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Comparison of polyphenol-rich wine grape seed flour-regulated fecal and blood microRNAs in high-fat, high-fructose diet-induced obese mice

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ABSTRACT

A biomarker, easily and noninvasively measured in feces, can be useful in monitoring efficacy of novel functional foods against complicated pathologies of obesity-related metabolic diseases. Fecal microRNA (miRNA)s were analyzed for potential noninvasive biomarkers in C57BL/6J mice fed a high-fat and high-fructose diet (HFrD) containing either microcrystalline cellulose (MCC) or 5% polyphenol-rich wine grape seed flour (GSF) for 8 weeks. GSF supplementation significantly reduced HFrD-induced body weight gain, visceral adiposity, and hyperlipidemia. miRNome analysis revealed that GSF supplementation significantly ($p < 0.05$; fold-change, $|FC| \geq 1.5$) regulated 82 and 4 miRNAs in the feces and blood, respectively. Among these miRNAs, 22 in feces and 4 in blood were strongly associated with obesity-related physiological biomarkers, and *miR-129-5p* in both blood and feces showed a strong correlation with obesity-related biomarkers. These results suggest that fecal *miR-129-5p* is a potentially useful noninvasive biomarker in monitoring personalized GSF efficacy against obesity and obesity-metabolic disease.

1. Introduction

Obesity is one of the leading global public health challenges, and its prevalence is increasing at an alarming rate (Collaboration, 2016). It represents a risk factor for multiple non-communicable diseases (NCDs), including cardiovascular disease (CVD), type 2 diabetes, hypertension, and cancer (Pi-Sunyer, 2009). Phenotypically, its surrogate markers include body mass index (BMI) and waist circumference (Nimptsch, Konigorski, & Pischon, 2019). Biomarkers of obesity risk include adipokines such as leptin, adiponectin, and resistin (Nimptsch et al., 2019). Lifestyle modification, including functional food consumption, may prevent obesity associated risk factors. The development of functional foods guided by noninvasive biomarkers may enable personalized response to effectiveness of functional foods that prevent obesity-related diseases.

MicroRNAs (miRNAs), which are small noncoding RNAs, are endogenous 19–23 nucleotide RNAs that play an important role in gene expression regulation at the post-transcription level via target mRNA pairing (Bartel, 2009). Recent studies have shown their role in obesity regulation (Arner and Kulyte, 2015a; Lacomino & Siani, 2017; Zaiou, El Amri, & Bakillah, 2018), energy homeostasis (Bjorndal, Burri, Staalesen, Skorve, & Berge, 2011), insulin sensitivity (Williams & Mitchell, 2012), and inflammation (Ge, Brichard, Yi, & Li, 2014). They could also serve as promising biomarkers in monitoring early responses to lifestyle modification such as dietary interventions (Pahlavani et al., 2018; Palmer et al., 2014) and stress (Leung & Sharp, 2010). Additionally, compared with other biomarkers, they are more stable during the preparation of test samples derived from blood, urine, saliva, and feces (Cortez & Calin, 2009). Also, they represent a category of biomarkers that can be applied in monitoring treatment response in colon cancer (Masuda et al., 2017).

Adipose tissue miRNAs are packaged into adipose-derived microvesicles and delivered to blood (Arner and Kulyte, 2015b). Circulating miRNAs have been suggested as promising diagnostic biomarkers for obesity-related metabolic diseases (Pan et al., 2019). miRNAs are also found in feces, possibly due to exosomal protection of microvesicles (Koga et al., 2011). Interestingly, fecal miRNAs are reportedly involved in the modulation of gut microbiota (Liu et al., 2016). However, their potential role as noninvasive biomarkers in the development of personalized functional foods remains unclear.

Our previous studies showed that supplementation with wine grape seed flour (GSF), a byproduct of winemaking, suppressed high-fat (HF) diet-induced body weight gain, hepatic steatosis, visceral adiposity, and hyperlipidemia by the modulation of oxidative stress-, inflammation-,

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cholesterol-, and fatty acid metabolism-related genes (Seo et al., 2015) (K. H. Seo et al., 2016), an effect that is associated with the polyphenols in GSF (K. H. Seo, et al., 2016).

This study aimed to identify novel miRNA biomarkers noninvasively in feces that can be applied to monitoring the anti-obesity property of GSF in high-fat and high-fructose diet (HFrD)-fed obese mice.

2. Materials and methods

2.1. Animals and diets

Four-week-old male C57BL/6J mice purchased from Orient Bio, Inc. (Sungnam, Korea) were individually housed under a 12 h/12 h light/dark cycle in an environmentally controlled room (temperature, 20–22 °C; relative humidity, 60%). All institutional and national guidelines for animal care and use were followed, and the study was approved by the Konkuk University Animal Experiment Ethics Committee (KU16046 and KU17020). Prior to experimental diet initiation, the mice were acclimated for 1 week, during which food (mouse chow diet; LabDiet 5015; PMI International, Redwood, CA, USA) and water were made available *ad libitum*. Thereafter, the mice (n = 10 per each group) were fed an HFrD diet containing either 5% microcrystalline cellulose (MCC, control diet; Dyets Inc, Bethlehem, PA) or 5% wine grape seed flour (GSF) for 8 weeks *ad libitum* (Table 1). MCC is an insoluble fiber that has little effect on sterol metabolism (Horton, Cuthbert, & Spady, 1994). HFrD was modified from the previous studies (Cho, Lee, Seo, Yokoyama, & Kim, 2018; Kun Ho Seo et al., 2016; Seo et al., 2015) by replacing sucrose with fructose. The composition of macronutrients and total dietary fiber, containing approximately 17%, 36%, and 47% calories from protein, carbohydrates (including 20% from fructose), and fat, respectively, were matched across the diets (Table 1). GSF was purchased from Apr esVin Enterprise, Inc. (Prosser, WA, USA), and its total phenolic content was analyzed using the Folin–Ciocalteu method (Lamuela-Raventós, 1994). GSF contains 30 g total phenolic content per 100 g. Body weight was measured weekly and food intake was monitored twice every week.

