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Circulating microRNAs in human obesity: A systematic review

Tesis que presenta

Alejandra Ortiz Dosal

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Director de la Tesis:

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Constancia de aprobación de la tesis

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Esta tesis fue elaborada en el Laboratorio de Biotecnología Médica y Pecuaria de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C., bajo la dirección del Dr. Luis A. Salazar Olivo.

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que se desarrolló bajo la dirección de

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A mi familia

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Resumen

MicroRNAs circulantes en obesidad humana: Una revisión sistemática

Contexto. Se han reportado diferencias en los perfiles de expresión de microRNAs circulantes y específicos de tejido (miRNAs) en la obesidad de humanos, lo cual sugiere un papel de miRNAs en el desarrollo de esta afección.

Objetivo. Revisar los miRNAs circulantes (c-miRNAs) desregulados en la obesidad de humanos y predecir sus posibles genes diana.

Material y métodos. (PROSPERO, CRD42017077742). Se buscaron trabajos originales en PubMed incluyendo c-miRNAs y obesidad en humanos; se registraron c-miRNAs con perfiles de expresión diferencial. Luego, con herramientas bioinformáticas, buscamos posibles genes diana y vías metabólicas de miRNAs reportados desregulados por lo menos en dos informes independientes.

Resultados. Veintidós c-miRNAs fueron reportaron como sobreexpresados, nueve como subexpresados y dos c-miRNAs desregulados en ambas direcciones en personas con obesidad en comparación con controles de peso normal. En los análisis bioinformáticos, estos c-miRNAs hacen diana en genes asociados con el metabolismo de los ácidos grasos y la vía PI3k/Akt.

Conclusiones. La literatura registra 33 c-miRNAs desregulados en la obesidad de humanos. Sus genes diana predichos están involucrados en vías que podrían explicar el desarrollo de la obesidad y sus comorbilidades. Investigaciones futuras aclararán el papel de estos miRNAs en las enfermedades metabólicas y su utilidad en la prevención, pronóstico, y tratamiento de la obesidad.

PALABRAS CLAVE: obesidad; microRNAs circulantes; análisis bioinformático; genes blanco.

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Abstract

Circulating microRNAs in human obesity: A Systematic Review

Context. Differential expression profiles of microRNAs have been reported in human obesity suggesting a miRNAs role in the development of obesity and associated disorders.

Objective. Identify circulating microRNAs (c-miRNAs) dysregulated in human obesity and predict their possible target genes.

Methods. We performed a systematic review on PubMed database (PROSPERO, CRD42017077742) for original research on c-miRNAs in human obesity and identify c-miRNAs with differential expression profiles. Based on a bioinformatic analysis, we searched for potential target genes of these dysregulated miRNAs with at least two independent reports.

Results. Twenty-two c-miRNAs are overexpressed, nine underexpressed and two c-miRNAs dysregulated in both directions in people with obesity compared to lean controls. Bioinformatic analysis suggest that these c-miRNAs target on genes associated with fatty acid metabolism and PI3k/Akt pathway.

Conclusion: This data shows that 33 c-miRNAs are dysregulated in human obesity. Their predicted target genes are involved in pathways that could explain the development of obesity and its comorbidities. Further research will clarify the role of these miRNAs on metabolic diseases as well as in prognosis, prevention and obesity treatment.

KEYWORDS**:** obesity; circulating microRNAs; bioinformatic analysis; target genes.

Introduction

Obesity is a worldwide public health problem. World Health Organization (WHO) estimates that by year 2020, 60 million children under 5 will develop obesity. Obesity has negative consequences such as hypertension, dyslipidaemia, fatty liver disease, insulin resistance and type 2 diabetes (Tanvig, 2014). Fat stored in visceral adipose depots makes individuals with obesity more prone to these complications than individuals with obesity by increased subcutaneous fat (Rev. in Schleinitz *et al*., 2014; Guglielmi and Sbraccia, 2018).

Although environmental factors have been associated with an increase in the rate of obesity, twin studies have shown that genetic and epigenetic traits could explain 40 to 90% of variation in body mass index (BMI) (Elks *et al*., 2012; Silventoinen *et al*., 2017). Identifying alterations that raise the risk of obesity will allow the understanding of the biology and physiopathology of the disease and recognize genes and metabolic pathways that could be therapeutic targets for this disease.

MicroRNAs (miRNAs) are small (19-24 nucleotides) non-coding single-stranded RNAs that act as post-transcriptional regulators of gene expression by specific binding to complementary regions of target mRNAs destabilizing and/or preventing their translation. miRNAs are expressed in virtually all human tissues. Currently, 2588 miRNAs have been described in humans (Kozomara *et al*., 2014) and it is thought that they regulate at least 60% of human genes. A high number of human miRNAs, 669, are detectable in circulation (cells, serum and plasma) with variable expression profiles (Shu *et al*., 2015, Freedman *et al*. 2016). Occurrence of miRNAs in blood may be due to disruption of cell membrane after damage or by

an active secretion process. Regardless of the involved mechanism, circulating miRNAs (c-miRNAs) act as signaling molecules that allow intercellular communication and can regulate metabolic processes in neighbouring or distant cells (Chen *et al*., 2012).

The role of miRNAs in obesity has not been clearly defined. Studies on experimental models and *in silico* analysis suggest miRNAs could play a regulatory role in many biological processes associated with obesity, including adipocyte differentiation, insulin signaling pathway and lipid or carbohydrate metabolism (McGregor *et al*., 2011; Peng *et al*., 2014; Amri and Scheideler, 2017). It has been reported that the expression profiles of c-miRNAs are variable in people with different clinical traits of metabolic syndrome (Karolina *et al*., 2012).

In this systematic review, we identify the c-miRNAs most frequently reported with an altered serum or plasma-expression levels in people with obesity and determine their possible target genes along with their regulated metabolic pathways by *in silico* bioinformatic analyses. These dysregulated miRNAs could be used as markers for prognosis, prevention and treatment of obesity.

Methods

Protocol Registration

The **P**referred **R**eporting **I**tems for **S**ystematic review and **M**eta-**A**nalysis (PRISMA) statement (Liberati *et al*., 2009) was followed as reference protocol standard. A PRISMA Protocol checklist is included as Supplemental Material 1. Our protocol was registered at the International Prospective Register of Systematic Reviews PROSPERO, with registration number CRD42017077742; available at https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=77742.

Bibliographic Search and Eligibility Criteria

We performed a systematic review in PubMed database for the following terms: circulating (All Fields) AND ("micrornas" (MeSH Terms) OR "micrornas" (All Fields) OR "microrna" (All Fields)) AND ("obesity" (MeSH Terms) OR "obesity" (All Fields)) without any limits. We did not establish date restrictions (last searched April 1st, 2018) and only research papers published in English were included

Study Selection

We screened all the selected abstracts and only original articles where there was a comparison between c-miRNAs expression profiles of people with obesity versus people with normal weight were included in the study. We verified that criteria for human obesity in the reviewed papers were in agreement with accepted criteria by health international agencies: BMI of 30.0 kg/m^2 or higher in adults and at or

above the 95th percentile in children and teens of the same age and sex. Only works satisfying these criteria were included in the review.

Data Collection Process

Two reviewers independently identify titles and abstracts of studies that met the inclusion criteria retrieved by our search . The full text of these studies were obtained and independently assessed for eligibility by two reviewers. All disagreements between the reviewers were resolved with a third reviewer by consensus.

Data Items

A standardized form was used to extract data from the studies. Extracted information included author, country and year of publication, study population and participant characteristics (age, gender and BMI), methods of profiling and quantification of miRNAs, miRNAs dysregulated and information for assessment of the risk of bias. We used bioinformatic tools to predict putative gene targets (and potential pathways) of the dysregulated miRNAs.

Risk of Bias in Individual Studies and across Studies

Two reviewers independently assessed the quality and risk of bias of individual studies using the standard scale "Quality assessment tool for observational cohort and cross-sectional studies" from the NHLBI, available at https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools.

Disagreements between the reviewers over the risk of bias in a particular study were resolved by discussion with a third reviewer. We made a summary assessment of the risk of bias within and across the studies, and decided by consensus to not consider studies with high risk of bias.

Summary Measures

With the extracted data, we considered as primary outcome a change in c-miRNAs expression for at least 1.5 fold in groups with obesity vs lean control, with a *p*-value < 0.05 as statistically significant, and the report by at least two independent publications to include any particular miRNA in the bioinformatic analyses.

Bioinformatic Analyses

To explore the regulatory mechanisms of c-miRNAs with altered expression levels in human obesity we searched for genes and metabolic pathways potentially targeted by them. We grouped the miRNAs in overexpressed or underexpressed. We use miRSystem (version 2016.05.13, analyses performed in May 2018), a database that integrates the target gene prediction programs DIANA, miRanda, miRBridge, PicTar, PITA, rna22 and TargetScan. This database also contains information from TarBase and myRecords regarding the interaction of miRNAs-target mRNA and allows the analyses of metabolic pathways involved. It has a miRConverter tool that allowed us to adjust different annotations of miRNAs to the latest version of miRBase (Lu *et al*., 2012). We performed the informatic analyses accordingly to the latest miRNA annotations (adjusting hsa-miR-126-5p, hsa-miR-125b-5p and hsa-miR-130b-3p). We defined the following parameters: Hit greater

than or equal to 4 (Target genes are shown if they are greater than or equal to 4 algorithms predicting the same miRNA-gene interaction pair and we choose to include validated target genes verified by biological experiments regardless of the number of hits), O/E ratio greater than or equal to 4 and total genes in a pathway greater than or equal to 25 and less than or equal to 500 and include only KEGG metabolic pathways. To increase the prediction specificity, we performed an analysis with DIANA miRPath v.3.0 (conducted in May 2018) and we use Tarbase, TargetScan and microT-CDS from the tool (Vlachos ***et al***., 2015).

Synthesis of Results and Additional Analyses

We provided a narrative synthesis of the findings from the selected data and a summary of the c-miRNAs dysregulated in the context of obesity, their putative target genes and metabolic pathways implicated.

Results

Bibliographic Search

The bibliographic search in PubMed retrieved 91 papers published between March 4, 2009 and April 1st, 2018. Based on titles and abstracts, we excluded 24 review articles, two editorial comments, one meta-analysis, one project register, two papers in language other than English, 13 studies in animal or *in vitro* model systems, 15 papers not including patients with obesity or comparison between obesity *vs* normal weight groups and four papers with no identification of c-miRNAs.

Supplemental Material 2a summarizes all expression profiles reported in the 29 papers that fulfill the inclusion criteria. From this set, we eliminated eight papers: three of them due to high risk of bias, three papers were eliminated because their results only included comparison between people with obesity and its comorbidities (lacking the comparison with lean group) and two papers because the determination of c-miRNAs was performed in whole blood. The 21 papers finally included in this review reported 158 dysregulated miRNAs in serum or plasma of people with obesity compared to people with normal weight. This analysis showed 59 c-miRNAs as underexpressed, 85 as overexpressed and 14 c-miRNAs reported as dysregulated in both directions (Supplemental Material 2b and Figure 1).

miRNAs Reported as Dysregulated in the Context of Obesity

To attain a clearer picture on the relation of c-miRNAs with obesity we selected those miRNAs reported with statistically significant differential expression by at least two independent groups. According to this criterion, we found c-miRNAs hsa-

miR-15b-5p, hsa-miR-23a-3p, hsa-miR-24-3p, hsa-miR-26b-5p, hsa-miR-30d-5p, hsa-miR-34a-5p, hsa-miR-122-5p, hsa-miR-126, hsa-miR-140-5p, hsa-miR-142-5p, hsa-miR-146a-5p, hsa-miR-148a-3p, hsa-miR-191-5p, hsa-miR-192-5p, hsa-miR-197-3p, hsa-miR-222-3p, hsa-miR-223-3p, hsa-miR-223-5p, hsa-miR-320a, hsa-miR-342-3p, hsa-miR-486-5p and hsa-miR-636 reported as overexpressed in patients with obesity (Table 1).

Conversely, c-miRNAs hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-125b, hsa-miR-151a-5p, hsa-miR-151a-3p, hsa-miR-199a-5p, hsa-miR-324-3p, hsa-miR-331-3p and hsa-miR-590-5p were reported as underexpressed in people with obesity (Table 2). Interestingly, hsa-miR-21-5p and hsa-miR-130b were reported as dysregulated in both directions in human obesity (Table 3).

Potential Target Genes and Metabolic Pathways

The tools used for bioinformatic analyses (miRSystem v.2016 and the three tools in DIANA miRPath v.3.0 [TarBase, TargetScan and micro-T CDS]), consistently predicted the PI3k/Akt signaling pathway and fatty acid metabolism as target pathways for the dysregulated c-miRNAs (Figure 2, Table 4 and Supplemental Material 3). One of the predicted genes was *PTEN* (Phosphatase and Tensin Homolog), a putative target for miRNAs miR-23a-3p, miR-26b-5p, miR-142-5p, miR-148a-3p, miR-320a and miR-486-5p, overexpressed in human obesity, as well as for miR-21-5p and miR-130b-3p reported as both overexpressed and underexpressed in obesity, respectively. *IRS1* (Insulin Receptor Substrate 1) was predicted as a putative target gene for miR-15b-5p, miR-30d-5p, miR-126-3p, miR-142-5p, miR-148a-3p, miR-223-3p; *AKT3* (AKT Serine/Threonine Kinase 3) was

predicted as a putative target for miR-15b-5p, miR-34a-5p, miR-122-5p and miR-320a; *FOXO3* (Forkhead box 3) was a putative target gene for miR-23a-3p, miR-30d-5p, miR-122-5p and miR-223-3p; all of this miRNAs was reported as overexpressed in human obesity. These genes participate in the PI3K-Akt signaling pathway, the inositol-phosphate metabolism and biological processes relevant to obesity-associated disorders like the insulin-receptor cascade. Other genes such as *ACSL1*, *ACSL3* and *ACSL4* (Acyl-CoA Synthetase Long-Chain family members 1, 3 and 4), predicted targets for miR-15b-5p, miR-26b-5p, miR-34a-5p, miR-130b-3p, miR-223-3p and miR-636, participate in the fatty acid metabolism (Supplemental material 3).

Assessment of Risk of Bias

Most of the analysed information came from studies at low or unclear risk of bias. In particular, we were interested in a cross-sectional measure: the difference between c-miRNAs expression profile in people with obesity *vs* people with normal weight. We identified some source of bias: a) some of the papers were “pilot studies”, b) only two clearly stated the justification for sample size, c) most of the analysed papers did not report any blinding, d) differences in baseline characteristics of the participants, e) lack of statistically adjusting for confounders, and f) preselection of some miRNAs already reported in obesity, diabetes or metabolic diseases. The results from our assessment of quality and risk of bias in individual studies and across the studies are detailed in Supplemental material 4.

Discussion

Summary of Evidence

In this review we found 158 c-miRNAs reported as dysregulated in human obese populations. Considering those c-miRNAs with two or more independent reports, we found 22 c-miRNAs reported as overexpressed, nine reported as underexpressed and two c-miRNAs dysregulated in both directions in humans with obesity compared to lean controls. Our bioinformatic analyses suggest that these miRNAs target genes involved in fatty acid metabolism and PI3K-Akt pathways. To the best of our knowledge, only a few of these microRNAs have been approached in intervention studies (Ortega *et al*., 2013; Tabet *et al*., 2016; Hernández-Alonso *et al*., 2017), so their usefulness as therapeutic targets remain to be evaluated. This is a promising field in the search for new therapeutic targets for obesity.

miRNAs Dysregulated in Obesity

miRNAs are present in blood either bound to high-density lipoproteins (HDL), forming complexes with proteins such as Argonaute 2 or inside extracellular vesicles (EV; exosomes, microvesicles or apoptotic bodies) (Baldasarre *et al*., 2017). Adipose tissue could be the source of EV and explain the increase in c-miRNAs reported as overexpressed in the context human obesity, characterized by excessive accumulation of excess body fat. In mice and in human adults with obesity it has been shown that EV are increased in blood, and this mechanism is associated with the cross-talk between adipocytes and other cell types, including immune and liver cells (Freeman, 2017). The miRNAs circulating in blood within

EVs are capable of modifying the gene expression in different cell types. Recent investigations suggest that this could be a therapeutic strategy to inhibit harmful signals or enhance a desired pathway (Gallo *et al*., 2016; Togliatto *et al*., 2016; Lv *et al*.; 2018). However, given the extraction methods reported in the analysed studies, it is difficult to assure the source of the c-miRNAs reported as dysregulated in human obesity.

Fourteen miRNAs appeared overexpressed in some studies and underexpressed in others (Supplemental Material 2b). We considered these miRNAs as overexpressed or underexpressed if they were reported in the same direction by at least two independent groups, as outlined before. In this scenario, only hsa-miR-21-5p and hsa-miR-130b were reported dysregulated in both directions. miR-130b was overexpressed in serum of Asian and Caucasian children with obesity and in plasma samples of Chinese men with obesity (Prats-Puig *et al*., 2013; Wang *et al*., 2013; Cui *et al*., 2018) and underexpressed in plasma of Brazilian adults and Caucasian adults with obesity (Ortega *et al*., 2013; Thomé *et al*., 2015). In same manner, miR-21-5p appeared overexpressed in serum and plasma of Asian and American children and in American and Caucasian adults with obesity (Ortega *et al*., 2013; Nuñez-Lopez *et al*., 2016; Thompson *et al*., 2017; Cui *et al*., 2018), and underexpressed in serum of Iranian adults and in plasma of Caucasian adults with morbid obesity when compared with controls (Ortega *et al*., 2013; Ghorbani *et al*., 2017).

The study by Ortega *et al*. (2013) found some of these miRNAs dysregulated in both directions. According to these results, some miRNAs were overexpressed in patients with moderate obesity and underexpressed in cases of morbid obesity.

The presence of comorbidity and the severity of obesity could explain the differences in the expression profiles found, in addition to the differences in the cohorts where they were reported. Other miRNAs that have been associated with obesity in the reviewed studies are miR-122-5p, miR-223-5p (over-expressed), miR-130b (reported dysregulated in both directions), and miR-125b (underexpressed).

