Modulation of human subcutaneous adipose tissue microRNA profile associated with changes in adiposity-related parameters

Simona Giardina[1,2], Pablo Hernández-Alonso[1,2], Jordi Salas-Salvadó[1,2], Antoni Rabassa-Soler[1], Mònica Bulló[1,2]

Author affiliations

[1] Human Nutrition Unit, Faculty of Medicine and Health Sciences, Institute of Health Pere Virgili, Universitat Rovira i Virgili, Reus, Spain.

[2] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

Corresponding author and person to whom reprint requests should be addressed:

Mònica Bulló, PhD, and Jordi Salas-Salvadó, PhD, Human Nutrition Unit, Faculty of Medicine, Universitat Rovira i Virgili, C/ Sant Llorenç, 21; 43201 Reus, Spain. Tel. +34 977759313 / Fax +34 977759322; e-mail address: monica.bullo@urv.cat and jordi.salas@urv.cat

Abbreviations: BMI, body mass index; **BW**, body weight; **C**t, threshold cycle; **HGI**, high glycemic index; **HOMA-IR**, homeostatic model assessment-insulin resistance; LGI, low glycemic index; **LF**, low fat; **miRNAs**, microRNAs; **SAT**, subcutaneous adipose tissue; **TLDS**, TaqMan Low Density Array; **WC**, waist circumference

Received: 11/07/2017; Revised: 22/09/2017; Accepted: 25/09/2017
This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mnfr.201700594.

Keywords: carbohydrates, diet, high-throughput, post-transcriptional regulation, obesity,

Clinical Trial Registry number and website:

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

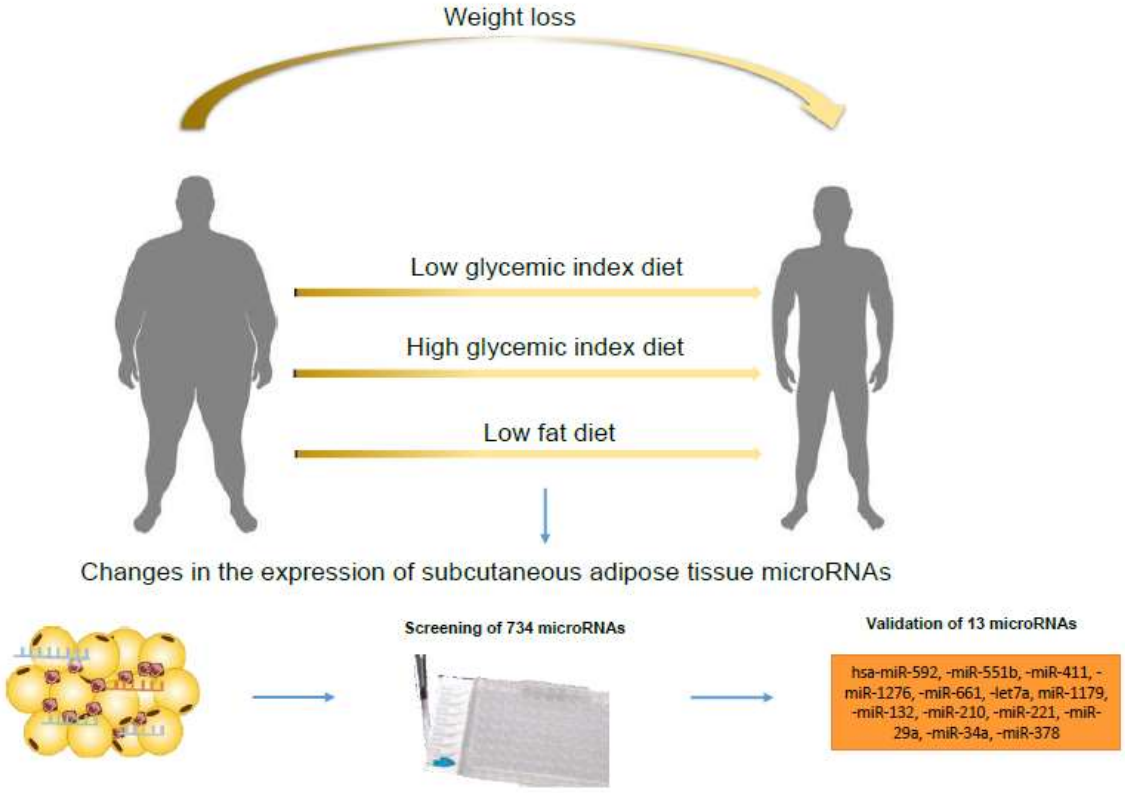
ABSTRACT

Scope: To analyze the effect of three calorie-restricted diets with different amount and quality of carbohydrates on subcutaneous adipose tissue (SAT) miRNA profile.

Methods and Results: 6-month, parallel, randomized trial conducted on overweight and obese subjects randomized to: 1) low glycemic index diet (LGI), 2) high glycemic index diet (HGI), and 3) low-fat (LF). The genome-wide SAT miRNA profile was assessed in 8 randomly selected participants and the most relevant changing miRNAs (n=13) were validated in 48 subjects. None of the miRNAs showed significant changes between the intervention groups. However, changes in some of them correlated with changes in biochemical and anthropometric variables. Stratifying our population according to tertiles of percentage change in body weight, we observed a significant down-regulation of miR-210 in those subjects in Tertile 1 compared to Tertile 3. When our population was stratified by tertiles of waist circumference, miR-132, miR-29a, miR-34a and miR-378 were found to be significantly down-regulated, in T2 compared to T3. Furthermore, when stratified by tertiles of fat mass, we also observed the significant down-regulation of miR-132 in T1.

Conclusion: The macronutrient composition of a calorie-restricted diet does not affect the expression of the miRNAs analyzed, while changes in adiposity play a primary regulatory role.

Adipose tissue microRNAs expression changes in response to weight loss. However, it is not clear whether the change is due to the magnitude of the weight loss or the way in which it is lost. To better clarify this issue, we analyzed the effect of three energy-restricted diets that differ in the amount and quality of carbohydrates, on subcutaneous adipose tissue miRNAs expression.