2.2. Sample collection

Following a 12 h fasting period, the mice were anesthetized in an induction chamber, using a vaporizer (VetEquip, Livermore, CA) containing 4% isoflurane (Phoenix Pharmaceutical, St. Joseph, MO, USA) with an oxygen flow of 1 L/min. Blood samples were collected using the cardiac puncture method with syringes previously rinsed with potassium EDTA solution (15% w/v), centrifuged at 4500 rpm for 10 min, and stored for further analysis. After weighing, liver and epididymal adipose tissues samples were immediately frozen in liquid nitrogen for further analysis.

Table 1
Animal diet composition.

Ingredient (g/kg diet)	HFrD	HFrD + 5% GSF
Lard fat	225	225
Soybean oil	27	23
Cholesterol	0.8	0.8
MCC	50	21.4
GSF	0	50
Casein	200	199
Corn starch	91.2	74.8
Fructose	355	355
L-cystine	3	3
Choline bitartrate	3	3
Mineral mix	35	35
Vitamin mix	10	10
Total diet (g)	1000	1000
Total dietary fiber content (g/kg diet)	50	50
Fat %	46.7	46.7
Protein %	16.7	16.7
Carbohydrate %	36.6	36.1

[a] MCC, microcrystalline cellulose; [b] GSF, chardonnay grape seed flour).

2.3. Plasma lipid analysis

Size exclusion chromatography was used to analyze plasma lipoprotein cholesterol. HPLC was performed using the Agilent 1100 HPLC chromatograph with a Superose 6HR HPLC Column (Pharmacia LKB Biotechnology, Piscataway, NJ, USA), consisting of a mixing coil (1615-50 Bodman, Aston, PA, USA) in a temperature-controlled water jacket (Aura Industrials, Staten, NY, USA). The cholesterol reagent (Roche Diagnostic, Indianapolis, IN, USA) was delivered using a Hewlett-Packard HPLC pump (79851-A; Agilent Technologies, Palo Alto, CA, USA), at a 0.2 ml/min flow rate. Signals based on peak areas were calibrated using the bovine cholesterol lipoprotein standard.

2.4. miRNA microarray expression analysis

Total RNA in feces and blood samples were extracted using the Stool Kit (Power Microbiome RNA Isolation Kit, MoBio, USA) and the QIAamp RNA Blood mini Kit (Qiagen, Germantown, MD, USA), respectively, Purity and integrity of RNA samples were analyzed using a spectrophotometer (ND-1000, NanoDrop, Wilmington, USA) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA), respectively. Thereafter, 745 ng of total RNA was fragmented and hybridized to the Affymetrix GeneChip miRNA 4.0 Array, washed, and stained using GeneChip Fluidics Station 450 (Affymetrix, Santa Clara, California, USA). The hybridized array was scanned immediately using the Affymetrix GCS 3000 Scanner (Affymetrix, Santa Clara, California, USA), and the acquired signals were analyzed using the Affymetrix GeneChip Command Console Software (Affymetrix, Santa Clara, California, USA).

2.5. qRT-PCR analysis

Total RNA was extracted from feces and blood samples using the Stool Kit (Power Microbiome RNA Isolation Kit, MoBio, USA) and the QIAamp RNA Blood mini Kit (Qiagen, Germantown, MD, USA), respectively. TaqMan™ microRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) was used to synthesize cDNA. Expression of *miR-129-5p* and *U6* was measured with the TaqMan MicroRNA assay kit (Applied Biosystems, Foster City, CA, USA). The expression level of *miR-129-5p* was normalized by a reference gene, U6 miRNA. The results were analyzed with the $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical analysis

All data are presented as mean ± SEM. Student *t*-test was used to determine significant control and treated group difference. The Pearson correlation analysis was used to determine the correlation between miRNA obtained from samples and metabolic obesity physiological biomarkers. All statistical analyses were performed using SPSS software v20.0 (IBM SPSS Statistics, Chicago, IL, USA).

3. Results

3.1. Effects of wine GSF supplementation on body weight, organ weight, and blood cholesterol concentrations

GSF contains 30 g total phenolic content per 100 g. Compared to consumption of chow diet for 8 weeks (body weight gain in gram, 5.30 ± 0.95; epididymal adipose tissue weight in gram, 0.27 ± 0.03; liver weight in gram, 0.99 ± 0.03), feeding C57BL/6J mice with HFrD significantly increased body weight gain (57%) and epididymal adipose tissue weight (80%). HFrD supplemented with 5% GSF for 8 weeks

