Aleksi Kuusinen

MICRORNAS IN ASSOCIATION WITH OBESITY IN YOUNG FINNS STUDY

Faculty of Medicine and Health Technology

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Aleksi Kuusinen: MicroRNAs in association with obesity in Young Finns Study
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Obesiteetti eli lihavuus on maailmaa uhkaava sairaus, joka aiheuttaa kasvavia kustannuksia. Obesiteetin, jossa painoindeksi eli BMI on yli 30, ja ylipainon, painoindeksi 25–30 välillä, ehkäisemiseksi epigeneettisten tekijöiden, kuten miRNA:iden, tunteminen on tärkeää. Lasten Sepelvaltimotaudin Riskitekijät (LASERI) on vuonna 1980 aloitettu tutkimus, jonka viimeisimpään 2012 valmistuneeseen seurantaan osallistui 2 063 33–48-vuotiasta tutkimushenkilöä. Tutkimusaineistossa 22 miRNA:ta oli yhteydessä joko painoindeksiin (BMI) tai vyötärö-lantio-suhteeseen riippumatta iästä ja sukupuolesta.

Varianssianalyysissä (ANOVA) selvisi, että miR-122 ja miR-144 olivat tilastollisesti merkitsevästi yhteydessä kehon eri fenotyyppeihin, jotka olivat normaalipainoinen, ylipainoinen tai obeesi. MicroRNA-122:n tiedetään olevan sidonnainen maksaan ja verenkierron miR-122-tasojen olevan koholla obeeseilla henkilöillä. MicroRNA-144-tasot olivat laskeneet ylipainoisilla. On mahdollista, että miR-144 assosioituu häiriintyneeseen glukoosiaineenvaihduntaan.

MicroRNA-144 saattaa siis olla merkittävä säätelytekijä ylipainon muodostumisessa. Uutena löydöksenä tutkimusryhmämme selvitti, että miR-223 oli yhteydessä Activator protein 1 (AP-1) metaboliareittiin Gene set enrichment analysis (GSEA) -tutkimuksen mukaan (FDR q-arvo < 0,25). Ylipainoisilla AP-1 metaboliareitti oli hiljentynyt terveisiin verrattuna.

Avainsanat: microRNA, ylipaino, lihavuus, obesiteetti, BMI, vyötärö-lantio-suhde, epigenetiikka, genetiikka, miR-223, miR-144, GSEA, painoindeksi

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Introduction

1.1 Background

Due to increasing prevalence rate obesity (body mass index, BMI>30) is leading to a public health crisis. Overweight, 25<BMI<30, is a simultaneously growing problem. A total of 1.9 billion or 39 % of the world's total population has been estimated to be overweight or obese in 2014 (WHO's statistics 2004 and 2015). Even in developing countries, obesity has grown into pandemic proportions and the crisis is worldwide (Wang, Beydoun, Liang, Caballero, & Kumanyika, 2008). Obesity is associated with several diseases such as several cancers, metabolic disease, diabetes, cardiovascular disease, osteoarthiritis, hypertension and hypercholesterolemia (do Carmo et al., 2016; Duclos, 2016; Hall, Crook, Jones, Wofford, & Dubbert, 2002; Hall, do Carmo, da Silva, Wang, & Hall, 2015; Mazzarella, 2015; Parhofer, 2015; Vucenik & Stains, 2012; Zimmet, Alberti, & Shaw, 2001). It is well-established that overweight would decrease individuals' life-expectancy by eight years (Grover et al., 2015; Ovaskainen et al., 2015).

Lagos-Quintana et al. describe miRNA formation as follows: "MicroRNAs are transcribed from DNA to primary transcripts (pri-miRNA), which are then cleaved to shorter hairpin structures (pre-miRNAs), exported to cytoplasm, and further processed to form mature one-stranded miRNAs. In most cases, miRNAs repress their targets by interaction with the 3'UTR of the target mRNA, inducing a detectable change in the mRNA levels." (Lagos-Quintana, Rauhut, Lendeckel, & Tuschl, 2001.) MicroRNAs can also be transported between cells and tissues via circulation. Exogenous miRNA can be transported in circulation via microparticles such as exosomes, microvesicles and apoptotic bodies (Valadi et al., 2007) or associated with lipoprotein particles (HDL) (Vickers, Palmisano, Shoucri, Shamburek, & Remaley, 2011a) or miRNA binding proteins like Argonaute2 (Arroyo et al., 2011)

stabilizing their structure. 2588 miRNA have been found in humans (miRBase 21) and it is estimated that they would regulate 60% of all genes (Friedman, Farh, Burge, & Bartel, 2009). MicroRNAs can be used as diagnostic or prognostic markers. In the future, altering miRNA expression levels could be utilized in therapeutics.

