A Metabolic Health Study of Pacific Adolescents

Investigating the metabolic health of Pacific adolescents in New Zealand: Environmental and genetic risk factors.

Jarrod Moors

Supervisor: Associate Professor Tony Merriman

A thesis submitted for the degree of Master of Science in Biochemistry

University of Otago, Dunedin, New Zealand

October 2015
Abstract

Adults of Pacific descent residing in New Zealand have more than double the prevalence of diabetes, obesity, gout and the metabolic syndrome (MS), compared to the general population. Genetic, biochemical and environmental factors play a crucial role in the development of these diseases. These factors that predispose people of the Pacific, particularly Pacific adolescents, are poorly understood.

Beginning 2013 the Pacific Trust Otago (PTO) in collaboration with the University of Otago recruited 80 Pacific Island young people (males = 33, females = 47) aged between 15 – 20 years residing in Dunedin. Participants, upon informed consent, were required to provide blood and urine samples to determine biochemical traits (e.g. serum urate, cholesterol, triglycerides etc.). Demographic and other lifestyle information were collated by means of questionnaires. DNA samples from this sample set were genotyped using Taqman genotyping. All (statistical) analyses were accomplished using STATA – a statistical software package.

The most significant findings from this study are the association of alcohol with several outcomes, particularly body mass index (BMI). Alcohol consumption was significantly associated with increased BMI (Beta_{adjusted} = 3.379, P_{adjusted} = 0.03), and serum triglycerides (Beta_{adjusted} = 0.236 mmol/L, P_{adjusted} = 0.04) when adjusted by age and sex. At the crude level, alcohol consumption significantly caused an increase to diastolic blood pressures (Beta = 5.762 mm Hg, P = 0.004), and an increase in serum urate (Beta = 0.036 mmol/L, P = 0.02). Furthermore, this study provides evidence of association between BMI and serum urate adjusted for age and sex (Beta = 0.002 mmol/L, P = 0.05) and further, an association of BMI with increased triglycerides (Beta 0.027 mmol/L, P = 0.01).

Physical activity correlated with reduced BMI, where moderately active participants had higher BMI (30.7 kg/m^2) than those who were highly active (27.6 kg/m^2). Genetics investigations provided no evidence of association of two variants of SLC2A9 (rs11942223 and rs3775948) with serum urate. Gene-environment interaction analysis also failed to provide any evidence of interaction between sugar and the genetic variants of the SLC2A9 for an influence on urate levels. The same account was made for the variants of the fat mass and obesity associated protein (FTO) (rs9922047 and rs9923233), tested for association with BMI. No evidence of association was established, with the gene-environment interaction test also unsuccessfully
producing evidence of interaction between physical activity and the variants of the FTO gene, for an influence on BMI.

Collectively, the findings from this research further support the notion that community based studies set out to evaluate risk factors for metabolic diseases ought to assess these factors in adolescents particularly those with high BMI values. The findings from this study are of importance for Pacific health by identifying pathways that could be targeted in treating and preventing metabolic disorders, to which Pacific people are more susceptible. These findings will also serve as a basis for allowing the PTO to set up intervention programs.
Acknowledgements

“Ou te vivi’i atu ia te oe, auā na faia a’u e ala ai ona mata’utia ma ofo tele ai; o mea na e faia, o mea mamana ia; ua ou matuā iloa e a’u nei mea” Salamo 139:14 (Psalm 134:14)

I would like to express my heart-felt appreciation to everyone who supported me through this research project. First and foremost, all glory and honor to God for the gift of life, and the continuous blessings bestowed upon me.

I express sincerest gratitude and appreciation for my supervisor, the incomparable Associate Professor Tony Merriman for his guidance and knowledge. For providing endless encouragement and his unequivocal support with patience while allowing me to work in my own way. I attribute the level of my Master’s degree to his encouragement and faith in me. One simply could not wish for a better and awesome supervisor. Fa’afetai tele lava, Tony. I also express my thanks to Dr. Mele Taumoepeau who through the length of this project provided continued support, expert advice and always made sure my head was above water. Malo ‘aupito, Mele.

To the Pacific Trust Otago, thank you for helping with recruitment and clinics for this project. I would also like to thank the participants that volunteered for this project. For without them, this would not have been possible.

My special thanks to the awesome staff and students of the Merriman Laboratory – Marilyn Merriman, Humaira Rasheed Mandy Phipps-Green, Ruth Topless, Tanya Flynn, Mansour Zamanpoor, Sara Altaf, Anna Gosling, James Boocock, Hoang Tan Nguyen, Cushla McKinney, Tahzeeb Fatima, Keresoma Leaupepe and Amara Umair. Extra special thanks goes to Humaira Rasheed, my own “STATA-expert”. Thank you for sharing your knowledge and being very patient as I try to learn and understand the statistical methods required for this project. I would also like to thank Tom Kelly for his expert assistance with some of my statistics and computer-related stuff.

I owe thanks to the Biochemistry department staff for the continuous support, and offering a great environment to work in.
A special mention is also warranted to my friends who have become family. My Samjian family - Talai and Eric who have offered unwavering support, precious advice and for keeping up with my insanity longer than anyone should ever have to. Thank you for the years. I am immensely grateful.

To Silia, Saofa’i and Niutao, who took me in as family and supported me throughout – thank you for being blessings in my life. Also to Grettel, Tumanu and Randy who have been supportive great friends and my source of happiness. Thank you for the endless support and for being my family away from home.

My thanks also to my awesome housemate Lenara Tuipoloa, for constantly supporting and reminding me of my purpose at this University. To my postgrad buddies, (Alapasa Teu and Melbourne Mauiliu-Wallis), thank you for the years! I couldn’t have done it without the unfailing support and for being my source of security – Triple threat.

I am grateful also to Elizabeth Tauati-Williams, my supporter from Samoa. I am immensely grateful for your prayers and support from afar. I’d also like to thank my great friend Dan Sharples for his superb aptitude of keeping up with my Kardashian-like lifestyle, the company during the many coffee breaks, and for being my source of comfort and reassurance when I was stressed, both academically, and non-academically. Thanks, pal.

Finally, I owe a tremendous thanks to my critics - my family. My parents (Christina and Setefano) who have supported me over the years, and desperately attempting to understand what exactly it is I’m doing. To my sisters (Jiltrice and Jazmyn) and my brothers (Jeddidiah and Andrew) who through good and bad, they have been my rock. Thank you family for your unconditional love and having faith in me when I had lost faith in myself.

And last but not the very least, a special thanks to those who gave me strength, honor and courage to go through the rough patches of life. Special thanks also to the naysayers who tried to bring me down. They, in a way, made me what and who I am today.
Table of Contents

Abstract ............................................................................................................................................. ii
Acknowledgements ........................................................................................................................ iv
Table of Contents .......................................................................................................................... vi
List of Figures .................................................................................................................................. xi
List of Tables ................................................................................................................................... xiii
List of Abbreviations ....................................................................................................................... xvi

CHAPTER 1: INTRODUCTION ........................................................................................................ 18
1.1 General Introduction to Pacific Health ..................................................................................... 19
1.2 Obesity ...................................................................................................................................... 20
  1.2.1 Generalizability of obesity standards across ethnic groups .............................................. 22
  1.2.2 Obesity and the fat mass and obesity associated (FTO) gene ........................................ 25
1.3 HbA1c – A measure of diabetes ............................................................................................... 27
1.4 Gout .......................................................................................................................................... 29
  1.4.1 Genetics of Gout; SLC2A9 – Urate Transporter ............................................................... 31
1.5 Lifestyle Factors ....................................................................................................................... 33
  1.5.1 Physical activity ................................................................................................................... 33
  1.5.2 Diet - Sugar ........................................................................................................................ 34
  1.5.3 Alcohol consumption ......................................................................................................... 39
1.6 The health of Pacific teenagers in Dunedin ............................................................................. 43

vi
1.7 Aims of this thesis ............................................................................................................. 44

CHAPTER 2: METHODOLOGY ................................................................................................ 45

1.8 Pacific Research Framework............................................................................................. 46

2.1 Parties involved and roles played in this study ............................................................... 50

2.1.1 The Role of the Candidate.......................................................................................... 50

2.1.2 The Pacific Trust Otago............................................................................................ 50

2.2 Ethical Approval ............................................................................................................ 51

2.3 Recruitment Strategies and Study Population ............................................................... 52

2.3.1 Selection and Criteria for recruitment ...................................................................... 52

2.3.2 Method of Recruitment ............................................................................................ 52

2.3.3 Study Population ...................................................................................................... 53

2.4 Clinical Procedure ........................................................................................................ 54

2.4.1 Informed Consent..................................................................................................... 54

2.4.2 Blood and Urine Samples: Collection and Testing .................................................. 56

2.5 Blood Pressure ............................................................................................................. 58

2.6 Anthropometric and Body Composition Procedures .................................................... 58

2.6.1 Anthropometrical Variables ..................................................................................... 60

2.7 Demographic, Biochemical risk factors and Sugar Consumption data ....................... 63

2.7.1 Biological Questionnaire (Appendix C) .................................................................. 63

2.8 Food Frequency Questionnaire ...................................................................................... 65

2.9 Breakfast ....................................................................................................................... 66
2.10 Debrief ........................................................................................................................................... 66
2.11 Koha ............................................................................................................................................... 66
2.12 Protocol for examining biochemical risk factors in this thesis .................................................. 67
  2.12.1 Extraction of human genomic DNA from whole blood .......................................................... 67
  2.12.2 SNP selection and design ........................................................................................................ 68
  2.12.3 TaqMan® SNP genotyping assay ............................................................................................ 68
  2.12.4 TaqMan® SNP genotyping using the Light Cycler ................................................................ 70
  2.12.5 Generation of cluster plot ....................................................................................................... 72
2.13 Data for analysis ............................................................................................................................. 73
  2.13.1 Data entry into Haploped Files ............................................................................................. 73
2.14 Statistical Analysis ......................................................................................................................... 74
  2.14.1 SNP Association analysis ....................................................................................................... 74
  2.14.2 Ordinary least square (linear) regressions ............................................................................. 75
  2.14.3 Adjustments ............................................................................................................................ 75

CHAPTER 3: RESULTS .......................................................................................................................... 76
3.1 Summary statistics on health measures ......................................................................................... 77
3.2 Comparison of Summary level data between two cohorts; the change in health measures between 2006 and 2013 ........................................................................................................ 80
3.3 Important Clinical Measures ......................................................................................................... 86
  3.3.1 HbA1c ...................................................................................................................................... 86
  3.3.2 BMI ........................................................................................................................................ 91
3.4 Lifestyle / Environmental Factors ................................................................. 95
  3.4.1 Physical Activity ....................................................................................... 95
  3.4.2 Smoking .................................................................................................. 98
  3.4.3 Alcohol Consumption ........................................................................... 99
  3.4.4 Sugar Consumption .............................................................................. 107

3.5 Genetics ........................................................................................................ 114
  3.5.1 SLC2A9 and serum urate ....................................................................... 115
  3.5.2 FTO and BMI ......................................................................................... 119

CHAPTER 4: DISCUSSION AND CONCLUSION .................................................. 123

4.1 Baseline findings ......................................................................................... 124

4.2 Comparison with Grant and associates’ findings ....................................... 125

4.3 Important Clinical Measure ....................................................................... 129
  4.3.1 HbA1c and diabetes .............................................................................. 129
  4.3.2 The effects of BMI on lipid concentrations ........................................ 131

4.4 Lifestyle Factors ......................................................................................... 137
  4.4.1 Physical activity ................................................................................... 137
  4.4.2 Alcohol Consumption ......................................................................... 140
  4.4.3 Sugar Consumption ............................................................................ 146

4.5 Genetics ....................................................................................................... 150
  4.5.1 SLC2A9 ................................................................................................. 150
  4.5.2 FTO ........................................................................................................ 151
List of Figures

Figure 1.1 shows OECD rankings with New Zealand’s obesity rates coming third highest. Update, 2014 (http://www.oecd.org/health/obesity-update.htm) .......................................................... 24

Figure 1.2 – HbA1c values from NZSSD ............................................................................................................. 29

Figure 2.1: Figure showing the logo representing the Pacific Trust Otago. ................................................. 51

Figure 2.2 - General Overview of protocol ...................................................................................................... 53

Figure 2.3: Equation utilized to determine whether a third measurement was necessary for anthropometrical measurements ............................................................................................................. 59

Figure 2.4: Example of an image provided to help participants estimate how much they usually drank ........................................................................................................................................... 65

Figure 2.5: Allelic differentiation is accomplished by the selective hardening of TaqMan® MGB probes. The VIC dye attaches to the major allele (target allele 1) whereas the FAM dye binds to the minor allele (target allele 2). Figure adapted from Applied Biosystems ............... 70

Figure 2.6: Cluster plot generated from TaqMan® SNP genotyping assay for SLC2A9, SNP rs3775948 ................................................................................................................................................. 72

Figure 2.7: Sample of Master Haploped ............................................................................................................. 73

Figure 2.8: Image showing SNP coded for risk allele (0, 1 or 2) .................................................................. 74

Figure 3.1: Age and gender distribution of the Pacific Island teenagers’ cohort (N=80) ..................... 77

Figure 3.2: Physical activity distribution in males, females and the aggregate cohort .......................... 95

Figure 3.3: Correlation between sugar intake in grams per day, and frequency per day ................... 108

Figure 4.1 shows percentage of each category and the number of MS components (Present study) ........................................................................................................................................ 126
Figure 4.2 shows percentage of each category and the number of MS components (Grant et al., 2008).
List of Tables

Table 1.1 - World Health Organization Worldwide Body Mass Index (BMI) definitions for adults. Adapted from WHO report (2000). ........................................................................................................21

Table 1.2 – Higher ethnic-specific BMI cut off points. ........................................................................................................21

Table 2.1: Blood results tabulated in this manner and returned to participants. .................56

Table 2.2 - Clinical measurements for biochemistry profile......................................................................................57

Table 2.3: TBE and TE Buffer formulae......................................................................................................................67

Table 2.4: List of genetic variants investigated in this study. ..........................................................................................68

Table 2.5: TaqMan SNP genotyping cocktail reaction ................................................................................................70

Table 2.6: Specification of protocol per cycle for Taqman assay for 40 cycles. .........................71

Table 3.1: Baseline characteristics of the Pacific Island teenager’s cohort (N = 80). ............78

Table 3.2: Demographic and clinical characteristics: the listed means and standard deviations (SD) are for the cohort separated by sex. .................................................................79

Table 3.3: Comparison of characteristics of the 80 participants for both studies, separated according to BMI status ........................................................................................................82

Table 3.4 - weight status, and prevalence of risk factors in the Grant and associates, and present cohorts. ..................................................................................................................................................84

Table 3.5 showing HbA1c mean estimates for males, females and the cohort as a whole. ....86

Table 3.6 showing summary demographic statistics of pre-diabetic participants. Data presented in mean and SD. .........................................................................................................................87

Table 3.7: Association of blood pressure with HbA1c .................................................................................................89

Table 3.8: Biochemical measurements and their associations with HbA1c ...............................90
Table 3.9: Association of biochemical traits with BMI .................................93

Table 3.10: Association of physical activity and blood pressure with BMI ................94

Table 3.11 demonstrates descriptive summary statistics according to levels of physical activity96

3.12 Association of Physical Activity and HbA1c..................................................97

Table 3.13: Data presented in mean and standard deviations of values between smokers and non-smokers .................................................................................................................................98

Table 3.14 illustrates the mean and standard deviations of different variables for drinkers and abstainers..............................................................................................................................................100

Table 3.15: Summary statistics of male drinkers vs. male non-drinkers presented in mean and standard deviation. ........................................................................................................................................103

Table 3.16: Summary statistics of female drinkers vs. female non-drinkers presented in mean and standard deviation...........................................................................................................................................105

Table 3.17 reflect number of problems related to alcohol separated according to sex: Self-reports of controlled study..................................................................................................................................................106

Table 3.18 - difference in BMI and serum urate across the four quartiles of sugar intake. Data presented in mean and standard deviations ..................................................................................................110

Table 3.19 Biochemical measures arranged by sugar sweetened beverage (SSB) quartiles .....111

Table 3.20 Biochemical measures arranged by sugar-sweetened food quartiles.................112

Table 3.21 Biochemical measures arranged by Natural sugar (from fruit) quartiles..........113

Table 3.22 illustrates the association analyses of rs11942223 and rs3775948 with serum urate116

Table 3.23: Interaction terms between sugar intake (g/day) and rs11942223 genotype for serum urate (mmol/L) ................................................................................................................................................117

Table 3.24: Interaction terms between sugary drink consumption (frequency per day) and rs11942223 genotype for serum urate (mmol/L) ................................................................................118
Table 3.25: Interaction terms between sugar intake (g/day) and rs3775948 genotype for serum urate (mmol/L)........................................................................................................................................118

Table 3.26: Interaction terms between sugary drink consumption (frequency per day) and rs3775948 genotype for serum urate (mmol/L) ..................................................119

Table 3.27 illustrates association analysis of FTO genetic variants with BMI.................................120

Table 3.28: Interaction terms between physical activity and rs9922047 genotype for BMI (kg/m²).................................................................................................................................121

Table 3.29: Interaction terms between physical activity and rs9923233 genotype for BMI (kg/m²).................................................................................................................................122
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>Adenosine Monophosphate</td>
</tr>
<tr>
<td>Apo B</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Diseases</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FTO</td>
<td>Fat mass and obesity-associated gene</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome Wide Association Studies</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated (A1c) Haemoglobin</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>HFCS</td>
<td>High Fructose Corn Syrup</td>
</tr>
<tr>
<td>HU</td>
<td>Hyperuricaemia</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low-density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>ISAK</td>
<td>The International Society for the Advancement of Ki anthropometry</td>
</tr>
<tr>
<td>MS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>NZSSD</td>
<td>New Zealand Society for the Study of Diabetes</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PTO</td>
<td>Pacific Trust Otago</td>
</tr>
<tr>
<td>SCL</td>
<td>Southern Community Laboratories</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide Polymorphism</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>2SLS</td>
<td>Two-Stage Least Squares</td>
</tr>
<tr>
<td>SSB</td>
<td>Sugar-Sweetened Beverages</td>
</tr>
<tr>
<td>SSF</td>
<td>Sugar-Sweetened Foods</td>
</tr>
<tr>
<td>SST</td>
<td>Serum Separator Tube</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION
1.1 General Introduction to Pacific Health

The health of Pacific people in New Zealand (NZ) compared to its overall population is disclosed to be of very poor status (Ministry of Health, 2012a, Ministry of Health, 2012b). Statistics reveal that adults of Pacific Island descent residing in New Zealand have more than double the incidence of diabetes (10% vs. 4% for the NZ population), cardiovascular disease (390 per 100,000 vs. 176 per 100,000 for the NZ population) and an increased risk of the metabolic syndrome (odds ratio, 2.54; 95% confidence interval [CI], 1.93-3.35), as opposed to the general European population in New Zealand. Despite these figures regarding the health of Pacific adults, there is a lack of knowledge as to the presence of risk factors particular to these disorders amongst Pacific Island adolescents.