INTRODUCTION

Since their discovery in 1993, microRNAs (miRNAs) have been widely studied as important regulators of gene expression. MiRNAs are small non-coding RNA molecules (18-25 nucleotide-long) that bind their target mRNAs and cause either translational repression or degradation [1]. Due to this interaction, miRNAs regulate several biological processes such as development, cell differentiation and apoptosis, thus playing an important role in the pathophysiology of several metabolic disorders [2].

Owing to their involvement in the regulation of energy metabolism [3], these molecules have received growing attention in the field of obesity research. In fact, a growing number of studies have pointed out their involvement in obesity and its related metabolic disorders [4, 5]. In particular, these studies focus on the integral role miRNAs have in the formation and function of adipose tissue [6–8]. Therefore, the traditional view of adipose tissue as a biologically inactive lipid storage depot has fundamentally changed and it is now well-known that it is a highly responsive endocrine and exocrine organ that influences and interacts with metabolic homoeostasis and inflammation [9]. In fact, in the fat cells of diet-induced obese mice 35 of the 574 miRNAs detected were expressed differently than in lean animals [10]. While in *in vitro* differentiated adipocytes and adipose tissue biopsies from human donors 40 miRNAs in pre-adipocytes and 31 miRNAs in adipocytes were expressed differently in the obese state. The same study also reported that the expression pattern of 22 miRNAs in human subcutaneous adipose tissue is associated with parameters of obesity [11]. However, the evidence collected on this topic still presents a high degree of inconsistency [12].

The extent to which miRNA adipose tissue expression changes in response to weight-reduction strategies is not well understood and whether expression is changed by the magnitude of the weight lost or the way in which it is lost has never been explored so far. In this regard, such dietary components as amino acids, carbohydrates, fatty acids and vitamins,

and different dietary patterns have been found to modulate the expression and functions of miRNAs [13]. Therefore, we hypothesized that the quality of a diet in a weight loss program might affect miRNAs expression, and that this effect is more prominent than the weight loss itself.

For this reason, our aim was to analyze the effect of three energy-restricted intervention diets that differ in the total carbohydrate amount and carbohydrate quality (high or low glycemic index) on changes in the miRNA profile of subcutaneous adipose tissue.

METHODS

Study population

We analyzed SAT from 48 participants in the GLYNDIET study. Briefly, the GLYNDIET study was a 6-mo randomized, parallel, controlled, clinical trial carried out between 2010 and 2012. It aimed to assess the effect of dietary GI on weight loss, satiety, glucose and insulin metabolism, lipid profile, inflammation and other metabolic risk markers. The participants were community-dwelling overweight and obese men and women with a body mass index (BMI) between 27 and 35 (in kg/m^2) aged between 30 and 60 years. Details of the study protocol have been described elsewhere [14]. The study protocol was approved by the institutional review board and registered in the International Standard Randomized Controlled Trial Number (ISRCTN54971867). All eligible candidates provided written informed consent.

Dietary interventions, and anthropometric and biochemical measurements

Participants were randomly allocated into three dietary intervention groups: 1) a moderate-carbohydrate and low glycemic index diet (LGI); 2) a moderate-carbohydrate and high glycemic index diet (HGI), and 3) a low-fat and high glycemic index diet (LF). Moderate carbohydrate diets contain approximately 40% E from fat, 42% E from carbohydrates and 18% E from protein, and the low-fat diet fulfilled the criteria defined by the American Heart association (30% E from fat, 52% E from carbohydrates and 18% E from protein) [15]. Individual examinations were scheduled at baseline, 15 d into the intervention, and monthly till the end of the study. Anthropometric measures were taken during the individual examinations, while blood samples were collected at baseline and the end of the dietary intervention periods. Data on body weight and height was obtained with subjects wearing light clothes. Waist circumference was measured midway between the lowest rib and the iliac crest. Body composition was assessed by using a bioelectrical impedance analysis (TANITA TBF-300; Tanita).

Plasma fasting glucose concentration was determined by using standard enzymatic automated methods (COBAS; Roche Diagnostics Ltd). Fasting insulin was determined by using an enzyme-linked immunosorbent assay commercial kit (Merck Millipore, Darmstadt, Germany). Insulin resistance was estimated by using homeostatic model assessment-insulin resistance (HOMA-IR).

Adipose tissue collection and RNA extraction

Subcutaneous adipose tissue samples were collected in participants at baseline and at the end of the study. Samples were taken by incisional biopsy on the right side of the abdomen under local anesthesia. The adipose tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until use.

We purified total RNA using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Briefly, a piece of tissue sample was placed into 700 µL QIAzol Lysis Reagent and homogenized using a rotor-stator homogenizer. After 5 minutes at room temperature, we added 140 µL chloroform and centrifuged the tube for 15 min at 12,000 xg at 4°C and transferred the upper aqueous phase to a new collection tube. After adding 100% ethanol, the sample was transferred into an RNeasy Mini spin column for washing and the RNA was collected in the final step by elution with 50 µL of RNase free water. Aliquots of the eluted samples were stored at -80°C until use.

Screening step: microRNAs retro-transcription and pre-amplification

In an initial screening step, we profiled the miRNAs expression in a randomly selected representative cohort of 8 subjects (3 LGI, 3 HGI and 2 LF), using a TaqMan Low Density Array (TLDA's) (Applied Biosystems, Darmstadt, Germany). A total of 754 miRNAs were reverse transcribed using TaqMan MicroRNA Reverse Transcription (RT) kit and Megaplex RT Primers, Human Pools A v.2.1 and B v.3.0 (Applied Biosystems, Darmstadt, Germany). Then, the RT products were preamplified with TaqMan PreAmp Master Mix and Megaplex PreAmp Primers (Human Pools A v.2.1 and B v.3.0). We ran both reactions in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Darmstadt, Germany).

Using a TaqMan Universal PCR Master Mix, no AmpErase UNG (2x) and the TLDA's Array (TLDAs card A v2.1 and B v3.0, Applied Biostystes, Darstadt, Germany), we carried out a quantitative polymerase chain reaction (qPCR) on the pre-amplified product. The TLDAs arrays were analyzed in a real-time thermal cycler (7900 Fast Real-Time PCR System; Applied Biosystems, Darstadt, Germany).