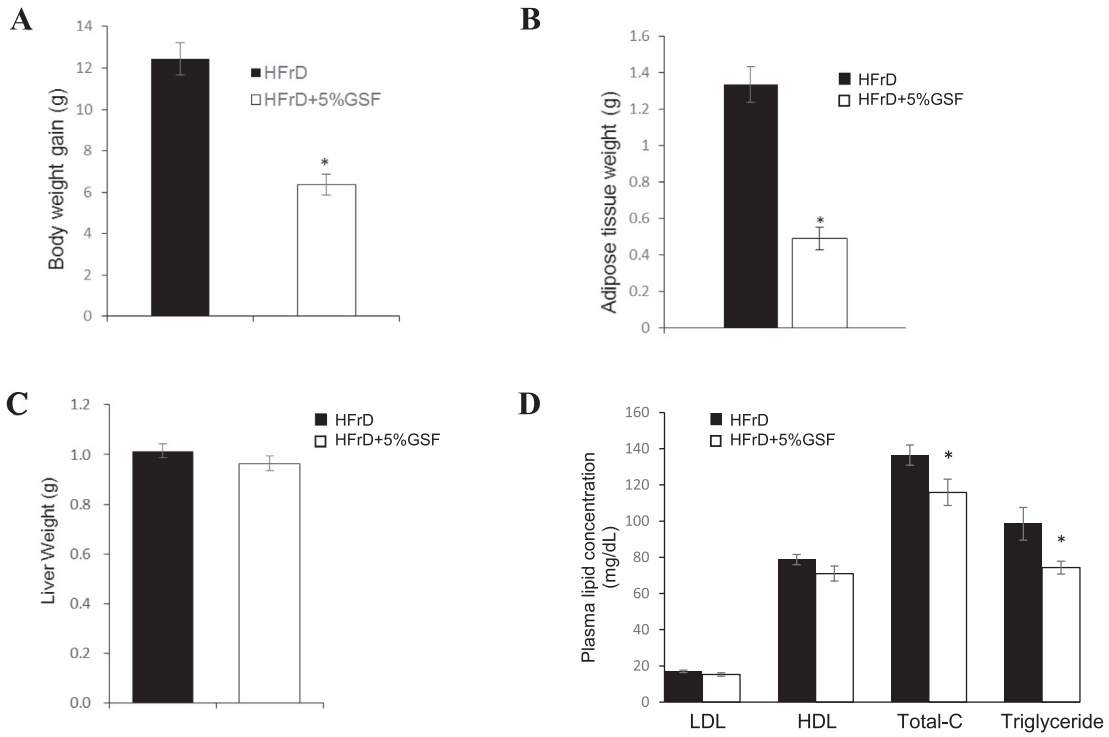

Fig. 1. Effect of 5% wine grape seed flour (GSF) on body weight gain (A), adipose tissue weight (B),liver weight (C), plasma lipid concentration (D) in C57BL/6J mice that were fed a high-fat and high-fructose diet for 8 weeks. Data are expressed as mean ± SE. *$p < 0.05$ vs. the control group.

significantly ($p < 0.05$) lowered body weight gain and epididymal adipose tissue weight by 49% and 63%, respectively, compared with the control group (Fig. 1). Daily food intake was not affected by diets (CON, 2.94 ± 0.33; HFrD, 2.82 ± 0.33 g/mouse). HFrD supplementation with 5% GSF also significantly reduced plasma total cholesterol and triglyceride by 15% and 25%, respectively ($p < 0.05$); however, it did not affect blood glucose levels (CON, 203 ± 19; 5% GSF, 224 ± 21), HDL (CON, 78.7 ± 3; 71 ± 4), or LDL (CON, 17 ± 0.7; 14 ± 1.1) concentrations.

3.2. Fecal miRNA microarray expression analysis

The overall expression of miRNAs in the feces of C57BL/6J mice that were fed either with HFrD or HFrD + 5% GSF was assessed using miRNA expression microarray analysis. A total of 82 fecal miRNAs were significantly affected by HFrD + 5% GSF, compared with the control group. Among these miRNAs, 29 were up-regulated while 53 were down-regulated (Table 2).

3.3. Blood miRNA microarray expression analysis

To assess blood miRNA expression, miRNA microarray analysis was used. After 8 weeks of 5% GSF treatment, 54 blood-derived miRNAs had $|FC| \geq 1.5$ (Supporting information Table S1). Out of 54 circulating miRNAs ($|FC| \geq 1.5$), *miR-129-5p* was significantly ($p < 0.05$) down-regulated, while *miR-6366*, *miR-6898-5p*, and *miR-6954-5p* were significantly ($p < 0.05$) up-regulated after GSF supplementation, compared with the control group (Table 3).

Expression level of *miRNA 129-5p* was measured by qRT-PCR analysis to confirm miRNA microarray results (Table 4). *miR-129-5p* was chosen since it is significantly regulated in both blood (FC −3.4) and in feces (FC 2.0). The *miRNA 129-5p* gene in feces and blood samples showed expression pattern comparable to the miRNA microarray data (Table 4).

3.4. Analysis of the correlation between expressed miRNA and metabolic obesity physiological biomarkers

To determine whether feces and blood miRNA regulation after 8 weeks of 5% GSF treatment was associated with changes in metabolic obesity physiological biomarkers, a correlation analysis between the miRNAs and 6 obesity physiological biomarkers (body weight gain, liver weight, adipose tissue weight, triglyceride, total cholesterol, and HDL) was performed (Tables 5 and 6). The results revealed that 23 miRNAs from feces and 4 miRNAs from blood were significantly ($p < 0.05$ or $p < 0.01$) correlated with obesity physiological biomarkers (Tables 5 and 6).

The fecal miRNAs *miR-1956* (FC 2.1), *miR-760-3p* (FC 1.8), *miR-7648* (FC 1.8), *miR-7119-5p* (FC 1.7), *miR-3473b* (FC 1.6), and *miR-714* (FC 1.6) showed a significantly ($p < 0.05$ or $p < 0.01$) negative correlation with body weight gain, liver weight, and adipose tissue weight, while with the same parameters, *miR-5100* (FC 6.4), *miR-7007-5p* (FC −6.5), and *miR-6979-5p* (FC −15.5) showed a significantly positive correlation (Fig. 2A, B, C and Table 5).

The correlation analysis also revealed that 4 C57BL/6J mice blood-derived miRNAs showed a significant correlation with metabolic obesity physiological biomarkers. *miR-129-5p* (FC −3.4) showed a significantly positive correlation with body weight gain and liver weight, while *miR-6366* (FC 2.6), *miR-6954-5p* (FC 2.4), showed a significantly negative correlation ($p < 0.01$ and $p < 0.05$, respectively) with body weight gain and adipose tissue weight (Fig. 2D, F, and Table 5). *miR-6898-5p* (FC 2.0) revealed a significantly ($p < 0.05$) negative correlation with liver weight and adipose tissue weight (Fig. 2E, F, and Table 6).

A correlation analysis was also performed between blood miRNAs ($|FC| \geq 1.5$) and obesity physiological biomarkers (Supporting information Table S2), which revealed that a total of 28 blood-derived miRNAs showed a significant ($p < 0.05$ or $p < 0.01$) correlation with obesity physiological biomarkers (Supporting information Table S2).