Obesity is a major risk factor for heart disease, type 2 diabetes and gout. The Ministry of Health (2012) in its account of significant findings from the New Zealand Health Survey reported a worrying concern as obesity rates continue to escalate. This trend has been observed to increase over the previous 15 years from 19% in 1997 to 28% in 2011/12 (Ministry of Health, 2012a).

From 1996 – 2000, cardiovascular diseases accounted for 3 of the 5 leading causes of death for Pacific people aged 25-44, and remained a significant cause of death in older age groupings (45-64 and 65+ years). Diabetes was found to be the 2\textsuperscript{nd} and 3\textsuperscript{rd} leading cause of death in Pacific women and men, respectively, aged 45-64 years and remained in the top 5 leading causes of death for those 65 and over. Based on death rates in New Zealand in 2012–14, life expectancy was 78.7 years for Pacific females and 74.5 years for Pacific males, compared with 83.2 years for females and 79.5 years for males in the total New Zealand population (Ministry of Health, 2014).

Furthermore, adults of Pacific Island descent have a significant burden due to chronic diseases and diabetes. The prevalence of these diseases in Pacific people is much higher than that of other ethnic populations in New Zealand. Thus, mortality rates for cardiovascular diseases and diabetes account for the sizeable difference between the overall mortality rates for Pacific people and those of dissimilar ethnicities in New Zealand. The high rates of diabetes within New Zealand have been continual. 10 percent of Pacific peoples aged 15 years and over were diagnosed with diabetes. A statistic approximately three times the number of diagnoses for the general New Zealand populations.
Provided the aforementioned, this literature review endeavors to explore the available research that investigates the health of Pacific people, particularly the risk factors associated with metabolic disorders of which Pacific people are susceptible to.

1.2 Obesity

A basic definition of obesity is the excess accumulation of body fat that poses a major risk to health (World Health Organization, 2014b). While human history dictated weight gain and fat storage to be a reflection of status - that of health and affluence - principles of living at the moment continue to escalate, thus rendering weight gain and obesity a global major health hazard (Brown, 1991, World Health Organization, 2000). Obesity contributes significantly to the prevalence of numerous adult physiologic conditions, of which include cardiovascular disease, different forms of cancers, kidney disease and type 2 diabetes and elevated levels of serum urate (Choi et al., 2005, James, 2008, Williams, 2008, Yusuf et al., 2005).

Multiple ways are employed to measure excess body fat in order to delineate obesity in individuals. The most frequently used measure of excess body weight in clinical practice and population studies is the body mass index (BMI), which is used as a proxy measure of adiposity and exists as the global standard for assessment of obesity. BMI may be calculated dividing total body weight in kilograms (kg) by height in meters (m) squared (unit = kg/m$^2$).

A great deal of debate has lingered on the accuracy of using a single measure of BMI as a measure of obesity for people of different ethnic groups. This has led to the use of different levels of BMI for ethnic groups that categorize ‘overweight’ and ‘obesity. Today, global BMI cut-points for classification of obesity amongst adults have been established, validated and were mainly derived from European population studies (Table 1.1). It is worthwhile to note that there is continuing debate with regard to these standards and whether they are applicable to non-European populations (Razak et al., 2007). However, a study carried out by the Ministry of health and the University of Auckland established higher BMI cut – off points for classification of overweight and obesity for individuals of Māori and Pacific Island descent (Table 1.2), due to Māori and Pacific adults having less body fat percentage compared to Europeans (Swinburn, 1998).
Table 1.1 - World Health Organization Worldwide Body Mass Index (BMI) definitions for adults. Adapted from WHO report (2000).

<table>
<thead>
<tr>
<th>BMI Categories</th>
<th>BMI ranges, kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5 kg/m²</td>
</tr>
<tr>
<td>Healthy weight</td>
<td>18.5 – &lt;25.0</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0 – 30.0 kg/m²</td>
</tr>
<tr>
<td>Obese</td>
<td>30.0 – 40.0 kg/m²</td>
</tr>
<tr>
<td>Morbid obesity</td>
<td>&gt;40.0 kg/m²</td>
</tr>
</tbody>
</table>

Table 1.2 – Higher ethnic-specific BMI cut off points.

<table>
<thead>
<tr>
<th>Classification</th>
<th>European and Other</th>
<th>Māori and Pacific People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight</td>
<td>25.0 – 29.9 kg/m²</td>
<td>26.0 – 31.9 kg/m²</td>
</tr>
<tr>
<td>Obese</td>
<td>≥30.0 kg/m²</td>
<td>≥32.0 kg/m²</td>
</tr>
<tr>
<td>Extreme Obesity</td>
<td>≥40.0 kg/m²</td>
<td>≥40.0 kg/m²</td>
</tr>
</tbody>
</table>
1.2.1 Generalizability of obesity standards across ethnic groups

The World Health Organization (WHO) (2000) declared obesity as a worldwide epidemic with a prevalence increasing perpetually in both developed and developing countries; a prevalence that has virtually doubled since 1980 (World Health Organization, 2000) giving rise to some obvious health complications, and some which are not so plain (Jean, 2000). Furthermore, in 2008 approximately 1.4 billion adults (aged 20 years and older) were classified as overweight and 500 million (over 200 million men and approximately 300 women) were classified as obese (World Health Organization, 2014a).

The increasing prevalence of obesity amongst children and young adults is a major health concern as evidence has signaled a correlation between childhood and adult obesity (Freedman et al., 2005, Serdula et al., 1993). Gordon and colleagues in their study of childhood obesity reported the exceptionally high levels of obesity amongst young Pacific adults residing in New Zealand (Gordon et al., 2003). Additionally, a more recent systematic analysis of the trends in overweight and obesity by country during 1980 – 2013 employment data from literature, surveys and reports to further document the worldwide burden of overweight and obesity (Ng et al., 2014). The authors in their analysis revealed a dramatic increase in the global prevalence of obesity both in developed and developing countries. Accounts of the rapid increase in prevalence of obesity suggest that the true health impact of obesity may intensify in the future, compelling public health ministries to declare obesity reduction as a priority population health objective.

The South Pacific region has the greatest degree of documented rates of obesity in the world (Cutris, 2004, Hodge et al., 1996, World Health Organization, 2003a). New Zealand encompasses a vast population of diverse ethnic groups. According to the 2013 census and it’s reflection of population statistics, 74.0% of adults (aged 15 years and over) identified as New Zealand European, 14.9% identified as Māori, 11.8% as Asians and 7.4% identified as Pacific Islanders (Statistics NZ, 2013b). More than half of the adult population in New Zealand (62.7%) and about a third (31.1%) of children aged between 5 – 15 years were classed as overweight and obese (Ministry of Health, 2003, Ministry of Health, 2008); these statistics are not necessarily specific to Pacific Islanders. In New Zealand, the aim to reduce obesity was recorded as the third
highest “Priority populations health objective” by the New Zealand Ministry of Health in 2000 (King, 2000).

Over the past 15 years in NZ, the prevalence of obesity emerged from 19% in 1997 to 20% in 2006/6007 and further increasing to 28% in 2011/2012. An approximation of one million adults in NZ are said to be obese (Ministry of Health, 2012a). The increase has been seen in both males (17% in 1997 to 30% in 2012/2013) and females (21% in 1997 to 32% in 2012/2013) (World Health Organization, 2014c).

The prevalence of obesity in Pacific males and females according to an earlier report was 26% and 47% respectively (Russell et al., 1999). Adults and children of Pacific Island descent residing in New Zealand are two and a half times more likely to be obese, and older Pacific adults are about eleven times more likely to be extremely obese as opposed to their European counterparts (Goulding et al., 2007). In the most recent account of statistics about obesity in New Zealand, percentages reflect comparatively high rates of obesity amongst Pacific adults (68%) and Pacific children (27%) (Ministry of Health, 2013).

Results from a study on 1011 Pacific people, carried out by Sundborn and colleagues in Auckland (2010) found that from their cohort, about 95% of Pacific men and 100% of Pacific women were classified as overweight or obese. A more detailed account for men who were classified as obese were: all Pacific 53%, Samoan 58%, Cook Island 23%, Tongan 60%, and Niuean 49%; and an account for obese women were: all Pacific 74%, Samoan 75%, Cook Island 69%, Tongan 78%, and Niuean 76% (Sundborn et al., 2010).

Studies have also shown that the rates of obesity are high in the Pacific Island countries in Oceania (Hawley and McGarvey, 2015, Lin et al., 2015). The WHO STEPwise approach surveillance (STEPS) survey data showed the prevalence of obesity in 14 Pacific Islands with obesity rates in American Samoa at 74.6%, with 80% of obese subjects being females. In 2011, Niuean obesity rates were at 60%, where the majority were also females. Of the 14 Pacific Islands studied, with the exception of Papua New Guinea where rates of overweight and obesity were considerably low, and perhaps due to the slow and gentle pace of economic development, more than half of the populations studies were obese. The rates in Samoa and Tonga were reported at 54% and 57%, and Nauru also at a high of 58% (World Health Organization, 2014d). Provided these statistics, it is safe to hypothesise that obesity is not a problem associated with living in NZ per se.
Further to these studies are the earlier reports that showed obesity was not previously prevalent in the Pacific Islands. About half a century ago Prior conducted epidemiological studies on metabolic health throughout the Pacific. The studies showed good metabolic health where the prevalence of obesity was low in the Pacific Islands. Thus the conclusion can be drawn, the high rates of obesity in Pacific people is not due solely to the change in environment i.e. living in New Zealand, nor a problem of genetic background but perhaps a combination of the two (Prior et al., 1981, Prior et al., 1966, Prior, 1981). The environmental risk factors associated with obesity include, lifestyle, physical inactivity and diet to name a few (risk factors discussed in detail in Section 2 of this chapter). Furthermore, the association of genetic variants of the fat mass and obesity-associated gene (FTO) have been established with obesity (as discussed in Section 1.2.2). Thus the interplay between genetics and environmental components may explain the high rates of obesity in Pacific peoples.

It is evident from these results that obesity is a major concern amongst Pacific Island people today. Furthermore, the comorbidities associated with obesity have particularly affected the occupants of the Pacific Islands, with some of the extreme levels of obesity in the world evident in the region.

Figure 1.1 shows OECD rankings with New Zealand’s obesity rates coming third highest. Update, 2014 (http://www.oecd.org/health/obesity-update.htm)
Gallagher and colleagues (2000) in their study to examine a new method for developing percentage body fat ranges claimed ethnicity to be a confounding factor of the international BMI definitions (Gallagher et al., 2000). Furthermore, although BMI is generally accepted as a rational measure of body fat, evidence however inconsistent, reflects an intermittent relationship between BMI and adiposity through populations and may vary significantly across ethnic groups, both in adults (Craig et al., 2001, Deurenberg et al., 1998, Norgan, 1994) and children (Daniels et al., 1997, Malina and Kayzmarzyk, 1999). Several studies carried out in New Zealand involving people from different ethnic groups have established disparities of body compositions; where people that identify as Māori and Pacific have a lower degree of body fatness as opposed to Europeans at any given BMI level (Rush et al., 2004, Swinburn, 1998, Swinburn et al., 1996, Swinburn et al., 1999). On the contrary, those of Asian descent have greater body fat for any given BMI and have more central or truncal adiposity than Europeans (Duncan et al., 2004).

Tyrell and colleagues’ study researching the correlation between BMI and body composition through ethnicity established no significant difference in the standard range of BMI (<30 kg/m²) between children of European, Pacific and Māori descent. They did however discover substantial differences at higher BMI levels (>30 kg/m²), with body fat percentage lower in Pacific children as opposed to children of European descent (Tyrell et al., 2001). Accounts as to the different associations amid BMI and body fat in dissimilar ethnic groups are poorly understood. The Ministry of Health reflects the necessity of having large-scale New Zealand based population studies to be embarked on, particularly to establish apt measures of body composition for New Zealand’s burgeoning multi-ethnic young (Ministry of Health, 2006a).

1.2.2 Obesity and the FTO gene

Variants within introns of the FTO gene have been found to be associated with an increased risk of obesity and diabetes in genome-wide association studies (Dina et al., 2007, Frayling et al., 2007, Scuteri et al., 2007). While the molecular underlying mechanisms associating these noncoding variants directly with obesity are still obscure, several studies using mice models have established that the expression levels of FTO have an influencing effect on body mass and composition phenotypes (Church et al., 2010, Fischer et al., 2009, Gao et al., 2010), and a potential role of FTO in energy homeostasis (Gerken et al., 2007). Despite the function of FTO in humans being poorly understood, it has been established that the obesity-associated non-coding genetic variants within the FTO gene are functionally linked at megabase distances with
the neighbouring IRX3 gene (Smemo et al., 2014). The IRX3 gene is a functional long-range target of obesity-associated variants within FTO, and obesity associated SNP’s have more robust associations with expression of IRX3 rather than FTO (Smemo et al., 2014).

While many studies have alluded to the associations between the variations in the FTO gene and the obesity phenotype, the majority have only been on European populations or European subjects (Andreasen et al., 2008, Dina et al., 2007, Field et al., 2007, Frayling et al., 2007, Wahlen et al., 2008). Conflicting association results were observed in studies on Asian populations (Li et al., 2008, Omori et al., 2008). To the candidate’s understanding, there have only been two reports that have investigated the association of FTO with BMI in Pacific populations. The first was carried out by Ohashi and associates in 2007 who found no association of BMI with three of their FTO SNPS of interest: rs1421085, rs17817449 and rs9939609. These findings were based on 516 Oceanic subjects including 116 Tongans. The second study was carried out by Karnes et al., (2012) who found no significant associations between BMI and 51 FTO genetic variants in their sample of 1089 Samoans (465 American Samoans and 624 Samoans) (Karns et al., 2012).

For the purposes of this study, two variants from the FTO gene were selected (rs9922047 and rs9923233) to investigate for an association with BMI in this particular cohort of Pacific adolescents. The C allele of the non-coding intronic variant within FTO, rs9922047, has been found to be associated with increased BMI in a Sorbian population residing in Germany (Tiwari et al., 2011, Tonjes et al., 2010). Furthermore, the rs9923233 variant is in linkage disequilibrium (LD) with rs9939609 – a genetic variant also located in the first intron of the FTO gene - was also found to be associated with an increased risk of obesity (Dina et al., 2007, Frayling et al., 2007). As variations within the FTO have been found to be associated with the obesity phenotype in several European populations, this is a first attempt to investigate these two FTO variants for an association with BMI in Pacific adolescents, with the hope to further illuminate the relationship between variants of FTO with obesity and BMI in a Pacific population.
1.3 HbA1c – A measure of diabetes

Diabetes is a major risk factor for CVD (Alberti and Zimmet, 1998). Glucose homeostasis and metabolism is regulated by insulin, an endocrine hormone produced by the pancreatic beta cells in the Islet of Langerhans. Insulin signals a cascade of physiological responses to transport glucose to the liver for storage as glycogen (Beckman et al., 2002). Impaired insulin synthesis or sensitivity is associated with elevated blood glucose levels and development of diabetes (Creager et al., 2003) Diabetes is classified into two major types, Type 1 and Type 2.

Type 2 diabetes (T2D) is the most prevalent form of diabetes (World Health Organization, 1980). It is a global epidemic with increasing rates of diabetes-related complications and mortality among indigenous populations (Bramley et al., 2005). T2D is typically a heterogeneous adult-onset disease (Gentles et al., 2006) associated with obesity, unhealthy lifestyle and having a genetic predisposition. In addition to the burgeoning prevalence among adults, the rising levels of T2D among children and young adults are a major public health concern (van Dieren et al., 2010). T2D is characterized by insulin resistance post-receptor, with increased hepatic glucose production and decreased insulin-mediated glucose transport in skeletal muscle and adipose tissue (Weyer et al., 1999). In addition an element of beta-cell dysfunction is observed, with loss of early phase insulin release in response to hyperglycemic stimuli (Alberti and Zimmet, 1998). Common risk factors associated with T2D are high BMI, hypertension, dyslipidaemia and albuminuria. The pathophysiological response caused by T2D includes the hyperglycemic-activated production of reactive oxygen species, free radical production by oxidation of fatty acids, inhibited nitric oxide production and increased synthesis of vasoconstrictive molecules such as ET-1, giving rise to increased atherosclerotic burden and ultimately CVD development (Creager et al., 2003, Luscher et al., 2003).

The prevalence of T2D is on the rise worldwide primarily due to the vicissitudes in lifestyle. The improvements in the standards of living, together with economic wealth are aspects for the increased burden of diabetes. This is predominantly seen in developing countries, where urbanization and adoption of a high caloric western diet are leading to the increased incidence of diabetes and obesity (Day, 2001). Early investigations have revealed that while T2D was almost absent in populations indigenous to the Pacific upholding traditional lifestyles, the contrary was true for the more urbanized Pacific populations (Prior et al., 1987). The prevalence of T2D among the people of Polynesia, Melanesia and Micronesia has grown dramatically over the
years: this is said for people still living in the Pacific and those who have migrated to New Zealand (McCarty and Zimmet, 2001). T2D is one of the crucial health problems in New Zealand. According to the Ministry of Health’s Virtual Diabetes Registry, 241,381 adults aged 15 years and older had diabetes as at the end of 2013. This report also revealed that the higher rates of diagnosed T2D were seen in Indian and Pacific adults. More recently, compared to the general population, 9% of the NZ Pacific adult population has been diagnosed with diabetes. When adjusted for age and sex differences, the rates of Pacific diabetes are said to be at least 2.8 times compared to non-Pacific counterparts (Ministry of Health, 2014). The average onset of T2D at young age is higher in Māori and Pacific people as opposed to Europeans (Josh et al., 2006). The prevalence of T2D increases with age, with an expected increase of 50% in prevalence amongst Māori and Pacific people in the next 20 years, compared to the 20% rise in Europeans. Simmons et al., (1996) reported that T2D is more common in Māori and Pacific men, than it is in women. Although, the diagnosis in women, although the diagnosis is more common in pregnant women due to gestational diabetes (Simmons et al., 1996).