Data from qPCR was obtained by SDS v.2.2 and processed by RQ Manager v1.2 software. Results were expressed as threshold cycle (Ct) values. Mean-centering method was used for

normalization, as described by Wylie et al [16]. We also selected a set of seven miRNAs related to the adipose tissue, obesity, insulin resistance, glucose metabolism and diabetes modulation, using updated reviews [8, 11, 17–19] and databases [20–22].

Analysis of individual miRNAs

Commercially available TaqMan hydrolysis probes were used to validate a total of 13 miRNAs (plus the endogenous control) in the 48 subjects of the GLYNDIET study. A fixed 3 μL volume of RNA eluate was used for RT using the TaqMan MicroRNA Reverse Transcription Kit in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Darmstadt, Germany).

We carried out various qPCR reactions using the following TaqMan MicroRNA Assays in a 7900HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany): hsa-miR-592 (MI0003604), -miR-551b (MI0003575), -miR-411 (MIMAT0003329), -miR-1276 (MIMAT0005930), -miR-661 (MI0003669), -let7a (MIMAT0000062), -miR1179 (MI0006272), -miR-132 (MIMAT0004594), -miR-210 (MIMAT0000267), -miR-221 (MIMAT0000278), -miR-29a (MIMAT0000086), -miR-34a (hsa-miR-34a-5p) and -miR-378 (MIMAT0000732). RNU48 was used as the endogenous control in each reaction for target miRNAs. All measurements were performed in duplicate, and qPCR data was acquired using Sequence detector software (SDS version 2.4, Applied Biosystems, Darmstadt, Germany)

The expression of the miRNAs analyzed was normalized by the mean of RNU48 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, a feature selection that combines miRNA data with gene ontology (GO) information is proposed in Supplementary Table2. For this purpose, we selected a list of genes for the 5 miRNA differentially expressed according to tertiles of body weight (BW), waist circumference (WC)

or fat mass (miR-210, miR-132, miR-29a, miR-34a and miR-378) 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. The enrichment of biological processes among these genes was evaluated by DAVID Bioinformatics Resources 6.7 considering the total human genome as a background and P values <0.05 after Bonferroni correction.

Statistical analysis

The descriptive data of study participants are shown as medians and IRQs for continuous measures and as a numbers and percentages for categorical variables.

Before the miRNA data was subject to statistical analysis, the normal distribution and homogeneity of the variances were evaluated using Lilliefors' and Levene's tests, respectively. All the miRNAs showed non-normal and skewed distribution, so normalized log2 ratios of expression relative to the baseline were used for statistical tests, and data are presented as medians [IQR (interquartile range)]. Therefore, values higher than 0 mean that the intervention leads to up-regulation and lower than 0 that it leads to down-regulation. Variables were adjusted using the residual method. Kruskal-Wallis and Mann-Whitney tests were used to assess differences between intervention groups or categorical variables (tertiles), while the Wilcoxon test was performed to compare baseline and end miRNA levels within each intervention period. The Bonferroni post-hoc test was used for multiple comparisons. We used Spearman's correlation coefficients to evaluate whether the changes (final/baseline of each intervention period) in fat tissue levels of different miRNAs correlated with anthropometric and biochemical data regardless of the intervention period. All analyses were carried out using SPSS 22.0 (SPSS Inc, Chicago, IL) and R 3.3.2. All the tests were 2-sided, and significance was set at $P<0.05$.

RESULTS

Of the total 122 subjects randomized in the original study, only 48 subjects agreed to have a SAT biopsy at baseline and at the end of the dietary intervention. **Supplemental table 1** shows the baseline characteristics of the study participants divided by intervention group, LGI (n=16), HGI (n=17), LF (n=15). The groups were well-balanced except for the diastolic and systolic blood pressure, which was higher in the LF group than the LGI group. **Table 1** shows the baseline and 6-month changes in body weight and composition and glucose-related variables. All the three dietary approaches succeeded in significantly decreasing the average body weight, BMI, waist circumference, fat mass, fat free mass and HOMA-IR of the participants, compared to their baseline values. Across the intervention, we observed a significantly higher reduction in BMI, fat free mass and fat mass in the LGI group than in the LF group, and higher decreases in the fat mass in the HGI group than in the LF group (**Table 1**). Baseline and 6-month changes in dietary variables are showed in the **Supplemental table 2**.

Study of subcutaneous adipose tissue miRNAs

The SAT of 734 miRNAs was profiled in a randomly selected subsample of 8 subjects using TLDAs system (3 LGI, 3 HGI and 2 LF) before and after the dietary intervention. Six miRNAs were differentially modulated by treatments (hsa-miR-592, -miR-551b, -miR-411, -miR-1276, -miR-661, -let7a). We also made a computerized search to select 7 miRNAs all of which had previously been linked to obesity, adipose tissue development and glucose metabolism (hsa-miR-1179, -miR-132, -miR-210, -miR-221, -miR-29a, -miR-34a, -miR-378). Selected miRNAs were validated n the total subjects. No significant changes were observed in any validated miRNA between the interventions (**Table 2**). However, the expression of miR-551b, miR-221, miR-378 and let7a was downregulated after the LGI

intervention; the expression of miR-1276, miR-132 and miR-29a was downregulated after the HGI intervention; and the expression of miR-661, miR-1179, miR-132, miR-221, miR-29a and miR-378 was downregulated after the LF intervention.

Association with clinical outputs/ Predictors of changes in miRNAs

Changes in the expression of miR-551b, miR-1179, miR-132, miR-221, miR-29a, miR-34a and miR-378 were positively correlated to increasing values of anthropometric variables BW, BMI, WC and/or Fat mass), indicating that increases in the levels of these miRNAs mirror an increase in adiposity (**Table 3**). Interestingly, we found a significant positive association between changes in the levels of miR-1179 and changes in BW, BMI, WC and fat free mass, but not with changes in fat mass.

As far as the glucose metabolism is concerned, changes in miR-210 and miR-34a were positively correlated to changes in glucose levels, but negatively correlated to changes in insulin levels (only in case of miR-34a). However, there were no significant correlations with HOMA-IR (data not shown).

