

Effect of positive pressure ventilation and bariatric surgery on extracellular vesicle microRNAs in patients with severe obesity and obstructive sleep apnea

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INTRODUCTION: Obstructive sleep apnea (OSA) and severe obesity share a common pathophysiological phenomenon, systemic and tissue hypoxia. Hypoxaemia modifies microRNA expression, particularly, extracellular vesicles microRNAs which are involved in the progression of cardiovascular diseases, metabolic syndrome and cancer. We aim to evaluate extracellular vesicle miRNAs among patients with severe obesity with and without OSA and the effect of OSA and severe obesity treatment: continuous positive airway pressure (CPAP) and bariatric surgery.

METHODS: Patients were selected from the Epigenetics Modification in Morbid Obesity and Obstructive Sleep Apnea (EPMOOSA) study (NCT03995836), a prospective observational study of patients undergoing bariatric surgery. Patients were divided into OSA (Apnea-hypnoea index (AHI) > 10) and non-OSA (AHI < 10). Patients with OSA were treated with CPAP for 6 months. Then, all patients had bariatric surgery and re-evaluated 12 months later. At each visit, blood samples were obtained for biobanking. Subsequently, extracellular vesicles were extracted, and then, miRNA expression was analysed.

RESULTS: 15 patients with OSA and 9 without OSA completed the protocol. At baseline, patients with OSA showed higher miR16, miR126 and miR320 ($p < 0.05$) and lower miR223 expression ($p < 0.05$) than those without OSA. In patients with severe obesity and OSA, after 6 months with CPAP, we observed a significant decrease in miR21 ($p < 0.01$), miR126 ($p < 0.001$) and miR320 ($p < 0.001$), with no changes in any miRNA in patients without OSA. No changes were detected in any miRNA after 6 months of bariatric surgery in patients with or without OSA.

CONCLUSION: Co-existence of OSA and severe obesity alters the profile of extracellular vesicle miRNAs. Bariatric surgery and weight loss did not reverse this effect meanwhile the treatment with CPAP in patients with severe obesity and OSA showed a recovery outcome in those extracellular vesicle miRNAs. Those facts remark the need for OSA screening in patients with severe obesity.

CLINICAL TRIAL REGISTRATION: The study has also been registered at ClinicalTrials.gov identifier: NCT03995836.

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INTRODUCTION

Severe obesity and obstructive sleep apnea (OSA) are closely related diseases. Obesity is considered by the World Health Organisation to be a pandemic that, in particular, affects first-world countries [1]. This pandemic is accompanied by an increase in the prevalence of OSA, affecting, based on a recent systematic review, between 10 and 38% of the general population [2].

Patients with OSA suffer multiple nocturnal episodes of partial (hypopnea) or total (apnea) obstruction of the upper airway by a multifactorial, i.e. anatomical, muscular and neurological, mechanism [3]. Patients with severe obesity have greater susceptibility to OSA due to involvement of anatomical and muscular components

as a consequence of fat accumulation at the cervical level. Apnea results in hypoxaemia/hypercapnia and produces arousal, closing the cycle known as chronic intermittent hypoxia and releasing inflammatory mediators [4].

MicroRNAs (miRNAs) are small noncoding RNA between 21 and 25 nucleotides that silence gene expression at the posttranscriptional level [5]. MiRNAs play a fundamental role in different cellular processes, such as differentiation, proliferation and apoptosis [6–8]. MiRNAs are released to the exterior bound to HDL cholesterol molecules either in free form or packaged in extracellular vesicles (EVs). Storage and transport in EVs protects miRNAs from external degradation and causes them to target

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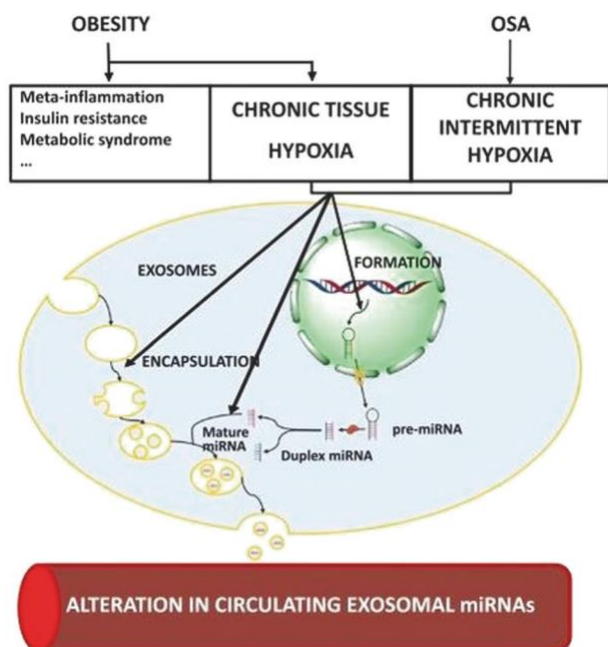


Fig. 1 Hypothetical relationship between chronic tissue hypoxia due to obesity and chronic intermittent hypoxia due to obstructive sleep apnea [16]. Both facts could participate in the modification of microRNA (miRNA) formation, maturation and encapsulation in EVs, which finally is translated into alterations of circulating exosomal miRNAs.

selectively cells based on EVs membrane proteins [9]. EVs are a heterogeneous group of nanosized membranous vesicles that participate in the cell-to-cell communication [10]. They are involved in both physiological and pathological processes [11].

Severe obesity is characterised by hyperplasia and hypertrophy of adipose tissue with poor blood supply that deteriorates in the postprandial period [12], causing chronic hypoxia. The relationship between chronic hypoxia and circulating miRNA is well studied. The presence of OSA produces repeated episodes of intermittent hypoxia. Both chronic and intermittent hypoxia have been shown to alter the expression of miR210 [13, 14] and miR21 [15], respectively.

We hypothesise that both: severe obesity and OSA, through respectively chronic and intermittent hypoxia, modify miRNA formation, maturation and their cargo on EVs, which finally alter the epigenetic profile of those patients (Fig. 1) [16]. Furthermore, we suggest that anomalies in the extracellular vesicle miRNAs that are inherent to the severe obesity state are modified after bariatric surgery and that there are changes in the exosomal load of miRNA secondary to the action of OSA that will be modified with continuous positive airway pressure (CPAP) treatment. Our objective, therefore, is to determine, in patients with severe obesity who are candidates for bariatric surgery, the differences in a battery of extracellular vesicle miRNAs associated with inflammation and cardiovascular risk between subjects with and without associated OSA and the changes in those miRNAs after treatment with CPAP and bariatric surgery.