The measure of diabetes used in this study is HbA1c, which refers to glycated haemoglobin (A1c), which identifies average plasma glucose concentrations. The recognition of haemoglobin A1c was first brought to light in the 1960s as glycated haemoglobin that is predominant in individuals with diabetes (Rahbar, 1968). Later, in the 1970s, HbA1c was suggested as an indicator of both glucose regulation and glucose tolerance in diabetes (Koenig et al., 1976). In 2009, the American Diabetes Association, together with the International Diabetes Federation, and the European Association for the study of Diabetes made the recommendation that HbA1c should be used for the diagnosis of diabetes (International Expert Committee, 2009). The simplistic concept of HbA1c is that erythrocyte life span is constant. Erythrocytes are freely permeable to glucose, haemoglobin glycation occurs nonenzymatically at a rate directly proportional to the ambient glucose concentration, and so HbA1c provides a glycemic history of the previous 120 days (Herman and Cohen, 2012). The New Zealand Society for the Study of Diabetics (NZSSD) provided the standards employed by this study (Figure 1.2).
Gout is caused by an inflammatory response to the build-up of monosodium urate crystals associated with elevated levels of serum urate, hyperuricaemia. It is a very common form of arthritis that may be increasing in prevalence due to changes in lifestyle, diet and environmental factors (Doherty, 2009). Urate is routinely produced in the body as a result of endogenous and dietetic purine metabolism (Doherty 2009, Richet and Bardin 2010). The production of urate depends on the balance between purine ingestion, de novo synthesis in cells, recycling and degradation by xanthine oxidase and rate of excretion (Doherty, 2009). It is synthesized via the purine metabolism pathway by the catalytic action of the enzyme xanthine oxidase. In most mammals, urate is catalysed further to allantonin by the enzyme uricase (also referred to as urate oxidase), thus maintaining low levels of serum urate (Doherty, 2009).

The underlying biochemical cause of gout is elevated levels of serum urate, known as hyperuricaemia (Perez-Ruiz, 2009). Several studies also supported that the ultimate predictor of gout risk and development is the concentration of urate in the serum (Richette and Bardin, 2010). Normally, urate levels fall into the range of 2.4-6.0mg/dL in women and 3.4-7.0mg/dL in men (Dincer et al., 2002). Clinically significant hyperuricaemia is diagnosed when serum urate levels surpass 7mg/dL and as a consequence forms needle shape crystals that deposit around the joint. When urate concentrations exceed the saturation point, the risk of monosodium urate crystal formation increases considerably (Richette and Bardin, 2010). According to Doherty (2009), only a minority (10%) of individuals with hyperuricaemia ever develop gout, highlighting the importance of other perilous factors involved in the formation of crystals (Doherty, 2009).
Epidemiology has played a crucial role in improving our understanding of the pathogenesis of gout. The distribution and determinants of gout in human populations are based on two important suppositions; 1) gout does not occur at random and 2) gout has causal and preventive factors that can be established through systemic studies of ethnically and geographically varied populations. Therefore, epidemiological studies of gout attempt to explain the manner in which the disease appears in a population (prevalence, incidence, co-morbidity, geographic distributions and clinical characterisations) and the quantification of assumed risk factors that influence the incidence of gout (Gabriel and Michaud, 2009).

The heritability of gout differs greatly between nations and ethnic groups. Doherty (2009) established that gout is most common in men and has a prevalence that has amplified dramatically over the years. All populations have a gender bias in gout, where men are affected 4 – 5 times more than premenopausal women (Merriman and Dalbeth, 2011). European men residing in developed countries have prevalence rate of 2% on average, a comparable figure in all studies carried out on Europeans thus far (Choi and Curhan, 2005, Klemp et al., 1997). Men and women at an elderly age are equally probable to suffer from gout. Oestrogen, a female hormone, increases renal uric acid excretion by effects of oestrogenic compounds, which are likely to reduce the number of active renal urate transporters, and as a result lowering renal tubular uric acid reabsorption and thereby increasing urate clearance (Terkeltaub, 2010).

It has been documented that severe and chronic gout are more frequent in some Polynesian populations. Modern Pacific island populations hold the highest frequencies of gout in the world (Gibson et al. 1984, Rose and Prior 1963, Silman and Hochberg 2001). This high prevalence of gout in Polynesian populations has therefore led to the proposal that “people of the Pacific belong to one large gout family” (Kellgren 1964, quoted in Prior 1981, pp. 214). Consistent with the global tendency of elevating levels of gout, Klemp et al. (1997) revealed that gout is more common in Māori and Pacific men in New Zealand with an estimated prevalence to range between 9.3-13.9% and 14.9% respectively. Furthermore, incidences of gout in European men within New Zealand are similar to other countries with a prevalence of 5.8% reflecting a significant difference between populations (Klemp et al., 1997). Gibson et al. (1984) proposed that the reason of high prevalence of gout in Polynesian populations is due to a genetic predisposition for hyperuricaemia. This predisposition may be intensified by several effects of urbanization but it is more likely to be due to a deficiency of urate excretion (Gibson et al., 1984).
Taiwanese aborigines share a common ancestry with Māori and Pacific Islanders with evidence indicating that the people of Polynesian partially originated from Taiwan (Jobling et al., 2004). Taiwan aborigines have one of the highest prevalence rates of gout (11.7%) (Chou and Lai, 1998).

Monogenic disorders that result in overproduction of urate via enzyme defects in purine metabolism are very rare. However, common primary gout in men often reveal a strong ancestral predisposition, although the genetic basis remains undetermined (Doherty, 2009). Twin studies have demonstrated high heritability for both uric acid renal clearance (60%) and uric acid: creatinine ratio (87%), and several susceptibility loci for this have been reported (Roddy et al., 2007). Hyperuricaemia and primary gout are a result of inadequate renal uric acid excretion, excessive urate production or the amalgamation of the two. Renal under – excretion is confirmed to be a primary and the most important determining factor of gout, particularly in Māori and Pacific populations.

1.4.1 Genetics of Gout; SLC2A9 – Urate Transporter

The leading mechanism of validating hyperuricaemia is by the regulation of urate renal excretion, but also involving the gut (Sorensen and Levinson, 1975). The regulation of serum urate is influenced by both genetic and environmental factors (Merriman and Dalbeth, 2011). The SLC2A9 gene has been highlighted to be the chief genetic regulator. It encodes the GLUT9 renal molecule, which passages urate facilitated by sugar molecules – glucose and fructose (Caulfield et al., 2008, Witkowska et al., 2012). Studies have confirmed that 3.70% of the phenotypic variance in serum urate levels of those with European descent is explained by genetic variants within SLC2A9. Furthermore, variants of the SLC2A9 gene have been documented to have a robust correlation with gout in European, New Zealand Māori, and New Zealand Pacific Island sample sets (Hollis-Moffatt et al., 2009, Kottgen et al., 2013). However, very little is known about the association of this locus with serum urate and gout in youth, certainly not adolescents of Pacific Island descent.

The SLC2A9 gene is an important locus due to its involvement in urate re-absorption (Caulfield et al., 2008, Doring et al., 2008, Vitart et al., 2008) and can explain 3.5% variation in serum urate concentration among Europeans (Kottgen et al., 2013). With its extremely large effect on serum
urate, the risk allele of this locus increases the gout risk by greater than 50% in Europeans (Hollis-Moffatt et al., 2009, Kottgen et al., 2013).

Batt and associates produced evidence for non-additive interaction between the genotypes of SLC2A9 and the consumption of sugar-sweetened beverages (SSB) and a role in gout development in three populations; Māori, Pacific and Europeans. Their initial observations revealed the increase in gout risk by SSB consumption which corroborated with other studies who revealed positive associations between high consumption of fructose-sweetened beverages and the increased risk of gout incidence in both males and females (Choi and Curhan, 2008, Choi et al., 2010, Choi et al., 2008). Interaction analyses demonstrated a significant interaction term in the collective ($P_{\text{interaction}} = 0.01$), but not when the data were meta-analyzed ($P_{\text{interaction}} = 0.99$). Furthermore, they also found a trend towards significant evidence for interaction between SLC2A9 genotype (rs6449173) and SSB consumption in determining serum urate levels ($P_{\text{interaction}} = 0.062$). Overall, epidemiological evidence of non-additive interaction between sugary sweetened beverages (SSB) consumption and a urate-associated variant of $SLC2A9$, generates a hypothesis that physiologically SSB derived fructose can influence the $SLC2A9$-mediated excretion of urate by interfering with the ability of SLC2A9 to transport uric acid (Batt et al., 2014).

Two genetic variants (rs11942223 and rs3775948) from this gene are being investigated in this study for direct evidence of association with serum urate. The T allele of the intronic non-coding variant rs11942223 was shown to be associated with elevated levels of serum urate and gout in Caucasian populations (Yang et al., 2010). Furthermore, Hollis-Moffatt and associates (2009) tested four variants of the SLC2A9 gene, one of which was rs11942223, for an association in Maori, Pacific and Caucasian cohorts. Findings showed that the C allele was protective against gout. The association of the rs3775948 non-coding variant has previously been reported to correlate with serum urate levels in East Asians (Okada et al., 2012).
Section 2

1.5 Lifestyle Factors

1.5.1 Physical activity

1.5.1.1 Physical activity, Obesity and Diabetes

Physical activity is defined as any movement of skeletal muscle associated with energy expenditure (Shephard and Balady, 1999, Thompson et al., 2003). Physical inactivity and obesity are known independent risk factors for T2D (Almdal et al., 2008). The excessive free fatty acid released by adipose tissue results in a decrease in insulin sensitivity of muscle, fat and liver, and subsequently raising blood glucose levels, insulin resistance and causing T2D (Bjorntorp and Rosmond, 1999, Boden, 1997, Lewis et al., 2002). The lack of physical activity accelerates the pathogenesis of T2D and consequently leading to morbidity and mortality (LaMonte et al., 2005).

Studies have proposed an interaction between obesity and physical activity; where the diabetogenic influence of obesity is counteracted by physical activity, by decreasing fat mass, increasing fat oxidative capability, or through supplementary biological pathways (Church et al., 2005, Sui et al., 2008). However, the true pathophysiological mechanisms are not fully understood. From public health and clinical viewpoints, the understanding of the interaction between obesity and physical activity is of importance to allow for identification of target groups and thus, implement primary prevention approaches for T2D (Qin et al., 2010).

1.5.1.2 Physical activity and Cardiovascular risk

Physical inactivity is also a known risk factor for cardiovascular disease (Ignarro et al., 2007, Thompson et al., 2003) that is associated with adverse levels of other cardiovascular and metabolic risk factors. Investigations have demonstrated evidence on the benefits of regular physical activity on cardiovascular risk factors and decreased cardiovascular mortality (Haskell et al., 2007, Lee et al., 2003, Paffenbarger et al., 1986, Powell et al., 1987, Thompson et al.,
The mechanisms underlying the pathophysiological response to decreased cardiovascular mortality through exercise remain obscure, but are thought to be primarily related to the changes in body mass, as well as improvements to heart function i.e. ejection fraction are also responsible for the decreased cardiovascular mortality with exercise (Ignarro et al., 2007).

Recommendations of physical activity required for health promotion and disease prevention are defined as approximately 30 minutes or more, five or more days a week of vigorous exercise (Haskell et al., 2007, Pate et al., 1995). The decline of physical activity with age is of concern (Haskell et al., 2007). A study in America showed that younger age groups were more physically active when compared to the older age groups. In New Zealand, the levels of physical activity recommended were reported by at least 50% in those aged between 16 and 65 years, where the most physically active individuals (54%) were in the 35 – 49 age bracket. The older age group (65 years and over) had considerably lower rates of physical activity (24%) (Sport and Recreation New Zealand, 2008).

### 1.5.2 Diet - Sugar

As aforementioned, the last three decades have seen a worldwide increase in the prevalence of non-communicable diseases such as obesity, T2D and the metabolic syndrome (Ruxton et al., 2010). The speed of this rise in disease prevalence suggests that environmental factors may contribute to this problem (Johnson et al., 2009). Corroborating with this notion is the significant change in the patterns and consumption of food and beverages that have taken place (World Health Organization, 2003a).

Diet, as a risk factor, comes under examination for its association with disease. As certain diseases, for example CVD, have the ability to be reversed or managed through modifiable changes of which include changes in diet. Over the past century, an increase in the consumption of sugar has been observed; lifting concern that sugar may play a somewhat partial influence for the increases in the prevalence of non-communicable diseases (Johnson et al., 2009, World Health Organization, 2003b).

The term “sugar” is very ubiquitous, thus making it problematic to compare literature. For the purposes of this review, sugar has been defined as monosaccharide’s such as glucose, added sugar such as sucrose (disaccharides) as well as sugar that is naturally present in fruit (Mann and Truswell, 2012)
Experimental and prospective cohort studies have produced inconsistent records of the effect of sugar on risk factors for metabolic diseases (Choi and Curhan, 2008, Sonestedt et al., 2012, Wang et al., 2013). However, international observational studies have provided evidence to support the idea that excess sugar intake results in high intake of dietary sugar fructose, and may be an important contributor to these diseases (de Koning et al., 2012, Johnson et al., 2009).

1.5.2.1 Sugar intake and body weight (obesity)

While numerous factors are known to contribute to weight gain, the most rudimentary of all is that weight gain strikes when energy intake surpasses energy expenditure (Mann and Truswell, 2012, van Dam and Seidell, 2007). Sugar sweetened beverages and foods tend to be energy-dense, tasty and very appetizing, thus easily resulting in excess consumption that contributes to positive energy balance (van Dam and Seidell, 2007). Furthermore, these sorts of foods provide very minimal dietary fibre and macronutrients, consequently providing little in the way of nutritional value, contributing merely ‘empty calories’ (Du and Feskens, 2010, Ruxton et al., 2010, van Dam and Seidell, 2007). Fructose, unlike glucose, has been specifically implicated in weight gain due to reducing satiety (Teff et al., 2004). Blood glucose levels would normally increase after a meal containing glucose is ingested, releasing insulin and the satiety hormone leptin. Conversely, fructose ingestion has been shown to decrease postprandial blood glucose levels causing a decline in serum insulin and leptin, thus attenuating the feeling of ‘fullness’ after a meal. A high fructose meal could therefore lead to over consumption more so than other sugars and the positive energy balance contribute to weight gain.

Literature surrounding the influence of sugar on metabolic conditions repeatedly focuses on SSB intake due to the fact that they are widely consumed, the intake amounts are quite often easily reported and the availability and consumption of SSB are highly unchanged by seasonality. Malik and associates carried out a systematic review and meta-analysis to assemble the claims of association between sugar-sweetened beverages and body weight in both children and adults (Malik et al., 2013). Findings from this study were comparable with, and supported the findings produced by Te Morenga et al., (2013) on the positive association between sugar consumption and body weight (Te Morenga et al., 2013).

A surplus of sugar intake that exceeds the requirements of daily energy is reserved as fat in the body. Although the mechanism is obscure, fructose (as opposed to glucose) has been reported to
stimulate de novo lipogenesis (Samuel, 2011). Interestingly, one intervention study in Denmark showed that the consumption of sugar might have an impact on the storage sites of fat in the body (Maersk et al., 2012). The findings from this study showed that over a period of six months, daily consumption of sugar sweetened beverages caused a significant increase in the accumulation of fat in the liver and high visceral adipose tissue, compared to the intake of water and artificially sweetened soft drinks.

### 1.5.2.2 Sugar and diabetes

With the growth in worldwide prevalence of diabetes, the role of sugars, particularly fructose is of certain attention.

Literature has identified an association of excess sugar intake with weight gain, an established risk factor for diabetes (Mokdad et al., 2003). Furthermore, a link has also been reported between the risk of diabetes and total sugar intake that impairs beta cell function in the pancreas and acute insulin responses (Davis et al., 2005b). The failure of beta cell function supposedly occurs across long-term high demand for the secretion of insulin to deal with the continuous exposure to high blood glucose levels (Ludwig, 2002).

To investigate the effect of particular sugars on the risk of diabetes, The Finnish Mobile Clinic Health Examination Survey investigated the effects of glucose, fructose, maltose, lactose, sucrose and total sugars intakes in a prospective cohort study conducted in Finnish men and women with 12 years of follow-up (Montonen et al., 2007). The only significant relationship that emerged with the risk of T2D was with glucose and fructose. In quartile analyses, the highest quartile had a 57% increased risk of developing T2D, when compared to the lowest quartile. It is noteworthy that glucose and fructose intakes were combined to form these quartiles. Furthermore, with the association established in this study, it is also important that their results are interpreted with caution. This is mainly due to the seeming limitation that diet history was dependent on memory recollection: participants were required to recall their dietary intake over the last year and may well produce poor reproducibility of sugar data.

Apart from increasing the risk of developing diabetes, sugar has also been reported to have an influence of the lifespan of patients already diagnosed with diabetes. For example, in a study of patients with diabetes \( n = 6192 \), increased mortality risk in normal weight subjects was significantly associated with high sugar consumption (Burger et al., 2012).
1.5.2.3 Sugar and serum urate (gout)

Over the past few decades, dietary habits have intensely changed. Dietary purine consumption is a significant contributor to serum urate concentrations. The consumption of purine-rich foods is habitually sufficient to trigger a gout attack in hyperuricaemic individuals. This makes diet a major risk factor. Diets rich in purines include: red meat, seafood and alcohol have been positively associated with increased serum urate levels (Choi et al., 2010, Saag and Choi, 2006). The international increase in prevalence of gout has also been correlated with greater consumption of fructose, more specifically, sugary drink intake (Hak and Choi, 2008).

A link between fructose and hyperuricaemia has been observed in cross-sectional studies conducted on nationally representative samples in the United States (US) (Choi et al., 2008, Gao et al., 2007). Choi et al. (2008) reported a significant association between serum urate levels and both SSB (p<0.001) and orange juice (p=0.009) consumption. The association tended to be stronger in men than women. In another study, Gao et al. (2007) found no association between SSB and serum urate in women (Choi et al., 2008, Gao et al., 2007).

As excessive fructose consumption can induce hyperuricaemia (Choi et al., 2008, Nakagawa et al., 2005), it may be an important risk factor for gout. Choi et al. have examined the relationship between consumption of fructose-containing beverages and foods and risk of incident gout in two large prospective cohort studies (Choi and Curhan, 2008, Choi et al., 2010). In both studies daily consumption of SSB was associated with increased risk of gout amongst the predominantly white, middle-aged cohorts (with the more recent study by Batt et al., (2014) extending these findings to Polynesians). Participants consuming SSB once daily had an increased risk of gout of 74% (95% C.I. 19% to 155%) in women and 45% (95% C.I. 2% to 108%) in men in comparison with those consuming less than one serving per month. Additionally, intakes of orange or apple juice and whole oranges or apples were also significantly associated with risk of incident gout over 12- and 22-year follow up periods.

1.5.2.4 Sugar and CVD-risk

Research into CVD has focused on sugar and in particular fructose because of its association with the risk factors of elevated blood pressure and dyslipidaemia (Brown et al., 2008, Brown et al., 2011, de Koning et al., 2012, Duffey et al., 2010, Jalal et al., 2010, Stanhope et al., 2011).
Fructose available in sugary drinks was evaluated in a prospective study. Results showed that participants in the highest quartile of sugar had an increased risk of 18% (p <0.01) for coronary heart disease when compared to the lowest quartile of sugar consumers (de Koning et al., 2012).