1.2 Aims of the study

The aim of the study is to find relation between peripheral miRNA expression levels and obesity. Finally we briefly try to investigate obesity associated dysregulated metabolic pathways targeted by miRNAs.

Materials and Methods

2.1 The Young Finns Study

The Cardiovascular Risk in Young Finns Study (YFS) is a large multicenter follow-up study on cardiovascular risk from childhood to adulthood. The YFS was launched in 1980, with over 3,500 children and adolescents (3–18 years old) from all around Finland participating in the baseline study (Raitakari et al., 2008). Thereafter the subjects have been followed with several examinations including comprehensive risk factor assessments. The 30-year follow-up was performed in 2011–2012, with 2,063 adults, now aged 33–48 years, participating in the study. The examinations have included comprehensive data collection using questionnaires, physical measurements and blood tests. The present study has been approved by the Ethical Review Committee of Turku University Hospital.

All study subjects have given an informed consent and the study has been conducted according to the principles of the Declaration of Helsinki. For the current study, miRNAs were successfully profiled from 871 individuals, which were selected on the basis of a) successful RNA isolation, b) availability of phenotypic data and c) inclusion in the genome wide gene expression profiling. The demographics of the study subpopulation are presented in table 1. (Raitoharju, 2014.)

Table 1. Demographics of the study population.

Amount of subjects	871
Age, years	43.0 (4.85)
Female %	54.6
Metabolic syndrome, %	19.4
Total cholesterol, mmol/l	5.1 (0.91)
HDL cholesterol, mmol/l	1.3 (0.33)
LDL cholesterol, mmol/l	3.2 (0.80)
Triglycerides, mmol	1.1 (0.72)
Diabetes	
Type-1-diabetes, %	0.6
Type-2-diabetes, %	1.8
Blood glucose	5.3 (0.87)
Blood pressure	
Systolic, mmHg	124.5 (14.7)
Diastolic, mmHg	76.8 (10.2)
Body mass index, kg/m2	25.6 (4.9)

2.2 Anthropometry and blood pressure

Weight was measured with a Seca weighing scale to the nearest 0.1 kg and height with a Seca anthropometer to the nearest centimeter. BMI was calculated as weight(kg)/(height(m))^2. Waist circumference was measured using an anthropometric tape at the midpoint between the iliac crest and the lowest rib to the nearest 0.1 cm. The average of two measurements was used. Hip circumference

was measured around the widest portion of the buttocks, tape parallel to the floor. Again the average of two measurements was used. Waist-to-hip ratio (WHRAT) was calculated waist circumference divided by hip circumference. Blood pressure was measured three times after a 5-minute rest with a random zero sphygmomanometer. Blood pressure was estimated as the average of the three measurements of systolic and diastolic blood pressure.

2.3 Blood samples

Venous blood samples were drawn from the right antecubital vein after an overnight fast, and serum was separated, aliquoted, and stored at -70°C until analysis. Serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma-glutamyltransferase (GT), glucose, cholesterol and triglyceride concentrations were measured by enzymatic methods (ALAT, ASAT, GGT, Glucose, Cholesterol and Triglycerides System Reagent, Beckman Coulter Biomedical, Ireland) on an automatic analyzer (AU400, Olympus, Japan). Apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and C-reactive protein (CRP) were determined immunoturbidimetrically (ApoA1 and B assay reagent, Orion Diagnostica, Finland and CRP Latex reagent, Beckman Coulter Biomedical) using the same analyzer. The serum triglyceride concentration was assayed using the enzymatic glycerol kinase–glycerol phosphate oxidase method (Triglyceride reagent, Beckman Coulter Biomedical). Serum total cholesterol levels were measured by the enzymatic cholesterol esterase–cholesterol oxidase method (Cholesterol reagent, Beckman Coulter Biomedical). The same reagent was used for estimating HDL cholesterol levels after the precipitation of LDL and VLDL with dextran sulfate- Mg^{2+}. Serum glucose concentrations were determined by the enzymatic hexokinase method (Glucose reagent, Beckman Coulter Biomedical). All the above-mentioned assays were performed on an AU400 instrument (Olympus). Glycated hemoglobin (GHbA1c) fraction in whole blood was measured by an Abbott Architect ci8200 analyzer (Abbott Laboratories). The concentration of total