Free fatty acids increase hepatic expression and secretion of miR-122 that regulates the balance between storage and energy expenditure in liver and peripheral tissues. miR-122 targets mRNAs of genes involved in insulin signaling (Abente *et al*., 2016). Overexpression of miR-122 is associated with hepatic injury and steatosis, non-alcoholic fatty liver disease, insulin resistance, type 2 diabetes, childhood obesity and adverse lipid profile (Iacomino *et al*., 2017). In an experimental rat model, antagomiR-122 leads to accumulation of triglycerides in liver and muscle (Chai *et al*., 2017). The use of antisense oligonucleotide to block the interaction between miR-122 and its target gene *HMGCR* (3-Hydroxy-3-Methylglutaryl-CoA Reductase) led to a dramatic decline in the serum total cholesterol level (Krutzfeldt *et al*., 2005).

miR-223 is one of the miRNAs that regulates lipid metabolism in the liver, targeting the mRNAs of *HMGCS1* (3-Hydroxy-3-Methylglutaryl-CoA Synthase 1) and *MSMO1* (Methylsterol Monooxygenase 1; also called *SC4MOL*, Sterol-C4-Methyl Oxidase) genes, both involved in cholesterol synthesis (Abente *et al*., 2016). miR-223 (associated with high density lipoproteins (HDL)), decreases significantly in people with obesity or overweight on a diet-induced weight loss (in 12-week intervention). HDL transport miR-16, miR-17, miR-126, miR-222 and miR-223.

miR-223 regulates glucose metabolism and GLUT 4 (glucose transporter) expression in rat cardiomyocytes. Furthermore, it is overexpressed in human hearts with insulin resistance and in adipocytes of obese mice. Interestingly, it is underexpressed in the plasma of patients with type 2 diabetes (Tabet *et al*., 2016).

miR-130b is closely related to blood glucose levels and insulin resistance. Circulating levels of miR-130b in blood shows an inverse relationship with glycated haemoglobin, insulin resistance index (HOMA-IR), triglycerides, low density lipoproteins (LDL) and blood urea nitrogen in patients with type 2 diabetes. miR-130b is also a possible marker of hypertriglyceridemia in patients with metabolic syndrome. The c-miR-130b expression is reduced in patients with morbid obesity and in those with kidney damage due to type 2 diabetes and this reduction is gradual according to the degree of diabetic nephropathy (Lv *et al*., 2015; Iacomino and Siani, 2017). In addition, it has been shown that miR-130b-loaded microvesicles can be transported into adipocytes and decrease expression of peroxisomal proliferation activator receptor gamma (*PPARG*), influencing the phenotype of the recipient cell (Pan *et al*., 2014).

miR-125b is involved in synthesis of triglycerides and targets the mRNA of *SCD-1* (Stearoyl-CoA Desaturase 1) gene, which encodes for an enzyme involved in lipid synthesis. In our review, we found miR-125b underexpressed in plasma of people with obesity (Ortega *et al*., 2013, Prats-Puig *et al*., 2013, Zhao *et al*., 2017). The overexpression of miR-125b in mammalian adipocytes confers protection to oxidative damage, decreases accumulation of triglycerides and their levels in subcutaneous adipose tissue of mice are inversely associated with caloric restriction (Brandão *et al*., 2017).

Bioinformatic Analyses

Obesity is a risk factor for metabolic and neoplasic diseases. In our analyses of c-miRNAs in human obesity, we found that dysregulated miRNAs probably target genes involved in pathways associated with metabolic processes and cancer. The tools we used for the bioinformatic analyses predicted that PI3k/Akt and fatty acids metabolism pathways could be the targets of c-miRNAs dysregulated in obesity. After meals, blood levels of glucose and insulin increase. Insulin phosphorylates its receptor, thus activating specific kinases, including PI3k and the "downstream" kinases Akt and mTOR (Yu *et al*., 2008). Dysregulation of these insulin-activated kinases is associated with both insulin resistance and tumorigenesis (Manning *et al*., 2004). Thus, the altered expression of miRNAs that target the PI3k/Akt and fatty acids metabolism pathways could explain the relationship of this condition with the subsequent development of type 2 diabetes and other comorbidities associated with obesity.

The PI3k/Akt pathway is a positive regulator of adipose differentiation (Yu *et al*., 2008; Lowe *et al*., 2011). Lipogenic enzymes can be regulated by multiple mechanisms, such as allosteric control and post-translational modifications, *v.gr*. phosphorylation-dephosphorylation (Wang *et al*., 2015b). Another mechanism could be the post-transcriptional silencing by miRNAs. In our analysis we found that 17 (hsa-miR-15b-5p, hsa-miR-122-5p, hsa-miR-126-3p, hsa-miR-142-5p, hsa-miR-148a-3p, hsa-miR-192-5p, hsa-miR-222-3p, hsa-miR-223-3p, hsa-miR-23a-3p, hsa-miR-24-3p, hsa-miR-26b-5p, hsa-miR-30d-5p, hsa-miR-34a-5p, hsa-miR-342-3p, hsa-miR-320a, hsa-miR-486-5p, hsa-miR-636) and 7 (hsa-miR-324-3p, hsa-

miR-590-5p, hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-151a-3p, hsa-miR-199a-5p, hsa-miR-331-3p) c-miRNAs overexpressed and underexpressed respectively, in human obesity targeting the PI3k/Akt pathway (Tables 4 and 5). We also found two c-miRNAs dysregulated in both directions, hsa-miR-21-5p and hsa-miR-130b, that targets the PI3k/Akt pathway (Table 4 and 5).

This *in silico* prediction showed that both overexpressed- and underexpressed-in-obesity c-miRNAs target the same metabolic pathway: the PI3k/Akt pathway. This apparent contradiction will be solved by the experimental confirmation of the interaction of dysregulated miRNAs in obesity with particular genes in this metabolic pathway and the determination of their mechanisms of action.

A determinant of metabolic health is the ability to store excess fat in the subcutaneous adipose tissue to avoid its accumulation in ectopic deposits, such as liver, muscle and heart; or in deposits of perivisceral fat, which favours metabolic complications of obesity like fatty liver disease, type 2 diabetes and cardiovascular diseases. The inability to recruit and differentiate precursor cells to adipocytes (adipogenesis) in subcutaneous tissue leads to development of hypertrophic, dysfunctional and insulin resistant adipose cells due to a reduced content of the glucose transporter GLUT4 (Smith and Kahn, 2016). Overexpression of Akt in skeletal muscle favours insulin sensitivity and skeletal muscle hypertrophy and increases fatty acid oxidation in liver, which decreases accumulation of fat (Chai *et al.*, 2017). The dysregulation of this route could lead to insulin resistance, increasing the risk of type 2 diabetes in individuals with obesity and a possible explanation of our results. Recently, it has been reported that activation of Akt in

animal models could be a therapeutic strategy to ameliorate insulin resistance. This was achieved by inhibiting IP6K1 (Inositol hexakisphosphate kinase 1), with RNAi, knockout mice or with chemical inhibition. The improved Akt activity contributes to elevated glucose uptake in skeletal muscle hence maintains euglycaemia (Zhang *et al*., 2017). The improved Akt activity contributes to increased glucose uptake in skeletal muscle cells hence maintaining euglycaemia

Furthermore, dysregulation of PI3k/Akt pathways are associated with some types of cancer (Haddadi *et al*., 2018). For example, *PTEN* is a tumour suppressor gene; the loss of function in this gene is associated with the constitutive activation of Akt and the increased risk of breast, thyroid, uterine, and other types of cancer (Hopkins *et al*., 2014). Eight of the miRNAs found dysregulated in obesity, probably target *PTEN* gene (hsa-miR-130b-3p, hsa-miR-142-5p, hsa-miR-148a-3p, hsa-miR-21-5p, hsa-miR-23a-3p, hsa-miR-26b-5p, hsa-miR-320a, hsa-miR- 486-5p; Supplemental Material 3).

The association of the dysregulated miRNAs in obesity that also contributes to the higher risk of cancer in this population is beyond the scope of this article, however, it is an interesting topic for further research.

Experimental analysis is needed to evaluate the role of the selected miRNAs on the regulation of the PI3k/Akt pathway and specially in view of its use as a therapeutic strategy for human obesity and its comorbidities.

Limitations

The analysis of expression profiles of circulating miRNAs in people with obesity is a complex endeavor. Among the limitations of this study we can include the lack of

accuracy, mainly due to the characteristics of the participants in the different studies, because it is difficult to assure for the homogeneity of the sample and to attribute the differences in c-miRNA expression only to obesity. To solve this issue requires adjusting for confounders, an approach not always considered. Some of the studies we analysed screened for differences in c-miRNA expression according to comorbidities, such as fatty liver disease, prediabetes or type 2 diabetes, endothelial dysfunction or heart failure (Supplemental Material 2). In reports that included women, it has been observed that even the menstrual cycle day in which the sample is taken has an effect in the expression profile of circulating miRNAs (Murri *et al*., 2013). In pregnant women, in addition to gestational age, the gender of the foetus is a factor to consider: having a male foetus has been associated with lower B cell function, higher postprandial glycaemia and higher risk of gestational diabetes. The baby's gender could also influence mother's metabolism (Retnakaran *et al*., 2015).

Another limitation to consider is that we only perform our search in PubMed and we only included profiles of people with obesity *vs* people with normal weight. This approach could be excluding important miRNAs involved in the pathophysiology of the disease. For example, other studies not included in our analysis (because they did not report comparisons between circulating miRNAs in obesity and healthy people), have found a positive correlation of miR-375 with plasma glucose levels, insulin and HOMA index in subjects with prediabetes (Hernández-Alonso *et al*., 2017).

Another important limitation of this work is the origin of the miRNAs. To homogenize the source of these miRNAs, we included studies where the

quantification was performed in serum or in plasma, thus, it is not possible to distinguish between small RNAs from exosomes and those circulating in complexes with proteins. The implications regarding the biological function of these differences are uncertain.

Most of the studies included in this review are based on the initial realization of microarrays and subsequent validation by RT-qPCR. It is possible hat this approach is excluding some miRNAs that could be involved in the metabolic alterations found in subjects with obesity. The use of RNA-seq as the initial screening technique could diminish this risk.

Finally, prediction tools not always yield consistent results. One possible explanation is because there are different versions and annotations of the isoforms of miRNAs. It has been suggested that combining the results of different prediction tools improves specificity [Oliveira et al., 2017].

Conclusions

Multiple studies have reported dysregulated expression profiles of circulating miRNAs in human obesity. In this review, we found 33 circulating miRNAs whose dysregulated expression in serum or plasma from people with obesity is reported by two or more independent research groups. Our bioinformatic analyses suggest these miRNAs could target mRNAs involved in the PI3k/Akt pathway. These findings could have implications in the understanding of gene silencing mechanisms in the pathophysiology of obesity. Further research on the role of c-miRNAs dysregulated in human obesity will convey a more detailed vision on their use as biomarkers of prognosis or therapeutic targets for obesity.

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Disclosure of Interest

The authors report no conflict of interest.

References

Abente EJ, Subramanian M, Ramachandran V, *et al*. MicroRNAs in obesity-associated disorders. *Archives of Biochemistry and Biophysics* 2016; 589: 108-119.

Amri EZ, Scheideler M. Small non coding RNAs in adipocyte biology and obesity. *Molecular and Cellular Endocrinology* 2017; 456: 87-94.

Auguet T, Aragonés G, Berlanga A, *et al*. miR-33a/miR-33b* and miR-122 as possible contributors to hepatic lipid metabolism in obese women with nonalcoholic fatty liver disease. *International Journal of Molecular Sciences* 2016; 17: E1620.

Baldassarre A, Felli C, Prantera G, *et al*. Circulating microRNAs and bioinformatics tools to discover novel diagnostic biomarkers of pediatric diseases. *Genes* (Basel) 2017; 8: 234.

Brandão BB, Guerra BA, Mori MA. Shortcuts to a functional adipose tissue: The role of small non-coding RNAs. *Redox Biology* 2017; 12: 82-102.

Can U, Buyukinan M, Yerlikaya FH. The investigation of circulating microRNAs associated with lipid metabolism in childhood obesity. *Pediatric Obesity* 2016; 11: 228-234.

Carreras-Badosa G, Bonmatí A, Ortega FJ, *et al*. Altered circulating miRNA expression profile in pregestational and gestational obesity. *The Journal of Clinical Endocrinology and Metabolism* 2015; 100: E1446-E1456.

Chai C, Rivkin M, Berkovits L, *et al*. Metabolic circuit involving free fatty acids, microRNA 122, and triglyceride synthesis in liver and muscle tissues. *Gastroenterology* 2017; 153: 1404-1415.

Chen H, Liang J, Zhang K, *et al*. Secreted microRNAs: a new form of intercellular communication. *Trends in Cell Biology* 2012; 22: 125-132.

Cui X, You L, Zhu L, *et al*. Change in circulating microRNA profile of obese children indicates future risk of adult diabetes. *Metabolism* 2018; 78: 95-105.

Elks CE, den Hoed M, Zhao JH, *et al*. Variability in the heritability of body mass index: a systematic review and meta-regression. *Frontiers in Endocrinology* 2012; 3: A29.

Enquobahrie DA, Wander PL, Tadesse MG, *et al*. Maternal pre-pregnancy body mass index and circulating microRNAs in pregnancy. *Obesity Research & Clinical Practice* 2017; 11: 464-474.

Freedman JE, Gerstein M, Mick E, *et al*. Diverse human extracellular RNAs are widely detected in human plasma. *Nature Communications* 2016; 7: 11106.

Freeman DJ. Extracellular vesicles from adipose tissue. A potential role in obesity and type 2 diabetes? *Frontiers in Endocrinology* 2017; 8: 202.

Gallo S, Gili M, Lombardo G, *et al*. Stem cell-derived, microRNA-carrying Extracellular Vesicles: A novel approach to interfering with mesangial cell collagen production in a hyperglycaemic setting. *PLoS One* 2016; 11: e0162417.

Ghorbani S, Mahdavi R, Alipoor B, *et al*. Decreased serum microRNA-21 level is associated with obesity in healthy and type 2 diabetic subjects. *Archives of Physiology and Biochemistry* 2017; 7: 1-6.

Guglielmi V, Sbraccia P. Obesity phenotypes: depot-differences in adipose tissue and their clinical implications. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity* 2018; 23: 3-14.

Haddadi N, Lin Y, Travis G, et al. PTEN/PTENP1: 'Regulating the regulator of RTK-dependent PI3K/Akt signalling', new targets for cancer therapy. *Molecular Cancer* 2018; 17: 37

Hernández-Alonso P, Giardina S, Salas-Salvadó J, *et al*. Chronic pistachio intake modulates circulating microRNAs related to glucose metabolism and insulin resistance in prediabetic subjects. *European Journal of Nutrition* 2017; 56: 2181-2191.

Hopkins BD, Hodakoski C, Barrows D, *et al*. PTEN function: the long and the short of it. *Trends in Biochemical Sciences* 2014; 39: 183-190.

Hubal MJ, Nadler EP, Ferrante SC, *et al*. Circulating adipocyte-derived exosomal microRNAs associated with decreased insulin resistance after gastric bypass. *Obesity* (Silver Spring) 2017; 25: 102-110.

Iacomino G, Russo P, Stillitano I, *et al*. Circulating microRNAs are deregulated in overweight/obese children: preliminary results of the I.Family study. *Genes & Nutrition* 2016; 11: 7.

Iacomino G, Siani A. Role of microRNAs in obesity and obesity-related diseases. *Genes & Nutrition* 2017; 12: 23.

Karolina DS, Tavintharan S, Armugam A, *et al*. Circulating miRNA profiles in patients with metabolic syndrome. *The Journal of Clinical Endocrinology and Metabolism* 2012; 97: E2271-E2276.

Khalyfa A, Kheirandish-Gozal L, Bhattacharjee R, *et al*. Circulating microRNAs as potential biomarkers of endothelial dysfunction in obese children. *Chest* 2016; 149: 786-800.

Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Research* 2014; 42: D68-D73.

Krützfeldt J, Rajewsky N, Braich R, *et al*. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005; 438: 685-689.

Liberati A, Altman DG, Tetzlaff J, *et al*. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Medicine* 2009; 6: e1000100.

Liu L, Li Q, Xiao X, *et al*. miR-1934, Underexpressed in obesity, protects against low-grade inflammation in adipocytes. *Molecular and Cellular Endocrinology* 2016; 428: 109-117.

Lowe CE, O'Rahilly S, Rochford JJ. Adipogenesis at a glance. *Journal of Cell Science* 2011; 124: 2681-2686.

Lu T-P, Lee C-Y, Tsai M-H, *et al*. miRSystem: An integrated system for characterizing enriched functions and pathways of microRNA targets. *PLoS One* 2012; 7: e42390.

Lv C, Zhou YH, Wu C, *et al*. The changes in miR-130b levels in human serum and the correlation with the severity of diabetic nephropathy. *Diabetes/Metabolism Research Reviews* 2015; 31:717-724.

Lv L, Wu W, Feng Y, *et al*. Therapeutic application of extracellular vesicles in kidney disease: promises and challenges. *Journal of Cellular and Molecular Medicine* 2018; 22: 728-737.

Manning BD. Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *Journal of Cell Biology* 2004; 167: 399-403.

Masotti A, Baldassarre A, Fabrizi M, *et al*. Oral glucose tolerance test unravels circulating miRNAs associated with insulin resistance in obese preschoolers. *Pediatric Obesity* 2017; 12: 229-238.

McGregor RA, Choi MS. MicroRNAs in the regulation of adipogenesis and obesity. *Current Molecular Medicine* 2011; 11: 304-316.

Murri M, Insenser M, Fernández-Durán E, *et al*. Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. *The Journal of Clinical Endocrinology and Metabolism* 2013; 98: E1835-E1844.

Murri M, Insenser M, Fernández-Durán E, *et al*. Non-targeted profiling of circulating microRNAs in women with polycystic ovary syndrome (PCOS): effects of obesity and sex hormones. *Metabolism* 2018; 86: 49-60.

Nuñez-Lopez YO, Garufi G, Seyhan AA. Altered levels of circulating cytokines and microRNAs in lean and obese individuals with prediabetes and type 2 diabetes. *Molecular BioSystems* 2016; 13: 106-121.

Oliveira AC, Bovolenta LA, Nachtigall PG, *et al*. Combining results from distinct microRNA target prediction tools enhances the performance of analyses. *Frontiers in Genetics* 2017; 8: 59.

Ortega FJ, Mercader JM, Catalán V, *et al*. Targeting the circulating microRNA signature of obesity. *Clinical Chemistry* 2013; 59: 781-792.

Pan S, Yang X, Jia Y, *et al*. Microvesicle-shuttled miR-130b reduces fat deposition in recipient primary cultured porcine adipocytes by inhibiting PPAR-γ expression. *Journal of Cellular Physiology* 2014; 229: 631-639.