Nakawaga et al., (2006) revealed that high blood pressure might be a result of the inhibition of (the vasodilator) nitric oxide, a consequence of fructose-induced hyperuricaemia. To support this notion, several cross sectional studies have provided evidence of this correlation. For example, a study in the UK established that the consumption of one sugary drink per day was correlated with a significant increase of 1.1 mm Hg in blood pressure (Brown et al., 2011). Furthermore, another cross-sectional study in the United States analysed the National Health and Nutrition Examination Survey (NHANES) data from 2003 – 2006. Their findings demonstrated an association of increased risk of high blood pressure with daily fructose intake of ≥ 74 g per day (Jalal et al., 2010).

Dietary intervention trials have established a link between blood pressure and fructose intake (Brown et al., 2008, Maersk et al., 2012), with conflicting results in another study (Stanhope et al., 2011). Maersk et al (2012) investigated the effects of sugar-sweetened drinks, isocaloric milk, artificially sweetened soft drink and water on cardiometabolic outcomes, including blood pressure. Findings from this study showed that at baseline, sugar-sweetened soft drinks had no significant effect on increasing blood pressure. However, consumption of isocaloric milk and artificially sweetened soft drink significantly decreased systolic blood pressure by 10-15% compared to consumption of SSB (p<0.05). Diastolic blood pressure also decreased, but this was not statistically significant.

Fructose ingestion is additionally thought to increase the rate of hepatic de novo lipogenesis and simultaneously decrease the activity of heparin lipoprotein lipase resulting in both increased hepatic fat stores and increased circulating plasma VLDL and triglycerides (Stanhope and Havel, 2010). Stanhope et al., (2011) investigated the effects of consuming glucose, fructose and high fructose corn syrup (HFCS) at 25% of total energy requirements on risk factors for cardiovascular disease. After two weeks of dietary intervention, the consumption of fructose and HFCS beverages significantly increased 24- hour triglyceride under the curve, concentrations of fasting LDL-C, non-HDL-C, apolipoprotein B (apo-B), and apo-B to apolipoprotein-A (apo-A) ratio, compared to baseline. Conversely, glucose had no significant effect on these outcomes, suggesting the mechanism in which fructose is metabolised differentially affects these risk factors. A recent randomised controlled trial in healthy, non-diabetic subjects further supports
these associations after a six-month intervention period (Maersk et al., 2012). Compared to baseline, their soft-drink intervention group developed an 11% increase in total cholesterol and 32% increase in triglycerides after consumption of one litre daily SSB (50% glucose to 50% fructose). In comparison to the consumption of isocaloric milk, artificially sweetened soft drink and water, this was a significant increase (p <0.01). The longer intervention period and more realistic modification of sugar intakes through SSB intake were strengths over the study conducted by Stanhope et al., (2011).

### 1.5.3 Alcohol consumption

#### 1.5.3.1 Alcohol intake and body weight (obesity)

Obesity is a multifactorial and complex condition that develops from genotype and environmental interactions (Marti et al., 2004). While numerous investigations have delved into examining the relationship between alcohol and body weight, most have been opportunistic, such that the main objective was an issue aside from the actual influence of alcohol on body weight. In modern times, there has been a lot of regard on the short- and long-term consequences associated with alcohol consumption among adolescents (Boden and Fergusson, 2011).

Research shows that alcohol consumption may contribute to obesity in subjects who consume alcohol by serving as an energy source, and by increasing appetite and food consumption (Breslow and Smothers, 2005). The onset of obesity is caused when energy intake consistently exceeds energy expenditure, and as a result leading to a fundamental chronic imbalance (Manson et al., 2004). The investigation of the relationship between alcohol consumption and obesity is vital, and alcohol is a multifaceted and exclusive constituent of the human diet (Sayon-Orea et al., 2011). Alcohol comes second to that of fat as a potential source of energy, as it provides 29 kJ per gram. Furthermore, alcohol has pharmacological effects on the nervous system where it activates the γ-aminobutyric acid receptors but with effects on several other neuro-chemical systems as well. Additionally, alcohol cannot be stored by the body; therefore the oxidation of alcohol is prioritized over fats and carbohydrates (Sayon-Orea et al., 2011, Yeomans, 2004, Yeomans et al., 2003).

The investigations of links between obesity and alcohol consumption in young people, has been driven by the datum of significant increase of adolescent obesity in Western civilizations, in
contemporary times (Hedley et al., 2004), an epoch by which a parallel increase in binge drinking has also been observed in adolescents (Farke and Anderson, 2007). Furthermore, longitudinal studies have also demonstrated the association of high alcohol consumption with the increased risk of overweight and obesity in adults. For example, Rissanen et al (1991) in their study of 12,699 Finnish adults found a positive correlation between the prevalence of obesity and alcohol consumption in males and partly in females (Rissanen et al., 1991).

Adolescents who indicate high levels of alcohol consumption are at greater risk of being overweight and obese (Croezen et al., 2009, Fonseca et al., 2009, Must et al., 2008), although there are gender differences – with regard to the extent by which alcohol intake is associated with increased body weight – with males being at greater risk (Barry and Petry, 2009). Furthermore, the association between alcohol intake and obesity may be long-term. This is cemented by a study by Oesterle et al., (2004) who reported that adolescents with high rates of alcohol intake, including binge drinking were at higher risk of obesity, and may lead to associated health complications in early adulthood (Oesterle et al., 2004). To the contrary, other studies have documented the opposite direction of association. Duncan and associates (2009) in their study on American women reflected that obesity was associated with a decreased possibility of ever drinking (Duncan et al., 2009). However, this could be due to the fact that obese adolescents may have fewer friendships and participate less in social gatherings (Boden and Fergusson, 2011).

### 1.5.3.2 Alcohol intake and diabetes

Recent meta-analyses studies suggested a protective effect of alcohol consumption, proposing a relation of moderate drinking (no more 5 – 30 g of alcohol per day) with a lower risk of T2D, with high risk associated with high alcohol consumption (more than 30 g of alcohol per day) (Baliunas et al., 2009, Carlsson et al., 2005, Koppes et al., 2005).

Koppes and associates (2005) conducted the first meta-analysis on the relationship between alcohol consumption and the risk of T2D. Their results indicated a U-shaped relationship with a significant approximate of 30% reduction in risk of T2D in alcohol consumers of 6 – 48 grams per day. These findings were consistent also with findings from a systematic review and meta-analysis that also confirmed moderate alcohol consumption is protective of T2D in both men and women (Baliunas et al., 2009). A more recent epidemiological study on Swedish subjects (2070
males and 3058 females) demonstrated total alcohol consumption and binge drinking increased the risk of pre-diabetes and T2D in men (OR 1.42, 95% CI 1.00 – 2.03 and OR 1.67, 95% CI 1.11 – 2.50, respectively). The authors also found an association between low alcohol consumption and a decreased diabetes risk in women (OR 0.41, 95% CI 0.22 – 0.79). In conclusion, the results from this study established high alcohol intake increases the risk of abnormal glucose regulation in men with more complex associations in women; decreased risk of diabetes with low or medium alcohol consumption, and increased risk with high alcohol consumption.

The postulated diabetogenic influence of alcohol consumption include its input to excessive caloric intake and obesity, inducing pancreatitis, the disturbance of glucose and carbohydrate metabolism, and also impairment of liver function (Avogaro and Tiengo, 1993, Manolio et al., 1990, Perry et al., 1998).

1.5.3.3 Alcohol intake and serum urate

Along with inherited genetic variants, hyperuricaemia (HU) and gout are significantly influenced by dietary factors that include purine-rich foods, seafood, sugary drinks and alcohol consumption (Batt et al., 2014, Choi et al., 2004, Choi and Curhan, 2004, Choi and Curhan, 2008). The correlation between alcohol consumption and increased risk of gout is long established. Over many years ago, Garrod believed the most powerful predisposing causes of gout to be fermented liquors. He declared that “The use of fermented liquors is the most powerful of all the predisposing causes of gout; nay, so powerful, that it may be a question whether gout would ever have been known to mankind had such beverages not been indulged in” (Garrod, 1876). Later on in the 1980’s, several investigations provided evidence that alcohol intake may induce hyperuricaemia (Drum et al., 1981, Faller and Fox, 1982, Puig and Fox, 1984), and by extension, increase the risk of developing gout (Abbott et al., 1988, Choi et al., 2004, Sharpe, 1984).

Alcohol metabolism - ethanol and its intermediate metabolite acetate (formed during ethanol oxidation) - can stimulate the production of urate as a result of ATP degradation to AMP, a precursor for serum urate (Puig and Fox, 1984). In the 1960’s an association between lactate in the blood and reduced urinary urate excretion was established (Lieber et al., 1962). Thus alcohol consumption may elevate serum urate levels by either decreased renal excretion of serum uric acid, or the reverse, overproduction of serum urate as a result of enhanced turnover of adenine dinucleotide (Faller and Fox, 1982). Additionally, alcohol consumption can increase the
depletion of ATP to ADP via the conversion of acetate to acetyl CoA in the metabolism of ethanol (Faller and Fox, 1982, Puig and Fox, 1984), thus the overproduction of serum urate.

1.5.3.4 Alcohol intake CVD-risk

Research shows that alcohol intake is analogous to the proverbial double-edged sword, and possibly no other factor in cardiovascular health is proficient enough in cutting so deeply in whichever direction it is used (O'Keefe et al., 2007). The influences of ethanol on people’s health depend on the amounts of alcohol consumed, and the patterns of drinking. The graphical relationship of alcohol intake and mortality shows a J or U-shaped curve; where a significant lower risk of cardiovascular mortality is observed in moderate drinkers, when compared to abstainers, light and heavy drinkers; thus indicative of a protective effect of moderate drinking on cardiovascular health (O'Keefe et al., 2007). O’Keefe and colleagues in their study found that moderate alcohol consumption is associated with cardio protective responses such as increased insulin sensitivity, raised levels of high lipoprotein cholesterol (HDL-C), and decreased levels of inflammatory markers such as the C-reactive protein, tumor necrosis factor alpha, fibrinogen and interleukin-6. To the contrary, the patterns of drinking have also been established to mediate risk of cardiovascular disease; predominantly, heavy and irregular consumption is linked with detrimental effects on cardiovascular outcomes (Rehm et al., 2003). While a protective effect of moderate alcohol consumption on cardiovascular health has been reported, abstainers are not encouraged to begin consuming alcohol (NZGG, 2003).

The erraticism of the definitions of light, moderate and/or heavy drinkers was highlighted in a review, contingent on the methodology and country the study commenced (Kloner and Rezkalla, 2007). For example, the equivalent of one standard drink in New Zealand is 10 g of alcohol (Seddon, 1999), while in America; one standard drink is 14 g of alcohol (Kloner and Rezkalla, 2007). Thus interpretation of international reports on the influence of alcohol intake on risk factor ought to be carefully considered. Another issue is the method by which alcohol consumption data is collected. The main methodology involved is the reliance on participants to self-report alcohol intake. Thus there is always the issue of under reporting, or over reporting, thus a limitation.
The health of Pacific teenagers in Dunedin

The University of Otago in collaboration with the Pacific Trust Otago (PTO) had previously carried out a study in Dunedin to investigate the health of Pacific teenagers. The previous study carried out in 2006, and published by Grant and colleagues (2008) was conducted on 80 teenagers between the ages of 15 and 18 residing in Dunedin. The prime aim of the study was to establish associations amid body composition, glucose and lipid metabolism, and components of the metabolic syndrome (MS) – a cluster of risk factors independently associated with type 2 diabetes and cardiovascular disease - in a community sample of individuals that identify with different Pacific Island ethnic groups. Findings from this study revealed 6 participants to have MS, 2 with high fasting blood glucose values (>7.0 mmol/L), 55 with high adiposity, and 21 with insulin resistance (Grant et al., 2008). Several components of MS were evaluated by body mass index (BMI) status, showing that the 29 participants who were obese had a high prevalence (86.2% had one or more component), while 10.5% of those with a healthy BMI status (n=19) had at least one MS component. Derived from these results is the proposal that community-based studies of Pacific Island adolescents ought to focus on evaluating risk factors whenever BMI values are of high range.

The study by Grant and colleagues is the basis of this present study with the following aims:
1.7 Aims of this thesis

The principle aim of this thesis was to explore and document the current health status of a Pacific Island cohort, with the main focus being:

1. Document the health status of Dunedin Pacific teenagers (comparison with Grant et al., 2008).
2. Evaluate the risk factors associated with obesity, type 2 diabetes, cardiovascular disease and gout in this particular cohort.
   a. Risk factors include:
      i. High BMI
      ii. Lipid concentrations
      iii. Physical activity
      iv. Alcohol consumption
      v. Sugary drink consumption
3. Establish associations between genetic variants of the SLC2A9 and FTO genes with selected variables.
   a. SLC2A9 (rs11942223 and rs3775948)
      i. Investigate for an association with serum urate
      ii. Test for possible gene-environment interaction with sugary drink consumption
   b. FTO (rs9922047 and rs9923233)
      i. Investigate for an association with BMI
      ii. Test for possible gene-environment interaction with physical activity
CHAPTER 2: METHODOLOGY
1.8 Pacific Research Framework

The University of Otago’s Pacific Peoples Reference Group (PPRG) was established in 2006 to advise the University on how to accomplish its goals and aims for Pacific people. PPRG devised a document that outlined proposed etiquettes regarding research that involved Pacific people in the Otago region, New Zealand and its entirety, and more generally, in the Islands of Oceania. The established protocol falls within the broad domain of the University of Otago’s Human Ethics Committee and other fundamental strategic documents.

The following protocol was offered by the University of Otago, Pacific Research Protocols to provide direction in facilitating research in different Pacific communities.

1. Maximizing benefits to humans

• The candidate made every effort to maximize the benefits of his project to the participants and avoid any harm done to them.

2. Relationships

• This was a very important value considered by the candidate. In every way, the candidate worked to develop, encourage and maintain meaningful relationships with the participants.

3. Respect

• “Respect is fundamental to all ethical relationships”. The candidate being of Pacific Island descent acknowledged the primacy of the group of participants and recognized that each individual is a valued member of the group. All participants were addressed and treated with the utmost respect from the beginning to the end of the project.

4. Cultural Competency

• As aforementioned, the participant was of Pacific Island descent himself and was cognizant of his own cultural beliefs, values, traditions, and understood that these may differ greatly from the participants values and principles, and this must be respected at all times.
• The candidate at all times supported the need to create a safe and culturally competent environment during this project.

5. Meaningful Engagement

• The protocol requires developing, maintaining and sustaining relationships that involve mutual trust to establish meaningful relationships between the researcher and participants.

• As a start, the candidate ensured face-to-face communication was effective whenever consultation was undertaken. The candidate undertook an approach that made the participants comfortable with the setting by which the study took place and most importantly, establishing “mutual trust” between the two parties. This approach included making conversation as to mutual interests the candidate and participants had.

6. Reciprocity

• The candidate and research team reciprocated with the participants and the wider Pacific communities in several ways. This included building the research knowledge of participants and educating both the participants and community on Pacific Health. The candidate attended Pacific community sports events and also other community activities as a way of extending gratitude for showing interest, supporting and participating our project. Furthermore, some research findings, particularly blood test results were returned to the participants. Numerous adults from the community regarded our tests as a “Free health blood test” for their children and were grateful. The final research findings will be presented to the community in aggregate form as a summary on the whole cohort. This will enlighten them as to the current health status of the Pacific young (and by extension their children) residing in Dunedin.

• Participants who volunteered were also offered a gift of thanks outlined later in this chapter.

7. Utility

• This project aims to address certain health issues on Pacific teenagers living in Dunedin. It will be an informative means to educate Pacific people on the current health and its status, and also generate knowledge that will help in updating health promotion strategies and health interventions.
8. Rights

- All participants were given the right and freedom to make an informed decision as to whether or not they wanted to participate or not. The candidate outlined all procedures that were to be undertaken for the purposes of this project and emphasized that they (the participants) could at any time withdraw from the project without any embedded disadvantage to them; their decision will be respected.

- The participants were well informed by the candidate, both by means of an information sheet and face-to-face consultation for clarity.

- Their rights to withdraw at any time of the research were reiterated.

- Confidentiality was outlined multiple times to ensure that the participants were comfortable to partake and entrust the candidate with their information.

9. Balance

- Balance is critical when working according to the ethical moralities of Pacific research. It applies to the mutuality of power, control and involvement.

- Research partnerships that were established for the purposes of this research were unbiased and fair for the organizations/parties involved, producing equilibrium in the balance of power.

- The research team and the organization involved were cognizant that this research will benefit the Pacific community and by extension contribute to the scientific literature particular to Pacific Health. The community was also informed of the benefits of this research project.

- Several meetings were held between the research team from the University of Otago and the organizations involved to ensure that the parties involved were aware of the progress of the study. This was a way for the research partnership to voice opinions and expectations as to how the project is progressing and whether there was a mutual understanding between the parties involved.

- These meetings were also a way for the candidate to update everyone involved on the status of the project, any issues raised by participants and also, generally to ensure the mutuality in power,
control and involvement in the research partnerships developed between the University and the organizations involved.

10. Protection

• Knowledge acquired first-hand from the participants were acknowledged as such.

• The research team ensured that the settings by which the project would take place were a safe environment that deterred any harm being imposed on the participants and all those involved in this study.

• The candidate was mindful of confidentiality when consulting with participants.

11. Capacity Building

• The Pacific Research Protocol supports the need for capacity building to refining the knowledge that would stem from Pacific-based research.
2.1 Parties involved and roles played in this study

The University of Otago and the Pacific Trust Otago have previously collaborated in addressing the issue of “Pacific Health”. This research was also a partnership between the two parties.

2.1.1 The Role of the Candidate

The candidate was the prime participant in designing the study methods. Such included arrangement and understanding of clinic protocols, and the organization of procedures and analyses with collaborating laboratories and parties. Further, the candidate was also a vital associate of the team at all clinics performing a range of roles that comprise assisting in recruitment processes, obtaining consent and interviewing, collecting blood from the associated laboratories – The Southern Community Labs, Dunedin – carrying out anthropometric measures, aiding in the answering of questionnaires provided to the participants and also assisted with data entry. While genomic DNA extractions were carried out in the Merriman Laboratory, Department of Biochemistry, University of Otago by courtesy of their technical staff, the candidate himself carried out SNP selection and genotyping assays.

2.1.2 The Pacific Trust Otago

“Health is defined holistically by Pacific people as a state of physical, social, mental, and spiritual well being”.

The Pacific Trust Otago is a disclosed charitable organization under the governance of a thirteen-member Board of Trustees representing people from the Cook Islands, Fiji, Kiribati, Niue, Tonga, Tuvalu, Samoa and the Melanesian countries. The Pacific Trusts vision stands as “Healthy Pacific Communities”. Derived from their vision are four principle aims or otherwise categorized as mission statements:

1). Create an environment that nurtures the development and well-being of all Pacific Island people in Otago.

2). Gather and analyze information about the health status of Pacific Island people living in Otago.
3). Deliver educational, support and diagnostic services that enhance the health and well being of Pacific Island communities.

