Consumption of Coconut Oil Affects adipose miRNA Profile in Pigs.

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Research Article

Keywords: miRNA, coconut oil, adipose, pig

Posted Date: November 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1063962/v1>

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Abstract

Circulating miRNA molecules are intensively studied for their usefulness as biomarkers of civilization diseases. At the same time, it is known that diet can influence the level of miRNA expression in tissues. Our research aimed to determine how a diet containing various sources of fat (rapeseed oil, beef tallow, coconut oil) and different amounts of cDDGS (corn Dried Distilled Grains with Solubles) affects the miRNA profile in pig fat – the main source of circulating miRNAs. For this purpose, we used Next Generation Sequencing of miRNA libraries. We observed the highest number of differentially expressed miRNAs in the samples from animals that were fed with coconut oil in the diet compared to all other treatments. In contrary, cDDGS appeared to have little effect on miRNA expression. We propose a subset of diet-related, adipose-specific, conservative miRNAs among mammals, namely: ssc-miR-99b, ssc-miR-4334-3p, ssc-miR-146b, ssc-miR-23a. Moreover, we observed that several miRNAs regulated by dietary fats are considered as biomarkers in human and animal diseases.

1. Introduction

A properly balanced diet, in addition to physical activity, is of colossal importance in maintaining health in humans and animals. One of the most critical components of the diet is the appropriate content of fatty acids. At the same time, it is believed that today we consume an excessive amount of saturated fatty acids and omega – 6- fatty acids. In general, vegetable fats are considered to be healthier than animal fats. The cardioprotective effect of some vegetable fats has already been very well documented, e.g. olive oil, but other vegetable fats in the diet, such as coconut oil, are still quite controversial.

Understanding the molecular mechanisms by which dietary fat affects health may help clarify these controversies. It is known that fatty acids affect many processes because they are the main component of cell membranes, substrates of important biological molecules, and are involved in transmitting signals between cells. We have recently found that diet enriched with cDDGS (corn Dried Distilled Grains with Solubles – additive often used in pig breeding) changes mRNA expression in porcine adipose tissues [1]. Moreover, we observed that the source of dietary fats changes the expression of mRNA connected to neurodegenerative, cardiovascular diseases, or cancer [2] in this tissue. In this paper, we focus on miRNA profiles of porcine adipose tissue from these two previous nutrigenomic experiments [1,2]. Nutrigenomics of miRNA is especially interesting given its crucial role in cancer and other non-communicable diseases. However, the effect of nutrients on miRNA expression is relatively poorly studied, especially for the whole miRNAome scale.

Nevertheless, using the NGS technique, a number of miRNAs with different expressions in the adipose tissue of pigs with divergent backfat deposition were identified [3]. Moreover, in mice, it was shown that the overall miRNA expression profile in adipose tissue is influenced by a high-fat diet and other factors like sex hormones [4]. In pigs, the addition of CLA (Conjugated Linoleic Acid) to the diet changed the expression of 14 miRNAs in adipose tissue [5], while in rats, consumption of distinct dietary lipids during early pregnancy modulated the expression of microRNAs in mothers and offspring [6]. Recently, it has

been established that adipose tissue is the primary source of circulating miRNAs in mice. Moreover, it has been shown that these miRNAs can alter gene expression in other organs [7]. Such observations make the adipose tissue a vital secretory organ and miRNA as endocrine molecules.

Our study aimed to establish the effect of two dietary factors: cDDGS and source of fat (rapeseed oil, beef tallow and coconut oil) on the miRNA profile in the backfat of pigs.

