Exploring the relationships between microRNA expression, body composition and metabolic risk in healthy New Zealand women

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Abstract

Background: Excess adipose tissue is associated with metabolic risk and developing obesity related diseases. A significant amount of people are unknowingly metabolically unhealthy, having a high body fat percentage (BF%) despite normal body mass index (BMI) classification. Evidence of microRNAs (miRNA; miR) as potential biomarkers of metabolic risk has emerged and may prove useful in identifying those at metabolic risk where BMI classification may fail them.

Objectives: To explore miRNAs expression levels in body composition of healthy New Zealand (NZ) women and its association with metabolic markers, dietary and physical activity factors.

Methods: Cross - sectional design investigating healthy NZ women (n = 406) of three ethnicities (Māori, Pacific, European) aged 16 to 45 years. Body mass index and BF% defined body profile groups; "NN" group - normal BMI (≥18.5 and <25kg/m^2) and normal BF% (≥18%, <30%); "NH" group - normal BMI (<25 kg/m^2) and high BF% (≥30%); "HH" group – high BMI (≥25 kg/m^2) and high BF% (≥30%), of which 382, met the criteria. Anthropometry, metabolic biomarkers, miRNA, dietary, and physical activity factors were evaluated.

Results: 105 (27.5%), 70 (18.3%), and 207 (54.2%) participants were classified as having NN, NH, and HH body profile, respectively. The adjusted (age, deprivation index and other miRNAs) odds of having higher miR-222-3p were increased in NH (OR = 1.92, 95% CI 1.13-3.26) and HH (OR = 2.58, 95% CI 1.58-4.21) versus NN group. The adjusted odds of having higher miR-29b-3p decreased in HH (OR = 0.09, 95% CI 0.02-0.37) versus NN group. Higher miR-222-3p (1.084-14.438 AU) was associated with HH body profile (p = 0.002), higher leptin levels (p = 0.04) and sucrose intake (p = 0.025) and lower protein intake (p = 0.017). Higher miR-29b-3p (0.202-1.851 AU) was associated with lower HbA1c (p = 0.016), TNF-α (p = 0.001), IL-6 (p = 0.016), IL-10 (p = 0.021) and higher sucrose intake (p = 0.049), and higher miR-17-5p (0.103-0.806 AU) was associated with higher TNF-α (p = 0.014) and lower IL-6 (p = 0.001).

Conclusion: Our findings for miR-222-3p strongly support previous research, making it the most promising biomarker for obesity of the selected miRNA analysed in this study. Our study identified a selection of specific metabolic (Leptin, HbA1c) and inflammatory markers (IL6, IL-10, TNF-α) as well as dietary factors (sucrose, carbohydrate, protein) and light physical activity, to be associated with miR-222-3p, miR-29b-3p, and miR-17-5p. These findings suggest that miRNAs are involved in metabolic processes and, with further research, may be used as biomarkers of metabolic risk.

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Abbreviations

ADP	Air displacement plethysmography
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
BF%	Body fat percentage
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CDC	Centres for disease control and prevention
CDKN1B	Cyclin dependent kinase inhibitor 1B
cmiRNA	Circulating microRNAs
CRP	C-reactive protein
CVD	Cardiovascular disease
DALYs	Disability-adjusted life years
DGCR8	DiGeorge syndrome critical region 8
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DXA	Dual-energy X-ray absorptiometry
EDTA	Ethylene diamine tetraacetic acid
EXPLORE	"EXamining Predictors Linking Obesity Related Elements"
FDR	False discovery rates
FFQ	Food frequency questionnaire
GBD	Global burden of disease
GDM	Gestational diabetes mellitus
GLUT4	Glucose transporter type 4
HbA1c	Glycated haemoglobin
HC	Hip circumference
HDL	High-density lipoprotein
HH	High BMI, high BF%
HOMA-IR	Homeostasis model of insulin resistance

HSCT	Hematopoietic stem cell transplantation
HSPH	Harvard T.H. Chan School of Public Health
IL	Interleukin
ISAK	International Society for the Advancement of Kinanthropometry
JNK	c-Jun N-terminal kinase
LDL	Low-density lipoprotein
M1 or M2	Macrophage1 or Macrophage2
MCP-1	Monocyte chemoattractant protein 1
MetS	Metabolic syndrome
miRNA; miR; microRNA	Micro ribonucleic acids
MOH	Ministry of Health
mRNA	Messenger ribonucleic acids
MUHNR	Massey University Human Nutrition Research
NCD	Non- communicable diseases
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGT	Normal glucose tolerance
NH	Normal BMI, high BF%
NN	Normal BMI, normal BF%
NWO	Normal weight obesity
NZ	New Zealand
NZE	New Zealand European
OECD	Organisation for Economic Co-operation and Development
OR	Odds ratio
PPARG	Peroxisome proliferator- activated receptor gamma
qPCR	Quantitative polymerase chain reaction
RISC	RNA-induced silencing complex
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
STEMI	Segment elevation myocardial infarction
T1D	Type 1 diabetes
T2D	Type 2 diabetes

TAG	Triacylglycerols
TC	Total cholesterol
TNF-α	Tumour necrosis factor alpha
USA	United States of America
VAT	Visceral adipose tissue
WAT	White adipose tissue
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WtHR	Waist-to-height ratio

Chapter 1 – Introduction

1.1 Background

Obesity is the accumulation of excess body fat or adipose tissue, that increases the likelihood of developing non-communicable diseases (NCD) such as cardiovascular disease (CVD), diabetes mellitus (DM), hypertension and hyperlipidaemia (Khan et al., 2018; Panuganti et al., 2020; Pi-Sunyer, 2009; World Health Organization [WHO], 2021c). These diseases have shown to increase mortality, reduce quality of life, and productivity and are an economic burden on countries (Campfield et al., 1999; Tremmel et al., 2017).

From 1975 – 2016, the prevalence of obesity has tripled, with 2.8 million people dying each year as a result of being overweight or obese (WHO, 2021c). Globally, obesity is higher in women (15%) than in men (13%) (Organisation for Economic Co-operation and Development [OECD], 2019; WHO, 2021c) with similar trends seen in NZ; 32.8% men and 35.9% women are obese (Ministry of Health [MOH], 2021a). There are ethnic variations in NZ with a higher prevalence of obesity found in the Pacific (71.3%) and Māori (50.8%) compared to NZ Europeans (NZE) (31.9%) adults (MOH, 2021a).

The most common cause of obesity is an energy imbalance (measured in kilocalories). When energy intake exceeds energy expenditure, weight gain occurs. An energy surplus is caused by multiple factors such as excess food intake, sedentary lifestyle, an individual's metabolism, or genetic predisposition (Landrier et al., 2019; Ritchie, 2017).

Accumulation of fat occurs at various sites in the body. These sites can be classified according to fat storage location. Subcutaneous adipose tissue (SAT) is found beneath the skin and visceral adipose tissue (VAT) is found within the abdominal cavity (Harvard T.H. Chan School of Public Health [HSPH], 2010). An increase in VAT compared to SAT, is associated with worse metabolic outcomes (Elffers et al., 2017; Shah et al., 2014). It is more metabolically and biologically active and can produce substances that can cause inflammation. Excess body fat therefore has a chronic inflammatory effect which is one of the main drivers that can cause metabolic disorders (HSPH, 2010; Landrier et al., 2019).

Body mass index is the most common anthropometric measure to define obesity (WHO, 2021c). The BMI formula uses a person's weight in kilograms divided by their height in metres squared to classify them into one of several categories (WHO, 2021a) (**Table 1.1**).

Table 1.1 Classification of adults according to BMI, adapted from the World Health Organization (2021a)

	BMI (kg/m^2)	**Obesity Class**
Underweight	< 18.5	
Normal	18.5–24.9	
Overweight	25.0–29.9	
Obesity	30.0–34.9	I
	35.0–39.9	II
Extreme Obesity	40.0 +	III

Although BMI is a useful tool and correlates with body fat accumulation and metabolic health in large populations, it has its limitations. It does not account for an individual's specific body composition (proportion of lean versus fat mass) or the regional distribution of fat in the body, influencing metabolic risk (android/abdominal region - high metabolic risk; gynoid/hips - low metabolic risk) (Kruger et al., 2015). The concept 'Normal Weight Obesity' (NWO) demonstrates this and is defined as normal weight individuals who are metabolically obese and at risk of metabolic disease due to having high BF% (>30%), or VAT (Oliveros et al., 2014). There is a significant proportion of these people who have this 'hidden body fat profile' yet are classified as having a normal BMI (Kruger et al., 2015). A meta - analysis found subjects with NWO are at higher cardiometabolic risk having increased odds of hyperglycaemia (50%) diabetes (42%), HTN (40%), dyslipidaemia (83%) and hypertriglyceridemia (90%) compared to subjects with a normal weight and normal BF% (Mohammadian Khonsari et al., 2022). Therefore, BMI measurements alone would not identify their metabolic risk (Rush et al., 2009; Sluyter et al., 2011). Using BF% would be a better indicator of metabolic risk than BMI; but lack of scientific validation when determining BF% cut-off points is a limitation (Oreopoulos et al., 2011; Romero-Corral et al., 2008; Wollner et al., 2017). Although there is no validated BF% threshold to define obesity (Oliveros et al., 2014), collective evidence-based research and guidelines have shown that cut-off points ranging from 30% to 38% in women are useful to identify those who are at risk for metabolic disease and/or possibly "misclassified" by their BMI (Oreopoulos et al., 2011).

To maintain normal weight and regulate energy homeostasis, the adipose endocrine system must function correctly (Landrier et al., 2019). Diet and physical activity can influence miRNA expression levels which can alter gene expression and energy metabolism (use/storage); this could lead to fat accumulation and metabolic disease (Kruger et al., 2015; Quintanilha et al., 2017; Ultimo et al., 2018).

MicroRNAs are short non-coding molecules consisting of 21-25 nucleotides. They can alter and regulate gene expression by turning them on and off (Landrier et al., 2019). MicroRNA can alter normal physiology and act as mediators of diseases by regulating multiple pathways such as insulin signalling, adipokine expression and differentiation, food intake and immune-mediated inflammation (Deiuliis, 2016; Plotnikova et al., 2019). This suggests that miRNAs can function as 'extracellular endocrine and paracrine messengers that allow metabolic organ crosstalk' (Hammond, 2015; Ji et al., 2019).

Numerous works have linked miRNA dysregulation with obesity and metabolic disease development, identifying differential miRNA expression between healthy people and people with metabolic disease (obesity/T2D) (Huang et al., 2018; Ji et al., 2019; Kim et al., 2020; Tsukita et al., 2017; Zhong et al., 2018). Although miRNA mechanisms are still unclear, miR-222, miR-29b and miR-17-5p are associated with obesity, T2D, and/or inflammation (Arner et al., 2015; Deiuliis, 2016).

MicroRNA-222-3p (miR-222) is involved in cytokine regulation, cellular processes (glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration), the regulation of apoptotic processes and inflammatory responses (Brazil et al., 2001; Song et al., 2019). Upregulation of miR-222 is associated with an increase in adiposity, inflammation and T2D, whilst weight loss reduces its expression (Chartoumpekis et al., 2012; Ortega et al., 2015; Prats-Puig et al., 2013; Sadeghzadeh et al., 2020; Xie et al., 2009). In diabetes, miR-222 is highly expressed in β-cell dysfunction, is associated with HbA1c levels, and negatively effects GLUT4 expression, which is an important regulator of glucose metabolism/homeostasis (Belongie et al., 2017; Deiuliis, 2016; Huang et al., 2018; Xiao et al., 2018).

MicroRNA-17-5p (miR-17) is part of the miR-17~92 cluster which consists of five other miRNAs (miR-18a; miR-19a; miR-20a; miR-19b-1; miR-92a-1) (Kozomara et al., 2019). Expression of the miR-17~92 family is involved in adipogenesis (Lin et al., 2009), adipocyte differentiation (Hilton et al., 2013; McGregor et al., 2011; Q. Wang et al., 2008), regulating

inflammatory phenotypes of adipose tissue macrophages (X. Zhang et al., 2020) and is differentially expressed in most cancers, obesity and atherosclerosis (Dellago et al., 2017). The cluster is also associated with impaired glucose metabolism (Xiao et al., 2018). By itself, miR-17-5p is involved in numerous processes, regulating genes involved in autophagy, apoptosis, and cell cycle regulation (Dellago et al., 2017). Its expression has been linked to inflammation, obesity, lipid accumulation, adipose tissue dysfunction and the development of obesity-related disorders such as T2D (Kloting et al., 2009; Tan et al., 2019; X. Zhang et al., 2020). Expression of miR-17 is reported to be down-regulated in obese subjects and correlates inversely with BMI (Heneghan et al., 2011; Kloting et al., 2009). Furthermore, a high fat, high sucrose diet has shown to alter miR-17 levels (Tan et al., 2019; Yerlikaya et al., 2019).

MicroRNA-29b-3p (miR-29b) is part of the miR-29 family which includes miR-29a and miR-29c (Kriegel et al., 2012). Expression of the miRNA-29 family is most associated with cancer (Y. Wang et al., 2013). The family is linked to the regulation of cell differentiation, apoptosis promotion, and antifibrotic effects in some organs, including the heart (Kriegel et al., 2012). Recently, it has been found to play a major role in T2D balancing differentiation and proliferation in pancreatic β-cell (Vienberg et al., 2017). Prediabetic pancreatic β-cell miR-29 has shown to be a main contributor to inflammatory signatures in diabetes by promoting macrophage-induced inflammation (Y. Sun et al., 2021). In a diabetic setting, miR-29 expression significantly differs in T2D (Hsieh et al., 2014) with an increase in expression of the miR- 29 family found in the liver, skeletal muscle, and fat (He et al., 2007; Kurtz et al., 2014; Liang et al., 2013; Massart et al., 2017). Specifically, miR-29a and miR-29b were up-regulated in hyperinsulinemia and hyperglycaemia (He et al., 2007). MicroRNA-29b specifically, is associated with pancreatic islet β cell function, glycaemic control and was dysregulated in obese prediabetic and T2D subjects and expressed in damaged/stressed human islets (Saravanan et al., 2019).

Significant progress has been made over the years in clarifying the role of miRNAs in a variety of diseases, including cancers, obesity, diabetes, inflammatory, and autoimmune conditions. Evidence on the three miRNAs above suggests that they may be strongly involved in obesity and T2D manifestations; however, more research is needed to consider these specific miRNAs as potential biomarkers and/or therapeutic treatment of obesity and T2D (Karolina et al., 2012; Liang et al., 2013).

There is little research on body composition in NZ women, miRNA and how they all may link to metabolic health. Various miRNAs are associated with energy expenditure and metabolic disorders, thus there is potential to use miRNA as biomarkers of health to help determine those who are metabolically at risk (Deiuliis, 2016). If we can identify specific markers for disease risk linked to adiposity, then it would be possible to determine the risk in people where BMI might misrepresent them (NWO). Although many studies reported significant differences in miRNAs between healthy and metabolic disease states in a variety of people (Bork-Jensen et al., 2015; Pescador et al., 2013; Sliwinska et al., 2017), there are noticeable inconsistencies in the findings (Deiuliis, 2016).

Overall, there is a need for more research and information on:

- MicroRNA expression in NWO; to determine if the BMI system is overlooking people who may be at risk.
- If miRNAs could be used for early detection of metabolic risk in NWO and as biomarkers of metabolic disease.
- Factors that influence miRNA expression.

Few studies have explored NWO (Di Renzo et al., 2010; Karelis et al., 2004; Marques-Vidal et al., 2008; Romero-Corral et al., 2010), and its link to miRNA and metabolic disease risk (Kruger et al., 2015). Furthermore, little is known about the environmental drivers of miRNA expression related to adipose tissue (diet and physical activity) in people with different body composition profiles. This could potentially help to identify appropriate biomarkers to assess metabolic health and contribute to research using biomarkers for prevention and treatment strategies. The study is also unique in that it can explore miRNA data from multiple premenopausal NZ women from different ethnicities with different body composition profiles (Kruger et al., 2015).

1.2 Aims, Objectives and Hypotheses

Aim

Investigating miRNA expression related to energy expenditure/storage as a predictive factor associated with hidden and apparent body fat profiles in 16- to 45-year-old women (post-menarcheal and pre-menopausal) NZE, Māori, and Pacific women.

Objectives

- To explore the differences in miRNA expression in women from different body profile groups (BPG).
- To assess which of the three miRNAs are associated with biomarkers of metabolic disease.
- To determine if women classified in the hidden fat (NH) group have had changes in miRNA expression associated with metabolic disease.
- To investigate whether selected miRNAs are associated with different dietary factors and levels of physical activity.
- To explore differences in miRNA expression in Māori, Pacific, and NZE women (NZE).

Hypothesis

Subjects in the NH group (normal BMI and high BF%) would be at a higher metabolic risk than those in the NN group (normal BMI and normal BF%) (Kruger et al., 2015).

The interactions between diet, physical activity, body composition and metabolic disease risk are, in part, mediated by changes in miRNA expression.

1.3 Structure of the thesis

This thesis comprises four chapters. Chapter 1 introduces the research topic providing the scope, aims and purpose of the study. Chapter 2 is a narrative literature review covering obesity, its prevalence and aetiology, anthropometric and body composition assessments, body fat profile groups, adipose tissue, and microRNAs relationship to metabolic disease. To conduct this review, databases such as PubMed, Google Scholar, SpringerOpen, PLOS one, and Elsevier-Science Direct were used. Keywords included: Obesity; body composition; BMI; body fat; adipose tissue; visceral fat; normal weight obesity; metabolic disease;

metabolic health; metabolic syndrome; inflammation; CVD; diabetes; insulin; microRNA; microRNA dysregulation; miR-222; miR-17; miR-29b; ethnicity. Searches were conducted between 2020-2022, ordered according to keyword relevance, and keywords were used by themselves and combined. Chapter 3 is the manuscript of the research study, which is a complete presentation of the research conducted. It includes an abstract, introduction, methods, results, discussion, conclusion, supplementary tables, and references. Chapter 4 includes a brief overview and achievement of the study objectives, strengths, limitations, and recommendations for future research. For all chapters involved in this thesis, the APA referencing style was used. This thesis does not redefine abbreviations in every chapter.

1.4 Contributors to the research

Contributor	**Contribution to Research /Thesis**
Tazyn Fini	Thesis author Data processing, statistical analysis, and interpretation of result, writing of the thesis
A/Prof Rozanne Kruger	Main thesis supervisor Concept and research design of the EXPLORE study Ethics application Execution of the study, Interpretation of results Revision and approval of thesis
Wendy O'Brien Martin Dickens	Thesis co-supervisors Thesis revision
Shakeela Jayasinghe Wendy O'Brien	Study recruitment, screening, and testing of participants
Pam von Hurst, Cathryn Conlon, Kathryn Beck, Richard Swift, Owen, Mugridge, Maria Casale, Andrea Fenner, Jenna Schrijvers, Adrianna Hepburn, Zara Houston, Sarah Philipsen, Carmel Trubuhovich, Rozanne Kruger	Study facilitation of participant testing: screening questionnaire, blood pressure, blood sample, BODPOD and DXA scanning

Chapter 2 – Literature Review

2.1 Introduction

Obesity is an excess accumulation of body fat or adipose tissue that may increase the risk of developing several NCD and poor metabolic health (Panuganti et al., 2020). Good metabolic health is defined as having an optimal waist circumference (WC), blood pressure (BP), blood sugar, triacylglycerols (TAG), high-density lipoprotein (HDL), and total cholesterol (TC) levels (Araujo et al., 2019; Sweet, 2018). Poor yet preventable lifestyle factors such as unhealthy diet, physical inactivity, tobacco use and alcohol abuse can affect metabolic health (WHO, 2020a). These behaviours can lead to metabolic abnormalities and physiological changes, increasing the risk of developing NCDs such as CVD, DM, hypertension, and hyperlipidaemia (Bell et al., 2015; Khan et al., 2018; Panuganti et al., 2020; Pi-Sunyer, 2009; Sweet, 2018; WHO, 2021c).

Obesity is classified as a complex NCD, as many factors can influence adipose tissue regulation (genetics, metabolic and hormonal processes, environment, diet, and lifestyle) (Campfield et al., 1999; Luo et al., 2007). This increased risk of impaired health can be a socioeconomic burden for the healthcare sector and the individual (Kjellberg et al., 2017), by reducing the quality of life and increasing the risk of morbidity and early mortality (Campfield et al., 1999).

Obesity is classified by a BMI of $\geq 30 kg/m^2$. Many large epidemiological studies have shown associations between an increased risk of chronic diseases, health complaints, physical disability, and a high BMI (Agrawal et al., 2016; Pi-Sunyer, 2009). Research however shows that body composition is a stronger indicator of chronic disease development than BMI (E. Thomas et al., 2012; Tomiyama et al., 2016). Currently, there are ongoing investigations into miRNA as potential drivers of body composition and metabolic dysregulation (Iacomino et al., 2017; Ramzan et al., 2021). This review aims to investigate the published literature on obesity, body composition, miRNA, and their relationships with metabolic health.

2.2 Prevalence of obesity

Obesity is one of the leading causes of premature death (Ritchie, 2017), resulting in at least 2.8 million deaths per year (WHO, 2021b). It is a persistent condition that is on the rise in both developed and developing countries; affects all ages, genders and socio-economic groups and can have serious social and psychological effects (WHO, 2021c). Between 1975 and 2016, obesity has tripled worldwide, with 1.9 billion adults classified as overweight (39%) of which 650 million (13%) are considered obese (WHO, 2021c).

Globally, the prevalence of obesity is higher in women than men, with the NCD Risk Factor Collaboration age-standardised prevalence of obesity reporting an increase from 3.2% to 10.8% in men and 6.4% to 14.9% in women between 1975-2014. Similar trends have been reported in GBD and OECD data (Collaborators et al., 2017; Inoue et al., 2018; OECD, 2019). New Zealand followed world trends, showing an increase from 10% in 1970 to 30% in 2013, then stabilising until 2019/2020 (MOH, 2015; MOH, 2021a; WHO, 2021c), when it increased to 31.2%. The 2020/2021 NZ Health Survey reported a significant increase in obesity ($p < 0.01$), with one in three adults (34.3%) older than 15 years of age being obese (MOH, 2021b). In 2016, NZ women had a higher average BMI (28.3 kg/m^2) than women globally (25 kg/m^2), which has continued to rise and is currently at 28.9 kg/m^2 (MOH, 2021a; Ritchie, 2017). The latest NZ health survey shows obesity among adults is more prevalent in Pacific (71.3%) and Māori (50.8%) compared to NZE (31.9%) adults. Extreme obesity (BMI $\geq$ 40kg/m^2) is also higher among Pacific (33.6%), Māori (17.1%) and NZE (6.1%) females than their male counterparts (13.3%, 8.8%, and 2.6%, respectively), indicating one of many ethnic health inequalities faced by these groups (MOH, 2021a).

2.3 Aetiology

A disruption in energy homeostasis, where energy intake exceeds energy expenditure (energy used to function and perform daily activities), is the most common cause of obesity (Ritchie, 2017). However, although this energy balance model is the generally accepted view, it has limitations to 'explain' obesity (Wells et al., 2011). The neuro-endocrine system is largely responsible for regulating energy homeostasis pathways (Ghanemi et al., 2018). If there is a

disruption in these pathways, it could be the starting point for obesity pathogenesis, rather than a consequence of adaptation (Ghanemi et al., 2018).

The body releases energy by breaking down and metabolising carbohydrates, fats, and protein consumed in the form of food. Transformed substances can be oxidized to release metabolic energy for biological processes, or they can be stored (Hall et al., 2012). Carbohydrates are stored in skeletal muscle and the liver in the form of glycogen; however, reserves are relatively small and turnover is rapid compared to fat (Hall et al., 2012). Fat is stored in adipose tissue as TAG, the largest source of stored excess energy and the main energy reserve of the body (Hall et al., 2012). An ongoing energy surplus will lead to changes in body composition, particularly fat accumulation (Hall et al., 2012; Spiegelman et al., 2001).

The World Health Organization (2021c) identifies excess food intake and physical inactivity as the main drivers of global weight gain. Over the past century, food supply available for consumption led to increased energy intake and physical inactivity, both of which increased globally. Over 25% of the world's adult population do not meet the recommended amount of moderate (≥150min) or vigorous (≥75min) physical activity each week, with no notable improvements in activity levels since 2001 (WHO, 2020c). The impact of individual foods/nutrients on metabolic health is not yet fully understood (Bell et al., 2015). Australian adults with unhealthy eating patterns (processed and refined foods) had a lower chance of being metabolically healthy, regardless of BMI (Bell et al., 2015). Studies investigating the interaction between physical activity and metabolic health in the general population found that physical activity was associated with a lower incidence of individual metabolic risk factors and metabolic syndrome (MetS) (Churilla et al., 2009; Healy et al., 2008).

The technology boom has facilitated the development of an obesogenic environment through enhanced accessibility and availability of energy-dense, nutrient-poor foods and has contributed to the sedentary nature of many forms of work via ease of transportation and performance of daily tasks (Abbade et al., 2015; Gilmore et al., 2014; WHO, 2021c). Despite populations living in the same obesogenic environment, not everyone is obese, suggesting that some people may be more susceptible to weight gain than others (Albuquerque et al., 2017). Other obesity drivers can be attributed to a person's metabolism/hormonal imbalances or genetic predisposition (Landrier et al., 2019; Sidhu et al., 2017). Various genetic studies have identified numerous genes associated with adiposity and weight gain, indicating that obesity is highly heritable (Albuquerque et al., 2015; Panuganti et al., 2020). A cohort

familial study found a high correlation between parental obesity and child BMI; children whose parents are both obese, have a higher risk of becoming obese themselves, compared to those from non-obese parents (Lake et al., 1997). However, it is difficult to distinguish whether the correlation is due to genetic or environmental factors. A meta-analysis on 31 twin studies showed that in adults, 47-80% of BMI variation can be explained by genetics (Elks et al., 2012). Another study by Silventoinen et al. (2016), analysed 87,782 twin-pairs from 45 cohorts, finding an association between BMI variation and genetic factors. These findings were supported by an adopted children study, in which BMI was strongly correlated with biological parents (Silventoinen et al., 2010).

Epigenetics is a set of mechanisms that regulate gene expression without altering the DNA sequence. One such mechanism is via miRNA. Although all aspects of miRNA are not yet fully understood, studies found that miRNAs were linked to obesity and metabolic health by playing a role in altering metabolic function and adipocyte differentiation, which increases metabolic risk (Deiuliis, 2016; Parrizas et al., 2016; Son et al., 2014). Although there is a well-established association between obesity and genetics, the prevalence of obesity is increasing far too quickly to blame genetics alone (Spiegelman et al., 2001). This notion is supported by a WHO report suggesting that an energy imbalance prompted by an obesogenic environment is a key driver in propelling the growth of the disease (WHO, 2020b).

Although metabolic abnormalities are commonly associated with obesity, there is evidence that weight alone cannot determine metabolic risk (Pajunen et al., 2011). Araujo et al. (2019) evaluated data from 8721 adults in the 2009-2016 National Health and Nutrition Examination Survey and found that one in eight adults (12.2%) in the USA had optimal metabolic health. Of these, less than one third (31.1%) of normal weight adults were metabolically healthy. Zheng et al. (2015) assessed the metabolic health of 5013 Chinese individuals with obesity, and found that 10-27% were metabolically healthy (Zheng et al., 2015), similarly Bluher (2010) study found 10-25% of Caucasians subjects with obesity to be metabolically healthy. These studies show that metabolic health is low in all adults, not just in those with obesity, suggesting that body weight shouldn't be the only indicator of metabolic disease risk, as people with normal weight can also develop obesity-related diseases (Bell et al., 2015; Sweet, 2018).