METHODOLOGY

Patients were recruited and selected from the Epigenetics Modification In Morbid Obesity and Obstructive Sleep Apnoea (EPIMOOSA) study (ClinicalTrials.gov identifier: NCT03995836) [16]. EPIMOOSA is an observational study of a cohort of patients with severe obesity who were candidates for bariatric surgery in a second-level hospital. All patients included in the study were older

than 18 years, diagnosed with severe obesity and in bariatric surgical waiting list. The recruitment of patients extended from July 1st, 2014 to July 1st, 2016. The included patients had BMI greater than 40 kg/m² without having achieved sustained weight loss through diet or a BMI between 35 and 40 kg/m² with severe comorbidities (diabetes mellitus, dyslipidaemia, high blood pressure, etc.). Patients with obesity hypoventilation syndrome; being treated with CPAP; pregnant; diagnosed with systemic disease; or having suffered a cardiovascular event (heart attack, stroke, atrial fibrillation, etc.) in the last 6 months or a malignant neoplasm of any origin in the last 5 years were excluded. The study was approved by the Regional Institutional Review Board of Aragon, Spain (IRB23052014). In accordance with recommendations from the Declaration of Human Rights, the Helsinki Conference patients must sign the informed consent form before being enrolled in the study. All patients signed informed consent.

Timeline

Data were obtained from a total of 3 visit: initial visit, 6 months after the initial visit and 1 year after bariatric surgery (Supplementary Table S1). At the first and last visits, a respiratory polygraph was performed (Apnoea Link ResMed®, Sydney, Australia). We defined apnea as the absence of flow for more than 10 s and hypopnea as a reduction in airflow by more than 50% for more than 10 s, accompanied by a decrease in oxygen levels greater than 4% [17]. The apnea-hypopnea index (AHI), expressed as the sum of apneas and hypopneas per hour of the recorded time period, was calculated. Data for the following variables were obtained for analysis: AHI, average oxygen saturation (SatO₂), minimum SatO₂, and percentage of time with

a SatO₂ below 90% (CT₉₀). At each visit, a blood sample was obtained for biobanking, and for the first and last visits, an arterial blood gas test was performed.

Patients who had an AHI above 10 events/h were diagnosed with OSA, and based on the clinical practice guidelines for the management of OSA of the Spanish Society of Respiratory System Pathology (Sociedad Española de Patología del Aparato Respiratorio—SEPAR) [17], treatment with CPAP was started. For those without OSA, no intervention was performed. At 3 months, CPAP pressure was titrated using an auto-CPAP (Autoset T; ResMed®, Sydney, Australia).

Extracellular vesicle isolation and miRNA expression

Whole blood samples were drawn using a 21G butterfly needle into EDTA BD Vacutainer blood collection tubes and were then centrifuged at 3000 × g at 4 °C for 15 min. Plasma obtained at each visit were frozen at -80 °C. EVs from frozen plasma were analysed based on the protocol published by our research group [18]. Once the plasma was thawed, a miRCURY Exosome Isolation Kit (Qiagen, Hilden, Germany) was used to isolate EVs. Then, RNA was extracted from the EVs using miRCURY RNA Exosome Isolation Kit (Qiagen, Hilden, Germany). RNA analysis was performed using quantitative RT-qPCR. RNA was reverse transcribed using a miRCURY LNA Universal RT microRNA RT-qPCR Kit (Qiagen, Hilden, Germany) and the miRCURY assay (Qiagen, Hilden, Germany) for each miRNA described in Table S2. RT-qPCR was used to quantify the miRNA following the specifications of the manufacturer cited above. Cycle threshold (C_t) values, the minimum number of RT-qPCR cycles necessary for a quantifiable signal to be produced, were acquired and processed using the $\Delta\Delta C_t$ method described by Livak and Schmittgen in 2001 [19]. Finally, based on the mathematical development proposed by Livak, the final value was obtained after exponential transformation of $\Delta\Delta C_t$, $2^{-\Delta\Delta C_t}$. This value expressed the results as a fold change (FC). The set of miRNAs described in the Supplementary Table 2 was selected based on the previous work of our group that described their stability along time in healthy subjects and by the relationship of those miRNAs with cardiovascular outcomes such as

atherosclerosis that are commonly associated with severe obesity and OSA.

Statistical analysis

Descriptive data of the sample are expressed as the mean \pm standard deviation when the variables are continuous. For the qualitative variables, frequency distributions were obtained. After assessment of normality distribution, the results obtained for patients with/without OSA were analysed using nonparametric statistics: Mann–Whitney *U* for intergroup comparisons and the Kruskal–Wallis *H* test for intragroup evolution throughout the visits. The statistical packages SPSS 20.0 (IBM) and GraphPad Prism 8 (GraphPad Software) were used for graphical expression.

RESULTS

Subjects characteristics

Figure 2 shows the flowchart of the patients recruited for the study. First, 124 patients were selected from the surgical waiting list. After applying the exclusion criteria, that number was reduced to 70, and, finally, 24 patients (15 OSA and 9 non-OSA) completed all study visits. The differences between patients with and without OSA were compared both in the group that only attended the initial visit ($n = 70$) and those who finished the study ($n = 24$) as described Table 1. In both groups, patients with and without OSA were morphologically similar, i.e. no difference in BMI, but the former had a greater neck circumference (mean and standard deviation: 44 ± 3.7 vs. 40 ± 3 , $p < 0.001$) and included a higher percentage of men (53% vs. 15%, $p < 0.001$). Patients with OSA were older (48 ± 8 years versus 40.5 ± 8 years, $p < 0.001$) and showed a higher prevalence of comorbidities. For the group of patients who completed the study, those in the OSA group were, on average, older (49 ± 9 years versus 42.5 ± 8 years), but the difference was not statistically significant. Same happened with abdominal circumference and systolic blood pressure (SBP), with higher values for both variables in the OSA group (abdominal circumference: 128 ± 13 cm versus 118 ± 15 cm; SBP: 135 ± 13 mmHg vs. 129 ± 10 mmHg).

