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Original article

Changes in circulating miRNAs in healthy overweight and obese subjects: Effect of diet composition and weight loss

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SUMMARY

Background: MicroRNAs (miRNAs) are small non-coding RNA molecules that can play an important role in several chronic metabolic conditions, including obesity. However, to date little is known about how they are regulated. Weight loss induced by surgical procedures has been effective at modulating specific circulating miRNAs, but the effect of energy-restricted diets with different macronutrient compositions on circulating miRNAs is not well understood. The objective of the present analysis was to explore the effect of three energy-restricted diets of different macronutrient composition and carbohydrate quality on plasma miRNA levels.

Methods: The GLYNDIET study is a 6-month, parallel, randomized clinical trial conducted on overweight and obese subjects who were randomized to one of three different dietary intervention groups: i) a moderate-carbohydrate and low glycemic index diet (LGI), ii) a moderate-carbohydrate and high glycemic index diet (HGI), and iii) a low-fat and high glycemic index diet (LF). We assessed the genome-wide circulating miRNA profile in a subsample of eight randomly selected participants. A total of 8 miRNAs (miR-411, miR-432, miR-99b, miR-340, miR-423, miR-361, let-7c) were differently quantified according to diet intervention, and were therefore longitudinally validated in 103 participants before and after the energy-restricted diets.

Results: Circulating miR-361 levels were lower in the LGI group than in the HGI group, even after adjusting for differences in weight loss. The intra-group analyses demonstrated a significant down-regulation of all miRNAs screened in our study subjects after the LGI intervention. Similarly, miR-139 and miR-340 were down-regulated after the HGI intervention, while miR-139, miR-432 and miR-423 were down-regulated after the low-fat diet. Changes in circulating miR-139 and let-7c were significantly associated with changes in lipid profile and insulin resistance.

Conclusion: An energy-restricted low-glycemic index diet down-regulates circulating miRNA-361 more than an energy-restricted high-glycemic index, regardless of the magnitude of the weight loss.

1. Introduction

In the last decade microRNAs (miRNAs) have opened up new frontiers in the knowledge of post-transcriptionally regulation of gene expression. They carry out key role in almost all cellular processes such as proliferation, differentiation, apoptosis and cell metabolism, so they are likely to be involved in many human diseases and metabolic disorders, including obesity and insulin resistance [1,2]. Specific miRNAs are involved in adipocyte differentiation, fat metabolism and insulin sensitivity [3] and different miRNA signatures have been found in the undifferentiated pre-adipocytes and the differentiated adipocytes, in both cell cultures [4] and humans [5]. Between 35 and 71 miRNAs are differentially expressed in adipocytes from wild type and diet-induced obesity (DIO) or *ob*/*ob* mice [6]. In humans, to date 41 miRNAs have been found to be differentially expressed in subcutaneous adipose tissue from severely obese and non-obese subjects [7]whereas 89 miRNAs have shown different expression in obese and lean visceral adipocytes [8].

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As well as having an intracellular function, miRNAs are also secreted into body fluids where they are abundant and relatively stable, so they are still useful as potential diagnostic and prognostic biomarkers for diseases [9]. Alterations in circulating miRNA (c-miRNAs) profiles have been described in several chronic metabolic conditions, including obesity [10,11]. Indeed, a specific 9 c-miRNA signature has been identified in morbidly obese subjects, most of these miRNAs being strongly associated with such adiposity measurements as BMI, total fat mass and waist circumference. Surgically-induced weight loss has proven to be effective at reversing this c-miRNA signature [11]. However, since the expression and secretion of miRNAs are tightly regulated at multiple levels [12] and strongly affected by physiological and environmental stimuli [13–15], dietary strategies designed for losing weight could differ in modulating c-miRNA beyond the weight loss achieved. In fact, dynamic changes in miRNA patterns caused by restriction or supplementation with different macro- and micronutrients have already been reported [16]. However, the effect of different energy-restricted diets on the modulation of c-mRNAs beyond the magnitude of weight loss has yet to be explored.

We have previously demonstrated that a low-glycemic index energy-restricted diet reduced weight more effectively than an energy-restricted low-fat diet and it was also more effective at controlling glucose and insulin metabolism [17]. In the same overweight and obese adult subjects, we aimed to analyze the effect of the three energy-restricted intervention diets that differ in the total carbohydrate amount and carbohydrate quality (high or low glycemic index) on changes in the c-miRNA profile.

