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Article

Analysis of Metabolites Changes on Obstructive Sleep Apnea Patients After Multilevel Sleep Surgery

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Abstract:

Background: Obstructive sleep apnea (OSA) is caused by partial or complete obstruction of the upper airways. Corrective surgeries aim at removing obstructions in the nasopharynx, oropharynx, and hypopharynx. OSA is associated with increased risk of various metabolic diseases. Our objective was to evaluate the effect of surgery on the plasma metabolome. Methods: This study included 39 OSA patients who underwent Multilevel Sleep Surgery (MLS). Clinical and anthropometric measures were taken at baseline and 5 months after surgery. Results: The mean Apnea Hypopnea Index (AHI) significantly dropped from 22.0 ± 18.5 events/hour to 8.97 ± 9.57 events/hour (p-Value <0.001). The Epworth's sleepiness Score (ESS) dropped from 12.8 ± 6.23 to 2.95 ± 2.40 (p-Value <0.001) indicating success of the surgery in treating OSA. Plasma levels of metabolites, phosphocholines (PC) PC.41.5, PC.42.3, ceremide (Cer) Cer.44.0, and triglyceride (TG) TG.53.6, TG.55.6 and TG.56.8 were decreased (p-Value<0.05) whereas lysophosphatidylcholines (LPC) 20.0 and PC.39.3 were increased (p-Value<0.05) after surgery. Conclusion: This study highlights the success of MLS in treating OSA. Treatment of OSA resulted in improvement in metabolic status that was characterized by decreased TG, PCs and Cer metabolites post-surgery indicating that the success of the surgery positively impacted the metabolic status of these patients.

Keywords: Obstructive sleep apnea, Metabolomics, Triglycerides, Phosphocholines, Ceramides, Apnea Hypopnea Index, Polysomnography, Lipid metabolism, Multilevel Sleep Surgery.

1. Introduction

The rise in worldwide obesity rates has also been paralleled by an increase in Obstructive sleep apnea (OSA) [1]. OSA is associated with disturbed sleep and intermittent hypoxia due to partial or complete cessation of breathing during sleep. OSA causes daytime lethargy and has been linked to road traffic accidents [2] as well as reduced productivity at workplace [3]. OSA rates, reported in literature, are extremely variable mainly due to differences in methods of assessment. The gold standard for diagnosis of OSA is the polysomnography (PSG) test that estimates the Apnea Hypopnea Index (AHI) [1]. AHI is a composite index that is made of Apnea index (AI) which is defined as the complete cessation for ≥10 seconds as well as hypopnea index (HI) which is defined as the reduction in respiratory effort with ≥4% oxygen desaturation. OSA can also be estimated using the Epworth Sleeping Scale (ESS) which is a subjective self-administered questionnaire that estimates the daytime sleepiness by responding to the likelihood (0-3 points) of falling asleep while involved in eight different daily activities. Multiple treatment modalities exist for OSA including weight loss in overweight people, continuous positive airway pressure (CPAP), oral devices as well as surgeries such as bariatric and upper airway surgeries [4-6]. OSA usually involves one or more upper airway levels. Thus, a multilevel sleep surgery (MLS) in a single-stage procedure has been developed as a surgical treatment method for OSA patients that require surgery. The success rate for the procedure is 60% [7,8].

Additionally, soaring rates of OSA are disconcerting as they are associated with increased risk of multiple chronic diseases including metabolic syndrome, Type 2 Diabetes (T2D) and cardiovascular diseases (CVD) [9-12]. It has been linked to the dysregulation of multiple metabolic related pathways such as inflammation, oxidative stress and insulin resistance [13-19]. OSA has also been demonstrated to dysregulate triglyceride metabolism which plays a pivotal role in linking inflammation, oxidative stress and insulin resistance [20]. Hypoxia is one of the hallmarks of OSA. Hypoxia, is marked by downregulation of the activity of lipoprotein lipase (LPL) and thereby regulating the hydrolysis of triglyceride-rich lipoprotein into fatty acids [21-23]. LPL activity is regulated by various factors, including angiopoietin-like (ANGPTL) proteins, such as ANGPTL4, and 8 that were increased in