Párrizas M, Brugnara L, Esteban Y, *et al*. Circulating miR-192 and miR-193b are markers of prediabetes and are modulated by an exercise intervention. *The Journal of Clinical Endocrinology and Metabolism* 2015; 100: E407-E415.

Pek SLT, Sum CF, Lin MX, *et al*. Circulating and visceral adipose miR-100 is down-regulated in patients with obesity and Type 2 diabetes. *Molecular and Cellular Endocrinology* 2016; 427: 112-123.

Peng Y, Yu S, Li H, *et al*. MicroRNAs: emerging roles in adipogenesis and obesity. *Cellular Signalling* 2014; 26: 1888-1896.

Pescador N, Pérez-Barba M, Ibarra JM, *et al*. Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. *PLoS One* 2013; 8: e7725.

Prats-Puig A, Ortega FJ, Mercader JM, *et al*. Changes in circulating microRNAs are associated with childhood obesity. The *Journal of Clinical Endocrinology and Metabolism* 2013; 98: E1655- E1660.

Retnakaran R, Kramer CK, Ye C, *et al*. Fetal sex and maternal risk of gestational diabetes mellitus: the impact of having a boy. *Diabetes Care* 2015; 38: 844-851.

Schleinitz D, Böttcher Y, Blüher M, *et al*. The genetics of fat distribution. *Diabetologia* 2014; 57: 1276-1286.

Shah R, Murthy V, Pacold M, *et al*. Extracellular RNAs are associated with insulin resistance and metabolic phenotypes. *Diabetes Care* 2017; 40: 546-553.

Shu J, Chiang K, Zempleni J, *et al*. Computational characterization of exogenous microRNAs that can be transferred into human circulation. *PLoS One* 2015; 10: e0140587.

Silventoinen K, Jelenkovic A, Sund R, *et al*. Differences in genetic and environmental variation in adult BMI by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. *American Journal of Clinical Nutrition* 2017; 106: 457-466.

Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, *de novo* lipogenesis and novel lipids. *Journal of Internal Medicine* 2016; 280: 465-475.

Tabet F, Cuesta Torres LF, *et al*. High-density lipoprotein-associated miR-223 is altered after diet-induced weight loss in overweight and obese males. *PLoS One* 2016; 11: e0151061.

Tanvig M. Offspring body size and metabolic profile. Effects of lifestyle intervention in obese pregnant woman. *Danish Medical Journal* 2014; 61: B4893.

Thomé JG, Mendoza MR, Cheuiche AV, *et al*. Circulating microRNAs in obese and lean heart failure patients: A case-control study with computational target prediction analysis. *Gene* 2015; 574: 1-10.

Thompson MD, Cismowski MJ, Serpico M, *et al*. Elevation of circulating microRNA levels in obese children compared to healthy controls. *Clinical Obesity* 2017; 7: 216-221.

Togliatto G, Dentelli P, Gili M, *et al*. Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing

miR-126 content: impact on clinical applications. *International Journal of Obesity* (London) 2016; 40: 102-111.

Vlachos IS, Zagganas K, Paraskevopoulou MD, *et al*. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. *Nucleic Acids Research* 2015; 43(Web Server issue):W460-W466. doi:10.1093/nar/gkv403.

Wander PL, Boyko EJ, Hevner K, *et al*. Circulating early- and mid-pregnancy microRNAs and risk of gestational diabetes. *Diabetes Research and Clinical Practice* 2017; 132: 1-9.

Wang R, Hong J, Cao Y, *et al*. Elevated circulating microRNA-122 is associated with obesity and insulin resistance in young adults. *European Journal of Endocrinology* 2015; 172: 291-300.

Wang Y, Viscarra J, Kim SJ, *et al*. Transcriptional regulation of hepatic lipogenesis. *Nature Reviews. Molecular Cell Biology* 2015; 16: 678-689.

Wang YC, Li Y, Wang XY, *et al*. Circulating miR-130b mediates metabolic crosstalk between fat and muscle in overweight/obesity. *Diabetologia* 2013; 56: 2275-2285.

Wen D, Qiao P, Wang L. Circulating microRNA-223 as a potential biomarker for obesity. *Obesity Research and Clinical Practice* 2015; 9: 398-404.

Willeit P, Skroblin P, Moschen AR, *et al*. Circulating microRNA-122 is associated with the risk of new-onset metabolic syndrome and type 2 diabetes. *Diabetes* 2017; 66: 347-357.

Xiong W, Lin Y, Xu L, *et al*. Circulatory microRNA 23a and microRNA 23b and polycystic ovary syndrome (PCOS): the effects of body mass index and sex

hormones in an Eastern Han Chinese population. *Journal of Ovarian Research* 2017; 10: 10-18.

Yu W, Chen Z, Zhang J, *et al*. Critical role of phosphoinositide 3-kinase cascade in adipogenesis of human mesenchymal stem cells. *Molecular Cell Biochemistry* 2008; 310: 11-18.

Zhang Z, Liu H, Liu J. Akt activation: a potential strategy to ameliorate insulin resistance. *Diabetes Research and Clinical Practice* 2017; pii: S0168-8227(17)30315-7.

Zhao H, Shen J, Daniel-MacDougall C, *et al*. Plasma microRNA signature predicting weight gain among Mexican-American women. *Obesity* (Silver Spring) 2017; 25: 958-964.

Table 1. Circulating miRNAs reported as overexpressed in human obesity by at least two independent working groups.

hsa-miRNA Source	Population			Expression level (+fold)	References
	Ethnics	Gender[1]	Age[2]		
miR-15b-5p Serum and plasma	American children Asian children Caucasian adults	Male / Female (40%) Male / Female (51%) Male / Female (47.8%)	13.5 4.9 54.6	3.42 8.79 0.99 (ROC)	Thompson et al 2017 Cui et al 2018 Pescador et al 2013
mir-23a-3p Serum and plasma	American children Caucasian adults	Male / Female (40%) Male / Female (66.6%)	13.5 28	5.3 1.39	Thompson et al 2017 Murri et al 2018
mir-24-3p Serum	American adults Caucasian adults	Male / Female (50%) Male / Female (66.6%)	68.8 28	0.032 (B-est) 1.29	Shah R et al 2017 Murri et al 2018
mir-26b-5p Serum and plasma	Asian children Caucasian children Caucasian children	Male / Female (51%) Male / Female (45%) Male / Female (50%)	4.9 10.6 4.5	3.43 25.37 1.63	Cui et al 2018 Iacomino et al 2016 Masotti et al 2017
mir-30d-5p Serum and plasma	American adults Asian children	Male / Female (50%) Male / Female (51%)	68.8 4.9	0.033 (B-est) 2.67	Shah R et al 2017 Cui et al 2018
mir-34a-5p Serum and plasma	American adults American children Caucasian children	Male / Female (48.8%) Male / Female (40%) Male / Female (50%)	41.5 13.5 4.5	1.18 (logFC) 5.09 2.41	Nuñez-Lopez* 2016 Thompson et al 2017 Masotti et al 2017
mir-122-5p Serum and plasma	American adults American children Caucasian adults Caucasian adults Mexican-American adults Caucasian adults Caucasian children Caucasian children Chinese adults	Male / Female (50%) Male / Female (40%) Male / Female (66.6%) Male / Female (50%) Female Male Male / Female (50%) Male / Female (50%) Male / Female (50%)	68.8 13.5 28 63 40 46.5 4.5 9.0 24	0.046 (B-est) 12.48 2.55 1.6 0.405 (Cox) 1.35 2.82 1.59 3.22 (QNFI)	Shah R et al 2017 Thompson et al 2017 Murri et al 2018 Willeit et al 2017 Zhao et al 2017 Ortega et al 2013 Masotti et al 2017 Prats-Puig et al 2013 Wang R et al 2015
mir-126-5p Serum and plasma	American adults Caucasian adults	Male / Female (48.8%) Male	41.5 46.5	0.32 (logFC) 1.63	Nuñez-Lopez* 2016 Ortega et al 2013
mir-140-5p Serum and plasma	Caucasian adults Caucasian adults Caucasian children	Male / Female (66.6%) Male Male / Female (50%)	28 46.5 9.0	1.66 2.59 1.41	Murri et al 2018 Ortega et al 2013 Prats-Puig et al 2013
mir-142-5p Serum and plasma	Caucasian adults Mexican-American adults	Male / Female (66.6%) Female	28 40	1.50 0.374 (Cox)	Murri et al 2018 Zhao et al 2017
mir-146a-5p Serum	American adults Asian children Caucasian children	Male / Female (48.8%) Male / Female (51%) Male / Female (50%)	41.5 4.9 4.5	0.73 1.01 (logFC) 1.48	Nuñez-Lopez* 2016 Cui et al 2018 Masotti et al 2017
mir-148a-3p Serum	Asian children Caucasian adults	Male / Female (51%) Male / Female (66.6%)	4.9 28	1.28 1.94	Cui et al 2018 Murri et al 2018
mir-191-5p Plasma	American adults American children	Male / Female (50%) Male / Female (40%)	68.8 13.5	0.037 (B-est) 7.21	Shah R et al 2017 Thompson et al 2017
mir-192-5p Serum and plasma	American adults American children Caucasian adults	Male / Female (50%) Male / Female (40%) Male / Female (66.6%)	68.8 13.5 28	0.053 (B-est) 3.78 1.68	Shah R et al 2017 Thompson et al 2017 Murri et al 2018

mir-197-3p Serum and plasma	American adults Caucasian adults	Male / Female (50%) Male / Female (66.6%)	68.8 28	0.038 (B-est) 1.95	Shah R et al 2017 Murri et al 2018
mir-222-3p Serum and plasma	American children Asian children	Male / Female (40%) Male / Female (51%)	13.5 4.9	2.14 1.15	Thompson et al 2017 Cui et al 2018
mir-223-3p Serum and plasma	American children Caucasian adults	Male / Female (40%) Male / Female (66.6%)	13.5 28	6.72 1.96	Thompson et al 2017 Murri et al 2018
mir-223-5p Serum	American adults Caucasian adults	Male / Female (48.8%) Male / Female (66.6%)	41.5 28	0.58 (logFC) 2.17	Nuñez-Lopez* 2016 Murri et al 2018
mir-320a Serum and plasma	American adults Caucasian children	Male / Female (50%) Male / Female (50%)	68.8 4.5	0.035 (B-est) 1.55	Shah R et al 2017 Masotti et al 2017
mir-342-3p Serum and plasma	American adults Caucasian children	Male / Female (50%) Male / Female (50%)	68.8 4.5	0.045 (B-est) 1.46	Shah R et al 2017 Masotti et al 2017
mir-486-5p Serum and plasma	American adults Asian children Caucasian children	Male / Female (50%) Male / Female (51%) Male / Female (50%)	68.8 4.9 9.0	0.030 (B-est) 1.21 2.20	Shah R et al 2017 Cui et al 2018 Prats-Puig et al 2013
mir-636 Serum and plasma	Caucasian adults Chinese adults	Male Male / Female (50%)	46.5 24	1.29 6.19 (QNFI)	Ortega et al 2013 Wang R et al 2015

[1]Women percentage in the total cohort

[2]Age average in years in lean and obesity groups

QNFI, quartile normalized fluorescence intensities; B-est, Beta-estimates; Cox, Cox-regression coefficient.

* et al.

Table 2. Circulating miRNAs reported as underexpressed in human obesity by at least two independent working groups.

hsa-miRNA Source	Population			Expression level (+fold)	References
	Ethnics	Gender[1]	Age[2]		
mir-30b-5p	Caucasian adults	Male / Female (66.6%)	28	0.77	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.020 (QNFI)	Wang R et al 2015
mir-30c-5p	Caucasian adults	Male / Female (66.6%)	28	0.79	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.029	Wang R et al 2015
mir-125b-5p	Caucasian adults	Male	46.5	3.31	Ortega et al 2013
Plasma	Caucasian children	Male / Female (50%)	9.0	0.5	Prats-Puig et al 2013
	Mexican-American adults	Female	40	0.316 (Cox)	Zhao et al 2017
mir-151a-5p	Caucasian adults	Male / Female (66.6%)	28	0.69	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.15	Wang R et al 2015
mir-151a-3p	Caucasian adults	Male / Female (66.6%)	28	0.74	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.18	Wang R et al 2015
mir-199a-5p	Caucasian adults	Male / Female (66.6%)	28	0.80	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.049	Wang R et al 2015
mir-324-3p	Chinese adults	Male / Female (50%)	24	0.24	Wang R et al 2015
Serum	PregnantCaucasian women	Female	30	2.00	Carreras-Badosa* 2015
mir-331-3p	Caucasian adults	Male / Female (66.6%)	28	0.67	Murri et al 2018
Serum	Chinese adults	Male / Female (50%)	24	0.11	Wang R et al 2015
mir-590-5p	Caucasian adults	Male	46.5	2.69	Ortega et al 2013
Serum	Chinese adults	Male / Female (50%)	24	0.03	Wang R et al 2015

[1]Women percentage in the total cohort
[2]Age average in years in lean and obesity groups
QNFI, quartile normalized fluorescence intensities; B-est, Beta-estimates; Cox, Cox-regression coefficient.
*et al.

Table 3. Circulating miRNAs reported as overexpressed and underexpressed in human obesity by at least two independent working groups.

hsa-miRNA Source	Population			Expression level (+fold)	References
	Ethnics	Gender[1]	Age[2]		
mir-21-5p	American adults	Male / Female (48.8%)	41.5	+0.33 (logFC)	Nuñez-Lopez* 2016
Serum and	American children	Male / Female (40%)	13.5	+4.89	Thompson et al 2017
plasma	Asian children	Male / Female (51%)	4.95	+1.55	Cui et al 2018
	Caucasian adults	Male	46.5	+1.17	Ortega et al 2013
mir-21-5p	Caucasian adults	Male	46.5	-0.73	Ortega et al 2013
Serum and	Iranian adults	Male / Female (57.5%)	52.05	-0.81	Ghorbani et al 2017
plasma					
mir-130b-3p	Asian children	Male / Female (51%)	4.95	+1.07	Cui et al 2018
Serum and	Caucasian children	Male / Female (50%)	9.0	+1.50	Prats-Puig et al2013
plasma	Chinese men	Male	50.5	+0.905 (ROC)	Wang et al 2013
mir-130b-3p	Brazilian adults	Male / Female (32%)	54.9	-2.0	Thomé et al2015
Serum and	Caucasian adults	Male	46.5	-3.13	Ortega et al 2013
plasma					

[1]Women percentage in the total cohort
[2]Age average in years in lean and obesity groups
QNFI, quartile normalized fluorescence intensities; B-est, Beta-estimates; Cox, Cox-regression coefficient.
*et al.

Table 4. Metabolic pathways predicted for c-miRNAs dysregulated in human obesity.

OVEREXPRESSED				
KEGG pathway	**DIANA miRPath**	**p-value**	**#genes**	**#miRNAs**
Fatty acid metabolism (MS Score 0.19)	Tarbase	2.5541E-006	37	21
	TargetScan[a]	3.0747E-015	7	8
Fatty acid biosynthesis	TargetScan[a]	3.5509E-030	3	4
	microT-CDS*	1.1188E-009	8	8
FoxO signaling pathway	Tarbase	4.7883E-005	113	23
	microT-CDS*	0.0014	73	22
mTOR signaling pathway (MS Score 1.349)	Tarbase	0.0023	54	22
	microT-CDS*	0.0395	35	20
Phosphatidylinositol signaling system (MS Score 0.97)	Tarbase	0.0041	63	22
Inositol phosphate metabolism (MS Score 0.654)	Tarbase	0.0115	51	22
PI3K-Akt signaling pathway	Tarbase	0.0343	229	23
	TargetScan[a]	0.0181	47	22
	microT-CDS*	0.0002	169	23
UNDEREXPRESSED				
KEGG pathway	**DIANAmiRPath**	**p-value**	**#genes**	**#miRNAs**
Fatty acid metabolism (MS Score 0.063)	Tarbase	3.6480E-008	22	10
	TargetScan[a]	4.0980E-005	6	2
Fatty acid biosynthesis	Tarbase	1.9573E-006	6	8
	TargetScan[a]	<1E-325	2	2
FoxO signaling pathway	Tarbase	9.3061E-006	73	11
	microT-CDS*	0.0005	38	8
Phosphatidylinositol signaling system (MS Score 0.843)	Tarbase	0.0034	41	11
mTOR signaling pathway (MS Score 0.808)	Tarbase	0.0108	34	11
PI3K-Akt signaling pathway (MS Score 0.636)	Tarbase	0.0368	127	11

FDR Correction, Conservative stats and significance level at $p<0.05$
[a]Context score -0.4
*microT-Threshold 0.8
MS: also predicted by miRSystem.

Table 5. Target genes in PI3k/Akt signaling pathway and regulatory miRNAs predicted

miRNA	Target gene	Description	Pathway*	Prediction tool**
hsa-miR-223-3p	*FOXO1*	Forkhead box protein O1	Insulin signaling pathway (K) PI3K-AKT Activation (R)	D, MR, MB, PI, TS
hsa-miR-223-3p	*FOXO3*	Forkhead box protein O3		MR, MB, PT, PI, TS
hsa-miR-21-5p **hsa-miR-15b-5p**	*PIK3R1*	Phosphoinositide-3-kinase Regulatory Subunit 1	PI3K-AKT Activation(R) Insulin signalign pathway (K) Type 2 Diabetes (K)	D, MR, MB, PI, TS D, MR, MB, PI, TS
hsa-miR-15b-5p	*INSR*	Insulin Receptor Precursor	Insulin signaling pathway (K) Type 2 Diabetes (K) PI3K-AKT Activation (R)	D, MR, MB, PT, PI, R2, TS
	IRS2	Insulin Receptor Sustrate 2		D, MR, MB, PT, PI, R2, TS
hsa-miR-15b-5p **hsa-miR-122-5p** **hsa-miR-320a**	*AKT3*	Serine/Threonine Kinase 3	Insulin signalign pathway (K) Adipocitokine signaling pathway (K) Carbohydratedigestion and absorption (K) PI3K-AKT (PID)	D, MR, MB, PT, PI, R2, TS D, MR, MB D, MR, PT, PI, R2

*K: KEGG, R: Reactome, PID: Pathway Interaction Database. **D: DIANA, miRanda: MR, miRBridge: MB, PicTar:PT, PITA:PI, Rna22: R2, TargetScan:TS
Created with miRSystem v.2016

FIGURE LEGENDS

Figure 1. PRISMA flow diagram for bibliographic search. *Modified from:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. *PLoS Med* 6: e1000097.

