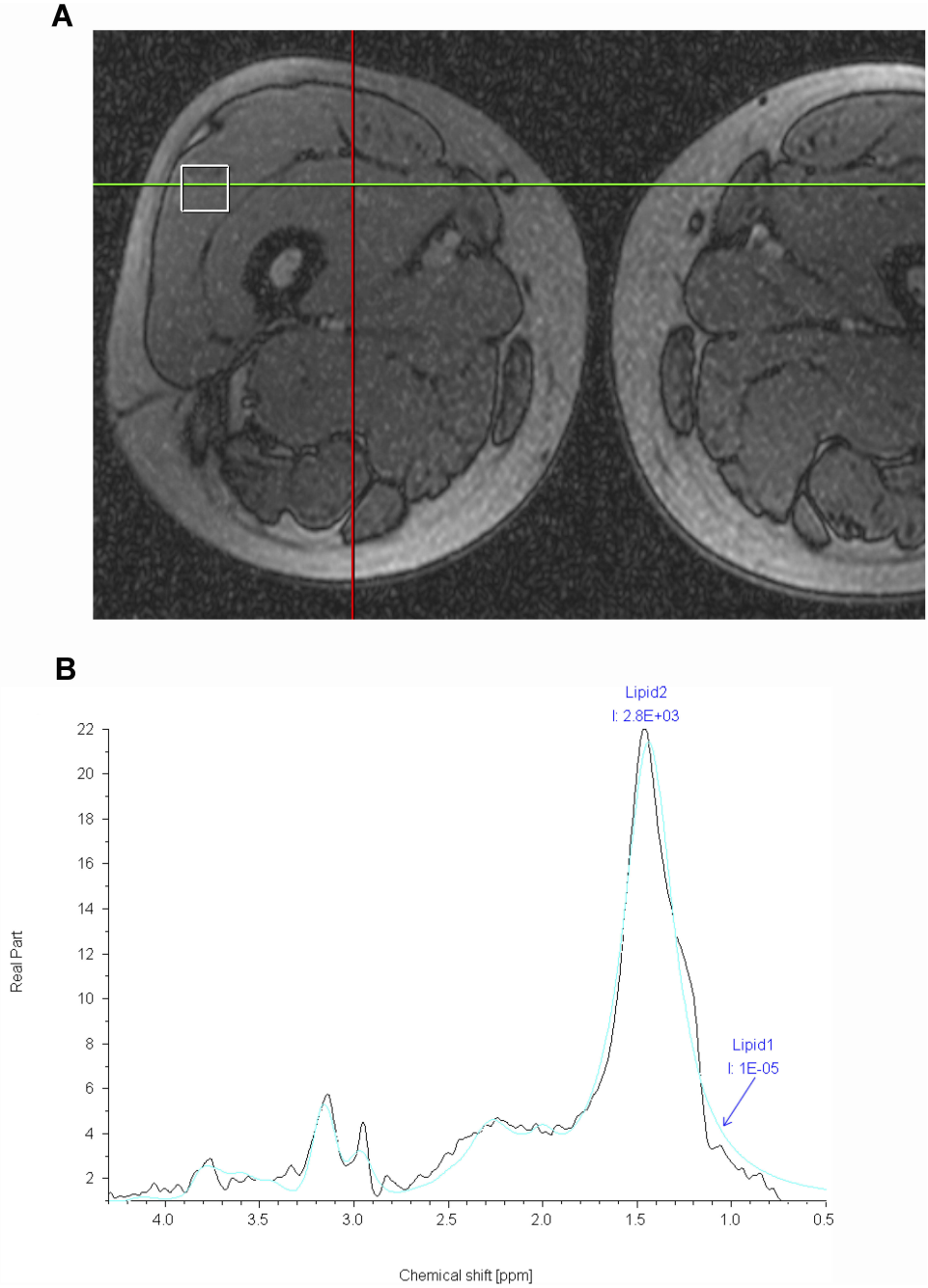

Figure 2 (**A**) ^{1}H-MRS voxel placement from the vastus lateralis muscle of a control participant. (**B**) The peak originating from the methylene protons of IMCLs of a control participant by an ^{1}H-MRS spectrum.

Indian patients with diabetes.[12,13,37] Recent studies have highlighted ethnic differences in the determinants of the relationship between IMCL and insulin resistance. Both Ingram et al[33] and Lawrence et al[38] reported that IMCL relates to insulin sensitivity and adiposity in European Americans, but not in African Americans.