2. Materials And Methods

2.1 Animals

Animals for the study were 24 crossbred fatteners originating from sows (Polish Landrace × White Large Polish) mated with a boar (Duroc × Pietrain) divided into four dietary groups: -cDDGS+rapeseed oil (group I, n=7), +cDDGS+rapeseed oil (group II, n=6), +cDDGS+beef tallow (group III, n=6), +cDDGS+coconut oil (group IV, n=5). All procedures relating to the use of live animals were in agreement with the local Ethics Committee for Experiments with Animals in Cracow (Resolution No. 912 dated 26 April 2012). All animals were healthy and as similar regarding weight as possible and kept in individual straw-bedded pens in uniform conditions. The ingredients, the nutritive value of the diets and the fatty acid compositions of the feed mixtures are presented elsewhere [8]. Briefly, all diets were isoenergetic and isoprotein, but differed in fatty acids composition: the group II feed mixture contained 80% of UFA content (44% MUFA and 36% PUFA), group III contained 67% of UFA (32% MUFA and 35% PUFA), and group IV encompassed 45% of UFA (16% MUFA and 29% PUFA). At the end of the experiment - when the animals reached the weight of 118 kg - all the pigs were slaughtered by stunning with high-voltage electric tongs (voltage 240–400 V), and samples of subcutaneous adipose tissue from the area between the last thoracic and the first lumbar vertebrae were collected for transcriptome analysis. All samples were stored in a freezer (–85 °C) until further analysis.

2.2 miRNA isolation, library construction and NGS.

Total RNA, including miRNA, was isolated from the samples of backfat using a Di-rect-zol RNA isolation Kit (Zymo Research). RNA of appropriate quality and quantity was used for library preparation. Next, miRNA-seq libraries were created using NEBNext Mul-tiplex Small RNA Library Prep Set for Illumina (New England Biolabs) according to the manufacturer protocol. The quantification of the obtained libraries was performed on a Qubit 2.0 spectrophotometer (Invitrogen, Life Technologies), while a quality control on a TapeStation 2200 instrument (D1000 ScreenTape; Agilent). 100 single-end cycle sequencing was performed on the HiScanSQ platform (Illumina) using TruSeq SR Cluster Kit v3- CBOT-HS and TruSeq SBS Kit v 3 - HS (Illumina).

2.3 Bioinformatic analysis of differentially expressed miRNA.

The obtained raw reads were converted to FastQ files, demultiplexed with the bcl2fastq software (Illumina), and quality controlled using the FastQC software [9]. The resultant sequences were analyzed with UEA sRNA Workbench V4.6 [10] to identify potentially novel and known miRNA sequences using the Sus scrofa reference genome (Sscrofa 10.2) and miRBase v22.1 [11,12]. The default animal parameters except for minimum abundance (6 reads), minimum length (17 bp), and maximum length (25 bp) were set [13]. Predicted novel microRNA precursors were analyzed to exclude those belonging to other non-coding RNA species using the RNA central database v14 [14]. Detected microRNAs were subjected to differential expression analysis with the DESeq2 software [15] to identify those affected by the addition of different fat in pigs' diets. miRNA-mRNA interactions were identified with miRNet [16], while functional analysis of miRNA was performed with Diana tools (mirPath v.3) [17].

3. Results

3.1 miRNA-seq statistics

In total, 99 861 653 reads were obtained (4 160 902 on average), which were further filtered to remove i.a. t/rRNA and low-quality sequences. This resulted in 26 456 757 high-quality sequences (1 102 364 on average). Further analysis allowed the identification of 165 unique known microRNAs and 299 potentially new miRNA sequences. 22 nucleotide long microRNAs were the most predominant. Results of the miRNA sequencing have been deposited to NCBI Gene Expression Omnibus database, accession number: GSE184177.

3.2 Coconut oil induces changes in the miRNA profile of backfat.

Principal component analysis revealed a relatively small variance between analyzed samples (12 and 15%). However, samples obtained from coconut oil-fed animals tended to separate from other samples (Figure 1).