2. Materials and methods

2.1. Study population

The GLYNDIET study is a 6-mo randomized, parallel, controlled, clinical trial designed to evaluate the effect on weight loss of energy-restricted diets with different glycemic index and glycemic load. Satiety, glucose and insulin metabolism, lipid profile, inflammation and other metabolic risk factors were considered to be secondary outcomes. The participants were recruited between 2010 and 2012 from community-dwelling men and women aged between 30 and 60 with a body mass index (BMI) between 27 and 35 kg/m^2. Among other excluding criteria were: 1) non-controlled T2D defined as glycated hemoglobin >8%; 2) systolic blood pressure >159 mmHg or diastolic blood pressure >99 mmHg; 3) plasma LDL-cholesterol>160 mg/dL; 4) plasma triglycerides>400 mg/dL; 5) suspicion of secondary obesity; 6) a restrictive diet three months before the study or a weight loss >5 kg. The detailed protocol and main outcomes have been published elsewhere [17,18].

2.2. Dietary interventions and anthropometric measurements

Participants were randomly assigned to three different energy-restricted dietary intervention groups: 1) a moderate-carbohydrate and low glycemic index diet, 2) a moderate-carbohydrate and high glycemic index diet, and 3) a low-fat diet. Individual examinations were scheduled at baseline, day 15 and once a month till the end of the study. Body weight and height were measured with subjects wearing light clothes and BMI was calculated. Waist circumference was measured twice midway between the lowest rib and the iliac crest. Body composition was measured by using a bioelectrical impedance analysis (TANITA TBF-300; Tanita). Blood pressure was measured in the non-dominant arm by using a validated semiautomatic oscillometer (Omron HEM-705CP; OMRON Corp).

2.3. Blood biochemical parameter measurements and RNA extraction

Blood samples were obtained by standard venipuncture after a 12-h fast at baseline and in the last visit of the study using K2EDTA-coated Vacutainer tubes. Plasma was obtained after centrifugation at 1800 × *g* for 15 min at 4 C and stored at −80 °C until use. Plasma fasting glucose, serum total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride and non-esterified fatty acid concentrations were determined by using Standard enzymatic automated methods (COBAS; Roche Diagnostics Ltd). LDL-cholesterol concentrations were estimated by using Friedewald's formula in subjects with triglycerides concentrations lower than 400 mg/dL. Fasting insulin was determined by using an enzyme-linked immunosorbent assay commercial kit (Merck Millipore, Darmstadt, Germany). HOMA-IR and homeostatic model assessment of b-cell function (HOMA-BCF) were estimated [19].

To perform total RNA isolation, thawed plasma samples were centrifuged again at 2000 × *g* for 10 min to discard any remaining blood cells. RNA was extracted using the mirVana PARIS isolation Kit (Applied Biosystems, Darmstadt, Germany) and in accordance with the manufacturer's instructions. We diluted a fixed volume of 400 μL of plasma in the same volume of 2× Denaturing Solution and, before the RNA isolation, 5 μL of 5 nM synthetic *Caenorhabditis elegans* miR-39 that do not exist in the human genome was added as an exogenous quality control as we have reported elsewhere [20]. We later added 800 μL of Acid-Phenol:Chloroform and centrifuged the samples to obtain separate phases. After various washing steps with 100% ethanol, miRNAs were finally eluted with 50 μL of preheated (95 °C) nuclease-free water and samples were stored at −80 °C until reverse transcription (RT).

2.4. Circulating miRNA profile evaluation

Using the TAqMan MicroRNA Reverse Transcription kit and the Megaplex RT Primers, Human Pools A v.2.1 and B v.3.0 (Applied Biosystems, Darmstadt, Germany) we reverse transcribed 734 miRNAs as allocated in the TaqMan Array microRNA for profiling a randomly selected sample of eight subjects (3 LGI, 3 HGI, 2 LF) at baseline and after the dietary intervention. Multiplex RT products were later pre-amplified with TaqMan PreAmp Master Mix and Megaplex PreAmp Primers (Human Pools A v.2.1 and B v.3.0). Both reactions were conducted in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Darmstadt, Germany).