Figure 2. Hierarchical clustering of miRNAs reported as dysregulated in human obesity. Heatmaps created directly from the DIANA-miRPath v3.0 interface using TarBase from DIANA as prediction tool show the level of enrichment in GO categories of miRNAs confirmedly reported as overexpressed (A) and underexpressed (B) in human obesity. The colour scale at the top illustrates the level of association of a miRNA with GO-Slim categories.

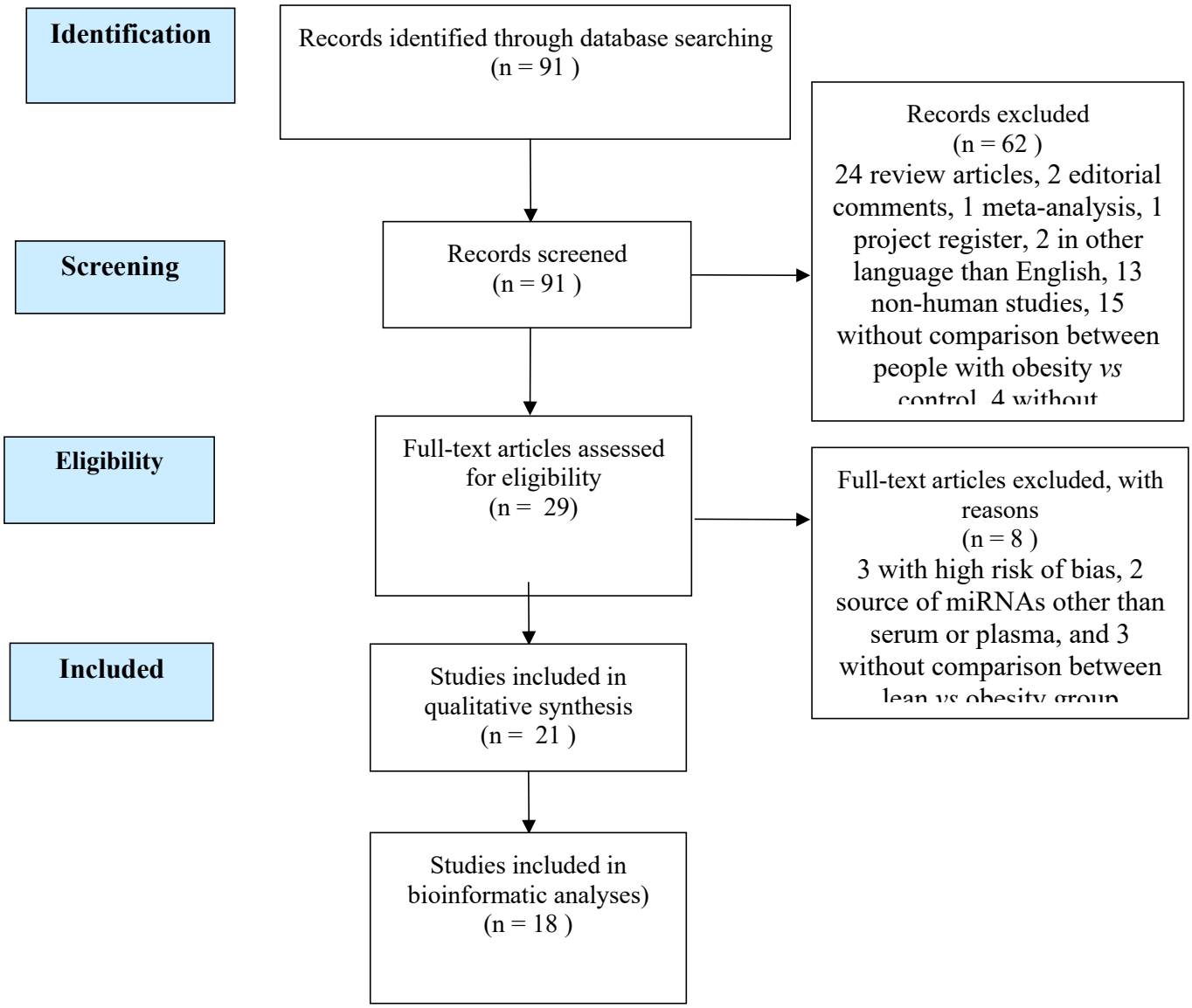
Identification
Records identified through database searching
(n = 91)
Screening
Records screened
(n = 91)
Records excluded
(n = 62)
24 review articles, 2 editorial comments, 1 meta-analysis, 1 project register, 2 in other language than English, 13 non-human studies, 15 without comparison between people with obesity vs control, 4 without
Eligibility
Full-text articles assessed for eligibility
(n = 29)
Full-text articles excluded, with reasons
(n = 8)
3 with high risk of bias, 2 source of miRNAs other than serum or plasma, and 3 without comparison between lean vs obesity group
Included
Studies included in qualitative synthesis
(n = 21)
Studies included in bioinformatic analyses)
(n = 18)

Figure 1.

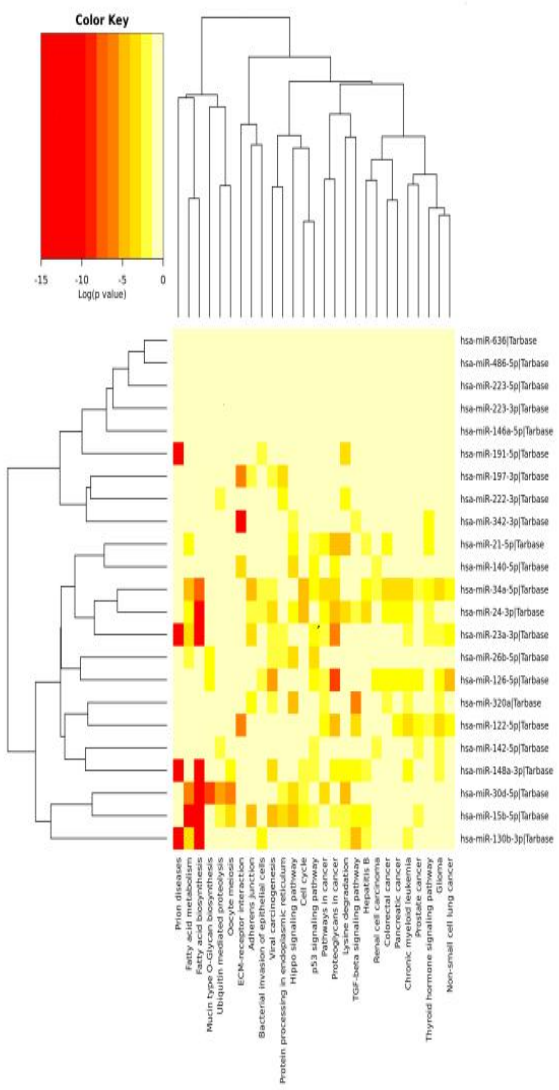

Figure 2a.

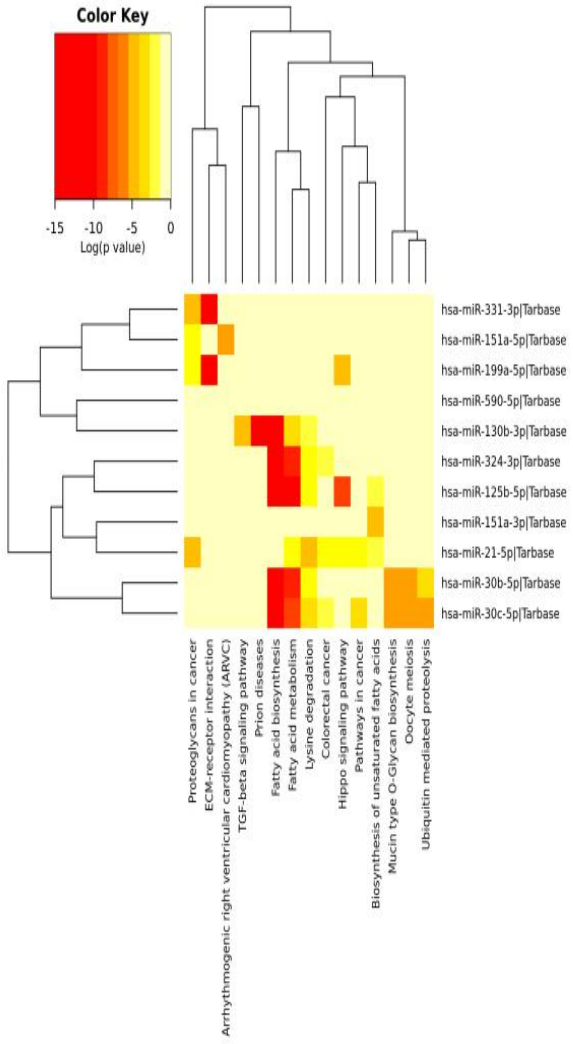

Figure 2b.

Supplemental Material 1. PRISMA Checklist

Section/topic	#	Checklist item	Page
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	i
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	xiii
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

Section/topic	#	Checklist item	Page
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Suppl. 2a
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Suppl. 4
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Suppl. 2b
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency.	7-8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Suppl. 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Suppl. 3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	19
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	20

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Supplemental Material 2.

Supplemental Material 2a. Summary of included studies.

Study	Cohort	Overexpressed miRNAs Obesity *vs* Lean	Underexpressed miRNAs Obesity *vs* Lean	miR profiling methods	Comments
Murri 2018 (Spain)	Serum samples. Adults. 12 women with PCOS (lean n=6, 27 sd 4 yr, BMI 22 sd 2; ob n=6, 27 sd 2 yr, BMI 39 sd 9), 12 control women (lean n= 5, 28 SD 3 yr, BMI 22 SD 2: ob n=6, 31 sd 6 yr, BMI 37 sd 3) and 12 men (lean n=6, 29 sd 3 yr, BMI 24 sd 1: ob n=6, 33 sd 6 yr, BMI 42 sd 8).	let-7b-3p (2.60), let-7g-3p (2.28), miR-16-1-3p (1.68), miR-23a-3p (1.39), miR-24-3p (1.29), miR-29c-3p (3.41), miR-30e-5p (1.33), miR-122-5p (2.55), miR-140-5p (1.66), miR-142-5p (1.50), miR-143-3p (1.67), miR-148a-3p (1.94), miR-181c-3p (4.92), miR-192-5p (1.68), miR-193a-5p (2.10), miR-197-3p (1.95), miR-203a (2.31), miR-223-3p (1.96), miR-223-5p (2.17), miR-302c-5p (2.96), miR-338-3p (1.87), miR-345-5p (1.65), miR-361-5p (1.47), miR-365a-3p (3.13), miR-378a-3p (1.47), miR-378a-5p (3.75), miR-424-5p (1.66), miR-425-3p (1.23), miR-425-5p (1.33), miR-582-5p (2.96), miR-651 (3.77), miR-671-5p (1.95), miR-877-5p (1.47), miR-1537 (2.77), miR-1539 (2.12)	let-7a-5p (0.73), let-7c (0.77), let-7d-5p (0.65), let-7f-5p (0.69), miR-18a-5p (0.82), miR-30b-5p (0.77), miR-30c-5p (0.79), miR-98-5p (0.62), miR-103a-3p (0.71), miR-107 (0.78), miR-151a-3p (0.74), miR-151a-5p (0.69), miR-181a-2-3p (0.42), miR-199a-3p (0.80), miR-199a-5p (0.61), miR-331-3p (0.67), miR-379-3p (0.50), miR-431-5p (0.41), miR-744-5p (0.70).	miRCURY LNA™ Universal RT microRNA PCR, 4x Human panel I+II (Exiqon, Denmark) (752). miRNA-specific real-time PCR (RT-PCR) using a LightCycler® 480 II	Results in fold-change Normalized to 5 reference miRNAs: miR-191-5p, miR-30c-5p, miR-423-3p, miR-423-5p and miR-93-5p.
Cui X 2018 (China)	Serum samples. discovery study: 9 children with obesity and 9 controls with normal weight were pooled into 3 pools, for miRNA profiling experiments cross-sectional validation study: the miRNAs of interest were validated in 352 individuals (100 children with obesity (61 sd 10.4 months, 51-5% girls, BMI 20.3 sd 2.20), 106 children with overweight (59.6 sd 11 months, 52.8% girls, BMI 17.4 sd 0.60) and 146 controls (60.4 sd 11.1 months, 49.6% girls, BMI 15.1 sd 1.06) 3) longitudinal validation study: the candidate miRNAs were estimated in newly diagnosed patients with T2D (n = 101, 57.5 sd 12.2 yr; BMI 26.8 sd 4.19) and controls with normal glucose tolerance	miR-146a-5p (+1.01) miR-130b-3p (+1.07) miR-222-3p (+1.15) miR-486-5p (+1.21) miR-148a-3p (+1.28) miR-21-5p (+1.55) miR-375 (+1.73) miR-146b-5p (+1.94) miR-99b-5p (+1.96) miR-30a-3p (+2.17) miR-27a-3p (+2.58) miR-30d-5p (+2.67) let-7d-5p (+2.85) miR-26b-5p (+3.43) miR-15b-5p (+8.79) miR-19a-3p (+9.06) miR-20a-5p (+9.64)	miR-197-3p (-1.41)	microRNA sequencing platform (Illumina Inc). FASTX-Toolkit software. RT-qPCR: ViiA7 Real-Time PCR System (Applied Biosystems)	Normalized to cel-miR-39. 177 miRNAs were detected in pooled samples. 94 miRNAs exhibited a >2-fold difference between groups. Based on the fold change and expression abundance, they selected 18 miRNAs as candidates for further study.

	(NGT) (n = 82, 49.3 sd 7.73 yr, BMI 24.3 sd 3.22)				
Ghorbani 2017 (Iran)	Serum samples. 45 T2D (29 female, 47.6 sd 5.8 yr, BMI 27.3 sd 3.9), 42 non-T2D (21 female, 56.5 sd 8.1 yr, BMI 28.2 sd 4.8)		miR-21 (0.81)	RT-qPCR: Rotogene Q (3)	Normalized to cel-mir-39 and miR-16 as internal control.
Thompson 2017 (USA)	Plasma samples. Children; 10 healthy controls (13.8 yr, 60% girls, BMI 20.11) vs 20 children with obesity (13.2 yr, 30% girls, BMI 34.7)	15b-5p(+3.42), 199a-5p (+17.18), 222-3p(+2.14), 223-3p(+6.72), 181b-5p (+3.29), 122-5p(+12.48), 23a-3p (+5.3), 27b-3p (+6.74), 21-5p (+4.89), 34a-5p (+5.09), 192-5p (+3.78), 29a-3p (+2.81), 214-5p (+2.73), 155-5p (+2.63), 191-5p (+7.21), 103a-5p (+3.38)		RT-qPCR: Exiqon Pick and Mix miRNA PCR panel (20)	Normalized to UniSp2. Reported data: fold change
Wander 2017 (USA)	Plasma samples. Among participants in the Omega study. Pregnant woman; 36 GDM cases, 34.3 yr, 16.5 weeks GA, ppBMI 25.5, 80 normal controls, 32.9 yr, 15.1 weeks GA, ppBMI 21.7.	GDM 155-5p (+2.11); 21-3p (+3.59); 146b-5p (+2.79); 223-3p(+1.89); 517-5p (+1.93); 29a-3p (+1.43) Obese + GDM 210-3p (+1.53)		RT-qPCR: 10 selected miR: Custom targeted panel Exiqon LNA primers (10)	Normalized to cel-miR-39; and endogenous miR-423-3p.
Zhao 2017 (USA)	Plasma samples. 300 Mexican-American women, mean 40 yr. Training set (arrays) n= 40, 47.5% with obesity at baseline, 72.5% at 5 yr of follow-up Testing set 1, n=160, 47.5% with obesity at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% with obesity at baseline, 63% at 5 yr.	Obesity at baseline (rho) 142(+0.374), 122(+0.405), 30a (+0.411), 519d (+0.313),	125b(-0.316)	Arrays: TaqMan Array Human Microarray Card Set v3.0 (384) RT-qPCR: Individual TaqMan miRNA Assays	Normalized to cel-miR-39 y cel-miR-54. Reported data: Cox-regression coefficient
Xiong 2017 (China)	Serum samples. Women from East China. 18 women with PCOS (25.8 yr, BMI 24) and 30 healthy women (25.5 yr, BMI 20)	+23a (obese women with PCOS) +23b (obese women)		RT-qPCR: miRCute miRNA qPCR detection kit (2)	Groups divided accordingly endometrial cycle. Normalized to U6.
Shah 2017 (USA)	Plasma samples. FHS Offspring Cohort, n=2317, 65.8 yr, 56% women, BMI27.7. Validation youth cohort: n= 90 ob/ow,15.5 yr, 60% women, BMI 33.8. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	122-5p(+0.046), 16-5p (+0.024), 191-5p(+0.037), 192-5p (+0.053), 194-5p (+0.033), 197-3p (+0.038), 19b-3p (+0.037), 24-3p (+0.032),, 301b-3p (+0.029), 30d-5p (+0.033), 320a (+0.035), 342-3p (+0.045), 486-5p (+0.030), 574-3p (+0.035), 616-5p (+0.040), 664b-3p (+0.015); snoRNA-1210: (+0.016)	320b (-0.016), 4446-3p (-0.033), piRNA-48383(-0.027)	Small-RNA sequencing: Ion Torrent Proton Sequencer High-troughput RT-PCR: miScript assay technologies, Fluidigm Biomark system (391 ex-RNA, 297 miR, 36 snoRNA, 58 piRNA)	122 and 192 validated in youth cohort Reported data: B-estimates p/HOMA-IR.
Willeit 2017 (Italy)	Serum and plasma samples. Bruneck Study: n= 810 Caucasian patients, 50% female, 63 yr; BMI 26, free of preexisting disease at baseline.	122 (+1.6)		RT-qPCR: TaqMan miRNA assays (1)	Normalized to U6 and cel-miR-39. They measure miR-122 at baseline and after 5 years. 100% follow-up.