Table 2
C57BL/6J mice feces-derived miRNAs, significantly regulated by 5% GSF supplementation.

miRNA	FC[a)]	miRNA	FC[a)]	miRNA	FC[a)]
mmu-miR-129-5p	2.0	mmu-miR-5622-3p	−1.6	mmu-miR-7016-5p	1.7
mmu-miR-194-5p	−9.5	mmu-miR-6238	−1.8	mmu-miR-7018-5p	−2.7
mmu-miR-204-3p	−1.9	mmu-miR-6239	−5.3	mmu-miR-7024-5p	1.5
mmu-miR-290a-5p	1.6	mmu-miR-6240	−1.6	mmu-miR-7033-5p	−2.6
mmu-let-7b-5p	−1.5	mmu-miR-6349	−2.1	mmu-miR-7038-5p	−1.7
mmu-let-7c-5p	−1.8	mmu-miR-6368	−1.5	mmu-miR-7047-5p	−2.7
mmu-miR-181b-5p	1.6	mmu-miR-6401	−2.3	mmu-miR-7051-5p	1.7
mmu-miR-363-5p	−2.3	mmu-miR-6405	−1.7	mmu-miR-7059-5p	1.6
mmu-miR-483-5p	−2.4	mmu-miR-6418-5p	−2.0	mmu-miR-7068-5p	−3.7
mmu-miR-1247-3p	−2.7	mmu-miR-6905-5p	1.8	mmu-miR-7075-5p	−3.1
mmu-miR-760-3p	1.8	mmu-miR-6906-5p	−1.9	mmu-miR-7082-5p	−3.1
mmu-miR-690	−2.3	mmu-miR-6929-5p	−6.5	mmu-miR-7115-5p	1.7
mmu-miR-698-5p	−1.8	mmu-miR-6941-5p	−1.5	mmu-miR-7119-5p	
mmu-miR-702-5p	1.6	mmu-miR-6945-5p		mmu-miR-7216-5p	
mmu-miR-714	1.6	mmu-miR-6958-5p		mmu-miR-7235-5p	
mmu-miR-652-5p	1.6	mmu-miR-6965-5p		mmu-miR-7648-5p	
mmu-miR-181d-3p	1.9	mmu-miR-6972-5p		mmu-miR-7665-5p	
mmu-miR-1894-3p	−1.6	mmu-miR-6974-5p		mmu-miR-7669-3p	
mmu-miR-1956	2.1	mmu-miR-6979-5p		mmu-miR-7671-3p	
mmu-miR-3072-5p	−2.1	mmu-miR-6982-5p		mmu-miR-7678-5p	
mmu-miR-3087-5p	−1.6	mmu-miR-6985-5p		mmu-miR-8100	
mmu-miR-3090-5p	1.5	mmu-miR-6989-5p		mmu-miR-8101	
mmu-miR-3092-3p	1.7	mmu-miR-6999-5p		mmu-miR-8104	
mmu-miR-3473b	−2.1	mmu-miR-7002-5p		mmu-miR-3473	
mmu-miR-5100mmu-miR-5107-5p	−6.4	mmu-miR-7007-5p		mmu-mir-7648	
	−3.0	mmu-miR-7012-5p			
mmu-miR-3572-5p	−3.7	mmu-miR-7014-5p			
mmu-miR-1231-5p	−1.6				

[a)] $p < 0.05$ and fold change, $|FC| > 1.5$ vs. the HFrD-fed control group.

Table 3
C57BL/6J mice blood-derived miRNAs significantly regulated by 5% GSF supplementation.

miRNA [a)]	FC
mmu-miR-129-5p	−3.4
mmu-miR-6366	2.6
mmu-miR-6898-5p	2.0
mmu-miR-6954-5p	2.4

[a)] mRNAs with $p < 0.05$ and fold change, $|FC| > 1.5$ vs. the HFrD-fed control group.

Table 4
qRT-PCR validation of *miR-129-5p* from miRNA array data in feces and blood.

	Feces		Blood	
Gene	miRNA microarray	q-RT PCR	miRNA microarray	q-RT PCR
miRNA-129-5p	2.0	3.6 ± 0.6	−3.4	−2.8 ± 0.3

4. Discussion

MicroRNAs could be potential biomarkers for the development of personalized functional foods against obesity and obesity-related diseases. Interestingly, they are very stable compared with mRNA and have a reproducible sensitivity (Wu et al., 2012). In this study, feces- and blood-derived miRNAs were systemically compared, and based on existing literature, we believe this study in HFrD diet-induced obese mice is the first to demonstrate feces-derived miRNAs that may serve as potential noninvasive biomarkers for monitoring the anti-obesity efficacy of flavonoid-rich GSF and other functional foods in humans as well as animal models.

Our previous studies showed that GSF supplementation inhibited HF-induced body weight gain, liver weight, and adipose tissue weight, as well as hyperlipidemia (Kim et al., 2014; Kun Ho Seo, et al., 2016; Seo et al., 2015). In this study, we show the anti-obesity effect of GSF on westernized diet-fed obese mice (high-fat and high-fructose). GSF modulated various blood and feces miRNAs, which were significantly associated with anti-obesity biomarkers.

Polyphenols regulate the expression of diverse intracellular and extracellular miRNAs related to obesity and obesity-related metabolic diseases (Correa & Rogero, 2019). The consumption of grape extract with resveratrol up-regulated miR-21, miR-181b, miR-663, and miR-30c2 and down-regulated miR-155, reportedly associated with inflammatory cytokine reduction in the blood of obese men (Tome-Carneiro et al., 2013). In the present study, GSF supplementation regulated several blood miRNAs previously linked to obesity such as let-7b-5p (Frost & Olson, 2011; Lacomino & Siani, 2017), miR-129-5p (Lv et al., 2017), miR-181a-5p (Lozano-Bartolome et al., 2018), miR-532-3p (Kuryłowicz et al., 2017), and miR-711 (Hsieh et al., 2015). Out of 54 circulating miRNAs ($|FC| \geq 1.5$), four miRNAs (miR-129-5p, miR-6366, miR-6898-5p, and miR-6954-5p) showed a significant correlation with body weight and/or adipose tissue weight, indicating the possible circulating biomarkers of the anti-obesity efficacy of GSF supplementation in the HFrD diet-induced obese mouse model.

Circulating mmu-miR-714 was up-regulated in diet-induced obese mice (Hsieh et al., 2015), while in adipose tissue obtained from obese and diabetic subjects, miR-181a-5p expression was lowered (Lozano-Bartolome et al., 2018). In 3T3L1 cells, miR-181a-5p was involved in cell proliferation inhibition, owing to G1-phase cell-cycle arrest (Quyang et al., 2016), and miR-483-5p enhanced adipogenesis by suppressing the RhoA/ROCK1/ERK1/2 pathway (Chen et al., 2015). In diet-induced obese (DIO) mice, miR-711 was up-regulated but was significantly down-regulated after consuming low-fat diet (Hsieh et al., 2015). Further, after adipose tissue weight loss in obese individuals, hsa-miR-194-5p expression was decreased (Kuryłowicz et al., 2017). Let-7 is considered an anti-adipogenic miRNA, which targets the FABP4 and PPARγ signaling pathways (Lacomino & Siani, 2017).