The most abundant miRNAs in the backfat of pigs identified in our experiment were: ssc-miR-148a-3p, ssc-miR-143-3p, ssc-miR-126-3p, ssc-miR-26a, ssc-miR-99a, ssc-miR-10b, ssc-miR-21, ssc-miR-30a-5p, ssc-let-7a, ssc-miR-199b-3p, ssc-miR-27b-3p. (Fig. 2)

The highest number of miRNAs with altered expression was identified in the comparison of animals fed the diet containing cDDGS and coconut oil (group IV) with animals from the –cDDGS + rapeseed oil (group I) (37 miRNAs, p adjusted <0.1). Moreover, in comparison between group IV and groups III and II, 29 (12 upregulated and 17 downregulated in +cDDGS+coconut oil group) and 28 (10 upregulated and 18

downregulated in +cDDGS+coconut oil group) miRNAs were identified, respectively (p adjusted <0.1) (Table 2). No differences were observed in any other comparisons, suggesting that coconut oil is the factor with the most substantial potential in modulating miRNA expression in adipose tissue, while cDDGS does not affect miRNA expression. Interestingly, almost half of the identified DE miRNAs has been shown previously as affected by diet or obesity in mice, humans or pigs (Table 1). Nearly 30% (15) of DE miRNAs was common for all comparisons, including the coconut oil group, and had the same direction of expression changes (Fig 2). Four of these common DE miRNAs (ssc-mir-148a-3p, ssc-mir-143-3p, ssc-mir-99a, ssc-mir-10b) were among the most abundant miRNAs expressed in the backfat of pigs (Fig. 1)

Table 1

Differentially expressed miRNAs identified in miRNAseq NGS experiment (p adjusted<0.1). Examples of miRNAs identified previously in experiments in pigs, mice and humans are shown. *indicates miRNAs identified previously as affected by CLA in adipose of pigs [5], † obesity (human homologs) in mice [18], ‡ - circulating miRNA in obesity (human homologs) [19], # neonatal diet in pigs [20].

Table 2

The number of differentially expressed genes (lower part) and differentially expressed miRNAs (upper part) was identified in this experiment and in our previous studies performed on the same material. I - (-cDDGS+rapeseed oil), II (+cDDGS+rapeseed oil), III (+cDDGS+beef tallow), IV (+cDDGS+coconut oil)

	Differentially expressed miRNA			
Differentially expressed genes	I	II	III	IV
I	*	0	0	37
II	93	*	0	28
III	13	29	*	29
IV	125	2	0	*

3.3 miRNA-mRNA interaction analysis

Next, we compared the obtained results with our previous RNAseq based identification of differentially expressed genes in the animals from the same nutritional experiment [1,2]. In these two previous experiments, we observed a strong effect of the addition of cDDGS on the gene expression in the backfat and gene expression differences between animals obtaining rapeseed oil and beef tallow in the diet