4). Develop and maintain a professional, viable and innovative operative structure.

The Pacific Trust Otago organization works primarily with and for Pacific people to increase participation, responsibility and an advanced sense of belonging to the community. In order to achieve this, health promotion activities are executed to encourage regular physical activity and healthy nutrition. Community sessions, well child Tamariki Ora and community support are all fortified to satisfy both parties, that of the Pacific Trust Otago and Pacific people.

Figure 2.1: Figure showing the logo representing the Pacific Trust Otago.

2.2 Ethical Approval

For this study the University of Otago, Human Ethics committee (12/349) approved the study protocol. Participants that were invited to take part were to be of Pacific Island descent between the ages of 15 to 20 years, and resided in Dunedin. The participants were made cognizant of the aims and objectives of the current research, and how their information will be gathered, used and securely stored. They were also informed that all the information provided by them would be completely confidential and would be available only to the researcher and defined users. Moreover, they were made aware that each of them would get assigned a “Participant Code” and
their results will be de-identified and stored under these codes and not their names. All participants were made aware of their voluntary participation, and could at any time withdraw from the study without any disadvantage to them, and their samples discarded. All participants of this study gave their informed consent to donate blood and urine samples for the purposes of this study, and also to provide extra information by means of completing questionnaires.

2.3 Recruitment Strategies and Study Population

2.3.1 Selection and Criteria for recruitment

In order to qualify and be able to participate in the study, potential participants were to be between the ages 15 – 20 years and also identify with at least one Pacific Island ethnic group. Those who were unable to provide full informed consent were excluded from the study. No one was excluded from this study.

2.3.2 Method of Recruitment

Multiple approaches were utilized to recruit potential participants for this study. Such included information and invitation letters to several high schools in the Dunedin region, multiple churches, an approach to the wider community in the form of presentations, phone calls, and mostly by direct approach. An invitation was also extended to University students. Other means were also used to advertise the project such as flyers and pamphlets. The Pacific Trust Otago holds several events during the year, which include an annual camp that caters to Pacific Island teenagers in Dunedin, weekly sports days and also other extracurricular activities. Finau Taungapeau, a Health Promoter at the Pacific Trust, and Eric Nabalagi the Pasefika Pathways (Youth) Coordinator, are very active members of the Pacific community were mainly involved in recruitment, as they have regular events that involves Pacific teenagers.
2.3.3 Study Population

The study population included subjects between the ages of 15 – 20 years and consisted of people that identified with at least one Pacific Island ethnic group and admixture. For this study, 80 Pacific Island volunteers, both males and females, were recruited. This magnitude of the cohort represents approximately one third of Pacific Island teenagers of this particular age group residing in Dunedin, and was deemed representative of the community (Grant et al., 2008). It is worth to note that this statistic does not encompass those who are visiting and, Pacific students attending the University of Otago.

---

**General Introductions**

**Information about the study and Informed Consent**

**Fasting Blood and Urine Sample Collection**

**Blood Pressure, Body Composition and Anthropometrical Measures**

**Breakfast (Pacific Trust Otago)**

**Completion of Questionnaires**

---

Figure 2.2 - General Overview of protocol.
2.4 Clinical Procedure

The clinic involved was the Southern Community Laboratory, South Dunedin. The candidate in his discretion operated at the clinic with a Pacific focus. Establishing meaningful relationships with and among Pacific people is a crucial practice that guarantees positive experiences and reinforces the engagement of participants and the researcher(s) involved. Upon arrival at the clinic, the candidate greeted all participants in a culturally competent manner, acknowledging that one’s own cultural beliefs, values and practices may differ to those of the participants. This role involved the Candidate in building meaningful relationships with participants.

2.4.1 Informed Consent

Participants were each informed of the objectives of the study by the Candidate. An information sheet (Appendix A) was then provided to each participant to read through for a few minutes. Following was guiding them through each of objectives of the study and answering any questions they may have had. The consent form (Appendix B) listed seven statements necessitating consent collectively. An additional three options were individually consented and required them to indicate whether they wanted a copy of their results and as to whom they would want their information returned to, an indication as to the method of disposal utilized for their sample upon conclusion of the study, and also asking permission to access and obtain medical records and information regarding their health from their General Practitioners (GPs) if necessary. The Candidate would check that the participants had given willing and informed consent before proceeding further with the study. Furthermore, the Candidate made sure that all participants were clear as to all methods and procedures that were to follow once they have provided consent. It is noteworthy, that the collection of samples was the initial part of the study and was followed by answering of questionnaires; participants were also made aware of this.

In addition, the subsequent culturally sensitive options were meticulously discussed at length with the participants.

2.4.1.1 Samples, Results and Confidentiality

All participants were reassured that samples and blood and urine results will not be shared with individuals external to the research team and all samples were de-identified and could only be
identified by their unique study code. Blood results (Table 2.1) however will be returned to the recipient; whoever that may be is according to the option selected by them on the consent form. Findings from the study would be presented only in summary form as an aggregate rather than an individual’s information. Only one participant did not consent to having their blood and urine samples taken and therefore withdrew from the study.

2.4.1.2 Method of Sample disposal

The consent form included two options of sample disposal. All participants were presented with a preference as to the mode of disposal either by standard disposal methods or have their samples disposed with appropriate karakia (prayer). Māori and Indigenous people recognize the body as tapu (sacred). Therefore, health or medical research that involved the use of donated samples, that inclusive of blood, human tissue and any part of the body are to be disposed in a respectful and appropriate manner (Health Research Council, 2010). Participants whom had selected the option of having their samples disposed with appropriate karakia had their blood samples marked with a “K” indicative of “disposed with appropriate karakia”.

2.4.1.2.1 Karakia Ceremony

Karakia are prayers or incantations. They are commonly used to safeguard a positive outcome to crucial occasions and undertakings such as tangihanga (the ritual of farewell to our deceased), hui (meetings), and unveilings, to name a few. Karakia, in their true essence, are ceremonial chants invoking spiritual guidance and protection.

In the case of this study, the Karakia ceremony is when all members of the Merriman Lab gather and have a prayer ceremony to dispose of the samples that have been donated for the purposes of our study. The University of Otago Chaplain, Greg Hughson leads the karakia ceremony where we prayed and gave thanks for the samples that have been donated for our studies.
Table 2.1: Blood results tabulated in this manner and returned to participants.

<table>
<thead>
<tr>
<th>TEST</th>
<th>TARGET</th>
<th>YOUR RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>&lt;5.0</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;2.0</td>
<td></td>
</tr>
<tr>
<td>HDL (good cholesterol)</td>
<td>&gt;1.0</td>
<td></td>
</tr>
<tr>
<td>LDL (bad cholesterol)</td>
<td>&lt;3.0</td>
<td></td>
</tr>
<tr>
<td>Urate for gout</td>
<td>&lt;0.42</td>
<td>(&lt;0.36 for people with gout)</td>
</tr>
<tr>
<td>Creatinine for kidney function</td>
<td>&lt;100</td>
<td></td>
</tr>
<tr>
<td>eGFR for kidney function</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>&lt;130/80</td>
<td></td>
</tr>
</tbody>
</table>

2.4.2 Blood and Urine Samples: Collection and Testing

During the course of recruitment, participants were informed, and reminded by telephone twenty-four hours prior to the day of testing, that they must undergo a ten-hour overnight fasting period. It was also reiterated that 30 milliliters (mL) of blood was to be drawn.

Upon completion of consent and provided all participants were happy to partake, each was handed a form to fill out, a prerequisite for taking blood at the Southern Community Laboratories, South Dunedin. Trained phlebotomists carried out all blood collecting. Blood was collected into two different tubes: 1) 10 mL of blood collected in Ethylenediaminetetraacetic acid (EDTA) and 2) 2x5 mL of blood collected into Serum Separator tubes (SST). Following blood collection, each participant was also required to provide a spot urine sample for urinalysis.

All blood and urine samples were left in the care of Southern Community Laboratories staff to be transported to the main Lab situated in the Dunedin Hospital for testing and analysis of basic blood biochemistry. This utilized 5 mL of plasma and test results of which included lipid profile, kidney function and urate (Table 2.2). These results were presented in a teenage friendly table (Table 2.1) and returned to the participants and/or their families. Furthermore, 10 mL of the sample was used for genomic DNA extraction and 5 mL of the remaining serum was stored for future measurement of other markers such as vitamin D and inflammatory molecules.
200 microlitres of whole blood samples were aliquoted into an eppendorf and put in storage for future measurement of HbA1c; a measure for glucose and diabetes.

Table 2.2 - Clinical measurements for biochemistry profile

<table>
<thead>
<tr>
<th>Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
</tr>
<tr>
<td>High density lipids (mmol/L)</td>
</tr>
<tr>
<td>Low density lipids (mmol/L)</td>
</tr>
<tr>
<td>Cholesterol: HDL ratio</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
</tr>
<tr>
<td>Serum creatinine (umol/L)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (ml/min/1.73 m²)</td>
</tr>
<tr>
<td>Urine creatinine (mmol/L)</td>
</tr>
<tr>
<td>Urine urate (mmol/L)</td>
</tr>
</tbody>
</table>
| Simkin index (mg/dl). (Urinary urate x serum creatinine)/urinary creatinine)/60 |}

Following all tests that were conducted at the Southern Community Laboratory, the candidate enquired about the participants’ opinion on how they found the initial part of the study. Questions were asked of them to check whether anyone felt unwell during and after the process of blood collection. This gesture was to provide continuity in building meaningful relationships with them. The candidate also at this point made sure that the participants were aware that there was another segment of the study that still required their participation and they were still enthusiastic to progress with the study.

All participants whom had participated agreed to carry on with the study. No participant withdrew upon completion of tests at the laboratory. At this point there was a change in scenery as to where the remains of the study would take place. The participants were informed that the final parts of the study were to take place at the Pacific Trust Otago headquarters, where they would be required to complete several questionnaires, have their body composition and anthropometric measurements recorded and also where breakfast will be provided.
2.5 Blood Pressure

Two seated blood pressure measures were taken by use of a purchased blood pressure monitor (Model: Omron Hem – 7200). Two cuff sizes were provided, one small and the other large. Participants were instructed to take a seat and relax as much as they possibly could. Initially, two blood pressure measurements were taken. The average of which was calculated and used for analyses. If large discrepancies between blood pressure measurements were evident, then a third measure was taken. At this point, the average of the two most similar readings was calculated and used for analyses.

2.6 Anthropometric and Body Composition Procedures.

The following method of acquiring anthropometric and body composition measurements was adapted from the International Standards for Anthropometric Assessment issued by the International Society for the Advancement of Kinanthropometry (The International Society for the Advancement of Kinanthropometry, 2001).

The candidate was well versed in the techniques of conducting the tests. The candidate attended training on how to take anthropometric measurements conducted by a trained and qualified level three anthropometrist of the International Society for the Advancement of Kinanthropometry (ISAK). The training took place at the Human Nutrition Department Clinic, University of Otago. The candidate’s skill and reliability were determined by test and retesting methods. The candidate took all measurements of anthropometrical variables, which is a reflection of acceptability and competency of skills required for testing.

All participants were informed as to what measurements that were going to be taken and were taken through the protocols that the candidate was to follow. Furthermore, it was also made clear that the protocol may involve them to assume various positions and allow access to bare areas of the skin for one particular measurement.
Due to all tests taking place in the morning (8 am), the majority of participants attended in their school uniforms. The participants were very cooperative when they were asked to disrobe as many layers as they could to keep clothing and thickness of layers to a minimum.

While common ground shared by the participants and candidate was that they collectively identified as Pacific Islanders, it was to the candidate’s knowledge to always be sensitive to the dissimilar cultural beliefs and traditions of each participant as these may vary between individuals.

All measurements were taken twice. If measurements differed by more than one percent, the result of which was determined a particular equation (Figure 2.3), a third measurement was performed. The mean of two measures was used mostly for data analysis. However, in cases where a third measurement was necessitated, the median of the three measures was calculated and the result of which was then used for data analysis.

Figure 2.3: Equation utilized to determine whether a third measurement was necessary for anthropometrical measurements.
2.6.1 Anthropometrical Variables

2.6.1.1 Body Weight

**Purpose:** To determine the body weight of each participant.

**Equipment Used:** Weighing scale.

**Protocol:** Participants were required to remove socks and shoes, and any excess clothing (e.g. jumpers, jackets and scarves) prior to stepping on to the scale. All subjects were advised to stand with minimal movement, hands to their side and head facing forward. The candidate checked that the scale read zero before allowing the participant to step on to it. The participant stands on the center of the scale, deprived of support and was instructed to stand straight with their weight evenly distributed on both feet, hands by their sides and looking straight ahead. Once the value appeared on the scale, the subject was instructed to step off.

**Scoring:** Body mass (weight) was recorded as a score in kilograms (kg).

2.6.1.2 Body Height

**Purpose:** To determine the body height of each participant.

**Equipment Used:** Stadiometer.

**Protocol:** Following taking their weight, subjects were advised to keep their shoes and socks off for measuring their height. The participant was instructed to stand with heels together, their weight evenly distributed on both legs, and have their heels, buttocks, and upper part of the back touching the vertical scale. They were also instructed to have their hands hang freely along their sides, with their palms facing towards their thighs. The head, when placed in the Frankfort plane should not touch the scale. The Frankfort plane is accomplished when the lower edge of the eye socket (orbitale) is in the same horizontal plane as the notch above the tragus of the ear (tragion). The participant was instructed to stand straight, continue to uphold the position of their heels, inhale as much as they can and then hold their breath, all the while maintaining the head in the Frankfort plane. The candidate placed the headboard firmly down on the most superior part of the skull (vertex) making sure that the participant’s hair was compressed as much as possible to
warrant an accurate measurement. The candidate proceeded by taking height measurements before the participant exhaled. The protocol was repeated twice for good measure.

**Scoring:** Standing height measurements were recorded as a score in centimeters (cm).

### 2.6.1.3 Waist Circumference

**Purpose:** To measure the waist girth of each participant.

**Equipment:** Lufkin anthropometric tape measure.

**Protocol:** The participants were instructed to assume a relaxed position with their arms folded across their chest. Measurement of waist circumference was taken at the level of the narrowest point between the lower costal border and the iliac crest. The candidate stood in front of the participant and passed the tape measure around the abdomen. The stub of the tape and the housing were then both held in the right hand, while the left hand was used to adjust the level of the tape at the back of the pronounced level of the narrowest point. The candidate resumed control of the stub with the left hand and then moved to right side of the participant to take the measurement. The candidate from the front of the participant, moved slightly to the right side to avoid any feeling of discomfort upon the subject. From here the participant employed the cross hand technique to position that tape at the target level. The participant was instructed to breathe normally and the measurement was recorded at the end of normal expiration. If there was no obvious narrowing, the measurement was taken at the mid-point between the lower costal border and the iliac crest.

**Scoring:** The waist circumference of each participant was recorded as a score in centimeters (cm).

**Obstacles:** This particular measure was in various ways slightly challenging with a few participants. In order to achieve accurate results, participants were required to be of minimal clothing. The measuring of the waist girth required the candidate to take measurements by standing in front of the subject and then passing the tape measure around the skin of the abdomen. The candidate appreciated the common knowledge that all people had a zone or area around their body perceived as “personal space”, and that when one’s personal space is invaded it is often when they feel uncomfortable or threatened. While measurements were taken in a
room that provided for privacy, some participants did not feel comfortable lifting their clothing to expose their abdomen for measurements. In order to satisfy and account for the participant’s discomfort, the candidate proceeded with the same protocol however the tape measure was passed over the participants clothing. Only a few subjects presented this as uncomfortable. Others did not seem to find this problematic.

### 2.6.1.4 Hip Circumference

**Purpose:** To measure the hip girth of each participant.

**Protocol:** Following the measurement of waist girth, the participant was instructed to continue to maintain a relaxed position with their arms kept folded across their thorax. They were advised to place their feet together and try to relax their buttocks, anatomically known as the gluteal muscles, as much as possible. The candidate at this point assumed a position on the side of the participant and then passed the tape around the hips from the side. The stub and housing of the measuring tape was then both held in the right hand, while the left hand was used to adjust the level of the tape at the targeted level for measurement. The hip circumference is taken at the level of the greatest posterior protuberance of the buttock, which normally corresponds anteriorly to approximately the level of the pubic symphysis. Control of the stub was resumed with the left hand and then, by way of the cross-hand technique, the candidate situated the measuring tape at the side, making sure that it was maintained in the horizontal plane at the target level. This measurement test required a great deal of concentration and skill from the candidate. The tape needed readjusting at times as it continued to slip off the target level. Furthermore, the candidate had to make sure that the necessary force was applied to avoid excessively indenting the skin.

**Scoring:** The hip circumference was recorded as a score in centimeters (cm).
2.7 Demographic, Biochemical risk factors and Sugar Consumption data.

2.7.1 Biological Questionnaire (Appendix C)

Upon completion of gathering anthropometrical measures, participants were then informed what will follow will be the last part of the study and will entail the completing of questionnaires. The candidate had explained the questions presented to them in the questionnaires and was available for any questions that required elucidation. Where language was a barrier for some participants, the candidate guided them through the questionnaire in the form of an interview.

Many of the questions included in the questionnaires were standardized questions adapted and some revised from several other questionnaires. The questionnaires were also made possible by collaborating with other academic researchers.

2.7.1.1 Demographics

All participants provided their date of birth, gender and ethnicity; because this project comprised a genetic component, participants were asked of their ancestral origin and the best way to do that was for them to report the ancestral origin of their grandparents. More than one ancestry could be selected.

2.7.1.2 Self-reports of any diagnosed personal and family medical history.

Any diagnoses previously made by a doctor regarding several co-morbidities were self-reported. Participants were also asked whether they were diagnosed of any condition other than what we had presented in the questionnaire.

Furthermore, specification of any regular medications was also self-reported. We also included questions as to whether they had been immobilized or had total bed rest for at least a month in 1 year. Females were also required to report on whether they have started menstruating. If their
indication dictates they are of age and have started menstruating, age of onset was also self-reported.

2.7.1.3 Lifestyle Risk Factors

2.7.1.3.1 Physical and Leisure Activities

This is a standardized question. Physical and leisure activities were asked of the participants. Self-reports on the number of hours spent on leisure activities during weekdays and weekend days were specified. Furthermore, participants self-reported their activity levels and exercise habits as strenuous, moderate or mild activity, or no physical activity.

2.7.1.3.2 Diet.

Participants were asked to indicate the number of sugar-sweetened drinks including juice they would consume per day. Participants had also self-reported the quantities of whole fresh fruit they consumed per day.

2.7.1.3.3 Cigarette smoking

Smoking status was self-reported. Participants were to identify as a current smoker, an ex-smoker or not a smoker.