In this study, miRNome analysis unexpectedly revealed that the effect of GSF supplementation on feces-derived miRNAs was more significant, compared with that of their blood-derived counterparts (82 feces-derived miRNAs compared with 4 blood-derived miRNAs, $p < 0.05$ and $|FC| \geq 1.5$). This observation may reflect the stability of fecal miRNAs (Wu et al., 2012). GSF supplementation significantly affected the level of obesity-related miRNAs in feces, including mir-let-7b-5p, miR-129-5p (Lv et al., 2017), miR-181a-5p (Lozano-Bartolome

Table 5
Correlation analysis between feces-derived miRNAs and metabolic obesity physiological biomarkers.

miRNA[a]	BW (g)	Liver weight (g)	Adipose tissue weight (g)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)
mmu-miR-129-5p	−0.79	−0.84*	−0.94#	−0.39	−0.18	0.06
mmu-miR-181d-3p	−0.95#	−0.76	−0.82*	−0.43	−0.16	−0.12
mmu-miR-194-5p	0.74	0.74	0.85*	0.17	0.12	0.00
mmu-miR-714	−0.96#	−0.92#	−0.85*	−0.34	−0.01	0.04
mmu-miR-760-3p	−0.93#	−0.97#	−0.86*	−0.44	0.01	0.14
mmu-miR-1956	−0.98#	−0.94#	−0.87*	−0.58	−0.06	0.11
mmu-miR-3092-3p	−0.83*	−0.87*	−0.76	−0.26	0.18	0.33
mmu-miR-3473	−0.93#	−0.85*	−0.90*	−0.59	−0.16	0.06
mmu-miR-5100	0.83*	0.81*	0.89*	0.23	0.13	0.05
mmu-miR-6368	0.89*	0.87*	0.78	0.16	−0.05	−0.03
mmu-miR-6401	−0.88*	−0.78	−0.92*	−0.33	−0.25	−0.17
mmu-miR-6905-5p	−0.78	−0.85*	−0.53	−0.01	0.46	0.44
mmu-miR-6979-5p	0.93#	0.91*	0.89*	0.43	0.03	−0.14
mmu-miR-6985-5p	−0.84*	−0.82*	−0.79	−0.12	−0.02	−0.04
mmu-miR-7007-5p	0.93#	0.87*	0.86*	0.29	0.09	0.07
mmu-miR-7018-5p	−0.67	−0.72	−0.90*	−0.31	−0.28	−0.07
mmu-miR-7024-5p	−0.88*	−0.92#	−0.78	−0.17	0.12	0.14
mmu-miR-7068-5p	−0.70	−0.67	−0.90*	−0.28	−0.33	−0.17
mmu-miR-7115-5p	−0.83*	−0.85*	−0.69	−0.02	0.19	0.12
mmu-miR-7119-5p	−0.92#	−0.92#	−0.86*	−0.30	0.04	0.13
mmu-mir-7648	−0.90*	−0.95#	−0.87*	−0.31	0.04	0.16
mmu-miR-7648-5p	−0.80	−0.81*	−0.94#	−0.38	−0.26	−0.08
mmu-miR-7678-5p	−0.88*	−0.89*	−0.78	−0.54	0.09	0.32

[a)] miRNAs with $p < 0.05$ and fold change, $|FC| > 1.5$ vs. the HFrD-fed control group.
[b)] $*p < 0.05$ and $^{\#}p < 0.01$.
[c)] BW, body weight gain; HDL, high density lipoprotein.

et al., 2018), miR-194-5p (Kuryłowicz et al., 2017), mir-483-5p (Chen et al., 2015), miR-714 (Hsieh et al., 2015), and miR-181d-3p (Yang, Min, & Lee, 2016). mmu-miR-129-5p, whose relevance in obesity has been suggested in previous studies, was up-regulated by 43-fold in insulin resistance adipocytes, compared with normal 3T3-L1 cell adipocytes (Ling et al., 2009). It inhibited preadipocyte proliferation by suppressing G3BP1 (GTPase-activating protein SH3 domain-binding protein 1) expression and activating the p38 signaling pathway in 3T3-L1 cells (Lv et al., 2017). GSF supplementation in HFrD fed mice showed that mmu-miR-129-5p, which is strongly associated with obesity physiological biomarkers, was significantly down-regulated in blood (FC −3.4) and significantly up-regulated (FC 2.0) in feces. Additionally, miR-129-5p showed a significantly inverse correlation with liver weight ($p < 0.05$) and adipose tissue weight ($p < 0.01$) in feces and a significantly positive correlation with body weight gain ($p < 0.01$) and liver weight ($p < 0.05$) in blood. These results suggest that mmu-miR-129-5p is a reliable and noninvasive novel biomarker in the monitoring of obesity and the efficacy of polyphenol-rich GSF against obesity.

Present study showed chardonnay GSF decreased a circulating *miR-129-5p* in the blood and increased levels of *miR-129-5p* in the feces. It has been suggested that miRNA 129-5p could be a potential obesity biomarker. Overexpression of *miR-129-5p* inhibited adipogenesis in primary stromal-vascular culture and serum miR-129-5p level was elevated in obese subjects (Fu et al., 2019). Dietary phytochemical components and short chain fatty acids have been also known to regulate expression levels of miRNAs (Abbasi et al., 2018; Bantscheff et al., 2011). Regulation of several miRNAs by epigallocatechin gallate (EGCG) and butyrate was reported, however, *miR-129-5p* was not reported (Quintanilha, Reis, Duarte, Cozzolino, & Rogero, 2017). Regulation of *miR129-5p* following GSF consumption could be explained by the intestinal production of short chain fatty acid and the presence of high amounts of flavonoids such as catechin, epicatechin, their 3-O-gallates, (epi) catechin dimers, oligomers, and polymers in GSF. In our previous study, GSF intake enhanced short chain fatty acid in the cecum (Cho et al., 2018) and total polyphenol content in the feces (not published data). Although there was a significant correlation between obesity biomarkers and *miR-129-5p*, it remains to be inconclusive whether miR-129-5p could be an indirect potential biomarker related to obesity or a direct biomarker of GSF intake. Further studies are required to explain the limitation on the present study.