Effect of OSA in extracellular vesicles miRNAs in patients with severe obesity

We evaluated a set of extracellular vesicles miRNAs (Supplementary Table S2) in the patients that completed the study ($n = 24$). At baseline, as shown Fig. 3, four miRNAs displayed significant changes between the group of severe obesity with and without OSA. Three of them, miR16, miR126 and miR320, were increased in patients with both conditions ($FC_{miR16} = 2.06$; $FC_{miR126} = 1.8$;

$FC_{miR320} = 2.1$; $p < 0.05$ for all) while miR223 was significantly lower in this group of patients ($FC_{miR223} = 0.60$; $p < 0.05$). When adjusting by sex, we also found that miR16, miR126 and miR320 were overexpressed in women (Table S3). We also evaluated the effect of age differences between groups but we did not find any correlation between miRNA expression and age (Table S4).

Changes in extracellular vesicles miRNAs after CPAP and bariatric surgery

After the cross sectional analyses of the extracellular vesicles miRNAs at baseline, we evaluated the effect of therapies for OSA and along time. Five of the miRNAs analysed showed significant changes in patients with severe obesity and OSA after 6 months of treatment with CPAP while no changes were observed in patients with severe obesity. As it is shown in Fig. 4, miR16 ($p < 0.001$), miR21 ($p < 0.01$), miR126 ($p < 0.001$), miR146 ($p < 0.05$) and miR320 ($p < 0.001$) were significantly reduced after CPAP therapy. As result of this, the differences observed between severe obesity with and without OSA at baseline disappeared for EVs miR16, miR126 and miR320.

In the third visit, after 12 month from surgery, no change was displayed in the group of patients with severe obesity. Patients with both conditions showed after bariatric surgery a significant increase for miR146 respect to visit after 6 months ($p < 0.01$). Bariatric surgery recover the values of baseline for this miRNA and that were reduced after 6 months of CPAP therapy ($p < 0.05$) showing no change respect baseline although CPAP treatment continues after bariatric surgery. On the other hand, changes respect to baseline for EVs miR16 ($p < 0.001$), miR126 ($p < 0.001$), and miR320 ($p < 0.01$) remain similarly to those produced after CPAP. Finally, EV miR223, which showed lower expression in patients with severe obesity and OSA at the initial visit, was unchanged at the follow-up visits as consequence of CPAP or bariatric surgery.

DISCUSSION

In this work, we present novel results that show differences, at the epigenetic level, between patients with severe obesity with and without OSA, suggesting the importance of diagnosing this pathology with high prevalence in this type of subject. In addition, we evaluated the impact that different treatments have on the expression of a battery of extracellular vesicles miRNAs related to atherosclerosis, with a major effect of CPAP rather than bariatric surgery.

OSA prevalence in our sample was close to 60%, and although it is within the range described in the literature, it below the value reported [20–24]. In this sample, patients with severe obesity and OSA suffer a greater number of comorbidities than severe obesity without OSA, similar to other studies with lean patients with/without OSA [25]. Though all participants were people with severe obesity, the overlap of severe obesity and OSA exhibited higher neck and abdominal circumference, comparable to lean patients with OSA [26].

Both in our study and in the publications of patients pending bariatric surgery, the majority are women [27]. In lean patients, female sex has been associated with a lower risk of OSA [20, 28, 29]. However, in women with severe obesity, OSA prevalence could be as high as 86% [24], even though it doesn't mean the same risk among both sex [30]. Age is higher in patients with OSA. The lower prevalence of OSA in our sample is not due to the number, women included, or age. We put these differences down to the inclusion and exclusion criteria, which implied the selection of patients who had never undergone a sleep study or CPAP treatment.

We described significant differences in miR16, miR126, miR223 and miR320 expression between patients with severe obesity with or without OSA at baseline. EV miR223 was lower in patients with

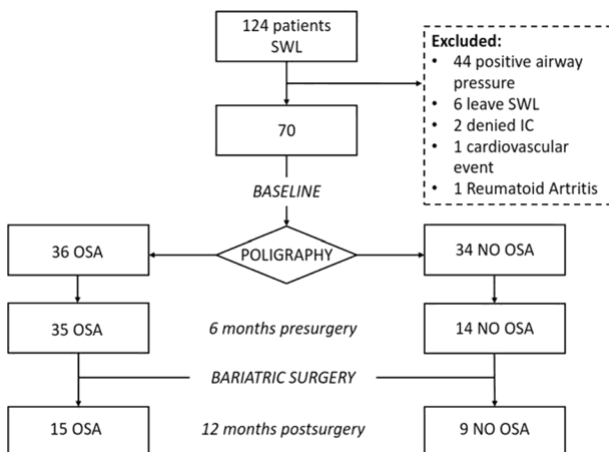


Fig. 2 Flowchart. SWL Surgical Waiting List. IC Inform consent. OSA Obstructive Sleep Apnea.

Table 1. Description of the sample.