	136 developed metabolic syndrome, 57 T2D after 10 years:1995-2005)				
Hubal 2017 (USA)	Plasma and serum samples. 6 African-American women with obesity (38.5 yr, BMI 51.2)	1227-3p (+2.23), 4691-5p (+1.98), 219a-5p (+1.8), 4728-3p (+1.64), 103-3p (+1.59), 3622a-3p (+1.54), 4749-3p (+1.53), 125b-3p(+1.50)	3926 (-1.52), 224-5p (-1.53), 4723-5p (-1.58), 16-5p (-1.59), 3690 (-1.73), 208a-3p (-1.82), 4716-3p (-1.87), 4525 (-1.91), 2355-5p (-1.93), 4782-5p (-2.29)	Affimetrix GeneChip microRNA 4.0 arrays (2578)	In circulating adipocyte-derived exosomes. 168miR differentially expressed after one year post-surgery (gastric bypass). They found 168 miR with differential expression, 56 human mature miR, and reported those associated with insulin signalling.
Enquobahrie 2017 (USA)	Plasma samples. Among participants from two cohorts: Omega and POUCH studies: Omega study: n=20, 29.75 yr, 16.62GA, BMI <18 n=1, 18-25 n=12, >25 n=7. POUCH study: n=20, 26.01 yr, 22.40GA, BMI<18 n=1, 18-25 n=9, >25 n=10	Pregestational Ob/ow (+) 28-3p(0.115, 0.101), let-7d*(0.117, 0.081), 3137(0.125, 0.105), 584(0.120, 0.110), 28-5p(0.130, 0.118), 4286(0.096, 0.104), 376a(0.174, 0.141), 423-5p(0.082, 0.079), 425(0.138, 0.099), 199a-5p(0.185, 0.147), 652(0.133, 0.117), 151-3p(0.130, 0.115), 221(0.185, 0.124), 891a(0.116, 0.093), 103-2(0.080, 0.076), 361-5p(0.126, 0.112), 151-5p(0.164, 0.145), 130b(0.119, 0.103), 146b-5p(0.161, 0.160), 377(0.153, 0.129), 128(0.129, 0.121), 139-5p(0.090, 0.070), 423-3p(0.116, 0.100), 487b(0.130, 0.114), 191(0.130, 0.130), 29c(0.112, 0.120), 26b(0.060, 0.165)		Arrays: Microarray based epigenome wide miRNA profiling (319)	Reported data: B-estimates from both cohorts (Omega and Pouch). All positively correlated.
Nunez-Lopez 2016 (USA)	Serum samples. From ORIGINS trial. Subjects Lean vs with obesity; health or with prediabetes or T2D: lean+healthy (n=10, 8 female, 32 yr, BMI 21.8) lean+prediabetes (n=10, 6 female, 42.5 yr, BMI 21.7), lean+T2D (n=2, 1 female, 41 yr, BMI 23.1); obesity+healthy (n=9, 7 female, 34 yr, BMI 35), obesity+prediabetes (n=11, 5 female, 42 yr, BMI 35.1), obesity+T2D (n=15, 5 female, 51 yr, BMI 36.5).	Obesity vs lean(logFC) 21 (+0.33), 24.1(+0.74), 27a (+0.6), 34a (+1.18), 126 (+0.32), 146a (+0.73), 148a (+0.87), 152 (+0.66), 223(+0.58)	25 (-0.44), 93 (-0.48), 150 (-0.67)	RT-qPCR: TaqMan Universal Master Mix and TaqMan microRNA Assay. (23)	A diabetes-related human miRNA panel was used. Normalized to cel-mir-39 and 3 endogenous miR: 191, 423-3p and 451. Reported data: differential abundant in circulation, logFC, median and interquartile rank.
Auguet 2016 (Spain)	Serum samples. Women. 62 with morbid obesity MO; 30 with moderate obesity ModO; 30 normal weight (41 yr, BMI 22.1). Both obesity groups divided according to liver	+33b* MO vs ModO +122 MO vs ModO		RT qPCR:miRNeasy Serum/Plasma kit (Qiagen) (3)	MO + NASH >122 than MO with SS Normalized to cel-mir-39 Reported data: arbitrary units

	disease. In -ModO: normal (n=9, 49.8 yr, BMI 35.4), SS (N=9, 49.06 yr, BMI 36.2, NASH n=12, 52.23 yr, BMI 35.1). In MO: normal (n=22, 46.3 yr, BMI 48.5), SS (n=18, 47.2 yr, BMI 48.9) NASH n=22, 48.8 yr, BMI 47.2)				
Iacomino 2016 (Italy)	Plasma samples. IDEFICS Cohort; 20 children selected from the Italian cohort of the "I.Family project": 2 groups: 1) normal weight (5 girls, 5 boys, 10.5 yr, BMI 16.45), 2) ow/ob (4 girls, 6 boys, 10.7 yr, BMI 31.68)	Arrays 26b-5p (+25.3723), 31-5p(+4.9499), 2355-5p(+6.5213) RT-qPCR 31-5p (+1.92), 2355-5p (+2.93)	206 (-0.52) With differences in microarray but without RT-qPCR validation: 1231 (-8.7217), 361-3p (-4.8918), 136-5p (-4.8356), 320a (-9.9692), 206 (-6.0515)	Arrays: Serum and Plasma 384HC miScript miRNA PCR Arrays (Qiagen) RTqPCR: SYBR Green PCR kit (Qiagen) (372)	Normalized to cel-miR-39-3p, SNORD61, SNORD68, SNORD72, SNORD95 and SNORD96A Reported data: fold change expression
Masotti 2017 (Italy)	Serum samples. 12 children with obesity selected from the cohort "Origin study" whose BMI switched from normal weight to ow/ob in the year prior to enrollment. 6 insulin-resistant (4.63 yr, BMI 20.87), 6 insulin-sensitive (4.35 yr, BMI 18.52). Age, sex and BMI matched.	505-3p(+3.11), 122-5p(+2.82), 34a-5p(+2.41), 26b-5p(+1.63), 320a(+1.55), 146a-5p(+1.48), 148b-3p(+1.47), 342-3p(+1.46)	660-5p(-1.50), 19a-3p(-1.55), 95(-1.72), 205-5p(-2.60), 200c-3p(-2.78), 190a(-3.04)	Arrays: Serum/Plasma Focus microRNA PCR Panel (Exiqon) (179)	Comparison between obese children, with or without insulin resistance. With differences statistically significant in weight, height, ALT and HDL-cholesterol between groups. Reported data: mean fold change expression. Normalized: two artificial spike-in miRNAs
Liu 2016 (China)	Serum samples. 25 control subjects (51.12 yr, BMI 21.94); 24 subjects with obesity, (46.96 yr, BMI 30.81). 50% women in both groups, sex and age matched.		-1934(-32.5%)	RT-qPCR: miScript SYBER Green PCR kit (1)	Normalized to RNU6B. Reported data: Relative expression
Carreras-Badosa 2015 (Spain)	Plasma samples. 70 pregnant Caucasian women, 24-32 sdg; 3 groups: 20 pregestational obesity (31 yr, BMI 1st to 3rd trim 29.4-32.3), 25 gestational obesity (30 yr, BMI 1st to 3rd trim 23-29.2), 25 normal pregnancies (30 yr, BMI 1st to 3rd trim 23-26.7). Arrays: 6 women were randomly selected, from each group. Validation RT-qPCR in the complete cohort.	GestOb vs control 30a-5p (+1.89), 130a(+1.63), 150(+1.75) Ob vs control 625(+1.82, +1.30) PregestOb vs GestOb 221 (+1.78)	GestOb vs control 29c(-1.32), 99b (-1.43), 103(-1.64), 221(-1.65), 340(-3) PregestOb vs GestOb 130a(-1.76) Ob vs control 122(-2.07,-1.45),324–3p(-1.75,-2.00), 375(-2.08, -1.56), 652(-1.19, -1.70)	TaqMan Low Density Arrays human mRNA Card Set version 3.0 RT-qPCR: Individual TaqMan miRNA Assays (723)	Normalized to U6 snRNA and 3 miR endogenous. 18 miR deregulated in microarrays, 13 confirmed by RT-qPCR Reported data: relative expression
Pek 2016 (China)	Whole blood samples. N=32 men, 4 groups n=8: 1) non-T2D, lean (42.3 yr, BMI 21.3), 2) non-T2D with, obesity (36.6 yr, BMI 33.3), 3 T2D-lean (50.5 yr, BMI 22.4), 4)		-125b, -181a, -210, -378 -100 (obese and diabetic vs control)	Arrays: Agilent SurePrint G3 Human miRNA microarray v.16 RT-qPCR: LNA-qPCR individual primers (Exiqon) (1205)	215 detected in microarrays; 31 significantly different between the 4 groups; 8 validated by RT-qPCR Reported Data: relative expression

	T2D-obesity(38.0, BMI 37.0)				Normalized to SNORD48
Khalyfa 2016 (USA)	Plasma samples. 16 children with obesity/overweight , 8 with endothelial dysfunction, 8 with normal endothelial function; (8.41 yr and 7.59 yr) Matched for age, sex, ethnicity and BMI (74% white, 60% male)	365b-3p (+1.41, +1.52)	125a-5p (-1.33, -1.27), 342-3p (-1.41, -1.22)	Pathway specific for human CVD miRNA PCR Array Qiagen RT-qPCR: SYBR Green Real-Time PCR (84)	Comparisons in obese/overweight children with or without endothelial function. No lean control. Normalized: cel-mir-39 and SNORD68
Can 2016 (Turkey)	Whole blood samples. Turkish children. 45 children with obesity (19 male, 26 female) and adolescents vs 41 controls (17 male, 24 females) (14.71 yr and 14.44 yr; BMI 41.31 and 18.94, respectively). Matched for age and sex.	27(+1.61), 378(+3), 370(+3.37)	335-5p(-4.46), 143(-3.78), 758-3p(-2.75)	RT-qPCR: miRCURY LNA Universal RT PCR kit (Exiqon) (7)	Reported data: medians; analysis ROC.
Thomé 2015 (Brazil)	Plasma samples. 57 age and gender matched subjets: 40 patients with heart-failure (65% men, 20 with obesity: 54.9yr, BMI 37.3; 20 lean: 54.9 yr BMI 21.8).17 healthy controls (52.1 yr, BMI 24.7, 71% men).		-130b	RT-qPCR:TaqMan miR Expression Array Probes (4)	miR-423-5p elevated in heart failure vs control; 221 and 21 ns Normalized: cel-miR-39 Reported data: fold change
Wen 2015 (China)	Serum samples. Asian population. 41 normal-weight (50.5 yr, BMI 21.7); 40 Ow(51.6 yr, BMI 25.7); 40 Ob(50.4 yr, BMI 30.2). 50% women each group		223(ow -7.11, ob -1.65)	RT-qPCR: SYBR Premix DimerEraser kit (1)	Normalized: cel-miR-39 miR-223 increased after 3-months lifestyle intervention Reported data: median and interquartile rank
Párrizas 2015 (Spain)	Serum samples. 92 men, 29 control group (54.39 yr, BMI 29.11), 22 individuals with prediabetes IFG (53.62 yr, BMI 29.46), and 21 individuals with prediabetes IGT, 56.79 yr, BMI 28.98 and 20 newly diagnosed T2D, 55.68 yr, IMC 30.08 A second cohort (exercise intervention, n=18, both sexes, 12 control, 6 with prediabetes (3 with IGT, 3 with IFG)	+192, +193b With differences in microarrays but without RT-qPCR validation: +125a-5p, +150	With differences in microarrays but without RT-qPCR validation: -191, -15b, -128	Arrays: Exiqon panels (176) RT-qPCR: Exiqon SYBRGreen primers	Reported data in dCt value. Normalized with endogenous miR (let-7b, let-7g and let-7i). All participants Ow/Ob. miR-192 and 193b overexpressed in prediabetes; 2nd cohort: baseline expression post-exercise intervention.
Wang R 2015 (China)	Serum samples. In Chinese people. Arrays: 56 patients with obesity (24.31 yr, 50% women, BMI 39.03) and 56 control subjects (24.47 yr, 50% women, BMI 20.97) RT-qPCR Validation: 107 lean control (23.97 yr, 53.3% women, BMI20.79).	122(+3.22), 636(+6.19), 933(+4.26), 574-5p(+3.05)	140-3p(-0.33), 20b(-0.31), 19b(-0.29), 550a(-0.29), 361-5p(-0.29), 17(-0.28), 30a(-0.27), 654-5p(-0.27), 324-3p(-0.24), 148b(-0.21), 10a(-0.19), 151-3p(-0.18), 335(-0.18), 126*(-0.18), 142-5p(-0.17), 494(-0.17), 151-5p(-0.15), 223*(-0.13), 331-3p(-0.11), 144*(-0.10), 199a-5p (-0.049), 195(-0.04), 301-a(-0.04), 338-3p(-0.04),	Microarray: Agilent Human miRNA 8*60K v.16 (1205) RT-qPCR miRCURY LNA Universal RT kit (Exiqon)	Reported data: quartile normalized fluorescence intensities. Normalized with algoritm Quantile miR-122 was the only miR validated in RT-qPCR 4 initial pools, 28 patients each (for microarrays)

	123 subjects with obesity (24.02 yr, 50% women, BMI 37.73)		590-5p(-0.03), 186(-0.03), 30c(-0.029), 30b(-0.020), 140-5p(-0.015)		
Pescador 2013 (Spain)	Serum samples. 13 patients with T2D (46% women, 69.4 yr, BMI 24.86); 20 patients with obesity (85% women, 41.7 yr, BMI 42.73); 16 patients with obesity+T2D (40% women, 67.55 yr, BMI 33.38), 20 controls (50% women, 42.9 yr, BMI 22.7).	+15b	-138, -376a, -503	Arrays: miRCURY LNA Universal RT cDNA Synthesis kit Human Panel I and II (742) RT-qPCR: miRCURY LNA microRNA PCR System (Exiqon)	(Initial pools for arrays) Reported data: ROC-AUC for each miRNA. Normalized to miR-30c, 103, miR-191 and miR-423-3p.
Murri 2013 (Spain)	Whole blood samples. 12 control women, 12 with PCOS, 12 men. 6 subjects per group with normal weight and 6 with obesity. Control: 29 yr, BMI 22 vs 37, PCOS: 27 yr, BMI 22 vs 39. Men: 30 yr, BMI 24 vs 43.		-21, -276, -103, -155	RT-PCR: TaqMan MicroRNA Reverse Transcription kit (4)	Reported data: B-estimates In whole blood, uncertain cellular origin of this miR. Normalized to exogenous miR: RNU44 and RNU6b
Prats-Puig 2013 (Spain)	Plasma samples. TaqMan array - discovery study: , 10 Caucasian boys, 5 with obesity (8.8 yr) vs 5 control (9.9 yr) RT-PCR - cross-sectional validation study: 85 control (9 yr, 49% girls) and 40 with obesity (9.2 yr, 55% girls) Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	+486-5p, +486-3p, +142-3p, +130b, +423-5p +532-5p, +140-5p, +16-1, +222, +363, +122	-221, -28-3p, -125b, -328	TaqMan miRNA Low Density Arrays (754) RT-PCR: TaqMan hydrolysis probes .	Reported data: relative Log10-ratios, correlation coefficients and B-estimates Normalized to 4 endogenous miR-106a, miR-146a, miR-19b and miR-223 (geometric mean)
Wang YC 2013 (China)	Serum samples. 21 Chinese lean men (48.95 yr, BMI 22.03) and 23 Chinese men with ow/ob (52.22 yr, BMI 27.25)	+130b		RT-PCR. Prime Script RT reagent kit. (1)	Normalized to miR-223. The study includes a validation in mouse and cell culture. Reported data: correlation coefficient and AUC-ROC.
Ortega 2013 (Spain)	Plasma samples. TaqMan array: , 32 white men (12 lean, 50 yr, BMI 23.7; 12 with obesity, BMI 33.1, 51 yr, 42% with T2D, 8 with morbid obesity, BMI 45.3, 46 yr, 50% T2D) (BMI 20-60) RT-qPCR: Replication in 80 patients: white men (49 lean: 49 yr, BMI 25.4; 19 with obesity: 51 yr, BMI 33.0, 37% with T2D; 12 with morbid obesity: 42 yr, BMI 45.5, 33% T2D). Longitudinal study: 5 men, 17 women, age 44 yr, BMI 42.9 (treated with surgery) and 9	Obesity vs Lean +140-5p, +142-3p, +222, +532-5p, +221, +423-5p, +21, +590-5p, +122, +483-5p, +636 Morbid obesity vs Lean ++140-5p, ++142-3p, +126, ++222	Obesity vs Lean -125b, -15a, -520c-3p, -193a-5p, -126, -625 , -130b Morbid obesity vs Lean -532-5p, -221, -423-5p, -21, -590-5p, -122, -483-5p, -636, --125b, -130b, --15a, --520c-3p, -193a-5p, --625	TaqMan miRNA Arrays (754) TaqMan Low Density Arrays RT-qPCR: TaqMan Hidrolysis Probes.	Normalized to 6 endogenous miR, analysing geometric means. Reported data: base log2-transformation. Results were validated in 80 patients, and in a longitudinal cohort, in obese patients treated with diet or surgery. We report results from comparisons in cross-sectional studies between obesity (BMI 30-40) vs non-obesity (BMI<30)

	treated with diet 5 men, 4 woman, age 47 yr, BMI 34.4.				

With statistical significance $p < 0.05$ in all cases.

Yr= years, BMI = body mass index, ppBMI: pre-pregnancy body mass index, GA: gestational age, GDM: gestational diabetes, PCOS: Polycystic ovarian syndrome, FHS: Framingham Heart Study, T2D: type 2 diabetes, NASH: non-alcoholic hepatic steatosis, SS: simple steatosis, IFG: impaired fasting glucose, IGT: impaired glucose tolerance, CVD: cardiovascular disease, RT-qPCR: real-time polymerase chain reaction.