2.7.1.3.4 Alcohol consumption

These questions were a standardized tool utilized by the Dunedin Multidisciplinary Health and Development study, which initially asked whether they consumed alcohol. If indication was that they did consume alcohol, other questions were to be answered. Such questions included the amount of alcohol consumed in different timeframes of the year. Furthermore, participants were asked to self-report if any of the listed problems in the questionnaire were due to them being under the influence of alcohol.
2.8 Food Frequency Questionnaire

This survey more specifically, is a sugar consumption questionnaire used to evaluate patterns of sugar consumption in a cohort of Pacific Island teenagers. The questionnaire was achieved through several meetings, and by collaboration of Associate Professor Tony Merriman and the candidate with Dr. Lisa Te Morenga from the University of Otago, Department of Human Nutrition.

The questionnaire was adapted by a questionnaire provided by Dr. Te Morenga. Initially, the original survey was entitled “The Pukapukan Kai Study” and was designed as part of a project that explored the development of a brief instrument to examine the eating patterns in Pukapuka Cook Islanders. Minor modifications were made where appropriate for the purposes of this study. The survey encompassed questions that required participants to report their sugar intake patterns over the past month from the date of participation. Questions were divided into several sections and each item in the questionnaire comprised of two questions: 1) How much? And 2) How often? Images were provided to aid participants with estimations as to the quantity of their sugar intake (Figure 2.4). The finalized questionnaire may be found in Appendix D.

![Figure 2.4: Example of an image provided to help participants estimate how much they usually drank.](image-url)
2.9 Breakfast

Upon completion of body composition and anthropometrical measures, the participants were informed that at this stage they could have breakfast. This was a time to fill in questionnaires, have breakfast and also a time permitted to share experiences. The participants relayed their interests in the field of Science and research, thus the candidate at this point shared his undergraduate journey and also his experiences in the field of research to enlighten and encourage the burgeoning young.

2.10 Debrief

The candidate debriefed all participants individually. Each participant was taken aside privately for debriefing. This allowed for the candidate to ensure that all questions were answered. This part was also an opportunity for the participants to raise any concerns or questions they had concerning the project, and to present any comments they had regarding things we could or could have done better.

2.11 Koha

All participants were awarded a $20 Warehouse voucher as a token of appreciation for their voluntary participation and time. As most of the participants were still in high school, this gift was very much appreciated and was reflected in their excitement when they were each handed a voucher.
2.12 Protocol for examining biochemical risk factors in this thesis

2.12.1 Extraction of human genomic DNA from whole blood.

Genomic DNA extraction from the blood samples were carried out in the Merriman Laboratory, Department of Biochemistry, University of Otago, by courtesy of their technical staff. Ten mL of whole blood was mixed with 40 mL blood lysis buffer (320 nM sucrose, 1% Triton-X-200, 4.9 M MgCl$_2$, 1M Tris (pH 7.4) and centrifugated at 2000 g for 15 minutes. Amalgamation of the pellet with 20 mL blood lysis buffer was undertaken and then centrifugated at 2000 g for 15 minutes. Three and a half mL of 6 M guanadinium hydrochloride was added to the pellet and mixed well prior to the addition of 250 µL 7.5 acetate, 50 µL of 100 mg mL$^{-1}$ proteinase K and 250 µL 20% sodium sarcosyl; 16 hours was allowed for incubation of the sample at 37°C. Two mL of pre-chilled chloroform was added before incubating the sample again at room temperature for 1 minute, followed by centrifugation at 2000 g for 3 minutes. The upper phase was gathered and 10 mL of 95% ethanol (4°C) was added prior to 15 minutes of centrifugation at 2500 g. The DNA pellet was washed twice with 4 mL of 70% ethanol through centrifugation at 2500 g for 5 minutes. Finally, re-suspension of the DNA pellet commenced in 200 µL TE buffer (pH 7.5) and was stored at -20°C. The concentration of DNA was established by optical densitometry at 260 nm and samples were maintained at a stock concentration of 200 ng mL$^{-1}$.

Table 2.3: TBE and TE Buffer formulae

<table>
<thead>
<tr>
<th></th>
<th>TBE Buffer</th>
<th>TE Buffer</th>
<th>Final (Conc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>10.778 gL$^{-1}$</td>
<td>-</td>
<td>89 mmolL$^{-1}$</td>
</tr>
<tr>
<td>Boric acid</td>
<td>5.485 gL$^{-1}$</td>
<td>-</td>
<td>89 mmolL$^{-1}$</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.744 gL$^{-1}$</td>
<td>1 mL</td>
<td>2 mmolL$^{-1}$</td>
</tr>
<tr>
<td>TRIS (1molL$^{-1}$)</td>
<td>-</td>
<td>20 µL</td>
<td>-</td>
</tr>
<tr>
<td>EDTA (0.5 molL$^{-1}$)</td>
<td>1 L</td>
<td>100 mL</td>
<td>0.1 mmolL$^{-1}$</td>
</tr>
</tbody>
</table>

67
**2.12.2 SNP selection and design**

Single nucleotide polymorphisms (SNPs), or otherwise known as genetic variants were selected through a literature review in order to establish genetic variants that demonstrated significant associations with mainly serum urate, and adiposity (weight and BMI). Genetic variants that showed some level of significant association with co-morbidities that include obesity, T2D, and elevated serum urate levels (gout) were identified and explored in this study.

The nominated variants were selected due to previous evidence that indicated their non-additive interactions with environmental exposures. Furthermore, SNP selection was also based on those that were the strongest independent predictors of the associated variables within European populations.

For urate, this included 2 SNPs found at the SLC2A9 renal transporter locus: rs3775948 and rs11942223. And for the FTO gene: rs9922047 and rs9923233. The variants examined for association with particular biochemical risk factors in this study are exemplified in Table 2.4.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTO</td>
<td>rs9922047</td>
<td>G/C</td>
</tr>
<tr>
<td></td>
<td>rs9923233</td>
<td>G/C</td>
</tr>
<tr>
<td>SLC2A9</td>
<td>rs11942223</td>
<td>C/T</td>
</tr>
<tr>
<td></td>
<td>rs3775948</td>
<td>C/G</td>
</tr>
</tbody>
</table>

**2.12.3 TaqMan® SNP genotyping assay**

The genotyping procedure utilized to assay the amplification of each genetic variant was the Taqman® pre-designed SNP genotyping assay. The assay is robust with genotyping precision. The PCR apparatus employed to achieve genotyping assays for this project was the Roche Light Cycler® 480, (Roche Applied Science, Indianapolis, USA). All assays comply with the universal reaction conditions (Applied Biosystems, 2006).
In allelic discrimination (refer to Figure 2.5) genotype assays, the polymerase chain reaction (PCR) utilizes a particular fluorescent, dye-labelled probe for every allele. The probes encompass dissimilar fluorescent reporter dyes (FAM and VIC) to distinguish the amplification of alleles (Applied Biosystems, 2006).

The TaqMan SNP Genotyping assay works in the same manner as a PCR reaction. Two fluorogenic probes (6FAM dye-MGB labelled probe and VIC dye-MGB labelled probe) are utilized by TaqMan assays. The FAM dye is connected to the 5’ prime end of the allele 2 probe, while the VIC dye is connected to the 5 primer end of the allele 1 probe. The TaqMan probe however is designated to the SNP, which is positioned between the two primers and has a melting temperature that is about 10º higher than that of the primers.

While the probe is intact, a nonfluorescent quencher at the 3’ end of the probe will remain in close proximity to the fluorophore (VIC/FAM), thus eradicating the fluorophore’s signal. If the allele-specific probe matches the SNP allele during PCR amplification, it will in turn bind to its target site, the DNA and subsequently undergoes degradation. During the PCR amplification step, if the allele-specific probe is perfectly complementary to the SNP allele, it will then bind to the target DNA strand, thus permitting degradation by the 5’-nuclease activity of the Taq polymerase, which extends the DNA from the PCR primers’ emitting signal due to the detachment of the reporter from the quencher. The accumulation of PCR products is detected by the increase fluorescence of the reporter dye (FAM and VIC). The frequency of each signal released is an indication of which allele(s) of the target region is present ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA) monitors the 384 reactions for each cycle and not removing any sample. By the end of 40 cycles all the data for quantitative analysis are stored for analysis.
Figure 2.5: Allelic differentiation is accomplished by the selective hardening of TaqMan® MGB probes. The VIC dye attaches to the major allele (target allele 1) whereas the FAM dye binds to the minor allele (target allele 2). Figure adapted from Applied Biosystems.

### 2.12.4 TaqMan® SNP genotyping using the Light Cycler

Plating layout was planned and labelled appropriately prior to aliquoting 2 μL of 6-10 ng/μL of genomic DNA into white Light Cycler, 384-well plates. Plates were vortexed and sealed to avoid DNA contamination, and left to dry at room temperature for a minimum of two days. The TaqMan Master Mix standard reaction was prepared accordingly; 2.73 μL 2x ABgene master mix, 2.2 μL of distilled water and 0.07 μL 40X SNP assay for each sample (Table 2.5).

Initially the Taqman probes were defrosted from the freezer. The ABgene mix was aliquoted into an eppendorf followed by distilled water. The mixture was vortexed and spun down before adding the defrosted probe. The final product, Master Mix, was vortexed and spun down again and was ready to be added to the samples.

<table>
<thead>
<tr>
<th>Table 2.5: TaqMan SNP genotyping cocktail reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent</strong></td>
</tr>
<tr>
<td>2x ABgene mix</td>
</tr>
<tr>
<td>H₂O</td>
</tr>
<tr>
<td>40X SNP assay</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
5uL of Master Mix was loaded into wells of a 384-well plate, for each sample using a dispenser. The plates were sealed prudently using optical adhesive seals (Roche Applied Science, Indianapolis, USA). Subsequently, the plates were spun at 1000rpm for one minute. Finally, before amplification the plates were stored and protected from light by means of tinfoil until they were ready to be run in the Light Cycler® version 480 (Roche Applied Science, Indianapolis, USA).

The plates were loaded into the Light Cycler apparatus to undergo ‘TaqMan run protocol SNP Genotyping’ and allelic discrimination. Standard amplification procedure for TaqMan genotyping is listed in Table 2.6.

Each plate loaded into the Light Cycler for analysis was created into a subset by selecting the subset editor function. Pre-existing templates had been created by the Merriman Lab staff and were applied where appropriate. However, new subsets were also created where necessary.

Upon completion of each cycle, analysis of the data was analyzed utilizing the Light Cycler 480 software. The colour compensation in database was initiated prior to analysis of each of the subsets. ‘Endpoint genotyping’ was selected for ‘Analysis type’ while program was set to ‘mini-melt’ before selecting the calculate function. The samples were organized in the appropriate order by toggling the ‘position’ button at the bottom left (i.e. A1, A2, and A3 to A1, C1, and E1).

Table 2.6: Specification of protocol per cycle for Taqman assay for 40 cycles.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre incubation</td>
<td>50</td>
<td>2 min</td>
<td>1</td>
</tr>
<tr>
<td>Activation</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>Cycling</td>
<td>95</td>
<td>15 sec</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Mini-melt</td>
<td>60</td>
<td>1 sec</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>40</td>
<td>30 sec</td>
<td>1</td>
</tr>
</tbody>
</table>
2.12.5 Generation of cluster plot

A cluster plot (refer to Figure 2.7) was produced automatically by a SNP auto-caller that automatically determined genotypes and generated graphic representation of results. The data was analyzed carefully, where manual calling of alleles, although very few, were carried out where advised and/or necessary. The results were tabulated into Haploped files, created by Microsoft word, Excel (refer to Figure 2.8)

![Cluster plot generated from TaqMan® SNP genotyping assay for SLC2A9, SNP rs3775948.](image-url)

**Figure 2.6:** Cluster plot generated from TaqMan® SNP genotyping assay for SLC2A9, SNP rs3775948.
2.13 Data for analysis

2.13.1 Data entry into Haploped Files

With the aid of the Merriman Lab staff members, a master Haploped file that encompassed relevant information regarding the data utilized for the purposes of this study was fabricated. Results collated from the genotype runs previously carried out were tabulated into the master Haploped (refer to Figure 2.8).

![Figure 2.7: Sample of Master Haploped](image-url)
2.14 Statistical Analysis

2.14.1 SNP Association analysis

Each SNP was coded 0 – 2 based on the number of risk alleles present; non-risk allele homozygotes were coded as 0, heterozygotes were coded as 1 and homozygotes of the risk allele were coded as 2 (refer to Figure 2.9).

Both phenotypic and genotypic raw data were stored within BC\textit{SNPmax}. BC\textit{SNPmax} is a secure data management system which stores genotype and phenotype information provided by Biocomputing Platforms Ltd, Espoo, Finland. Moreover, access to an array of statistical analyses facilitating genetic research is upheld within this data system. For the purposes of this study, phenotypic and genotypic data were exported from BC\textit{SNPmax} into Microsoft Excel enabling necessary filtering, adjustments and other calculations to be performed. Once files were modified to meet the selected criteria, they were saved as text files to allow for statistical analysis to be carried out in STATA version 13.0 (StataCorp, College Station, Texas, USA), as described in the next section.

Figure 2.8: Image showing SNP coded for risk allele (0, 1 or 2)
2.14.2 Ordinary least square (linear) regressions

Exposure-outcome associations, $r^2$ and F-statistic values, quartile data and supplementary analyses supporting 2SLS regression estimates were carried out by employment of linear regression models in STATA 13.0. Linear regression is based on fitting a linear equation to observed data in order to demonstrate the association between two or more of the variables of interest. While this does not automatically indicate causation, regression models are advantageous in examining links and associations amid different variables. The outcome estimator is characterized as the beta coefficient. This presents the estimated change in the outcome apiece one category increase in the exposure. Linear regression analyses functioned in STATA 13.0 also concedes summary statistics within the outcome table, of which include information on F-statistic and $r^2$ values. Linear regression analyses were performed by utilizing the `regress` function, present in STAT 13.0 where a p-value of $<$0.05 was used for nominal significance.

2.14.3 Adjustments

In order to minimalize possible biases and counterfeit correlations caused by confounding factors, alterations were made for potential confounders in multivariate regression models. Common confounders that were controlled for across all analyses comprised of age and sex and BMI. Physical activity, weight and height were also accounted for contingent on the variables that were examined. It is worth to note that these methods were applied across other data that was collected and analyzed, not only for SNP analysis.
CHAPTER 3: RESULTS
3.1 Summary statistics on health measures

Participants who self-identified with at least one Pacific ethnic group and within the age bracket of 15 – 20 years of age, qualified for this study. A total of 80 participants volunteered and participated in this study. Participants attended screening clinics at the Southern Community Laboratories and Pacific Trust Otago, South Dunedin. The overall mean age of the cohort was 17.4 (SD 1.67), where age distribution averages were 17.2 years (SD 1.52) for males and 17.26 years (SD 1.79) for females (Figure 3.1).

The aggregate summary anthropometric and biochemical results of the cohort are presented in Table 3.1. There was a higher proportion of females than males. Summary level data of anthropometric measures showed that the average height of this particular cohort was 170.25 cm, and the average weight was 83.87 kg. The mean BMI for the cohort is 28.89 kg/m², which places the cohort in a classification category of overweight. The mean waist circumference for the cohort was 86.13 cm, hip girth was 107.62 cm, and the mean waist to– hip ratio was 0.80.

![Figure 3.1: Age and gender distribution of the Pacific Island teenagers’ cohort (N=80).](image)

The average results of the biochemical variables were well within reference ranges. The results of the manifold variables that were measured are as follows: total cholesterol (3.76 mmol/L), serum triglycerides (0.91 mmol/L), HDL-C (1.19 mmol/L), LDL-C (2.15 mmol/L) and serum urate (0.37 mmol/L), as presented in Table 3.1 (Reference values are presented in Table 2.1).
A comparison was also made for all measures between males and females as seen in Table 3.2. A significant difference was observed in systolic and diastolic blood pressures between males and females.

Table 3.1: Baseline characteristics of the Pacific Island teenager’s cohort (N = 80).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Males = 41.25%, Females = 58.75%</th>
<th>Range (min – max)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>15 - 20</td>
<td>17.24 ± 1.67</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>150.00 – 188.20</td>
<td>170.25 ± 8.87</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.20 – 170.00</td>
<td>83.87 ± 18.83</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.60 – 57.46</td>
<td>28.89 ± 5.93</td>
<td></td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>63.40 – 117.00</td>
<td>86.13 ± 11.60</td>
<td></td>
</tr>
<tr>
<td>Hip girth (cm)</td>
<td>78.00 – 174.00</td>
<td>107.62 ± 13.45</td>
<td></td>
</tr>
<tr>
<td>Waist to –hip ratio</td>
<td>0.48 – 1.21</td>
<td>0.80 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>83.00 – 147.00</td>
<td>112.25 ± 11.39</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>40.00 – 89.00</td>
<td>61.78 ± 8.94</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.60 – 5.60</td>
<td>3.76 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.10 – 3.70</td>
<td>2.15 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.81 – 2.36</td>
<td>1.19 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.40 – 2.40</td>
<td>0.91 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.21 – 0.56</td>
<td>0.37 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>47.00 – 226.00</td>
<td>74.78 ± 29.57</td>
<td></td>
</tr>
</tbody>
</table>

- Summary level statistics presented in mean and standard deviations (SD) with the ranges for the 80 participants that have made up this cohort
Table 3.2: Demographic and clinical characteristics: the listed means and standard deviations (SD) are for the cohort separated by sex.

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 33)</th>
<th>Females (n = 47)</th>
<th>T - test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age, years</td>
<td>17.21</td>
<td>1.52</td>
<td>17.26</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.17</td>
<td>7.68</td>
<td>166.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.44</td>
<td>21.46</td>
<td>79.96</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.90</td>
<td>7.35</td>
<td>28.88</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>85.46</td>
<td>10.49</td>
<td>86.59</td>
</tr>
<tr>
<td>Hip girth (cm)</td>
<td>106.90</td>
<td>16.61</td>
<td>108.14</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.81</td>
<td>0.08</td>
<td>0.80</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116.64</td>
<td>8.76</td>
<td>109.36</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>59.00</td>
<td>8.58</td>
<td>63.91</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.79</td>
<td>1.38</td>
<td>3.73</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.20</td>
<td>0.89</td>
<td>2.11</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.17</td>
<td>0.46</td>
<td>1.20</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.91</td>
<td>0.47</td>
<td>0.91</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.36</td>
<td>0.13</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Table 3.2 illustrates main demographic and clinical characteristics of participants obtained within this study, stratified according to sex. Age distribution was akin across both males and females. Height, BMI, systolic and diastolic blood pressures were comparable across both sexes, with diastolic pressure higher in females. Also presented above are the mean estimates of biochemical traits of which included total cholesterol, LDL-C, HDL-C and triglycerides.

3.2 Comparison of Summary level data between two cohorts; the change in health measures between 2006 and 2013.

A similar study to that of the present study was carried out in 2006 (Grant et al., 2008), which aimed to establish associations amid body composition, glucose and lipid metabolism, and constituents of the metabolic syndrome. The cohort was also a Pacific Island sample population of 80 teenagers between the ages of 15 and 18 years, living in Dunedin. Due to this study being an attempt on a very similar cohort with very similar health measures being analyzed, this section will present a comparison between key findings achieved from the present study and that of the previous study.