hemoglobin was first determined colorimetrically, after which the concentration of GHbA1c was measured immunoturbidimetrically using the microparticle agglutination inhibition method (Hemoglobin A_{1c} reagent, Fisher Diagnostics). These two concentrations were used to calculate the GHbA1c percentage. (Raitoharju et al., 2014; Suomela et al., 2014.)

2.4 RNA isolation and quality control

Whole blood (2.5ml) was collected into PaXgene Blood RNA Tubes (PreAnalytix). The tubes were inverted 8–10 times then stored at room temperature for at least 2 hours. The tubes were frozen (-80°C) and thawed overnight before RNA isolation with a PAXgene Blood microRNA Kit (Qiagen) including the DNase Set using the QiaCube. The concentrations and purity of the RNA samples were evaluated spectrophotometrically (BioPhotomer, Eppendorf). The RNA isolation process was validated by analyzing the integrity of several RNAs with the RNA 6000 Nano Chip Kit (Agilent). RNA integrity number (RIN) and the shape of the electropherogram were evaluated. The presence of the small RNA fraction was confirmed by the Agilent Small RNA Kit (Agilent). (Raitoharju et al., 2014.)

2.5 MicroRNA expression profiling

MicroRNA expression profiling was performed with the TaqMan® OpenArray® MicroRNA Panel (Life Technologies) containing 758 microRNAs, the functionality of which has been previously validated with our samples (Raitoharju et al., 2014). Briefly, 100 ng of RNA was used to run both A and B pools of Megaplex (Life Technologies) preamplification for cDNA synthesis. In the OpenArray Sample Loading Plate, 22.5 µl of each preamplified pool was mixed 1:1 with TaqMan OpenArray Real-Time PCR Master Mix. MicroRNA panels were loaded using the AccuFill System and run with

the QuantStudio 12K Flex (Life Tehnologies). Original data analysis was performed with Expression Suite Software version 1.0.1. RNU6, RNU44 and RNU48 were used as housekeeping small RNAs. Principal component analysis was performed in R (http://www.r-project.org/) and data was adjusted for 10 principal components. 60 failed samples/outliers were removed. MicroRNAs expressed in at least 75% of the samples were included in further analysis. Only assays with Amplification score >1 and Cq Confidence >0.7 were accepted. (Raitoharju et al., 2016.)

2.6 Genome-wide expression analysis

The expression levels were analyzed with an Illumina HumanHT-12 version 4 Expression BeadChip (Illumina Inc.) containing 47,231 expression and 770 control probes. In brief, 200 ng of RNA was reverse-transcribed into cDNA and biotin-UTP-labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion); 1,500 ng of cDNA was then hybridized to the Illumina HumanHT-12 v4 Expression BeadChip. The BeadChips were scanned with the Illumina iScan system. Raw illumina probe data was exported from Beadstudio and analyzed in R (http://www.r-project.org/) using the Bioconductor (http://www.bioconductor.org/) packages. The expression data was processed using nonparametric background correction, followed by quantile normalization with control and expression probes, using the neqc function in the limma package and log2 transformation. The expression analysis was successful in 772 of the 908 samples with miRNA expression profile. (Raitoharju et al., 2014.)

2.7 Statistical analysis

All analyses were performed with SPSS statistics version 22, R-language or bioinformatics tools identified below. First we discovered all miRNAs that were related with BMI classes (lean BMI<25,

overweight 25≤BMI<30 and obese BMI≥30) below Bonferroni adjusted p-level [i.e. p<0.05/243] using one-way analysis of variance, ANOVA, (due to non-gaussian population). MicroRNA levels were then correlated with BMI and WHRAT with gaussian correlation (due to abnormal distributions as well). Correlations were considered significant if fdr-value < 0.05 (independently of sex and age). MicroRNA expression levels were also correlated with metabolite levels and physiological features previously associated with metabolic dysfunction (i.e. plasma lipoprotein subclass concentrations, glucose and glycosylated hemoglobin levels, branched and aromatic amino acid concentrations, anthropometric data, and blood pressure variables) using linear regression model to clarify the association between miRNAs and obesity. All phenotypes or physiological features besides metabolites that were independently (of sex, age, BMI and WHRAT) associated with obesity related with miRNAs (at p<0.05 level) were considered significant.