A quantitative PCR (qPCR) was carried out using a pre-amplified product, TaqMan Universal PCR Master Mix, no AmpErase UNG (2×) (Applied Biosystems, Darmstadt, Germany) and the TaqMan miRNAs low-density arrays (TLDAs cards A v2.1 and B v3.0; Applied Biosystems, Darmstadt, Germany). The TLDA arrays were analyzed in a real-time thermal cycler (7900HT Fast Real-Time PCR System; Applied Biosystems, Darmstadt, Germany).

Data from qPCR were obtained by SDS v.2.2 and processed by RQ Manager v.1.2 software. Results were expressed as threshold cycle (Ct) values. Ct values were normalized to obtain the normalized threshold cycle (normCt) values using the mean of the four most stable miRNAs (used as normalizer) in the samples analyzed. These normalizing miRNAs were identified using the concordance correlation restriction (CCR) method described elsewhere [21].

2.5. Analysis of individual miRNAs

We used commercially available TaqMan hydrolysis probes to assess the presence in plasma of 8 individual miRNAs plus the exogenous control in 103 study subjects, as described elsewhere [19]. Briefly, cDNA was synthesized using the TaqMan MicroRNA

Reverse Transcription Kit in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Darmstadt, Germany).

Real time PCR was performed in a 7900HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) using the following TaqMan MicroRNA Assays: hsa-miR-139-3p (MIMAT0004552), hsa-miR-411 (MIMAT0003329), hsa-miR-432 (MIMAT0002814), hsa-miR-99b (MIMAT0004678), hsa-miR-340 (MIMAT0000750), hsa-miR-423-5p (MIMAT0004748), hsa-miR-361 (MIMAT0000703), hsa-let7c (MIMAT0000064) and cel-miR-39 as exogenous control. All measurements were performed in duplicate, and qPCR data were acquired using Sequence Detector Software (SDS v.2.2). The expression of the miRNAs analyzed was normalized by the mean of cel-miR-39 and the normalized expression was calculated for individual samples using $2^{-\Delta Cq}$ methods. Changes in expression were shown as the ratio between final and baseline values.

Finally, miR-361 gene ontology (GO) information is proposed in Supplementary Table 2. For this purpose, we selected a list of candidate genes using the DIANA TOOL software. Filters applied were *Homo sapiens*, Normal and High Throughput method, Direct validation type, Tarbase 7.0 source and a prediction score of 0.

2.6. Statistical analysis

The descriptive data of participants at baseline and 6-mo changes are shown as means (±SEMs) or medians and IRQs for continuous measures, and as numbers and percentages for categorical variables. The normal distribution of variables was tested with the Kolmogorov–Smirnov test. In variables with normal distribution, ANCOVA and the pair t-test were used to assess differences between groups and intra-groups. Non-parametric statistical Kruskal–Wallis, Mann–Whitney and Wilcoxon tests were used to assess differences between intervention groups and changes within each intervention in terms of miRNA expression. Normal distributed biochemical variables were adjusted for their baseline values, while variables without normal distribution were adjusted using the residual method. Data of c-miRNAs was log2 transformed (ratio end/baseline values) and it is shown as mean (95% CI). Therefore, values higher than 0 mean up-regulation throughout the intervention period and lower than 0, down-regulation. The Bonferroni post-hoc test was used for multiple comparisons. We used Pearson's or Spearman's correlation coefficients to evaluate whether plasma levels of different miRNAs correlated with biochemical parameters. Analyses were performed using SPSS v.22.0 and R v.3.3.0 software and significance was set at $P < 0.05$.

3. Results

3.1. Study population

We analyzed the circulating miRNA profile in a subcohort of 103 participants from the GLYNDIET study [17,18]. Subjects were divided into three groups as follows: LGI (n = 36), HGI (n = 36) and LF (n = 31). Table 1 shows the baseline characteristics of the study participants. There were no differences in any baseline characteristics between intervention groups. Table 2 describes baseline and 6-monthly changes in parameters of the glucose metabolism and lipid profile. Baseline and 6-monthly changes in dietary parameters are shown in Supplemental Table 1. We observed a significant reduction in body weight and BMI in the LGI and HGI groups compared to the LF group. We also reported a significant decrease in fasting plasma insulin and HOMA-IR levels between the LGI and LF groups and in HOMA-BCF between the LGI group and both the HGI and LF groups.