The comparisons made in this section were a direct comparison of the data from this study to the data presented in the published paper by Grant and associates (Grant et al., 2008).

Key findings being contrasted were based only on measures that both studies measured. Other measures analyzed and presented by Grant and colleagues were not measured in this study, hence being omitted from the comparison segment of this chapter. Such measures were fat mass, fat percentage, lean mass, fasting insulin, adiponectin and several stages of puberty.

Grant and associates found 40% of their cohort to be overweight and a further 36% were obese. Similarly, this study also reflected that of the 80 participants, 40% were overweight and 34% were obese. Results from the present study showed that 15 participants weighed more than 100 kg, and 7 participants had BMI values higher than 35 kg/m². These values were lower than that of Grant and colleagues who reported that of their 80 participants, 22.50% (n = 18) weighed more than 100 kg, and 16.25% (n = 13) had BMI values that were greater than 35 kg/m².
It is worthwhile to note, that all participants that weighed more than 100 kg and those with BMI values greater than that of 35 kg/m$^2$ for this present study, were all females. The data presented in Grant’s paper did not reveal whether those with higher BMI and weighed over 100 kg were male or female.
Table 3.3: Comparison of characteristics of the 80 participants for both studies, separated according to BMI status

<table>
<thead>
<tr>
<th></th>
<th>Current study</th>
<th>Grant et al., 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal weight (n = 21)</td>
<td>Overweight (n = 32)</td>
</tr>
<tr>
<td>M, F (n, %)</td>
<td>10 (12.50%), 11 (13.75%)</td>
<td>11 (13.75%), 21 (26.25%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>16.9 ± 1.4</td>
<td>17.8 ± 1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.5 ± 9.45</td>
<td>169.7 ± 8.25</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.9 ± 7.78</td>
<td>78.6 ± 7.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 1.44</td>
<td>27.3 ± 1.5</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>75.2 ± 5.8</td>
<td>83.8 ± 8.6</td>
</tr>
<tr>
<td>Hip girth (cm)</td>
<td>96.7 ± 6.0</td>
<td>106.7 ± 14.8</td>
</tr>
<tr>
<td>Waist to–hip ratio</td>
<td>0.77 ± 0.04</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>107.7 ± 9.9</td>
<td>110.7 ± 10.5</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>56.1 ± 7.9</td>
<td>62.3 ± 8.6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.73 ± 1.02</td>
<td>3.64 ± 1.33</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.11 ± 0.69</td>
<td>2.06 ± 0.88</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.25 ± 0.34</td>
<td>1.22 ± 0.49</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.83 ± 0.33</td>
<td>0.79 ± 0.47</td>
</tr>
</tbody>
</table>

- Results are presented in mean ± standard deviation. ANOVA signifies the analysis of variance between the three categories within EACH cohort.
- *Significant associations demonstrated in bold
Table 3.3 shows the comparisons made between parameters accomplished from this study, against those achieved by Grant and colleagues. The data were delineated according to BMI categories as carried out by Grant and associates. Significant trends of change between each BMI category within each cohort are demonstrated in bold by way of an ANOVA test. Note: the ANOVA test does not signify the analysis of variance between the results from this study and Grant’s (i.e. not present study obese results vs. Grant’s study obese results).

For the present study, 26.25% (n = 21) of the 80 participants were classified as normal weight, 40% (n = 32) were overweight and 33.75% (n = 27) were obese. Grant and associates showed 19 healthy participants (23.75%) of the 80 in their cohort, 32 (40%) were overweight, and 29 (36.25%) obese subjects.

A significant increasing trend in weight from the healthy to obese populations (p value = 2.2 x 10^{-16}) was observed in this study. A significant trend alike was shown in the study by Grant et al., (p value = 0.001), however our p value was more significant. Consistent with the results from Grant and associates, increasing trends were also observed with significance, from healthy to obese groups in our study upon comparison of waist (p value = 5.5 x 10^{-15}) and hip girths (p value = 6.3 x 10^{-08}). The p values from this study reflected more robust significance, than the nominal significances established by Grant et al., (p value = 0.001 for waist girths, and p value = 0.001 for hip girths). Further, both studies demonstrated substantial increasing trends in waist to – hip ratios, diastolic blood pressures and also serum triglycerides. A significant increase from healthy individuals to those who were classified as obese, in systolic blood pressure (p value = 0.005) was found in this study. To the contrary, while there was an observed increase in systolic blood pressure from healthy to obese individuals in Grant et al., study, the analysis of variance demonstrated no significance (p value =0.15). Grant et al., also found a significant decreasing trend in HDL cholesterol from healthy to obese participants (p value = 0.001).

For the present study, the data show that participants with higher BMI values had higher degrees of waist and hip girths, waist to – hip ratios, higher SBP and DBP, and also greater concentrations of triglycerides as opposed to those with a healthy BMI. These were consistent with the findings provided by Grant and associates in their study where they also found that participants with higher BMI values had higher waist and hip girths, high DBP and also greater concentrations of triglycerides. They also found that those with a high BMI had lower HDL cholesterol; as opposed to the present study, the values of which did not reflect this same finding.
Table 3.4 - weight status, and prevalence of risk factors in the Grant and associates, and present cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Present Study</th>
<th></th>
<th>Grant and associates (2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females (N = 47)</td>
<td>Males (N = 33)</td>
<td>Both (N = 80)</td>
</tr>
<tr>
<td>Healthy BMI, overweight, obese (n)</td>
<td>11, 21, 15</td>
<td>10, 11, 12</td>
<td>21, 32, 27</td>
</tr>
<tr>
<td>*Current alcohol</td>
<td>19 (23.75%)</td>
<td>15 (18.75%)</td>
<td>34 (42.50%)</td>
</tr>
<tr>
<td>*Current smoker</td>
<td>3 (3.75%)</td>
<td>1 (1.25%)</td>
<td>4 (5.00%)</td>
</tr>
<tr>
<td>Low physical activity</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Components of the metabolic syndrome (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High waist</td>
<td>22 (46.8)</td>
<td>1 (3.0)</td>
<td>23 (28.8)</td>
</tr>
<tr>
<td>High triglycerides</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Low HDL</td>
<td>6 (12.7)</td>
<td>4 (12.1)</td>
<td>10 (12.5)</td>
</tr>
<tr>
<td>Raised systolic blood pressure</td>
<td>3 (6.3)</td>
<td>2 (6.0)</td>
<td>5 (6.2)</td>
</tr>
<tr>
<td>Raised diastolic blood pressure</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Any component of Metabolic Syndrome</td>
<td>23 (48.9)</td>
<td>7 (21.2)</td>
<td>30 (37.5)</td>
</tr>
<tr>
<td>Full Metabolic syndrome</td>
<td>4 (8.5)</td>
<td>1 (3.0)</td>
<td>5 (6.2)</td>
</tr>
</tbody>
</table>

- High waist > 88 cm for females and >102 cm for males
- High triglycerides = > 2 mmol/L
- Low high density lipoprotein (HDL) = < 1 mmol/L
- High blood pressure = > 130 mm/Hg systolic and > 85 mm/Hg diastolic
- Full MS = three or more risk factors
- * Participants who reported to be current smokers and current consumers of alcohol during screening.
Table 3.4, adapted from Grant et al (2008), represents the findings from this study compared to findings from Grant and colleagues. In this present study a total of 34 participants (42.50%) reported to be alcohol consumers. Self-reports of alcohol drinking for this study reflected that 23.75% (n = 19) of these reports were female drinkers, and 18.75% (n = 15) were males. The numbers of alcohol drinkers in this study were also very high (n = 34) compared to figures reported by Grant and associates who established in their study a total of 14 drinkers (17.5%) in their study population. To the contrary, Grant and associates reported a higher number of smokers (n = 15, 18.75%) where the majority were females (n = 11, 13.75%). This study population only had a total of 4 self-reports (5%) of smoking with 3.75% of the 4 self-reports of smoking being female (3 females vs. 1 male). An assessment on physical activity was also made. Methods of attaining information were slightly different. Grant and associates had a scale of 1 to 5: where 1 was least active and 5 the most active. This study employed a shorter scale; where 1 was sedentary (inactive) and 4 was being very active (high physical activity [competition standards]). The discovery by Grant and colleagues demonstrated seventeen subjects of their cohort reported low physical activity. This present study reports no subjects with low physical activity according to the scale used. Given the scale employed in this study and the levels of physical activity self-reported, my data were divided into only two categories: moderately active and highly active. The physical activity data attained from this study are discussed later in this chapter.

Results regarding the metabolic syndrome for this project were consistent with, and echoed similar findings to the reports made by Grant and associates where high waist circumference was the most common component of the metabolic syndrome in both studies. The second most common component of the metabolic syndrome was low levels of HDL-C, followed by elevated systolic blood pressure and high triglyceride concentration. High diastolic pressure was more evident in the study by Grant, 3.75% (n = 3) compared to this study 1.25% (n = 1). Grant also reported a higher number of high levels of triglyceride 8.75% (n = 7), as opposed to the 2.50% (n = 2) from this study. Findings from this study reported 37.5% of the cohort had at least one or two components of the metabolic syndrome (n = 30), with only 6% meeting the full criteria for the metabolic syndrome. Grant and colleagues reported a slightly higher percentage of individuals having at least one or two components of the metabolic syndrome. Almost half of their study population (n = 39, 49% respectively) was established to have any component of MS, with 7% of their cohort meeting full criteria for MS.
Grant et al (2008) included a measure of fasting glucose by means of an oral glucose tolerance test (OGTT), in this section of their study. This study, however, undertook a different measure of determining diabetes, which was a measure of glycated haemoglobin, or otherwise known as HbA1c, which was omitted from this comparison section (but discussed in the next section of this chapter). No statistical analysis to investigate significant differences between the two comparisons was carried out due to the complete individual data from the previous study not being available.

3.3 Important Clinical Measures

3.3.1 HbA1c

Whole blood samples for HbA1c measurement were stored in the freezer and were later transported on ice to the University of Otago, Human Nutrition Department for testing. Only 77 participant samples were available for testing. The results demonstrate the mean HbA1c for this particular cohort was 35.20 mmol/mol (SD 2.82).

Table 3.5 showing HbA1c mean estimates for males, females and the cohort as a whole.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Total</th>
<th>Males (n = 30)</th>
<th>Females (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>35.21 (2.82)</td>
<td>35.10 (3.23)</td>
<td>35.27 (2.57)</td>
</tr>
</tbody>
</table>

Table (3.5) illustrates the mean HbA1c for the 77 participants whose samples were available for testing. There were a higher proportion of female samples available than males. No significant difference was observed between the levels of HbA1c between males and females in this study. Of the 77 HbA1c samples, 6.5% of these samples were indicative of being pre-diabetic according to the standards provided by the New Zealand Society for the Study of Diabetes (NZSSD). The standards provided by the NZSSD state that an individual with an HbA1c that lies between 41 – 49 mmol/mol ought to be classified as pre-diabetic or otherwise termed as “dysglycaemia” or “borderline diabetes”. It is worth to note, that these standards are provided for adults; where no standards have been specifically established for children and/or teenagers. Thus, the participant with an Hb1Ac of 40 mmol/mol was included as being pre-diabetic due to his young age and having such a high HbA1c.
Table 3.6 presents the summary findings of the 5 pre-diabetic participants in this population listed in mean and standard deviations. All mean estimates for the five participants appeared to be within reference range. The BMI mean estimate however, is indicative of overweight.

An interesting finding from these results was that the participant with the highest HbA1c (45 mmol/M) was indeed obese (a very high BMI = 38.74 kg/m²) and had a very high level of serum urate (0.41 (mmol/L) for someone at that age (15 years old) and also being of the female gender. All other results were well within the normal reference ranges. These included a normal blood pressure and biochemical traits (triglycerides, cholesterol, HDL-C and LDL-C). It was evident that the most common factor that was out of range for those who were pre-diabetic was serum urate and also BMI. It is however notable that the participant with the second highest HbA1c level (43 mmol/mol) had a BMI of 19.60 kg/m², which is indicative of normal weight, and all other measures were normal. The only value that appeared to be high and out of range for this particular individual was that of the HbA1c. This participant was a male of 15 years of age.

Table 3.6 showing summary demographic statistics of pre-diabetic participants. Data presented in mean and SD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>5 (2 males, 3 females)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.4 (1.82)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>86.63 (32.19)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.13 (14.12)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.69 (8.01)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>112.75 (12.95)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>62.0 (10.03)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>42.0 (2.0)*</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.8 (0.47)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.13 (0.36)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.19 (0.63)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.10 (0.63)</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.38 (0.05)</td>
</tr>
</tbody>
</table>

- HbA1c of pre-diabetic values range from 40 – 45 mmol/M
- BMI = Body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HbA1c = glycated haemoglobin, HDL = high density lipoprotein, LDL = low density lipoprotein
3.3.1.1. Association of HbA1c with blood pressure.

The coexistence of diabetes and hypertension in the same patient is stressful to the cardiovascular system. Mean glucose concentrations have been reported to be higher in hypertensive subjects, and also the general consensus is that diabetes increases the risk of cardiovascular disease by a factor of two to three at every level of systolic pressure (Filipovsky et al., 1996).

Table 3.7 presents testing for evidence of a linear relationship or association between HbA1c and systolic, and diastolic blood pressures. Association analyses were carried out by employment of a linear regression model in STATA.

In the test for association with systolic blood pressure, no evidence of a linear relationship with HbA1c was observed in the unadjusted model (Beta = 0.644 P = 0.19). Common confounders were accounted for, and a trending significant relationship emerged in the adjustment made for age and sex (Beta 0.975, P = 0.05). Thus inferring an increase of 0.975 mm/Hg in systolic pressure per every unit increase in HbA1c levels, considering the confounding effects of age and sex. In a second adjustment model where sugar (g/day), biochemical measures and BMI were included to the first adjustment, although trending, the association was no longer significant (B = 0.996, P = 0.08).

The regression of diastolic blood to assess a relationship with HbA1c in unadjusted model showed no significant linear relationship (Beta = 0.352 P = 0.33). This model when adjusted for age and sex, like systolic blood pressure also produced an expected significant increase of 0.729 mmg/Hg (P = 0.04) in every unit of increase of HbA1c. In the second model, evidence also suggests, that diastolic pressure is expected to increase by 0.908 mmg/Hg (P = 0.02) for every unit of increase in HbA1c in this population when age and sex, sugar consumption, BMI, and biochemical traits were adjusted for as confounders.
Table 3.7: Association of blood pressure with HbA1c

<table>
<thead>
<tr>
<th>Variable:</th>
<th>Unadjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted (2) β-Coefficient (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>0.644 [-0.343 - 1.632]</td>
<td>0.19</td>
<td>0.975 [-0.019 - 1.969]</td>
<td>0.05</td>
<td>0.96 [-0.124 - 2.117]</td>
<td>0.08</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>0.352 [-0.372 - 1.078]</td>
<td>0.33</td>
<td>0.729 [0.023 - 1.435]</td>
<td>0.04</td>
<td>0.908 [0.146 - 1.671]</td>
<td>0.02</td>
</tr>
</tbody>
</table>

- Adjusted 1 = Age and sex
- Adjusted 2 = Collective adjustment (adjusted 1+ BMI, sugar intake (g/day), high density lipoprotein, low density lipoprotein, triglycerides and total cholesterol)
- The beta coefficient represents the change in blood pressure attributed to one unit of change in HbA1c. A p-value of ≤ 0.05 was employed for significance.

### 3.3.1.2 A Test for association of HbA1c with biochemical traits

The associations of several biochemical traits were tested for association with HbA1c and are tabulated in Table 3.8. The beta coefficient represents the change in each biochemical trait attributed to one unit of change in HbA1c. No significant associations were established between serum urate, high-density lipoprotein, low, triglycerides and also total cholesterol with HbA1c. An adjustment for common confounders such as age and sex was made and no associations were evident. Following this adjustment a second adjustment was made to include BMI as a potential confounder, and still no associations were apparent. The test for LDL-C demonstrated no evidence of association in the unadjusted model, however it did show a trend towards being significantly associated with HbA1c after adjusting for age and sex (Beta = - 0.051 P = 0.05), and also the second adjustment where BMI was included (Beta = -0.054 P = 0.04). These results for LDL-C after adjustments suggest, that there is an observed decrease in LDL-cholesterol concentrations per unit of increase of HbA1c in this specific population. However, this p-value was not significant after adjustment for multiple testing (P>0.05).
Table 3.8: Biochemical measurements and their associations with HbA1c

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted (1) β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted (2) β-Coefficient (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urate</td>
<td>0.002 [-0.003 - 0.007]</td>
<td>0.38</td>
<td>0.002 [-0.003 - 0.008]</td>
<td>0.36</td>
<td>0.001 [-0.004 - 0.007]</td>
<td>0.58</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>-0.006 [-0.029 - 0.016]</td>
<td>0.55</td>
<td>-0.007 [-0.032 - 0.168]</td>
<td>0.52</td>
<td>-0.003 [-0.027 - 0.021]</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>-0.039 [-0.086 - 0.007]</td>
<td>0.09</td>
<td>-0.051 [-0.104 - 0.001]</td>
<td>0.05</td>
<td>-0.054 [-0.108 - (-) 0.001]</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.014 [-0.035 - 0.063]</td>
<td>0.57</td>
<td>0.039 [-0.015 - 0.093]</td>
<td>0.15</td>
<td>0.029 [-0.024 - 0.084]</td>
<td>0.27</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.039 [-0.091 - 0.012]</td>
<td>0.13</td>
<td>-0.038 [-0.098 - 0.021]</td>
<td>0.20</td>
<td>-0.042 [-0.103 - 0.018]</td>
<td>0.17</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted 1 = Age and sex
*Adjusted 2 = Age, sex, and BMI
*The beta coefficient represents the change in each variable (mmol/L) attributed to one unit of change for HbA1c. A p-value of <0.05 was employed for significance (e.g. for every 1 unit of change in HbA1c, there is a 1.41 mmol/L increase in serum urate concentrations).
3.3.2 BMI

The associations of biochemical measures taken from blood samples were evaluated with BMI (Table 3.9) by means of a linear regression model. There was a positive correlation of serum urate with BMI (Beta = 0.002, P = 0.05), which infers that the higher the BMI, the higher the serum urate levels. Or, for every one unit increase of BMI, there is an increase of 0.002 mmol/L in serum urate levels, at the crude level. The association was adjusted for age, sex and sugar consumption, to ensure that this association was not masked or due to confounding, and it revealed that the variables adjusted for are not confounding. This association is consistent with the masses of studies that have well established the association of serum urate with BMI, by which have also inferred, the higher the BMI, specifically the levels of obesity are strongly associated with elevated serum urate levels (Ishizaka et al., 2010, Lyngdoh et al., 2012b).