In summary, GSF supplementation significantly inhibited HFrD-induced body weight gain, adipose tissue weight, and liver weight. In consistent with previous study (Cho et al., 2018), it did not affect food intake and inhibited total plasma cholesterol and triglyceride concentrations. Further, obesity associated physiological biomarkers were significantly correlated with blood- and feces-derived miRNAs. In blood and feces, 9 and 7 miRNAs, respectively, up- or down-regulated after the GSF diet, were known obesity biomarkers. Furthermore, 3 (*miR-6366, miR-6898-5p, miR-6954-5p*) and 16 miRNAs (Table 4) in blood and feces, respectively, were identified as novel biomarkers, which are significantly associated with obesity physiological biomarkers. GSF

Table 6
Correlation analysis between blood-derived miRNAs and metabolic obesity physiological biomarkers.

miRNA[a]	BW (g)	Liver weight (g)	Adipose tissue weight (g)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)
mmu-miR-129-5p	0.95#	0.91*	0.77	0.59	0.11	−0.17
mmu-miR-6366	−0.93#	−0.81	−0.97#	−0.71	0.31	0.47
mmu-miR-6898-5p	−0.81	−0.92*	−0.82*	−0.32	−0.17	0.07
mmu-miR-6954-5p	−0.89*	−0.67	−0.90*	−0.59	0.15	0.22

[a)] mRNAs with $p < 0.05$ and fold change, $|FC| > 1.5$ vs. the HFrD-fed control group.
[b)*] $p < 0.05$ and $^{\#}p < 0.01$.
[c)] BW, body weight gain; HDL, high density lipoprotein.

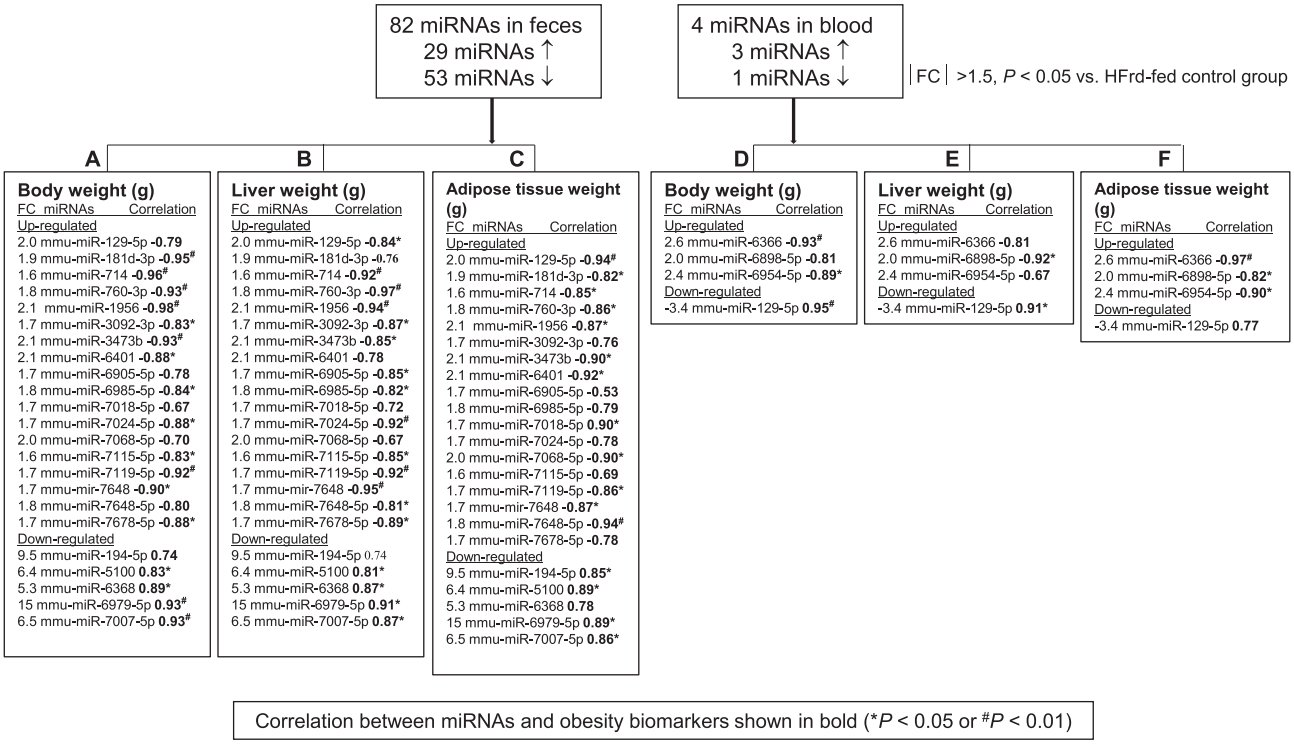

Fig. 2. Correlations between feces-derived miRNAs and body weight (A), liver weight (B), adipose tissue weight (C), and blood-derived miRNAs and body weight (D), liver weight (E), adipose tissue weight (F) in C57BL/6J mice fed a high-fat and high-fructose diet (HFrD) for 8 weeks. *$p < 0.05$ or #$p < 0.01$ vs. the control group. miRNAs have |fold change| > 1.5 with $p < 0.05$ vs. the control group.

supplementation significantly regulated miR-129-5p in both blood and feces, which is significantly associated with obesity physiological biomarkers (body weight gain, liver weight, and adipose tissue weight). Given the novel importance of miR-129-5p, further study will be required to confirm its expression in the blood, feces as well as tissues including liver and adipose tissue by qPCR analysis.

5. Conclusion

The results of this study suggest that fecal miR-129-5p is a potential novel biomarker that can be easily measured noninvasively for monitoring functional food efficacy in the management of the complicated pathologies of obesity-related metabolic diseases. However, clinical studies are needed to confirm these findings and facilitate the development of personalized functional foods in the dietary management of obese individuals.

CRediT authorship contribution statement

Kun-Ho Seo: Conceptualization, Investigation, Methodology, Writing - review & editing. **Wallace Yokoyama:** Writing - review & editing. **Hyunsook Kim:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104147.