Supplemental material 2b. miRNAs reported dysregulated in obesity.

miRNA		Sample	Population	Reference
let-7a-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
let-7b-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
let-7c	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
let-7d-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
let-7f-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
let-7g-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-10a	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-15a	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-15b	o	Serum	13 patients with T2D, 20 patients with obesity; 16 patients with obesity+T2D, 20 controls	Pescador 2013
miR-15b-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-16-1	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-16-1-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-16-5p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-17	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-18a-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-19a-3p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-19b	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-19b-3p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-20a-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-20b	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-21	d	Serum	Adults. 45 T2D, 42 non-T2D	Ghorbani 2017
	d	Plasma	*in morbid obesity vs control. Array: , 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-21-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-23a	o	Serum	Women from East China. 18 women with PCOS, 30 control	Xiong 2017
miR-23a-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-23b	o	Serum	Women from East China. 18 women with PCOS, 30 control	Xiong 2017
miR-24	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-24-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-25	d	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-26b-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study) 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-27a	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-27a-3p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study), 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-27b-3p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-28-3p	d	Plasma	Discovery study:10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-29a-3p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-29c-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-30a	o	Plasma	300 Mexican-American women, mean 40 yr. Training set (arrays) n= 40, 47.5% obese at baseline, 72.5% at 5 yr of follow-up. Testing set 1, n=160, 47.5% obese at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% obese at baseline, 63% at 5 yr.	Zhao 2017
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-30a-3p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-	Cui X 2018

			sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	
miR-30b	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-30b-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-30c	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-30c-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-30d-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-30e-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-31-5p	o	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
miR-33b	o	Serum	62 Women with morbid obesity, 30 with moderate obesity and 30 normal weight	Auguet 2016
miR-34a	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-34a-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-93	d	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-95	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-98-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-99b-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-103a-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-103a-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-107	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-122	o	Plasma	300 Mexican-American women, mean 40 yr. Training set (arrays) n= 40, 47.5% obese at baseline, 72.5% at 5 yr of follow-up. Testing set 1, n=160, 47.5% obese at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% obese at baseline, 63% at 5 yr.	Zhao 2017
	o	Serum	62 Women with morbid obesity, 30 with moderate obesity and 30 normal weight	Auguet 2016
	o	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-122-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
	o	S/P	810 Caucasian adults, 50% female, 63 yr; BMI 26, free of preexisting disease at baseline. 136 developed metabolic syndrome, 57 T2D after 10 years:1995-2005)	Willeit 2017
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
	d	Plasma	70 pregnant Caucasian women; 20 pregestational obesity, 25 gestational obesity, 25 normal pregnancies	Carreras-Badosa 2015
miR-125b	d	Plasma	300 Mexican-American women. Training set (arrays) n= 40, 47.5% obese at baseline, 72.5% at 5 yr of follow-up Testing set 1, n=160, 47.5% obese at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% obese at baseline, 63% at 5 yr.	Zhao 2017
	d	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-126	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
	o	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-126*	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-130b	d	Plasma	57 age and gender matched subjects: 40 heart-failure patients, 17 healthy controls	Thomé 2015
	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Serum	21 Chinese men with normal weight, 23 Chinese men with ow/ob	Wang YC 2013
miR-130b-3p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study); 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-136-5p	d	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
miR-138	d	Serum	13 patients with T2D, 20 patients with obesity; 16 patients with obesity+T2D, 20 controls	Pescador 2013
miR-140-3p	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with	Wang R

			obesity	2015
miR-140-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-142	o	Plasma	300 Mexican-American women.. Training set (arrays) n= 40, 47.5% obese at baseline, 72.5% at 5 yr of follow-up. Testing set 1, n=160, 47.5% obese at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% obese at baseline, 63% at 5 yr.	Zhao 2017
miR-142-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-142-3p	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-143-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-144*	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-146a	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-146a-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-146b-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-148a	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-148a-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
miR-148b	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-148b-3p	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-150	d	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-151-3p	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-151-5p	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-151a-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-151a-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-152	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
miR-155-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-181a-2-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-181b-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-181c-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-186	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-190a	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-191-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-192-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-193a-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-194-5p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-195	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-197-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-199a-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-199a-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-200c-3p	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-203a	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-205-5p	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-206	d	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016

miR-210-3p	o	Plasma	Pregnant women, 36 with GDM , 80 normal controls	Wander 2017
miR-214-5p	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-221	d	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: , 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-222	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-222-3p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-223-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	o	Plasma	10 healthy children; 20 children with obesity	Thompson 2017
miR-223-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-223	o	Serum	57 adults, Lean vs obesity groups ; healthy or with prediabetes or T2D	Nunez-Lopez 2016
	d	Serum	41 Asian adults with normal-weight; 40 with overweight; 40 with obesity	Wen 2015
miR-223*	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-301a	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-301b-3p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-302c-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-320a	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
	d	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-320b	d	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-324-3p	d	Plasma	70 pregnant Caucasian women; 20 pregestational obesity, 25 gestational obesity, 25 normal pregnancies	Carreras-Badosa 2015
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-328	d	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-331-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-335	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-338-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-342-3p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-345-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-361-3p	d	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
miR-361-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-363	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-365a-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-375	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	d	Plasma	70 pregnant Caucasian women; 20 pregestational obesity, 25 gestational obesity, 25 normal pregnancies	Carreras-Badosa 2015
miR-376a	d	Serum	13 patients with T2D, 20 patients with obesity; 16 patients with obesity+T2D, 20 controls	Pescador 2013
miR-378a-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-378a-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-379-3p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-423-5p	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-424-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-425-3p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-425-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018

miR-431-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-483-5p	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-486-5p	o	Serum	9 children with obesity and 9 controls (discovery); 352 children with obesity, 106 with overweight, 146 controls (cross-sectional validation study). 101 adults with T2D and 82 controls (longitudinal validation study)	Cui X 2018
	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-486-3p	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
miR-494	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-503	d	Serum	13 patients with T2D, 20 patients with obesity; 16 patients with obesity+T2D, 20 controls	Pescador 2013
miR-505-3p	o	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-519d	o	Plasma	300 Mexican-American women. Training set (arrays) n= 40, 47.5% obese at baseline, 72.5% at 5 yr of follow-up Testing set 1, n=160, 47.5% obese at baseline, 54.4% at 5 yr. Testing set 2: n=100, 57% obese at baseline, 63% at 5 yr.	Zhao 2017
miR-520c-3p	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-532-5p	o	Plasma	Discovery study: 10 Caucasian boys, 5 with obesity. Cross-sectional validation study: 85 control and 40 with obesity. Longitudinal evaluation: same children, 23 boys and 22 girls, lean at baseline	Prats-Puig 2013
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-550a	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-574-3p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-574-5p	o	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-582-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-590-5p	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-616-5p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-625	o	Plasma	70 pregnant Caucasian women; 20 pregestational obesity, 25 gestational obesity, 25 normal pregnancies	Carreras-Badosa 2015
	d	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-636	o	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
	o	Plasma	Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
	d	Plasma	*in morbid obesity vs control. Array: 32 white men (12 control; 12 with obesity, 8 with morbid obesity). RT-qPCR: Replication in 80 patients: white men: 49 control; 19 with obesity; 12 with morbid obesity. Longitudinal study: 5 men, 17 women treated with surgery and 9 treated with diet 5 men, 4 woman.	Ortega 2013
miR-651	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-652	d	Plasma	70 pregnant Caucasian women; 20 pregestational obesity, 25 gestational obesity, 25 normal pregnancies	Carreras-Badosa 2015
miR-654-5p	d	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-660-5p	d	Serum	12 Italian children with obesity, 6 with insulin resistance, 6 with insulin sensitivity	Masotti 2017
miR-664b-3p	o	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017
miR-671-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-744-5p	d	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-877-5p	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-933	o	Serum	Chinese adults. Arrays: 56 patients with obesity and 56 control. RT-qPCR Validation: 107 control and 123 subjects with obesity	Wang R 2015
miR-1231	d	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
miR-1537	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-1539	o	Serum	12 women with PCOS, 12 control women, 12 men	Murri 2018
miR-2355-5p	o	Plasma	20 Italian children, 10 with normal weight, 10 ob/ow	Iacomino 2016
miR-4446-3p	d	Plasma	FHS Offspring Cohort, n=2317, Validation youth cohort: n= 90 ob/ow. Initial study RNAseq: n=40, 68.8 yr, BMI 28.2, 50% female, 50% with CVD	Shah 2017

o= overexpressed

d= underexpressed

Supplemental Material 3. Results from bioinformatic analyses. The results from

I: DIANA miRPath v. 3.0 – with TarBase, TargetScan and micro-T CDS-

II: KEGG Pathway summary report in miRSystem v.2016

III: miRNAs and predicted target genes in PI3k / Akt and fatty acid metabolism in KEGG and Reactome. miRSystem v.2016

I: DIANA miRPath v. 3.0 – with TarBase, TargetScan and micro-T CDS

a) DIANA miRPath. KEGG pathways, overexpressed miRNAs. Tarbase. FDR p 0.05, conservative stats.

KEGG pathway	p-value	#genes	#miRNAs
MicroRNAs in cancer	6.0800430848E-053	139	24
Proteoglycans in cancer	4.47238028384E-013	163	23
Renal cell carcinoma	9.4444158425E-009	61	23
Hepatitis B	1.43239063324E-008	117	23
Pancreatic cancer	5.69057185952E-008	61	22
Protein processing in endoplasmic reticulum	7.0057960447E-008	137	23
Cell cycle	2.46101467727E-007	107	23
Fatty acid metabolism	2.55419793394E-006	37	21
Ubiquitin mediated proteolysis	2.55419793394E-006	114	24
Pathways in cancer	0.000006714	295	24
TGF-beta signaling pathway	1.31397551801E-005	66	21
Endocytosis	1.80587561185E-005	162	24
Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate	2.02571096558E-005	17	12
Prostate cancer	2.48763679999E-005	77	22
p53 signaling pathway	2.5241332282E-005	63	23
Transcriptional misregulation in cancer	2.74535181528E-005	135	23
FoxO signaling pathway	4.78834978898E-005	113	23
Chronic myeloid leukemia	4.78834978898E-005	66	23
Small cell lung cancer	8.45151241523E-005	74	22
Adherens junction	9.67690752731E-005	62	22
Non-small cell lung cancer	9.67690752731E-005	48	22
Colorectal cancer	0.0001300855	54	23
Glycosaminoglycan biosynthesis - keratan sulfate	0.0001857917	14	12
Hippo signaling pathway	0.0004545794	110	22
Other types of O-glycan biosynthesis	0.0005215505	23	18
Prion diseases	0.0005215505	25	21
Epstein-Barr virus infection	0.0007014133	156	22
Viral carcinogenesis	0.0008359908	165	23
Glioma	0.0009771701	52	22
Endometrial cancer	0.0015250984	45	22

ErbB signaling pathway	0.0015250984	68	23
MAPK signaling pathway	0.0021346344	188	23
RNA transport	0.0022359145	129	23
mTOR signaling pathway	0.0023013499	54	22
Fatty acid degradation	0.0033484053	28	16
Oocyte meiosis	0.0033484053	84	22
Neurotrophin signaling pathway	0.0034569619	93	23
Phosphatidylinositol signaling system	0.0041427146	63	22
Acute myeloid leukemia	0.0046756119	48	22
Spliceosome	0.0047303608	95	22
Thyroid hormone signaling pathway	0.0047303608	92	22
Bacterial invasion of epithelial cells	0.0047303608	61	23
N-Glycan biosynthesis	0.0048857356	38	18
Central carbon metabolism in cancer	0.006128675	54	22
Fatty acid elongation	0.0064130254	18	16
Lysine degradation	0.0064163531	39	21
Focal adhesion	0.0092435769	155	23
Signaling pathways regulating pluripotency of stem cells	0.0096360024	104	23
Inositol phosphate metabolism	0.0115070838	51	22
Shigellosis	0.0115070838	52	23
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0.0117354742	22	16
Thyroid cancer	0.0155024708	25	22
Insulin signaling pathway	0.0170152572	106	23
Melanoma	0.0209452615	55	23
Apoptosis	0.0209554027	69	22
Progesterone-mediated oocyte maturation	0.0224035802	69	22
Sphingolipid signaling pathway	0.0299312683	87	23
Axon guidance	0.0329859637	93	22
TNF signaling pathway	0.0329859637	84	24
Pyrimidine metabolism	0.0331857043	76	22
Lysosome	0.0331857043	88	22
PI3K-Akt signaling pathway	0.0343396695	229	23
DNA replication	0.0343836113	29	15
Chagas disease (American trypanosomiasis)	0.0443312449	77	22

b) DIANA + TargetScan. Overexpressed miRNAs. Context score -0.4, FDR, conservative stats and p 0.05

KEGG pathway	**p-value**	**#genes**	**#miRNAs**
Fatty acid biosynthesis	3.55093059276E-030	3	4
Prion diseases	2.51619786355E-022	4	6
Fatty acid metabolism	3.07474808279E-015	7	8
Glycosphingolipid biosynthesis - lacto and neolacto series	0.0131221549	3	3
Mucin type O-Glycan biosynthesis	0.0181820424	6	7
Cytokine-cytokine receptor interaction	0.0181833996	31	17

PI3K-Akt signaling pathway	0.0181833996	47	22
Glycosaminoglycan biosynthesis - heparan sulfate / heparin	0.0446098753	3	6

c) DIANA + microT-CDS. Overexpressed miRNAs. MicroT Threshold 0.8

KEGG pathway	**p-value**	**#genes**	**#miRNAs**
Fatty acid biosynthesis	1.11889144973E-009	8	8
Mucin type O-Glycan biosynthesis	2.84293839002E-008	17	15
Pathways in cancer	1.15223904047E-006	199	23
Proteoglycans in cancer	1.16457575633E-006	103	23
MAPK signaling pathway	1.70916043456E-005	137	24
Signaling pathways regulating pluripotency of stem cells	1.96231012205E-005	79	24
Axon guidance	2.13652796158E-005	75	22
Thyroid hormone signaling pathway	3.212068906E-005	65	23
Rap1 signaling pathway	4.56119262949E-005	114	23
Prion diseases	0.000058103	12	14
Renal cell carcinoma	6.35216735341E-005	42	22
Ras signaling pathway	0.0001178121	114	22
Long-term depression	0.0001399584	37	20
ErbB signaling pathway	0.000297166	52	22
PI3K-Akt signaling pathway	0.000297166	169	23
Prostate cancer	0.0004776205	53	23
Glioma	0.0005583944	37	22
Hippo signaling pathway	0.0007176661	77	22
Melanoma	0.0007176661	44	22
Glutamatergic synapse	0.0007283007	60	22
Non-small cell lung cancer	0.0010426908	34	19
TGF-beta signaling pathway	0.0010426908	45	21
FoxO signaling pathway	0.0014185679	73	22
Colorectal cancer	0.0014283738	35	20
Gap junction	0.0014283738	49	22
Adherens junction	0.0014659518	44	21
Oxytocin signaling pathway	0.0017371508	83	23
Focal adhesion	0.0023912746	106	23
Neurotrophin signaling pathway	0.0026911488	66	23
Regulation of actin cytoskeleton	0.0029738217	111	22
Choline metabolism in cancer	0.0029738217	57	22
N-Glycan biosynthesis	0.0036893768	24	16
Endometrial cancer	0.0050747077	32	20
Tight junction	0.0061730605	72	23
Adrenergic signaling in cardiomyocytes	0.0070711804	71	23
p53 signaling pathway	0.0081876308	39	20
Pancreatic cancer	0.009425463	38	20
Chronic myeloid leukemia	0.0096394863	41	23
Prolactin signaling pathway	0.0110343244	39	20
Ubiquitin mediated proteolysis	0.0168482958	71	24
Sphingolipid signaling pathway	0.0257373139	61	22

Wnt signaling pathway	0.0335578825	69	22
ECM-receptor interaction	0.0377411885	38	20
Endocytosis	0.0377411885	98	22
mTOR signaling pathway	0.0395918749	35	20

d) Diana + Tarbase. KEGG. Underexpressed miRNAs.

KEGG pathway	**p-value**	**#genes**	**#miRNAs**
MicroRNAs in cancer	1.04218336742E-061	104	11
Proteoglycans in cancer	1.91745701273E-011	104	11
Fatty acid metabolism	3.64806861495E-008	22	10
Lysine degradation	1.36782648056E-007	29	11
Protein processing in endoplasmic reticulum	4.37410913424E-007	88	11
Colorectal cancer	7.22137750536E-007	39	11
Cell cycle	1.15136074135E-006	67	11
Adherens junction	1.25206305599E-006	41	11
Fatty acid biosynthesis	1.95739861294E-006	6	8
Hepatitis B	2.78125145325E-006	72	11
Hippo signaling pathway	2.93793121997E-006	65	11
FoxO signaling pathway	9.30614264531E-006	73	11
Pancreatic cancer	1.02678918883E-005	41	11
Glioma	1.17652567901E-005	36	11
Prostate cancer	2.93598021567E-005	52	11
Endometrial cancer	3.67443525449E-005	32	11
Central carbon metabolism in cancer	3.67443525449E-005	36	11
Viral carcinogenesis	5.12394914699E-005	93	11
Spliceosome	9.19036992581E-005	64	11
Thyroid hormone signaling pathway	9.19036992581E-005	58	11
Neurotrophin signaling pathway	0.000101363	63	11
Ubiquitin mediated proteolysis	0.0001119049	72	11
Estrogen signaling pathway	0.0001120435	50	11
Chronic myeloid leukemia	0.0001462347	41	11
Non-small cell lung cancer	0.0001472632	32	11
p53 signaling pathway	0.0001850886	41	11
Renal cell carcinoma	0.0006005235	37	11
Biosynthesis of unsaturated fatty acids	0.0009605068	12	9
TGF-beta signaling pathway	0.0011070787	39	11
ErbB signaling pathway	0.001211141	42	11
RNA transport	0.001548655	78	11
Pathways in cancer	0.001548655	163	11
Regulation of actin cytoskeleton	0.0030158197	91	11
Oocyte meiosis	0.0034169746	52	11
Phosphatidylinositol signaling system	0.0034169746	41	11
Focal adhesion	0.0034169746	94	11
Small cell lung cancer	0.0050852206	43	11
Thyroid cancer	0.0065945316	16	11
mRNA surveillance pathway	0.0080242407	44	11
TNF signaling pathway	0.0080242407	54	11
Epstein-Barr virus infection	0.0087088388	93	11

Bladder cancer	0.0096383971	23	11
Progesterone-mediated oocyte maturation	0.0106482898	43	11
Bacterial invasion of epithelial cells	0.0106482898	36	11
mTOR signaling pathway	0.0108534042	34	11
Prolactin signaling pathway	0.0134001283	34	11
AMPK signaling pathway	0.0135419823	57	11
Fatty acid elongation	0.0160639187	11	8
HIF-1 signaling pathway	0.0226623394	49	11
Choline metabolism in cancer	0.025074804	46	11
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.0345817991	24	11
PI3K-Akt signaling pathway	0.0368458226	127	11
Endocytosis	0.0410506425	84	11
Wnt signaling pathway	0.0410506425	59	11
HTLV-I infection	0.0423373556	106	11
MAPK signaling pathway	0.0437025212	101	11
Insulin signaling pathway	0.0450318224	61	11
Signaling pathways regulating pluripotency of stem cells	0.0478400082	59	11
Melanoma	0.0478400082	32	11

e) DIANA + Targetscan. Underexpressed microRNAs. Context score -0.4

KEGG pathway	**p-value**	**#genes**	**#miRNAs**
Fatty acid biosynthesis	<1E-325	2	2
ECM-receptor interaction	<1E-325	19	5
Mucin type O-Glycan biosynthesis	3.109805E-006	1	2
Fatty acid metabolism	4.098023E-005	6	2
Glycosphingolipid biosynthesis - lacto and neolacto series	0.008277265	1	1
Proteoglycans in cancer	0.008487167	38	2
Steroid hormone biosynthesis	0.01982273	1	2
Adherens junction	0.03674103	14	3

f) DIANA + microT-CDS. Underexpressed microRNAs. MicroT Threshold 0.8

KEGG pathway	**p-value**	**#genes**	**#miRNAs**
Mucin type O-Glycan biosynthesis	2.07137645133E-005	8	6
FoxO signaling pathway	0.0005386679	38	8
Neurotrophin signaling pathway	0.0005386679	37	10
MAPK signaling pathway	0.0005386679	64	11
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.0331443028	21	5
Axon guidance	0.0331443028	33	7
Long-term depression	0.0331443028	17	7
Amphetamine addiction	0.0331443028	16	7
N-Glycan biosynthesis	0.0331443028	12	8
B cell receptor signaling pathway	0.0331443028	22	9
Non-small cell lung cancer	0.0392900667	16	8
Ras signaling pathway	0.0392900667	46	10

Hippo signaling pathway	0.0408059962	25	7
Ubiquitin mediated proteolysis	0.0408059962	35	8
Glioma	0.0408059962	16	8
Prostate cancer	0.0408059962	22	8
Signaling pathways regulating pluripotency of stem cells	0.0408059962	33	10
Regulation of actin cytoskeleton	0.0412588654	47	8

II: KEGG Pathway summary report in miRSystem v.2016

a) MiRSystem. Overexpressed miRNAs. KEGG Pathway ranking summary report.