Variable	All patients enrolled		Completed the study	
	Without OSA	With OSA	Without OSA	With OSA
<i>n</i>	34	36	9	15
Sex [female; <i>n</i> (%)]	29 (85.3)	17 (47.2)***	8 (88.9)	6 (40)*
Age (years)	40.5 ± 7.9	47.9 ± 8.2***	42.4 ± 7.9	49.6 ± 9
Weight (kg)	113.4 ± 13.8	121.1 ± 16.4*	116.3 ± 13.2	117 ± 13.1
BMI (kg/m ²)	41.6 ± 3.8	43 ± 4.8	42 ± 1.5	42 ± 3.1
Neck circumference (cm)	40.2 ± 3.1	43.9 ± 3.7***	40.4 ± 4.2	44.6 ± 4.1**
Abdominal circumference (cm)	119.1 ± 10.6	129.3 ± 13.3***	118.4 ± 14.7	127.8 ± 12.8*
Hip circumference (cm)	134.1 ± 9.1	134.4 ± 14.1	136.1 ± 10.9	129.9 ± 10
Waist-to-hip ratio	0.89 ± 0.09	0.96 ± 0.11**	0.87 ± 0.14	0.99 ± 0.12*
Epworth	7.2 ± 3.5	7.9 ± 4.4	8.8 ± 4	9.2 ± 4.8
AHI (events/hour)	3.8 ± 2.3	32.9 ± 23.6***	4.5 ± 3	35.5 ± 21.7***
CT ₉₀ (%)	4 ± 7.5	33.2 ± 27.9***	1.7 ± 1.7	30.4 ± 23.9***
SatO ₂ (%)	93.8 ± 1.2	91 ± 2.6***	94.3 ± 1.1	91.2 ± 2.1***
Active smoker	5 (14.7)	10 (27.8)	4 (16)	7 (33.3)
Ex-smoker	11 (32.4)	19 (52.8)	8 (32)	9 (42.9)
Never smoker	18 (52.9)	7 (19.4)*	13 (52)	5 (23.8)
High blood pressure	14 (41.2)	23 (63.9)*	6 (66.7)	10 (66.7)
Dyslipidaemia	6 (17.6)	20 (55.6)***	1 (11.1)	10 (66.7)**
Diabetes mellitus	9 (26.5)	20 (55.6)**	1 (11.1)	9 (60)**
Cardiovascular event	3 (8.8)	4 (11.1)	1 (11.1)	2 (13.3)
Asthma	2 (5.8)	3 (8.3)	0	1 (6.7)
Consumption of sedative drugs	8 (23.5)	7 (19.4)	1 (11.1)	2 (13.3)
FEV ₁ (l)	3.19 ± 0.84	2.99 ± 0.9	3.36 ± 0.98	2.86 ± 0.75
FEV ₁ (%)	100.9 ± 13.8	93.8 ± 17.4*	103.2 ± 5.4	88.5 ± 15.3**
SBP (mmHg)	129.5 ± 13.7	140.6 ± 16.2**	129.4 ± 10	135.5 ± 13.6
DBP (mmHg)	85.4 ± 8.4	87.9 ± 11.4	84.4 ± 8.7	90 ± 12.2

OSA obstructive sleep apnoea, *BMI* body mass index, *AHI* apnoea-hypopnea index, *CT₉₀* percentage of time with oxygen saturation below 90%, *SatO₂* oxygen saturation, *FEV₁* forced expiratory volume in the first second, *SBP* systolic blood pressure, *DBP* diastolic blood pressure.

p* < 0.05; *p* < 0.01; ****p* < 0.001.

both conditions than those without OSA, while for the other 3 miRNAs, expression in patients with severe obesity and OSA was between 1.8 and 2.1 times higher. As seen in Table 2, the vast majority of studies analyse a wide variety of circulating miRNAs from different tissues, but none of them take into account the comorbidity OSA in severe obesity neither the miRNAs from extracellular vesicles.

The increase in miR320e in patients with severe OSA (AHI > 30) has been described in a case-control study in male patients with obesity [31]. Additionally, miR320b, together with miR126-3p, presents lower expression in subjects with OSA with and without hypertension than in healthy individuals [32]. miR16 plays an antiapoptotic role in tumour processes [33]. Its in vitro overexpression has been studied in situations of hypoxia and reoxygenation in myocardial cell cultures, which increases cellular apoptosis [34]. In our patients with severe obesity and OSA, miR16 was overexpressed, which could contribute to the deleterious effects that intermittent hypoxia has in these patients.

The lower expression of miR223 in patients with severe obesity and OSA has not been described in other clinical studies. However, in vitro and in vivo studies that analysed the effect of chronic hypoxia in pulmonary artery smooth muscle cell cultures and in mice have shown that hypoxia triggers a decrease in miR223 expression, favouring the remodelling and thickening of pulmonary vascular smooth muscle [35]. miR223 overexpression in

patients with severe obesity without OSA could be preferentially driven by an outlier, however, after applied the adequate statistical test, this hypothesis was negative. To support the decreasing effect of OSA on this miRNA, additional assays should be performed in the future.

The increase in blood pressure decreases the expression of miR126 [36, 37]. Yang et al. examined whether the expression of miR126 was different in patients without obesity and OSA with and without hypertension [32]. The authors concluded that there was a decrease in the expression of circulating miR126 in patients with OSA and, therefore, independent of hypertension. In our study, patients with both conditions, severe obesity and OSA, exhibited higher expression than those without OSA. This finding is consistent with the conclusion of that work.

Regarding miR320, in an article by Karolina et al. [38] the behaviour of a battery of miRNAs, was analysed in healthy individuals and patients with metabolic syndrome, dyslipemia, diabetes or hypertension. In that study, they found that miR320 was part of the *miRNA cluster* related to diabetes but not to hypertension. In the study by Yang et al. [32] the expression of circulating miR320 was lower in patients with OSA without hypertension than in healthy controls but not in patients with hypertension and OSA. Thus, we propose that the results of our study may be due to the effect that diabetes has on the expression of miR320, independent of hypertension.