-cDDGS+rapeseed oil vs +cDDGS+coconut oil			+cDDGS+coconut oil vs +cDDGS+beef tallow			+cDDGS+rapeseed oil vs +cDDGS+coconut oil		
miRNA	log2FC	padj	miRNA	log2FC	padj	miRNA	log2FC	padj
ssc-miR-339-5p#	1.121	0.001	ssc-miR-195	-0.854	0.005	ssc-miR-10a-5p	-0.79	0.003
ssc-miR-30d	-0.824	0.008	ssc-miR-10b	0.569	0.005	ssc-miR-339-5p#	0.997	0.003
ssc-miR-574-3p#	0.991	0.008	ssc-miR-30a-5p†	0.617	0.005	ssc-miR-10b	-0.561	0.008
ssc-miR-214-3p	1.105	0.008	ssc-miR-30d	0.891	0.005	ssc-miR-7#	-0.981	0.008
ssc-miR-99b†#	-0.728	0.008	ssc-miR-339-5p#	-0.869	0.008	ssc-miR-151-3p†	-0.842	0.013
ssc-miR-7-5p#	-0.835	0.017	ssc-miR-148a-5p†	0.947	0.009	ssc-miR-30d	-0.782	0.013
ssc-miR-139-5p	-0.683	0.023	ssc-miR-10a-5p	0.638	0.012	ssc-miR-4334-3p*#	0.798	0.013
ssc-miR-10b	-0.46	0.023	ssc-miR-99b†#	0.706	0.012	ssc-miR-106a	4.385	0.013
ssc-miR-148a-3p†	-0.75	0.024	ssc-miR-532-5p#	0.652	0.023	ssc-miR-148a-3p†	-0.818	0.018
ssc-let-7f†	-0.436	0.024	ssc-miR-148a-3p†	0.771	0.023	ssc-miR-95	-1.015	0.019
ssc-miR-30a-3p	-0.866	0.028	ssc-miR-151-3p†	0.755	0.024	ssc-miR-29c	0.623	0.019
ssc-miR-10a-5p	-0.549	0.028	ssc-miR-7#	0.784	0.024	ssc-miR-221-3p†	1.513	0.019
ssc-miR-199a-5p#	0.774	0.028	ssc-miR-143-3p†	0.854	0.027	ssc-miR-214	0.997	0.019
ssc-miR-20a-5p	0.634	0.031	ssc-miR-214	-0.887	0.042	ssc-miR-143-3p†	-0.863	0.028
ssc-miR-199a-3p#	0.686	0.034	ssc-miR-455-3p	-1.091	0.047	ssc-miR-99b†#	-0.641	0.028
ssc-miR-17-5p	0.771	0.035	ssc-miR-17-5p	-0.761	0.049	ssc-miR-30a-5p†	-0.485	0.032
ssc-miR-143-3p†	-0.778	0.039	ssc-miR-374a-5p	-0.827	0.062	ssc-miR-126-3p#	-0.62	0.043
ssc-miR-	-0.662	0.041	ssc-miR-365-	-0.646	0.062	ssc-miR-	-0.629	0.045

151-3p‡			3p*‡			139-5p		
ssc-miR-195	0.614	0.042	ssc-miR-199a-3p#	-0.629	0.067	ssc-miR-146b*†	-1.724	0.057
ssc-miR-1468#	-0.884	0.046	ssc-miR-29c	-0.492	0.067	ssc-miR-148b-5p	-1.415	0.06
ssc-miR-99a#	-0.582	0.046	ssc-miR-7134-3p	0.826	0.067	ssc-miR-17-5p	0.753	0.06
ssc-miR-455-3p	1.024	0.046	ssc-miR-16	-0.505	0.072	ssc-miR-365-3p*‡	0.649	0.067
ssc-miR-365-3p*‡	0.62	0.055	ssc-miR-139-5p	0.556	0.072	NAbta-miR-6529a	-1.137	0.085
ssc-miR-150‡	-0.596	0.057	ssc-miR-20a	-0.55	0.077	ssc-miR-125b	0.616	0.085
ssc-miR-146b*†	-1.534	0.058	ssc-miR-100#	0.558	0.084	ssc-miR-455-3p	0.982	0.096
ssc-miR-29c	0.475	0.058	ssc-miR-21*	0.611	0.084	ssc-miR-378	-0.561	0.098
ssc-miR-128#	-0.662	0.062	ssc-miR-455-5p	0.611	0.084	ssc-miR-99a#	-0.547	0.098
ssc-miR-148a-5p	-0.654	0.062	ssc-miR-340	0.645	0.089	ssc-let-7f‡	-0.354	0.098
ssc-miR-23a‡#	0.652	0.069	ssc-miR-107	-0.808	0.094			
ssc-miR-126-3p#	-0.507	0.071						
ssc-miR-16	0.47	0.071						
ssc-miR-374a-5p	0.725	0.071						
ssc-miR-19b	0.788	0.071						
ssc-miR-4334-3p*#	0.535	0.074						
ssc-miR-532-5p	-0.465	0.089						
ssc-miR-140-5p	0.528	0.098						
ssc-miR-361-5p	0.604	0.098						

(Table 2). We expected a correlation between the number of identified differentially expressed genes and

differentially ex-pressed miRNAs in each comparison; however, contrary to the gene expression study, we observed no differentially expressed miRNAs after comparison between the group which obtained cDDGS in the feedstuff and without it. On the other hand, the number of DEGs was the highest in the comparison between group I and IV and the same was noted for miRNAs (Table2).