Changes in subcutaneous adipose tissue miRNAs profile by tertiles of changes in body weight, waist circumference and body weight composition

In order to check whether the changes in miRNA levels were influenced by the magnitude of weight lost due to the dietary intervention we stratified our population according to changes in body weight. We observed a significantly lower down-regulation of miR-210 in those participants with a lower weight loss and a non-significant trend for miR-132 (p=0.057) (**Table 4**). These trends were maintained according to changes in body weight distribution (**Table 5**). Those participants with a smaller decrease in waist circumference had a lower downregulation of miR-132 and an increased expression of miR-210. Similarly, miR-29a,

miR-378 and miR-34a were significantly less down- or up-regulated in the upper tertile of percentage change of waist circumference. According to these findings the expression of miR-132 was less down-regulated and miR-210 was up-regulated in participants with the lowest changes in fat mass during the weight loss intervention.

The *in silico* analysis of the significantly modulated microRNAs by tertiles of anthropometry parameter showed a total of 17 significantly enriched GO processes (**Supplemental table 3**).

DISCUSSION

The influence of diet on microRNA expression has been assessed in animals and in vitro studies. However, to the best of our knowledge, this is the first human feeding trial to analyze the effect of three weight-loss energy restricted diets with different carbohydrate and fat contents on the expression of miRNAs in adipose tissue. The results of our study show that weight loss and changes in body composition have such a great effect on miRNA expression that they may mask any potential role of the diet composition.

MiRNAs have emerged as important regulatory determinants in many biological processes, including adipogenesis and obesity [1, 2]. Several miRNAs with potential physiological functions [8, 11, 12] are deregulated in obesity. Accordingly, weight-reduction strategies induced with a low-fat diet in mice [23] and surgical procedures or an energy-restricted diet plus exercise in humans [24][25][26] modulate both circulating and adipose tissue miRNA expression. In our study, we failed to found major changes in miRNAs expression in response to the three different dietary interventions in the context of a weight-loss program, supporting that not all physiological effects necessarily have epigenetic mechanisms (through miRNA changes). These lack of dietary intervention effect suggested that the magnitude of the weight lost and the changes in body composition or adipose tissue distribution could be the main

drivers in changing the expression of SAT miRNAs, rather that the dietary composition. Accordingly, when stratifying the population by tertiles of changes in body weight, waist circumference or % of fat mass, we observed a consistent down-regulation of miR-210 in the lowest tertiles of changes compared to the highest. MiR-210 is an adipogenic miRNA that acts on Transcription factor 7-like 2 (*TCF7L2*), which is a transcription factor modulating WNT signaling pathway [27] and it is also one of the most consistently and ubiquitously induced miRNAs under hypoxia in hypoxic cell and tissue types [28]. Since hypoxia is one of the mechanisms involved in the development of inflammation and metabolic dysfunction of the adipose tissue in obesity [29], the down-regulation of miRNA-210 observed in our study could be an indirect biomarker of reduction of inflammation due to weight and fat loss.

Changes in miR-132 were also found to be correlated with changes in fat mass, and participants with a greater change in fat mass showed a greater down-regulation of miR-132. This miRNA was found to promote the production of pro-inflammatory secretion factors in human adipose tissue by targeting sirtuin 1, so inducing the NF-Kb mediated activation of interleukin-6 (*IL-6*) and *CP1* expression [30]. Therefore, the down-regulation observed in our study could help to improve the inflammation associated to obesity. We also observed the down-regulation of miR-29a, miR-34a and miR-378 expression associated with changes in waist circumference, although in this case with significant differences between the second and the third tertile.

These miRNAs have all been found to be related to either obesity or obese related complications. MiR-34a and miR-29a seem to be involved in the development of the insulin resistance associated to obesity [4, 31, 32], while miR-378 is mostly responsible for adipogenesis and lipid accumulation in adipocytes [33]. In parallel to the down-regulation of these miRNAs, an improvement in insulin resistance has been observed in our patients. Therefore, our data suggest a change towards a better metabolic profile secondary to total

body fat loss. Moreover, despite no significant differences were observed in miR-1179adipose tissue expression according to tertiles of body weight, waist circumference or percentage of body fat, the positive correlation observed with fat free mass could infer a potential role of this miRNA in muscle mass.

The results of this study highlight the importance of waist circumference itself together with the extent to which it is reduced as an important modulator of SAT miRNA expression, as suggested by the significant reduction observed in T2 compared to T3. Waist circumference is an anthropometric measure that reflects abdominal obesity and, according to the WHO, is related to increased risk of several chronic diseases and all-cause death [34]. In fact, waist circumference has been demonstrated as a better predictor of obesity-related metabolic conditions than BMI [35].

Although a potential limitation of our study was the relative small sample size, we used a high throughput method to cover the expression of the maximum number of miRNAs in a well-designed clinical trial with a medium-term of follow-up.

In conclusion, according to our results, the composition of the diet does not seem to be a factor that modulates the expression of the miRNAs analyzed here. However, some of the parameters of adiposity, especially how the fat is distributed, may play a significant role in regulating the expression of miRNAs that have been related to obesity-related complications.

Author contributions:

MB and JS-S had full access to all the data in the study and take full responsibility for the integrity and accuracy of the data analysis. Study concept and design: Acquisition of data: SG. Analysis and interpretation of data: SG, MB, PH-A. Drafting of the manuscript: SG, MB, PH-A. Critical revision of the manuscript for important intellectual content: MB and JS-S.

Statistical analysis: PH-A, SG and MB. Obtained funding: MB. Administrative, technical, or material support. All the authors read and approved the final manuscript.

Acknowledgements

The authors thank the participants for their enthusiastic collaboration and the GLYNDIET personnel for their excellent assistance. The Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN) is an initiative of Instituto de Salud Carlos III, Spain. The Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, the Instituto de Salud Carlos III - Fondo de Investigación Sanitaria (PI12/0153) and cofounded by the Fondo Europeo de Desarrollo Regional (FEDER).

Conflict of interest statement:

I certify that neither I nor my co-authors have a conflict of interest as described above that is relevant to the subject matter or materials included in this Work.