3.2. Study of circulating miRNAs

Using the TLDAs method, eight miRNAs were differently expressed in the three dietary interventions (hsa-miR-139-3p, -miR-411, -miR-432, -miR-99b, -miR-340, -miR-423-5p, -miR-361 and -let7c). This set of miRNAs was analyzed and validated individually in all the 103 participant completers in the GLYNDIET study.

Of all the miRNAs studied in the whole population, miR-361 was found to be significantly down-regulated in the LGI group compared to the HGI group (mean (95% CI): 0.18 (−0.41, 0.77) vs −1.09 (−1.85, −0.33), $P = 0.012$) (Fig. 1). The intra-group analysis showed a significant down-regulation of all the miRNAs screened in subjects after the LGI intervention. We also found decreased levels of miR-139 and miR-340 between the baseline values and the end of the HGI intervention, and a significant decrease in the expression of miR-139, miR-432 and miR-423 in the LF group after the intervention. The *in silico* analysis of miR-361 showed a total of 27 significantly enriched GO processes (Supplemental Table 2).

3.3. Correlations between circulating miRNAs and biochemical parameters

A significant positive correlation was found between miR-139-3p, miR-423-5p and let-7c and LDL-C or total cholesterol, as well as between miR-340 and let-7c and triglyceride levels (Table 3).

4. Discussion

As far as we know, this is the first study to have evaluated the effect of energy-restricted diets with different carbohydrate and fat contents on the c-miRNA profile. We provide the first evidence of a dietary modulation of miR-361 independently of the weight loss achieved. With these results, we add to our previous finding regarding the effectiveness of a low-glycemic index diet at reducing body weight and controlling glucose and insulin metabolism, the specific modulation of miR-361 which is related with cardiovascular diseases.

Table 1
Baseline characteristics of study participants.

Variable	LGI diet (n = 36)	HGI diet (n = 36)	LF diet (n = 31)	*P*-value
Sex (females) [n (%)]	28 (77.8%)	30 (83.3%)	24 (77.4%)	0.794
Age (y)	42.1 ± 1.2	44.9 ± 1.3	44.9 ± 1.4	0.238
Systolic blood pressure (mm Hg)	128.0 ± 2.4	129.0 ± 2.5	133.3 ± 2.4	0.270
Diastolic blood pressure (mm Hg)	80.0 ± 1.4	82.1 ± 1.6	83.8 ± 1.7	0.154
Hypercholesterolemia [n (%)]	2 (5.5%)	2 (5.5%)	5 (16.1%)	0.224
Hypertension [n (%)]	5 (13.9%)	5 (13.9%)	5 (16.1%)	0.958
Current smokers [n (%)]	7 (19.4%)	5 (13.9%)	4 (13%)	0.730
Leisure-time physical activity (kcal/d)	227.0 ± 52.6	293.3 ± 51.2	201.5 ± 40.4	0.400

Mean ± SEM (all such values). *P* value of differences between intervention groups (ANOVA was used for continuous variables, and the chi-square test was used for categorical variables). HGI, high-glycemic index; LF, low fat; LGI, low glycemic index.

Table 2
Baseline and 6-monthly changes in glucose metabolism and lipid profiles.