We also attempted to identify the miRNA-mRNA interactions in our datasets from the present and previous experiments. For this purpose, we chose the comparison between group I (-cDDGS + rapeseed oil) and group IV (+ cDDGS + coconut oil) because this comparison showed the largest number of both altered genes and miRNAs (Table 2). Interestingly, we observed the predominance of upregulated genes in the (+cDDGS+coconut oil) group[1] and the predominance of downregulated miRNA in the same group. This agrees with the assumption that although some exceptions exist, miRNAs downregulate the expression of target genes.

Using the miRNet 2.0 database [16], we identified 23 interactions between the identified differentially expressed miRNAs and mRNAs (Table 3). Four identified genes were targeted by more than one miRNA, while five miRNAs targeted more than one gene. Two genes: RCSD1 and CCDC93, were targeted by three different miRNAs, but only in the case of CCDC93, an increase of mRNA expression was accompanied by a decrease in expression of targeting miRNA (Table 3).

Table 3

miRNA-gene interactions identified by analysis of differentially expressed genes and differentially expressed miRNAs in (-cDDGS+rapessed oil) vs (+cDDGS+coconut oil) comparison. Shaded interactions in which increase in miRNA expression was accompanied by increase in mRNA expression.

DE miRNA	Human homolog	miRNA sequence	miR logFC (Group IV vs I)	DE target gene	Gene logFC (Group IV vs I)
ssc-miR-16	hsa-mir-16	uagcagcacguaauauuggcg	0.470	<i>ARGLU1</i>	-0.73
ssc-miR-17			0.471	<i>RCSD1</i>	-0.66
ssc-miR-365-3p	hsa-mir-365-3p	uaaugccccuaaaaauccuuau	0.620	<i>CCDC93</i>	-0.47
ssc-miR-4334-3p	hsa-mir-4334-3p	ucccuguccuccaggagcuc	0.535	<i>CCDC93</i>	-0.47
ssc-miR-4334-3p	hsa-mir-4334-3p	ucccuguccuccaggagcuc	0.536	<i>MPHOSPH8</i>	-0.68
ssc-miR-17-5p	hsa-mir-17-5p	caaagugcuuacagugcagguag	0.771	<i>GDF11</i>	-0.59
ssc-miR-20a-5p	hsa-mir-20a-5p	uaaagugcuuauagugcaggua	0.634	<i>GDF11</i>	-0.59
ssc-miR-199a-3p	hsa-mir-199a-3p	acaguagucugcacauugguua	0.686	<i>HSPB6</i>	-0.84
ssc-miR-214-3p	hsa-mir-214-5p	acagcaggcacagacaggcag	1.105	<i>LAD1</i>	-0.83
ssc-miR-361-5p	hsa-mir-361-5p	uuaucagaaucuccagggguac	0.604	<i>MAP4K4</i>	-0.50
ssc-miR-339-5p	hsa-mir-339-5p	ucccuguccuccaggagcucac	1.121	<i>MYO1E</i>	-0.56
ssc-miR-339-5p	hsa-mir-339-5p	ucccuguccuccaggagcucac	1.122	<i>CCDC93</i>	-0.47
ssc-miR-199a-5p	hsa-mir-199a-5p	cccaguguucagacuaccuguuc	0.774	<i>OCN</i>	-0.67
ssc-miR-30d	hsa-mir-30d	uguaaaacauccccgacuggaagcu	-0.824	<i>TAF4B</i>	0.63
ssc-miR-30a-3p	hsa-mir-30a-3p	cuuucagucggauguuugcagc	-0.866	<i>UGP2</i>	0.55
ssc-miR-140-5p	hsa-mir-140-5p	agugguuuuaccuauugguag	0.528	<i>ZC3H4</i>	-0.72
ssc-miR-195	hsa-mir-195	uagcagcacagaaauauuggc	0.614	<i>LOC100521680</i>	0.53
ssc-miR-139-5p	hsa-mir-139-5p	ucuacagugcacgugucuccag	-0.683	<i>MAGI1</i>	-0.47