2.4 Body composition

Accumulation of body fat and its metabolic risk depend strongly on where fat is distributed in the body (Luong et al., 2018). Subcutaneous adipose tissue accounts for 90% of body fat. Visceral adipose tissue surrounds vital organs and accounts for 10% of body fat yet may increase with poor diet and sedentary lifestyle (HSPH, 2010; HSPH, 2019; Luong et al., 2018). Both SAT and VAT differ in location and the cardiometabolic risk they pose (Sam, 2018). Accumulation of VAT leads to truncal (android) obesity and is highly associated with metabolic risk and developing MetS. In contrast, SAT accumulation leads to peripheral obesity, accumulating fat in the buttocks, hips, and thighs, which appears to be protective against MetS (M. Zhang et al., 2015; Zhu et al., 2022). Fat can also be deposited into non-adipose tissue such as the liver, muscle, and pancreas, known as ectopic fat. Although deposition of small amounts of ectopic fat is relatively normal, excess accumulation can impair the normal function of these tissues and increase metabolic risk (E. Thomas et al., 2012).

Due to health-related consequences of excess body fat, it is important to find ways to identify those at risk. The most common tool to assess weight status and associated health risk is BMI (Panuganti et al., 2020; Ritchie, 2017). The BMI is valued globally, as it is simple, inexpensive, and strongly correlated with more direct measures of body fat, such as bioelectrical impedance (BIA), dual-energy x-ray absorptiometry (DXA), air displacement plethysmography (ADP) and underwater weighing (Centers for Disease Control and Prevention [CDC], 2020b; HSPH, 2021; Q. Sun et al., 2010; Willett et al., 2006). A persons BMI is calculated using their body mass (kg) divided by the square of their body height (m) to categorise weight status **(Table 2.1).** However, its merits are contested, as BMI cannot directly measure body fatness, fat location, or health status (CDC, 2020a), nor does it account for sex, age, ethnicity, muscle, or bone mass, all of which can influence metabolic risk. (Borga et al., 2018; CDC, 2020a; Panuganti et al., 2020).

Table 2.1 Classification of overweight and obesity by BMI and associated disease risks

Classification by BMI	BMI (kg/m^2)	Obesity Class	Comorbidity Risk
Underweight	< 18.5		Low
Normal	18.5–24.9		Average
Overweight	25.0–29.9		Increased
Obesity	30.0–34.9 35.0–39.9	I II	Moderate Severe
Extreme Obesity	≥40.0	III	Very Severe

Adapted from: World Health Organisation (2000)

Increased BMI is associated with increased metabolic risk (Khan et al., 2018). The GBD study attributed high BMI to increased global deaths and disability-adjusted life years (DALYs), both of which have more than doubled between 1990-2017 (Dai et al., 2020).

Truncal obesity and its associated metabolic risk are assessed using indirect measures of fat distribution; WC and waist-to-hip ratio (Borga et al., 2018; WHO, 2000) (**Table 2.2**).

Table 2.2 Waist circumference cut-off points and association with disease risk

Indicator	Cut-off points		Risk of metabolic complications
	Men	Women	
Waist circumference	>94 cm	>80 cm	Increased
Waist circumference	>102 cm	>88 cm	Substantially increased
Waist–hip ratio	≥0.90	≥0.85	Substantially increased

Adapted from: World Health Organization (2000)

A combination of BMI and WC are a good predictor of metabolic abnormalities as they correlate with BF% (Jensen, 2009; Ross et al., 2020). However, some argue they are insensitive to the actual distribution of body fat, thus are poorer predictors of metabolic health risk (Borga et al., 2018; E. Thomas et al., 2012). In obese and normal-weight individuals, metabolic abnormalities have a stronger link to visceral or ectopic fat (E. Thomas et al., 2012), and are only somewhat reflected by the overall abdominal fat (Schulze, 2019). A study by Camhi et al. (2011), found that both WC and BMI were more highly correlated with fat mass and SAT than with VAT. In the study, the relationship between WC, BMI, VAT, SAT, and fat mass also differed significantly depending on sex and race, suggesting that WC and BMI cut-off points may not reflect the same level of fat mass or abdominal obesity in men and women of different ethnicities. This indicates that although these indirect measurements have their place in a clinical setting, adding a more direct measure of body fat, ideally from DXA or ADP may help to better determine the risk related to adiposity,

especially in non-obese groups (Boneva-Asiova et al., 2008; Borga et al., 2018; Hames et al., 2014; Jean et al., 2014; Wingfield et al., 2014). However, these machines are expensive and availability is low (Kuriyan, 2018).

Normal weight obesity, a term introduced by Dr Neil Ruderman in 1981, refers to a subgroup of individuals with normal BMI who are metabolically obese and at risk of metabolic dysregulation/disease due to a high BF% (>30%), VAT or high total body fat mass (Oliveros et al., 2014). Research shows that a significant proportion of adults have NWO or a 'hidden body fat' profile' (normal BMI but high BF%) (Kruger et al., 2015), and increases in BF% can cause similar health risks as those classified as obese (Bosomworth, 2019; Coutinho et al., 2011; Jean et al., 2014). A pilot study consisting of 116 NZE women, aged 18-44 years, found 21.4% had this hidden body fat profile, along with increased biomarkers indicative of metabolic risk (fasting plasma leptin and insulin concentrations. These women also led a sedentary lifestyle, making it unclear whether the fat was related to environmental and/or genetic factors (Kruger et al., 2015).

To date, there is no validated BF% threshold to define obesity (Oliveros et al., 2014; Oreopoulos et al., 2011). Determining BF% cut-off points whilst accounting for ethnic variations has been problematic and lacking scientific validation (Oreopoulos et al., 2011; Wollner et al., 2017). However, research has shown that cut-off points for body fat of between 30-38% for women are useful in identifying those who may be at risk for metabolic disease and/or possibly misclassified by their BMI (e.g., Pacific or Māori ethnicities) (Oreopoulos et al., 2011).

There are an increasing number of cohort studies assessing obesity and NWO and its impact on metabolic health (Caleyachetty et al., 2017; Eckel et al., 2018; Eckel et al., 2016; Fan et al., 2013; Kramer et al., 2013; Lassale et al., 2018; Zheng et al., 2016). More epidemiological research is needed in the classification of metabolic health status for clinicians to arrive at an agreement in defining obesity and metabolic health to optimally target and monitor obese and normal weight individuals (Schulze, 2019).

2.5 Adipose tissue

2.5.1 Adipose Tissue Function and Cell Types

Adipose tissue, specifically white adipose tissue (WAT) plays an integral role in energy storage, insulin sensitivity and as an endocrine gland, producing hormones and substances that can affect health and increase risk of metabolic disease (HSPH, 2019; Richard et al., 2020). White adipocytes have a large capacity to store lipids and can continuously expand in size (hypertrophy) and number (hyperplasia or adipogenesis) (Richard et al., 2020).

In an energy surplus state, body fat is stored as TAG in adipose tissue. When required (during fasting, exercising or cold exposure), adipocytes mobilise their TAG stores and release free fatty acids to fuel the body (Richard et al., 2020). Adipose tissue is vital for regulating lipid metabolism. Hormones are used to balance lipid storage and lipolysis (e.g., leptin and adrenaline stimulate lipolysis, while insulin inhibits it). This balance between lipogenesis and lipolysis in adipocytes is critical to maintain energy homeostasis and insulin sensitivity (Richard et al., 2020).

2.5.2 Adipose Tissue and Inflammation

Obesity and dysfunctional adipocytes cause a chronic inflammatory effect in the body by altering immune cell number and function, which can lead to metabolic disorders (Heredia et al., 2012; Longo et al., 2019). If, during an energy surplus, adipose tissue expands beyond its limit, adipocytes rupture and cause adipocyte cell death (Monteiro et al., 2006; Richard et al., 2020). This triggers pro-inflammatory macrophages to travel to the damaged site, release inflammatory cytokines, and remove debris (Cinti et al., 2005). An increase in circulating levels of inflammatory cytokines such as C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) has been strongly correlated to excess adipose tissue, insulin resistance, CVD and obesity-related metabolic disorders (Bullo et al., 2003; Festa et al., 2001; Park et al., 2005). With this strong association between adipocyte size and death (Cinti et al., 2005), a normal inflammatory response becomes chronic in obesity and cytokine production ceases to resolve, causing impaired adipocyte insulin signalling, further inflammation, and adipose tissue dysfunction (Richard et al., 2020).

Hypoxia, free fatty acids, and mechanical stress of adipocytes are some proposed mechanisms of obesity-associated inflammation. The free fatty acids bind to toll-like receptor

4 and 2, leading to activation of the JNK1 and NF-kB pathways that regulate the secretion of chemokines like MCP-1, which cause pro-inflammatory macrophage infiltration (Saltiel et al., 2017). Hypoxia can induce a heterodimeric basic helix-loop-helix transcription factor called Hypoxia Inducible Factor 1, that regulates a gene programme involved in the initiation of inflammation. Adipocyte expansion in the extracellular matrix also increases adipocyte mechanical stress (Saltiel et al., 2017). Mechanisms behind obesity-associated inflammation are not fully understood, but macrophages are always involved (H. Shi et al., 2006; K. Sun et al., 2011; Trayhurn, 2013).

Higher macrophage infiltration in omental fat versus subcutaneous fat has been found and exaggerated by central obesity, potentially linking abdominal fat accumulation with metabolic disease (Harman-Boehm et al., 2007; Yamada et al., 2018). The main purpose of adipose tissue macrophages is to clear dead adipocytes (D. Thomas et al., 2017). There are two types of adipose tissue macrophages: M1 and M2, classified by their cell surface markers and secretory profile (Rodriguez et al., 2015). The M1 is responsible for secreting pro-inflammatory cytokines (TNF-α, IL-6, MCP-1), while M2 is associated with anti-inflammatory responses and produces immunosuppressive factors such as IL-10 (Rodriguez et al., 2015). In murine studies obesity altered the M1:M2 ratio, favouring an increase in M1 production. Adipose tissue macrophage accumulation is also positively correlated with increased adiposity, providing a credible mechanism for increased inflammation associated with obesity (D. Thomas et al., 2017; Weisberg et al., 2003). Fjeldborg et al. (2014) also found enhanced anti- and pro-inflammatory cytokines with increased macrophage numbers in adipose tissue from obese subjects; however, in contrast, an unexpected shift toward the M2 profile was favoured. The shift suggested a protective mechanism to counteract the increased inflammation associated with obese subjects. However, evidence of M2 subtypes secreting inflammatory cytokines including TNF-α and IL-6 has been found (Guttman et al., 2016; L. X. Wang et al., 2019; Zeyda et al., 2007). This suggests various forms of adipose tissue macrophages exist, which may be polarised and can be reprogrammed with appropriate stimuli such as local environment changes (cytokines present) (Tarique et al., 2015; Xu et al., 2013).

Strong correlations of CRP, TNF-α and IL-6 have been found in obese subjects; with CRP significantly associated with BMI and IL-6 and highly correlated with visceral fat (Park et al., 2005). A study focusing on women similarly found CRP and body composition to be strongly correlated (BMI; WC; adipose body mass) and more strongly correlated in women than men

(Festa et al., 2001; Maachi et al., 2004). Weight loss has shown to reduce TNF-α, IL-6 and CRP, and increase the anti-inflammatory molecule adiponectin, all supporting the idea that excess body fat and associated environmental changes drive inflammation (Bianchi, 2018).

2.5.3 Adipose Tissue Dysfunction and Insulin

Insulin is the main hormone responsible for energy storage. It is tissue specific and works by binding to insulin receptors on the cell surface in muscle, heart, liver, vascular endothelium, and adipose tissue. In the vascular endothelium and heart, vasodilation is triggered, and in the liver, gluconeogenesis is inhibited, and glycogenesis is stimulated (Vargas et al., 2020). In adipose tissue, skeletal muscle, and the heart, cells are triggered to take up and metabolise glucose, and in adipose tissue, lipolysis is inhibited (Richard et al., 2020).

Obesity can cause insulin resistance (Hardy et al., 2014), which in adipose tissue can lead to increased lipolysis and increased levels of plasma free fatty acids in the fed and fasted state. Excess free fatty acids promote storage as visceral fat and uptake/ectopic storage in vital organs such as the liver and muscle impairing their metabolic function. (Petersen et al., 2018; Richard et al., 2020). Ectopic and visceral fat can impair insulin signalling and contribute to whole body insulin resistance and metabolic risk; more so than SAT (Hsieh et al., 2014; Richard et al., 2020). Other studies have found that truncal SAT plays a major role in obesity-related insulin resistance (Abate et al., 1995; Maffeis et al., 2008).

Mechanisms of insulin resistance related to adipose tissue, can be linked to adipose tissue inflammation. Decrease in adiponectin, leptin, IL-6, and other adipokine concentrations and increase in free fatty acid secretion are thought to be the most important mechanism of insulin resistance development (Mlinar et al., 2006). This reduces insulin's ability to supress lipolysis, thus free fatty acids continue to be released (Jung et al., 2014; Panuganti et al., 2020). The excess free fatty acids are then processed by the liver, causing an overproduction of very low-density lipoproteins that can alter lipid metabolism and contribute to dyslipidaemia (Jung et al., 2014). Positive correlations between BF%, peripheral SAT, truncal SAT, WC, and insulin resistance have been found (Dwimartutie et al., 2010). Body fat percentage is seen as a good indicator of insulin resistance especially in normal weight individuals (Zegarra-Lizana et al., 2019). Further longitudinal studies are needed to validate findings. Overall altered adipokine secretion and low-grade inflammation can therefore lead to altered glucose and lipid metabolism, increasing visceral obesity and cardiometabolic risk (Panuganti et al., 2020).

2.5.4 Adipose Tissue an Endocrine Organ

The central nervous system is responsible for sending neural signals that directly target tissues to control metabolism and energy balance. The brain gathers information about energy stores via peripheral hormones which then act to regulate neuronal circuits in the hypothalamus (Faintuch et al., 2020).

Adipocytes secrete various bioactive molecules that affect many physiological and pathological processes. They exclusively produce 'adipokine' hormones such as leptin and adiponectin (Guilherme et al., 2008; Stern et al., 2016). These hormones along with ghrelin can regulate these neural circuits in the hypothalamus (Faintuch et al., 2020).

During fasting when energy reserves are low, ghrelin is secreted by the stomach to stimulate hunger/ food intake and supresses energy expenditure (Faintuch et al., 2020). In obese subjects, studies have found higher density and expression of ghrelin-forming cells (Maksud et al., 2011) and a dysfunction in ghrelin reducing after meals, potentially triggering hyperphagia (English et al., 2002).

Leptin serum concentration is proportional to body fat (Sidhu et al., 2017), enabling it to regulate energy homeostasis. Higher levels of leptin should supress energy intake and increase expenditure; however, in overweight or obese individuals, this is not always the case, suggesting leptin resistance (Iqbal et al., 2020; Sidhu et al., 2017). A study observing circulating soluble leptin receptors, found obese and overweight subjects had fewer compared to lean subjects (Elmquist et al., 1999; Ogier et al., 2002), suggesting a lack of leptin and/or soluble leptin receptors can lead to obesity (Iqbal et al., 2020).

Leptin can influence the central nervous system and peripheral organs (liver, skeletal, muscle, pancreatic β and adipose cells) by participating in endocrine, autocrine and paracrine energy regulation (Sidhu et al., 2017). Leptin and the hypothalamus work together to maintain energy homeostasis, with leptin travelling across the blood-brain barrier acting on the hypothalamus which supresses appetite centres (Iqbal et al., 2020; Sidhu et al., 2017). However, in obesity, leptin transport across the blood-brain barrier is reduced (Elmquist et al., 1999; Levin et al., 2004) and leptin's ability to modulate hypothalamic activity is also lower (Caro et al., 1996; El-Haschimi et al., 2000; Lartigue et al., 2011; Rhee et al., 2011). This can result in weight gain and obesity (Sidhu et al., 2017), as the lack of leptin or its resistance mimics starvation, causing the hypothalamus to stimulate appetite centres, as it

perceives body fat stores as being low (Gautron et al., 2011; Iqbal et al., 2020). Leptin also regulates thyrotropin-releasing hormone and thyroid-stimulating hormone, within the hypothalamus to stimulate lipogenesis, lipolysis and regulating energy storage, expenditure, and glucose/lipid metabolism (Mullur et al., 2014; Sidhu et al., 2017). In human and animal studies, an increase in free leptin is associated with an increase in BMI and leptin resistance, causing thyrotropin-releasing hormone inactivation, allowing for euthyroidism in diet-induced obesity (Mullur et al., 2014).

Adiponectin has anti-inflammatory, anti-hyperglycaemic and anti-atherogenic effects; however, when body fat mass is high, adiponectin levels drop, diminishing these effects (Liu et al., 2016; Richard et al., 2020). In obese patients, insulin resistance, dyslipidaemia and atherosclerosis are associated with lower levels of adiponectin (Sidhu et al., 2017). Studies have found links between adiponectin levels and fat types, such as adiponectin concentrations being determined by intra-abdominal fat mass (Cnop et al., 2003), decreases in adiponectin levels with increased adipose tissue (Reneau et al., 2018), and VAT being negatively correlated with adiponectin gene expression and adiponectin secretion. This may explain why lower adiponectin levels are observed in centrally obese people (Reneau et al., 2018; Sirbu et al., 2018). A conflicting study found that SAT rather than VAT is inversely correlated with adiponectin levels (Frederiksen et al., 2009). A study of obese subjects, found a 46% increase of mean plasma adiponectin levels with a 21% reduction in mean BMI, showing weight loss to improve adiponectin levels (W. S. Yang et al., 2001).

Adipocytes can also produce oestrogen, which plays an integral role in body weight, fat distribution, energy homeostasis, and metabolism (Nelson et al., 2001; Sidhu et al., 2017), (Nelson et al., 2001). Adiposity can influence oestrogen levels (Sidhu et al., 2017). Studies have shown a non-linear association between oestradiol levels and body fat in healthy women; that both very low and high body fat levels are associated with decreased oestradiol and that the relationship between oestradiol and body fat was strongly influenced by women's energy balance (Colleluori et al., 2018; Marchand et al., 2018; Nelson et al., 2001; Ziomkiewicz et al., 2008). Balance between oestrogen production is important, as both oestrogen deficiency, or surplus, can lead to metabolic dysfunction (Colleluori et al., 2018; Sidhu et al., 2017).

Overall, leptin, adiponectin, oestrogens, and thyroid-stimulating hormone are strongly associated with adipose tissue levels. Findings suggest that dysregulation of expression in any

of these hormones may contribute to metabolic dysfunction/diseases (Mauvais-Jarvis et al., 2013; Richard et al., 2020).

2.6 MicroRNA overview

Short non-coding RNA or miRNAs consist of 21-25 nucleotides and regulate various metabolic processes by altering the expression of specific genes/proteins (Landrier et al., 2019; Treiber et al., 2019).

For miRNA biogenesis, firstly, miRNAs are transcribed as precursor molecules, which are then converted into mature miRNA by the enzymes Drosha and Dicer, including a process where a member of the Argonaute protein family binds to the mature miRNAs to form a RNA-induced silencing complex (RISC). The RISC is then released and binds to messenger RNA (mRNA), blocking translation and causing degradation or cleavage of mRNA, which ultimately determines whether genes are turned on or off (Hammond, 2015; Treiber et al., 2019) (**Figure 2.1)**. This process has been thoroughly reviewed elsewhere (Hammond, 2015; Krol et al., 2010; Treiber et al., 2019; Winter et al., 2009).

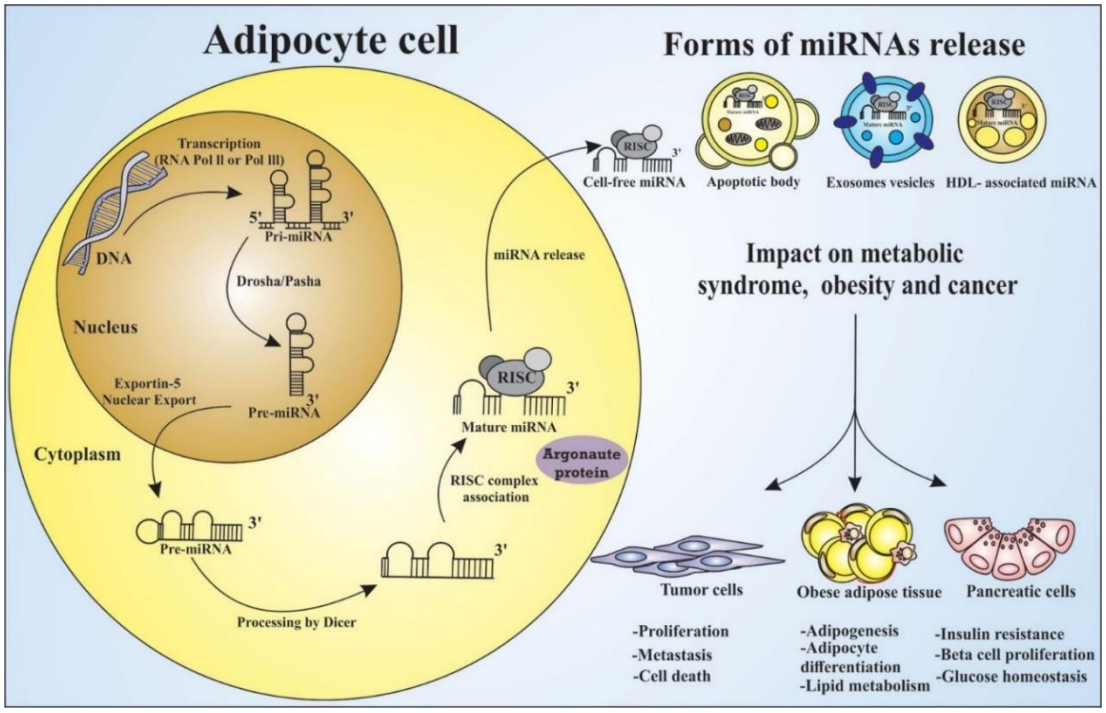

Figure 2.1 Biogenesis and release of miRNA from adipocytes and their influence on metabolic syndrome, obesity, and cancer. Image retrieved and adapted from Heyn et al. (2020). Permission of reuse from Frontiers.

Clarification of the mechanisms that drive these molecular machines and the biological importance of miRNA has been obtained through structural analyses of the Drosha and Dicer complexes (Hammond, 2015; Treiber et al., 2019). Tissue-specific knockout of Dicer or DGCR8 (an enzyme more specific for miRNA biogenesis compared to Drosha) in mice, resulted in developmental defects in that tissue and embryonic lethality (Hammond, 2015). Other mouse studies found that if a cardiac specific miRNA (miR-208) was missing, no problems in cardiac tissue specification were found (the mice still had a heart); however it did affect cardiac tissue homeostasis, with mice experiencing defects in cardiac stress response and cardiac hypertrophy (van Rooij et al., 2007), suggesting that this miRNA is needed for maintaining tissue differentiation (Hammond, 2015).

There are currently 2654 potential miRNAs encoded in the human genome (Kozomara et al., 2019), and an estimated ~45 000 miRNA-targeting sites which may affect 60% of gene expression (Deiuliis, 2016). The miRNA's ability to alter normal physiology can make them mediators of disease as they may regulate multiple pathways/cellular processes, such as proliferation, differentiation and apoptosis (Plotnikova et al., 2019), insulin signalling, adipokine expression, food intake regulation and immune-mediated inflammation etc (Deiuliis, 2016).

Most cells in the body, including adipocytes, can secrete exosomes which have been shown to be a major site for exosomal miRNAs that can impact adipose tissue and influence the adipocyte phenotype and function of WAT (Karbiener et al., 2014). Adipocyte exosomes encapsulate miRNA and allow extracellular miRNA to be stabilized and protected in plasma (Hammond, 2015; Kim et al., 2020). Several studies have suggested that these extracellular miRNAs are transferred into target cells and can inhibit or stimulate the expression of certain genes, thereby controlling gene expression (Hammond, 2015; Hergenreider et al., 2012; Kim et al., 2020; Mittelbrunn et al., 2011; Pegtel et al., 2010; Thomou et al., 2017). This suggests that they may promote disease progression by functioning as extracellular endocrine and paracrine messengers, allowing metabolic organ crosstalk (Hammond, 2015; Ji et al., 2019). Studies have shown that miRNA-containing exosomes are involved in numerous pathological processes, such as insulin resistance, dyslipidaemia, endocrine disorders, chronic inflammation, MetS and obesity progression (Bae et al., 2019; Castaño et al., 2018; Yao et al., 2018) (**Figure 2.2**).

Evidence of miRNA's role in the development of metabolic disease and obesity progression have been reported (Huang et al., 2018; Ji et al., 2019; Tsukita et al., 2017; Zhong et al., 2018). Some suggested mechanisms of miRNA dysregulation involve deletion, amplification or, mutation of miRNA genes, as well as changes in miRNA transcription, (Croce, 2009), whilst others have shown epigenetic changes specifically involving DNA methylation and histone modifications (Gulyaeva et al., 2016; Saito et al., 2006). Diet and physical activity can also influence miRNA expression (Guller et al., 2010; Gulyaeva et al., 2016; Quintanilha et al., 2017; Ultimo et al., 2018). Davidsen et al. (2011) found resistance training- induced hypertrophy in human skeletal muscle was associated with changes in miRNA expression, suggesting select miRNA regulate gene networks responsible for skeletal muscle growth. In mice, diets high in fat and sugar have also shown to change the expression of certain miRNAs compared to control groups (Yerlikaya et al., 2019). Therefore, due to miRNA ability to regulate genes, dysregulation of miRNAs could be problematic and have the potential to cause endocrine dysfunction. Mechanisms behind miRNA dysregulation are very complex and need further research (Ji et al., 2019; McGregor et al., 2011; R. Zhang et al., 2013).

Many miRNAs are closely associated with adipocyte differentiation through acceleration or inhibition during adipogenesis, thus regulating adipocyte development and possibly adipocyte numbers (McGregor et al., 2011). However, in obesity, these miRNAs are dysregulated (Ji et al., 2019). The adipose tissue-derived miRNAs are released from both adipose tissue macrophages and adipocytes (Thomou et al., 2017; Ying et al., 2017) and their levels correlate with the degree of obesity and its complications (Ji et al., 2019). Accumulating evidence also shows that the circulating miRNA (cmiRNA) profile is distinctly different between healthy individuals and patients with obesity and T2D (Guay et al., 2013; Iacomino et al., 2016; Ji et al., 2019; Ortega et al., 2014; Pescador et al., 2013). Adipose tissue-derived and/or obesity-associated cmiRNAs also have the potential to be used as biomarkers in obesity and metabolic disease prevention and management (Ji et al., 2019).

