Elevated levels of circulating miR-92a are associated with impaired glucose homeostasis in patients with obesity and correlate with metabolic status after bariatric surgery

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Abstract

Introduction

miRNAs are small non-coding RNAs, some of which are expressed in adipose tissues, are present in the circulation, and are regulated in obesity. Bariatric surgery (BS) has been proposed to lead to activation of brown adipose tissue, an effect that may be related to beneficial effects of BS on systemic metabolism. Here, we evaluated circulating levels of miR-92a and miR-99b, two miRNAs proposed as biomarkers of brown fat activity, in a cohort of patients with severe obesity before and after BS, and studied their potential relationship with BS-associated improvements in metabolic parameters.

Methods

Circulating levels of miR-92a and miR-99b were quantified in a cohort of 26 patients (age, 48 ± 10 years; BMI, 45 ± 7 kg/m^2) before and 6 months after BS. Clinical parameters were determined at different time points and correlations among them were studied.

Results

Basal levels of miR-92a were significantly increased in patients with obesity relative to lean controls. Serum miR-92a levels were strongly reduced at 6 months after BS, reaching levels similar to those in controls. Serum miR-99b levels were unchanged in relation to both the obese condition and BS. Elevated levels of miR-92a were directly correlated with worsened glucose homeostasis parameters and poor BS outcome.

Conclusions

Our findings show that miR-92a is elevated in conditions of obesity, and its reduction after BS correlates with metabolic improvement. Further studies would be necessary to establish miR-92a as serum biomarker and potential predictor of the BS success in improving the metabolic status of patients with obesity.

Introduction/Purpose

Brown adipose tissue (BAT) is the main site of non-shivering thermogenesis in mammals and serves to protect against obesity and associated metabolic diseases in rodent models [1-3]. BAT has been shown to act as a source of endocrine factors (so-called brown adipokines or secreted 'batokines') that can act on distant organs and contribute to a healthy metabolic state [4]. In 2009, several studies confirmed that functional BAT is present in adult humans and patients with obesity have less active BAT [5-8].

Currently, the most effective therapy to avert obesity and obesity-related type 2 diabetes is bariatric surgery (BS) [9, 10]. Several reports have indicated that BS in patients with obesity leads to BAT activation, which may contribute to increase energy expenditure, weight loss, and overall improvement in glucose and lipid metabolism [11, 12]. The "gold-standard" technique for quantifying BAT in humans is positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro-D-glucose integrated with computed tomography (18F-FDG PET–CT) [13]. However, this is an expensive and time-consuming method, so biomarker-based approaches should be considered as surrogate alternatives to determine BAT activation.

Micro RNAs (miRNAs) are small non-coding RNAs involved in the regulation of gene transcription, and are therefore able to modulate protein turnover [14-16] and other fundamental metabolic processes. Many miRNAs are present in the circulation and in different tissues [17]. Some of these miRNAs are expressed in adipose tissue and their levels are modulated in subjects with obesity compared to healthy controls [reviewed in 18]. Moreover, several miRNAs have been reported to be involved in brown adipocyte differentiation [19-21], and changes in miRNA expression patterns have been observed after cold exposure of mice [19].

Circulating miR-92a levels, in particular, are reported to be inversely associated with the extent of BAT activity in both mice and humans [22]. In fact, researchers have found that inhibition of miR-92a leads to a much healthier metabolic phenotype in mice [23]. Circulating levels of miR-99b have also been linked to the activity of BAT, which is proposed to release miR-99b into the circulation; from there, it may target the liver and cause beneficial systemic effects on obesity, diabetes, and aging [24].

Here, we provide the first evaluation of circulating levels of miR-92a and miR-99b in a cohort of patients with severe obesity before and after BS, and assess the potential association of these biomarkers with parameters indicative of BS-related metabolic improvements.

Materials and methods

Subjects cohort

A cohort of 26 patients with severe obesity (16 female/10 male) were included in this study and were evaluated by the same endocrinology specialist (S.P.) according to criteria formulated in the Spanish Position Statement on the relationship between Obesity, Endocrinology, Diabetes and Surgery Societies [25]. A sleeve gastrectomy (SG; n = 13) or a Roux-en-Y gastric bypass (RYGB; n = 13) was performed as surgical therapy for these patients. Demographic and clinical data, including age, history of diabetes and hypertension, as well as other co-morbidities were recorded for all subjects before and 6 months after BS. A group of 7 age-matched, lean (BMI, 20.8 ± 1.6 kg/m^2) women was included in the study as a control group. The Institutional Ethics Committee of Germans Trias i Pujol Hospital approved the study (PI16-025), in accordance with the Declaration of Helsinki. All participants gave their written informed consent before the collection of clinical data and samples.