Funding sources:

Supported by the Institut d'Investigació Sanitaria Pere Virgili (PV11059S) and the Fondo de Investigación Sanitaria (PI12/0153).

References

[1] Bartel, D.P., MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 2004, *116*, 281–297.

[2] Rottiers, V., Näär, A.M., MicroRNAs in metabolism and metabolic disorders. *Nat. Rev. Mol. Cell Biol.* 2012, *13*, 239–250.

[3] Vienberg, S., Geiger, J., Madsen, S., Dalgaard, L.T., MicroRNAs in metabolism. *Acta Physiol.* 2016.

[4] Xie, H., Sun, L., Lodish, H.F., Targeting microRNAs in obesity. *Expert Opin. Ther. Targets* 2009, *13*, 1227–1238.

[5] Park, S.-Y.Y., Jeong, H.-J.J., Yang, W.-M.M., Lee, W., Implications of microRNAs in the pathogenesis of diabetes. *Arch. Pharm. Res.* 2013, *36*, 154–166.

[6] Mudhasani, R., Puri, V., Hoover, K., Czech, M.P., Imbalzano, A.N., Jones, S.N., Dicer is required for the formation of white but not brown adipose tissue. *J. Cell. Physiol.* 2011, *226*, 1399–406.

[7] Wang, Q., Li, Y.C., Wang, J., Kong, J., Qi, Y., Quigg, R.J., Li, X., miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. *Proc. Natl. Acad. Sci.* 2008, *105*, 2889–2894.

[8] Hilton, C., Neville, M.J., Karpe, F., MicroRNAs in adipose tissue: their role in adipogenesis and obesity. *Int. J. Obes.* 2013, *37*, 325–332.

[9] Coelho, M., Oliveira, T., Fernandes, R., Biochemistry of adipose tissue: an endocrine organ. *Arch. Med. Sci.* 2013, *9*, 191–200.

[10] Xie, H., Lim, B., Lodish, H.F., MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes* 2009, *58*, 1050–1057.

[11] Ortega, F.J., Moreno-Navarrete, J.M., Pardo, G., Sabater, M., Hummel, M., Ferrer, A., Rodriguez-Hermosa, J.I., Ruiz, B., Ricart, W., Peral, B., Fernandez-Real, J.M., Fernández-Real, J.M., MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. *PLoS One* 2010, *5*, e9022.

[12] Arner, P., Kulyté, A., MicroRNA regulatory networks in human adipose tissue and obesity. *Nat. Rev. Endocrinol.* 2015, *11*, 276–28825.

[13] García-Segura, L., Pérez-Andrade, M., Miranda-Ríos, J., The Emerging Role of MicroRNAs in the Regulation of Gene Expression by Nutrients. *J. Nutrigenet. Nutrigenomics* 2013, *6*, 16–31.

[14] Juanola-Falgarona, M., Salas-Salvadó, J., Ibarrola-Jurado, N., Rabassa-Soler, A., Díaz-López, A., Guasch-Ferré, M., Hernández-Alonso, P., Balanza, R., Bulló, M., Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. *Am. J. Clin. Nutr.* 2014, *100*, 27–35.

[15] American Heart Association Nutrition Committee, A.H., Lichtenstein, A.H., Appel,

L.J., Brands, M., Carnethon, M., Daniels, S., Franch, H.A., Franklin, B., Kris-Etherton, P., Harris, W.S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Van Horn, L., Winston, M., Wylie-Rosett, J., Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006, *114*, 82–96.

[16] Wylie, D., Shelton, J., Choudhary, A., Adai, A.T., A novel mean-centering method for normalizing microRNA expression from high-throughput RT-qPCR data. *BMC Res. Notes* 2011, *4*, 555.

[17] Kloting, N., Berthold, S., Kovacs, P., Schon, M.R., Fasshauer, M., Ruschke, K., Stumvoll, M., Bluher, M., MicroRNA expression in human omental and subcutaneous adipose tissue. *PLoS One* 2009, *4*, e4699.

[18] Parts, L., Hedman, Å.K., Keildson, S., Knights, A.J., Abreu-Goodger, C., van de Bunt, M., Guerra-Assunção, J.A., Bartonicek, N., van Dongen, S., Mägi, R., Nisbet, J., Barrett, A., Rantalainen, M., Nica, A.C., Quail, M.A., Small, K.S., Glass, D., Enright, A.J., Winn, J., Deloukas, P., Dermitzakis, E.T., McCarthy, M.I., Spector, T.D., Durbin, R., Lindgren, C.M., Extent, causes, and consequences of small RNA expression variation in human adipose tissue. *PLoS Genet.* 2012, *8*.

[19] Brandão, B.B., Guerra, B.A., Mori, M.A., Shortcuts to a functional adipose tissue: The role of small non-coding RNAs. *Redox Biol.* 2017, *12*, 82–102.

[20] Lu, M., Zhang, Q., Deng, M., Miao, J., Guo, Y., Gao, W., Cui, Q., An Analysis of Human MicroRNA and Disease Associations. *PLoS One* 2008, *3*, e3420.

[21] Jiang, Q., Wang, Y., Hao, Y., Juan, L., Teng, M., Zhang, X., Li, M., Wang, G., Liu, Y., miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res.* 2009, *37*, D98-104.

[22] Kozomara, A., Griffiths-Jones, S., miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* 2011, *39*, D152-7.

[23] Hsieh, C.-H., Rau, C.-S., Wu, S.-C., Yang, J.C.-S., Wu, Y.-C., Lu, T.-H., Tzeng, S.-L., Wu, C.-J., Lin, C.-W., Weight-reduction through a low-fat diet causes differential expression of circulating microRNAs in obese C57BL/6 mice. *BMC Genomics* 2015, *16*, 699.

[24] Nardelli, C., Iaffaldano, L., Pilone, V., Labruna, G., Ferrigno, M., Carlomagno, N., Dodaro, C.A., Forestieri, P., Buono, P., Salvatore, F., Sacchetti, L., Changes in the MicroRNA Profile Observed in the Subcutaneous Adipose Tissue of Obese Patients after Laparoscopic Adjustable Gastric Banding. *J. Obes.* 2017, *2017*, 1–6.