Variable	LGI diet (n = 36)	*P* within group	HGI diet (n = 36)	*P* within group	LF diet (n = 31)	*P* within group	*P* between groups
Weight (kg)							
Baseline	83.6 ± 1.6		82.6 ± 1.7		83.9 ± 1.9		0.856
6-mo changes	−7.2 ± 0.7b	<0.001	−7.1 ± 0.7b	<0.001	−4.3 ± 0.8	<0.000	0.012
BMI							
Baseline	31.4 ± 0.4		30.8 ± 0.4		30.7 ± 0.4		0.317
6-mo changes	−2.7 ± 0.3b	<0.000	−2.5 ± 0.3b	<0.001	−1.5 ± 0.3	<0.000	0.006
Waist circumference							
Baseline	101.4 ± 1.3		100.6 ± 8.7		103.9 ± 1.2		0.202
6-mo changes	−8.01 ± 0.9	<0.000	−7.1 ± 0.9	<0.000	−5.8 ± 1.8	<0.000	0.241
Fat mass (%)[a]							
Baseline	40.8 (37.7; 43.7)		41.1 (36.5; 43.5)		41.0 (31.9; 43.7)		0.955
6-mo changes	−5.4 (−6.4; −3.4)b	<0.000	−3.9 (−7.6; −2.1)b	<0.000	−2.3 (−3.8; −1.1)	<0.000	0.001
Fat free mass (%)[a]							
Baseline	59.2 (56.1; 62.8)		58.6 (56.4; 63.2)		58.9 (55.9; 67.9)		0.980
6-mo changes	−2.7 (−3.4; −1.8)b	<0.000	−2.1 (−4.9; −1.4)b	<0.000	−1.4 (−2.1; −0.4)	<0.000	0.005
Glucose (mg/dL)[a]							
Baseline	99 (65; 104)		99 (93; 105)		102 (96; 110)		0.280
6-mo changes	−3.4 (−10.8; 3.6)	0.085	−6.2 (−13.7; 2.7)	0.193	−3.7 (−8.4; 2.7)	0.086	0.634
Total cholesterol (mmol/L)[a]							
Baseline	4.9 (4.5; 5.6)		4.9 (4.5; 5.5)		4.7 (4.4; 5.3)		0.303
6-mo changes	−0.1 (−0.6; 0.6)	0.660	0.04 (−0.4; 0.6)	0.577	−0.04 (−0.4; 0.5)	0.451	0.516
HDL cholesterol (mmol/L)							
Baseline	1.4 ± 0.04		1.5 ± 0.1		1.3 ± 0.04		0.186
6-mo changes	0.03 ± 0.04	<0.001	0.1 ± 0.04	0.003	0.05 ± 0.04	<0.000	0.510
LDL cholesterol (mmol/L)							
Baseline	3.1 ± 0.1		3.1 ± 0.1		3.0 ± 0.1		0.709
6-mo changes	−0,01 ± 0.1	<0.001	0.1 ± 0.09	0.043	−0.01 ± 0.1	0.005	0.467
Triglycerides (mmol/L)[a]							
Baseline	1.02 (0.8; 1.3)		1.03 (0.7; 1.3)		0.97 (0.7; 1.5)		0.758
6-mo changes	0.15 (−0.1; 0.3)a	0.001	−0.1 (−0.2; 0.1)b	0.026	0.1 (−0.1; 0.4)	0.047	<0.001
Non-esterified fatty acids (μmol/L)							
Baseline	507.6 ± 35.2		503.0 ± 29.0		503.1 ± 29.3		0.993
6-mo changes	−39.5 ± 26.6	0.335	−45.7 ± 26.3	0.577	−87.1 ± 28.3	0.015	0.422
Fasting insulin (mU/mL)							
Baseline	4.7 (3.3; 7.5)		3.8 (3.2; 5.6)		4.5 (3.3; 6.8)		0.254
6-mo changes	−1.8 (−2.6; −0.4)	<0.001	−2.0 (−2.8; −1.0)b	<0.001	−0.8 (−2.1; −0.2)	0.005	0.044
HOMA IR[a]							
Baseline	1.2 (0.8; 1.8)		0.95 (0.6; 1.4)		1.1 (0.7; 1.8)		0.203
6-mo changes	−0.5 (−0.6; −0.2)	<0.001	−0.6 (−0.7; −0.3)b	<0.001	−0.2 (−0.6; −0.04)	0.005	0.014
HOMA-BCF[a]							
Baseline	50.5 (36.7; 69.3)		39.7 (31.5; 61.4)		42.5 (33.8; 66.3)		0.237
6-mo changes	−13.2 (−24.1; −4.1)	<0.001	−11.9 (−25.7; −2.1)	0.001	−5.7 (−14.7; 11.0)	0.327	0.076

Data on 6-monthly changes were adjusted for the baseline values of each variable. Abbreviations: BMI, body mass index; HDL, high density lipoprotein; HGI, high glycemic index; HOMA-BCF, homeostatic model assessment of β cell function; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; LF, low fat; LGI; low glycemic index.

[a] Data are given as mean ± SEM or median (interquartile range) in those variables without normal distribution. Analysis of Covariance models were used to assess differences between intervention groups in those variables with normal distribution and Kruskal–Wallis and Mann–Whitney tests in those without normal distribution. The paired-t-test or the Wilcoxon test was used to assess intra-group differences. a: significantly different from HGI; b: significantly different from LF.