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people with OSA [24-27]. Other classes of metabolites have been demonstrated to be affected by OSA. For example, acylcarnitines, glycerophospholipids and sphingomyelins were found to be increased in the urine of moderate and severe OSA patients compared to controls [28]. In an earlier study, Ferrarini et al., utilized metabolomics to quantify various phospholipids in people diagnosed as severe and non-severe OSA [29]. Metabolomics is an emerging technique that has been fundamental to enhancing our understanding of global changes in metabolic pathways by allowing the quantification of various metabolites [30-32]. Metabolomics is mainly focused at the quantification and identification of low molecular weight metabolites that can be used for disease diagnosis, drug targets as well as better understanding of cellular pathways involved in disease pathophysiology [30-32]. In order to better understand the role of various classes of metabolites in OSA, we have analyzed the metabolome of people with OSA before and after MLS.

2. RESULTS

Study population characteristics

The study population was composed of 39 patients that underwent MLS. Population characteristics are shown in Table 1. The average time of the repeated investigations were five months after the surgery. Overall, there was no significant changes in BMI and Blood pressure. No significant changes were observed in total cholesterol, HDL, LDL, FG or HbA1c. Slight reduction in the TG level was observed though not significant.

Table 1: Characteristics of all people included in the study before and after surgery.

	Before surgery (N=39)	After surgery (N=39)	p-value
AGE Year			
Mean (SD)	40.0 (10.5)		NA
Median [Min, Max]	40.0 [24.0, 65.0]		
BMI Kg/m2			
Mean (SD)	30.2 (4.41)	29.6 (4.61)	0.121
Median [Min, Max]	30.0 [20.2, 38.3]	30.0 [19.0, 38.5]	
SBP			
Mean (SD)	125 (12.7)	124 (13.0)	0.686
Median [Min, Max]	125 [97.0, 149]	124 [99.0, 151]	

DBP			
Mean (SD)	73.1 (10.2)	76.7 (9.79)	0.248
Median [Min, Max]	72.0 [55.0, 92.0]	77.5 [57.0, 94.0]	
AHI Events/Hour			
Mean (SD)	22.0 (18.5)	8.97 (9.57)	<0.001
Median [Min, Max]	17.0 [5.70, 93.5]	6.10 [0, 42.8]	
AI Events/Hour			
Mean (SD)	2.30 (2.62)	3.64 (6.86)	0.862
Median [Min, Max]	0.900 [0.100, 7.10]	0.700 [0.100, 15.9]	
HI Events/Hour			
Mean (SD)	15.8 (10.1)	6.30 (4.93)	0.193
Median [Min, Max]	13.5 [4.50, 50.0]	5.80 [0, 15.4]	
ESS			
Mean (SD)	12.8 (6.23)	2.95 (2.40)	<0.001
Median [Min, Max]	13.5 [2.00, 24.0]	3.00 [0, 8.00]	
TOTAL CHOL mmol/L			
Mean (SD)	4.96 (1.30)	4.96 (1.30)	0.935
Median [Min, Max]	5.15 [2.90, 9.20]	4.80 [3.30, 9.90]	
HDL mmol/L			
Mean (SD)	1.10 (0.238)	1.10 (0.260)	0.848
Median [Min, Max]	1.05 [0.770, 1.64]	1.11 [0.320, 1.54]	
LDL mmol/L			
Mean (SD)	3.19 (1.21)	3.24 (1.37)	0.976
Median [Min, Max]	3.30 [1.30, 7.50]	3.10 [1.40, 8.70]	
TG mmol/L			
Mean (SD)	1.47 (0.945)	1.36 (0.411)	0.99
Median [Min, Max]	1.26 [0.470, 5.75]	1.25 [0.650, 2.06]	
FG mmol/L			
Mean (SD)	5.77 (1.23)	5.77 (1.08)	0.794
Median [Min, Max]	5.50 [4.70, 11.4]	5.55 [4.70, 9.70]	
HbA1c %			
Mean (SD)	5.69 (0.698)	5.59 (0.552)	0.76
Median [Min, Max]	5.60 [4.60, 8.40]	5.50 [4.60, 7.70]	

BMI: Body Mass index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, AHI: Apnea Hypopnea Index, AI: Apnea Index, HI: Hypopnea index, ESS: Epworth Sleepiness Scale, TOTAL CHOL: Total Cholesterol, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglycerides, FG: Fasting Glucose, HbA1C: Glycosylated Hemoglobin A1C.