[25] Nunez Lopez, Y.O., Coen, P.M., Goodpaster, B.H., Seyhan, A.A., Gastric bypass surgery with exercise alters plasma microRNAs that predict improvements in cardiometabolic risk. *Int. J. Obes.* 2017.

[26] Parr, E.B., Camera, D.M., Burke, L.M., Phillips, S.M., Coffey, V.G., Hawley, J.A., Circulating MicroRNA Responses between "High" and "Low" Responders to a 16-Wk Diet and Exercise Weight Loss Intervention. *PLoS One* 2016, *11*, e0152545.

[27] McGregor, R.A., Choi, M.S., microRNAs in the regulation of adipogenesis and

obesity. *Curr. Mol. Med.* 2011, *11*, 304–16.

[28] Huang, X., Le, Q.-T., Giaccia, A.J., MiR-210--micromanager of the hypoxia pathway. *Trends Mol. Med.* 2010, *16*, 230–7.

[29] Trayhurn, P., Alomar, S.Y., Oxygen deprivation and the cellular response to hypoxia in adipocytes - perspectives on white and brown adipose tissues in obesity. *Front. Endocrinol. (Lausanne).* 2015, *6*, 19.

[30] Strum, J.C., Johnson, J.H., Ward, J., Xie, H., Feild, J., Hester, A., Alford, A., Waters, K.M., MicroRNA 132 regulates nutritional stress-induced chemokine production through repression of SirT1. *Mol. Endocrinol.* 2009, *23*, 1876–84.

[31] Roggli, E., Britan, A., Gattesco, S., Lin-Marq, N., Abderrahmani, A., Meda, P., Regazzi, R., Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells. *Diabetes* 2010, *59*, 978–86.

[32] Meerson, A., Traurig, M., Ossowski, V., Fleming, J.M., Mullins, M., Baier, L.J., Human adipose microRNA-221 is upregulated in obesity and affects fat metabolism downstream of leptin and TNF-α. *Diabetologia* 2013, *56*, 1971–9.

[33] Civelek, M., Hagopian, R., Pan, C., Che, N., Yang, W. -p., Kayne, P.S., Saleem, N.K., Cederberg, H., Kuusisto, J., Gargalovic, P.S., Kirchgessner, T.G., Laakso, M., Lusis, A.J., Genetic regulation of human adipose microRNA expression and its consequences for metabolic traits. *Hum. Mol. Genet.* 2013, *22*, 3023–3037.

[34] Nishida, C., Ko, G.T., Kumanyika, S., Body fat distribution and noncommunicable diseases in populations: overview of the 2008 WHO Expert Consultation on Waist Circumference and Waist-Hip Ratio. *Eur. J. Clin. Nutr.* 2010, *64*, 2–5.

[35] Janssen, I., Katzmarzyk, P.T., Ross, R., Waist circumference and not body mass index explains obesity-related health risk. *Am. J. Clin. Nutr.* 2004, *79*, 379–84.

Table1. Baseline and 6-month changes in body composition and glucose-related parameters by intervention group

Variable	LGI diet (n=16)	P-value within group	HGI diet (n=17)	P-value within group	LF diet (n=15)	P-value within group	P-value between groups
Weight (kg)							
Baseline	79.5 [76.7; 90.9]		81.6 [75.5; 86.2]		86.8 [75.5; 90.3]		0.801
6-mo changes	-8.2 [-10.7; -6.5]	<0.001	-10.3 [-12; -5.6]	<0.001	-5.0 [-9.3; -3.4]	0.003	0.060
BMI							
Baseline	31.5 [29.7; 32.5]		29.7 [28.6; 31.9]		30.1 [29.4; 31.0]		0.156
6-mo changes	-3.0 [-4.0; -2.4][a]	<0.001	-3.4 [-4.4; -1.6]	<0.001	-1.5 [-2.9; -1.2]	0.002	0.029
Waist circumference							
Baseline	99.3 [94; 104.3]		98 [93; 107]		104 [98; 106.5]		0.349
6-mo changes	-9.3 [-11.2; -6.7]	<0.001	-8.9 [-11.0; -6.5]	<0.001	-8.1 [-9.8; -4.4]	0.001	0.220
Fat mass (%)							
Baseline	40.9 [37.8; 45]		40.5 [36.5; 42.6]		38.4 [30; 41.5]		0.164
6-mo changes	-5.7 [-6.6; -3.8][a]	<0.001	-5.9 [-8.4; -3.1][a]	0.001	-2.9 [-4.3; 1.9]	0.001	0.017
Fat free mass (%)							
Baseline	59.1 [54.7; 62.2]		59.1 [57.4; 63.2]		61.5 [58; 70]		0.183
6-mo changes	-3.5 [-4.0; -2.7][a]	<0.001	-2.3 [-4.4; -1.6]	0.001	-1.7 [-2.2; -0.7]	0.001	0.007
Glucose (mg/dL)							
Baseline	96 [91; 101]		100 [93; 104]		99 [93; 108]		0.395
6-mo changes	-5.9 [-10.4; -1.7]	0.108	-4.3 [-13.8; 3.7]	0.449	-3.2 [-8.4; 1.3]	0.348	0.710
Fasting insulin (mU/mL)							
Baseline	5.6 [4.0; 7.1]		3.5 [2.6; 5.4]		4.3 [3.9; 6.8]		0.303
6-mo changes	-1.8 [-2.5; -1.3]	0.002	-2.3 [-2.7; -1.4]	0.001	-1.8 [-2.2; -0.7]	0.011	0.303
HOMA IR							
Baseline	1.3 [0.9; 1.8]		0.9 [0.7; 1.3]		1.1 [0.9; 1.8]		0.351
6-mo changes	-0.5 [-0.6; -0.4]	0.002	-0.6 [-0.7; -0.3]	0.001	-0.4 [-0.6; -0.2]	0.011	0.360

Data are given as median±IQRs. *P* values for intra-group differences by Wilcoxon. P values for differences between intervention group by Kruskal-Wallis and Mann-Whitney tests. Data on 6-month changes were adjusted for the baseline values of each variable.