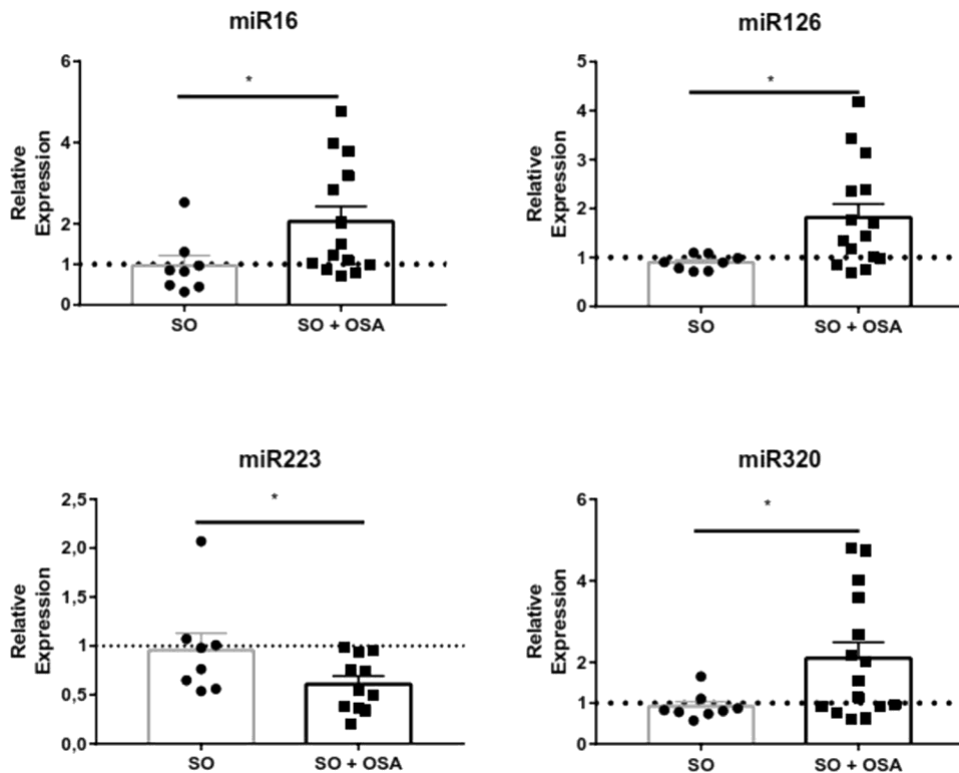


Fig. 3 Circulating EV-miRNA relative expression differences in patients with severe obesity with/without obstructive sleep apnea. SO severe obesity. SO + OSA severe obesity with obstructive sleep apnea. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Patients with severe obesity and OSA who were treated with CPAP showed a significant decrease in EV miR16, miR21, miR126 and miR320. This modification in miRNA expression induced by CPAP removed the differences observed at the initial visit. The effect of CPAP treatment on EVs and the expression of circulating miRNAs has already been studied [39–43]. Data from the randomised control trial “Effect of CPAP treatment in the Control of Refractory Hypertension” (HIPARCO study) [40, 42] showed that significant variations were produced with 3 and 6 months of treatment with CPAP. Although the results from this study are similar, it must be taken into account that the HIPARCO study analysed free circulating miRNA, while ours analysed miRNAs contained in extracellular vesicles. On the other hand, only two studies analysed the effect that CPAP has on people with severe obesity [41, 43]. Both studies had as main objectives to analyse the behaviour of circulating extracellular vesicles in subjects with hypoventilation and obesity syndrome. The work of Bhattacharjee et al. [43], that was conducted with 12 patients, mostly African Americans, indicated that treatment with a CPAP or bi-level positive airway pressure (BiPAP) device modified EV miRNA expression, including miR16.

After bariatric surgery, the greatest weight loss occurred, with a decrease of 11 kg/m² in the sample as a whole, representing a loss of 25% of excess weight. Patients with OSA experienced a greater decrease than patients without OSA, although these differences were not significant. The greatest weight loss is achieved in the first year after bariatric surgery, and although it continues into the second year, weight loss should plateau over the years [44, 45]. At the last visit, there were significant differences in miR16, miR21, miR126 and miR320 expression compared to the first visit in patients with severe obesity and OSA. However, we cannot attribute these differences to weight loss because treatment with CPAP contributed. In case of miR146, this miRNA showed a behaviour that it has not previously described, showing a significant decrease after CPAP therapy but after weight loss with

bariatric surgery, the expression level of this miRNA was recovered to those observed at baseline.

Two works have previously analysed extracellular vesicles miRNAs in patients with severe obesity before and after bariatric surgery [46, 47]. The work by Hubal et al. [46] was carried out in 6 African American women and analysed adipocyte-derived extracellular vesicles miRNAs and their influence on insulin resistance. Unlike our study, they analysed EVs derived from culture of human adipocytes while our study was performed in circulating plasma EVs. Of all the miRNAs that showed significant changes after bariatric surgery, miR16 and miR125 stood out. Both acted selectively on 37 mRNAs related to insulin signalling, but a significant correlation was only established between miR16 variation and improvement in insulin resistance 1 year after bariatric surgery. Another study carried out by Bae et al. [47], the behaviour of 12 patients with nondiabetic nor hypertensive with severe obesity was analysed before bariatric surgery and 6 months later. They used a group of healthy volunteers as controls. In their analysis, they showed that weight loss induced by bariatric surgery modified the expression of EV-miRNA. Neither study analysed the influence of OSA on their results. Another study analysed the temporal evolution of circulating miRNAs in the first 12 months after bariatric surgery [48]. Among the modified miRNAs were miR16, which was modified at 9 months, and miR21 and miR320, which were modified at the last visit, coinciding with the greatest weight loss. Once again, the existence of OSA was not taken into account. In our review, the expression of multiple circulating miRNAs was altered by weight loss induced by bariatric surgery, but miR320 expression was the most frequently altered [48–51].

This study obtained interesting and previously undescribed results in a scarcely studied field: the influence of OSA on the expression of EV-miRNA in patients with severe obesity. However, we must highlight some limitations that could affect the interpretation of the results. Although the objective of including