References

Abbasi, B. A., Iqbal, J., Mahmood, T., Khalil, A. T., Ali, B., Kanwal, S., ... Ahmad, R. (2018). Role of dietary phytochemicals in modulation of miRNA expression: Natural swords combating breast cancer. *Asian Pacific Journal of Tropical Medicine, 11*(9), 501–509.

Arner, P., & Kulyte, A. (2015a). MicroRNA regulatory networks in human adipose tissue and obesity. *Nature Reviews Endrocrinology, 11*, 276–288.

Arner, P., & Kulyte, A. (2015b). MicroRNA regulatory networks in human adipose tissue and obesity. *Nature Reviews Endrocrinology, 11*(5), 276–288. https://doi.org/10.1038/nrendo.2015.25.

Bantscheff, M., Hopf, C., Savitski, M. M., Dittmann, A., Grandi, P., Michon, A. M., ... Drewes, G. (2011). Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. *Nature Biotechnology, 29*(3), 255–U124.

Bartel, D. P. (2009). MicroRNAs: Target recognition and regulatory functions. *Cell, 136*(2), 215–233. https://doi.org/10.1016/j.cell.2009.01.002.

Bjorndal, B., Burri, L., Staalesen, V., Skorve, J., & Berge, R. K. (2011). Different adipose depots: Their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *International Journal of Obesity, 2011*, Article 490650. https://doi.org/10.1155/2011/490650.

Chen, K., He, H., Xie, Y., Zhao, L., Zhao, S., Wan, X., ... Mo, Z. (2015). miR-125a-3p and miR-483-5p promote adipogenesis via suppressing the RhoA/ROCK1/ERK1/2 pathway in multiple symmetric lipomatosis. *Scientific Reports*. https://doi.org/10.1038/srep11909.

Cho, Y. J., Lee, H. G., Seo, K. H., Yokoyama, W., & Kim, H. (2018). Antiobesity Effect of Prebiotic Polyphenol-Rich Grape Seed Flour Supplemented with Probiotic Kefir-Derived Lactic Acid Bacteria. *Journal of Agriculture and Food Chemistry, 66*(47), 12498–12511. https://doi.org/10.1021/acs.jafc.8b03720.

Collaboration, N. C. D. R. F. (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet, 387*(10026), 1377–1396. https://doi.org/10.1016/S0140-6736(16)30054-X.

Correa, T. A., & Rogero, M. M. (2019). Polyphenols regulating microRNAs and inflammation biomarkers in obesity. *Nutrition, 59*, 150–157. https://doi.org/10.1016/j.