Gene set enrichment analysis (GSEA) provided by Broad Institute was conducted to obtain differences in miRNA levels between normal and overweight phenotypes (MSigDB database v5.0) for those miRNAs that were significantly associated with BMI/WHRAT. Specifically the study population was separated into two subgroups by BMI: normal (BMI<25) and overweight (BMI≥25). All those predicted mRNA targets that were identified by at least two target predictors (miRecords, TarBase, mirTarbase, TargetScan, miRNAorg, Microcosm Targets, PITA, Pictar and miRDB) in miRGator v3.0 were included in pathway analysis (Cho et al., 2013). Combining miRNAs' predicted mRNA targets and genome-wide expression data we studied differentially expressed pathways between normal and overweight groups. Our analysis included all canonical pathways. Significance was predetermined to include all pathways below frd q-value 0.25.

Results

3.1 The miRNA quality control

The RNA quality and functionality of the TaqMan OpenArray microRNA expression panels have been previously validated (Raitoharju et al., 2014). After quality control and removal of outlier miRNA profiling was successful on 871 samples. 772 of these had successful genome wide gene-expression data available analyzed from the same blood sample/RNA isolation. 276 miRNAs were expressed in the whole blood in 75% of the samples and qualified in the further analysis.

3.2 MicroRNAs and obesity

A total of 22 miRNAs were associated with either or both BMI and WHRAT (independently of sex and age) shown in table 1 below. MicroRNAs 19a-3p, 19b-3p, 144-5p, 25-5p and 106b-3p were inversely correlated with BMI. MicroRNAs 122-5p, 23a-3p, 769-5p and 223-3p were directly correlated with BMI. Patients were also grouped into lean, overweight and obese individuals and in ANOVA miRNA-122 and miRNA-144 were significantly associated with these groups. Results from ANOVA analysis can be seen in table 2 below. MicroRNA-122 was found to be directly correlated (figure 1) to BMI whereas miRNA-144 (figure 2) had an inverse correlation to BMI. MicroRNA-223 targeted Activator protein 1 (AP-1) pathway was down-regulated in overweight class (table 3). MicroRNAs were also found to be related with sex and miRNAs may regulate different gene expressions in opposite sexes.

Table 1. Mirna trait results: MicroRNAs that are related to obesity independently of sex and age.

Trait	Mirna	mirna_p_bon	mirna_p_fdr
bmi	19a-3p	0,030	7,51E-03
bmi	122-5p	0,064	9,98E-03
bmi	19b-3p	0,181	1,77E-02
bmi	144-5p	0,207	1,77E-02
bmi	25-5p	0,300	1,87E-02
bmi	106b-3p	0,684	3,80E-02
bmi	23a-3p	0,905	4,11E-02
bmi	769-5p	0,894	4,11E-02
bmi	223-3p	0,864	4,11E-02
whrat	144-5p	0,000	3,91E-04
whrat	19b-3p	0,010	5,10E-03
whrat	103a-3p	0,029	7,51E-03
whrat	19a-3p	0,070	9,98E-03
whrat	363-3p	0,068	9,98E-03
whrat	185-5p	0,099	1,24E-02
whrat	183-3p	0,230	1,77E-02
whrat	320a	0,200	1,77E-02
whrat	1275	0,213	1,77E-02
whrat	30a-3p	0,270	1,87E-02
whrat	211-5p	0,289	1,87E-02
whrat	744-5p	0,334	1,97E-02
whrat	636	0,738	3,88E-02
whrat	199a-3p	1,000	4,57E-02
whrat	1227-3p	1,000	4,63E-02
whrat	324-5p	1,000	4,84E-02

Table 2. ANOVA. Phenotypes were clustered into three classes due to BMI: lean, overweight and obese. Only miRNA-122 and miRNA-144 were significantly associated with these phenotypes.