Abbreviations: BMI, body mass index; HGI, high glycemic index; HOMA-IR, homeostatic model assessment of insulin resistance; LF, low fat; LGI; low glycemic index. [a], significantly different from LF; [b], significantly different from HGI.

Table 2. Changes versus baseline of miRNAs by intervention diet

miRNA	Low GI (n=16)	High GI (n=17)	Low Fat (n=15)	P-value
miR-592	0 [-0.61, 0.51]	0 [-0.43, 0.53]	-0.23 [-1.93, 1.1]	0.752
miR-551b	-0.95 [-2.31, 0.41]*	-0.13 [-1.77, 0.69]	0.16 [-0.23, 0.43]	0.348
miR-411	-0.28 [-0.62, 0.11]	0.2 [-0.72, 0.35]	0.14 [-0.09, 0.82]	0.175
miR-1276	-0.35 [-0.93, 1.06]	-0.68 [-1.5, 0.14]*	-0.52 [-1.46, 0.19]	0.408
miR-661	0.14 [-1.65, 0.81]	-0.34 [-2.04, 0.65]	-1.8 [-1.95, -0.1]*	0.325
let7a	-0.39 [-3.89, 0]*	0 [0, 0]	0 [-1.25, 0]	0.155
miR-1179	-1.76 [-2.7, -0.5]	-0.89 [-2.22, 0.23]	-1.24 [-2.17, 0.15]*	0.728
miR-132	-1.32 [-2.78, 0.11]	-0.62 [-2.19, 0.05]*	-0.94 [-2.88, -0.15]*	0.751
miR-210	-0.39 [-0.97, 0.07]	-0.59 [-1.58, 0.71]	-0.52 [-2.04, 0.5]	0.916
miR-221	-2.28 [-4.63, -0.45]*	-1.43 [-3.45, 1.15]	-2.13 [-4.58, -0.18]*	0.486
miR-29a	-0.92 [-1.99, 0.61]	-0.42 [-1.72, 0.07]*	-0.65 [-2.41, -0.14]*	0.888
miR-34a	-1.46 [-2.45, 0.69]	-0.57 [-1.38, 0.4]	-0.18 [-2.82, 0.02]	0.939
miR-378	-1.77 [-4.76, -0.87]*	-0.45 [-2.94, 0.56]	-1.33 [-3.77, -0.54]*	0.695

Data shows median [IQR]. Kruskal-Wallis was performed between the three groups and Mann-Whitney between pairs. *P<0.05 shows significant results when comparing baseline versus end in each period, as calculated by Wilcoxon test. P-value refers to the Kruskal-Wallis test.
Abbreviations: miRNA, microRNA; GI, glycemic index.

Table 3. Correlations between changes in miRNA expression and changes in anthropometric and biochemical variables related to glucose and lipid metabolism

miRNA	Body weight	BMI	WC	Fat mass	Fat free mass	Glucose	Insulin
miR-592	0.114	0.007	0.227	0.261	-0.336	0.232	-0.050
miR-551b	0.621 *	0.539 *	0.570 *	0.418	0.511	-0.125	-0.536 *
miR-411	-0.146	-0.171	-0.291	-0.454	0.271	-0.229	-0.189
miR-1276	-0.039	-0.089	-0.097	0.146	-0.043	0.246	0.064
miR-661	0.000	0.036	-0.200	-0.039	0.100	-0.214	0.193
let7a	0.077	0.044	-0.107	0.081	-0.314	0.085	-0.096
miR-1179	0.65 *	0.682 *	0.640 *	0.464	0.604 *	-0.182	-0.436
miR-132	0.696 *	0.650 *	0.727 *	0.604 *	0.407	0.379	-0.554 *
miR-210	0.471	0.475	0.561 *	0.346	0.368	0.579 *	-0.350
miR-221	0.568 *	0.561 *	0.436	0.521 *	0.118	0.279	-0.589 *
miR-29a	0.807 **	0.782 **	0.794 **	0.693 *	0.479	0.354	-0.664 *
miR-34a	0.539 *	0.482	0.609 *	0.457	0.243	0.536 *	-0.561 *
miR-378	0.579 *	0.593 *	0.483	0.604 *	0.175	0.482	-0.371

Single associations were tested between the end/baseline ratio by Spearman's correlation coefficient for the whole cohort (n=48). Results represent the coefficient of correlation. Significant associations: *P<0.05, **P<0.001. -C, cholesterol;

Abbreviations: miRNA, microRNA; BMI, body mass index; WC, waist circumference.

Table 4. Changes versus baseline of miRNAs by tertiles of percentage change of body weight

miRNA	Tertile 1 -14.33 [-15.78, -13.05] (n=16)	Tertile 2 -9.34 [-10.39, -7.7] (n=16)	Tertile 3 -4.29 [-4.74, -3.8] (n=16)	P-value
miR-592	0 [-0.47, 0.31]	-0.07 [-2.01, 0.78]	0 [-0.52, 1.49]	0.634
miR-551b	0.05 [-1.43, 0.71]	-1.03 [-2.54, -0.07]	0.25 [-0.15, 0.55]	0.116
miR-411	-0.09 [-0.3, 0.27]	-0.16 [-0.64, 0.71]	0.09 [-0.27, 0.7]	0.899
miR-1276	-0.48 [-1.07, 0.98]	-0.79 [-1.16, 0.02]	-0.32 [-1.45, 0.15]	0.845
miR-661	-0.3 [-2.71, 0.81]	-0.52 [-1.93, 0.39]	-0.56 [-1.9, 0.51]	0.988
let7a	0 [-2.88, 0.13]	0 [-2.27, 0]	0 [-0.55, 0]	0.476
miR-1179	-1.8 [-2.82, -0.02]	-1.76 [-2.44, -0.52]	-0.08 [-1.65, 0.24]	0.113
miR-132	-1.08 [-2.72, -0.55]	-2.23 [-3.4, 0.02]	-0.37 [-0.97, 0.2]	0.057
miR-210	-0.64 [-1.63, -0.35]**a**	-0.98 [-2.39, 0.86]	-0.02 [-0.47, 0.78]	0.044
miR-221	-1.4 [-3.71, 0.04]	-3.33 [-5.32, -1.07]	-0.96 [-2.64, 0.43]	0.186
miR-29a	-0.9 [-2.16, 0.09]	-1.78 [-2.75, -0.29]	-0.22 [-0.83, 0.01]	0.111
miR-34a	-0.63 [-2.77, 0.11]	-1.63 [-3.11, 0.01]	-0.16 [-1.24, 0.17]	0.183
miR-378	-1.59 [-3.99, -0.03]	-3.17 [-5.04, -1.02]	-0.68 [-2.3, 0.51]	0.169

Data shows median [IQR]. Kruskal-Wallis was performed among the three tertiles and Mann-Whitney between pairs. P-value refers to the Kruskal-Wallis test. Mann-Whitney test between paired tertiles: a, Tertile 1 vs Tertile 3.