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Polysomnographic data

AHI score was used for the diagnosis for OSA. On average, AHI and ESS score were significantly lower after surgery. AHI dropped from 22.0 ± 18.5 events/hour to 8.97 ± 9.57 events/hour (p-Value <0.001) while ESS score dropped from 12.8 ± 6.23 to 2.95 ± 2.40 (p-Value <0.001) (Figure 1).

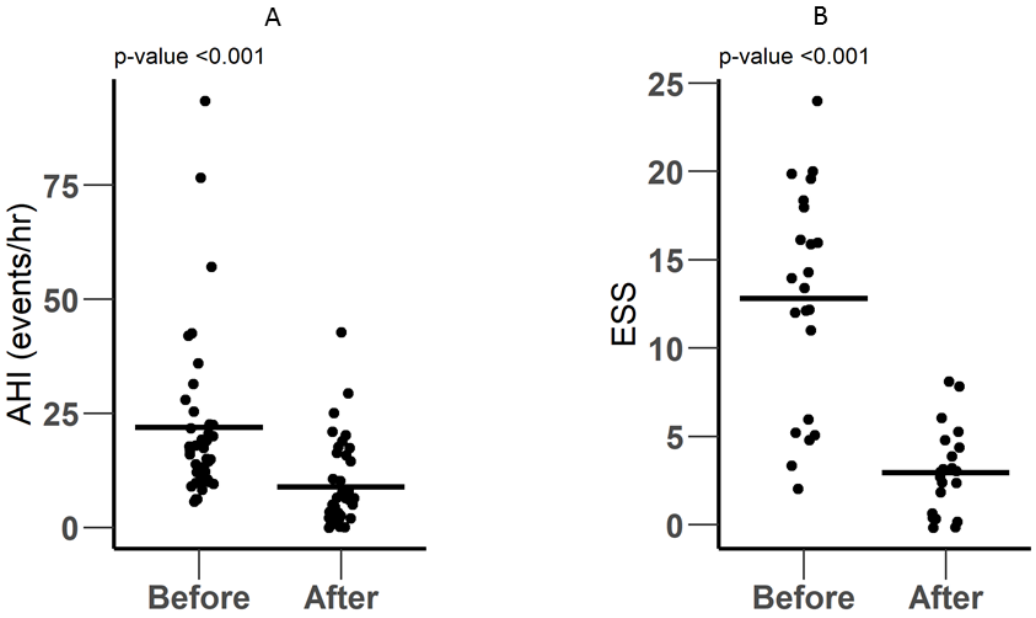

Figure 1: Polysomnographic data showing the AHI score before and after the surgery **A** and the ESS score **B**. OSA was diagnosed based on an AHI >5 events/hour. * p-Value < 0.05 as determined using paired student's t-test.

Metabolomics Analysis of People with OSA after MLS

The biocrates kit p400 was used to quantify a total of 408 plasma metabolites. Out these metabolites, a total number of 256 were identified from all classes of metabolites. The quantified metabolites are shown in Supplementary Table 1. The quantified metabolites included amino acids, biogenic amines, acyl carnitines, glycerophospholipids phosphatidylcholines and lysophosphatidylcholines, glycerides (triglycerides and

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diglycerides), hexoses (including glucose), cholesterol esters and glycerides (triglycerides and diglycerides).

Differentially Expressed Triglyceride Metabolites

The Biocrates P400 kit includes 60 glycerides (42 triglycerides and 18 diglycerides). A total of 51 metabolites were quantified as shown in Supplementary table 1 including 36 triglycerides and 15 diglycerides. Three TG metabolites were significantly decreased after surgery TG.53.6, TG.55.6, and TG.56.8. TG.53.6 was reduced from 0.375 ± 0.242 µM to 0.302 ± 0.162 µM (p-Value=0.01) after surgery (Figure 2A). TG.55.6 was also decreased from 1.27 ± 1.10 µM to 1.05 ± 0.554 µM after surgery (p-Value=0.042) (Figure 2B). The third TG metabolite was TG.56.8 which was decreased from 9.00 ± 5.24 to 7.89 ± 4.57 after surgery (p-Value=0.04) as shown in Figure 2C.