MicroRNA	Sig.	Bonferroni adjusted p-level
miR-122	8,43E-08	2,03E-05
miR-144	2,03E-04	0,05
miR-296	9,25E-04	0,22
miR-19b	1,17E-03	0,28
miR-432	1,66E-03	0,40
miR-142	4,87E-03	1,17
miR-106b	5,11E-03	1,23

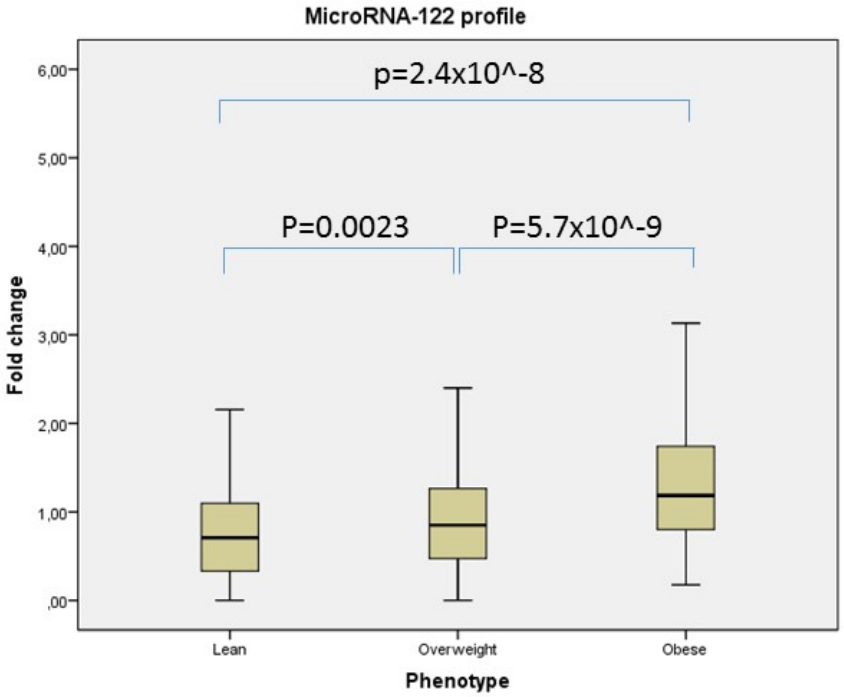

Figure 1. MicroRNA-122 levels are elevated in overweight.

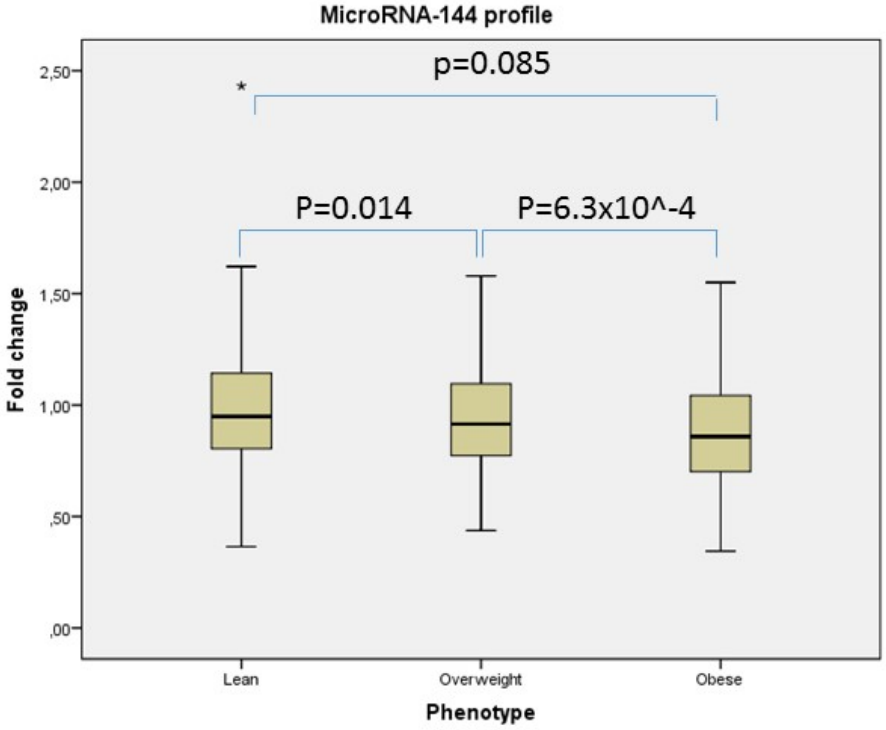

Figure 2. MicroRNA-144 profiles are inversely correlated with BMI.