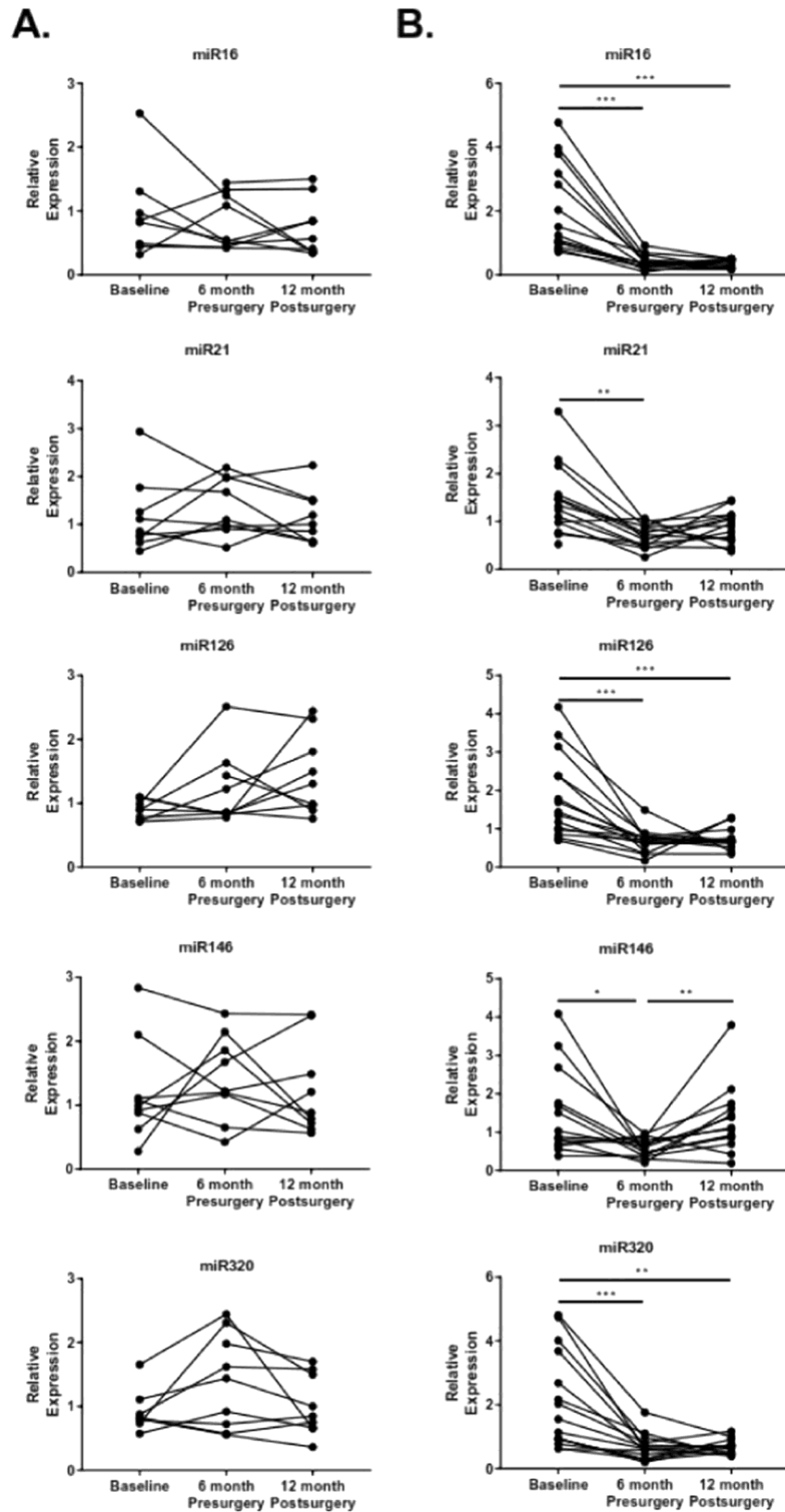


Fig. 4 Evolution of circulating EV-miRNA relative expression. Positive airway pressure and bariatric surgery effect. A Severe obesity without obstructive sleep apnea. B Severe obesity with obstructive sleep apnea. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2. Previous studies of miRNAs in severe obesity.

Population	n	OSA	miRNA	Source of miRNA	BS	Objective	Ref
Asians with obesity	21	No	125	SCT Omentum	No	Sex differences in miRNA expression	[52]
Asians with obesity	12	No	29, 122, 124, 320	Circulating	Yes	Impact on type 1 DM one year after BS	[53]
Women with obesity	9	No	221, 222, 155	SCT Cell culture	Yes	Variation in miRNA and inflammation 2 years after BS	[54]
Asians with obesity	124	No	448	Circulating	Yes	Prognostic ability of miR448 with respect to the success of BS	[55]
Women with obesity versus lean	48	No	143, 652	SCT	Yes	Insulin resistance and increased lipogenic action	[56]
Women with obesity	3	No	212, 299, 370, 487, 519, 671	SCT	Yes	Variation in miRNA at 3 years after BS	[57]
Obese	22	No	7, 15, 106, 135	Circulating	Yes	Metabolic benefit of physical exercise after surgery	[58]
Women with obesity versus lean	51	No	155, 130, 221, 339, 345, 483, 501, 575, 1973, 1295, 4286	SCT Cell culture	Yes	The inflammatory mRNA and miRNA improves 2 years after BS	[59]
Obese	54	No	10	Circulating	Yes	Variation after BS	[49]
Obese	9	No	125, 378, 192, 629, 22, 15	Circulating	Yes	Variation 1 year after BS	[48]
Obese	21	No	122/451	Circulating	Yes	Variation at 3 months from BS and endothelial and oncological risk	[60]
Obese versus lean	15	No	103, 107, 146, 24, 26, 320, let-7	Circulating	Yes	Impact of BS on type 2 DM and circulating miRNAs	[50]

miRNA microRNA, SCT subcutaneous cell tissue, BS bariatric surgery, OSA Obstructive Sleep Apnea.

30 patients (36 with OSA and 34 without OSA) was attained, the subsequent follow-up had a dropout rate greater than the 10% expected, especially in the group of patients with severe obesity without OSA. The small sample, therefore, allowed us to determine the direction of differences but not to establish accurate conclusions. We admit other limitations such as the lack of control lean group and the limited number of miRNA analyzed. Furthermore, daily medication of each patient could affect to the EV-miRNA cargo and therefore it is necessary to evaluate those relationships in studies that include a large population.

In conclusion, our study highlights the differences in the expression of circulating EV-miRNAs in patients with severe obesity as a function of the coexistence of OSA. This allows us, therefore, to accept our working hypothesis that there is an epigenetic profile of patients with severe obesity and OSA and that modifications in the expression of these miRNAs observed after weight loss induced by bariatric surgery is actually due to treatment with CPAP. The results confirm and reinforce the need for OSA screening in patients with severe obesity. Finally, the results open the door for future studies, both clinical and basic research, to elucidate the disparity in the activity of miRNAs depending on whether they are transported in EVs or bind to proteins or lipoproteins.

DATA AVAILABILITY

The data are available upon request.