Adipose tissue-derived cmiRNAs are considered a new form of adipokine. The profile of cmiRNA is altered in obesity, and these alterations are significantly associated with BMI and/or BF%, waist-to-height ratio and plasma adipokine levels (Al-Rawaf, 2019; Castaño et al., 2018; Ji et al., 2019; Prats-Puig et al., 2013). In patients with lipodystrophy, cmiRNA levels were substantially lower compared to healthy individuals (Ji et al., 2019; Mori et al., 2014). Similar findings were observed in mice with an adipose tissue specific deficiency in

the Dicer enzyme, which displayed a lipodystrophic phenotype (Thomou et al., 2017). These findings support the notion that adipose tissue is a major source of cmiRNA, and that cmiRNAs are influenced by the degree of obesity and related complications (Ortega et al., 2013; Ortega et al., 2014).

A prospective study investigating both obese- and obese-T2D subjects, found that the expression levels of 25 miRNAs differed between the two groups. Obese-T2D subjects had 53 dysregulated miRNAs compared to healthy subjects (Kim et al., 2020). Exosomal miRNA profiles of obese-T2D and obese subjects overlapped (similar) compared to healthy subjects, and some miRNAs differed in the presence of T2D. This suggests that obesity has a major effect on exo-miRNAs, contributes to metabolic complications, and may cause pathological mechanisms where subjects with insulin resistance go on to develop diabetes (Kim et al., 2020).

Bariatric surgery has also been shown to change the cmiRNA profile. For example, in one study, miR-140-5p and miR-142-3p decreased significantly after surgery (Hubal et al., 2017). In another study, miR-122, miR-885-5p or miR-192 were positively correlated with BMI, BF% and blood glucose levels, however, these levels were reversed three months after surgery to non-obese control levels. On the contrary, adipose tissue-derived miRNAs, miR-99b remained the same while miR-221, miR-222 increased, suggesting that WAT might contribute to cmiRNA levels (Sangiao-Alvarellos et al., 2020).

These findings suggest, further study on the impact and role of adipose tissue-derived miRNAs is needed (Ji et al., 2019; Ogawa et al., 2010). Multiple miRNAs have been investigated in obesity and metabolic disorders (**Figure 2.2**).

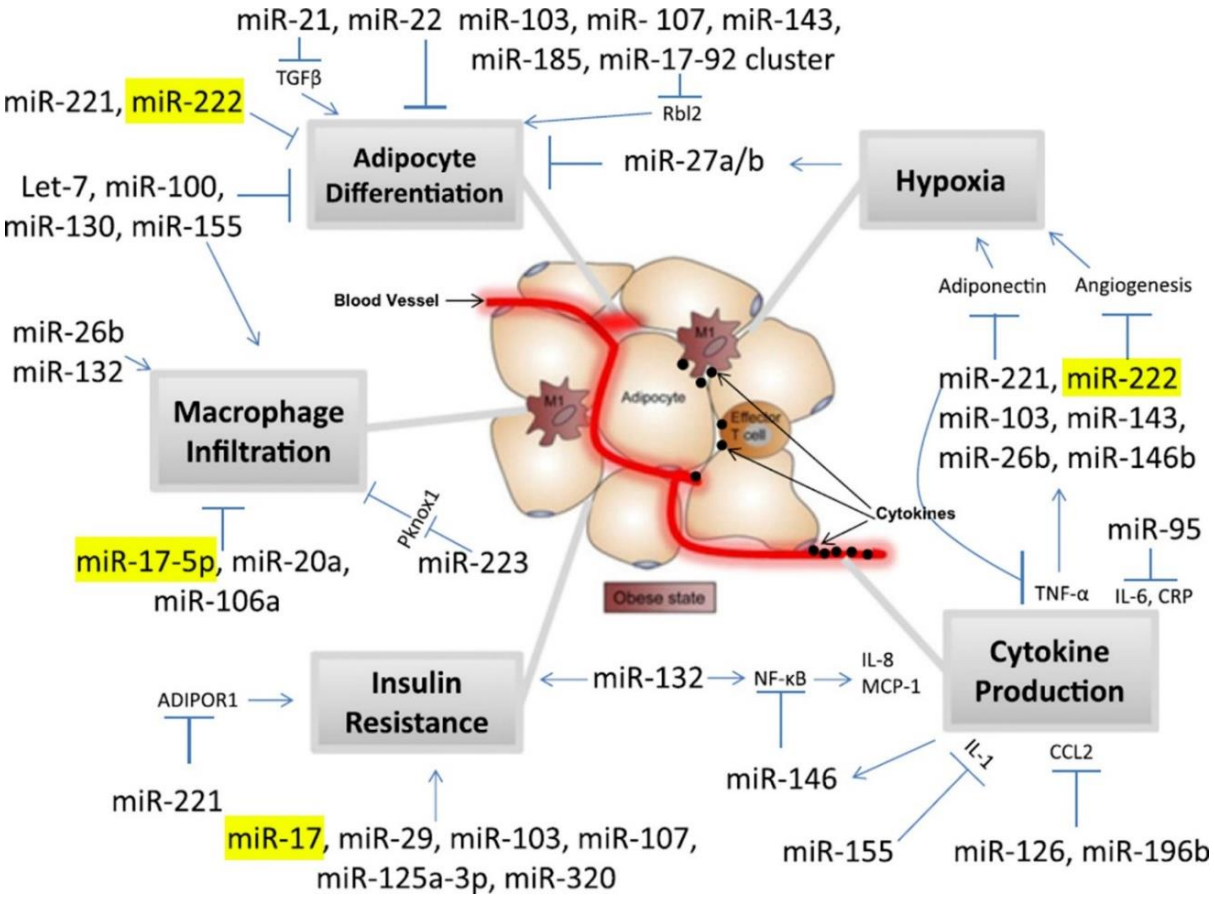

Figure 2.2 Summary of miRNAs involved in obesity pathophysiology and related inflammation. This image summarises miRNA involved in obesity and miRNA target sites. Arrows indicate activation, blunted arrows, inhibition. Grey boxes indicate the characterised process of obesity-associated inflammation. Highlighted miRNAs are discussed further in this review. Image retrieved from Marques-Rocha et al. (2015). Permission of reuse from John Wiley & Sons.

A global meta-analysis consisting 26 studies found seven miRNA were linked to obesity and ten linked to T2D; miR-17 and miR-29b were linked to T2D while miR-222 was associated with both obesity and T2D (Villard et al., 2015).

2.7 miR-222-3p (miR-222)

MicroRNA-222-3p plays many roles in the body, with 78 annotations currently identified on the miRNA database (miRbase) (Kozomara et al., 2019). MicroRNA-222 is involved in cytokine regulation and inflammatory responses. It further regulates signalling molecules such as protein kinase B, which is involved in control of glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration (Brazil et al., 2001; Song et al., 2019). It is also associated with an increase in adiposity (Prats-Puig et al., 2013; Xie et al., 2009). Numerous studies comparing obese and non-obese children, adolescents and adults have all found significant increases in miR-222 concentrations (**Table 2.3**) (Al-Rawaf, 2019; Bao et al., 2018; Cui et al., 2018; Ortega et al., 2013; Prats-Puig et al., 2013; Thompson et al., 2017).

Links to miRNA and adipose tissue have also been assessed in bariatric studies to determine the effect weight loss may have on miRNA expression. Sangiao-Alvarellos et al. (2020), investigated liver- and adipose tissue-derived miRNAs in patients before and 12 months post-surgery. They found miR-222 was high in the SAT of healthy subjects, not elevated in morbidly obese patients, and either unchanged or increased after surgery. With a 40% reduction in average BMI after 12 months, these results suggested that adipose tissue might not be the main source of cmiRNA, as there would have been a greater change in miR-222 levels if it were. To confirm these findings, an independent validation cohort of obese patients with samples before and after bariatric surgery was examined (Sangiao-Alvarellos et al., 2020). Results were compared between the discovery and validation cohort, reporting no significant changes in putative adipose tissue-derived miRNAs (including miR-222). The study concluded that although morbid obesity was associated with significant increases in liver-derived miRNAs, which dropped 3 months after surgery (20% reduction in BMI), no further changes in any miRNA were seen with additional losses of WAT, suggesting that miRNA changes were due to metabolic improvement rather than WAT loss (Sangiao-Alvarellos et al., 2020). Unlike Sangiao-Alvarellos et al. (2020), Ortega et al (2013) found that morbidly obese patients had significantly higher levels of miR-222 compared to non-obese subjects. However part of the study's findings were similar to Sangiao-Alvarellos et al. (2020), reporting no significant changes in miR-222 after one year of bariatric surgery weight loss (30% decrease in initial bodyweight).

In murine and human studies, miR-222 played a role in inflammation. In WAT, miR-222 negativity correlated with adiponectin expression. TNF-α exposure also led to up-regulation of miR-222. This indicates miRNA may mediate inflammation in WAT by regulating macrophages and/or adipokine release (Arner et al., 2015; Landrier et al., 2019; Xie et al., 2009). These findings are supported in a study investigating differentiated adipocytes, which found a 2.5-fold increase of miR-222 in inflamed human adipocytes in which its expression lowered after weight loss (Ortega et al., 2015). Therefore, miRNA-222 significantly increased when inflammation was present. Similarly, miR-222 was down-regulated in adipogenesis and up-regulated in obesity (Xie et al., 2009). This suggests that miR-222 up-regulation may participate in the crosstalk between obesity-related inflammation, insulin resistance, and obesity-associated morbidities (Chartoumpekis et al., 2012; Ortega et al., 2010; Ortega et al., 2015).

Several studies identified miR-222 involvement in T2D (**Table 2.3**). Herrera et al. (2010), found miR-222 was up-regulated in the adipose tissue of diabetic rats. A study looking at omental adipose tissue in gestational diabetes found that miR-222 was up-regulated and negatively correlated with GLUT4 protein expression, which is important for glucose homeostasis/metabolism (Z. Shi et al., 2014). In humans, miR-222 was also highly expressed in those with β-cell dysfunction (Belongie et al., 2017) and positively correlated with HbA1c levels (Akerman et al., 2018). In the Ortega et al. (2014) cross-sectional study, miR-222 levels increased in T2D but decreased significantly upon metformin and insulin infusion during clamp, while an intralipid/heparin mixture increased cmiR-222, suggesting that there may be a close association between specific cmiRNAs, T2D and insulin sensitivity.

Table 2.3 Associations found between miR-222-3p (miR-222) and obesity related diseases.

Author Year DOI	**Disease/ Process**	**miRNA Sample Type**	**Expression**	**Population (n)**	**Study Outcome**
Human – Experimental Studies					
Bao et al., 2018 10.1016/j.physbeh.2018.09.011	Obesity	222 Plasma	↑ in obese	n = 24 (18 - 35 years) 12 untrained normal weight controls: Sex 6M/6F Age: 23.27 ± 2.14 BMI: 22.11 ± 1.62 12 untrained obese: Sex: 5M/7F Age: 22.67 ± 4.31 BMI: 35.46 ± 4.08 Country: USA	Higher baseline of miR-222 in obese subjects versus normal – weight subjects. miR-222 increased in response to acute aerobic exercise in obese subjects
Hess et al., 2020 10.1002/oby.22704	Obesity	222-3p Serum	↑ after weight loss	n = 85 obese/overweight BMI = 28-45 kg/m^2 Age: 18 -60y Sex: both Country: Denmark (Copenhagen)	In overweight and obese subjects prescribed an energy deficit of ~500 kcal/d; miR-222 increased in response to weight loss
Human – Observational Studies					
Al-Rawaf et al., 2019 10.1016/j.clnu.2018.09.024	Obesity/MetS	222 Plasma	↑ in adolescents with obesity	n = 250 adolescents 150 Boys 100 Girls Age: 12-18y Normal weight: (n = 50, BMI; 16.2–17.3 kg/m^2), Overweight: (n = 100, BMI; 17.4–21.45 kg/m^2),	miR-222 was positively correlated with BMI, waist-to-height ratio (WHtR), adipokines; adiponectin, leptin, L/A ratio, and other biomarkers related to MS such as FBG, insulin, HOMA-IR, and circulated plasma lipids such as TG, HDL-C, and LDL-C

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Obese: (n = 100, BMI; ≥ 22 kg/m^2). Country: Egypt (Mansoura)	
Belongie et al., 2017 10.1371/journal.pone.0182932	T2D	222 Plasma	↑ in pre-diabetes and β-cell dysfunction	n = 1384 Control: Age mean: 45.2+- 7.5 BMI mean:26.2+-3.7 Sex: 17M/26F Cases: Age mean: 44.8 +-8.0 BMI average 26.2+-4.0 Sex :18M/25F Country: pan- Europe (13 countries)	miR-222 was up-regulated in pre-diabetic subjects and was a prognostic miRNA biomarkers of β-cell dysfunction
Cui et al., 2018 10.1016/j.metabol.2017.09.006	Obesity/T2D	222 Plasma	↑ in subjects with obesity versus healthy controls	Discovery Study- Randomly selected sample N = 9 controls children N = 9 obese children (pooled into 3 pools, for miRNA profiling experiments) Cross-sectional validation study (children) N = 352 100 obese 106 overweight 146 normal weight controls Longitudinal validation study (adults)	miR-222 was positively correlated with BMI and abdominal fat mass index.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				101 newly diagnosed diabetes patients 82 normal glucose tolerance (NGT) controls Country: China (Nanjing)	
González-Arce et al., 10.1002/ajhb.23540 2020	Obesity	222 Plasma	↑ in children with obesity	n = 99 Mayan children 50 obese 49 control Sex: 50.51% F /49.49% M Age: 6-12 Country: Mexico	Higher levels of miR-222 were significantly associated with BMI, WtHR, BF%, serum HDL, TAG, and metabolic index
Gonzalo-Calvo et al., 2016 10.1016/j.arteri.2016.05.005	Atherosclerosis.	222-3p Human coronary artery smooth muscle cell – derived microparticles + Plasma	↓ in those with familial hypercholesterolemia versus normocholesterolemic controls.	n = 24 12 controls 12 familial hypercholesterolemia (FH) matched for age, sex, and cardiovascular risk factors. Country: n/a	Hypercholesterolemia induced a decrease in miR-222-3p expression in microparticles, not in cells. In circulating microparticles miR-222-3p was down-regulated in FH patients compared to normocholesterolemic controls. Microparticles from atherosclerotic plaque areas showed down-regulation of miR-222-3p compared to non-atherosclerotic areas.
Li et al., 2016 10.4238/gmr.15	T2D/ Obesity	222 Serum	↑ in T2D versus healthy controls	n = 110 20 controls 30 T2D and obesity 30 post-menopausal breast cancer 30 both T2D and post-menopausal	miR-222 was positively correlated with BMI, HOMA-IR, HbA1c, and TAG.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
027259				breast cancer Country: China (Binzhou)	
Ortega et al., 2013 10.1373/clinchem.2012.195776	Obesity	222 Plasma	↑ in morbidly obese BMI ≥40 kg/m^2	Plasma samples to profile circulating miRNAs 32 Men BMI: 20-60 kg/m^2 Relevant miRNAs validated cross-sectionally: N = 80 Men Before and After Surgery induced weight loss N = 6 morbidly obese (3M/3F) Subpopulation randomly selected from cohort of 22 Caucasian morbidly obese patients: BMI mean (SD): 42.9 (5.9) kg/m^2 Age: 44 (14) years, Sex: 5M/17F Conventional diet induced weight loss: N = 9 obese patients. BMI: 32.4 (3.8) kg/m2, Age: 47 (12) years, Sex: 5M/4F Ethnicity for all: Caucasian Country: Spain	miR-222 was positively correlated with BMI and other measures of obesity such as fat mass and waist circumference.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
Ortega et al., 2014 10.2337/dc13-1847	T2D	222 Plasma	↑ in T2D subjects	Discovery study -observational (Spain) n = 12 (age-matched men with or without T2D) 6 obese NGT: BMI [mean ± SD]: 33.3 ± 3.9 kg/m^2], Age [mean ± SD]: 47 ± 5 years) 6 obese T2D: BMI [mean ± SD]: 41.9 ± 10.4 kg/m^2, Age [mean ± SD]: 43 ± 3 years Plasma specimens cross sectionally validated (Spain) n = 93 Men (including Discovery subjects) 45 Normal glucose tolerance (NGT) controls 48 T2D (65 nonobese and 28 obese men) previous 6 months, as defined by stable Longitudinal validation study - randomized, placebo-controlled, and double-blinded validation study n = 35 T2D 18 placebo 8M/10F 17 metformin treated T2D 8M/9F Total: 16M/19F Age: 53 ± 8 years	miR-222 is up-regulated in subjects with T2D. Metformin treatment down-regulated miR-222. Insulin infusion during clamp down-regulated miR-222. The intralipid/heparin mixture up-regulated miR-222.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Independent Cohort Clamp and Intralipid/Heparin Infusion Studies N = 7 healthy men Age 28 ± 4 years BMI 25 ± 3 kg/m^2	
Sadeghzadeh 2020 10.2147/DMSO.S263883	T2D	222 Plasma	↑in pre T2D subjects versus control subjects	n = 90 (35 – 80 years) 30 Control: Sex: 21M/9F BMI: 27.60±3.9 Age: 51.44±6.04 30 pre-T2D: Sex: 22M/8F BMI: 28.02±4.14 Age: 49.98±9.25 30 T2D: Sex: 20M/10F BMI: 28.17±5.46 Age: 52.42±8.77 Country: Iran (Shahid Sadoughi)	No significant differences in miR-222 expression were found between pre-T2D and T2D subjects. Pre T2D: miR-222 positively correlated with FG. LDL-C negative correlation with miR-222.
Sangiao-Alvarellos et al., 2020 10.1136/bmjdrc-2020-001441	Obesity/MetS	222 Serum	↑ in healthy human SAT ↑ after bariatric surgery in morbidly obese	Discovery cohort (Spain) n = 155 47 Control: healthy, non-obese Age [mean ±SD]: 39.7 ± 10.90 BMI [mean ±SD]: 24.3 ± 3.49 Sex: 20M/27F 115 morbidly obese:	miR-222 was not elevated in morbidly obese patients (pre-op) however expression of miR-222 increased after bariatric surgery.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Before surgery: Age [mean ±SD]: 45.3 ± 9.21 BMI [mean ±SD]: (49.2 ± 8.54) Sex: 47M/108F After 3 m surgery: 10M/19F (BMI 39.4 ± 7.53) 6m surgery: 10M/17F (BMI 35.9 ± 6.10) 12m surgery: 7M/21F (BMI 30.6 ± 4.8) Validation cohort (Austria): Before surgery (n = 33) After surgery: 12 months (n = 14) 18 months (n = 19)	
Telkoparan-Akillilar et al., 2021 10.1007/s11033-021-06352-7	Atherosclerosis.	222 Plasma	↓ in atherosclerosis subjects versus healthy controls	n = 51 26 healthy controls Sex:9F/17M Mean age: 54,69 ± 8,2 25 atherosclerosis. Sex: 2F/23M Mean age: 63,2 ± 8,8 Country: Turkey	In the hypercholesterolemia and diabetes subgroups, miR-222 was significantly down regulated in both subgroups.
In Vitro Studies					
Nardelli et al., 2017	Obesity	222-3p Human amniotic	↑ in obese hA-MSCs	n = 20 pregnant women: 7 control 13 obese	miR-222-3p was overexpressed in Obese-hA-MSCs versus Control hA-MSCs.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
10.1089/scd.2016.0127		mesenchymal stromal cells (hA-MSCs)		Country: n/a	
Animal – Experimental Studies					
Chartoumpekis et al., 2012 10.1371/journal.pone.0034872	Obesity	222 Adipose Tissue	↑ in high-fat diet fed mice versus standard diet fed mice	Mice Country: Greece (Patras)	A high-fat diet versus standard diet up-regulated miR-222 in WAT of mice
Herrera et al., 2010 10.1007/s00125-010-1667-2	T2D	222 Adipose, liver and muscle tissue	↑ in adipose tissue of diabetic rats versus normoglycaemic rats	Gyoto–Kakizaki rat Country: UK	miR-222 is up-regulated in hyperglycaemia
Combined Study Design					
Ortega et al., 2010 10.1371/journal.pone.0009022	Obesity	222 human adipocytes and subcutaneous fat	↓ during adipogenesis	n = 28 adipose tissue biopsies BMI :20 - 55Kg/m^2 Sex: Female Ethnicity: Caucasian 6 controls 9 obese with T2D 13 obese without T2D Cell culture: Commercially available cryopreserved human subcutaneous preadipocytes from two non-diabetic male subjects:	**In vitro and In vivo:** miR-222 was associated with BMI in human adipose tissue samples.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Age>40 1 lean: BMI <25 1 obese: BMI>30Kg/m2 Country: Spain	
Ortega et al., 2015 10.1210/jc.2015-2357	Obesity/ Inflammation	222 Abdominal SAT	↑ in inflamed adipocytes and in their supernatants ↓ in adipose tissue after weight loss	Controls (age- and sex-matched samples): n = 26 lean Females BMI [mean ±SD] = 24.2 ± 2.3 kg/m2, Age [mean ±SD] = 45 ± 5 years Roux-en-Y gastric bypass: n = 16 morbidly obese women This subsample was randomly selected from an extended cohort of 25 women: BMI [mean ±SD] = 43.1 ± 4.9 kg/m2 Age [mean ±SD] = 48 ± 10 years Ethnicity: Caucasian Country: Spain	**Human observational and in vitro:** miR-222 were up-regulated in obese adipose tissue and inflamed adipocytes.
Shi et al., 2014 10.1210/en.2013-2046	Gestational diabetes mellitus (GDM)	222 Omental adipose tissue	↑ in GDM versus normal pregnant women	13 GDM patients Age: 27.62 ± 3.10 BMI: 25.51 ± 0.93 13 NGT healthy pregnant women Age: 27.85 ± 3.36 BMI: 24.91 ± 1.23 Gestation: 38–39 wk.	**In Vivo and in Vitro:** miR-222 negatively correlated with expression of ERα protein and GLUT4 protein miR-222 significantly correlated with serum oestradiol levels.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Caesarean delivery Indication for both groups: breech presentation or foetal macrosomia. Age Range:23 - 33y BMI range: 23.2 -27.2 kg/m^2 Country: China (Nanjing)	
Xie et al., 2009 10.2337/db08-1299	Obesity	222 Pre-adipocyte 3T3-L1 cells and adipocytes	↑ in obese adipocytes	Leptin deficient ob/ob diet-induced obese Mice. Country: USA (Massachusetts)	**In Vivo and in Vitro:** Decreased during adipogenesis but up-regulated in obese adipocytes. TNF-α induced miR-222 expression.
Review					
Arner et al., 2015 10.1038/nrendo.2015.25	Obesity/ Inflammation	222	Systematically searched PubMed: miR-222 positively correlated with TNF-a & negatively correlated with adiponectin in WAT of Mice in response to diet regimes TNF ↑ miR-222 miR-222 function to inhibit adipogenesis in human, mouse, and porcine cells. ectopic expression of miR-222 in human mesenchymal stem cells resulted in inhibition of adipogenesis PPARG, CEBPA and CDKN1B transcripts targeting.		
Huang et al., 2018 10.1155/2018/7372636	MetS	222	Upregulated in Omental adipose tissue from GDM patients Negatively correlated with the expression of the ERa and GLUT4 proteins Upregulated in the diabetic rats adipose tissue Regulates oestradiol concentrations of PCOS patients characterized by intrinsic insulin resistance and dysfunctional glucose metabolism in adipose tissue Positively associated with HbA1c		
Landrier et al.,	Obesity	222	miRNAs 222 expression correlate with diet and lifestyle TNF-α ↑ miR-222 expression in murine adipocyte model		

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
2019 10.3390/cells8080859					
Withers et al., 2020 10.3390/ncrna6010005	Obesity /Cancer	222	↑ in the circulation of obese patients and up-regulated in SAT of obese subjects Linked to a diverse range of cancers and their response to therapy Elevated in obesity and cancer		

2.8 miR-17-5p (miR-17)

MiR-17-5p is part of the miR-17/92 cluster which consists of a further five miRNA members (miR-18a; miR-19a; miR-20a; miR-19b-1; miR-92a-1) (Kozomara et al., 2019). This cluster is reported to increase adipogenesis (Lin et al., 2009), and to be significantly up-regulated during adipocyte differentiation as it targets Rb2 and p130 which are negative regulators of differentiation. If the miR-17-92 cluster is overexpressed, Rb2 and p130 will be repressed and rapid adipocyte differentiation will occur (Hilton et al., 2013; McGregor et al., 2011; Q. Wang et al., 2008).

A review by Dellago et al. (2017), covering miRNA-17-5p role in aging, age-related diseases and cancer, found miR-17-5p was involved in numerous processes, regulating genes involved in autophagy, apoptosis and cell cycle regulation (**Table 2.4**). High levels of miR-17-5p were found in almost all cancers, atherosclerosis, and obesity, indicating it may be a useful biomarker for those conditions.

Heneghan et al. (2011) investigated the differential expression of miRNA in omental adipose tissue and blood (cmiRNA) of obese patients (BMI > 40 kg/m^2). They found that miR-17-5p was inversely correlated with BMI and levels were significantly lower in both omental adipose tissue and blood from obese individuals, highlighting the potential use of miR-17-5p as a metabolic biomarker.

GLUT4, an insulin-responsive glucose transporter has been shown to be a direct target gene of miR-17, suggesting a mechanism by which this miRNA may influence insulin resistance (Xiao et al., 2018). The study showed that miR-17 expression in skeletal muscle tissues of rats with T2D was significantly elevated, and that miR-17 levels negatively correlated with GLUT4 expression. Glucose metabolism was also impaired when miR-17 was overexpressed. Further associations between miR-17-5p and T2D have been shown in a study by Kloting et al. (2009), which found significantly higher levels of miR-17-5p expression in omental fat of normal glucose tolerance controls, compared to T2D subjects. They also found that miR-17-5p was negatively associated with visceral fat, significantly related to lower HbA1c levels, and improved insulin sensitivity (glucose infusion rate). This suggests that miR-17-5p expression plays a role in adipose tissue dysfunction and the development of obesity related disorders including T2D (Kloting et al., 2009). These results however, should be reviewed

with caution as the study did not correct for body fat levels which can contribute to insulin resistance (Johnson et al., 2013).

A study looking at cmiRNA profiles of MetS patients similarly found that miR-17 was significantly down-regulated in patients with T2D (Karolina et al., 2012) and another study found that its expression negatively correlated with HbA1c (Arner et al., 2015; McGregor et al., 2011). These results suggest that miR-17-5p may play an important role in fully manifested diabetes (Karolina et al., 2012). Diet and miRNA expression have also been explored (Fontalba-Romero et al., 2021; MacDonald-Ramos et al., 2021; Palmer et al., 2014; Slattery et al., 2016; J. Wang et al., 2021; W. M. Yang et al., 2014). In a study by Yerlikaya et al. (2019), miR-17-5p and miR-29b-3p were found to be down-regulated in rats consuming a high-fat and high-sucrose diet, suggesting that the expression of these miRNAs in the high-fat- high sucrose fed rat, may be associated with hyperlipidaemia and insulin resistance (Yerlikaya et al., 2019).

Table 2.4 Associations found between miR-17-5p (miR-17) and obesity related diseases.