Serum samples

Serum samples were collected from study participants (controls and obesity patients) after an 8-hour fasting period; in the case of patients, samples were collected before and 6 months after BS. All samples were stored at -80°C in the Biobank of the Germans Trias i Pujol Research Institute Foundation.

Human serological analysis

Glucose, insulin and glycated hemoglobin (Hb1Ac) levels, as well as lipid profile (total cholesterol, HDL and LDL cholesterol, and triglycerides), were measured in the core clinical laboratory of the Germans Trias i Pujol Hospital after an overnight fasting period. Homeostatic model Assessment-insulin resistance (HOMA-IR) was calculated using the following formula:

$$HOMA - IR = \frac{\left[Glucose\,\frac{mg}{dL}\right] * \left[Insulin\,\frac{\mathrm{m.u.int}}{dL}\right]}{405}$$

RNA extraction

Circulating total RNA was extracted from 100 µL of serum using the MagMAX mirVana Total RNA Isolation Kit (Thermo Fisher Scientific), a Thermomixer incubator (Eppendorf) and DynaMag-2 magnet (Thermo Fisher Scientific), applying a manual large-volume procedure adapted for 1.5 mL tubes, as described by the manufacturer. RNA was recovered in 50 µL of elution buffer.

miRNA expression assay

Expression of individual miRNAs was determined with TaqMan Advanced miRNA Assays (Thermo Fisher Scientific) using an automated protocol developed on a Sciclone NGS (PerkinElmer) liquid handler for polyA tailing, integrated adapter ligation, reverse transcription, miR-Amp amplification reactions, preamplified library dilution, and quantitative polymerase chain reaction (qPCR). Cycling steps were performed on a Tetrad thermocycler (Bio-Rad) using 2 µL of RNA as a template. Real-time qPCR was performed in 384-well plates (10 µL/well) on an Applied Biosystems 7900HT system using the standard settings recommended by the manufacturer and TaqMan Fast Advanced PCR Master mix and Advanced Probes (assay IDs: 477992_mir, 477952_mir, 477827_mir, 478343_mir for human miRNAs, miR-24-3p, miR-191-5p, miR-92a-3p, and miR-99b-5p, respectively). miR-451a (478107_mir) and miR-23a-3p (478532_mir) were also assayed, and hemolysis was assessed as the ΔCt between miR-451a and miR-23a-3p.

Statistical analysis

Unless otherwise stated, data are presented as means ± sem. Normality was tested using the Shapiro-Wilk test. Two-tailed test, Mann-Whitney U test for non-normal distributed data, or one-way ANOVA with Tukey's post-hoc test for more than two groups were applied to compare groups. Non-normal distributed variables were log-transformed to achieve normality before analyzing potential associations. Raw data were generated using SDS 2.3 software (Thermo Fisher). Relative miR-92a-3p and miR-99b-5p expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method using miR-24-3p and miR-191-5p as housekeeping reference miRNAs. Correlation analyses were implemented in SPSS statistics (IBM). Statistical tests were performed using GraphPad Prism 6.0. Grubbs test was used to remove outliers prior to statistical analyses. A p-value < 0.05 was considered the threshold of statistical significance for all analyses.

Results

Clinical data from obesity patients are shown in Table 1. Ten of them had been diagnosed of type 2 diabetes, and medication was suspended after the surgery. BS in our cohort resulted in a significant decrease in anthropometric and metabolic parameters, including BMI, waist circumference, glucose levels, insulin levels, glycated hemoglobin (Hb1Ac), HOMA-IR, and LDL-cholesterol levels (Table 1).

Under basal conditions, patients with obesity showed higher circulating levels of miR-92a than healthy lean controls, whereas miR-99b levels were similar in patients with obesity and lean individuals. In patients with obesity, miR-92a levels were significantly decreased 6 months after BS, at which point they attained levels similar to those in lean controls (Figure 1). This change occurred regardless of sex and type of surgery (SG or RYGB).

Levels of miR-92a did not correlate with BMI, weight loss, biochemical parameters related to lipidemia, or blood pressure. However, miR-92a levels were significantly associated with impaired glucose homeostasis in patients with obesity at baseline, showing a positive association with insulin levels, Hb1Ac, and HOMA-IR (Table 2). Moreover, patients with higher miR-92a levels before BS also showed the highest glucose and Hb1Ac levels 6 months after surgery. Finally, when assessing associations of the variation of miR-92a levels with the changes in the distinct parameters (after and before BS), a significant correlation was found with circulating insulin. Those patients showing a lesser reduction in miR-92a levels after BS were also those who showed the smallest decrease in insulin levels after surgery (Table 2).