REFERENCES

- Prevalence of obesity among adults, BMI \geq 30 (crude estimate) (%). In. [https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-obesity-among-adults-bmi-30-\(crude-estimate\)-\(-\)](https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-obesity-among-adults-bmi-30-(crude-estimate)-(-)).
- Senaratna CV, Perret JL, Lodge CJ, Lowe AJ, Campbell BE, Matheson MC, et al. Prevalence of obstructive sleep apnea in the general population: a systematic review. *Sleep Med Rev.* 2017;34:70–81.
- Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. *Physiol Rev.* 2010;90:47–112.
- Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Cardiol.* 2004;43:678–83.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004;116:281–97.
- Feng S, Cong S, Zhang X, Bao X, Wang W, Li H, et al. MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells. *Nucleic Acids Res.* 2011;39:6669–78.
- Gagan J, Dey BK, Layer R, Yan Z, Dutta A. MicroRNA-378 targets the myogenic repressor MyoR during myoblast differentiation. *J Biol Chem.* 2011;286:19431–8.
- Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 2005;65:6029–33.
- Chen JJ, Zhao B, Zhao J, Li S. Potential roles of exosomal MicroRNAs as diagnostic biomarkers and therapeutic application in Alzheimer's disease. *Neural Plastic.* 2017;2017:7027380.
- Kalra H, Drummen GP, Mathivanan S. Focus on extracellular vesicles: introducing the next small big thing. *Int J Mol Sci.* 2016;17:170.
- Hugel B, Martinez MC, Kunzelmann C, Freyssonet JM. Membrane microparticles: two sides of the coin. *Physiology.* 2005;20:22–7.
- Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol Rev.* 2013;93:1–21.
- Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, et al. A microRNA signature of hypoxia. *Mol Cell Biol.* 2007;27:1859–67.
- Ivan M, Harris AL, Martelli F, Kulshreshtha R. Hypoxia response and microRNAs: no longer two separate worlds. *J Cell Mol Med.* 2008;12:1426–31.
- Lacedonia D, Scioscia G, Palladino GP, Gallo C, Carpagnano GE, Sabato R, et al. MicroRNA expression profile during different conditions of hypoxia. *Oncotarget.* 2018;9:35114–22.
- Lázaro J, Clavería P, Cabrejas C, Fernando J, Daga B, Ordoñez B, et al. Epigenetics dysfunction in morbid obesity with or without obstructive sleep apnoea: the EPIMOSA study. *Respir Res.* 2020;21:42.
- Lloberes P, Durán-Cantolla J, Martínez-García M, Marín J, Ferrer A, Corral J, et al. Diagnóstico y tratamiento del síndrome de apneas-hipopneas del sueño. *Archiv Bronconeumol.* 2011;47:143–56.