KEGG pathway	Total Genes Of The Term	Union Targets In The Term	miRs In The Term	Score
Pathways_In_Cancer	325	145	22	3.487
Axon_Guidance	129	68	20	2.682
MAPK_Signaling_Pathway	272	114	21	2.636
Focal_Adhesion	199	78	20	2.196
Prostate_Cancer	89	45	20	2.166
Neurotrophin_Signaling_Pathway	127	66	20	2.105
Melanoma	71	38	21	2.036
Wnt_Signaling_Pathway	150	73	21	2.033
Pancreatic_Cancer	70	39	21	1.923
Renal_Cell_Carcinoma	70	38	17	1.908
Glioma	65	33	21	1.884
TGF-Beta_Signaling_Pathway	84	44	19	1.873
Chronic_Myeloid_Leukemia	73	40	22	1.868
Regulation_Of_Actin_Cytoskeleton	213	89	21	1.849
Endocytosis	201	83	20	1.832
Ubiquitin_Mediated_Proteolysis	135	53	19	1.632
Erbb_Signaling_Pathway	87	43	19	1.628
T_Cell_Receptor_Signaling_Pathway	108	48	20	1.569
Insulin_Signaling_Pathway	137	52	19	1.567
Small_Cell_Lung_Cancer	84	36	20	1.531
Adherens_Junction	73	36	18	1.443
Non-Small_Cell_Lung_Cancer	54	29	21	1.39
mTOR_Signaling_Pathway	52	23	18	1.349
Colorectal_Cancer	62	33	18	1.33
P53_Signaling_Pathway	68	33	18	1.324
Cell_Cycle	124	47	21	1.316
Progesterone-Mediated_Oocyte_Maturation	86	34	20	1.308
Oocyte_Meiosis	112	45	19	1.305
Long-Term_Potentiation	70	33	17	1.253
Fc_Gamma_R-Mediated_Phagocytosis	94	40	19	1.22
Chagas_Disease	104	43	18	1.185
Vegf_Signaling_Pathway	76	29	17	1.173
Aldosterone-Regulated_Sodium_Reabsorption	42	21	17	1.168
Melanogenesis	101	45	18	1.16
Cytokine-Cytokine_Receptor_Interaction	275	61	21	1.114
Jak-Stat_Signaling_Pathway	155	45	21	1.105
Acute_Myeloid_Leukemia	57	27	18	1.104
Chemokine_Signaling_Pathway	189	63	19	1.09
Protein_Processing_In_Endoplasmic_Reticulum	166	46	17	1.069

Gap_Junction	90	35	16	1.048
B_Cell_Receptor_Signaling_Pathway	75	27	17	1.035
Calcium_Signaling_Pathway	177	46	20	0.993
Adipocytokine_Signaling_Pathway	68	23	15	0.974
Bladder_Cancer	42	21	16	0.973
Phosphatidylinositol_Signaling_System	78	29	17	0.97
Tight_Junction	132	51	18	0.968
Amyotrophic_Lateral_Sclerosis_(Als)	54	23	16	0.939
Bacterial_Invasion_Of_Epithelial_Cells	70	24	19	0.931
Dilated_Cardiomyopathy	90	36	16	0.928
Gastric_Acid_Secretion	74	30	16	0.922
Type_Ii_Diabetes_Mellitus	47	19	17	0.92
Endometrial_Cancer	52	22	15	0.91
Apoptosis	88	31	20	0.906
Shigellosis	61	26	17	0.89
Toxoplasmosis	132	38	18	0.89
Hypertrophic_Cardiomyopathy_(Hcm)	87	31	17	0.851
Vascular_Smooth_Muscle_Contraction	126	39	18	0.827
Leukocyte_Transendothelial_Migration	116	34	19	0.825
Notch_Signaling_Pathway	47	20	15	0.815
Gnrh_Signaling_Pathway	101	37	16	0.805
Fc_Epsilon_Ri_Signaling_Pathway	79	29	16	0.803
Hepatitis_C	134	39	19	0.794
Salivary_Secretion	89	28	14	0.79
Toll-Like_Receptor_Signaling_Pathway	102	28	16	0.78
Pancreatic_Secretion	103	27	15	0.763
Long-Term_Depression	70	25	15	0.755
Olfactory_Transduction	388	7	8	0.753
Arrhythmogenic_Right_Ventricular_Cardiomyopathy_(Arvc)	74	27	14	0.731
Huntington's_Disease	183	37	20	0.729
Hedgehog_Signaling_Pathway	56	22	12	0.722
Cell_Adhesion_Molecules_(Cams)	133	30	17	0.69
Natural_Killer_Cell_Mediated_Cytotoxicity	140	28	16	0.667
Amoebiasis	105	30	17	0.666
Inositol_Phosphate_Metabolism	57	19	12	0.654
Neuroactive_Ligand-Receptor_Interaction	318	46	16	0.644
Ecm-Receptor_Interaction	84	25	15	0.633
Snare_Interactions_In_Vesicular_Transport	36	16	11	0.632
Epithelial_Cell_Signaling_In_Helicobacter_Pylori_Infection	68	22	16	0.6
Thyroid_Cancer	29	12	11	0.599
Viral_Myocarditis	70	19	11	0.586
Vasopressin-Regulated_Water_Reabsorption	44	17	12	0.582
Basal_Cell_Carcinoma	55	21	16	0.58
Rna_Transport	144	30	17	0.551
Spliceosome	127	22	15	0.537
Nod-Like_Receptor_Signaling_Pathway	62	16	16	0.524
Carbohydrate_Digestion_And_Absorption	43	11	15	0.522

Protein_Digestion_And_Absorption	80	20	15	0.494
Rna_Degradation	57	12	12	0.487
Lysosome	121	24	14	0.482
Alzheimer's_Disease	168	34	15	0.481
Basal_Transcription_Factors	35	12	13	0.468
Phagosome	154	29	15	0.45
Cardiac_Muscle_Contraction	77	19	15	0.449
Purine_Metabolism	161	23	15	0.448
Rig-I-Like_Receptor_Signaling_Pathway	71	19	14	0.444
N-Glycan_Biosynthesis	49	14	10	0.429
Prion_Diseases	36	11	10	0.414
O-Glycan_Biosynthesis	30	10	9	0.412
Glycosaminoglycan_Biosynthesis_Heparan_Sulfate	26	7	6	0.404
Leishmaniasis	72	17	12	0.396
Glycosphingolipid_Biosynthesis_Lacto_And_Neolacto_Series	26	8	7	0.366
Hematopoietic_Cell_Lineage	88	18	12	0.356
PPAR_Signaling_Pathway	70	13	11	0.353
Parkinson's_Disease	130	14	13	0.349
Peroxisome	79	13	12	0.349
Vibrio_Cholerae_Infection	54	14	9	0.339
Nucleotide_Excision_Repair	44	9	9	0.302
Pathogenic_Escherichia_Coli_Infection	56	12	10	0.301
Type_I_Diabetes_Mellitus	43	9	7	0.3
Sphingolipid_Metabolism	40	12	10	0.299
Regulation_Of_Autophagy	34	7	8	0.293
Cysteine_And_Methionine_Metabolism	36	7	11	0.288
Abc_Transporters	44	7	9	0.265
Allograft_Rejection	37	8	6	0.265
Systemic_Lupus_Erythematosus	136	9	8	0.264
Maturity_Onset_Diabetes_Of_The_Young	25	7	6	0.257
Malaria	51	8	9	0.255
Lysine_Degradation	44	10	10	0.251
Graft-Versus-Host_Disease	41	7	6	0.25
Intestinal_Immune_Network_For_Iga_Production	48	7	7	0.244
Pyrimidine_Metabolism	99	9	8	0.224
Antigen_Processing_And_Presentation	76	11	8	0.214
Glycerophospholipid_Metabolism	79	14	6	0.214
Citrate_Cycle_(Tca_Cycle)	31	5	7	0.205
Galactose_Metabolism	26	4	7	0.205
Fructose_And_Mannose_Metabolism	34	7	8	0.202
Selenoamino_Acid_Metabolism	26	3	9	0.195
Fatty_Acid_Metabolism	43	6	6	0.19
Collecting_Duct_Acid_Secretion	27	5	4	0.171
Autoimmune_Thyroid_Disease	52	5	4	0.164
Glycerolipid_Metabolism	49	7	6	0.16
Oxidative_Phosphorylation	132	9	6	0.16
Starch_And_Sucrose_Metabolism	53	4	7	0.153
Amino_Sugar_And_Nucleotide_Sugar_Metabolism	47	7	6	0.149

Complement_And_Coagulation_Cascades	69	5	6	0.139
Taste_Transduction	52	5	6	0.139
Dna_Replication	36	5	5	0.133
Pentose_Phosphate_Pathway	26	3	4	0.133
Homologous_Recombination	28	4	3	0.125
Arginine_And_Proline_Metabolism	54	8	5	0.123
Base_Excision_Repair	33	4	4	0.119
Glycolysis_Gluconeogenesis	65	4	5	0.116
Retinol_Metabolism	65	5	4	0.107
Cytosolic_Dna-Sensing_Pathway	56	4	5	0.096
Proteasome	44	4	4	0.096
Ether_Lipid_Metabolism	35	4	2	0.087
Alanine_Aspartate_And_Glutamate_Metabolism	32	5	3	0.085
Valine_Leucine_And_Isoleucine_Degradation	44	3	3	0.075
Phototransduction	29	2	3	0.073
Glutathione_Metabolism	50	3	3	0.067
Pyruvate_Metabolism	41	3	3	0.062
Propanoate_Metabolism	32	2	2	0.059
Staphylococcus_Aureus_Infection	55	2	2	0.047
RNA_Polymerase	29	2	2	0.04
Glycosylphosphatidylinositol(Gpi)-Anchor_Biosynthesis	25	1	2	0.037
Asthma	30	2	2	0.036
Butanoate_Metabolism	30	1	1	0.032
Glycine_Serine_And_Threonine_Metabolism	32	2	1	0.029
Drug_Metabolism_Other_Enzymes	52	2	1	0.026
Primary_Immunodeficiency	35	1	1	0.025
Aminoacyl-Trna_Biosynthesis	63	1	1	0.019
Steroid_Hormone_Biosynthesis	56	1	1	0.019
Arachidonic_Acid_Metabolism	57	1	1	0.018

b) MiRSystem. Underexpressed miRNAs. KEGG Pathway ranking summary report.

KEGG Pathways	**Total_genes_of_the_term**	**Union_targets_in_the_term**	**miRs_in_the_term**	**Score**
Axon_guidance	129	39	8	3.215
Neurotrophin_signaling_pathway	127	32	9	2.002
Ubiquitin_mediated_proteolysis	135	29	8	1.972
TGF-beta_signaling_pathway	84	27	7	1.956
MAPK_signaling_pathway	272	54	9	1.946
Pathways_in_cancer	325	62	9	1.89
Endocytosis	201	43	8	1.758
Protein_processing_in_endoplasmic_reticulum	166	30	9	1.691
Pancreatic_cancer	70	21	8	1.647
Cytokine-cytokine_receptor_interaction	275	36	7	1.597
Amyotrophic_lateral_sclerosis_(als)	54	19	7	1.587
Chronic_myeloid_leukemia	73	21	9	1.581

Erbb_signaling_pathway	87	25	10	1.553
Regulation_of_actin_cytoskeleton	213	39	9	1.525
Jak-stat_signaling_pathway	155	22	9	1.403
Bacterial_invasion_of_epithelial_cells	70	19	8	1.388
Prostate_cancer	89	22	8	1.354
Glioma	65	19	8	1.315
Colorectal_cancer	62	16	7	1.225
Melanoma	71	18	7	1.196
Long-term_potentiation	70	19	6	1.194
Insulin_signaling_pathway	137	28	10	1.192
Focal_adhesion	199	33	10	1.162
Adherens_junction	73	19	8	1.144
Shigellosis	61	15	7	1.118
Olfactory_transduction	388	3	3	1.117
T_cell_receptor_signaling_pathway	108	21	7	1.105
Non-small_cell_lung_cancer	54	14	8	1.086
Chemokine_signaling_pathway	189	26	10	1.048
Chagas_disease	104	20	7	1.045
Renal_cell_carcinoma	70	18	8	1.041
Small_cell_lung_cancer	84	14	7	1.03
Toxoplasmosis	132	22	8	1.014
Oocyte_meiosis	112	24	6	1.01
Wnt_signaling_pathway	150	29	7	1.009
Apoptosis	88	17	7	1.001
p53_signaling_pathway	68	17	5	0.996
Dilated_cardiomyopathy	90	19	8	0.989
Calcium_signaling_pathway	177	26	8	0.954
Notch_signaling_pathway	47	12	7	0.941
Type_II_diabetes_mellitus	47	11	8	0.921
Progesterone-mediated_oocyte_maturation	86	17	7	0.914
B_cell_receptor_signaling_pathway	75	11	6	0.91
Cell_cycle	124	21	7	0.909
Arrhythmogenic_right_ventricular_cardiomyopathy_(arvc)	74	12	7	0.882
O-glycan_biosynthesis	30	8	6	0.853
VEGF_signaling_pathway	76	12	7	0.849
Phosphatidylinositol_signaling_system	78	16	7	0.843
Hypertrophic_cardiomyopathy_(hcm)	87	16	8	0.827
N-glycan_biosynthesis	49	10	6	0.813
mTOR_signaling_pathway	52	13	7	0.808
Aldosterone-regulated_sodium_reabsorption	42	11	7	0.789
Natural_killer_cell_mediated_cytotoxicity	140	15	7	0.773
Adipocytokine_signaling_pathway	68	14	7	0.768
Amoebiasis	105	14	8	0.752
Fc_gamma_r-mediated_phagocytosis	94	14	7	0.711
Bladder_cancer	42	9	7	0.694
Hepatitis_c	134	20	7	0.672
Inositol_phosphate_metabolism	57	11	6	0.636
Endometrial_cancer	52	9	8	0.632

Melanogenesis	101	17	5	0.621
Rig-i-like_receptor_signaling_pathway	71	10	7	0.616
Spliceosome	127	11	7	0.592
Gap_junction	90	14	4	0.591
Vasopressin-regulated_water_reabsorption	44	8	7	0.573
Glycosphingolipid_biosynthesis_lacto_and_neolacto_series	26	5	5	0.564
Neuroactive_ligand-receptor_interaction	318	22	7	0.559
Viral_myocarditis	70	9	5	0.551
GNRH_signaling_pathway	101	13	6	0.544
Huntington's_disease	183	21	6	0.543
Toll-like_receptor_signaling_pathway	102	9	7	0.54
Vascular_smooth_muscle_contraction	126	14	7	0.533
Hedgehog_signaling_pathway	56	11	5	0.529
Prion_diseases	36	7	5	0.529
Leukocyte_transendothelial_migration	116	11	7	0.514
Acute_myeloid_leukemia	57	8	7	0.5
RNA_degradation	57	8	6	0.499
RNA_transport	144	12	6	0.497
Fc_epsilon_RI_signaling_pathway	79	11	7	0.495
Pancreatic_secretion	103	12	6	0.492
Tight_junction	132	15	6	0.46
Basal_cell_carcinoma	55	9	5	0.452
ECM-receptor_interaction	84	8	5	0.448
Long-term_depression	70	11	4	0.442
Alzheimer's_disease	168	17	7	0.44
Maturity_onset_diabetes_of_the_young	25	4	3	0.418
Salivary_secretion	89	9	4	0.416
ABC_transporters	44	5	5	0.414
Cell_adhesion_molecules_(cams)	133	12	5	0.405
Lysosome	121	11	5	0.404
Phagosome	154	14	6	0.403
Nod-like_receptor_signaling_pathway	62	7	7	0.394
Type_I_diabetes_mellitus	43	5	5	0.377
Purine_metabolism	161	13	5	0.373
Epithelial_cell_signaling_in_helicobacter_pylori_infection	68	7	6	0.358
Basal_transcription_factors	35	4	5	0.342
Starch_and_sucrose_metabolism	53	7	4	0.341
Galactose_metabolism	26	3	5	0.336
Gastric_acid_secretion	74	8	4	0.335
Cardiac_muscle_contraction	77	6	6	0.328
Leishmaniasis	72	7	6	0.328
Lysine_degradation	44	6	4	0.325
Thyroid_cancer	29	5	4	0.317
Allograft_rejection	37	4	3	0.312
Hematopoietic_cell_lineage	88	9	5	0.312
Malaria	51	5	5	0.31
Parkinson's_disease	130	7	5	0.306