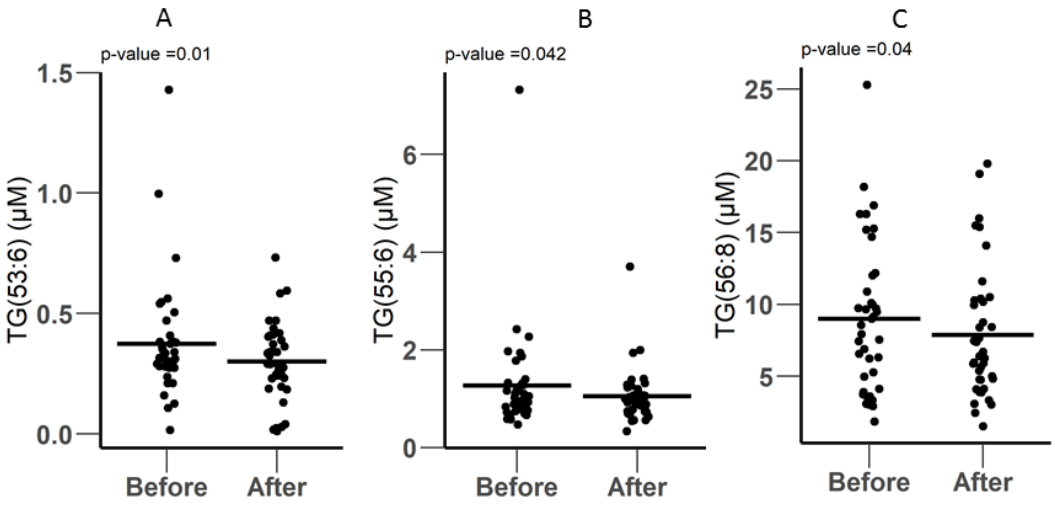

Figure 2: Differentially expressed TGs (A, B, C) before and after surgery as measured by LC-MS using the biocrates P400 kit.

Differentially Expressed Phospholipid Metabolites

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Most of the metabolites included in the Biocrates P400 kit were glycerophospholipids including 172 phosphatidylcholines and 24 lysophosphatidylcholines. A total of 123 metabolites were quantified including 102 PCs and 21 LPCs. Three PCs were differentially expressed including PC.39.3 that was significantly increased in people after the surgery from 0.756 ± 0.277 µM to 0.871 ± 0.307 µM after surgery (p-Value=0.04) (Figure 3A). The other two PCs decreased after surgery were; PC.41.5 which was reduced from 0.367 ± 0.0769 µM to 0.338 ± 0.0657 µM after surgery (p-Value= 0.016) and PC.42.3 was reduced from 0.368 ± 0.269 µM to 0.251 ± 0.242 µM (p-Value=0.043) after surgery (Figure 3B and 3C, respectively). The only LPC metabolite that was differently expressed was LPC.20.0 which increased after surgery from 0.156 ± 0.0347 µM to 0.173 ± 0.0475 µM (p-Value=0.02), Figure 3C.