18. Sanz-Rubio D, Martin-Burriel I, Gil A, Cubero P, Forner M, Khalyfa A, et al. Stability of circulating exosomal miRNAs in healthy subjects. *Sci Rep.* 2018;8:10306.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods.* 2001;25:402–8.
20. Dixon JB, Schachter LM, O'Brien PE. Predicting sleep apnea and excessive day sleepiness in the severely obese: indicators for polysomnography. *Chest.* 2003;123:1134–41.
21. Kositanurit W, Muntham D, Udomsawaengsup S, Chirakalwasan N. Prevalence and associated factors of obstructive sleep apnea in morbidly obese patients undergoing bariatric surgery. *Sleep Breath.* 2018;22:251–6.
22. Lecube A, Sampol G, Lloberes P, Romero O, Mesa J, Morell F, et al. Asymptomatic sleep-disordered breathing in premenopausal women awaiting bariatric surgery. *Obes Surg.* 2010;20:454–61.
23. Peromaa-Haavisto P, Tuomilehto H, Kossi J, Virtanen J, Luostarinen M, Pihlajamaki J, et al. Obstructive sleep apnea: the effect of bariatric surgery after 12 months. A prospective multicenter trial. *Sleep Med.* 2017;35:85–90.
24. Sharkey KM, Machan JT, Tosi C, Roye GD, Harrington D, Millman RP. Predicting obstructive sleep apnea among women candidates for bariatric surgery. *Journal of women's health.* 2010;19:1833–41.
25. Dewan NA, Nieto FJ, Somers VK. Intermittent hypoxemia and OSA: implications for comorbidities. *Chest.* 2015;147:266–74.
26. Dacal Quintas R, Tumbeiro Novoa M, Alves Perez MT, Santalla Martinez ML, Acuna Fernandez A, Marcos Velazquez P. Obstructive sleep apnea in normal weight patients: characteristics and comparison with overweight and obese patients. *Archiv Bronconeumol.* 2013;49:513–7.
27. Lecube A, de Hollanda A, Calanas A, Vilarrasa N, Rubio MA, Breton I, et al. Trends in bariatric surgery in Spain in the twenty-first century: baseline results and 1-month follow up of the RICIBA, a National Registry. *Obesity Surg.* 2016;26:1836–42.
28. Durán J, Esnaola S, Rubio R, Rubio R, Iztueta A. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. *Am J Respir Crit Care Med.* 2001;163:685–9.
29. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med.* 1993;328:1230–5.
30. Kolotkin RL, LaMonte MJ, Walker JM, Cloward TV, Davidson LE, Crosby RD. Predicting sleep apnea in bariatric surgery patients. *Surg Obes Relat Dis.* 2011;7:605–10.
31. Freitas LS, Silveira AC, Martins FC, Costa-Hong V, Lebkuchen A, Cardozo KHM, et al. Severe obstructive sleep apnea is associated with circulating microRNAs related to heart failure, myocardial ischemia, and cancer proliferation. *Sleep Breath.* 2020;24:1463–72.
32. Yang X, Niu X, Xiao Y, Lin K, Chen X. miRNA expression profiles in healthy OSAHS and OSAHS with arterial hypertension: potential diagnostic and early warning markers. *Respir Res.* 2018;19:194.
33. Tian R, Wang J, Yan H, Wu J, Xu Q, Zhan X, et al. Differential expression of miR16 in glioblastoma and glioblastoma stem cells: their correlation with proliferation, differentiation, metastasis and prognosis. *Oncogene* 2017;36:5861–73.
34. Zhang HJ, Zhang YN, Teng ZY. Downregulation of miR16 protects H9c2(21) cells against hypoxia/reoxygenation damage by targeting CIAPIN1 and regulating the NFkappaB pathway. *Mol Med Rep.* 2019;20:3113–22.
35. Zeng Y, Zhang X, Kang K, Chen J, Wu Z, Huang J, et al. MicroRNA-223 attenuates hypoxia-induced vascular remodeling by targeting RhoB/MLC2 in pulmonary arterial smooth muscle cells. *Sci Rep.* 2016;6:24900.
36. Hijmans JG, Diehl KJ, Bammer TD, Kavlich PJ, Lincenberg GM, Greiner JJ, et al. Association between hypertension and circulating vascular-related microRNAs. *J Hum Hypertens.* 2018;32:440–7.
37. Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. MicroRNA-9 and microRNA-126 expression levels in patients with essential hypertension: potential markers of target-organ damage. *J Am Soc Hypertens.* 2014;8:368–75.
38. Karolina DS, Tavintharan S, Armugam A, Sreeramani S, Pek SL, Wong MT, et al. Circulating miRNA profiles in patients with metabolic syndrome. *J Clin Endocrinol Metab.* 2012;97:E2271–6.
39. Khalyfa A, Marin JM, Qiao Z, Rubio DS, Kheirandish-Gozal L, Gozal D. Plasma exosomes in OSA patients promote endothelial senescence: effect of long-term adherent continuous positive airway pressure. *Sleep.* 2020;43:zsz217.
40. Santamaria-Martos F, Benitez I, Ortega F, Zapater A, Giron C, Pinilla L, et al. Circulating microRNA profile as a potential biomarker for obstructive sleep apnea diagnosis. *Sci Rep.* 2019;9:13456.
41. Khalyfa A, Gozal D, Masa JF, Marin JM, Qiao Z, Corral J, et al. Sleep-disordered breathing, circulating exosomes, and insulin sensitivity in adipocytes. *Int J Obes.* 2018;42:1127–39.
42. Sanchez-de-la-Torre M, Khalyfa A, Sanchez-de-la-Torre A, Martinez-Alonso M, Martinez-Garcia MA, Barcelo A, et al. Precision medicine in patients with resistant hypertension and obstructive sleep apnea: blood pressure response to continuous positive airway pressure treatment. *J Am Coll Cardiol.* 2015;66:1023–32.
43. Bhattacharjee R, Khalyfa A, Khalyfa AA, Mokhlesi B, Kheirandish-Gozal L, Almen-dros I, et al. Exosomal cargo properties, endothelial function and treatment of obesity hypoventilation syndrome: a proof of concept study. *J Clin Sleep Med.* 2018;14:797–807.
44. O'Brien PE, Hindle A, Brennan L, Skinner S, Burton P, Smith A, et al. Long-term outcomes after bariatric surgery: a systematic review and meta-analysis of weight loss at 10 or more years for all bariatric procedures and a single-centre review of 20-year outcomes after adjustable gastric banding. *Obes Surg.* 2019;29:3–14.
45. Adams TD, Davidson LE, Hunt SC. Weight and metabolic outcomes 12 years after gastric bypass. *N Engl J Med.* 2018;378:93–6.
46. Hubal MJ, Nadler EP, Ferrante SC, Barberio MD, Suh JH, Wang J, et al. Circulating adipocyte-derived exosomal MicroRNAs associated with decreased insulin resistance after gastric bypass. *Obesity.* 2017;25:102–10.
47. Bae YU, Kim Y, Lee H, Kim H, Jeon JS, Noh H, et al. Bariatric surgery alters microRNA content of circulating exosomes in patients with obesity. *Obesity.* 2019;27:264–71.
48. Alkandari A, Ashrafian H, Sathyapalan T, Sedman P, Darzi A, Holmes E, et al. Improved physiology and metabolic flux after Roux-en-Y gastric bypass is associated with temporal changes in the circulating microRNAome: a longitudinal study in humans. *BMC Obes.* 2018;5:20.
49. Hohensinner PJ, Kaun C, Ebenbauer B, Hackl M, Demyanets S, Richter D, et al. Reduction of premature aging markers after gastric bypass surgery in morbidly obese patients. *Obes Surg.* 2018;28:2804–10.
50. Lirun K, Sewe M, Yong W. A Pilot Study: the effect of Roux-en-Y gastric bypass on the serum microRNAs of the type 2 diabetes patient. *Obes Surg.* 2015;25:2386–92.
51. Wu Q, Li JV, Seyfried F, le Roux CW, Ashrafian H, Athanasiou T, et al. Metabolic phenotype-microRNA data fusion analysis of the systemic consequences of Roux-en-Y gastric bypass surgery. *Int J Obes.* 2015;39:1126–34.
52. Yeh CL, Cheng IC, Hou YC, Wang W, Yeh SL. MicroRNA-125a-3p expression in abdominal adipose tissues is associated with insulin signalling gene expressions in morbid obesity: observations in Taiwanese. *Asia Pac J Clin Nutr.* 2014;23:331–7.
53. Zhu Z, Yin J, Li DC, Mao ZQ. Role of microRNAs in the treatment of type 2 diabetes mellitus with Roux-en-Y gastric bypass. *Braz J Med Biol Res.* 2017;50:e5817.
54. Ortega FJ, Moreno M, Mercader JM, Moreno-Navarrete JM, Fuentes-Batllevell N, Sabater M, et al. Inflammation triggers specific microRNA profiles in human adipocytes and macrophages and in their supernatants. *Clin Epigenet.* 2015;7:49.
55. Wang Y, Wang DS, Cheng YS, Jia BL, Yu G, Yin XQ, et al. Expression of microRNA-448 and SIRT1 and prognosis of obese type 2 diabetic mellitus patients after laparoscopic bariatric surgery. *Cell Physiol Biochem.* 2018;45:935–50.
56. Dahlman I, Belarbi Y, Laurencikiene J, Pettersson AM, Arner P, Kulyte A. Comprehensive functional screening of miRNAs involved in fat cell insulin sensitivity among women. *Am J Physiol Endocrinol Metab.* 2017;312:E482–94.
57. Nardelli C, Iaffaldano L, Pilone V, Labruna G, Ferrigno M, Carlomagno N, et al. Changes in the microRNA profile observed in the subcutaneous adipose tissue of obese patients after laparoscopic adjustable gastric banding. *J Obes.* 2017;2017:6754734.
58. Nunez Lopez YO, Coen PM, Goodpaster BH, Seyhan AA. Gastric bypass surgery with exercise alters plasma microRNAs that predict improvements in cardiometabolic risk. *Int J Obes.* 2017;41:1121–30.
59. Ortega FJ, Mercader JM, Moreno-Navarrete JM, Nonell L, Puigdecane E, Rodriguez-Hermosa JI, et al. Surgery-induced weight loss is associated with the downregulation of genes targeted by microRNAs in adipose tissue. *J Clin Endocrinol Metab.* 2015;100:E1467–76.
60. Blum A, Yehuda H, Geron N, Meerson A. Elevated levels of miR-122 in serum may contribute to improved endothelial function and lower oncologic risk following bariatric surgery. *Isr Med Assoc J.* 2017;19:620–4.

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AUTHOR CONTRIBUTIONS

JL conceived, designed, wrote and approved the final version. DSR contributed to the design, writing, lab work and revised the paper. PC, CC, JF and AC contributed to acquire data. JMM contributed to the design, writing and revision of the paper.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study has been approved by the Aragon Institute of Health Sciences (Ethics Committee for Clinical Research of Aragon number: 23/2014). In accordance with recommendations from the Declaration of Human Rights, the Helsinki Conference and the IACS, patients must sign the informed consent form before being enrolled in the study.