ssc-miR-150	hsa-mir-150	ucucccaacccuuguaccagug	-0.596	<i>MAP4K4</i>	-0.50
ssc-miR-7-5p	hsa-mir-7-5p	uggaagacuagugauuuuguuguu	-0.835	<i>RCSD1</i>	-0.66
ssc-miR-374a-5p	hsa-mir-374a-5p	uuauaaauacaaccugauaagug	0.725	<i>UGP2</i>	0.55
ssc-miR-214-3p			1.106	<i>LOC100522725</i>	0.53
ssc-miR-151			-0.597	<i>RCSD1</i>	-0.66

Next, we constructed a miRNA-mRNA network to summarize the relationship between the selected miRNAs and their target genes. Identified miRNA-mRNA interactions are graphically presented in figure 4.

3.4 Functional analysis of identified differentially expressed miRNA.

Functional analysis of all DE miRNAs, from all comparisons, showed that they are overrepresented in 45 metabolic pathways: (Table 4), of which the highest statistical sig-nificance was noted for Proteoglycans in cancer, Pathways in cancer, Hippo signaling pathway, Fatty acid biosynthesis, Signaling pathways regulating pluripotency of stem cells.

Table 4

KEGG pathways overrepresented after analysis of differentially expressed miRNAs from all comparisons revealed by Diana tools software.

KEGG pathway	p-value	#genes	#miRNAs
Proteoglycans in cancer	2.66E-09	96	20
Pathways in cancer	1.34E-07	171	20
Hippo signaling pathway	1.97E-07	71	19
Fatty acid biosynthesis	3.79E-07	6	5
Signaling pathways regulating pluripotency of stem cells	4.34E-07	71	19
Axon guidance	4.34E-07	63	20
GABAergic synapse	9.94E-07	38	18
Morphine addiction	1.97E-06	43	19
Prion diseases	5.40E-06	7	7
Ras signaling pathway	5.71E-06	99	19
ECM-receptor interaction	1.08E-05	36	17
TGF-beta signaling pathway	1.25E-05	40	18
FoxO signaling pathway	2.38E-05	66	20
Renal cell carcinoma	3.82E-05	37	19
PI3K-Akt signaling pathway	4.77E-05	145	21
Rap1 signaling pathway	7.71E-05	94	18
Thyroid hormone signaling pathway	0.0003099	51	17
Glioma	0.0003099	32	17
cGMP-PKG signaling pathway	0.0003099	75	19

4. Discussion

In recent years, adipose tissue has ceased to be treated only as a covering that insulates internal organs and protects against heat loss and has begun to be considered as a vital metabolic and endocrine organ due to the multitude of substances it secretes. It also turned out that adipose tissue is the main source of circulating miRNAs and low-grade adipose inflammation is one of the main factors responsible for atherosclerosis, metabolic diseases and cancer. Therefore, the identification of miRNAs formed in the fat is of great importance in humans and model animals such as pigs. In our NGS-based experiment, we identified eleven miRNAs with more than 10 000 read counts in porcine backfat, among which the most abundant were: ssc-miR-148a-3p, ssc-miR-143-3p, ssc-miR-126-3p (Fig. 1). This result is in good agreement with the report of Wang et al., [21], who observed that the same miRNAs are highly expressed in porcine adipose tissue. Similarly, Solexa sequencing revealed abundant expression of ssc-miR-143,

ssc-let-7a and ssc-miR-148 in porcine adipose [22]. Some concordance is also maintained compared to the results obtained by Gaffo et al. [23], although the authors observed a significant enrichment of ssc-miR-10b, while in our study, miRNAs with the highest expression in adipose tissue was miR-148a-3p.