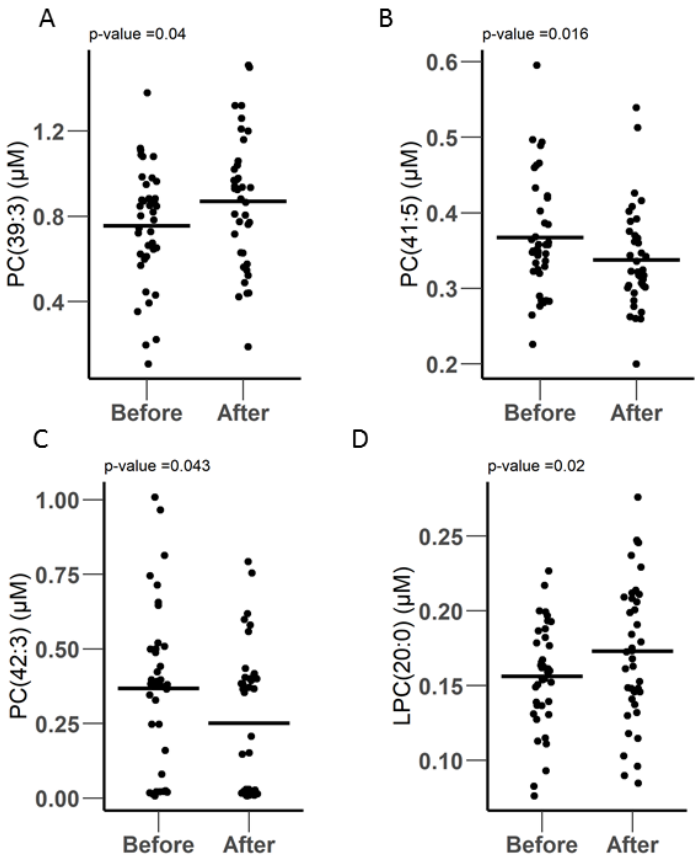

Figure 3: Differentially expressed PCs (A, B, C) and LPC (D) before and after surgery as measured by LC-MS using the biocrates P400 kit.

Differentially Expressed Sphingolipid Metabolites

A total of 55 acyl carnitines were included in the Biocrates P400 kit. Out of them, 48 acyl carnitines were quantified. The only significantly expressed metabolite was Cer.44.0 which was reduced from 0.11 ± 0.03 μM to 0.09 ± 0.02 μM after surgery (p-Value=0.023), Figure 4.

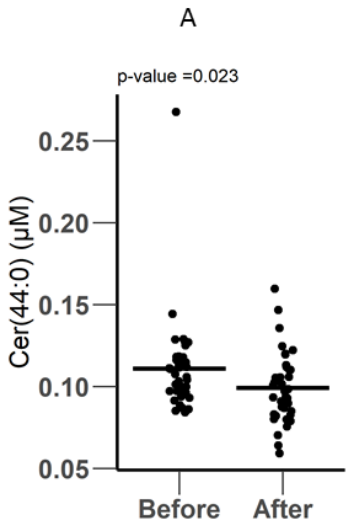

Figure 4: Differentially expressed Ceramides before and after surgery as measured by LC-MS using the biocrates P400 kit.

3. DISCUSSION

OSA is increasingly becoming a major health problem that is aggravated with the increase obesity rates worldwide. Its proper diagnosis requires the use of an expensive and lengthy procedure known as PSG test. Metabolites are showing great promise in advancing our understanding of pathophysiology as well as diagnostic biomarkers.

In this study, we analysed a panel of metabolites of patients were treated for OSA through MLS. The surgical procedures that the participants received is a different

combination of surgeries as indicated by their level of upper airway obstruction, which included tonsillectomy, adenoidectomy, septoplasty (complete list of surgeries is in method section). There was a significant reduction in AHI values following surgery. We compared the metabolome profile of people with successful surgical outcome before and after surgery. Most metabolites did not show any significant change after surgery. Nonetheless, the majority of the differentially expressed metabolites showed a reduction in their level after surgery. These metabolites were triglycerides, sphingolipids as well as phosphocholines. It is important to note that the BMI of the people understudy did not change before and after surgery and thus excluding the impact of obesity on this finding.

Several epidemiological studies have emphasised increased risk for T2D in people diagnosed with OSA independent of obesity [9,10,16,17]. It is known that hypoxia leads to increased inflammation as well as increased insulin resistance. It also mediates the activation of the hypothalamic-adrenal axis and reduce β-cell function [33]. This is indicated in the high prevalence of OSA amongst people with T2D, which has been reported to be at minimum as 24% and can reach about 86% [34]. This is particularly alarming as OSA has been associated with increased vascular complications as well as worse glycaemic control [35-38]. On the other hand, T2D has also been identified as a risk factor for the development of OSA suggesting a bidirectional relationship between T2D and OSA. In a retrospective study examining 360,250 people with T2D and 1,296,489 people without T2D, Subramanian et al., showed that T2D patients are at increased risk for OSA especially male patients with high BMI with diabetic foot diseases, depression, hypertension, CVD as well as patients taking insulin [34].