Table 3. MicroRNA-223 is associated with AP1 (activator protein 1) pathway in GSEA (Gene set enrichment analysis).

Phenotype	Normal versus overweight
Upregulated in class	Normal
GeneSet	AP1-PATHWAY
Nominal p-value	0.00384
FDR q-value	0.167
FWER p-Value	0.089

Statistically significant (FDR q-value below 0.25 and nominal p-value below 0.05).

3.3 Gene Set Enrichment analysis

Combining the predicted miRNA target genes from miRGator v3.0 (genes that were identified by at least two databases) and gene expression data we tried to identify all metabolic pathways that would be dysregulated in obesity. All predicted or validated target genes that were statistically correlated with gene expression data ($p<0.05$) were taken into Gene set enrichment analysis (GSEA) provided by the Broad Institute. GSEA is a computational method that provides information of differences between different phenotypes. In our analysis patients were classified to lean or overweight/obese classes according to their BMI. In GSEA we found that miR-223 related AP-1 pathway was downregulated in obese phenotypes (FDR q-value < 0.25). In trajectory analysis we did not find differences between those groups that had gained or lost weight relatively from childhood to adulthood.

Discussion

4.1 Overview

MicroRNA-122 and miRNA-144 circulatory levels were associated with individual's phenotype (lean/overweight or obese). A total of 22 miRNAs were associated with either or both BMI and WHRAT (independently of sex and age). MicroRNA-122 is known to be liver associated from previous studies performed by our group. MicroRNA-144 is potentially associated with impaired glucose tolerance. MicroRNA-223 targeted AP-1 pathway was down-regulated in overweight group in GSEA.

MicroRNA-223 was positively correlated with BMI and has been linked to HDL in previous studies (Vickers, Palmisano, Shoucri, Shamburek, & Remaley, 2011b). MicroRNA-223 has been predicted to target cholesterol synthesis (Abente, Subramanian, Ramachandran, & Najafi-Shoushtari, 2015). MicroRNA-223 targeted AP-1 pathway was down-regulated in overweight class. Previous studies suggest that AP-1 activation would be down-regulated in liver tissue (Videla, 2010). AP-1 transcription factor has been inflammation linked in previous studies (Latruffe et al., 2015). This study is the first to our knowledge that links miR-223 with AP-1 pathway. This finding could be utilized in the future but more studies are needed to demonstrate causality between miRNA-223 expression and function of AP-1 pathway.

4.2 MicroRNA-122 is liver associated

MicroRNA-122 correlated with many liver variables (ASAT, ALAT, GT, apolipoproteins and

lipoprotein fractions etc.) showing it is clearly liver tissue specific miRNA. A previous refined study performed by our group shows its association with the liver (Raitoharju et al., 2016). It is very likely that obesity related fatty liver disease explains elevated miRNA-122 levels.

4.3 MicroRNA-144-5p possibly associated with glucose intolerance

Glucose intolerance plays a key role in obesity. MicroRNA-144-5p has been previously linked to obesity and its related diseases such as diabetes (Karolina et al., 2011). It is thought to regulate cell proliferation (usually by inhibiting) and tumorigenesis for example (Chen et al., 2015). Interestingly it was inversely regulated with BMI so according to our study it is possible that obesity leads to excess cell proliferation via miR-144-5p expression levels.

The main disadvantage of this study is that results are mainly descriptive. In addition, we did not find differences in miRNA expression between those groups that had gained or lost weight from childhood to adulthood so miRNAs may be very variating in circulation. Hence, this study does not establish any support that dysregulated miRNA levels in childhood would lead to overweight in adulthood. Furthermore, it could be interesting to study miRNA expression levels from previous blood samples from study populations' childhood. Some previous studies claim that miRNAs are unspecific in diseases as single miRNAs may target a number of genes. Results from previous miRNA and obesity related studies have been ambivalent and difficult to replicate. According to some studies methodological differences in circulatory miRNA profiling exacerbates comparison of results (Endzelins et al., 2016). Use of Caucasian study population limits generalization of our results to some extent. MicroRNA extractions from blood samples do not necessarily represent miRNA levels in tissues such as white adipose tissue. More studies are needed to establish possible mechanisms in which miR-223 acts in the potentially obesity related AP-1 pathway.