Both human and animal studies have highlighted the important role of miRNAs in the development of such chronic conditions as obesity. miRNAs are involved in several processes at the adipocyte level, with important implications for the progression of obesity, adipogenesis and cell functionality [11]. Recently, a meta-signature of 42 miRNAs related to key gene targets of the insulin receptor signaling pathways, MAPK signaling, cell cycle and lipid metabolism was found to be differentially expressed in mature human adipocytes compared to stromal vascular cells and mesenchymal stem cells [22]. Although the secretory mechanism remains largely unknown, miRNAs secreted from the original donors are readily detected in plasma and may serve as endocrine signals for regulatory purposes in target cells [23]. Therefore, a better understanding of their expression and secretion would be helpful to target obesity and related comorbidities. In this regard, diet is a powerful candidate for modulating the miRNA profile since macronutrients and other dietary components, such as vitamins, have been found to differentially affect the expression profile of miRNAs and their functions [24]. However, the modulatory effect of a full dietary pattern on the miRNA profile has been poorly explored. Thus, whereas a surgically-induced weight loss exerted a significant modulatory effect on several c-miRNAs, no changes were observed when weight loss was achieved by an energy-restricted diet in humans [12]. In contrast, the majority of miRNAs down-regulated in the obesity state were reversed in a mouse model of diet-induced obesity (DIO), with weight reduction being achieved by a low-fat feeding diet [6]. In our study, only the c-miR-361 was significantly down regulated in the LGI group compared to the HGI group, even after adjusting for changes in weight loss during the intervention. The miR-361 has been associated with the suppression of tumor cell proliferation [25,26], but it could also play a protective role in cardiovascular diseases by suppressing endogenous vascular endothelial growth factor (VEGF) expression [27]. A recent study found that miR-361 directly target SH2B1 gene [28] which has been associated with obesity [29,30], and which expression seems to be regulated by the quality of the macronutrients of the diet, included the GI. SH2B1 is an adapter protein that binds a large number of kinases such as Janus kinase (JAK-2) and JAK1, and insulin receptors (IRS-1, IRS-2). SH2B1 has been demonstrated to regulate multiple cellular processes both at

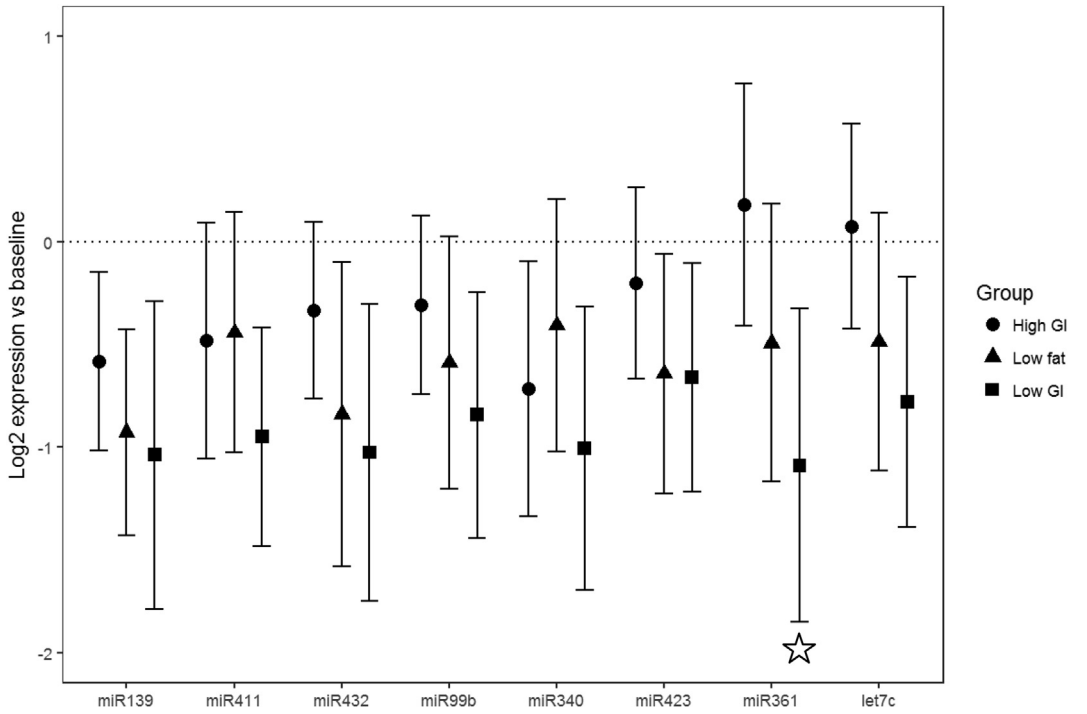

Fig. 1. Changes in circulating microRNA levels according to dietary intervention. Data is shown as mean and 95% CI. GI, glycemic index. *P*-value for the miR-361 between low-GI and high-GI is 0.012.