Conclusions

Circulating miRNAs have been proposed to act as biomarkers of metabolic diseases, including obesity [22]. It has been reported that miR-92a negatively regulates the metabolic status of mice, and that its inhibition is sufficient to cause an improvement in metabolic phenotype [23]. In our study, we show for the first time that levels of miR-92a are increased in serum from patients with obesity—both those with and without diabetes—compared with control lean individuals, and that these levels are positively correlated with indicators of impaired glucose metabolism, such as hyperinsulinemia, Hb1Ac, and HOMA-IR. Moreover, we show that miR-92a decreases after BS, a finding consistent with a previous report of reduced levels miR-92a after gastric bypass [26]. We further found that high levels of miR-92a before surgery predispose toward decreased improvement in glycemia and Hb1Ac levels 6 months after BS. Taken together, these observations suggest that miR-92a plays a role in the impaired metabolic status associated with obesity, providing a potential mechanism of action for the metabolic benefit of BS on glucose homeostasis, as suggested by other studies [26].

In addition, since insulin resistance contributes to fatty liver progression [27], it is tempting to suggest that the improvement of glucose homeostasis associated to miR-92a levels could be involved in the reduction of fatty liver index 6 months after BS. The lack of direct correlation between miR-92a and fatty liver index may be due to the limited number of samples, but further studies should address this issue.

Given previous reports that miR-92a is released by brown adipocytes and is inversely correlate with BAT activity [22], and that BAT is activated after BS [11, 12], it is tempting to speculate that high levels of miR-92a in obesity are attributable to impaired BAT activity and that normalization of miR-92a levels after BS might may reflect re-activation of BAT by this surgical intervention, as previously reported [11, 12]. However, further studies are necessary to

clarify the tissue sources responsible for changes in systemic miR-92a levels in obesity and after BS, as well as the causal relationship between elevated miR-92a in serum and changes in glucose homeostasis.

Finally, we found no changes in miR-99b before or after BS in patients with severe obesity. Altered miR-99b levels have been reported in lipodystrophy [24] and gestational obesity [28]. In this regard, our study is not exempt of limitations, and the relatively low number of participants may have prevented the detection of smaller differences in miR-99b.

It is noteworthy that previous studies have proposed a regulation of these miRNAs in obesity [26, 28], but here we report some associations that suggest a specific link of miR-92a with glucose homeostasis in obesity. Moreover, our analysis of miR-99b levels, which to the best of our knowledge is the first such an analysis in patients with severe obesity, suggests that miR-99b does not play a role in the severely obese condition, either as an actor or biomarker.

We conclude that serum levels of miR-92a are elevated in patients with severe obesity, are correlated with dysfunctional glucose homeostasis parameters at baseline, and are normalized after BS. Therefore, miR-92a can be considered a promising serum biomarker of the metabolic status of individuals with obesity that changes upon BS treatment. Although results of the current study are consistent with the previously proposed inverse association between miR-92 levels and BAT activity, further studies are needed to decipher the role of miR-92 in relation to BAT activity in humans.

Conflict of Interest

The authors declared no conflict of interest.