4.4 Conclusion

In conclusion, according to our study miRNA expression levels vary depending on a person's phenotype. MicroRNA-122 and miR-144 were related to a person's phenotype in ANOVA analysis. MicroRNA-144 is potentially related with glucose metabolism and impaired glucose tolerance. In a novel finding we noticed, that MicroRNA-223 was related to previously obesity linked AP-1 pathway that was down-regulated in overweight group. More studies are required to obtain a causal relation of miRNAs in obesity.

References

Abente, E. J., Subramanian, M., Ramachandran, V., & Najafi-Shoushtari, S. H. (2015). MicroRNAs in obesity-associated disorders. *Archives of Biochemistry and Biophysics,* doi:S0003-9861(15)30069-2 [pii]

Arroyo, J. D., Chevillet, J. R., Kroh, E. M., Ruf, I. K., Pritchard, C. C., Gibson, D. F., . . . Tewari, M. (2011). Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proceedings of the National Academy of Sciences of the United States of America, 108*(12), 5003-5008. doi:10.1073/pnas.1019055108 [doi]

Chen, S., Li, P., Li, J., Wang, Y., Du, Y., Chen, X., . . . Zhang, G. (2015). MiR-144 inhibits proliferation and induces apoptosis and autophagy in lung cancer cells by targeting TIGAR. *Cellular Physiology and Biochemistry : International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology, 35*(3), 997-1007. doi:10.1159/000369755 [doi]

Cho, S., Jang, I., Jun, Y., Yoon, S., Ko, M., Kwon, Y., . . . Lee, S. (2013). MiRGator v3.0: A microRNA portal for deep sequencing, expression profiling and mRNA targeting. *Nucleic Acids Research, 41*(Database issue), D252-7. doi:10.1093/nar/gks1168 [doi]

do Carmo, J. M., da Silva, A. A., Wang, Z., Fang, T., Aberdein, N., de Lara Rodriguez, C. E., & Hall, J. E. (2016). Obesity-induced hypertension: Brain signaling pathways. *Current Hypertension Reports, 18*(7), 58-016-0658-1. doi:10.1007/s11906-016-0658-1 [doi]

Duclos, M. (2016). Osteoarthritis, obesity and type 2 diabetes: The weight of waist circumference. *Annals of Physical and Rehabilitation Medicine, 59*(3), 157-160. doi:10.1016/j.rehab.2016.04.002 [doi]

Endzelins, E., Melne, V., Kalnina, Z., Lietuvietis, V., Riekstina, U., Llorente, A., & Line, A. (2016). Diagnostic, prognostic and predictive value of cell-free miRNAs in prostate cancer: A systematic review. *Molecular Cancer, 15*(1), 41-016-0523-5. doi:10.1186/s12943-016-0523-5 [doi]

Friedman, R. C., Farh, K. K., Burge, C. B., & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research, 19*(1), 92-105. doi:10.1101/gr.082701.108 [doi]

Grover, S. A., Kaouache, M., Rempel, P., Joseph, L., Dawes, M., Lau, D. C., & Lowensteyn, I. (2015). Years of life lost and healthy life-years lost from diabetes and cardiovascular disease in overweight and obese people: A modelling study. *The Lancet.Diabetes & Endocrinology, 3*(2), 114-122. doi:10.1016/S2213-8587(14)70229-3 [doi]

Hall, J. E., Crook, E. D., Jones, D. W., Wofford, M. R., & Dubbert, P. M. (2002). Mechanisms of obesity-associated cardiovascular and renal disease. *The American Journal of the Medical Sciences, 324*(3), 127-137. doi:S0002-9629(15)34401-3 [pii]

Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E. (2015). Obesity-induced hypertension: Interaction of neurohumoral and renal mechanisms. *Circulation Research, 116*(6), 991-1006. doi:10.1161/CIRCRESAHA.116.305697 [doi]

Karolina, D. S., Armugam, A., Tavintharan, S., Wong, M. T., Lim, S. C., Sum, C. F., & Jeyaseelan, K. (2011). MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin

receptor substrate 1 in type 2 diabetes mellitus. *PloS One, 6*(8), e22839. doi:10.1371/journal.pone.0022839 [doi]

Lagos-Quintana, M., Rauhut, R., Lendeckel, W., & Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. *Science (New York, N.Y.), 294*(5543), 853-858. doi:10.1126/science.1064921 [doi]