Remarkably, seven highly abundant miRNA were differentially expressed depending of the source of fat in the diet. Ssc-miR-148a-3p was downregulated in pigs obtaining coconut oil compared to all other groups. Interestingly, it was reported recently that oleanolic acid induces upregulation of miR-148a-3p in chondrocytes [24] and consequently protects against IL1beta induced chondrocyte inflammation and dysfunction. Moreover, miR-143 (downregulated in coconut oil group) was downregulated in adipocytes from obese mice and upregulated during adipogenesis [18]. In our experiment, animals of all groups received isocaloric diets, which differed only slightly in the amount of fat but substantially in a fatty acid composition [1,2]. Dietary regimes did not affect animal's body weight or backfat thickness. However, the fatty acid composition of adipose tissue collected from the animals was strongly correlated with fatty acid composition in the feedstuff. Backfat from animals receiving coconut oil contained the lowest iodine value and the highest ratio of saturated to unsaturated fatty acids [8], which are known to induce a pro-inflammatory phenotype. Moreover, morphologically, it had the most solid consistency reflecting the high content of saturated fatty acids.

Generally, a high proportion of miRNAs identified by us as differentially expressed, has been previously shown as affected by CLA feeding, neonatal diet, or mother diet in pigs [5,20] (Table 1). Moreover, a huge part of them was over- or underexpressed in obesity in mice and humans [18,19]. This indicates on a subset of diet-related, adipose-specific, conservative miRNAs among mammals, namely: ssc-miR-99b, ssc-miR-4334-3p, ssc-miR-146b, ssc-miR-23a (Table 1). On the other hand, however, we found poor agreement with the results of the analysis of the effect of the diet on the miRNA expression in the cattle and sheep [25,26].

The adipose samples taken from the animals in the current analysis were subjected to detailed transcriptome studies beforehand [1,2]. Therefore, we decided to try to integrate the results obtained now regarding miRNAs with those from earlier analyzes. We identified 23 potential interactions between miRNAs and mRNAs, of which, in 16 cases, an increase in the level of miRNA expression was accompanied by a decrease in the level of expression of target genes (Table 2). Among the genes with the highest number of miRNA interactions, we identified CCDC93, RCSD1, MPHOSPH8, OCLN and MAP4K4. These are important regulators of inflammation, proliferation, epithelial barriers and metabolism. Thus, further investigation on diet regulation of these genes through miRNA may be of great clinical significance.

Among the KEGG pathways overrepresented by miRNAs with a different expression, we observed pathways associated with pathways in cancer, fatty acids biosynthesis, and stem cell pluripotency (Table 4). The latter pathway is interesting because pluripotency, and thus the ability to proliferate adipose cells, may influence the course of metabolic diseases associated with adipose tissue, such as diabetes. It is assumed that the proliferation of adipose cells is a favourable phenomenon in insulin resistance as

opposed to adipocyte hypertrophy [27]. It would be of clinical importance if a diet high in certain fatty acids could modulate adipose stem cell pluripotency. Another overrepresented pathway - ECM (Extracellular matrix) receptor interaction - is strictly connected with adiposity hyperphagia/hyperplasia balance. Excess extracellular matrix deposition in the form of fibrosis can limit adiposity hyperphagia and, in some cases, may be adaptive to counteract diabetes [28]. Thus, knowing the effect of different dietary fats on adipose ECM through differential miRNA expression is crucial.