It is still unknown why African Americans and European Americans exhibit different relationships between IMCL and insulin sensitivity. One possible explanation is that IMCL could be compartmentalized differently in different ethnicities.[39] Another possible explanation is a difference in skeletal muscle fiber type between different ethnicities. Insulin resistance has been related to a higher IMCL content in type I (oxidative) muscle fibers, compared with type II (glycolytic) muscle fibers.[40] It was reported that OSA would lead to a slow-to-fast muscle fiber-type

Table 3 Correlation Coefficients Between Biochemistrical Data and Proton Magnetic Resonance Spectroscopy Results (n=40)

	IMCL	EMCL	IMCL/ EMCL
Apnea-hypopnea index (/hr)	−0.27	−0.10	0.02
Oxygen desaturation index (/hr)	−0.27	−0.09	0.05
$TSpO_2$ < 90% (%)	−0.35*	−0.23	0.01
Arousal index (/hr)	−0.15	−0.18	0.24
Sleep efficiency (%)	0.19	0.42*	−0.41*
Lowest SpO_2 (%)	0.33*	0.18	−0.04
Body mass index (kg/m^2)	0.24	0.14	−0.13
Body fat (%)	−0.27	0.04	−0.03
Fat-free mass (kg)	−0.07	−0.11	0.01
VO_{2peak} (mL/kg/min)	0.19	0.12	−0.04
Strength (N-m)	0.25	−0.03	0.19
Endurance (N-m)	0.25	0.24	−0.12
Energy expenditure (kcal/kg/day)	0.14	−0.02	0.13
Insulin resistance index	−0.40*	−0.25	0.04
Fasting glucose (mg/dL)	−0.33*	−0.30	0.17
Insulin (μU/mL)	−0.36*	−0.11	−0.10
hsCRP (mg/dL)	−0.11	−0.22	−0.14
Total cholesterol (mg/dL)	−0.30	−0.29	0.07
Triglyceride (mg/dL)	0.01	−0.13	0.11
High-density lipoprotein (mg/dL)	−0.24	−0.27	0.17
Low-density lipoprotein (mg/dL)	−0.22	−0.11	−0.01

Notes: *Indicates statistically significant as $P < 0.05$. $TSpO_2$ <90% is the cumulative time with nocturnal pulse oximetric saturation lower than 90%.
Abbreviations: IMCL, intramyocellular lipid contents; EMCL, extramyocellular lipid contents; hsCRP, high-sensitivity C-reactive protein.

transformation. Thus, it is plausible that more type II fibers within the vastus lateralis muscle in the OSA group than in the control group might contribute to this phenomenon.

Because of the unique features of intermittent hypoxia and their chronic, low-grade inflammatory status, patients with OSA were considered at a high risk for developing metabolic syndrome. It was speculated that elevated IMCL levels and IR would be present in patients with OSA. Trenell et al[17] reported no change in IMCL content and insulin sensitivity scores after 12-week treatment of CPAP in patients with OSA. Our study demonstrated that patients with severe OSA exhibited a higher insulin level and IR, but there was a reduction in both IMCL and EMCL. Therefore, a significantly negative correlation between IMCL and both $TSpO_2$ and IR was reported. In addition to ethnic differences and muscle fiber-type variations, insufficient energy supply in OSA patients is another possible mechanism, based on the reduced VO_{2peak} observed in OSA patients. As previously mentioned, the relationships between IMCL, glucose, and insulin are complex and depend on several factors. More research is required to further investigate these relationships.

^{1}H-MRS of human skeletal muscle has become more commonly investigated in clinical practice, primarily due to the relationship between IMCL levels and insulin sensitivity.[41] Several longitudinal studies have highlighted the utility of ^{1}H-MRS to quantify IMCL over time. ^{1}H-MRS has been validated in humans and the error of this method is reported to be as low as approximately 6%.[42,43] The test–retest reliability of ^{1}H-MRS IMCL measurements in humans is acceptable, and changes in IMCL greater than 15% could be detected between ^{1}H-MRS measurements.[43] However, there are limitations of using ^{1}H-MRS to quantify IMCL. First, IMCL is typically expressed in relative terms as the ratio between the signal peak areas in the respective spectra, resulting from IMCL and creatinine or water. Secondly, the IMCL signal highly depends on the orientation of the muscle fibers so that only the lipid of selected voxels within certain muscle groups can be reliably measured.[42] Therefore, the leg must be carefully positioned to ensure muscle fibers are correctly oriented. Also, the regions of interest containing voxels must be carefully selected to avoid contamination.

There are several study limitations that should be considered. First, given the cross-sectional nature of this study, any causal relationship between these constructs remains to be determined. Future longitudinal studies are required to verify the sequential changes and relationships reported in this study. Second, evidence showed that IMCL content was not stable and might change over time. Factors such as diet, obesity state, and level of physical activity have profound effects on IMCL.[10] As the groups were matched for the anthropometric parameters and no significant difference of daily energy expenditure was found between two groups, the difference in IMCL could not be attributed to these factors. However, future studies should control the dietary factors, as these were not considered in the present study. Third, our participants were limited to middle-aged men. Thus, the results could not be extrapolated to women or other ethnicities.

Conclusion

The present study demonstrated that patients with severe OSA exhibited a reduction in IMCL and EMCL, poor aerobic and muscular performance, and impaired biochemical data, including glucose and insulin levels and lipid profiles. It appeared that both systemic and skeletal muscle metabolism dysfunction occurs in the OSA group, but not

in the control subjects. Additionally, IMCL positively correlated with aerobic capacity and muscular performance, but negatively correlated with AHI and IR. For patients with OSA that did not have obesity, lower IMCL might indicate insufficient energy storage in muscles compared with their counterparts. However, additional large-scale clinical trials are required to further explore the complex mechanism between OSA, muscle metabolism, and insulin action.

Data Sharing Statement

The authors do not intend to share individual deidentified participant data.

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Disclosure

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript. The authors report no conflicts of interest in this work.

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