Author Year DOI	**Disease/ Process**	**miRNA Sample Type**	**Expression**	**Population (n)**	**Study Outcome**
Human -Observational Study					
Chen et al., 2015 10.1016/j.ijcard.2015.06.037	Coronary artery disease (CAD)/atherosclerosis	miR-17-5p Plasma	↑ in CAD subjects compared to healthy controls	Consecutive series of n = 112 20 controls 59 CAD 33 Nonsignificant CAD Sex: Male Age 45–83 years, Country: China (Wuhan)	Severity of coronary atherosclerosis, was significantly correlated with miR-17-5p .
Heneghan et al., 2011 10.1210/jc.2010-2701	Obesity/Met S	17-5p Omentum tissue and SAT/serum/ plasma	↓ in obese omentum versus non-obese omental fat ↓Met S	Pilot Study N = 50 nonobese N = 50 obese Split into 3 phases: *Phase I:* adipose tissue biomarker discovery Cohort: 2 = nonobese BMI < 25 kg/m2 underwent elective laparoscopic Nissen's fundoplication. 3 = morbidly obese (had a sleeve gastrectomy). BMI > 40 kg/m2 Phase II: miRNA biomarker selection and validation in adipose tissue	miR-17-5p was down-regulated in omental fat of obese subject. miR-17 had an inverse correlation with BMI in both adipose tissue and blood samples.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Larger cohort: 19 bariatric surgery patients BMI > 40 kg/m2 That underwent sleeve gastrectomy Sex: 13W/6M Age = 42 [37-53] years BMI = 48.8 [43-52] kg/m2 10 controls with BMI < 25 kg/m2 that underwent Nissen's fundoplication, cholecystectomy, paraoesophageal hernia repairs: Sex: 6W/4M Age = 37 [29-49] years BMI = 24.1 [24-25] kg/m2 <u>Phase III: candidate miRNA evaluation as circulating metabolic biomarkers (age-matched)</u> Expanded cohort 30 obese 20 non-obese Country: Ireland	
Karolina et al., 2012 10.1210/jc.2012-1996	MetS	17 Blood and exosome	↓ in T2D subjects	n = 265 46 controls 50 MetS 50 T2D 89 hypercholesterolemia 30 hypertension	A 2-fold down-regulation of miR-17 was observed in subjects with T2D

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Country: Singapore	
Klöting et al., 2009 10.1371/journal.pone.0004699	Obesity/T2D	17-5p Abdominal subcutaneous and intraabdominal omental Adipose tissue	↑ in NGT omental fat	n = 15 Caucasian Sex: 8 M/7 F Age: 50 - 73 years BMI 25.4 - 38.1 kg/m^2. 9 NGT controls 6 newly diagnosed T2D Country: Germany (Leipzig) The study was approved University of Leipzig	Significant correlations were observed in miRNA-17-5p and adipose tissue morphology, visceral fat, HbA1c, fasting plasma glucose, and circulating leptin, adiponectin, interleukin-6 Expression of miR-17-5p was up-regulated in omental fat of NGT compared to T2D The expression of miR-17-5p was negatively associated with visceral fat area
Lirun et al., 2015 10.1007/s11695-015-1711-x	Obesity /T2D	17 Serum	↓ in low BMI group after gastric bypass surgery (RYGB)	A Pilot Study before and after study 3 controls 15 T2D: High BMI group (13 = BMI ≥ 27.5 kg/m2, Age ≤ 65 years) Low BMI group (2 = BMI ≥ 25.0 kg/m2 and ≤27.5 kg/m2) Divided into: high-BMI (n = 8) low-BMI (n = 7), (Reference point used BMI = 30 kg/m^2) Country: China (Shengjing)	The expression of miR-17 was down-regulated in the low-BMI postoperative group versus the high-BMI postoperative group
Ramzan et al.,	MetS	17-5p	↓ in MetS	Discovery Phase:	miR-17-5p was down-regulated in MetS

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
2020 10.1007/s00592-019-01406-6		Plasma	subjects versus healthy controls	N = 79 40 Control: Sex: 20F/20M Age: Female 63.4 ± 1.00 ; Male 49.2 ± 0.97 BMI: Female 24.3 ± 0.70; Male 24.8 ± 0.72 39 Met S: Sex: 20F/19M Age: Female62.5 ± 1.25 ; Male 52.8 ± 0.85 BMI: Female 28.6 ± 0.67; Male 30.4 ± 0.73 Validation phase (independent cohort) N = 20 10 healthy controls 10 MetS Sex: Female Country: New Zealand (Auckland)	subjects compared to healthy controls. Significant sex differences were observed; miR-17-5p was down-regulated in women with MetS, yet in men there were no significant associations. The regression model adjusted for sex and age found that miR-17-5p was a significant predictor of MetS
Wang, L et al., 2021 10.1159/000511772	Obesity	17 -3p Abdominal SAT samples	↓ in morbidly obese subjects versus normal weight controls	N = 8 Chinese Females 5 controls (normal weight) (admitted for elective abdominal surgical procedures) Age: 47.20 ± 2.58 years BMI 22.03 ± 0.78 3 morbidly obese (had received	miR-17-3p was down-regulated in the SAT of morbidly obese subjects versus normal weight controls.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				laparoscopic adjustable gastric banding) Age: 42.00 ± 7.57 years BMI: 47.53 ± 1.53) <u>Validation Study</u> N = 17 Females 9 controls (2 from the microarray group included) Age: 42.11 ± 2.23 years; BMI: 22.42 ± 0.54 8 morbidly obese (3 obese from microarray group included) Age: 34.88 ± 3.42 years; BMI 45.45 ± 2.40 Country: China	
Williams et al., 2019 10.1007/s40200-019-00404-3	T2D	17 Serum	↓ in subjects with elevated HbA1c	n = 69 African American Females Age: > 40 years old 23 Control (normal HbA1c) Age: 60.11 ± 1.89 BMI 30.55 ± 1.29 46 T2D (high HbA1c) Age 62.27 ± 1.23 BMI: 38.31 ± 1.27 Country: USA (North Carolina)	miR-17 levels are down-regulated in obese, African American women with elevated HbA1 compared the control group. MiR-17 was also correlated with serum calcium in participants with normal HbA1c
Wu et al., 2015	Obesity	17 Serum	↑ in subjects with obesity	n = 100 25 controls: Age: 45.5 ± 10.2	Significantly higher miR-17 was observed in the obese group when compared to controls, T2D, or T2D+obesity group.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
10.1111/apm.12389				BMI: 22.6 ± 2.6 25 T2D: Age:46.7 ± 8.6 BMI: 23.5 ± 2.1 25 obese: Age: 42.6 ± 11.8 BMI: 34.2 ± 1.5 25 obese + T2D: Age: 46.1 ± 12.6 BMI: 33.1 ± 1.9 Country :China (Guangzhou)	Although miR-17 levels in the T2D group were relatively lower than in the control and obesity + T2D groups, no significant associations were found.
Xue et al., 2019 10.3389/fphys.2019.00123	Acute myocardial infarction (AMI)	17-5p Plasma	↑ in AMI subjects versus healthy controls	21 healthy controls: Sex: 16M/5F Age: 58.5+-14.3 BMI 23.9 +-4.2 29 AMI Sex: 23M/6F Age 68.0 +-10.4 BMI: 26.2 +-2.6 -15 from segment elevation myocardial infarction (STEMI) -14 non- STEMI Country: China (Qingdao)	High levels of miR-17-5p were associated with AMI (before and after percutaneous coronary intervention (PCI). MiR-17-5p levels were also correlated with LDL cholesterol.
Zhu et al., 2015	GDM	17-5p Plasma	↑ in subjects with GDM versus controls	The two groups were matched for age,	In comparison to healthy controls, miR-17 was differentially expressed in pregnant women who subsequently developed

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
10.1016/j.ijgo.2015.01.010				10 controls BMI: 19.24 ± 1.07 Mean age: 26.67 years (range 23–34 years) 10 GDM BMI: 23.94 ± 2.98 Mean Age: 30.03 years (range 23–35 years) Country: China	GDM.
In Vitro Studies					
Lin et al., 2009 10.1111/j.1742-4658.2009.06967.x	Adipogenic differentiation	17/92 cluster adipose tissue	↑ during differentiation	Mice	During differentiation ↑ expression of miR-17/92 cluster, including miR-17-5p,
Wang Q et al., 2008 10.1073/pnas.0800178105	Obesity	17-92 Cell cultures	↑ during adipocyte differentiation	Mice Country: USA	Up-regulation of miR-17-5p accelerates adipocyte differentiation.
Animal - Experimental Study					
Yerlikaya et al., 2019 10.1016/j.yclnex.2019.07.001	Obesity	17-5p Plasma	↓ high-fat and high-sucrose fed rats	N = 28 male Wistar rats 7 standard rat chow, 7 high-fat diet, 7 high-sucrose diet 7 high-fat & high-sucrose diet	Both miR-17-5p and miR-29b-3p were down-regulated in rats fed a high-fat and high-sucrose diet

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Country Turkey (Konya)	
Combined Study Design					
Tan et al., 2019 10.1016/j.jphs.2018.11.012	Atherosclerosis/Obesity	17 -5p Peripheral blood leucocytes (subjects) cell cultures (Mice	↑ in atherosclerosis versus controls	30 controls (non- atherosclerosis) Age: 41–80 years 30 Atherosclerosis Age: 41–84 years 24 Mice Country: China (Shenyang)	**In vivo and in vitro study design:** miR-17-5p was up-regulated in subjects with atherosclerosis, as well as in macrophages of ApoE−/− mice that were on a high-cholesterol diet. Downregulation of miR-17-5p reduced inflammatory cytokine production.
Xiao et al., 2018 10.1016/j.ejphar.2018.08.036	T2D	17 Skeletal muscle + L6 cell	↑ skeletal T2D rats	20 Male Wistar rats 10 controls 10 T2D (high-fat diet: 55% fat, 33% CHO, 12% protein) Country: China (Harbin)	**In vitro + animal study design:** MiR-17 is the most up-regulated skeletal-enriched miRNA in T2D rats. GLUT4 is a direct target gene of miR-17 and a critical regulator of glucose metabolism. The miR-17/GLUT4 axis is, at least in part, responsible for insulin resistance
Review					
Arner et al., 2015 10.1038/nrendo.2015.25	Obesity	17–92 cluster	miR-17-5p inversely correlated with circulating levels of HbA1c miR-17–92 cluster drives adipogenesis by negatively regulating Rbl2 transcripts ↓miR-17-5p in obesity		
Dellago et al., 2017	Cancer/ Aging/ Obesity	17-92 cluster	The miR-17-92 cluster is led by its most prominent member (miR-17-5p) has oncogenic potential. miR- 17-5p – regulates genes involved in autophagy, apoptosis, and cell cycle regulation. ↑ miR- 17-5p causes lifespan extension by promoting autophagy in mouse models		

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
10.1159/000447773			↑miR- 17-5p in most cancers, atherosclerosis, and obesity, ↓ miR- 17-5 associated with aging miR- 17-deficient mouse is neonatally lethal. miR- 17-5 in play a role in cardiac aging in transgenic Mice and cells in vitro miR- 17-5 in play a role in bone formation,		
Guo et al., 2019 10.1038/s41574-019-0260-0	Obesity	17-5p	↓miR-17-5p significantly in blood samples from 30 obese patients compared to 20 non-obese controls miR-17-5p expression correlates with visceral fat tissue biopsy samples miR-17-5p is negatively correlated with BMI in patients with obesity		
Karolina, DS et al., 2014 10.4172/1747-0862.S1-011	Obesity T2D Endothelial Dysfunction	miR-17-92 cluster	TNF-α treatment induces the expression of miR-17 Specific inhibition of miR-17-3p increased neutrophil adherence to TNF-α stimulation Cluster known to promote cell proliferation, suppress cancer cell apoptosis and induce tumour angiogenesis miR-17 targets the expression of the anti-angiogenic metalloproteinase inhibitor 1 (TIMP1) which then affects endothelial cell proliferation and motility Over-expression of cluster (miR-17, miR-18a, miR-19a and miR-20a) inhibits endothelial sprouting in vivo		

2.9 miR-29b-3p (miR-29b)

The miR-29 family consists of miR-29a, miR-29b, and miR-29c encoded by two gene clusters. It also has a common seed region sequence and is predicted to target overlapping sets of genes. However, it exhibits differential regulation and subcellular distribution indicating their function is not identical (Kriegel et al., 2012).

The miRNA-29 family has strong antifibrotic effects in the heart, kidney, and other organs, it is also pro-apoptotic and likely involved in the regulation of cell differentiation (Kriegel et al., 2012).

Increased expression of the miRNA 29 family is shown in three target tissues of insulin action: muscle, fat, and liver of Goto-Kakizaki diabetic rats, with an overexpression in 3T3-L1 adipocytes repressing insulin-stimulated glucose uptake (He et al., 2007) (**Table 2.5**). These data indicate that miR-29 plays a major role in T2D and that hyperinsulinemia together with hyperglycaemia cause an increase in expression of miR-29a and miR-29b (He et al., 2007). The miRNA 29 family also plays an important role in maintaining the balance between differentiation and proliferation in pancreatic β-cells (Vienberg et al., 2017).

In a study of more than 800 individuals with T2D, 13 miRNAs were associated with T2D and all subjects with T2D had lower plasma levels of miR-29b (Zampetaki et al., 2010). It was noted that the associated miRNA levels were already modified 5–10 years before the onset of the disease, suggesting that the 13 cmiRNA including miR-29b may be useful as predictors of early T2D (Guay et al., 2013).

Another study examining the differential expression and release of exosomal miRNAs by human islets under inflammatory and hypoxic stress, found 29 miRNAs with significant differential expression. Eleven of these were expressed under stress and eight, including miR-29b-3p, were shown by qPCR, to be differentially released in exosomes when exposed to a proinflammatory cytokine cocktail and/or hypoxia (Saravanan et al., 2019). These results show that in response to damage and stress, exosomal miRNAs are differentially expressed and released by islets, and may be useful as a biomarker for islet dysfunction. A supporting study measuring circulating levels of cytokines and miRNAs in lean and obese subjects with prediabetes, found a dysregulation of circulating cytokines and miRNAs (including miR-29b) in obese prediabetic, and T2D subjects (Nunez Lopez et al., 2016). The study showed that the

expression levels of circulating miR-29b were significantly reduced ($p < 0.05$, FDR < 0.13) in prediabetic subjects, in which Nunez Lopez et al. (2016) indicated its potential to be used as a biomarker for prediabetes. A contradictory study that evaluated the expression of five diabetes-associated miRNAs including miR-29b, in normal, susceptible, and diabetic patients, found miR-29b was undetectable in any group, suggesting absence or very low expression levels of miR-29b in the samples collected (T. Zhang et al., 2013). The conflicting results indicate that the variability and lack of reproducibility and validity in miRNA results might act as a barrier for miRNA biomarker use.

Overall, the link between obesity and miRNAs is not well defined. Although there has been a substantial amount of research in this field, with findings that suggest that adipose tissue contributes, and may be a major source of cmiRNA (Ji et al., 2019), further research is needed to clarify specific miRNA function and role in obesity and associated metabolic disorders. This will help narrow down and determine which miRNAs are best suited as potential biomarkers.

Table 2.5 Associations found between miR-29b-3p (miR-29b) and obesity related diseases

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
Human – Experimental Studies					
Russell et al., 2013 10.1113/jphysiol.2013.255695	Physical Activity	29b Skeletal muscle samples	↑ after-acute exercise	n = 9 Males Age, 23 ± 5 years Country: Australia	29b elevated after acute training No change with Acute endurance exercise
Human – Observational Studies					
Collares et al., 2013 10.1186/1756-0500-6-491	T1D, T2D and GDM	29b Peripheral blood mononuclear cell RNAs	↓ in T1D, T2D and GDM	N = 20 7 T1D Age:18–27 years Sex: 4M/3F 7 T2D Age: 41–61 years 3M/5F 6 GDM Age: 29 -39 years Country: Brazil	Expression shared among T1D, T2D and gestational diabetes
Grabmaier et al., 2017 10.1016/j.ijcard.2017.06.054	Acute Myocardial infarction (AMI)	29b Plasma	↑ in AMI subjects versus controls	18 controls Age [y], median (IQR) 59 (55.25–70.00) Female Sex %: 5 (27.8) 44 AMI Age [y], median (IQR) 56 (48.00–66.00) Female Sex %: 7 (15.9)	6-months after AMI, miR-29b showed a significant inverse correlation with the absolute change in infarct volume and end-diastolic volume of the left ventricular.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Country: Germany (Munich)	
Lopez et al., 2017 **10.1039/C6MB00596A**	T2D	29b Plasma	↓ in pre diabetic subjects	N = 60 20 healthy controls: (11 lean + 9 with obesity): Lean : Sex :8F/3M Age 32 (23, 67.5) BMI: 21.8 (20.3, 24.7) Obese : Sex: 7F/2M Age 34 (19.4, 54 BMI: 35 (26.5, 37.4) 21 prediabetes (10 lean + 11 with obesity) Lean: Sex: 6F/4M Age 42.5 (22.7, 58.6) BMI 21.7 (18.8, 24.4) Obese Sex: 5F/6M Age 42 (35, 62.2) BMI 35.1 (31.5, 52.7) 17 with T2D (2 lean + 15 with obesity). Lean: Sex: 1F/1M Age 41 (30.5, 51.5) BMI: 23.1 (22.3, 23.9) Obese	miR-29b was positively correlated with Il-12

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
				Sex: 5F/10M Age :51 (36.4, 66.6) BMI: 36.5 (30.8, 52.1) Country: USA (Florida)	
Wang, L et al., 2021 10.1159/000511772	Obesity	29a-3p 29b-2-5 29c-5p Abdominal SAT samples	↓ in morbidly obese subjects versus normal weight controls	Microarray: N = 8 Chinese Females 5 controls (normal weight) (admitted for elective abdominal surgical procedures) Age: 47.20 ± 2.58 years BMI 22.03 ± 0.78 3 morbidly obese (had received laparoscopic adjustable gastric banding) Age: 42.00 ± 7.57 years BMI: 47.53 ± 1.53) Validation Study N = 17 Females 9 controls (2 from the microarray group included) Age: 42.11 ± 2.23 years; BMI: 22.42 ± 0.54 8 morbidly obese (3 obese from microarray group included) Age: 34.88 ± 3.42 years; BMI 45.45 ± 2.40 Country: China	miR-29b was down-regulated in morbidly obese subjects versus normal weight controls.

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
Wang, X 2014 10.1371/journal.pone.0086792	T2D	29b Plasma	↑in subjects with T2D	n = 152 84 Iraqis (19 T2D; 65 controls) Sex: F39/45M Age mean/%: 54.64 68 Swedes (14 T2D; 54 controls) Sex: 30F/38M Age mean/%: 57.15 Inclusion: 45 to 65 years; Born: Sweden or Iraq were randomly selected from the Malmö city census register and recruited to the study. Country: Sweden (Lund)	From the total study population, adjusted for age, sex, WC, family history of DM and sedentary lifestyle; miR-29b expression increased in T2D subjects versus controls
Zampetaki et al., 2010 10.1161/CIRCRESAHA.110.226357	T2D	29b Plasma	↓ in subjects with T2D	N = 160 (40 - 79 years) 80 Controls 80 80 T2D Sex: 49.9% Female Mean Age: 62.9 years Country Italy	miR-29b levels were lower in diabetic subjects. In subjects who were normoglycemic then went on to develop T2D over a 10-year period; baseline miR-29b levels were already significantly lower in those subjects.
In Vitro Studies					
He et al., 2007 10.1210/me.2007-0167	T2D	29a, b, c Tissue cells	↑ diabetic rats ↑ in target tissues of insulin action: muscle, fat, and liver of diabetic rats.	Animals, Cells, and Reagents Country: China (Beijing)	overexpression in 3T3-L1 adipocytes, repressed insulin-induced glucose uptake by cells miR-29a and miR-29b, but not miR-29c, in 3T3-L1 adipocytes can be up-regulated

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
					by hyperinsulinemia together with hyperglycaemia
Animal – Experimental Studies					
Esteves et al., 2018 10.3389/fendo.2018.00536	DM	29b-3p Muscle sample	↑ in diabetic rats	Male Wistar rats Country: Brazil (São Paulo)	miR-29b-3p was up-regulated in muscle of diabetic male rat's vs control miR-29b was negatively correlated with GLUT4, HK2 Insulin treatment completely restored miR-29b-3p expression.
Combined Study Design					
Domingo-Gonzalez et al., 2015 10.1152/ajplung.00283.2014	Inflammation	29b BAL cell samples	↑ in alveolar macrophages after bone marrow transplant	Humans: 6 controls 14 hematopoietic stem cell transplantation (HSCT) autologous and allogeneic. Mice Country: USA (Michigan)	**In Vitro + in Vivo + Animal** miR-29b drives the responses of defective alveolar macrophages Compared to control groups, higher was found of miR-29b expression in alveolar macrophages was found in bone marrow transplantation mice and in patients with HSCT. High expression of miR-29b was observed in patients even 2 years after HSCT.
Niderla-Bielińska et al., 2021 **10.3390/ijms22042197**	Met S	29b-3p Cardiac Tissue and cells	↓ in db/db myocardium-derived macrophages	Nine-week-old male mice Country: Poland (Warsaw)	**In Vitro and animal study** miR-29b is possibly associated with MetS pathology in cardiac tissue. miR-29b was found to be down-regulation in db/db myocardium-derived macrophages.
Saravanan et al., 2019	Inflammation/hypoxic stress	29b-3p Human islets	↑ in when exposed to cytokines and	N = 6 Blood samples Human islets in vivo	Ex vivo and in vivo mouse and human studies

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
10.1007/s00125-019-4950-x		+ Plasma exosomal miRNAs	hypoxia	Mice (islet transplantation) Country: USA	miR-29b-3p differentially released in exosomes when exposed to a proinflammatory cytokine cocktail and/or hypoxia Release of exo-miRNAs hsa-miR-29b-3p was detected within 6 h of exposure to cytokines and hypoxia.
Silambarasan et al., 2016 **10.3390/ijms17040518**	T2D	29b-3p Human Umbilical vein endothelial cells Human and Rat Samples	↑ in subjects who had Impaired fasting glucose or T2D	Cell Cultures Humans Rats Country: Singapore	**In Vivo + in Vitro** In both in vitro and in vivo studies, up-regulation of miR-29b-3p was observed. In humans and mice with T2D and in humans with impaired fasting glucose, a positive correlation was found between hyperglycaemia-induced endothelial dysfunction and miR-29b expression. miR-29b-3p gradually increased with increasing glucose concentration at 24 and 48 h treatments and miR-29b-3p correlated to endothelial cell apoptosis.
Review					
Guay et al., 2013 10.1038/nrendo.2013.86	DM	29 29a	Systematically searched PubMed ↑miR-29 in islets of prediabetic nonobese diabetic mice (a model of T1D) which has deleterious effects on β-cell function ↑miR-29a in patients to T2D compared with prediabetes or T2D susceptible to T2D; however, no differences were seen between NGT and prediabetes, patients-questioning the validity of the biomarker. Deregulated of miR-29a in women developing GDM before changes in blood levels of glucose were detectable		
McGregor et al.,	Obesity	29 family	miR-29 family has leptin binding sites in mouse studies miR-29 induced by exposure to high extracellular glucose		

Author Year DOI	Disease/ Process	miRNA Sample Type	Expression	Population (n)	Study Outcome
2011 10.2174/1566524 11795677990					
Vienberg et al., 2017 **10.1111/apha.126 81**	Obesity/ T2D	29 family	In the pancreatic β-cell: ↑miR-29 family in the young vs. the mature islets The miR-29 family is important in maintaining the balance between differentiation and proliferation		

Chapter 3 – Research Manuscript

3.1 Abstract

Background: Excess adipose tissue is associated with metabolic risk and developing obesity related diseases. Many people are unknowingly metabolically unhealthy, having a high BF% despite normal BMI classification. Evidence of miRNAs as potential biomarkers of metabolic risk may prove useful in identifying those at metabolic risk where BMI classification may fail them.

Objectives: To explore miRNAs expression levels in healthy NZ women with different body composition profiles and its association with metabolic markers, dietary and physical activity factors.

Methods: Cross-sectional design investigating healthy NZ women (n = 406) of three ethnicities (Māori, Pacific, NZE) aged 16 to 45 years. Body mass index and body fat % defined body profile groups; "NN" group - normal BMI (≥18.5 and <25kg/m^2) and normal BF% (≥18%, <30%); "NH" group - normal BMI (<25 kg/m^2) and high BF% (≥30%); "HH" group – high BMI (≥25 kg/m^2) and high BF% (≥30%), of which 382, met the criteria. Anthropometry, metabolic biomarkers, miRNA, dietary, and physical activity factors were evaluated.

Results: Of the 406 participants, 105 (27.5%), 70 (18.3%), and 207 (54.2%) were classified as having NN, NH, and HH body profiles, respectively. The adjusted (age, deprivation index and other miRNAs) odds of having higher miR-222-3p were increased in NH (OR = 1.92, 95% CI 1.13-3.26) and HH (OR = 2.58, 95% CI 1.58-4.21) versus NN group. The adjusted odds of having higher miR-29b-3p decreased in HH (OR = 0.09, 95% CI 0.02-0.37) versus NN group. Higher miR-222-3p (1.084-14.438 AU) was associated with HH body profile (p = 0.002), higher leptin levels (p = 0.04) and sucrose intake (p = 0.025) and lower protein intake (p = 0.017). Higher miR-29b-3p (0.202-1.851 AU) was associated with lower HbA1c (p = 0.016), TNF-α (p = 0.001), IL-6 (p = 0.016), IL-10 (p = 0.021) and higher sucrose intake (p =

0.049), and higher miR-17-5p (0.103-0.806 AU) was associated with higher TNF-α (p = 0.014) and lower IL-6 (p = 0.001).

Conclusion: Our findings for miR-222-3p strongly support previous research, as the most promising biomarker for obesity of the selected miRNA analysed in this study. Our study identified a selection of specific metabolic (Leptin, HbA1c) and inflammatory (IL6, IL-10, TNF-α) markers, dietary factors (sucrose, carbohydrate, protein) and light physical activity, to be associated with miR-222-3p, miR-29b-3p, and miR-17-5p. These findings suggest that miRNAs are involved in metabolic processes and, with further research, may be used as biomarkers of metabolic risk.

3.2 Introduction

Globally the prevalence of obesity is rising, increasing morbidity and mortality, and causing an enormous burden on our health care system (Tremmel et al., 2017). Obesity is associated with metabolic risk which is characterised by having a sub-optimal waist circumference, BP, blood sugar, and lipid profile, all which increase the risk of developing metabolic diseases (Araujo et al., 2019). A key driver behind metabolic disorders is chronic inflammation which is associated with excess body fat (Landrier et al., 2019). Metabolic risk also depends on where body fat is stored. Worse metabolic outcomes are associated with the accumulation of visceral compared to subcutaneous fat (Elffers et al., 2017; Shah et al., 2014).

Body mass index is the most common anthropometric measure that used the weight of a person in kilograms divided by their height in metres squared to determine obesity (BMI ≥30 kg/m^2) (WHO, 2021a). However, its merits are contested as it cannot directly measure BF% or its regional location, both which greatly influence metabolic risk (CDC, 2020a; Gomez-Ambrosi et al., 2011). More direct measures of adiposity, such as such as BIA, DXA, ADP, and underwater weighing, would be a better indicator of metabolic risk (CDC, 2020b; HSPH, 2021; Q. Sun et al., 2010; Willett et al., 2006). However, these too have limitations due to a lack of validation and no established BF% cut-off points to define obesity (Oreopoulos et al., 2011; Romero-Corral et al., 2008). However, previous research has indicated that 30-38% body fat is useful to determine metabolic risk (Oliveros et al., 2014; Oreopoulos et al., 2011).