nut.2018.08.010.
Cortez, M. A., & Calin, G. A. (2009). MicroRNA identification in plasma and serum: A new tool to diagnose and monitor diseases. *Expert Opinion on Biological Therapy, 9*(6), 703–711. https://doi.org/10.1517/14712590902932889.
Frost, R. J. A., & Olson, E. N. (2011). Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. *Proceedings of the National Academy of Sciences of the United States of America (Cell Biology), 108*(52).
Fu, X., Jin, L., Han, L., Yuan, Y., Mu, Q., Wang, H., Yang, J., Ning, G., Zhou, D., & Zhang, Z. (2019). miR-129-5p inhibits adipogenesis through autophagy and may be a potential biomarker for obesity. *International Journal of Endocrinology, 2019*, 1–11. https://doi.org/10.1155/2019/5069578.
Ge, Q., Brichard, S., Yi, X., & Li, Q. (2014). microRNAs as a new mechanism regulating adipose tissue inflammation in obesity and as a novel therapeutic strategy in the metabolic syndrome. *Journal of Immunology Research, 2014*, Article 987285. https://doi.org/10.1155/2014/987285.
Horton, J. D., Cuthbert, J. A., & Spady, D. K. (1994). Regulation of hepatic 7 alpha-hydroxylase expression by dietary psyllium in the hamster. *Journal of Clinical Investigation, 93*(5), 2084–2092. https://doi.org/10.1172/JCI117203.
Hsieh, C. H., Rau, C. S., Wu, S. C., Yang, J. C., Wu, Y. C., Lu, T. H., ... Lin, C. W. (2015). Weight-reduction through a low-fat diet causes differential expression of circulating microRNAs in obese C57BL/6 mice. *BMC Genomics, 16*, 699. https://doi.org/10.1186/s12864-015-1896-3.
Kim, H., Bartley, G. E., Arvik, T., Lipson, R., Nah, S. Y., Seo, K., & Yokoyama, W. (2014). Dietary supplementation of chardonnay grape seed flour reduces plasma cholesterol concentration, hepatic steatosis, and abdominal fat content in high-fat diet-induced obese hamsters. *Journal of Agriculture and Food Chemistry, 62*(8), 1919–1925. https://doi.org/10.1021/jf404832s.
Koga, Y., Yasunaga, M., Moriya, Y., Akasu, T., Fujita, S., Yamamoto, S., & Matsumura, Y. (2011). Exosome can prevent RNase from degrading microRNA in feces. *Journal of Gastrointestinal Oncology, 2*(4), 215–222. https://doi.org/10.3978/j.issn.2078-6891.2011.015.
Kurylowicz, A., Wicik, Z., Magdalena, O., Jonas, M. I., Kotlarek, M., Swierniak, M., . . . Puzianowska-Kuznicka, M. (2017). NGS Reveals Molecular Pathways Affected by Obesity and Weight Loss-Related Changes in miRNA Levels in Adipose Tissue. International Journal of Molecular Science, 19.
Lacomino, G., & Siani, A. (2017). Role of microRNAs in obesity and obesity-related diseases. *BioMed Central Genes & Nutrition*. https://doi.org/10.1186/s12263-017-0577-z.
Lamuela-Raventós RM, W. A. (1994). A direct HPLC separation of wine phenolics. American Journal of Enology and Viticulture, 45, 1–5.
Leung, A. K., & Sharp, P. A. (2010). MicroRNA functions in stress responses. *Molecular Cell, 40*(2), 205–215. https://doi.org/10.1016/j.molcel.2010.09.027.
Ling, H. Y., Ou, H. S., Feng, S. D., Zhang, X. Y., Tuo, Q. H., Chen, L. X., ... Liao, D. F. (2009). CHANGES IN microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. *Clinical and Experimental Pharmacology and Physiology, 36*(9), e32–e39. https://doi.org/10.1111/j.1440-1681.2009.05207.x.
Liu, S., da Cunha, A. P., Rezende, R. M., Cialic, R., Wei, Z., Bry, L., ... Weiner, H. L. (2016). The Host Shapes the Gut Microbiota via Fecal MicroRNA. *Cell Host & Microbe, 19*(1), 32–43. https://doi.org/10.1016/j.chom.2015.12.005.
Lozano-Bartolome, J., Llaurado, g., Portero-Otin, M., Alutan-Coy, A., Rojo-Martinez, G., Vendrell, J., . . . R. Chacon, M. (2018). Altered Expression of miR-181a-5p and miR-23a-3p Is Associated With Obesity and TNFα-Induced Insulin Resistance. Endocrin Society, 103, 1447–1458.
Lv, S., Ma, M., Sun, Y., Wang, X., Qimuge, N., Qin, J., & Pang, W. (2017). MicroRNA-129-5p inhibits 3T3-L1 preadipocyte proliferation by targeting G3BP1. *Animal Cells and Systems (Seoul), 21*(4), 269–277. https://doi.org/10.1080/19768354.2017.1337046.
Masuda, T., Hayashi, N., Kuroda, Y., Ito, S., Eguchi, H., & Mimori, K. (2017). MicroRNAs as Biomarkers in Colorectal Cancer. *Cancers (Basel), 9*(9), https://doi.org/10.3390/cancers9090124.
Nimptsch, K., Konigorski, S., & Pischon, T. (2019). Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. *Metabolism, 92*, 61–70. https://doi.org/10.1016/j.metabol.2018.12.006.
Pahlavani, M., Wijayatunga, N. N., Kalupahana, N. S., Ramalingam, L., Gunaratne, P. H., Coarfa, C., ... Moustaid-Moussa, N. (2018). Transcriptomic and microRNA analyses of gene networks regulated by eicosapentaenoic acid in brown adipose tissue of diet-induced obese mice. *Biochimica et Biophysica Acta, Molecular and Cell Biology of Lipids, 1863*(12), 1523–1531. https://doi.org/10.1016/j.bbalip.2018.09.004.
Palmer, J. D., Soule, B. P., Simone, B. A., Zaorsky, N. G., Jin, L., & Simone, N. L. (2014). MicroRNA expression altered by diet: Can food be medicinal? *Ageing Research Reviews, 17*, 16–24. https://doi.org/10.1016/j.arr.2014.04.005.
Pan, Y., Hui, X., Hoo, R. L. C., Ye, D., Chan, C. Y. C., Feng, T., ... Xu, A. (2019). Adipocyte-secreted exosomal microRNA-34a inhibits M2 macrophage polarization to promote obesity-induced adipose inflammation. *Journal of Clinical Investigation, 129*(2), 834–849. https://doi.org/10.1172/JCI123069.
Pi-Sunyer, X. (2009). The medical risks of obesity. *Postgraduate Medicine, 121*(6), 21–33. https://doi.org/10.3810/pgm.2009.11.2074.
Quintanilha, B. J., Reis, B. Z., Duarte, G. B. S., Cozzolino, S. M. F., & Rogero, M. M. (2017). Nutrimiromics: Role of microRNAs and nutrition in modulating inflammation and chronic diseases. *Nutrients, 9*(11).
Quyang, D., Xu, L., Zhang, L., Guo, D., Tan, X., Yu, X., ... Li, Y. (2016). MiR-181a-5p regulates 3T3-L1 cell adipogenesis by targeting Smad7 and Tcf7l2. *Acta Biochimica et Biophysica Sinica, 48*(11), 1034–1041.
Seo, K. H., Bartley, G. E., Tam, C., Kim, H.-S., Kim, D.-H., Chon, J.-W., ... Yokoyama, W. (2016). Chardonnay grape seed flour ameliorates hepatic steatosis and insulin resistance via altered hepatic gene expression for oxidative stress, inflammation, and lipid ceramide synthesis in diet-induced obese mice. *Public Library of Science*. https://doi.org/10.1371/journal.pone.0167680.
Seo, K. H., Bartley, G. E., Tam, C., Kim, H. S., Kim, D. H., Chon, J. W., ... Yokoyama, W. (2016). Chardonnay grape seed flour ameliorates hepatic steatosis and insulin resistance via altered hepatic gene expression for oxidative stress, inflammation, and lipid and ceramide synthesis in diet-induced obese mice. *PLoS ONE, 11*(12), Article e0167680. https://doi.org/10.1371/journal.pone.0167680.
Seo, K. H., Kim, H., Chon, J.-W., Kim, D.-H., Nah, S.-Y., Arvik, T., & Yokoyama, W. (2015). Flavonoid-rich Chardonnay grape seed flour supplementation ameliorates diet-induced visceral adiposity, insulin resistance, and glucose intolerance via altered adipose tissue gene expression. *Journal of Functional Foods, 17*, 881–891.
Tome-Carneiro, J., Larrosa, M., Yanez-Gascon, M. J., Davalos, A., Gil-Zamorano, J., Gonzalvez, M., ... Garcia-Conesa, M. T. (2013). One-year supplementation with a grape extract containing resveratrol modulates inflammatory-related microRNAs and cytokines expression in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease. *Pharmacological Research, 72*, 69–82. https://doi.org/10.1016/j.phrs.2013.03.011.
Williams, M. D., & Mitchell, G. M. (2012). MicroRNAs in insulin resistance and obesity. *Journal of Diabetes Research, 2012*, Article 484696. https://doi.org/10.1155/2012/484696.
Wu, C. W., Ng, S. M. S., Dong, Y. J., Ng, S. C., Leung, W. W., Lee, C. W., ... Sung, J. J. Y. (2012). Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. *Gut; London, 61*, 739.
Yang, W. M., Min, K. H., & Lee, W. (2016). MicroRNA expression analysis in the liver of high fat diet-induced obese mice. *Data Brief, 9*, 1155–1159. https://doi.org/10.1016/j.dib.2016.11.081.
Zaiou, M., El Amri, H., & Bakillah, A. (2018). The clinical potential of adipogenesis and obesity-related microRNAs. *Nutrition, Metabolism & Cardiovascular Diseases, 28*(2), 91–111. https://doi.org/10.1016/j.numecd.2017.10.015.