OSA is an overly concerning underdiagnosed diseases especially in a population with high rates of obesity and diabetes. For example, in the Arabian Gulf region obesity and overweight rates can reach as high as 90% in countries like Kuwait and T2D is around 20% [39,40]. As a result, improved understanding of OSA associated risk with T2D and other chronic diseases is critical because OSA can be treated with various procedures. Multiple treatment modalities exist for OSA including weight loss in overweight people, CPAP, oral

devices as well as surgeries; such as bariatric and upper airway surgeries, such as MLS. [4-6]. The most effective surgical procedure to reduce AHI in MLS is tonsillectomy. Studies have shown that MLS with tonsillectomy was effective in reducing the AHI in 58% of the patients, while if MLS was performed without tonsillectomy, it was effective in reducing the AHI in only 19% [7,8]. All our patients underwent tonsillectomy, combined with other surgical procedures. Tonsillectomy is a very common surgical procedure, particularly in children, to treat OSA as it improves the airflow and improves breathing [41].

Metabolomics analysis showed a reduction in multiple triglyceride species after surgery. OSA has been linked to triglyceride metabolism particularly through intermittent hypoxia (IH), which is one of the hallmarks of OSA [42,43]. In people with OSA, the repeated apnea and hypopnea events, which result in complete or partial cessation in breathing due to the collapse of the upper airway, induce hypoxia [42,43]. The duration of such events determines the reduction in oxygen saturation and the severity of diurnal consequences of OSA. IH has been linked to dysregulated triglyceride metabolism through the inhibition of LPL in adipose tissue [1,14,15,44]. It was also postulated that the inhibition of adipose tissue LPL rather than elevated hepatic TG secretion was responsible for the dysregulated TG metabolism under hypoxic conditions [22]. LPL is responsible for the hydrolysis of TG from TG rich chylomicrons and VLDL to generate energy [45,46]. We have recently shown that two of the important regulators of LPL activity, ANGPTL4 and 8 were increased in people with OSA [24]. Others have also showed that ANGPTL4 was increased in people with OSA [21]. ANGPTL3, 4 and 8 are inhibitors of LPL activity [45,47]. ANGPTL4 is increased under hypoxia through the master regulator of the hypoxic response; hypoxia inducible factor 1 alpha (HIF-1α) [48-50]. Drager et al., showed that the IH driven increase in ANGPTL4 expression has led to atherosclerosis in apolipoprotein E (apoE) knockout mouse model [51].

Furthermore, the same group has recently shown that people with severe OSA exhibited delayed lipoprotein remnants removal as well as decreased lipolysis of TG rich particles. Both processes were positively correlated with the severity of IH and were enhanced by CPAP treatment [52]. Our data are also pointing in the same direction and

highlighting the beneficial impact of MLS in the treatment of OSA. The main advantage of this procedure is the permanent correction for the OSA problem in people undergoing the surgery. The current study is the first report to shed light on the impact of MLS on the metabolic profile of people before and after the surgery highlighting the impact of this surgery on TG and other metabolites.

In our current study, the second class of metabolites that were shown to be reduced are the phospholipids. Phospholipids, also called glycerophospholipids, are important structural components in the lipid bilayer of the plasma membrane. Phosphocholines, are a class of phospholipids that are also part of the plasma membrane and play an important role as signaling molecules [53-55]. Interestingly, two PCs were increased following the surgical treatment of OSA. Previously, Lebkuchen et al., showed that people with OSA had a reduction in PCs [56]. They linked the observed reduction in PCs to the increased damaging activity of various phospholipase A1 (PLA1), A2 (PLA2) and C (PLC), which are activated under hypoxic conditions. PLA2 is required for the remodeling and repair of cell membranes. The activation of PLA2 in children with OSA was connected to endothelial dysfunction [57].

Finally, in our study one species of Lysophosphatidylcholine (LPC) was increased in OSA people after surgery. LPC is LPCs related to PCs as they are derived from their turnover in circulation by PLA2. Generally, LPCs have been positively associated with cardiovascular and neurodegenerative diseases. Species of this family have been recognized as diagnostic markers for myocardial infarction ((LPC 17:0 and LPC 18:2) and were suggested to associate with systemic inflammation [58]. They were also linked to promoting the fatty acid induced insulin resistance. In line with our data, Lebkuchen et al., showed that species of LPC were upregulated by OSA [56]. This finding could possibly show a different pattern of expression of LPCs or could be due to one of the main limitations of this study, which is the limited number of participants dictated by the nature of our study with surgery intervention.