In recent times, circulating miRNAs are increasingly considered biomarkers of various diseases and physiological conditions in humans and animals [29-31]). Most studies of this type concern the search for biomarkers for various types of cancer, but there are also many reports on biomarkers in Alzheimer's disease[32], atherosclerosis [33] and diabetes [34]. Recently, such markers have also been analyzed in veterinary medicine [35-37]. It is difficult not to notice the benefits of using circulating miRNAs in the diagnosis of civilization diseases, as it is a non-invasive method that allows for the detection of very early lesions. However, the clinical value of miRNA biomarkers will ultimately only be estimated when we consider the effects of extracorporeal factors affecting the expression of circulating miRNA: sex, age or physical exercise. Our research and others show that diet is also of great importance when it comes to influencing the level of miRNA expression in adipose tissue - the main source of circulating miRNA [7]. Among the miRNAs we identified as potentially sensitive to dietary fatty acid composition, several are considered as miRNA biomarkers of civilization diseases. Recently, miR-21 and miR-210 were established as novel non-invasive biomarkers for (CRC) Colorectal cancer diagnosis and prognosis [38]. Furthermore, miR-133b and miR-21 were proposed as possible candidates of novel biomarkers in the early prediction of (CAD) Coronary Artery Disease [39].

Similarly, plasma miR-126 and miR-143 are potential novel biomarkers for cerebral atherosclerosis [40]. The progression of civilization diseases is closely related to the type of fats consumed. Therefore, at times it can be difficult to distinguish whether the changes in the miRNA expression are due to the diet or the development of a pathologic state.

5. Conclusions

In this paper, we describe a subset of miRNA molecules whose expression changes after consuming a diet rich in coconut oil. Many of them have been identified previously as over-or under-expressed after a different diet. What is more, some of them are proposed as biomarkers of civilization diseases. Our results suggest caution and the need for further studies before miRNAs are used routinely as biomarkers in medicine.

Declarations

Author Contributions:

Conceptualization, M.O., M.Ś.; methodology, M.O., M.Ś., K.P.; software, T.S, K.P.; validation, M.O., K.P.; formal analysis, M.O.; investigation, K.P.; resources, M.Ś.; data curation, K.P. T.S.; writing—original draft preparation, M.O, K.P.; writing—review and editing, K.P, MŚ., T.S.; visualization, M.O., K.P.; supervision, M.O.; project administration, M.O.; fund-ing acquisition, M.O. All authors have read and agreed to the published version of the manu-script.

Funding:

This research was funded by National Science Center, Poland, grant number: 2014/13/B/NZ9/02134

Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Ethics Committee for Experiments with Animals in Cracow (Resolution No. 912 dated 26 April 2012).

Availability Statement:

Results of miRNAs sequencing have been deposited to NCBI Gene Expression Omnibus database, accession number: GSE184177.

Conflicts of Interest:

The authors declare no conflict of interest

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Figures

Figure 1

PCA – Principle component analysis of samples after RNA-seq.

Figure 2

The most abundant miRNAs identified in adipose tissue of pigs. ** - differentially expressed miRNAs in all comparisons after DEseq2 analysis, * - miRNAs identified in at least one comparison after DEseq2 analysis.

+cDDGS+coconut oil vs +cDDGS+beef tallow

+cDDGS+rapeseed oil vs +cDDGS+coconut oil

Figure 3

Venn diagram showing common miRNAs identified in many comparisons. 15 common miRNAs are listed; the red arrow indicates miRNA upregulated in the coconut oil group, a green arrow indicates miRNAs downregulated in the coconut oil group.

Figure 4

Graphical representation of differentially expressed miRNAs-mRNAs interaction identified in the adipose of pigs fed different diets (-cDDGS+rapeseed oil) vs (+cDDGS+coconut oil). Purple circles indicate DEGs identified in [2] while the green ones indicate those targeted by DE miRNAs identified in this study. Green squares indicate DE miRNAs identified in the present study, while blue squares indicate all miRNAs which may target identified DEGs [2].