Table 3
Significant correlations between changes in circulating-microRNAs and changes in biochemical parameters.

	miR-139	miR-340	miR-423	let-7c
Total cholesterol (mmol/L)	0.254 (0.010)	NS	0.230 (0.020)	0.254 (0.010)
LDL cholesterol (mmol/L)[1]	0.235 (0.017)	NS	NS	0.207 (0.037)
Triglycerides (mmol/L)	NS	0.223 (0.024)	NS	0.209 (0.035)
Insulin (mU/mL)	0.216 (0.029)	NS	NS	NS
HOMA IR	0.216 (0.028)	NS	NS	NS

Single associations were tested between the end/baseline ratio and changes in biochemical variables by Spearman's correlation coefficient or [1]Pearson for normally distributed variables. Correlation coefficient (*P* value). miR, microRNA; NS, non-significant correlations.

peripheral and central nervous system [31]. A genetic deletion of SH2B1 results in severe obesity and type 2 diabetes in mice [32,33]. At peripheral tissues, SH2B1 promotes pancreatic β cell expansion and insulin secretion, and regulates the hepatic content of triacylglycerol and the secretion of very low-density lipoproteins [31]. According to these previous described molecular mechanisms, the decreased c-miR-361 observed in our study after the LGI diet could contribute to explain the beneficial role of the LGI diets in the management of body weight, insulin resistance and other cardiovascular risk factors in comparison to HGI diets. We postulated that the lower glycemia and hyperinsulinemia responses after the consumption of low-GI diets could modulates c-miR-61 levels.

All the other c-miRNAs analyzed in our study were significantly down regulated after a LGI diet, whereas only a few were down regulated after the HGI diet. Since changes in body weight were not substantially different between the two intervention groups and no correlations were observed between changes in c-miRNAs and changes in body weight, we cannot discard a potential modulatory effect of the LGI diet on miR-411, miR-99b and let-7c. Moreover, the positive relationship observed between changes in circulating let-7c levels and changes in total cholesterol, LDL-cholesterol and triglycerides could contribute to support the beneficial role of LGI diets on lipid profile and cardiovascular risk. We postulated that let-7 could improve the lipid profile throughout the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) [34] as the use of HMG-CoA reductase inhibitor drugs decrease plasma total cholesterol, HDL-cholesterol and TAG [35]. Further experimental studies are needed to clarify our hypothesis.

Similarly, the down-regulation observed in miR-139, miR-432 and miR-423 after both LGI and LF diets irrespective of the weight loss achieved means that a residual modulatory effect of diet cannot be discarded.

Our study has some limitations since it was carried out in a small sample of participants. Although the sample was larger than previous studies in this field, it is too small for subgroup analysis. Moreover, because the study was conducted in overweight and obese subjects free of other metabolic conditions, such as type 2 diabetes, our findings cannot be generalized to all obese subjects. Also, having conducted the analysis in circulating miRNAs without carrying out in vitro or functional analysis, we cannot identify a cause–effect relationship between the miRNAs analyzed and the possible targeted pathways. Taken together, the results of our study stress the importance of weight loss on c-miRNA modulation but also highlight that miRNAs may respond to the physiological stimuli that diet induces. In this regard, a low-glycemic index diet seems to provide a better c-miRNA profile that could help to protect against cardiovascular diseases. Thus, our results encourage the need for further investigations to clarify the effect dietary factors have on circulating miRNAs and their overall functional impact on cells.

Statement of authorship

The authors' responsibilities were as follows—MB and JS-S: contributed to the conception, design, and implementation of the project; MB, SG, PH-A and AD-L: contributed to data collection and analytical procedures; SG, PH-A, and MB: conducted the statistical analysis, interpreted data, and wrote the manuscript; and all authors: read and approved the final version of the manuscript. Each author certifies that this material or similar material has not previously been published elsewhere.

Conflict of interest

The authors do not report any conflict of interest.

Clinical Trial Registry number and website

This trial was registered at Current Controlled Trials (www.controlled-trials.com) as ISRCTN54971867.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2017.11.014.

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