In conclusion, the current study demonstrated the positive impact of MLS on the treatment of OSA, where AHI values were dramatically reduced after the surgery. It also

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exhibited reduction in TG metabolites that could be indicative of the improved metabolic state after OSA treatment.

4. MATERIALS AND METHODS

Study population and ethical statement

The study was approved by the ethical review board of the Dasman Diabetes Institute and conducted in accordance with the Declaration of Helsinki ethical guideline Study number RA 2015-043. Written informed consent was obtained from all subjects prior to participation in the study. All patients who underwent MLS were followed-up for at least 6 months. Inclusion criteria was those who underwent MLS and completed a pre-operative and post-operative level 1 polysomnography (PSG), pre-operative and post-operative ESS, Pre-operative and post-operative blood metabolites, and we recorded their medical history, and patient's data, such as Body Mass Index (BMI). Exclusion criteria were patients with medical diseases, such as Diabetes, Hypertension, and cardiovascular disorders. The total participants who met our inclusion criteria were 39 participants.

OSA Assessment and The Surgery Procedures

The participants were diagnosed with OSA according to a level 1 polysomnography (PSG), if their Apnea-Hypopnea Index (AHI) was more than 5 events/hr. Furthermore, we evaluated other parameters of the PSG, such as apnea events and hypopnea events. The PSG sleep was performed at baseline and at least three months post-operatively. Moreover, the participants completed the ESS during the time of their PSG sleep study. The BMI was calculated using the standard BMI formula: body weight (kg) / height (m^2). All participants were undergoing surgery were carefully selected and an individualized procedure was performed, according to the site of the obstruction (oropharynx, hypopharynx, and/or nasopharynx). The principal author (ALT) was the sole sleep apnea surgeon for all the patients. After the individualized MLS was planned for the patient, the procedure was started with Drug Induced Sleep Endoscopy (DISE), to further identify the obstruction sites.

Afterwards, we proceeded with MLS, aiming to relieve the site of obstruction that was present in the patients (Oropharynx, Hypopharynx, and/or nasopharynx). Upon completion of the surgical procedures, the patients were kept in the hospital for further evaluation and monitoring and were discharged once they were stable. They were followed in the sole sleep surgeon outpatient clinic two weeks, six weeks, three months, and six months post-operatively. If any further follow-up visits were needed, the patients scheduled their appointments. They repeated the level 1 PSG, ESS, and blood investigations at least 3 months after the surgical procedure.

Blood collection and anthropometric and biochemical measurements

A fasting blood sample was collected twice from each participant, before the MLS operation and after five months from the operation. Blood samples were collected in a vacutainer EDTA tube where the plasma was separated by centrifugation at 400 ×g for 10 min. The plasma was then aliquoted and stored at −80°C until assayed as previously reported [47,59,60]. Blood pressure was measured using an Omron HEM-907XL digital sphygmomanometer. The mean blood pressure of three readings was recorded. Clinical parameters including the Fasting blood glucose (FBG), triglyceride (TG), total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured using the Siemens Dimension RXL chemistry analyzer (Diamond Diagnostics, Holliston, MA). The Glycated hemoglobin, HbA1c, was measured using the Variant^{TM} device (Bio-Rad, Hercules, CA).

Metabolomics analyses

Plasma metabolites were analyzed by quantitative targeted metabolic analysis method using the Absolute IDQ- p400 HR kit (Biocrates Life Science AG, Austria). This kit provides quantitative data on 408 metabolites that span 11 classes of metabolites including 21 amino acids, 21 biogenic amines, 55 acyl carnitines, 196 glycerophospholipids (172 phosphatidylcholines and 24 lysophosphatidylcholines), 60 glycerides (42 triglycerides and 18 diglycerides), hexoses (including glucose), 14 cholesterol esters. Quantification is based on a combination of Liquid Chromatography-High Resolution Mass Spectrometry (LC-

HRMS) that is used for the quantitation of amino acids and biogenic amines. The second part is used to quantify all other metabolites using a Flow Injection Analysis- HRMS (FIA-HRMS). Quantification of the LC-HRMS metabolites is based on a 7-point calibration curve unlike the FIA-HRMS which uses a single point calibrator. Quality control samples were included in each plate that included by injecting zero sample, three blanks and three quality control (QC) levels (QC 1-3), of which the QC2 medium level was injected five times.