There is a significant proportion of people who have normal weight obesity, a term used to describe those with normal BMI who are suffering or at risk of metabolic dysregulation, due to having a high BF% (>30%), or excess visceral fat (Kruger et al., 2015; Oliveros et al., 2014). For these individuals BMI measurements alone would not identify their metabolic risk.

To combat the limitations stated above, there has been a rise in research to find other potential biomarkers of health to determine disease risk, such as miRNA. MicroRNAs are short noncoding RNA molecules that can alter gene expression by turning genes on and off (Landrier et al., 2019). This allows them to regulate numerous metabolic processes like insulin signalling, adipokine expression and differentiation, food intake and immune-mediated inflammation (Deiuliis, 2016; Landrier et al., 2019). MicroRNAs ability to alter normal physiological processes allow them to act as mediators of diseases. This, along with

the benefits of circulating miRNAs being stable and easily accessible, makes them good biomarker candidates (Etheridge et al., 2011).

Although the exact mechanisms by which miRNAs influence metabolism are still unclear, several miRNAs show differential expression between healthy subjects and people with obesity and metabolic diseases (e.g. obesity, T2D) (Heneghan et al., 2011; Huang et al., 2018; Ji et al., 2019; Kim et al., 2020; Tsukita et al., 2017; Zhong et al., 2018). Up-regulation of miR-222-3p (miR-222) is associated with increased adiposity, inflammation, and T2D. In diabetes, miR-222 has been highly expressed in subjects with β-cell dysfunction (Belongie et al., 2017), positively associated with HbA1c levels and negatively affects GLUT4 expression, which is an important regulator for glucose metabolism/homeostasis (Deiuliis, 2016; Huang et al., 2018; Xiao et al., 2018).

MicroRNA-17-5p (miR-17) is one of six miRNAs in the miR-17~92 family (Kozomara et al., 2019). These miRNAs have been involved in adipogenesis (Lin et al., 2009), adipocyte differentiation (Hilton et al., 2013; McGregor et al., 2011; Q. Wang et al., 2008), and pro-inflammatory and anti-inflammatory macrophage regulation (X. Zhang et al., 2020). Expression of miR-17-5p specifically has been associated with inflammation, obesity, and related disorders (Kloting et al., 2009; Tan et al., 2019; X. Zhang et al., 2020). In obese subjects, miR-17-5p has been down-regulated and inversely correlated with BMI (Heneghan et al., 2011; Kloting et al., 2009). Significant differences in miR-17-5p expression have also been found in patients with T2D and miR-17-5p negatively correlated with HbA1c (Arner et al., 2015; McGregor et al., 2011), suggesting it may be a regulator of insulin resistance in T2D (Karolina et al., 2012).

MicroRNA-29b-3p is one of three miRNAs in the miR-29 family (Kriegel et al., 2012). Expression of miRNA-29 is mostly associated with cancer (Y. Wang et al., 2013), however, recently it has been found to play a major role in T2D, balancing differentiation and proliferation in pancreatic β-cells (Vienberg et al., 2017). Its increased expression has been a key contributor to inflammation in diabetes (Y. Sun et al., 2021). MicroRNA-29b is specifically associated with pancreatic islet β-cell function, glycaemic control, and is dysregulated in obese subjects with prediabetes and T2D. Upregulation of miR-29b-3p has been found in hyperinsulinaemic and hyperglycaemic states (He et al., 2007), affecting insulin secretion and increasing the risk of T2D (Y. Sun et al., 2021). Research has also

shown that miR-29 suppression improves insulin resistance and reduces islet inflammation (Y. Sun et al., 2021).

Diet and physical activity can influence miRNA expression, leading to altered energy metabolism (Tan et al., 2019). This can result in an increased risk of fat accumulation and metabolic disease (Kruger et al., 2015; Quintanilha et al., 2017; Ultimo et al., 2018). There is a substantial amount of research investigating the impact of genetics, diet, and physical activity on body composition and metabolic risk, which, in turn, may provide insight into future disease prevention and treatment strategies (Gulyaeva et al., 2016; Ji et al., 2019; Martinez et al., 2008; Quintanilha et al., 2017; Ultimo et al., 2018).

The differences in expression of miR-222-3p, miR-29b-3p, and miR-175p mentioned above and the association with metabolic markers strongly suggest that they may be involved in the manifestations of obesity and T2D. This is promising and suggests that miRNAs could be used not only as potential biomarkers of health, but also as therapeutic agents (Deiuliis, 2016).

The aim of this study was to explore miRNA expression levels in body composition of healthy NZ women and its association with metabolic markers, dietary and physical activity factors. Early identification for those at risk of metabolic disease associated with obesity is crucial for early interventions and management to prevent disease onset. If we can find specific markers for disease risk related to adiposity, we can determine the risk, especially in people whose BMI classification may fail them, such as those with NWO.

3.3 Methodology

3.3.1 Study design

The current study is a sub-analysis that presents data on one of the primary outcomes from the cross-sectional EXPLORE study, which investigated predictors and risks of body fat profiles in young (post-menarche, premenopausal) NZE, Māori, and Pacific women (Kruger et al., 2015). Written informed consent was obtained from all subjects. Ethical approval was obtained from the Massey University Human Ethics Committee: (Southern A), Reference No.13/13.

3.3.2 Subjects:

A total of 408 healthy premenopausal women, aged 16 to 45 years, were recruited from Auckland, NZ. The women were of Māori (n = 84), Pacific (n = 91) or NZE (n = 233) descent. Exclusion criteria were being pregnant or breastfeeding (lactation), irregular menstrual cycles, diagnosed with any chronic illness, particularly those that affect metabolic health (diabetes). Women were recruited according to three body composition profile groups (BPG): "Normal fat" (NN) group - normal BMI (≥18.5 and <25kg/m^2) and normal BF% (≥18%, <30%); "Hidden Fat" (NH) group - normal BMI (<25 kg/m^2) and high BF% (≥30%),; "Apparent Fat" (HH) group – high BMI (≥25 kg/m^2) and high BF% (≥30%).

3.3.3 Procedures

All data collection occurred at the Human Nutrition Research Unit at Massey University in Auckland. The full methodology of the EXPLORE study is described elsewhere (Kruger et al. (2015), however, the procedures related to this study are described below. The study design involved three stages of data collection: Stage 1 (screening for inclusion criteria and body composition), Stage 2 (site assessment), and Stage 3 (home assessments) (**Figure 3.1).**

Stage 1 (screening)

A screening questionnaire was used to ensure inclusion and exclusion criteria were met. Appointments were scheduled to conduct basic anthropometric measures to categorise women into one of the three body composition profile groups (described above). The anthropometric measures obtained were weight (kg) and height (m) to calculate BMI (kg/m^2)

and BF% using a bioelectric impedance machine (Biospace, Inbody 230, Cerritos, California, USA). Eligible subjects were recruited and progressed to the assessment stages.

Stage 2 (onsite assessments)

Participants visited the MUHNR facility following an overnight fast for collection of further anthropometric measures, body composition and blood samples to determine metabolic health markers. Participants also completed a dietary questionnaire to determine dietary intake (described below).

Stage 3 (home assessments)

Participants had to track their physical activity for seven days at home using an accelerometer and to complete a physical activity diary.

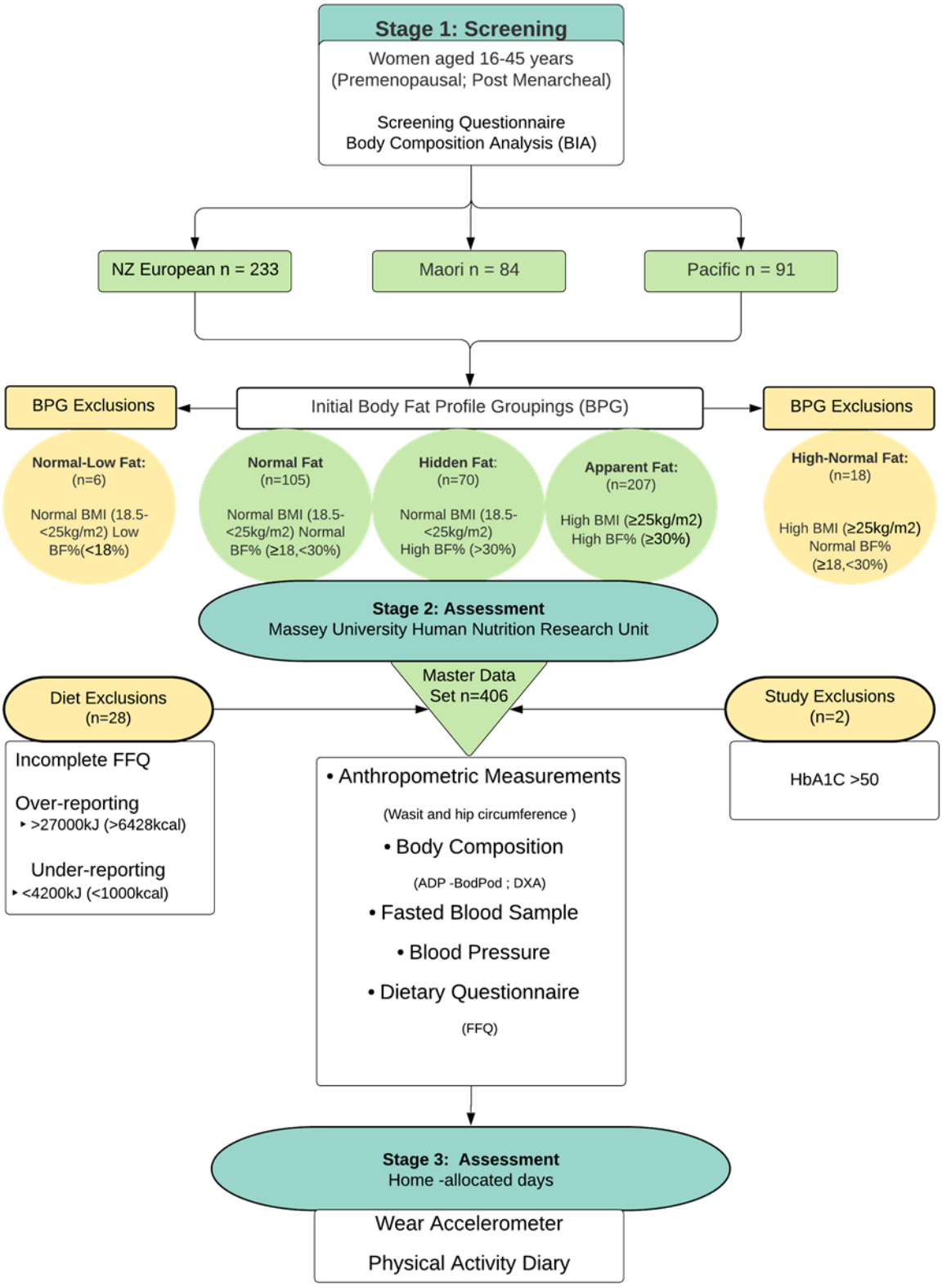

Figure 3.1 The EXPLORE study design and procedures

3.4 Metabolic health methodology

3.4.1 Body composition

All anthropometric assessments were performed by trained researchers, using the International Society for the Advancement of Kinanthropometry (ISAK) protocol and standards (Marfell-Jones et al., 2006). Waist/hip circumferences (cm) was measured using Lufkin steel tapes and height was measured using a Harpenden stadiometer and recorded to the nearest 0.1 cm. Waist-hip-and waist-height ratios were derived from these measurements. Total body fat content was measured using ADP, using the thoracic gas volume method and appropriate attire (swimwear and cap) (BodPod, 2007A, Life Measurement Inc., Concord, California, USA) (Kruger et al., 2015; Oliveros et al., 2014; Wingfield et al., 2014). Weight was measured with an electronic scale attached to the ADP machine. To assess metabolic risk regional body composition, including lean and body fat mass from the gynoid and android regions were measured using DXA (Hologic Discovery A, serial number 85296, with Hologic Discovery QDR software)(Kruger et al., 2015).

3.4.2 Blood sample collection

Metabolic biomarkers associated with lipid profile (TC, HDL, LDL, TAG), glucose control (insulin, glucose, HbA1c), inflammation (CRP, IL-6, IL-10, TNF-α) and appetite regulation (ghrelin, leptin) were measured. Overnight fasted serum and plasma (EDTA and heparin) samples were collected in vacutainers between 7-10 am by registered phlebotomists. Vacutainers were centrifuged at 3500g at 4° C for 15 minutes within an hour of sample collection. Separate aliquots in Eppendorf tubes were frozen at -80° C until analysis. Analyses were performed by fully accredited laboratories (with IANZ to the ISO 15189) or qualified laboratory technicians (Kruger et al., 2015).

3.4.3 Blood pressure

Resting BP was measured after a 10-minute rest period. One of two arm cuff sizes (22-32 cm or 32-48 cm) was attached to the arm not involved in venepuncture. Three measurements were taken with a one-minute rest period between each measure using a Ri-Champion N automated BP monitor (Rudolf Riester GmbH, Jungingen, Germany).

3.4.4 MicroRNA analysis

The miRNAs were selected based on their association with metabolic disease risk and energy homeostasis. Plasma miRNAs were analysed in Aaron Russell's research laboratory at Deakin University, Australia, using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). For miR-222-3p, miR-29b-3p, and miR-17-5p analysis, extracted RNA was reverse transcribed using target-specific primers followed by qPCR using target-specific probes as described previously (Guller et al., 2010; Russell, Lamon, et al., 2013; Russell, Wada, et al., 2013; Zacharewicz et al., 2014). All qPCR analysis were performed using the Stratagene MX3000p thermocycler (La Jolla, CA); miRNA results were normalized to RNA input and log transformed if not normally distributed.

3.5 Energy intake and expenditure methodology

3.5.1 Diet

All subjects completed a semi-quantitative validated NZ women's food frequency questionnaire (NZWFFQ) that captured energy and nutrient intake (Beck et al., 2020). The FFQ was adapted from the Adult National Nutrition Survey in NZ (MOH, 1997). All subjects recorded the frequency of single foods consumed during the previous month based on standard portion sizes. All NZWFFQ data were entered using Foodworks 7 2010 (Xyris Software Pty Ltd, Queensland, Australia). Data entered monthly and weekly were converted into daily equivalent frequencies (never, <1x/month, 1–3x/month, 1x/week, 2–3x/week, 4–6x/week, once/day, 2–3x/day, 4+ x/day) and assessed.

3.5.2 Physical activity

Waist-worn wGT3X triaxial accelerometers (Actigraph, Pensacola, FL) were worn over seven consecutive days. Physical activity intensity and duration were recorded. To be considered to have valid data, subjects must have worn the accelerometer for at least 10 h/day for 4 days, including one weekend day (Kruger et al., 2015). Physical activity levels (counts/min) were identified using validated cut-off points: sedentary (0-99), light (100-2019), moderate (2020-5998), vigorous (≥5999) and moderate-vigorous (≥2020); time was averaged across all valid days. All non-wear and sleep times were determined and removed

using MATLAB algorithm computer software (R2011b 7.13.0.564, The MathWorks Inc., Natick, MA)(O'Brien, 2018).

3.6 Data analysis

To detect a medium effect size *f* of 0.25 (G*Power 3.1.2) with 80% power when $p < 0.05$, there needed to be 225 women per ethnic group with 75 women per body fat profile group to compare the "hidden fat" profile with the other two body composition profiles (Kruger et al., 2015). To detect an effect size of 0.8 with 80% power, each group needed 26 subjects. In the data from the EXPLORE study the aim was to compare characteristic differences in BPG and ethnicities. There were enough NZE women to place into each of the three BPG however not enough Pacific and Māori women were able to be recruited within the study timeframe to place into each BPG; specifically, the "NN" and "NH". Due to this, NZE women were the only group that could be analysed according to these three BPG. To compare the differences in the BPG, the women were collectively assessed excluding ethnicity. To compare ethnic groups, differences were assessed excluding the BPGs.

All statistical analyses were carried out using IBM SPSS Statistics 27 analysis program (IBM Corporation, New York, USA). Assumption of normality was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests, Q-Q plots, box plots, and histograms. All scale data including non-normally distributed data were reported as mean ± standard deviation in agreement with the central limit theorem (Field, 2013). Differences between the EXPLORE profile groups and ethnic groups were analysed using an ANOVA test and Pearson's chi-square analysis (for categorical data such as lower and upper range of miRNA and metabolic markers). Significance was assumed when $p \leq 0.05$. Homogeneity of variance was tested using the Levenes F test. One-way ANOVA was used when homogeneity of variances was assumed ($p > 0.05$). When homogeneity of variances was violated, Welch's ANOVA was used. Significant differences observed between groups were assessed using the Bonferroni and Games Howell post hoc tests. Analysis of covariance (ANCOVA) was used to assess the association of BPG, BF%, BMI and ethnicity with selected miRNA. Bonferroni correction for significant variables was used for multiple comparisons. The ANCOVA body composition analyses were adjusted for ethnicity. Independent sample t-test compared the means of diet, physical activity, metabolic and inflammatory markers, in lower and upper range of selected miRNAs. Pearson's bivariate correlation was conducted to assess the

correlations between miRNAs and body composition, metabolic biomarkers, diet, and physical activity variables. Multiple binary logistic regression analysis was used. Models were investigated to ensure assumptions (multi-collinearity, homoscedasticity, linearity, influential outliers etc), weren't violated. Multinominal regression controlling for age and NZ deprivation was used to determine odds ratios of increased miRNA as predictors of BPG, BF%, BMI and ethnicity. Regression on BF% and BMI was conducted to ensure no associations were neglected due to BPG categorisation and to ensure results were comparable with other studies. To explore the clinical, dietary, and physical activity characteristics as predictors of selected miRNAs, binomial backward (LR) and forward (LR) regression consisting of 29 variables was used and compared. The forward method correlated with some significant variables found in other statistical tests; however, the backward method was favoured to minimise suppressor effects and lower the risk of Type II error (missing a predictor that does predict the outcome)(Field, 2018). Predictors selected for regression were based on significant associations found in our statistical tests, as well as significant variables from other studies. Pearson and Deviance statistics were used to assess goodness-of-fit indicated by $p > 0.05$. Associations were described using adjusted odds ratios (OR) and 95% confidence intervals (CI). A $p \leq 0.05$ was considered statistically significant in all statistical tests conducted.

3.7 Results

A total of 406 subjects were included in the analyses in this study, of which 382 subjects met the BPG criteria. Of those subjects there were 223 NZE, 80 Māori and 93 Pacific Island women (**Table 3.1).** On average, the total subjects were young (31 ± 9 years), overweight (BMI 27.3 ± 6.29 kg/m2), had a high BF% (34.2 ± 8.28%) and above normal WC (82.5 ± 13.2 cm).

3.7.1 Anthropometric and body composition measurements across body profile and ethnic groups

As seen in **Table 3.1** the BMI mean for the NN, NH, and HH group was 21.8, 23.2, and 31.8 kg/m^2, respectively. BF% was 25.1%, 33.5% and 40.2% for the NN, NH, and HH group, respectively, showing positive correlations between BMI and BF%. WHR, total lean mass and gynoid lean mass were significantly higher in NH and HH groups than the NN group ($p \leq 0.05$). Also, the NH group had significantly higher total fat% than the NN group ($p < 0.05$). Anthropometric measures were highest in Pacific women, followed by Māori then NZE ($p < 0.05$).

A larger percentage of the HH group than NH and NN groups **(Figure 3.2A)** and of Pacific ethnicities than NZE ethnicity **(Figure 3.2B)** were from lower socio-economic status (as measured by NZ deprivation index 9-10 versus 1-2, respectively, $p < 0.05$).

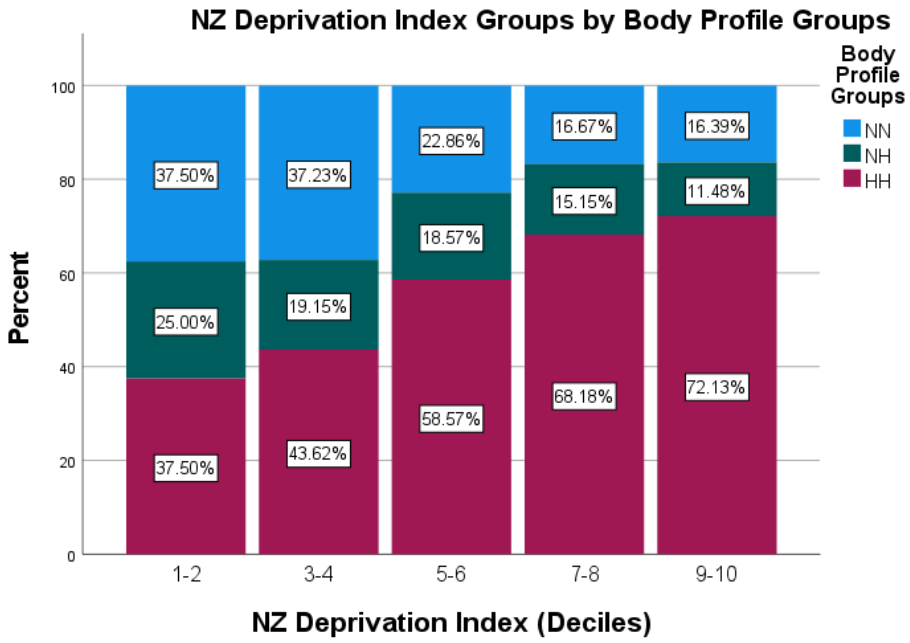

Figure 3.2A NZ Deprivation Index by body profile groups. Decile 1 represents areas with the least deprived scores and decile 10 represents areas with the most deprived scores (Environmental Health Intelligence New Zealand, n.d.).

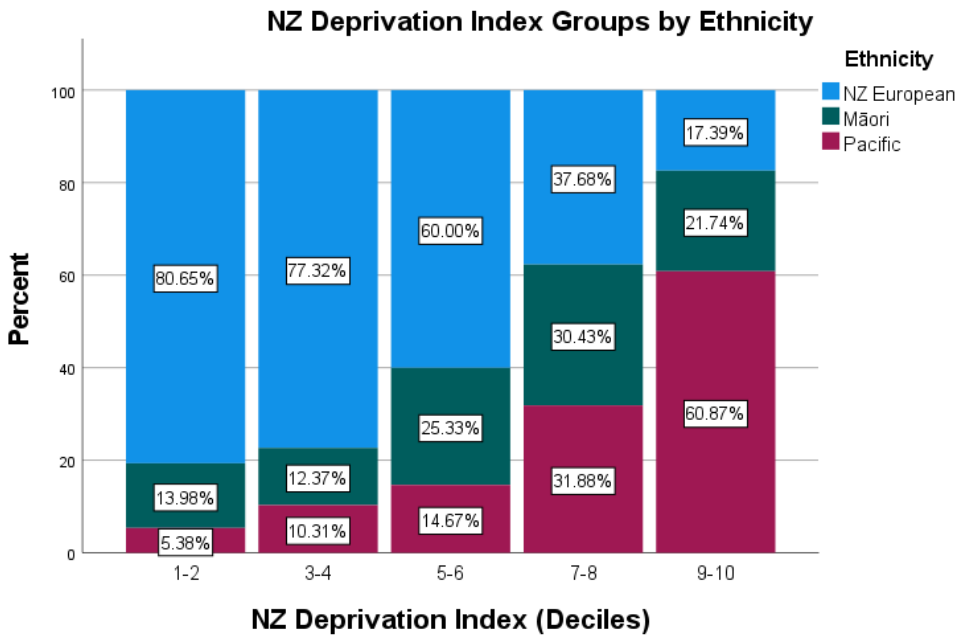

Figure 3.2B NZ deprivation index by ethnicity. Decile 1 represents areas with the least deprived scores and decile 10 represents areas with the most deprived scores (Environmental Health Intelligence New Zealand, n.d.).