Antigen_processing_and_presentation	76	8	5	0.304
Carbohydrate_digestion_and_absorption	43	3	5	0.274
Pyrimidine_metabolism	99	4	4	0.274
Retinol_metabolism	65	5	2	0.271
Peroxisome	79	5	5	0.27
Cysteine_and_methionine_metabolism	36	5	5	0.254
Graft-versus-host_disease	41	4	4	0.237
Regulation_of_autophagy	34	5	4	0.237
PPAR_signaling_pathway	70	5	4	0.235
Protein_digestion_and_absorption	80	4	4	0.235
Glycerophospholipid_metabolism	79	6	4	0.226
Nucleotide_excision_repair	44	3	4	0.222
Pentose_phosphate_pathway	26	2	4	0.219
Glycosaminoglycan_biosynthesis_heparan_sulfate	26	3	3	0.21
Glycolysis_gluconeogenesis	65	2	4	0.208
Amino_sugar_and_nucleotide_sugar_metabolism	47	4	3	0.194
Ascorbate_and_aldarate_metabolism	26	3	1	0.19
Snare_interactions_in_vesicular_transport	36	4	3	0.187
Drug_metabolism_other_enzymes	52	5	2	0.179
Pathogenic_escherichia_coli_infection	56	4	3	0.179
Glutathione_metabolism	50	4	3	0.177
Steroid_hormone_biosynthesis	56	4	1	0.175
Pentose_and_glucuronate_interconversions	31	3	1	0.171
Selenoamino_acid_metabolism	26	3	4	0.169
Fructose_and_mannose_metabolism	34	2	3	0.165
Sphingolipid_metabolism	40	3	4	0.163
Complement_and_coagulation_cascades	69	2	3	0.16
Arginine_and_proline_metabolism	54	3	3	0.151
Systemic_lupus_erythematosus	136	4	2	0.151
Metabolism_of_xenobiotics_by_cytochrome_p450	71	4	1	0.147
Glycerolipid_metabolism	49	2	3	0.144
Porphyrin_and_chlorophyll_metabolism	43	3	1	0.139
Intestinal_immune_network_for_iga_production	48	3	3	0.127
Glycine_serine_and_threonine_metabolism	32	2	2	0.125
Alanine_aspartate_and_glutamate_metabolism	32	2	3	0.123
Glycosylphosphatidylinositol(gpi)-anchor_biosynthesis	25	1	3	0.12
Oxidative_phosphorylation	132	4	2	0.115
Proteasome	44	2	2	0.107
Homologous_recombination	28	2	2	0.102
Autoimmune_thyroid_disease	52	2	1	0.1
Drug_metabolism_cytochrome_p450	73	3	1	0.095
*Vibrio_cholerae*_infection	54	3	1	0.087
Collecting_duct_acid_secretion	27	2	1	0.084
Ether_lipid_metabolism	35	1	2	0.083
Pyruvate_metabolism	41	1	2	0.083
Taste_transduction	52	2	2	0.08
Base_excision_repair	33	1	2	0.078
Citrate_cycle_(tca_cycle)	31	1	2	0.078

Fatty_acid_metabolism	43	2	1	0.063
Primary_immunodeficiency	35	1	1	0.044
Tryptophan_metabolism	42	1	1	0.041
Tyrosine_metabolism	41	1	1	0.041
Asthma	30	1	1	0.04
Phototransduction	29	1	1	0.04
Arachidonic_acid_metabolism	57	1	1	0.039
DNA_replication	36	1	1	0.039
*Staphylococcus_aureus*_infection	55	1	1	0.039

III: miRNAs and predicted target genes in pI3k / Akt and fatty acid metabolism in KEGG and Reactome. MiRSystem v.2016

REACTOME. PI3K-AKT ACTIVATION. 19 miRNAs TARGET 19 GENES FROM 37 TOTAL GENES IN THE PATHWAY. SCORE 1.371

Target Gene	**Gene Description**	**Observed miRNA**
PTEN	phosphatase and tensin homolog	130b-3p, 142-5p, 148a-3p, 21-5p, 23a-3p, 26b-5p, 320a, 486-5p
IRS1	insulin receptor substrate 1	126-3p, 142-5p, 148a-3p, 15b-5p, 223-3p, 30d-5p
RICTOR	RPTOR independent companion of MTOR, complex 2	142-5p, 148a-3p, 15b-5p, 192-5p, 342-3p, 636
CHUK	conserved helix-loop-helix ubiquitous kinase	130b-3p, 148a-3p, 15b-5p, 223-3p, 23a-3p
AKT3	v-akt murine thymoma viral oncogene homolog 3	122-5p, 15b-5p, 320a, 34a-5p
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	148a-3p, 222-3p, 24-3p, 34a-5p
FOXO1	forkhead box O1	15b-5p, 223-3p, 320a, 486-5p
FOXO3	forkhead box O3	122-5p, 223-3p, 23a-3p, 30d-5p
IRS2	insulin receptor substrate 2	142-5p, 15b-5p, 23a-3p, 30d-5p
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	15b-5p, 21-5p, 222-3p, 486-5p
CREB1	cAMP responsive element binding protein 1	122-5p, 223-3p, 30d-5p
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	130b-3p, 21-5p
FOXO4	forkhead box O4	23a-3p, 24-3p
PDPK1	3-phosphoinositide dependent protein kinase-1	223-3p, 23a-3p
PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (beta)	126-3p, 30d-5p
GSK3B	glycogen synthase kinase 3 beta	26b-5p
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	148a-3p
RHOA	ras homolog family member A	142-5p
TRIB3	tribbles pseudokinase 3	24-3p

KEGG:INOSITOL_PHOSPHATE_METABOLISM. 18 miRNAs target 25 genes of 57 total in the pathway. Score 0.893

Target Gene	Gene Description	Observed miRNA
PTEN	phosphatase and tensin homolog	130b-3p, 142-5p, 148a-3p, 21-5p, 23a-3p, 26b-5p, 320a, 486-5p
PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)	122-5p, 130b-3p, 148a-3p, 21-5p, 26b-5p, 34a-5p, 636
SYNJ1	synaptojanin 1	142-5p, 146a-5p, 148a-3p, 15b-5p, 23a-3p, 34a-5p
PIP5K3	Phosphatidylinositol 4-phosphate 5-kinase 3	130b-3p, 15b-5p, 21-5p, 23a-3p, 26b-5p
INPP5B	inositol polyphosphate-5-phosphatase, 75kDa	223-3p, 24-3p, 26b-5p
ITPK1	inositol-tetrakisphosphate 1-kinase	130b-3p, 148a-3p, 30d-5p
OCRL	oculocerebrorenal syndrome of Lowe	122-5p, 130b-3p, 15b-5p
PIP4K2B	phosphatidylinositol-5-phosphate 4-kinase, type II, beta	146a-5p, 23a-3p, 30d-5p
PIP5K1B	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	142-5p, 146a-5p, 30d-5p
INPP5A	inositol polyphosphate-5-phosphatase, 40kDa	197-3p, 23a-3p
PI4KB	phosphatidylinositol 4-kinase, catalytic, beta	15b-5p, 34a-5p
PIP5K1A	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	23a-3p, 34a-5p
PLCB4	phospholipase C, beta 4	23a-3p, 636
PLCG1	phospholipase C, gamma 1	30d-5p, 34a-5p
INPP5J	inositol polyphosphate-5-phosphatase J	15b-5p
IPPK	inositol 1,3,4,5,6-pentakisphosphate 2-kinase	23a-3p
ITPKB	inositol-trisphosphate 3-kinase B	130b-3p
PI4KA	phosphatidylinositol 4-kinase, catalytic, alpha	148a-3p
PIK3C2B	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 beta	30d-5p
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	148a-3p
PIK3CD	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta	30d-5p
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	122-5p
PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	30d-5p
PIP4K2C	phosphatidylinositol-5-phosphate 4-kinase, type II, gamma	142-5p
PLCD1	phospholipase C, delta 1	191-5p

KEGG Fatty acid metabolism. 8 miRNAs target 8 genes from 43 genes total in the pathway. Score 0.229

Target Gene	Gene Description	Observed miRNA

ACSL1	acyl-CoA synthetase long-chain family member 1	130b-3p, 15b-5p, 26b-5p, 34a-5p, 636
ACSL3	acyl-CoA synthetase long-chain family member 3	15b-5p, 223-3p, 26b-5p
ACSL4	acyl-CoA synthetase long-chain family member 4	130b-3p, 15b-5p, 34a-5p
ACADSB	acyl-CoA dehydrogenase, short/branched chain	26b-5p
ACOX1	acyl-CoA oxidase 1, palmitoyl	15b-5p
ACSL5	acyl-CoA synthetase long-chain family member 5	15b-5p
ACSL6	acyl-CoA synthetase long-chain family member 6	24-3p
ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide	23a-3p

UNDEREXPRESSED>REACTOME. PI3K-AKT ACTIVATION. 9 miRNAs TARGET 14 GENES FROM 37 TOTAL GENES IN THE PATHWAY. SCORE 0.91

Target Gene	**Gene Description**	**Observed miRNA**
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	21-5p, 324-3p, 590-5p
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	130b-3p, 21-5p
CREB1	cAMP responsive element binding protein 1	30b-5p, 30c-5p
FOXO3	forkhead box O3	30b-5p, 30c-5p
IRS1	insulin receptor substrate 1	30b-5p, 30c-5p
IRS2	insulin receptor substrate 2	30b-5p, 30c-5p
PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (beta)	30b-5p, 30c-5p
PTEN	phosphatase and tensin homolog	130b-3p, 21-5p
AKT3	v-akt murine thymoma viral oncogene homolog 3	151a-3p
CHUK	conserved helix-loop-helix ubiquitous kinase	130b-3p
FOXO1	forkhead box O1	590-5p
GSK3B	glycogen synthase kinase 3 beta	199a-5p
PHLPP	pleckstrin homology domain leucine-rich repeat protein p	331-3p
RICTOR	RPTOR independent companion of MTOR, complex 2	324-3p

UNDEREXPRESSED
REACTOME:FATTY_ACID_TRIACYLGLYCEROL_AND_KETONE_BODY_METABOLISM. 9 mir regulates 22 genes from 112 total genes in the pathway.

Target Gene	**Gene Description**	**Observed miRNA**
CHD9	chromodomain helicase DNA binding protein 9	130b-3p, 30b-5p, 30c-5p
GRHL1	grainyhead-like 1 (Drosophila)	125b-5p, 30b-5p, 30c-5p
NCOR2	nuclear receptor corepressor 2	125b-5p, 30b-5p, 30c-5p
TBL1XR1	transducin (beta)-like 1 X-linked receptor 1	130b-3p, 21-5p, 590-5p
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	130b-3p, 324-3p
ELOVL5	ELOVL fatty acid elongase 5	30b-5p, 30c-5p
ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	30b-5p, 30c-5p
NCOA2	nuclear receptor coactivator 2	151a-3p, 199a-5p
PPARA	peroxisome proliferator-activated receptor alpha	21-5p, 590-5p
RGL1	ral guanine nucleotide dissociation stimulator-like 1	30b-5p, 30c-5p
TBL1X	transducin (beta)-like 1X-linked	30b-5p, 30c-5p

ACSL1	acyl-CoA synthetase long-chain family member 1	130b-3p
ACSL4	acyl-CoA synthetase long-chain family member 4	130b-3p
CTGF	connective tissue growth factor	199a-5p
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	125b-5p
ELOVL1	ELOVL fatty acid elongase 1	125b-5p
ELOVL6	ELOVL fatty acid elongase 6	125b-5p
GPD1	glycerol-3-phosphate dehydrogenase 1 (soluble)	199a-5p
LPIN2	lipin 2	21-5p
NCOA1	nuclear receptor coactivator 1	130b-3p
PRKAA2	protein kinase, AMP-activated, alpha 2 catalytic subunit	130b-3p
PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	199a-5p

KEGG. Fatty acid metabolism: mir-130b-3p targets ACSL1 and ACSL4, acyl-CoA synthetase long-chain family member 1 and 4, 2/43 genes of the pathway.

Supplemental Material 4. QUADAS

Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?			

*CD, cannot determine; NA, not applicable; NR, not reported; Y=yes, N=no

Rating: good / fair / poor

Question number	Murri 2018 (Spain)	Cui 2018 (China)	Ghorbani 2017 (Iran)	Thompson 2017 (USA)	Wander 2017 (USA)	Zhao 2017 (USA)	Xiong 2017 (China)	Shah 2017 (USA)	Willeit 2017 (Italy)	Huba l 2017 (USA)	Nunez-Lopez 2016 (USA)	Enquob ahrie 2017 (USA)	Auguet 2016 (Spain)	Iacomino 2016 (Italy)	Masotti 2017 (Italy)	Liu 2016 (China)	Pek 2016 (China)	Carreras-Badosa 2015 (Spain)	Khalyf a 2016 (USA)	Can 2016 (Turk ey)	Thomé 2015 (Brazil)	Wen 2015 (China)	Párriza s 2015 (Spain)	Wang R 2015 (China)	Pescador 2013 (Spain)	Murri 2013 (Spain)	Prats-Puig 2013 (Spain)	Wang YC 2013 (China)	Ortega 2013 (Spain)
1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD
4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	CD	Y	Y	CD	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	CD	Y
5	Y	N	N	N	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	Y	Y	N	N	Y	Y	Y	N	N	Y	N	Y
6	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
7	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	N	N	N	N	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	N	N	N	Y	Y	N	N	N	Y	N	Y
11	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
12	NR	NR	NR	NR	NR	NR	NR	NR	Y	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
13	NA	NA	NA	Y	Y	Y	NR	Y	Y	NR	NR	NR	NR	NR	NR	NR	NR	N	NA	NA	NA	Y	NR	NR	NA	NA	NR	NR	NR
14	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Rating	G	G	G	G	G	F	G	G	G	P	G	P	F	F	F	P	G	G	G	G	G	G	G	G	G	G	G	G	G

Good=22/29 (75.86%); Fair=4/29 (13.79%); Poor=3/29 (10.34%)

Appendix

A. Manuscript Submitted. Cover Page

A. PROSPERO Registration

PROSPERO
International prospective register of systematic reviews

NHS
National Institute for Health Research

Circulating microRNAs in obesity. A systematic review

Alejandra Ortiz-Dosal, Luis Antonio Salazar-Olivo

Citation

Alejandra Ortiz-Dosal, Luis Antonio Salazar-Olivo. Circulating microRNAs in obesity. A systematic review. PROSPERO 2017 CRD42017077742 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017077742

Review question

Which microRNAs (miRNAs) present a deregulated expression in obese/overweight individuals compared with lean controls ?

Searches

We will search in PubMed. The search strategy will include the following MeSH terms: circulating[All Fields] AND ("micromas"[MeSH Terms] OR "micromas"[All Fields] OR "microma"[All Fields]) AND ("obesity"[MeSH Terms] OR "obesity"[All Fields]). We will include only articles in English.

Types of study to be included

We will include original articles that report circulating microRNAs in obese/overweight people (with no limitations for age, gender or ethnicity) and the comparison with a control group of normal-weight people.

Condition or domain being studied

Obesity is a growing public health concern now reaching epidemic status worldwide for children and adults; it is associated with various complications such as diabetes mellitus, hypertension and cardiovascular diseases. Obesity is determined by genetics as well as obesogenic environment. Various studies showed genetics may contribute towards obesity as much as 50-80%.
A microRNA (miRNA) is a small non-coding RNA molecule functioning in RNA silencing and post-transcriptional regulation of gene expression. It has been demonstrated that some miRNAs can exist in serum stably and is closely related to various diseases, including cancer, cardiovascular diseases and type 2 diabetes mellitus.

Participants/population

We will include studies evaluating circulating microRNAs from plasma of obese/overweight people, without age, race or gender restrictions. To be included in this review, articles should have a comparison between circulating miRNA expression profile in obese/overweight people vs lean control.

Intervention(s), exposure(s)

Weight that is higher than what is considered as a healthy weight for a given height is described as overweight or obese. Body Mass Index (BMI) is calculated by dividing a person's weight in kilograms by the square of height in meters; this measure is used as a screening tool for overweight or obesity. In adults, a BMI 25.0 to <30 falls within the overweight range. If BMI is 30.0 or higher, it falls within the obese range. BMI is a measure used to determine childhood overweight and obesity too. In children and teens, overweight is defined as a BMI at or above the 85th percentile and below the 95th percentile for peers of the same age and sex. Obesity is defined as a BMI at or above the 95th percentile for children and teens of the same age and sex. For children and teens, BMI is age- and sex-specific and is often referred to as BMI-for-age.

Comparator(s)/control

Control group is defined as normal weight population. In adults, BMI 18.5 to <25 is considered normal. In children and teens, normal or healthy weight is defined as BMI above the 5th percentile to less than the 85th percentile for peers of the same age and sex.

Primary outcome(s)
Change in microRNAs expression for at least 1.5 fold in obese / overweight vs lean control will be considered significant

Secondary outcome(s)
None

Data extraction (selection and coding)
Titles and abstracts of studies retrieved using the search strategy will be screened independently by two review authors to identify studies that potentially meet the inclusion criteria outlined above. The full text of these potentially eligible studies will be retrieved and independently assessed for eligibility by two review team members. Any disagreement between them over the eligibility of particular studies will be resolved through discussion with a third reviewer. A standardised form will be used to extract data from the included studies. Extracted information will include: author, country and year of publication; study population and participant characteristics (age, gender and BMI); microRNAs included in the study; methods of profiling and quantification of microRNAs; microRNAs deregulated and information for assessment of the risk of bias. Two review authors will extract data independently, discrepancies will be identified and resolved through discussion (with a third author where necessary). With the extracted data, we will use bioinformatic tools to predict putative gene targets (and pathways probably implicated) of the miRNAs deregulated.

Risk of bias (quality) assessment
Two review authors will independtly assess the risk of bias in included studies using the standard scale "Quality assessment tool for observational cohort and cross-sectional studies" from the NHLBI. Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion with involvement of a third review author when necessary.

Strategy for data synthesis
We will provide a narrative synthesis of the findings from the included studies and a summary of the miRNAs deregulated in the context of obesity. The miRNAs deregulated will be included for bioinformatic analysis.

Analysis of subgroups or subsets
None planned.

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Conflicts of interest
None known

Language
English

Country
Mexico

Stage of review
Review_Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Circulating MicroRNA; Humans; MicroRNAs; Obesity

Date of registration in PROSPERO
01 December 2017

Date of publication of this version
01 January 1900

Details of any existing review of the same topic by the same authors

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	Yes	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Versions
01 January 1900

C. Submission Confirmation