According to manufacturing instructions, calibrators, quality controls and the internal standard were diluted to required concentration. After that all samples including the plasma samples and QCs were centrifuged at 2750 x g at 4°C. Briefly,10 µL of plasma was pipetted into a 96-well kit plate containing the internal standards and the samples were dried for 30 min using Nitrogen Evaporator. Samples were then derivatized with 50 µL of 5% PITC solution in water:ethanol:pyridine at a ratio of 1:1:1. The plate was incubated for 20 min before drying again under nitrogen for 60 minutes. After that, samples were extracted by the addition of 300 ul of 5 mM ammonium acetate in methanol and shaking at 450 rpm for 30 min at room temperature and collected in a capture plate for FIA-HRMS run, then 250 µL of FIA mobile phase was added to each well of the original capture plate. A total of 150 µL from the capture plate was then transferred to another plate and diluted with 150 µL LC-MS grade water for the LC-HRMS run. The samples were then analysed on high-resolution Q-Exactive HF hybrid quadrupole -Orbitrap mass spectrometer (Thermo Scientific) which is equipped with electrospray ionization source coupled to Vanquish Duo UHPLC system. instruments were controlled using XCalibur 4.1 and Q-Exactive HF tune software V 2.9 SP4All. All parameters were set according to the Biocrates instructions. All solvents used were LC-MS hyper grade from Thermo Fisher Scientific. Analysis was done in the positive and negative ionization modes for both LC-HRMS and FIA-HRMS respectively. The mobile phase A was 0.2% formic acid in H_2O and mobile phase B 0.2% formic acid in Acetonitrile. The LC-HRMS chromatographic program was 5.8 min gradient at 0.8 mL/min flowrate at 50 °C. In the FIA-HRMS run, 20 µL of the sample was injected and analyzed for 3 min at 0.05 mL/min for the first 1.6 min and then increased to 0.2 mL/ min for 1.2 min and then back to 0.05 mL/min for the rest of the program. The LC-HRMS data was pre-processed via

XCalibur Quan 4.1 software. All data from the three runs for each sample was processed using the Biocrates Met*I*DQ Nitrogen software. Statistical analysis was performed with the Met*I*DQ StatPack module.

Statistical analysis

For assessing the normality of the data, Shapiro Wilk test was performed. Based on the results of normality test, Paired Student's t-test or Wilcoxon rank-sum test was used for comparisons between subjects before and after upper airway surgery. All data were reported as mean ± standard deviation. Statistical assessments were two-sided and considered significant at $p < 0.05$. All analyses were performed using R: A Language and Environment for Statistical Computing (version 3.6.1)

5. LIST OF ABBREVIATIONS

AI: Apnea Index

HI: Hypoapnea Index

AHI: Apnea/hypopnea index

BMI: Body mass index

CV: Coefficients of variation

HDL: High-density lipoprotein

LDL: Low-density lipoprotein

ELISA: Enzyme-linked immunosorbent assay

TC: Total cholesterol

FBG: Fasting blood glucose

HbA1C: Glycated haemoglobin

ANGPTL: Angiopoietin-like protein

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

TG: Triglyceride

IH: Intermittent hypoxia

MLS: Multilevel Sleep Surgery

PSG: Polysomnography

CPAP: Continuous Positive Airway Pressure

AUTHOR CONTRIBUTIONS: ALT: Study design, Surgeon, Critically revised manuscript, SJ: Metabolomics Analysis, TAT: Data analysis and critically revised manuscript, IA & PC: Sample Analysis, AC & DS: Data analysis, ME & AI: Clinical data analysis and compilation, AT: Metabolomic analysis, FA: Study design and critically revised manuscript, JA: Study design and wrote the manuscript, MA: Study design, Metabolomic analysis and wrote the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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