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Figure legends

Figure 1

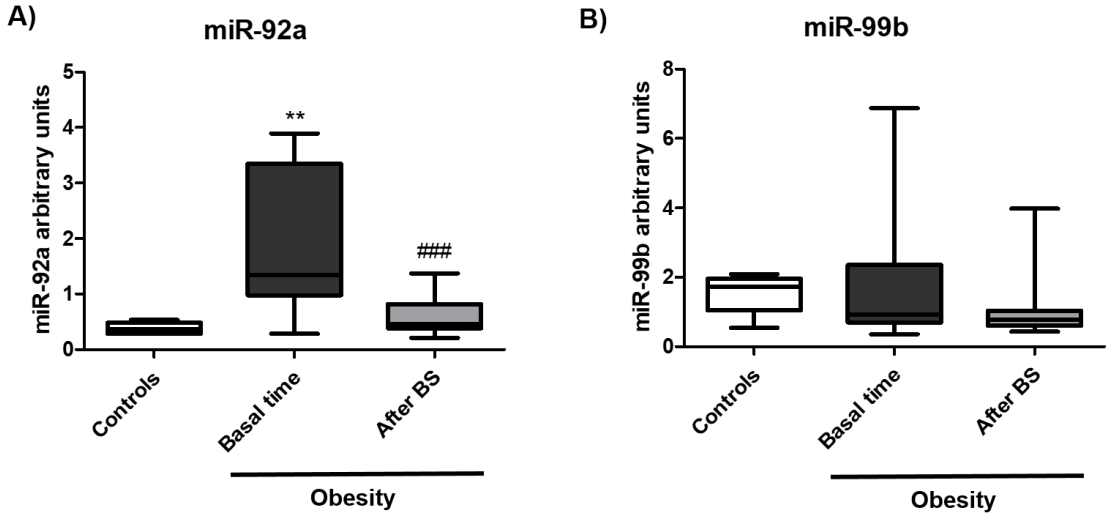

Figure 1. miR-92a levels, but not miR-99b levels, are elevated in patients with obesity and are reduced after BS. miR-92a (A) and miR-99b (B) levels in lean controls and in patients with obesity before and 6 months after BS. miR-92a and miR-99b levels are expressed in arbitrary units relative to levels of the housekeeping transcripts, miR-24-3p and miR-191-5p. Data are presented as means ± sems (**$P < 0.01$ for comparisons with controls; $^{###}P < 0.001$ for comparisons with 6 months after surgery).

	Basal		6 month	
	mean	Range	mean	Range
Age (years)	48.92	[20-64]		
Body mass index (kg/m^2)	45.66	[35-65]	33.11	[23-51]*
Waist circumference (cm)	137.49	[112-181]	111.57	[80-137]*
Excess weight loss (PSP) (%)	5.76	[0-12]	51.53	[25-82]*
Systolic blood pressure (mmHg)	139.18	[104-179]	124.69	[93-156]*
Diastolic blood pressure (mmHg)	79.68	[59-96]	74.50	[59-84]
Glucose (mg/dl)	112.62	[79-259]	93.78	[70-146]*
Glycated hemoglobin (%)	6.02	[5-8.7]	5.3	[4.8-6.6]*
Insulin (m.u.int./l)	12.63	[1.9-49.7]	9.23	[3.2-35.1]*
Homeostatic model assessment (HOMA-IR)	3.35	[0.38-13.32]	2.56	[0.62-18.29]*
Triacylglycerides (mg/dl)	133.50	[72-211]	119.57	[40-208]
Total cholesterol (mg/dl)	146.77	[85-202]	163.05	[82-282]
LDL -cholesterol (mg/dl)	84.29	[29-138]	108.71	[47-213]*
HDL-cholesterol (mg/dl)	40.27	[27-61]	50.45	[35-130]*
Fatty liver index (FLI)	97.99	[74-100]	62.64	[3-99]*
Creatinine (mg/dl)	0.94	[0.44-3.19]	0.83	[0.50-2.98]*
Urea (mg/dl)	39.60	[17-138]	35.60	[12-117]
C-reactive protein (mg/l)	12.43	[1.8-68.9]	8.18	[0.8-48.8]
Leucocytes (^10^9/l)	8.12	[3.5-17.8]	7.20	[3.4-10.1]

Table 1. Anthropometric and metabolic parameters from patients with morbid obesity before and after bariatric surgery. Data for normally distributed variables are shown as mean [CI]. Two-tail Student's t-test was applied to compare groups. * indicates $p<0.05$. LDL-Cho. Low-density lipoprotein; HDL-Cho, High-density lipoprotein.

	Basal time		Basal time-6 months		Δ6-0 months	
Variables	*r*	*p val*	*r*	*p val*	*r*	*p val*
Body mass index	-0.23	0.337	0.06	0.787	0.19	0.43
Excess weight loss	0.41	0.087	-0.09	0.713	-0.21	0.424
Insulin	0.48	**0.041**	0.25	0.394	0.61	**0.026**
HbA1c	0.64	**0.003**	0.56	**0.038**	0.28	0.326
Glucose	0.37	0.129	0.61	**0.016**	-0.10	0.723
HOMA-IR	0.65	**0.006**	0.37	0.192	-0.05	0.882
Triglycerides	-0.29	0.908	0.20	0.492	0.04	0.883
Total cholesterol	0.08	0.748	-0.26	0.375	0.37	0.190
HDL-cholesterol	-0.41	0.087	-0.15	0.65	0.80	0.789
LDL-cholesterol	-0.31	0.234	-0.29	0.320	0.37	0.235
Systolic blood pressure	-0.05	0.864	0.161	0.636	0.51	0.157
Diastolic blood pressure	0.02	0.918	-0.18	0.586	0.18	0.635

Table 2. Correlations between miR-92a levels and clinical variables.

Multivariate linear regression was used to assess potential associations between miR-92a levels and physiologic variables. Bold font indicates $p<0.05$. Hb1Ac; Glycated hemoglobin (%). HOMA-IR; Homeostatic model assessment.