Table 3.1 Demographic and anthropometric characteristics classified by body profile groups and ethnicity

		Body Profile Groups			***p*-value**	**Ethnic Groups**			***p*-value**
	Total	**NN**	**NH**	**HH**		**NZE**	**Māori**	**Pacific**	
	(*n* = 406)	(*n* = 105)	(*n* = 70)	(*n* = 207)		(*n* = 233)	(*n* = 80)	(*n* = 93)	
Age (years)	31±9	29±8 [a,b]	32±9 [a]	32±9 [b]	0.014	32±8 [a]	30±9	29±9 [a]	0.003
NZ Deprivation Index	5.11±2.85	4.14±2.51 [b]	4.37±2.67 [c]	5.78±2.87 [b,c]	**<0.001**	3.95±2.34 [a,b]	5.84±2.72 [b,c]	7.48±2.50 [a,c]	**<0.001**
Height (cm)	167±6.34	167 ±6.11	167±6.1	166±6.53	0.105	167±6.63	166±6.13	167±5.74	0.387
Weight (kg)	76.1±17.9	61.5±6.77 [a,b]	65.3±5.51 [a,c]	88.1±17.1 [b,c]	**<0.001**	70.2±14.3 [a,b]	77.7±17.6 [b,c]	89.7±19.1 [a,c]	**<0.001**
BMI (kg/m2)	27.3±6.29	21.8±1.66 [a,b]	23.2±1.25 [a,c]	31.8±5.76 [b,c]	**<0.001**	25.2±5.15 [a,b]	28.2±6.14 [b,c]	32.0±6.35 [a,c]	**<0.001**
Body Fat %	34.2±8.28	25.1±3.06 [a,b]	33.5±2.88 [a,c]	40.2±6.09 [b,c]	**<0.001**	32.6±8.05 [a]	34.5±8.44 [c]	38.0±7.51 [a,c]	**<0.001**
WC (cm)	82.5±13.2	70.5±4.62 [a,b]	74.6±3.80 [a,c]	91.9±11.6 [b,c]	**<0.001**	78.3±11.1	84.0±12.49 [c]	91.8±13.6 [c]	**<0.001**
HC (cm)	107.3±11.5	97.6±4.87 [a,b]	101.7±4.08 [a,c]	114.8±10.8 [b,c]	**<0.001**	104.1±9.72 [a,b]	107.7±12.0 [b,c]	115.0±11.4 [a,c]	**<0.001**
WHR	0.77±0.06	0.72±0.04 [b]	0.73±0.04 [c]	0.8±0.06 [b,c]	**<0.001**	0.75±0.06 [a,b]	0.78±0.06 [b]	0.8±0.07 [a]	**<0.001**
WHtR	0.50±0.08	0.42±0.03 [a,b]	0.45±0.02 [a,c]	0.55±0.07 [b,c]	**<0.001**	0.47±0.07 [a,b]	0.51±0.08 [b,c]	0.55±0.08 [a,c]	**<0.001**
Total Fat (kg)	6.62±2.53	4.37±0.66 [a,b]	5.60±0.75 [a,c]	8.33±2.43 [b,c]	**<0.001**	6.01±2.13 [a,b]	6.87±2.8 [b,c]	8.03±2.67 [a,c]	**<0.001**
Total Lean (kg)	11.13±2.33	9.60±1.257 [b]	9.65±1.06 [c]	12.46±2.34 [b,c]	**<0.001**	10.4±1.86 [a,b]	11.59±2.24 [b,c]	12.68±2.66 [a,c]	**<0.001**
Total Mass (kg)	17.75±4.61	13.97±1.65 [a,b]	15.24±1.53 [a,c]	20.78±4.48 [b,c]	**<0.001**	16.4±3.71 [a,b]	18.46±4.8 [b,c]	20.7±5.10 [a,c]	**<0.001**
Total Fat %	36.4±5.32	31.3±3.28 [a,b]	36.7±3.10 [a,c]	39.7±4.26 [b,c]	**<0.001**	35.9±5.17 [a]	36.1±6.05	38.1±4.69 [a]	**0.002**
Trunk Fat (kg)	11.06±5.35	6.24±1.30 [a,b]	8.71±1.60 [a,c]	14.74±5.05 [b,c]	**<0.001**	9.68±4.73 [a,b]	11.75±5.59 [b,c]	14.1±5.39 [a,c]	**<0.001**
Trunk Lean (kg)	24.38±4.29	21.54±2.46 [a,b]	21.7±2.06 [a]	26.82±4.28 [b]	**<0.001**	23.08±3.48 [a,b]	25.06±4.22 [b,c]	27.2±4.81 [a,c]	**<0.001**
Trunk Mass (kg)	35.44±9.08	27.8±3.10 [a,b]	30.39±2.86 [a,c]	41.55±8.64 [b,c]	**<0.001**	32.76±7.53 [a,b]	36.82±9.26 [b,c]	41.35±9.70 [a,c]	**<0.001**
Trunk Fat %	29.8±7.08	22.4±3.57 [a,b]	28.6±3.77 [a,c]	34.8±5.26 [b,c]	**<0.001**	28.4±6.89 [a]	30.4±7.57 [c]	33.1±5.91 [a,c]	**<0.001**
Android Fat (kg)	1.86±1.04	0.94±0.22 [a,b]	1.37±0.3	2.57±0.99 [b,c]	**<0.001**	1.57±0.87 [a,b]	2.05±1.1 [b,c]	2.46±11.02 [a,c]	**<0.001**

		Body Profile Groups			*p*-value	Ethnic Groups			*p*-value
	Total	**NN**	**NH**	**HH**		**NZE**	**Māori**	**Pacific**	
	(*n* = 406)	(*n* = 105)	(*n* = 70)	(*n* = 207)		(*n* = 233)	(*n* = 80)	(*n* = 93)	
Android Lean (kg)	3.34±0.83	2.74±0.39 [a,b]	2.88±0.32 [a,c]	3.83±0.838 [b,c]	**<0.001**	3.11±0.65 [a,b]	3.52±0.85 [b]	3.8±0.97 [a]	**<0.001**
Android Mass (kg)	5.20±1.79	3.685±0.518 [a,b]	4.25 ±0.52 [a,c]	6.40±1.74 [b,c]	**<0.001**	4.68±1.44 [a,b]	5.57±1.88 [b]	6.26±2.02 [a]	**<0.001**
Android Fat %	33.7±7.72	25.5±4.07 [a,b]	31.9±4.40 [a,c]	39.2±5.37 [b,c]	**<0.001**	31.8±7.50 [a,b]	0.03±0.01 [b,c]	37.8±6.20 [a,c]	**<0.001**
Gynoid Fat (kg)	4.76±1.57	3.43±0.50 [a,b]	4.23±0.57 [a,c]	5.76±1.55 [b,c]	**<0.001**	4.44±1.33 [a]	4.83±1.78 [c]	5.57±1.66 [a,c]	**<0.001**
Gynoid Lean (kg)	7.79±1.55	6.85±0.93 [b]	6.76±0.80 c	8.63±1.57 [b,c]	**<0.001**	7.29±1.26 [a,b]	8.06±1.46 [b,c]	8.87±1.74 [a,c]	**<0.001**
Gynoid Mass (kg)	12.55±2.91	10.28±1.22 [a,b]	10.99±1.17 [a,c]	14.39±2.86 [b,c]	**<0.001**	11.73±2.36 [a,b]	12.89±3.04 [b,c]	14.44±3.19 [a,c]	**<0.001**
Gynoid Fat %	37.4±4.92	33.4±3.44 [a,b]	38.5±3.2 [a,c]	39.7±4.36 [b,c]	**<0.001**	37.4±4.77	36.6±5.64	38.1±4.53	0.166

Abbreviations: BPG body profile group; BMI body mass index; BF% body fat percentage; NN normal fat group; NH hidden fat group; HH apparent fat group; WC waist circumference; HC hip circumference; WHR waist-to-hip ratio; WHtR waist to height ratio. NZ Deprivation Index (1 = least deprived, 10 = most deprived)
Values are mean±SD. The mean difference is significant when $p = \leq 0.05$
Differences between BPG were analysed by one- way ANOVA and Welch test
a-c Values with the same superscript letters are significantly different according to Games Howell and Bonferroni post-hoc tests ($p = \leq 0.05$).

3.7.2 Metabolic markers in body profile and ethnic groups

Among metabolic markers, leptin and LDL cholesterol were significantly higher in NH than NN group; no other metabolic markers were significantly different between these two groups (**Table 3.2)**. The mean for metabolic biomarker levels, were all within normal ranges per BPG except for insulin which was above normal in the HH group (16.04 mU/mL). The HH group had significantly higher insulin, CRP, ghrelin, leptin, TNF-α, glucose, TAG and BP, and lower HDL cholesterol than NN group ($p < 0.05$). Compared to NH, the HH group had significantly higher TNF-α, glucose, TAG and BP and lower HDL cholesterol.

Compared to NZE women, Māori and Pacific had higher insulin, TNF-α, HbA1c and lower HDL cholesterol ($p \leq 0.05$). Pacific women also had higher cholesterol, LDL cholesterol, BP, ghrelin, and leptin than NZE women ($p < 0.05$). Insulin levels in Pacific (19.52 mU/mL) were more than double those of NZE (9.21 mU/mL) and were above the normal reference range (>13 mU/mL).

When assessing the association of metabolic and inflammatory markers with lower and upper range of selected miRNA, women in the upper range group of miR-222-3p had higher leptin level than those in the lower range group (11695±8216 vs. 9870±8305, $p = 0.04$). Women in the upper range group of miR-29b-3p had lower HbA1c ($p = 0.016$), TNF-α ($p = 0.001$), IL-6 ($p = 0.016$) and IL-10 ($p = 0.021$) than those in the lower range group. Metabolic and inflammatory markers did not show statistically significant associations with miR-17-5p ($p \geq 0.05$) (supplementary **Table 3.A).**

3.7.3 Diet and physical activity across body profile and ethnic groups

The mean energy, protein, fat, saturated fat, and monounsaturated fat intake was higher in HH than NN group ($p < 0.05$), but the percentage energy from protein and fat and the percentage of total fat from monounsaturated fat and saturated fat was not significantly different across these BPGs (**Table 3.3)**. Similarly, the mean energy, protein, carbohydrate, fat, polyunsaturated fat, and monounsaturated fat intake was higher in Pacific than NZE women ($p < 0.05$), but the percentage of energy from protein, carbohydrate and fat and percentage of fat from polyunsaturated and monounsaturated fat was not different these ethnicities.

The NH and HH groups spent less time doing moderate ($p = 0.002$), moderate-vigorous ($p = < 0.001$) physical activity, while HH spent the least amount of time doing vigorous physical activity ($p = < 0.001$) compared to NN. Pacific women spent the least amount of time in moderate ($p = < 0.001$) and vigorous ($p = 0.028$) physical activity and most time being sedentary ($p = 0.004$), respectively, compared to NZE. Both Māori and Pacific women spent less time doing moderate-vigorous physical activity ($p = < 0.001$).

Women in the higher range group of miR-222-3p had higher sucrose intake ($p = 0.025$) and lower percentage energy from protein than those ($p = 0.023$) in the lower range group. Furthermore, women with higher miR-29b-3p had higher sucrose intake ($p = 0.049$). No association was found between miR-17-5p and any dietary factors. Apart from miR-29b-3p in which higher levels were associated with lower level of light physical activity, ($p = 0.032$), no other associations were found between miRNAs and physical activity levels (supplementary **Table 3.B**).

Table 3.2 MicroRNA and clinical characteristics of participants classified by body profile groups and ethnicity

	Reference Range	**Total** (*n* = 406)	**Body Profile Groups**			***p*-value**	**Ethnic Groups**			***p*-value**
			NN (*n* = 105)	**NH** (*n* = 70)	**HH** (*n* = 207)		**NZE** (*n* = 233)	**Māori** (*n* = 80)	**Pacific** (*n* = 93)	
MicroRNA										
miR-17-5p		0.14±0.13	0.13±0.11	0.18±0.15	0.14±0.12	0.128	0.14±0.127	0.16±0.16	0.14±0.12	0.655
miR-222-3p		1.4±1.29	1.1±0.77 [a,b]	1.54±1.30 [a]	1.54±1.52 [b]	**<0.001**	1.34±1.08	1.45±1.79	1.51±1.27	0.573
miR-29b-3p		0.29±0.28	0.30±0.26	0.35±0.31	0.27±0.28	0.141	0.33±0.31 [a]	0.25±0.26	0.20±0.16 [a]	**<0.001**
Inflammatory markers										
CRP (mg/L)	< 5	4±3.39	3.26±0.81 [b]	3.96±3.92	4.52±4.08 [b]	**<0.001**	4.14±4.01	3.91±2.6	3.71±1.99	0.584
TNF-α (pg/mL)		6.8±2.44	6.17±2.13 [b]	6.39±2.29 [c]	7.25±2.51 [b,c]	**<0.001**	6.27±2.2 [a,b]	7.69±2.94 [b]	7.42±2.19 [a]	**<0.001**
IL-6 (pg/mL)		2.46±2.49	2.58±3.69	2.36±1.46	2.47±2.06	0.850	2.2±1.29	2.78±2.89	2.84±3.99	**0.050**
IL-10 (pg/mL)		16.4±23.9	13.7±10.7	17.4±31.1	17.3±26.5	0.531	14.7±19.2	23.2±40.0	14.7±11	0.170
Metabolic markers										
HbA1c (mmol/mol)	≤ 40	28.6±3.63	28±3 [b]	27±3 [c]	29±4 [b,c]	**<0.001**	27±3[a,b]	30±4 [b]	31±3 [a]	**<0.001**
Glucose (mmol/L)	> 5.4	4.68±0.4	4.5±0.4 [b]	4.6±0.3 [c]	4.8±0.4 [b,c]	**<0.001**	4.6±0.4 [a]	4.7±0.4	4.8±0.4 [a]	**0.003**
Insulin (mU/mL)	< 13	12.4±8.74	8.29±5.26 [b]	8.66±4.07 [c]	16.04±10 [b,c]	**<0.001**	9.21±5.38 [a,b]	13.49±7.73 [b,c]	19.52±11.71 [a,c]	**<0.001**
Triglyceride (mmol/L)	< 2.0	0.97±0.64	0.79±0.28 [b]	0.89±0.42 [c]	1.11±0.8 [b,c]	**<0.001**	0.92±0.68	1.12±0.58	0.97±0.55	0.063
Cholesterol (mmol/L)	< 5.0	4.58±0.89	4.55±0.93	4.79±0.85	4.54±0.89	0.112	4.76±0.92 [a]	4.45±0.7	4.21±0.82 [a]	**<0.001**
Calc. LDL (mmol/L)	< 3.4	2.58±0.82	2.44±0.82 [a]	2.76±0.73 [a]	2.62±0.86	0.039	2.69±0.88 [a]	2.48±0.68	2.4±0.74 [a]	**0.010**
HDL (mmol/L)	> 1.0	1.55±0.41	1.75±0.45 [b]	1.63±0.38 [c]	1.41±0.35 [b,c]	**<0.001**	1.66±0.41 [a,b]	1.46±0.39 [b]	1.37±0.32 [a]	**<0.001**
Systolic BP (mmHg)	120	116±10	113±9 [a,b]	114±9 [a,c]	118±11 [b,c]	**<0.001**	115±10 [a]	116±11	118±11 [a]	**0.046**
Diastolic BP (mmHg)	80	72.8±8.3	70±7 [b]	71±8 [c]	75±9 [b,c]	**<0.001**	72±7 [a]	74±9	74±9 [a]	**0.012**
Ghrelin (pg/mL)		46.8±39.3	53.9±42.8 [b]	48.1±31.5	40.5±35.6 [b]	**0.010**	52.9±42.1 [a]	44.3±31.2	32.8±34.5 [a]	**<0.001**
Leptin (pg/mL)		10488±8168	4552±2897 [a,b]	7910±4407 [a]	15041±8672 [b]	**<0.001**	8903±7417 [a]	10705±7916 [c]	14481±8958 [a,c]	**<0.001**

Abbreviations: BPG body profile group; NN normal fat group; NH hidden fat group; HH apparent fat group; HbA1c glycated haemoglobin; HDL high density lipoprotein; Calc LDL Calculated low density lipoprotein; CRP C-reactive protein; TNF-α tumor necrosis factor α; IL interleukin; BP blood pressure.

Values are mean±SD. Differences between BPG were analysed by one- way ANOVA and Welch test. The mean difference is significant at the ≤0.05 level.

a-c Values with the same superscript letters are significantly different according to Games Howell and Bonferroni post-hoc tests (p = ≤0.05)

Table 3.3 Diet and physical activity characteristics of body profile groups and ethnicity

	Reference Range	Total (n = 406)	Body Profile Groups			p-value	Ethnic Groups			p-value
			NN (n = 105)	NH (n = 70)	HH (n = 207)		NZE (n = 233)	Māori (n = 80)	Pacific (n = 93)	
Diet										
Energy (kJ)		9724±3733	9046±2674 [b]	9276±3199	10265±4320 [b]	**0.012**	9121±2866 [a]	9785±3748	11403±5189 [a]	**0.001**
Protein (g)	46g/day	103±40.2	95.7±30.6 [b]	98.1±37.6 [c]	109±44.5 [b,c]	**0.008**	96.8±32.3 [a]	107±44.2	118±51.1 [a]	**0.001**
kJ from protein %	15-25%	18.3±3.58	18.1±3.0	18.3±3.8	18.5±3.8	0.714	18.3±3.4	18.8±3.9	18.0±3.7	0.425
Carbohydrates (g)		246±112	234± 79	229±88	259±135	0.075	230±87 [a]	238±109	297±158 [a]	**0.003**
kJ from carbohydrates %	45-65%	41.3±7.6	42.6±6.8	40.4±7.1	40.8±7.8	0.087	41.5±7.5	39.5±7.0	42.6±7.6	0.034
Total fat (g)		93.1±39.7	84.0±29.9 [b]	90.0±35.7	99.4±43.3 [b]	**0.002**	86.8±32.6 [a]	95.4±39.6	109.0±52.3 [a]	**0.001**
kJ from fat %	20-35%	35.4±6.5	34.3±6.1	35.7±6.2	36.1±6.8	0.055	35.0±6.5	36.2±6.8	35.4±6.4	0.390
Saturated Fat (g)		36.1±17.4	32.3±13.6 [a,b]	34.3±15.3 [a,c]	39.4±19.1 [b,c]	**0.003**	34.9±16.1	35.2±15.5	42.9±23.4	0.083
Fat as saturated %		44.4±6.1	43.9±6.1	43.9±6.7	45.1±5.7	0.194	44.5±6.2	44.4±5.5	43.7±6.6	0.710
Polyunsaturated fat (g)		13.2±5.29	12.6±4.27	13±4.95	13.6±5.89	0.285	12.8±4.77 [a]	12.8±5.36	15.5±6.85 [a]	0.042
Fat at polyunsaturated %		17.0±4.3	17.9±4.4	17.3±4.7	16.1±3.8	**0.006**	17.1±4.3	16.6±4.2	16.9±4.6	0.722
Monounsaturated fat (g)		30.9±12.8	27.6±9.33 [b]	29.9±10.5	33.4±14.6 [b]	**0.001**	29.5±10.9 [a]	30.8±12.7	37.7±18.2 [a]	**0.013**
Fat as monounsaturated %		38.66±3.32	38.2±3.1	38.9±3.8	38.7±3.3	0.408	38.4±3.2	39.0±3.1	39.5±4.1	0.104
Physical Activity										
Sedentary (mpd)		464±73	462±77	468±71	463±71	0.881	459±76 [a]	454±62 [c]	490±65 [a,c]	**0.004**
Light (mpd)		318±78	320±81	303±74	322±77	0.255	320±84	327±73	305±64	0.140
Moderate (mpd)		31±17	36±19 [a,b]	29±14 [a]	29±17 [b]	**0.002**	34±17 [a]	29±15	25±18 [a]	**<0.001**
Moderate-Vigorous (mpd)		36±21	44±23 [a,b]	33±18 [a]	31±18 [b]	**<0.001**	39±21 [a,b]	32±18 [b]	28±20 [a]	**<0.001**
Vigorous (mpd)		4±8	8±11 [b]	4±6	2±5 [b]	**<0.001**	5±90 [a]	4±7	3±6 [a]	**0.028**

Abbreviations: BPG body profile group; NN normal fat group; NH hidden fat group; HH apparent fat group; mpd minutes per day; NZE New Zealand European.
Values are mean±SD. The mean difference is significant at the ≤0.05 level.
Differences between BPG were analysed by one- way ANOVA and Welch test
a-c Values with the same superscript letters are significantly different according to Games Howell and Bonferroni post-hoc tests (p = ≤0.05)
Note: Energy was calculated inclusive of fibre.

3.7.4 miRNAs across body profile and ethnic groups

From BPGs, NH on average had the highest miRNA levels of those analysed (Table 3.2). However, despite this only miR-222-3p was significantly higher in NH than NN group (1.54±1.30 vs. 1.1±0.77, $p < 0.05$). Similarly, miR-222-3p was significantly higher in the HH than NN group (1.54±1.52 vs. 1.1±0.77, $p < 0.05$), but was not significantly different across ethnic groups ($p = 0.57$). MicroRNA-29b-3p was lower, though not significantly, in HH group than NN group (0.27±0.28 vs. 0.30±0.26, $p = 0.141$) and significantly lower in Pacific women than NZE women (0.20±0.16 vs. 0.33±0.31, $p < 0.001$). MicroRNA-17-5p was not significantly different between body profile and ethnic groups (Figure 3.3).

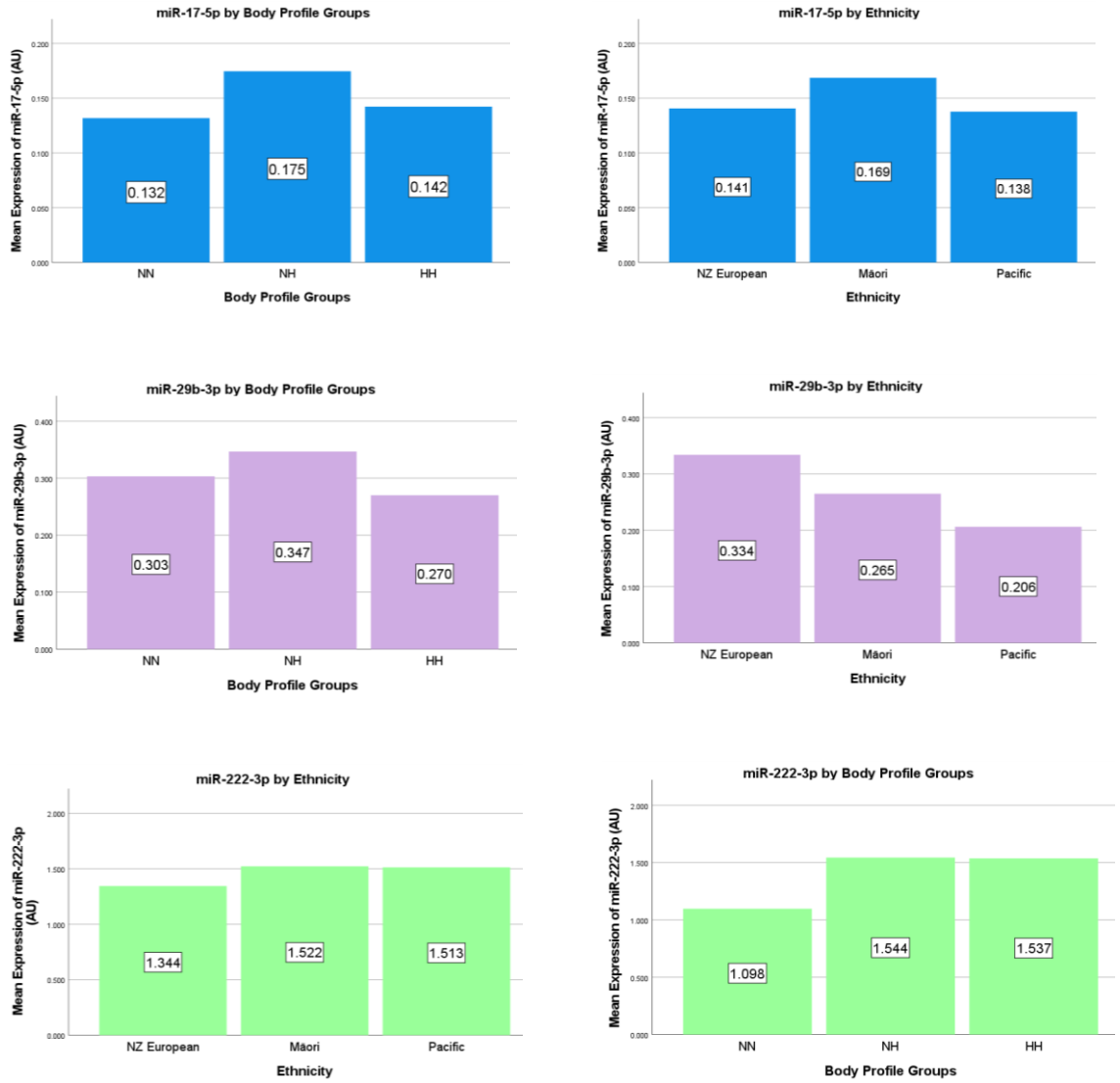

Figure 3.3 miRNA expression across BPGs and ethnicity. Body mass index and BF% defined body profile groups; "NN" group - normal BMI (≥18.5 and <25kg/m2) and normal BF% (≥18%, <30%); "NH" group - normal BMI (<25 kg/m2) and high BF% (≥30%); "HH" group – high BMI (≥25 kg/m2) and high BF% (≥30%)

The odds ratio of having a higher miR-222-3p increased in NH (OR = 1.92) and HH groups (OR = 2.58) (**Table 3.4)**. The odds ratio of having higher miR-29b-3p decreased in HH (OR = 0.09). MicroRNAs as predictors for BF% and BMI **(**supplementary **Table 3.C)** correlated with these results (data not shown). Higher miR-222-3p significantly increased in subjects categorised as overweight (OR = 1.764), obese (OR = 1.704) or having high BF% (BF% 30 to <35%, OR = 1.662; ≥35%, OR = 2.133) compared to normal BMI and BF% groups. When compared to those in the normal BMI and BF% groups, the odds of having high miR-29b-3p decreased in the overweight (OR = 0.217) and obese (OR = 0.181) BMI groups, as well as in those with a BF% >35% (OR = 0.125). A higher NZ deprivation Index was also a predictor of obesity (OR = 1.416). A similar analysis was conducted for ethnic groups **(**supplementary **Table 3.D).** When compared to NZE, the odds ratio of having higher miR-17-5p increased for Māori (OR = 8.305). The odds ratio of having higher miR-222-3p decreased in Māori compared to Pacific (OR = 0.292).

Table 3.5 shows predictors of selected miRNAs. With having higher miR-17-5p, the odds ratio of having higher TNF-α increased (OR = 1.176) whereas the odds of higher IL-6 decreased (OR = 0.683). The odds of being in the HH group increased with higher miR-222-3p levels (OR = 2.583). The odds of higher IL-6 (OR = 0.722) and protein (OR = 0.991) decreased with higher miR-222-3p levels. The odds of higher sucrose (OR = 1.014) and IL-10 (OR = 1.028) increased with higher miR-222-3p levels. For increased levels of miR-29b-3p the odds of higher HbA1c, IL-6, carbohydrate and light physical activity decreased. Lastly the chance of having high sucrose in the diet increased (OR = 1.024) when miR-29b-3p expression increased. The same data was assessed using a forward regression (supplementary **Table 3.E**) which showed similar significant predictors which followed the same trajectory as **Table 3.5**. The final model in the forward regression showed only one strong predictor for miR-17-5p (IL-6), three for miR-222-3p (IL-6, Il-10, leptin) and five for miR-29b-3p (HbA1c, TNF α, carbohydrates, sucrose, light physical activity). Leptin appeared as a new predictor with the odds of high leptin levels increasing with high miR-222-3p. Another new predictor which replaced IL-6 was TNF-α, for which the odds decreased with high 29b-3p levels. Although we favoured the backwards regression (**Table 3.8**) to minimise suppressor effects and lower the risk of Type II error, it was worth mentioning the new predictors generated from the forward regression, as those variables proved to be significant in other statistical tests.

Table 3.4 Multinominal logistic regression – miRNAs as predictors of body profile groups controlled for age and NZ deprivation

Body Profile Groups		b (SE)	95% CI for Odds Ratio		
			Lower	Odds Ratio [c]	Upper
NH vs. NN [a]					
	Intercept	-2.854 (-0.755)			
	Age (years)	0.057 (0.02)**	1.019	1.059	1.1
	NZ Deprivation Index	0.029 (0.063)	0.911	1.03	1.164
	miR-222-3p	0.651 (0.271)*	1.127	1.917	3.258
	miR-17-5p	1.014 (1.942)	0.061	2.757	124.068
	miR-29b-3p	-1.326 (0.723)	0.064	0.266	1.096
HH vs. NN [a]					
	Intercept	-2.567 (0.632)			
	Age (years)	0.06 (0.016)**	1.029	1.062	1.097
	NZ Deprivation Index	0.218 (0.05)**	1.127	1.244	1.373
	miR-222-3p	0.947 (0.251)**	1.576	2.577	4.214
	miR-17-5p	-1.683 (1.834)	0.005	0.186	6.767
	miR-29b-3p	-2.36 (0.691)**	0.024	0.094	0.366
NH vs. HH [b]					
	Intercept	-0.287 (0.688)			
	Age (years)	-0.003 (0.017)	0.964	0.997	1.031
	NZ Deprivation Index	-0.189 (0.055)**	0.743	0.828	0.923
	miR-222-3p	-0.296 (0.21)	0.493	0.744	1.123
	miR-17-5p	2.697 (1.714)	0.515	14.831	426.784
	miR-29b-3p	1.034 (0.676)	0.748	2.813	10.583

Abbreviations: NN normal fat group; NH hidden fat group; HH apparent fat group

[a] The reference category is NN ; [b] The Reference category is HH; [c] Odds ratios for a 1-SD unit increase of miRNA.