Abbreviations: miRNA, microRNA.

Table 5. Changes versus baseline of miRNAs by tertiles of percentage change of waist circumference

miRNA	Tertile 1 -12.06 [-13.45, -10.73] (n=16)	Tertile 2 -8.86 [-9.13, -7.96] (n=16)	Tertile 3 -4.75 [-6.14, -4.21] (n=16)	P-value
miR-592	0 [-0.48, 0.46]	0 [-1.69, 0.78]	-0.07 [-0.52, 1.19]	0.976
miR-551b	-0.13 [-2.31, 0.56]	-0.39 [-1.43, 0.31]	0.25 [-1.29, 0.42]	0.669
miR-411	0.03 [-0.36, 0.3]	0.1 [-0.38, 0.41]	-0.06 [-0.78, 0.7]	0.858
miR-1276	-0.32 [-0.9, 0.99]	-0.63 [-1.16, -0.03]	-0.56 [-1.57, 0.15]	0.482
miR-661	-0.19 [-2, 0.87]	-0.9 [-2.09, -0.07]	-0.56 [-2.03, 0.51]	0.471
let7a	0 [-0.38, 0]	0 [-3.93, 0.13]	-0.15 [-1.3, 0]	0.912
miR-1179	-1.55 [-2.34, -0.46]	-1.72 [-3.02, -0.18]	0.01 [-1.75, 0.66]	0.093
miR-132	-0.98 [-1.87, -0.4]	-2.51 [-3.26, -0.85]b	-0.01 [-1.02, 0.66]	**0.012**
miR-210	-0.64 [-1.08, -0.27]a	-1.67 [-2.06, -0.3]b	0.5 [-0.31, 1.2]	**0.008**
miR-221	-1.43 [-3.5, 0.04]	-3.15 [-4.94, -1.79]	-0.15 [-2.67, 1.33]	0.053
miR-29a	-0.74 [-1.53, 0]	-2.04 [-2.78, -0.69]b	-0.14 [-0.52, 1.09]	**0.007**
miR-34a	-1.12 [-2.37, 0.09]	-2 [-3.26, -0.52]b	0.02 [-0.58, 0.87]	**0.015**
miR-378	-1.45 [-2.93, -0.3]	-3.73 [-5.04, -1.45]b	-0.09 [-1.93, 1.71]	**0.021**

Data shows median [IQR]. Kruskal-Wallis was performed between the three tertiles and Mann-Whitney between pairs. P-value refers to the Kruskal-Wallis test. Mann-Whitney test between paired tertiles: a, Tertile 1 vs Tertile 3; b, Tertile 2 vs Tertile 3.
Abbreviations: miRNA, microRNA

Table 6. Changes versus baseline of miRNAs by tertiles of percentage change of fat mass

miRNA	Tertile 1 -28.68 [-33.03, -25.12] (n=16)	Tertile 2 -18.2 [-20.62, -15.18] (n=16)	Tertile3 -7.62 [-8.48, -5.68] (n=14)	P-value
miR-592	-0.12 [-0.97, 0.29]	0 [-0.7, 0.23]	0.58 [-0.47, 1.95]	0.363
miR-551b	-0.68 [-1.86, 0.77]	-0.23 [-2.01, 0.32]	0.25 [-0.12, 0.55]	0.273
miR-411	0.13 [-0.3, 0.57]	-0.35 [-0.73, 0.49]	0.09 [-0.39, 0.21]	0.542
miR-1276	-0.34 [-1.57, 0.98]	-0.88 [-1.11, -0.32]	-0.13 [-0.49, 0.28]	0.365
miR-661	-1.09 [-2.71, -0.2]	-0.15 [-2.01, 0.85]	-0.27 [-1.43, 0.45]	0.290
let7a	0 [-2.88, 0.02]	-0.4 [-1.85, 0]	0 [-0.39, 0]	0.554
miR-1179	-1.75 [-3.02, -0.02]	-1.17 [-2.25, -0.02]	-0.42 [-1.7, 0.22]	0.442
miR-132	-1.32 [-3.26, -0.55]**a**	-1.02 [-2.69, -0.29]	-0.01 [-0.93, 0.5]	**0.048**
miR-210	-0.78 [-1.98, -0.35]**a**	-0.74 [-1.93, 0.7]**b**	0.15 [-0.23, 1.06]	**0.010**
miR-221	-2.08 [-4.54, -0.45]	-1.97 [-4.73, -0.4]	-0.19 [-3.2, 1.01]	0.309
miR-29a	-1.17 [-2.78, -0.12]	-0.82 [-2.33, -0.02]	-0.18 [-0.74, 0.74]	0.113
miR-34a	-0.51 [-2.81, 0.11]	-1.4 [-2.82, -0.23]	-0.06 [-1.14, 0.65]	0.113
miR-378	-1.73 [-4.71, -0.34]	-1.7 [-4.15, -0.19]	-0.66 [-2.04, 0.77]	0.235

Data shows median [IQR]. Kruskal-Wallis was performed between the three tertiles and Mann-Whitney between pairs. P-value refers to the Kruskal-Wallis test. Mann-Whitney test between paired tertiles: a, Tertile 1 vs Tertile 3; b, Tertile 2 vs Tertile 3.
Abbreviations: miRNA, microRNA