Latruffe, N., Lancon, A., Frazzi, R., Aires, V., Delmas, D., Michaille, J. J., . . . Cherkaoui-Malki, M. (2015). Exploring new ways of regulation by resveratrol involving miRNAs, with emphasis on inflammation. *Annals of the New York Academy of Sciences, 1348*(1), 97-106. doi:10.1111/nyas.12819 [doi]

Mazzarella, L. (2015). Why does obesity promote cancer? epidemiology, biology, and open questions. *Ecancermedicalscience, 9*, 554. doi:10.3332/ecancer.2015.554 [doi]

Ovaskainen, M. L., Tapanainen, H., Laatikainen, T., Mannisto, S., Heinonen, H., & Vartiainen, E. (2015). Perceived health-related self-efficacy associated with BMI in adults in a population-based survey. *Scandinavian Journal of Public Health, 43*(2), 197-203. doi:10.1177/1403494814566263 [doi]

Parhofer, K. G. (2015). Interaction between glucose and lipid metabolism: More than diabetic dyslipidemia. *Diabetes & Metabolism Journal, 39*(5), 353-362. doi:10.4093/dmj.2015.39.5.353 [doi]

Raitakari, O. T., Juonala, M., Ronnemaa, T., Keltikangas-Jarvinen, L., Rasanen, L., Pietikainen, M., . . . Viikari, J. S. (2008). Cohort profile: The cardiovascular risk in young finns study. *International Journal of Epidemiology, 37*(6), 1220-1226. doi:10.1093/ije/dym225 [doi]

Raitoharju, E. (2014). *MicroRNAs in the etiology and pathophysiology of atherosclerosis* (ACADEMIC DISSERTATION). Retrieved from http://tampub.uta.fi

Raitoharju, E., Seppala, I., Lyytikainen, L. P., Viikari, J., Ala-Korpela, M., Soininen, P., . . . Lehtimaki, T. (2016). Blood hsa-miR-122-5p and hsa-miR-885-5p levels associate with fatty liver and related lipoprotein metabolism-the young finns study. *Scientific Reports, 6*, 38262. doi:10.1038/srep38262 [doi]

Raitoharju, E., Seppala, I., Oksala, N., Lyytikainen, L. P., Raitakari, O., Viikari, J., . . . Lehtimaki, T. (2014). Blood microRNA profile associates with the levels of serum lipids and metabolites associated with glucose metabolism and insulin resistance and pinpoints pathways underlying metabolic syndrome: The cardiovascular risk in young finns study. *Molecular and Cellular Endocrinology, 391*(1-2), 41-49. doi:10.1016/j.mce.2014.04.013 [doi]

Suomela, E., Oikonen, M., Virtanen, J., Parkkola, R., Jokinen, E., Laitinen, T., . . . Raitakari, O. T. (2014). Prevalence and determinants of fatty liver in normal-weight and overweight young adults. the cardiovascular risk in young finns study. *Annals of Medicine,* , 1-7. doi:10.3109/07853890.2014.966752 [doi]

Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J., & Lotvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology, 9*(6), 654-659. doi:ncb1596 [pii]

Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D., & Remaley, A. T. (2011a). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biology, 13*(4), 423-433. doi:10.1038/ncb2210 [doi]

Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D., & Remaley, A. T. (2011b). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biology, 13*(4), 423-433. doi:10.1038/ncb2210 [doi]

Videla, L. A. (2010). Liver NF-kappaB and AP-1 activation and PPAR-alpha expression are negatively correlated in obese patients: Pro-inflammatory implications. *Clinical Nutrition (Edinburgh, Scotland), 29*(5), 687-688. doi:10.1016/j.clnu.2010.03.006 [doi]

Vucenik, I., & Stains, J. P. (2012). Obesity and cancer risk: Evidence, mechanisms, and recommendations. *Annals of the New York Academy of Sciences, 1271*, 37-43. doi:10.1111/j.1749-6632.2012.06750.x [doi]

Wang, Y., Beydoun, M. A., Liang, L., Caballero, B., & Kumanyika, S. K. (2008). Will all americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring, Md.), 16*(10), 2323-2330. doi:10.1038/oby.2008.351 [doi]

Zimmet, P., Alberti, K. G., & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature, 414*(6865), 782-787. doi:10.1038/414782a [doi]