Note. R2 = 0.319 (Cox-Snell), 0.376 (Nagelkerke). Model $\chi 2$ (10) = 140.527, *p<0.05,**p<0.01

Table 3.5 Binomial backwards regression – clinical characteristics as predictors of selected miRNAs controlled for age and NZ deprivation

	b(SE)	95% CI for Odds Ratio			*p* -value
		Lower	Odds Ratio [c]	Upper	
miR-17-5p Model 25 [a]					
Included					
CRP (mg/L)	-0.075 (0.046)	0.847	0.927	1.015	0.103
HbA1c (mmol/mol)	-0.061 (0.034)	0.88	0.941	1.006	0.074
TNF-α (pg/mL)	0.162 (0.066)	1.033	1.176	1.338	*0.014
IL-6 (pg/mL)	-0.382 (0.117)	0.542	0.683	0.859	**0.001
Ghrelin (pg/mL)	-0.006 (0.003)	0.987	0.994	1	0.055
Constant	2.17 (1.019)		8.762		0.033
	Note R^2 = 0.674 (Hosmer-Lemeshow), 0.070 (Cox -Snell), 0.094 (Nagelkerke). Model X2 (5) = 21.201, *p* = <0.001, *p<0.05,**p<0.01				
miR-222-3p - Model 23[a]					
Included					
Body Profile Groups (NN)					**0.007
Body Profile Groups(1)(NH)	0.592 (0.362)	0.888	1.807	3.675	0.102
Body Profile Groups(2)(HH)	0.949 (0.3)	1.434	2.583	4.652	**0.002
Triglyceride (mmol/L)	-0.485 (0.271)	0.362	0.615	1.048	0.074
IL-6 (pg/mL)	-0.325 (0.116)	0.576	0.722	0.907	**0.005
IL-10 (pg/mL)	0.028 (0.013)	1.002	1.028	1.054	*0.032
Protein (g)	-0.009 (0.004)	0.984	0.991	0.998	*0.017
Sucrose (g)	0.014 (0.005)	1.003	1.014	1.024	**0.009
Light Physical Activity (mpd)	-0.003 (0.002)	0.993	0.997	1	*0.04
Constant	1.577 (0.722)		4.84		0.029
	Note R^2 = 0.657 (Hosmer-Lemeshow), 0.106 (Cox -Snell), 0.141 (Nagelkerke). Model X2 (8) = 32.62, *p* = <0.001, *p<0.05, **p<0.01				

	b(SE)	95% CI for Odds Ratio			*p* -value
		Lower	Odds Ratio [c]	Upper	
miR-29b-3p - Model 21[a]					
Included					
CRP (mg/L)	-0.088 (0.057)	0.819	0.916	1.024	0.122
HbA1c (mmol/mol)	-0.089 (0.036)	0.853	0.915	0.983	*0.014
IL-6 (pg/mL)	-0.198 (0.091)	0.686	0.821	0.981	*0.03
Ghrelin (pg/mL)	-0.006 (0.003)	0.988	0.994	1.001	0.087
Carbohydrate (g)	-0.004 (0.002)	0.992	0.996	1	*0.049
Sucrose (g)	0.024 (0.008)	1.008	1.024	1.041	**0.003
Light Physical Activity (mpd)	-0.004 (0.002)	0.993	0.996	0.999	*0.016
Do you smoke cigarettes? (Y/N)	1.062 (0.64)	0.825	2.893	10.145	0.097
Do you drink alcohol? (Y/N)	0.58 (0.298)	0.997	1.787	3.203	0.051
Constant	4.359 (1.265)		78.18		0.001
	Note R^2 = 0.810 (Hosmer-Lemeshow), 0.129 (Cox -Snell), 0.173 (Nagelkerke). Model X2 (9) = 40.351 p = <0.001, *p<0.05, **p<0.01				

Abbreviations: BMI body mass index; BF% body fat percentage; NN normal fat group; NH hidden fat group; HH apparent fat group; BPG body profile groups; HbA1c glycated haemoglobin; Calc LDL Calculated low density lipoprotein; CRP C-reactive protein; TNF-α tumor necrosis factor a; IL interleukin; mpd minutes per day.

Backwards regression included 29 variables: BPG; ethnicity; NZ deprivation index; age (years); BMI (kg/m2); BF%; cholesterol (mmol/L); triglyceride (mmol/L); glucose (mmol/L); insulin (mU/mL); calc. LDL (mmol/L); CRP (mg/L); HbA1c (mmol/mol); TNF-α (pg/mL); IL-6 (pg/mL); IL-10(pg/mL); leptin (upper and lower range); ghrelin (pg/mL); protein (g); total fat (g); carbohydrate (g); sucrose (g); sedentary, light, moderate, moderate-vigorous and vigorous physical activity (mpd); Do you smoke cigarettes? Y/N; Do you drink alcohol? (Y/N)

3.8 Discussion

In the current study we aimed to determine whether specific miRNAs that are associated with obesity may be predictive of metabolic risk and to verify the involvement of selected miRNAs in obesity and factors influencing obesity and metabolic health. Furthermore, to identify if specific miRNAs may be used as a biomarker for metabolic risk. In the current study, plasma miR-222-3p, miR-29b-3p, and miR-17-5p were analysed by body profile and ethnic groups. The miRNAs were tested for associations with various anthropometric measures, metabolic biomarkers, dietary factors, and physical activity levels in which significant expression differences were observed in the three miRNAs. MicroRNA-222-3p was associated with obesity, sucrose, protein, leptin, IL-6, and IL-10; miR-29b-3p with HbA1c, TNF-α, IL-6, IL-10, sucrose, carbohydrate, and light physical activity; miR-17-5p with TNF-α, IL-6, and was a predictor of Māori ethnicity. Our findings are suggestive that with more research, miRNAs could be used as biomarkers of obesity and related disease risk.

MicroRNA-222-3p arguably shows the greatest promise as a clinical biomarker of metabolic disease, as up-regulation of miR-222-3p has been associated with obesity, inflammation, and T2D (Deiuliis, 2016). Our study found that circulating levels of miR-222-3p were significantly higher in the NH and HH group than NN and that higher levels of miR-222-3p were a significant predictor of the body composition profiles of these groups. Higher levels of miR-222-3p were associated with a 1.9 greater likelihood of being in the NH group and a 2.6 greater likelihood of being in the HH group. Furthermore, obesity (HH) was a significant predictor of higher miR-222-3p levels. Similarly, Ortega (2013) found that miR-222 was up-regulated in obese adults compared to healthy controls, with significant associations found between miR-222, BMI, WC, and total fat mass. Ortega (2013) also found higher miR-222 had a diagnostic accuracy of 94% when predicting morbid obesity, with a 1.8-fold change in expression in morbidly obese men (BMI ≥40) versus nonmorbidly obese men. In agreement with other studies, our study found that up-regulation of miR-222 was associated with excess adiposity (Al-Rawaf, 2019; Cui et al., 2018; González-Arce et al., 2021; Prats-Puig et al., 2013).

To our knowledge, miR-222 expression has only been compared between obese and non-obese subjects (categorised solely by BMI). No research has been conducted on miR-222 expression in NWO. Despite BMI classifying the NH group as normal weight, this group had significantly higher android fat compared to NN, and the highest LDL and miR-222 levels of the three body composition groups. Visceral adipose tissue is more metabolically active than SAT and its accumulation is associated with cardiometabolic risk (Sam, 2018), suggesting that the NH group may be at greater metabolic risk than the NN group. The HH group had the highest WC (91.9cm), making them the only group with a WC above the recommend range (<88cm) which is indicative of cardiometabolic risk (WHO, 2000). A recent review has shown that some miRNAs are differentially expressed in VAT compared to SAT (Kurylowicz, 2021), this may explain the up-regulation of miR-222-3p in both the NH and HH groups in our study. Overall clinical differences observed in the NH versus NN group suggest that body fatness is the link to higher metabolic risk.

In our study higher miR-222 levels were a predictor of Pacific ethnicity compared to Māori. However, Pacific subjects were the only ethnic group to exceed the recommended WC (91.8cm) and WHR (0.8) measurements and had the highest anthropometric measures of the ethnic groups. Despite adjusting for ethnicity, due to the strong association between miR-222-3p and body fatness, one could suggest adiposity is the main driver of higher miR-222 versus ethnicity, due to all Pacific subjects having the highest levels of adiposity. The evidence from our study strengthens the notion that miR-222 is up-regulated in obesity and has the potential to be used as a biomarker for metabolic health. However, it is important to explore ethnic variations when considering these miRNAs, as studies have identified differential expression of miRNA in European, Asian, African, and Middle Eastern ethnicities (Rawlings-Goss et al., 2014; X. Wang et al., 2014).

Adipokines and proinflammatory cytokines are strongly associated with metabolic risk and obesity-related diseases such as T2D (Kang et al., 2016; Popko et al., 2010). Leptin, an adipokine involved in insulin sensitivity and long-term regulation of energy balance and suppression of food intake (Landrier et al., 2019), was a significant predictor of miR-222-3p expression and was significantly higher in the NH and HH group compared to NN. A study assessing miRNAs and adipokines as markers of MetS in adolescents with obesity, similarly found up-regulated miR-222 levels were positively associated with leptin in overweight and obese subjects (Al-Rawaf, 2019). Both IL-6 and TNF-α are pro-inflammatory cytokines secreted by adipocytes and their concentration is correlated with increased adiposity (Popko

et al., 2010). CRP and IL-6 have also been shown to correlate with each other (Popko et al., 2010). In the current study TNF-α and CRP were the only inflammatory markers to differ significantly between body composition and ethnic groups. Higher levels of TNF-α and CRP were found in the HH BPG (p = <0.001), and IL-6 and IL-10 were significant predictors of miR-222-3p. In human and murine studies, miR-222 was found to play a role in inflammation (Ortega et al., 2015; Xie et al., 2009). Although little has been reported on IL-6 and miR-222 in relation to obesity, a study evaluating their relationship in intracerebral haemorrhage inflammation, found that miR-222 promoted an increase in IL-6 and TNF-α levels (Bai et al., 2020). It may be hypothesised that, since IL-6 and TNF-α are strongly correlated and research has shown that TNF-α exposure induces the up-regulation of miR-222 (Xie et al., 2009), that higher concentrations of IL-6 concentrations would also up-regulate miR-222. However, in contrast, the current study found IL-6 to down-regulate miR-222-3p.

Proinflammatory cytokines and inflammatory diseases have also been associated with the miR-29 family (Botta et al., 2018; Horita et al., 2021). However, recent findings on regulatory roles of miR-29b-3p in the inflammatory response have been inconsistent. Our study found that higher levels of IL-6, TNF-α and IL-10 were associated with down-regulated miR-29b expression, in contrast with Huang et al. (2017), who found miR-29b-3p to be up-regulated in subjects with atherosclerosis and positivity correlated with IL-6. J. Wang et al. (2020) found that miR-29b-3p expression was up-regulated in the lungs of mice exposed to particulate matter, and that miR-29b was associated with higher levels of pro-inflammatory cytokines such as IL-6. In addition, inhibition of miR-29b reduced the secretion of pro-inflammatory cytokines. Similarly, Saravanan et al. (2019) found cytokines including TNF-α, and hypoxia induced the release of miR-29b-3p in human islets. In contrast Botta et al. (2018), found an up-regulation of miR-29b expression counteracted pro-inflammatory pathways in multiple myeloma. Similarly overexpression of miR-29b was shown to dampen irradiation-related vascular inflammatory responses (Eken et al., 2019) and inhibit the production of pro-inflammatory cytokines (TNF-α, IL-6) (Li et al., 2020). Despite conflicting results, the associations found between miR-29b and inflammatory cyctokines suggest that miR-29b is involved in the regulation of inflammation, and our results suggest that higher levels of miR-29-3p are protective against inflammation.

Studies have also shown that miR-17-5p plays a role in inflammation; however, these findings, too, are inconsistent. For example, a study on atherosclerosis found down-regulation of miR-17-5p, significantly decreased inflammatory cytokines (IL-6, TNF-α)(Tan et al.,

2019), whilst a study on rheumatoid arthritis found up-regulation of miR-17-5p decreased IL-6 production and had anti-inflammatory effects (Najm et al., 2020). Predictors of miR-17-5p in our study were TNF-α and IL-6. TNF-α up-regulated miR-17-5p, partially supporting Tan, 2019 findings. However, IL-6 down-regulated miR-17-5p expression in our study, supporting Najm, 2019 findings.

Inflammatory cytokines can interfere with insulin signalling, sensitivity and secretion, contributing to T2D, and diseases associated with obesity (Landrier et al., 2019). Several studies have identified miRNA dysregulation in T2D. Expression of miR-222 and miR-29b were up-regulated and miR-17-5p down-regulated in T2D subjects (Belongie et al., 2017; Cui et al., 2018; Kloting et al., 2009; Nunez Lopez et al., 2016; Ortega et al., 2014; Sadeghzadeh et al., 2020; X. Wang et al., 2014; Williams et al., 2019). However, a study by Zampetaki et al. (2010) found that miR-29b was down-regulated in subjects with T2D (OR = 1.00, 95% CI). In our study, besides leptins association with miR-222-3p, neither miR-222-3p nor miR-17-5p were significantly associated with metabolic markers. However, although our study population were all healthy, having higher HbA1c was a significant predictor of reduced miR-29b-3p expression (OR = 0.92, 95% CI), favouring Zampetaki, 2010 findings.

Several reviews have indicated that diet, physical activity, and lifestyle choices can influence miRNA expression (Guller et al., 2010; Gulyaeva et al., 2016; Quintanilha et al., 2017; Ultimo et al., 2018). Both miR-17-5p and miR-29b-3p have been down-regulated in rats fed a high-fat and high-sucrose diet (Yerlikaya et al., 2019) and miR-222 up-regulated in mice solely on a high fat diet (Chartoumpekis et al., 2012). In our study, whilst no significant associations were found with fat intake, sucrose intake significantly up-regulated miR-222-3p and miR-29b-3p, whilst carbohydrate intake down-regulated miR-29b-3p. Interestingly enough, while research on sucrose and miR-29b-3p is limited, a study by Silambarasan et al. (2016), which exposed human cell cultures to different glucose concentrations found that miR-29b increased significantly with higher glucose concentrations which is in line with our findings. Protein intake (kJ) has also been associated with the higher levels of miR-222 (González-Arce et al., 2021). In agreement with González-Arce et al. (2021), our study found that a higher intake of protein (kJ) was a predictor of lower miR-222 expression. The differential expression of miR-29b has been associated with patterns of physical activity. Short-term endurance training increased miR-29b expression according to Russell, Lamon, et al. (2013), while Barber et al. (2019) found that regular exercise in healthy adults decreased miR-29b expression. In agreement with Barber et al. (2019), our study found light physical

activity significantly down-regulated miR-29b-3p. Regarding ethnic comparisons, higher miR-29b-3p was found in NZE and higher miR-222-3p in the Pacific group. The most significant predictor of ethnicity was miR-17-5p; a person was 8.3 times more likely to be Māori than NZE if they had higher levels of miR-17-5p. The NZ deprivation index differed significantly between ethnic groups (NZE, Māori, and Pacific). Areas of greater deprivation generally reflect inequalities in diet, due to a disproportion of fast-food outlets selling unhealthy foods, which is overrepresented in low-income, high-ethnic minority, and more socially deprived areas (Pearce et al., 2009). Although adjusting for ethnicity, since diet and lifestyle have shown to influence miRNAs, and Māori and Pacific groups live in more deprived areas, more research would be required to determine if the differences in miRNAs between these groups are attributable to ethnicity, diet and or lifestyle choices. Studies have found a high degree of variability in miRNA expression within European, Mexican-American, and Spanish populations, suggesting that expression differences could not solely be attributable to genetic ancestry, but rather to environmental and lifestyle factors (González-Arce et al., 2021; Iacomino et al., 2019; Prats-Puig et al., 2013).

Three meta-analyses found limitations in using miRNAs as biomarkers; evidence is variable and results inconsistent. However, the three meta-analyses agreed that despite limitations, miRNAs do have the potential to be used as biomarkers in the future (Deiuliis, 2016; Ding et al., 2017; Guo et al., 2018; Zhou et al., 2020). Our study also showed some miRNA associations that were different from those reported by others, suggesting more research would be required to use miR-29b-3p and miR-17-5p as metabolic biomarkers. However, these miRNAs are predominately known for their influence in specific diseases, whilst our subjects were all healthy and relatively young (16 to 45 years), which could explain the variability in the findings.

The strengths of our study included its contribution to the extensive interest in recent years in determining whether miRNAs have the potential to be used as a biomarker of disease. We evaluated a variety of body composition measurements, metabolic markers, diet, and physical activity patterns. Our study provided new information on miRNAs in NWO and in the NZE, Māori and Pacific ethnicities that represent the NZ population. The use of plasma miRNA was also beneficial, as this method is more accessible to clinicians and less invasive than tissue-derived miRNA. Recruitment of healthy subjects was also a strength in that it enabled us to determine if specific miRNAs could be predictive of disease risk. MicroRNA-222-3p

was predictive of obesity, highlighting its value as a screening biomarker that could aid in the prevention of obesity-related disorders (T2D or MetS, etc.).

As illustrated in our study, miRNAs were differently expressed amongst BPGs and ethnicities. Some discrepancies were observed in miRNA expression levels between our study and others. Methodological differences, such as miRNA source, quality control, recruitment, RNA isolation, qRT-PCR strategy, or data normalisation, could contribute to these differences. Other limitations were the cross-sectional study design and lower recruitment of Māori and Pacific women, especially in the NN and NH groups, which reduced the statistical power to accurately assess the differences between ethnic groups stratified by BPG. This made it difficult to interpret miRNA differences, as the selected miRNAs were associated with obesity. All recruited subjects were also healthy, on average relatively young (~31 years) and presented a low prevalence of abnormal biomarkers among BPGs, again proving problematic, as selected miRNAs, especially miR-29b and miR-17-5p, are significantly associated with disease states (T2D/MetS). Another limitation potentially could be that too few miRNAs were assessed. The selected miRNAs are part of a miRNA family that work in a coordinated manner to control metabolism, thus selecting the family panel (miR-221/222; miR-29a/29b/29c; miR-17/92) may be better suited to predict metabolic risk.

In future studies, for miRNA to be used as biomarkers, there is a need for standardised procedures with detailed measures and methodologies to enhance the reproducibility and validity of miRNA results, as several studies convey conflicting findings. It would also be beneficial to establish miRNA reference ranges to provide clarity on if expression levels seen are considered high or low. Since our study, numerous emerging miRNAs have also been associated with obesity and metabolic disease; therefore, it would be worth evaluating a panel of these miRNAs, as recently this has shown to significantly improve the reliability of disease diagnosis (Min et al., 2019; Xiong et al., 2017).

As NZE women were the only ethnic group to have the acquired statistical power in all three BPGs, it would be worth assessing miRNA and BPGs from NZE women only, to strengthen the findings of miRNA differentiation in BPGs. This would also remove any influence ethnicity might have had on miRNA expression in the BPGs and allow one to determine miRNAs specific to NZE women. A follow up on the EXPLORE study's subjects later in life and/or a future study following similar protocols to the EXPLORE study with participants

diagnosed with metabolic disease, may provide valuable insight into miRNA and their predicted metabolic risk, and validate our findings.

Research has shown miRNAs have a variety of often overlapping functions. The expression of miRNA is variable and dependent on numerous factors (adiposity, disease states, diet, physical activity, and lifestyle). There are also many miRNA target sites, many of which are unknown. This is problematic as there is a keen interest in using miRNA-based therapeutics in the future, which may increase the risk of adverse off-target effects (Bartoszewski et al., 2019), thus precise mechanisms of action of miRNAs require further investigation.

Despite these limitations, research continues to expand the knowledge on miRNAs and a considerable amount of progress has been made in this field thus far. Overall, our study identified differences in the expression of selected miRNAs in BPGs and ethnicities. In agreement with some studies, we have identified miRNA 222-3p as a potential biomarker of metabolic health and have identified a selection of specific metabolic (Leptin, HbA1c) and inflammatory (IL6, IL-10, TNF-α) markers as well as dietary factors (sucrose, carbohydrate, protein) and light physical activity, to be associated with our selected miRNAs. These findings may help researchers determine which variables to investigate in the future. Further research and validation are needed before specific miRNAs can be used as biomarkers in clinical practice, but our results support the idea that miRNAs vary across different BPGs and ethnicities and that they have the potential to be used as a minimally invasive biomarker to determine metabolic health risk.

Chapter 4 – Conclusions and Recommendations

4.1 Overview and conclusion

The aim of this research was to explore miRNA expression in NZ women with different body composition profiles and their association with metabolic risk. Furthermore, to confirm the differential expression of specific miRNAs in obesity, as demonstrated in previous findings, and to investigate if miR-222-3p, miR-17-5p, and miR-29b-3p are associated with diet and physical activity. These findings may provide additional information on possible variables associated with these miRNAs, thus providing potential avenues for future research.

Researchers are increasingly interested in finding new health biomarkers that identify disease risk factors, people at risk of disease, and predict disease trajectory, to help prevent chronic disease development. MicroRNAs have been identified that are involved in the development of certain diseases and are differentially expressed or dysregulated in many states of diseases, including obesity (Heyn et al., 2020). These may be potential biomarkers of health but may also be used as therapeutic agents. MicroRNAs, specifically circulating miRNAs, are stable and readily accessible, through minimally invasive methods (fluid samples versus biopsy), making them good candidates for biomarkers (Etheridge et al., 2011).

The *first objective* was to explore differences in miRNA expression in women from different body profile groups (BPGs). This would establish if those with higher body fat percentage regardless of being of healthy BMI, had differentially expressed miRNA associated with metabolic disease risk. Of the selected miRNAs examined in this study, only miR-222-3p was significantly different between BPGs. Research has found that up-regulation of miRNA-222-3p is significantly associated with increased adiposity, which can cause a state of chronic inflammation; which is a key driver in developing metabolic disorders (HSPH, 2010; Landrier et al., 2019). Our study also found that higher levels of miR-222-3p were associated with increased adiposity. A study evaluating miRNAs and adipokines as MetS markers in obese adolescents, found that up-regulated miR-222 levels were positively associated with leptin in overweight and obese subjects (Al-Rawaf, 2019). MicroRNA-222-3p was significantly higher in the NH and HH groups compared to the NN group ($p < 0.05$). Leptin also presented itself as a predictor of miR-222-3p expression and was significantly higher in

NH and HH compared to the NN group, not surprisingly, since leptin has a strong positive association with body fat levels (Obradovic et al., 2021).

The *second objective* was to assess which miRNAs were associated with biomarkers of metabolic disease. Unexpectedly, our study found that up-regulation of miR-222-3p was associated with higher IL-10 (anti-inflammatory cytokine) and lower IL-6 (pro-inflammatory cytokine). As inflammation is associated with obesity and miR-222 is up-regulated in obesity, we expected a higher level of IL-6 to be associated with higher miR-222-3p. Although no significant associations were found in IL-10 and IL-6 for BPGs, mean IL-10 concentrations were higher in the NH and HH group compared to the NN group (17.4±31.1; 17.3±26.5 vs. 13.7±10.7 pg/mL). IL-10 has been found to correlate positively with BMI (Acosta et al., 2019). Fjeldborg et al. (2014), suggesting that the shift in higher IL-10 in those with higher adiposity, may be a protective mechanism to counteract the increased inflammation associated with obesity. This provides a potential reason why higher miR-222-3p was associated with higher IL-10 levels.

The miRNA-29 family, including miR-29b, has been associated with proinflammatory cytokines and inflammatory diseases (Botta et al., 2018; Horita et al., 2021). However, the findings on the regulatory roles of miR-29b-3p in inflammatory response are inconsistent. MicroRNA-29b-3p was shown to be positivity correlated with IL-6 and TNF-α (Huang et al., 2017; Saravanan et al., 2019; J. Wang et al., 2020). On the contrary, studies have found that up-regulation of miR-29b counteracted pro-inflammatory pathways, inhibiting IL-6 and TNF-α production in multiple myeloma and cardiac dysfunction (Botta et al., 2018; Li et al., 2020). Our study agreed with the latter, showing that higher levels of miR-29b-3p were associated with a decrease in IL-6 and TNF-α levels, suggesting that higher levels of miR-29-3p protect against inflammation. Low-grade inflammation associated with obesity is also a common feature in T2D (Velikova et al., 2021). Some studies have found that miR-29b is down-regulated in prediabetic and T2D subjects (Collares et al., 2013; Nunez Lopez et al., 2016; Zampetaki et al., 2010) as well as in obesity (L. Wang et al., 2021). Similarly, our study found higher miR-29b-3p was associated with a decrease in both HbA1c concentrations and adiposity, which supports the notion that miR-29b could be a potential biomarker, with lower levels predicting T2D risk.

Previously, miR-17-5p has been associated with inflammation; however, these findings are also inconsistent. A study on atherosclerosis found that down-regulation of miR-17-5p

significantly decreased IL-6 and TNF-α (Tan et al., 2019), while another study on rheumatoid arthritis found up-regulation of miR-17-5p decreased IL-6 production and had anti-inflammatory effects (Najm et al., 2020). Our study found higher levels of miR-17-5p were associated with increased TNF-α and decreased IL-6 levels, agreeing partly with Tan, 2019 and Najm, 2020. The inconsistencies found in miR-17-5p expression may be problematic for its potential use as a biomarker; however, regardless of the differences found in expression between studies, the association between miR-17-5p and inflammation is still present, suggesting that with future research, miR-17-5p may still be a contender as a biomarker for metabolic disease.

The *third objective* was to determine whether women classified in the hidden fat (NH) group (i.e., those with high BF% but normal BMI), had changes in miRNA expression associated with metabolic disease. On average, the NH group had the highest miRNA levels of all the BPGs. Specifically, miR-222-3p was significantly higher in the NH than NN group. One could argue that our recruitment of healthy subjects could be the reason why no significant associations were observed in miR-29b and miR-17-5p, since previous studies found differences in these miRNAs in chronic inflammatory and metabolic disease states (Karolina et al., 2012; Saravanan et al., 2019; Xiao et al., 2018; Zampetaki et al., 2010).

Overall, these findings suggest that miR-222 may have a regulatory role in metabolic pathways such as energy homeostasis and potentially inflammation. Strong associations found between miR-222 and adiposity, and the highest levels of miR-222-3p in the NH group, support the idea that as a clinical biomarker of metabolic disease and disease risk, miR-222 arguably shows the greatest promise (Deiuliis, 2016).

The *fourth objective* was to investigate whether selected miRNAs were associated with different patterns of diet and physical activity. According to studies, miR-17-5p and miR-29b-3p were both down-regulated in rats fed a high-fat and high-sucrose diet, whereas miR-222 was up-regulated in mice solely on a high fat diet (Chartoumpekis et al., 2012; Yerlikaya et al., 2019). Higher protein intake (kcal) has also been associated with higher levels of miR-222 (González-Arce et al., 2021). In our study, no dietary factors or physical activity levels were associated with miR-17-5p. No associations with fat intake and the selected miRNAs were found either. MicroRNA-222-3p however was significantly up-regulated with higher sucrose and lower protein (kJ) intake, suggesting that high sugar and low protein intake may be characteristics of a diet that contributes to obesity. In contrast to Yerlikaya et al. (2019),

our study found that a higher intake of sucrose was significantly associated with miR-29b-3p up-regulation, whilst increased total carbohydrate intake down-regulated miR-29b-3p. Exercise has also been shown to cause differential expression in miR-29b. Short-term endurance training increased miR-29b expression according to Russell, Lamon, et al. (2013), while Barber et al. (2019) found that regular exercise in healthy adults decreased miR-29b expression. Consistent with Barber et al. (2019), our study found that light physical activity significantly down-regulated miR-29b-3p. It is difficult to interpret the dietary and physical activity factors associated with miR-29b-3p due to the inconsistencies found in whether higher levels of miR-29b are protective or not. Conflicting studies have found that high and low levels are associated with T2D (Hung et al., 2019). If up-regulation was found to be protective, as we observed in our study, it would indicate that high sucrose, but low total carbohydrate intake and low levels of light physical activity may be beneficial. Although associations have found diet and physical activity to alter expression patterns, research on specific dietary and physical activity factors influencing miRNA expression is still limited. Caution should be taken when interpreting these results, as the mechanisms by which diet and physical activity affect miRNA are still unclear. Our results highlight specific variables that impact miRNA expression and provide potential avenues for future research. Should future research find strong associations between miRNA and certain dietary and physical activity factors, specific dietary and physical activity adjustments could be made to enhance preventative measures for obesity.

The *last objective* was to explore differences in miRNA expression in Māori, Pacific, and NZE women. Our study found that miR-29b-3p was the only miRNA that significantly different between ethnic groups. Lower levels were present in Pacific women compared to NZE women (0.20±0.16 vs. 0.33±0.31, $p < 0.001$ respectively). However, regression analysis showed higher levels of miR-17-5p and miR-222-3p were predictors of ethnicity. MicroRNA-17-5p was the strongest predictor of Māori ethnicity (OR = 8.305) and the odds of higher miR-222-3p levels, decreased in Māori compared to Pacific (OR = 0.292). Although miRNA ethnic variations have been observed in previous studies (Rawlings-Goss et al., 2014; X. Wang et al., 2014; Zampetaki et al., 2010), numerous variables, including diet and lifestyle factors, can influence miRNA expression. This could make it difficult to determine if the differences in miRNAs between these groups are solely associated with ethnicity, diet and or lifestyle choices. Several studies that assessed Mexican-American and Spanish populations, as well as subjects from eight European countries, found a high degree

of variability in miRNAs, suggesting that expression differences are not solely caused by genetic ancestry, but rather related to environmental and lifestyle factors (González-Arce et al., 2021; Iacomino et al., 2019; Prats-Puig et al., 2013).

Our selected miRNAs in the EXPLORE study, were chosen due to their associations with obesity and metabolic diseases. Thus, it is difficult to say whether the miRNA associations seen in Māori and Pacific groups were purely genetic, or were related to increased adiposity, as these ethnic groups on average had a higher fat mass compared to NZE.

Overall, these results met our aim by providing valuable insight into the miRNA differences that exist between BPGs and ethnic groups in NZ. They also supported our hypothesis that subjects with "hidden fat" (i.e., those in the NH group) would be at a higher metabolic risk than those in the NN group, therefore helping to identify women with normal BMI who are at metabolic disease risk. Furthermore, interactions between diet, physical activity, body composition, and metabolic disease risk are, in part, mediated by changes in miRNA expression. The findings support the notion that, as a clinical biomarker of metabolic disease, miR-222 holds the greatest promise; however, the current variability in miR-29b and miR-17-5p data may present challenges for their use as biomarkers.

4.2 Research strengths

Our research included a wide range of body compositional, metabolic, diet, and physical activity variables that were analysed and evaluated against selected miRNAs. To our knowledge this has not previously been done with NWO and ethnic groups. The recruitment of pre-menopausal women ensured that menopause and post-menopausal factors did not play a role in obesity, strengthening the findings of whether biomarkers could help predict and thus prevent obesity and associated co-morbidities in the future. The study provided valuable information to guide future research, for example, identifying that miR-222 is differentially expressed in the NH group, increases its potential for use as a biomarker for metabolic disease risk. In addition, we identified that specific dietary factors are associated with miRNA. Our analytical approaches used were also a strength as we adjusted for age, NZ deprivation and ethnicity.

4.3 Research limitations

As with any cross-sectional study, the outcome and exposure cannot be determined, as both are examined at the same time, thus the effects of miRNA, body composition, metabolic markers, diet, and physical activity on each other could not be established. A significant challenge was experienced in the recruitment of Māori and Pacific women, especially in the NN and NH BPGs. New Zealand Europeans were the only ethnic group to have enough subjects in each BPG to conduct the body profile analysis. Lower recruitment of Māori and Pacific subjects reduced the statistical power to accurately assess ethnic group differences stratified by BPG. As Māori and Pacific were predominantly in the HH group, respectively, it made interpreting miRNA differences difficult, especially since the selected miRNA are associated with obesity. An equal sample size between ethnic groups per BPG would have been beneficial in determining whether significant miRNAs were specifically related to adiposity or ethnicity. Recruitment of healthy subjects, who were on average relatively young (~31 years), also proved problematic as miR-29b-3p and miR-17-5p are specifically dysregulated in T2D and MetS; diseases that commonly occur more often as age increases. It was difficult to determine the association of miRNAs with metabolic risk, when there was a low prevalence of abnormal metabolic biomarkers, despite subjects being classified as overweight/obese. Other challenges that presented were the lack of evidence/information on normal miRNA reference ranges. Most reference ranges available are for miRNAs from tissue samples (Peltier et al., 2008). Many variables can alter/influence miRNA expression, making it difficult to interpret and provide the rationale behind specific findings. Common miRNAs also appear in different diseases, some show similar expression levels, others the opposite. As a result, using miRNAs as biomarkers for a specific disease may be problematic, as they can reflect multiple diseases.

4.4 Recommendations for future research

MicroRNAs have numerous functions and target cells. A better understanding of miRNAs, their mechanisms of action, and the role they play in obesity and associated diseases is essential if they are ever to be used as a biomarker.

Due to the overlapping functions of miRNAs and the common appearance in different diseases, it can be difficult to use a single miRNA as a biomarker (Chevillet et al., 2014). Specific reference ranges for miRNAs may also provide clarity and precision in determining the risk of metabolic disease. Future research may benefit from determining specific miRNA ranges and sets associated with a specific disease, as this has already shown to significantly improve the reliability of disease diagnosis (Min et al., 2019; Xiong et al., 2017). There is also a need for standardised procedures in future biomarker studies, with detailed measures and methodologies to enhance the reproducibility and validity of miRNA results.

From the EXPLORE study, it might be worth assessing the miRNAs in different BPGs from NZE women only, as they had the statistical power in all three BPGs. This would remove any ethnic variations/influence on the selected miRNAs and provide insight into expression differences of miRNAs in NZE women from different BPGs versus our current study which evaluated women collectively regardless of ethnicity.

It would also be worth conducting a similar study in the future in which, subjects also had abnormal metabolic/inflammatory biomarkers or who had miRNA related diseases (T2D, CVD). Potentially even a longitudinal EXPLORE study, conducting similar tests, may provide valuable insight into miRNA and their predicted metabolic risk and validate our findings.

Concluding remarks: Although technical limitations are currently present, our study provides evidence that miRNAs are variable between different BPGs and ethnicities and that metabolic and inflammatory markers (Leptin, HbA1c, IL6, IL-10, TNF-α), as well as levels of physical activity and dietary factors (light physical activity, sucrose, carbohydrates, proteins) are associated with miRNA expression. Furthermore, of the selected miRNAs, miR-222-3p showed the greatest promise as a biomarker of health. In the future, miRNAs may serve as minimally invasive biomarkers to predict metabolic health risk.

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Appendix A: Chapter 3 Supplementary Tables

Table 3.A The association of metabolic and inflammatory markers with lower and upper range of selected miRNAs

Biomarkers	miRNA-17-5p			miRNA-222-3p			miRNA-29b-3p		
	Lower range (0.009-0.102 AU)	Upper range (0.103-0.806 AU)	*p*-value†	Lower range (0.024-1.079 AU)	Upper range (1.084-14.438 AU)	*p*-value†	Lower range (0.011-0.198 AU)	Upper range (0.202-1.851 AU)	*p*-value†
Metabolic markers									
Cholesterol (mmol/L)	4.57±0.93	4.61±0.87	0.676	4.61±0.93	4.56±0.88	0.55	4.49±0.93	4.68±0.87	0.051
LDL (mmol/L)	2.58±0.84	2.61±0.78	0.661	2.61±0.82	2.59±0.81	0.82	2.54±0.82	2.65±0.8	0.227
HDL (mmol/L)	1.55±0.41	1.56±0.41	0.863	1.57±0.43	1.54±0.39	0.4	1.52±0.42	1.59±0.4	0.148
Triglyceride (mmol/L)	0.96±0.5	0.95±0.46	0.82	0.96±0.45	0.96±0.51	0.99	0.94±0.44	0.98±0.52	0.404
Glucose (mU/mL)	4.67±0.39	4.68±0.41	0.818	4.67±0.39	4.68±0.41	0.74	4.67±0.37	4.68±0.43	0.763
Insulin (mU/mL)	12.9±8.64	12.0±9.15	0.37	11.7±8.23	13.2±9.5	0.1	13.2±9.01	11.7±8.75	0.101
HbA1c (mmol/mol)	28.7±3.71	28.4±3.53	0.475	28.2±3.71	28.9±3.51	0.08	29.0±3.5	28.1±3.69	**0.016**
Leptin (pg/mL)	10636±8089	10886±8520	0.773	9870±8305	11695±8216	**0.04**	11420±8720	10101±7819	0.126
Ghrelin (pg/mL)	46.6±41.0	45.14±33.7	0.713	43.5±38.0	48.3±37.0	0.22	48.2±39.7	43.5±35.2	0.224
Inflammatory markers									
CRP (mg/L)	4.29±4.06	3.82±2.83	0.193	4.1±3.12	4.02±3.85	0.82	4.36±4	3.75±2.88	0.098
TNF-α (pg/mL)	6.89±2.87	6.73±1.8	0.531	6.7±2.84	6.93±1.85	0.37	7.24±2.7	6.38±1.96	**0.001**
IL-6 (pg/mL)	2.7±2.26	2.27±2.8	0.1	2.62±2.22	2.36±2.85	0.33	2.81±3.4	2.17±1.13	**0.016**
IL-10 (pg/mL)	18.25±32.14	13.54±10.72	**0.06**	15.5±22.03	16.3±26.0	0.75	18.78±32.13	13.01±10.49	**0.021**

Abbreviations: HbA1c, glycated haemoglobin; HDL high density lipoprotein; Calc LDL Calculated low density lipoprotein; TAG triacylglycerols; CRP C-reactive protein; TNF-α tumor necrosis factor a; IL interleukin; C, cholesterol; AU arbitrary units
All Values are reported as mean±SD
† Independent sample t-test; Significant association: $p \leq 0.05$

Table 3.B The association of diet and physical activity with lower and upper range of selected miRNAs.

	miRNA-17-5p			miRNA-222-3p			miRNA-29b		
	Lower range (0.009-0.102 AU)	Upper range (0.103-0.806 AU)	*p*-value†	Lower range (0.024-1.079 AU)	Upper range (1.084-14.438 AU)	*p*-value†	Lower range (0.011-0.198 AU)	Upper range (0.202-1.851 AU)	*p*-value†
Diet									
Energy (kJ)	9691±3552	9631±3623	0.876	9522±3130	9817±3968	0.446	9531±3409	9784±3746	0.513
Protein (g)	105±41.3	101±38	0.334	105±38.2	102±41	0.497	102±39.6	104±39.8	0.666
Total fat (g)	91.5±36.1	93.4±38.9	0.641	91.4±34.9	93.7±39.9	0.574	91±37	93.9±38.1	0.479
Saturated fat (g)	35.1±15.9	36.8±16.9	0.38	35.1±15.3	36.9±17.5	0.356	34.5±16	37.3±16.7	0.15
PUFA (g)	12.9±5.06	13.2±5.2	0.649	13.1±4.88	13.1±5.37	0.99	12.8±5.05	13.3±5.19	0.33
MUFA (g)	30.3±11.9	30.9±11.8	0.656	30.6±11.5	30.6±12.2	0.977	30±12.3	31.1±11.5	0.433
Carbohydrates (g)	246±109	241±108	0.677	236±88.6	252±125	0.182	241±98.7	246±118	0.646
Cholesterol (mg)	324±178	322±176	0.911	327±166	319±186	0.694	313±166	332±185	0.317
Sugars (g)	126±57.6	126±63.2	0.994	120±46.1	132±71.1	0.066	120±50.6	131±68.2	0.094
Glucose (g)	25.5±13.8	25.1±15	0.761	23.9±11	26.7±17	0.077	24.1±12.6	26.4±15.8	0.13
Fructose (g)	27.3±15.8	26±15.3	0.433	25.1±12.4	28.1±18	0.071	25.7±14.7	27.4±16.4	0.309
Sucrose (g)	49.2±27	51±31.8	0.563	46.5±21.2	53.6±35.4	**0.025**	46.9±22.6	53.1±34.6	**0.049**
Lactose (g)	20.8±17.2	20.8±16.4	0.972	21.4±16.7	20.3±16.9	0.558	20.5±17	21.1±16.6	0.71
Maltose (g)	2.73±1.57	2.8±1.78	0.713	2.7±1.51	2.83±1.83	0.47	2.81±1.6	2.72±1.74	0.632
Alcohol (g)	5.58±7.68	6.42±8.56	0.339	6.24±8.85	5.81±7.42	0.628	5.52±8.16	6.46±8.11	0.288
kJ from Alcohol %	1.76±2.36	2.04±2.69	0.314	2±2.8	1.82±2.26	0.527	1.77±2.56	2.02±2.51	0.368
Dietary Fibre (g)	30.8±12.2	30.6±13.3	0.853	30.2±10.3	31.3±14.7	0.419	30.1±11.6	31.3±13.7	0.35
Thiamine (mg)	1.79±1.01	1.74±1.05	0.646	1.77±0.99	1.75±1.08	0.847	1.82±1.05	1.7±1.01	0.268
Riboflavin (mg)	2.75±1.3	2.64±1.36	0.442	2.7±1.2	2.69±1.45	0.973	2.7±1.33	2.68±1.33	0.882
Niacin (mg)	26.1±13.1	23.9±11.3	0.103	25.4±12.1	24.7±12.4	0.557	25.9±13.3	24.1±11.1	0.177
Vitamin C (mg)	164±93.9	157±95.2	0.501	157.1±82.7	164±105	0.493	158±93.9	162±95.3	0.691
Vitamin E (mg)	13.5±5.64	14.1±5.81	0.38	13.6±5.45	14±5.96	0.499	13.5±5.8	14.1±5.65	0.288
Vitamin B6 (mg)	3.13±2.44	2.8±2.17	0.183	2.95±2.15	2.99±2.47	0.873	3.14±2.68	2.79±1.9	0.163

	miRNA-17-5p			miRNA-222-3p			miRNA-29b		
	Lower range (0.009-0.102 AU)	Upper range (0.103-0.806 AU)	*p*-value†	Lower range (0.024-1.079 AU)	Upper range (1.084-14.438 AU)	*p*-value†	Lower range (0.011-0.198 AU)	Upper range (0.202-1.851 AU)	*p*-value†
Vitamin B12 (mg)	5.82±3.49	5.39±3.23	0.227	5.58±2.98	5.64±3.7	0.858	5.6±3.25	5.6±3.48	0.99
Total folate (µg)	433±167	442±211	0.666	432±161	443±214	0.591	428±167	446±210	0.358
Total vitamin A equivalent (µg)	1609±851	1547±695	0.459	1576±745	1585±804	0.909	1578±846	1577±705	0.987
Sodium (mg)	2882±1537	2756±1374	0.422	2789±1355	2852±1552	0.692	2824±1503	2813±1415	0.943
Potassium (mg)	3924±1372	3837±1400	0.557	3853±1223	3917±1523	0.67	3789±1325	3966±1438	0.237
Magnesium (mg)	396±134	403±149	0.651	399±124	400±156	0.948	385±130	413±150	0.066
Calcium (mg)	1182±562	1161±524	0.715	1179±540	1168±546	0.852	1138±561	1203±523	0.263
Phosphorus (mg)	1759±639	1739±627	0.77	1771±602	1733±659	0.576	1704±623	1792±640	0.194
Iron (mg)	13.4±5.11	13.4±5.75	0.986	13.3±4.65	13.6±6.1	0.639	13.1±4.84	13.7±5.95	0.343
Zinc (mg)	12.9±5.1	12.3±4.63	0.269	12.7±4.67	12.5±5.06	0.725	12.5±4.81	12.7±4.94	0.754
Selenium (µg)	86±55.1	85.4±47.3	0.914	86.9±54.7	84.8±47.7	0.702	80.9±54.1	90.2±48	0.092
kJ from protein (%)	18.6±3.67	18.1±3.52	0.222	18.8±3.73	18±3.42	0.023	18.4±3.65	18.4±3.55	0.893
kJ from fat (%)	35.1±6.66	35.8±6.52	0.337	35.4±6.61	35.4±6.61	0.966	35.2±6.48	35.7±6.7	0.53
kJ from saturated fat (%)	13.9±3.63	14.3±3.65	0.327	14±3.59	14.3±3.7	0.418	13.9±3.47	14.4±3.79	0.267
kJ from carbohydrate (%)	41.4±7.41	41±7.62	0.573	40.7±7.09	41.6±7.9	0.254	41.6±7.21	40.9±7.78	0.396
Fat as Mono (%)	38.7±3.28	38.4±3.1	0.371	38.9±3.06	38.2±3.28	0.052	38.9±3.23	38.3±3.13	0.074
Fat as poly (%)	17.1±4.77	16.7±3.71	0.482	17.1±4.3	16.7±4.21	0.501	17.1±4.34	16.8±4.18	0.559
Fat as saturated (%)	44.2±6.39	44.9±5.48	0.325	44±5.93	45±5.91	0.127	44±6.18	45±5.7	0.168

	miRNA-17-5p			miRNA-222-3p			miRNA-29b		
	Lower range (0.009-0.102 AU)	Upper range (0.103-0.806 AU)	*p*-value†	Lower range (0.024-1.079 AU)	Upper range (1.084-14.438 AU)	*p*-value†	Lower range (0.011-0.198 AU)	Upper range (0.202-1.851 AU)	*p*-value†
Physical Activity									
Sedentary (mpd)	462±67.1	466±77	0.638	463±72.4	465±72.1	0.744	459±74.8	469±69.5	0.226
Light (mpd)	319±74.5	315±81.5	0.623	321±75	313±80.9	0.35	327±77.4	308±77.8	**0.032**
Moderate (mpd)	31.7±16.8	30.4±17.9	0.493	32±16.9	30.1±17.8	0.323	31.4±18.6	30.7±16.2	0.739
Vigorous (mpd)	4.42±8.57	4.19±7.29	0.795	5.01±8.88	3.61±6.85	0.116	4.45±8.45	4.18±7.46	0.76
Moderate-Vigorous (mpd)	36.1±19.9	34.6±21.4	0.514	37±20.4	33.7±20.8	0.156	35.8±22	34.9±19.3	0.71

Abbreviations kJ, kilojoules; g, grams; mg, milligrams; mpd, minutes per day; µg, microgram; %, percentage; PUFA, Polyunsaturated Fat; MUFA, Monounsaturated Fats
† Independent sample t-test; Significant association: $p \leq 0.05$
Note: Energy includes fibre

Table 3.C Multinominal logistic regression - miRNAs as predictors for BF% and BMI controlled for age and NZ deprivation.

		b(SE)	95% CI for Odds Ratio		
			Lower	Odds Ratio	Upper
	BF% Model				
High BF% (30 - <35%) vs. Normal BF%(22 - <30%) [a]	Intercept	-1.677 (0.702)			
	Age (years)	0.039 (0.019)*	1.003	1.04	1.079
	NZ Deprivation Index	0.044 (0.06)	0.93	1.046	1.176
	miR-222-3p	0.508 (0.26)*	0.999	1.662	2.765
	miR-17-5p	-0.061 (1.884)	0.023	0.941	37.763
	miR-29b-3p	-0.924 (0.719)	0.097	0.397	1.625
Very High BF% (≥35%) vs. Normal BF% (22 - <30%) [a]	Intercept	-3.135 (0.702)			
	Age (years)	0.07 (0.018)**	1.036	1.073	1.111
	NZ Deprivation Index	0.28 (0.056)**	1.186	1.324	1.477
	miR-222-3p	0.757 (0.257)**	1.289	2.133	3.531
	miR-17-5p	-1.065 (1.906)	0.008	0.345	14.456
	miR-29b-3p	-2.076 (0.747)**	0.029	0.125	0.543
High BF% (30 - <35%) vs. Very High BF% (≥35%) [b]	Intercept	1.459 (0.656)			
	Age (years)	-0.031 (0.016)	0.939	0.969	1.001
	NZ Deprivation Index	-0.236 (0.052)**	0.713	0.79	0.875
	miR-222-3p	-0.249 (0.201)	0.525	0.779	1.157
	miR-17-5p	1.004 (1.67)	0.103	2.728	72.04
	miR-29b-3p	1.152 (0.667)	0.856	3.165	11.708

Note. R^2 = 0.179 (Cox-Snell), 0.204 (Nagelkerke). Model $\chi^2(10)$ = 68.787, *p<0.05,**p<0.01

Reference category [a] = (22 - <30 BF%); [b] = (≥35 BF%)

		b(SE)	95% CI for Odds Ratio		
			Lower	Odds Ratio	Upper
BMI Model					
Overweight vs. Normal [a]	Intercept	-1.726 (0.601)			
	Age (years)	0.026 (0.016)	0.996	1.026	1.058
	NZ Deprivation Index	0.068 (0.05)	0.97	1.07	1.181
	miR-222-3p	0.567 (0.207)**	1.176	1.764	2.646
	miR-17-5p	-2.131 (1.694)	0.004	0.119	3.287
	miR-29b-3p	-1.527 (0.678)*	0.057	0.217	0.82
Obese vs. Normal [a]	Intercept	-3.8 (0.702)			
	Age (years)	0.044 (0.017)**	1.011	1.045	1.079
	NZ Deprivation Index	0.348 (0.055)**	1.272	1.416	1.577
	miR-222-3p	0.533 (0.221)*	1.104	1.704	2.629
	miR-17-5p	-1.867 (1.9)	0.004	0.155	6.402
	miR-29b-3p	-1.707 (0.744)*	0.042	0.181	0.779
Overweight vs. Obese [b]	Intercept	2.074 (0.746)			
	Age (years)	-0.018 (0.018)	0.949	0.983	1.017
	NZ Deprivation Index	-0.28 (0.059)**	0.674	0.755	0.847
	miR-222-3p	0.035 (0.211)	0.685	1.035	1.564
	miR-17-5p	-0.264 (2.029)	0.014	0.768	40.935
	miR-29b-3p	0.18 (0.822)	0.239	1.197	6.002

Note. R^2 = 0.184 (Cox-Snell), 0.209 (Nagelkerke). Model $\chi^2(10)$ = 74.458, *p<0.05,** p<0.01

Reference category [a] = Normal BMI; [b] = Obese BMI

Table 3.D Multinominal logistic regression - miRNAs as predictors for ethnicity controlled for age and NZ deprivation

Ethnic Groups		b (SE)	95% CI for Odds Ratio		
			Lower	Odds Ratio [c]	Upper
Māori vs. NZE [a]					
	Intercept	-2.137 (0.688)			
	Age (years)	-0.014 (0.018)	1.02	0.953	0.953
	NZ Deprivation Index	0.304 (0.059)**	1.52	1.208	1.208
	miR-17-5p	5.813 (1.886)**	13483	8.305	8.305
	miR-222-3p	-0.212 (0.241)	1.296	0.505	0.505
	miR-29b-3p	-2.314 (0.881)**	0.556	0.018	0.018
Pacific vs. NZE [a]					
	Intercept	-2.892 (0.773)			
	Age (years)	-0.027 (0.019)	1.01	0.938	0.938
	NZ Deprivation Index	0.514 (0.067)**	1.909	1.466	1.466
	miR-17-5p	2.683 (2.247)	1196	0.179	0.179
	miR-222-3p	0.475 (0.252)	2.633	0.982	0.982
	miR-29b-3p	-5.217 (1.346)**	0.076	0	0
Māori vs. Pacific [b]					
	Intercept	0.755 (0.858)			
	Age (years)	0.013 (0.02)	1.054	0.973	1.054
	NZ Deprivation Index	-0.211 (0.071)**	0.931	0.704	0.931
	miR-17-5p	3.13 (2.291)	2041	0.256	2040.78
	miR-222-3p	-0.687 (0.278)*	0.868	0.292	0.868
	miR-29b-3p	2.903 (1.414)*	291.7	1.14	291.688

[a] The reference category is NZE

[b] The Reference category is Pacific

[c] Odds ratios for a 1-SD unit increase of miRNA.

Note. R2 = 0.319 (Cox-Snell), 0.376 (Nagelkerke). Model $\chi 2$ (10) = 140.527, * $p<0.05$,** $p<0.01$

Table 3.E Forwards binomial regression – clinical characteristics as predictors of selected miRNAs controlled for age and NZ deprivation

miR-17-5p Model 1[a]	**b(SE)**	**95% CI for Odds Ratio**			
Included		**Lower**	**Odds Ratio [c]**	**Upper**	**P-Value**
IL-6 (pg/mL)	-0.246 (0.094)	0.651	0.782	0.939	**0.008
Constant	0.609 (0.239)		1.839		0.011
	Note R^2 = 0.371 (Hosmer-Lemeshow), 0.031 (Cox -Snell), 0.041 (Nagelkerke). Model X2 (1) = 9.183, p = <0.001, *p <0.05,**p <0.01				
	a Variable(s) entered on step 1: IL-6 (pg/mL).				
miR-222 - Model 3[c]					
Included					
IL-6 (pg/mL)	-0.291 (0.11)	0.602	0.747	0.928	**0.008
IL-10 (pg/mL)	0.024 (0.012)	1	1.025	1.05	*0.047
Leptin Lower and Upper Range (1)	0.651 (0.243)	1.19	1.917	3.088	**0.007
Constant	0.066 (0.247)		1.069		0.788
	Note R^2 = 0.747 (Hosmer-Lemeshow), 0.057 (Cox -Snell), 0.076 (Nagelkerke). Model X2 (3) = 17.072 p = <0.001, *p <0.05, **p <0.01				
	[a] Variable(s) entered on step 1: Leptin Lower and Upper Range; [b] Variable(s) entered on step 2: IL-6 (pg/mL); [c] Variable(s) entered on step 3: IL-10 (pg/mL).				
miR-29b-3p - Model 5[e]					
Included					
HbA1c (mmol/mol)	-0.084 (0.035)	0.859	0.92	0.985	*0.017
TNF-α (pg/mL)	-0.133 (0.053)	0.789	0.875	0.972	*0.012
Carbohydrate (g)	-0.005 (0.002)	0.991	0.995	1	*0.028
Sucrose (g)	0.024 (0.008)	1.008	1.024	1.04	**0.003
Light Physical Activity (mpd)	-0.004 (0.002)	0.993	0.996	0.999	*0.014
Constant	4.61 (1.143)		100.484		0
	Note R^2 = 0.997 (Hosmer-Lemeshow), 0.098 (Cox -Snell), 0.131 (Nagelkerke). Model X^2 (5) = 30.064 p = <0.001, * p <0.05, ** p <0.01				
	[a] Variable(s) entered on step 1: HbA1c (mmol/mol).; [b] Variable(s) entered on step 2: Light Physical Activity; [c] Variable(s) entered on step 3: TNF-α (pg/mL); [d] Variable(s) entered on step 4: Sucrose (g); [e] Variable(s) entered on step 5: Carbohydrate (g).				

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Figure B.1

Figure B.2

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