A significant positive correlation was also evident with triglycerides across the unadjusted model. The beta coefficient was 0.027, thus also implying a 0.027 mmol/L increase in triglyceride concentration per every unit of increase in BMI (P = 0.01). This model was also adjusted for age and sex and the trend, although decreased from the unadjusted model by 0.002 was still significant (Beta = 0.025, P = 0.02). A further adjustment was made to include sugary drink consumption as a potential confounding factor. Findings from this adjusted model, which also included age and sex, demonstrated that for every one-unit increase in BMI, there is a significant increase of 0.208 mmol/L in serum triglyceride concentrations in this population (Beta = 0.208, P = 0.01). The analysis for evidence of a linear relationship between BMI and HDL-C, LDL-C and HbA1c showed no significant associations across the unadjusted, and both adjusted models.

A linear regression model was also employed to investigate for evidence of a linear relationship between physical activity and BMI, where physical activity was the independent variable (Table 3.10); evidence of a linear relationship was observed between BMI and physical activity (Beta = -1.700, P= 0.02). This beta coefficient translates as such; in every one unit increase in the level of physical activity, a significant 1.700 kg/m² decrease is expected in BMI levels. The model was also adjusted for age, sex and in a second adjustment, sugary drink consumption in grams per day. The significance in association remained consistent where an expected decrease of 1.980 kg/m² (p = 0.01) in BMI per every unit of increase of physical activity was established upon adjustment for age and sex, and also a significant decrease of 1.856 kg/m² when sugar
Consumption was added to the model (P = 0.02). Conversely, from this model we can also deduce that as the level of physical activity declines, BMI levels are expected to increase. This makes perfect sense as common knowledge dictates, the more inactive the individual, the higher the chances of gaining weight and by extension an increase in BMI. And vice-versa, where more active individuals are expected to have a lower BMI.

High blood pressure among teenagers causes distress to their health and as a consequence causing additional health complications later in life (Goon et al., 2013, Jafar et al., 2005). Despite this, the majority of studies available on high blood pressure are focussed on adults. There has been consistent observations as to the associations between BMI and blood pressure, however, the association is ill understood.

A linear regression model was employed to test for an association of blood pressure with BMI in this study population. Results demonstrated that BMI was positively associated with systolic blood pressure (P = 0.03) in the unadjusted model. Age and sex were accounted for in an adjusted model and the association remained (Beta = 0.430, P = 0.03). A second model was made adding physical activity to the first model, with BMI still having an influence on raising systolic pressure (Beta = 0.427, P = 0.04).

Diastolic pressure was shown to be associated with BMI, with an increase of 0.514 mm/Hg per every unit of increase in BMI (P = 0.02). The model was adjusted by age and sex and the influence of BMI lowered, however significant (Beta = 0.490, P = 0.001). The final adjustment showed, that for every unit of increase in BMI, diastolic pressure was expected to increase by 0.509 mm/Hg in this sample (P = 0.002).
Table 3.9: Association of biochemical traits with BMI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted (2) β-Coefficient (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.002 [-0.000 – 0.005]</td>
<td>0.05</td>
<td>0.002 [0.000 – 0.005]</td>
<td>0.02</td>
<td>0.003 [0.000 – 0.005]</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.027 [0.005 – 0.049]</td>
<td>0.01</td>
<td>0.025 [0.003 – 0.047]</td>
<td>0.02</td>
<td>0.208 [0.003 – 0.037]</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.013 [-0.011 – 0.038]</td>
<td>0.28</td>
<td>0.011 [-0.014 – 0.037]</td>
<td>0.38</td>
<td>0.005 [-0.021 – 0.032]</td>
<td>0.67</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>-0.009 [-0.020 – 0.002]</td>
<td>0.10</td>
<td>-0.009 [-0.020 – 0.002]</td>
<td>0.12</td>
<td>-0.008 [-0.020 – 0.003]</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.009 [-0.012 – 0.031]</td>
<td>0.39</td>
<td>0.007 [-0.014 – 0.030]</td>
<td>0.48</td>
<td>0.004 [-0.019 – 0.028]</td>
<td>0.72</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>0.067 [-0.044 – 0.179]</td>
<td>0.23</td>
<td>0.072 [-0.034 – 0.180]</td>
<td>0.17</td>
<td>0.082 [-0.032 – 0.197]</td>
<td>0.15</td>
</tr>
</tbody>
</table>

- Adjusted 1 = Age and sex
- Adjusted 2 = Age, sex, SSB
- The beta coefficient represents the change in each biochemical trait attributed to one unit of change in BMI. A p-value of <0.05 was employed for significance
- Significant associations demonstrated in bold.
Table 3.10: Association of physical activity and blood pressure with BMI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted β-Coefficient (CI)</th>
<th>P-value</th>
<th>Adjusted (2) β-Coefficient (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.700 [-3.192 - (-) 0.209]</td>
<td>0.02</td>
<td>-1.980 [-3.594 - (-)0.367]</td>
<td>0.01</td>
<td>-1.856 [-3.446 - (-) 0.266]</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>0.454 [0.037 - 0.872]</td>
<td>0.03</td>
<td>0.430 [0.034 - 0.826]</td>
<td>0.03</td>
<td>0.427 [0.003 – 0.852]</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>0.514 [0.201 – 0.827]</td>
<td>0.02</td>
<td>0.490 [ 0.195 – 0.785]</td>
<td>0.001</td>
<td>0.509 [0.192 – 0.825]</td>
<td>0.002</td>
</tr>
</tbody>
</table>

- <sup>a</sup>Adjusted 1 = Age and sex
- <sup>a</sup>Adjusted 2 = Physical activity (age, sex, SSB), Systolic and Diastolic (age, sex, and PhysAct).
- <sup>b</sup>The beta coefficient represents the change in BMI (kg/m²) attributed to one unit of change for Physical activity. A p-value of <0.05 was employed for significance.
- <sup>b</sup>The beta coefficient represents the change in blood pressure attributed to one unit of change in BMI. A p-value of <0.05 was employed for significance.
3.4 Lifestyle / Environmental Factors

3.4.1 Physical Activity

Self-reports of exercise habits (Figure 3.2) reflected 42.5% of the cohort is moderately active. Approximately 57.5% of this cohort reported that they partake in at least 30 plus minutes of regular strenuous or hard physical activity on more than 5 days a week. With this criterion, 76% of the self-reports for moderate activity were females (32.5% of the whole cohort), while the remaining were males, approximately 23%. By the same token, of the 57.5% that reported regular strenuous exercise and hard physical training, approximately 54% of the reports were shown to be males (31% of the 80 participants), and approximately 46% were females.

![Physical Activity Distribution](image)

*Figure 3.2: Physical activity distribution in males, females and the aggregate cohort*
Table 3.11 demonstrates descriptive summary statistics according to levels of physical activity

<table>
<thead>
<tr>
<th></th>
<th>Moderately Active (n = 34)</th>
<th>Highly Active (n = 46)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17.50 ± 1.84</td>
<td>17.04 ± 1.51</td>
<td>0.22</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.50 ± 22.98</td>
<td>82.65 ± 15.20</td>
<td>0.50</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>86.97 ± 11.92</td>
<td>85.50 ± 11.45</td>
<td>0.57</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>110.15 ± 15.50</td>
<td>105.11.52</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.68 ± 7.36</td>
<td>27.56 ± 4.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>111.02 ± 12.80</td>
<td>113 ± 10.28</td>
<td>0.41</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>63.35 ± 8.11</td>
<td>60.60 ± 9.41</td>
<td>0.17</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.09 ± 0.48</td>
<td>4.04 ± 0.73</td>
<td>0.76</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.90 ± 0.31</td>
<td>1.05 ± 0.46</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.35 ± 0.29</td>
<td>1.24 ± 0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.33 ± 0.47</td>
<td>2.32 ± 0.64</td>
<td>0.92</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.37 ± 0.06</td>
<td>0.36 ± 0.07</td>
<td>0.83</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>30.73 ± 11.66</td>
<td>30.62 ± 12.77</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Table 3.11 displays a summary of outcomes separated according to physical activity. Those who were highly active had lower anthropometric measures compared to those who were moderately active. As established earlier in section 3.3.2, BMI showed a significant association with physical activity. Evidence in the test for association of BMI with physical activity showed significant decrease in BMI per every unit of increase in physical activity (\( \beta_{\text{unadjusted}} = -1.700 \text{ kg/m}^2, P = 0.02 \), \( \beta_{\text{adjusted1}} = 1.980 \text{ kg/m}^2, P = 0.01 \), \( \beta_{\text{adjusted2}} = 1.856 \text{ kg/m}^2, P = 0.02 \)) as depicted earlier in Table 3.10. The mean estimates of BMI illustrated in table 3.11 also show that those who were highly active had significantly lower BMI as opposed to those who were moderately active (27.56 kg/m\(^2\) versus 30.68 kg/m\(^2\)). These mean estimates for BMI however, were still high. Biochemical traits were similar with no statistical significant differences observed between the two groups.

### 3.12 Association of Physical Activity and HbA1c

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted ( \beta ) (CI)</th>
<th>( P )</th>
<th>Adjusted (1) ( \beta ) (CI)</th>
<th>( P )</th>
<th>Adjusted (2) ( \beta ) (CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>0.342 (-0.449 - 1.134)</td>
<td>0.39</td>
<td>0.299 (-0.523 - 1.122)</td>
<td>0.47</td>
<td>0.454 (-0.390 - 1.300)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

- Adjusted 1 = age and sex
- Adjusted 2 = age, sex and BMI (body mass index)
- The beta coefficient represents the change in HbA1c (kg/m\(^2\)) attributed to one unit of change for Physical activity. A p-value of <0.05 was employed for significance
The association test by way of a linear regression model (Table 3.12) to find a correlation between physical activity and HbA1c showed no significant association between PA and HbA1c at the crude level ($P = 0.39$), the age and sex adjusted model ($P = 0.47$) and also the combined adjustment model ($P = 0.28$).

### 3.4.2 Smoking

Gathered from self-reports, 95% of this particular cohort were non-smokers, with only 5% ($n = 4$) of the cohort that reported to be current smokers (Table 3.12), with three females and one male, reporting smoking. The non-smokers category includes those who don’t smoke and ex-smokers. The average age of the current smokers was calculated at 18.28 (SD 1.70), and the average of non-smokers was 17.18 (SD 1.60) with no significant difference ($p = 0.22$). The calculated mean BMI for current smokers was 32.78 kg/m$^2$ (SD 8.90), which places this particular cohort at a classification category of borderline obesity. Moreover, the mean BMI for non-smokers was 28.69 kg/m$^2$ (SD 5.8), which places this group in a classification category of overweight (according to the standards provided by WHO). Comparing the aforementioned means for BMI for the two groups, no significant differences were observed between the BMI values ($p = 0.18$).

No significant differences were observed in systolic blood pressure for smokers 107.75 (SD 2.8) mmHg, and non-smokers 112.49 (SD 11.6) mmHg ($p = 0.42$). Similarly, no significant differences were evident in diastolic blood pressure; where the mean for smokers was calculated at 64.5 (SD 6.2) mmHg versus non-smokers 61.6 (SD 9.1) mmHg, ($p = 0.54$).

Table 3.13: Data presented in mean and standard deviations of values between smokers and non-smokers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers</th>
<th>Non - smokers</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n, %</td>
<td>4, 5%</td>
<td>76, 95%</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>18.28 ± 1.70</td>
<td>17.18 ± 1.60</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>32.78 ± 8.90</td>
<td>28.69 ± 5.80</td>
<td>0.18</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>107.75 ± 2.88</td>
<td>112.45 ± 11.63</td>
<td>0.42</td>
</tr>
</tbody>
</table>
3.4.3 Alcohol Consumption

At screening, 42.5% of the cohort reported that they consumed alcohol within the last 12 months. Of this 42.5%, the reported average of weeks out of 52 where they had consumed an alcohol beverage was 17 weeks. 44.12% of this group was males (n=15) and 55.88% were females (n=19). 8% of alcohol drinkers reported to have at least one alcohol beverage in a typical working week (Monday to Thursday) varying from 5 – 8 drinks. All those whom reported to consume alcohol had a drink from Friday through to Sunday and it ranged from 0 – 30 drinks in total from Friday to Sunday, on weekends. The average however was approximately 11 drinks (SD 8.80). Of the 34 participants that reported consuming alcohol, 11% (n=4) reported to have a drink during a typical working week (Monday to Thursday). 61% (n=21) reported to have more than 6 drinks in total; the amount they would usually have over the course of three days (from Friday through to Sunday, on weekends). The range of drinks reported from Friday – Sunday varied from 0 – 30 drinks.

Table 3.13 presents the data divided into two categories: those who reported to have consumed alcohol and those who did not. Findings reflect that current drinkers were on average, approximately two years older than non-drinkers (P <0.001). The average age for current drinkers was 18.3 (SD 1.44) years and non-drinkers were 16.4 (SD 1.41) years. Furthermore, serum urate levels of those who consumed alcohol were significantly higher than those of non-drinkers (0.39 mmol/L versus 0.35 mmol/L, P = 0.02). The mean BMI for those whom consumed alcohol showed no significant difference from that of the average BMI of abstainers (30.21 kg/m² versus 27.91 kg/m², P = 0.09).
Table 3.1 indicates the mean and standard deviations of different variables for drinkers and abstainers

<table>
<thead>
<tr>
<th></th>
<th>Drinkers (n = 34)</th>
<th>Non-drinkers (n = 46)</th>
<th>P – Value¹</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beta (CI)</td>
<td>P Value²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% males</td>
<td>35.30%</td>
<td>39.10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>18.30 ± 1.44</td>
<td>16.40 ± 1.41</td>
<td>3.82 x 10⁻⁰⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.21 ± 5.20</td>
<td>27.91 ± 6.37</td>
<td>0.09</td>
<td>2.290 (-0.356 - 4.955)</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>113.85 ± 12.28</td>
<td>111.07 ± 10.67</td>
<td>0.28</td>
<td>2.787 (-2.337 - 7.913)</td>
<td>0.28</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>68.09 ± 8.85</td>
<td>59.33 ± 8.27</td>
<td>0.003</td>
<td>5.762 (1.925 - 9.598)</td>
<td>0.004</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>35.54 ± 2.97</td>
<td>34.85 ± 2.77</td>
<td>0.33</td>
<td>-0.917 (-2.303 – 0.467)</td>
<td>0.19</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.14 ± 0.72</td>
<td>4.01 ± 0.57</td>
<td>0.39</td>
<td>0.130 (-0.172 - 0.432)</td>
<td>0.39</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.30 ± 0.35</td>
<td>1.28 ± 0.24</td>
<td>0.77</td>
<td>0.198 (-0.115 - 0.155)</td>
<td>0.77</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.36 ± 0.62</td>
<td>2.30 ± 0.55</td>
<td>0.68</td>
<td>0.055 (-0.217 - 0.328)</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.39 ± 0.06</td>
<td>0.35 ± 0.07</td>
<td>0.02</td>
<td>0.036 (0.004 - 0.068)</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.07 ± 0.40</td>
<td>0.94 ± 0.42</td>
<td>0.19</td>
<td>0.130 (-0.063 - 0.325)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

- P – Value¹ of ≤ 0.05 was employed for significant difference between drinkers and non drinkers
- P – Value² of ≤ 0.05 was employed to determine significant associations between several variables with alcohol
- Beta-coefficient represents the change in each variable attribute to one unit of change in alcohol
- BP = Blood pressure, BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein.
The two-tailed pair test established that alcohol consumers were approximately two years older than non-drinkers ($P = 3.82 \times 10^{-07}$), thus implying a possible age confounding effect. By way of a linear regression model in STATA, measures presented in Table 3.13 were regressed and adjusted by age and sex. As aforementioned, the BMI between the two groups were trending towards significance ($P = 0.09$), as well as the unadjusted regression model by STATA ($P = 0.08$). However, the regression model adjusted by age and sex showed a significant increase of 3.379 kg/m² ($P = 0.03$) in BMI per unit increase of alcohol, thus inferring that the age and sex were confounding and by extension masking the original association of alcohol and BMI. For diastolic pressure, findings showed that drinkers had relatively higher diastolic blood pressure than non-drinkers (68.09 mm Hg vs. 59.33 mm Hg, $P = 0.003$).

To test for association of alcohol and diastolic blood pressure, the unadjusted model showed a significant increase of 5.676 mm Hg ($P = 0.004$) in diastolic blood pressure per unit increase of alcohol. Moreover, the adjustment by age and sex also conferred a greater increase of 5.868 mm Hg in diastolic blood pressure per unit increase of alcohol ($P = 0.01$). Given that a significant association was still evident when adjusted by age and sex, a conclusion can be drawn, that diastolic blood pressure was significantly higher in drinkers than non-drinkers, and this was not because drinkers were significantly older than non-drinkers or of a particular sex (male or female). No differences were observed in total cholesterol, HDL-cholesterol, and LDL-cholesterol between drinkers and abstainers. Raw data demonstrated that alcohol drinkers had significantly higher levels of serum urate (0.39 mmol/L) compared to non-drinkers (0.35, $P = 0.02$). Testing for association showed a significant increase of 0.36 mmol/L in serum urate per unit increase of alcohol. When adjusted by age and sex, the significance remained. The regression model showed that for every unit increase of alcohol, there is a significant increase in serum urate levels (Beta = 0.043 mmol/L, $P = 0.02$). Serum triglyceride levels were higher in drinkers than non-drinkers, although not significant (1.07 mmol/L vs. 0.94 mmol/L, $P = 0.19$). The test for association of serum triglycerides and alcohol revealed no association between these variables. However, the regression model when adjusted by age and sex showed a significant increase in serum triglycerides per unit of change of alcohol (Beta = 0.236 mmol/L, $P = 0.04$). This suggests that the confounding effects of age and sex masked the association of serum triglycerides with alcohol consumption.

Table 3.14 depicts that male drinkers had significantly higher ($P = 0.05$) BMI levels (31.52 kg/m²) than abstainers (26.71 kg/m²). By way of a linear regression model, the association of
alcohol and male drinkers was trending towards significance (P = 0.06), and when adjusted for age a significant increase of BMI was evident (P = 0.05), suggesting that for every unit increase of alcohol, there is a significant 5.203 kg/m² increase in BMI. It was evident, although not significant that male drinkers had high systolic and diastolic blood pressures than male non-drinkers. No association of blood pressure was established with alcohol in both regression models. Male drinkers had higher levels of total cholesterol than males that did not drink (4.58 mmol/L vs. 3.86 mmol/L, P = 0.003). A significant association was found between cholesterol and alcohol (P = 0.003), suggesting an increase of 0.719 mmol/L of cholesterol per every unit increase of alcohol. When the model was adjusted for age the significance remained (Beta = 0.745 mmol/L, P = 0.003). Triglyceride concentrations were evidently higher in drinkers than abstainers (1.20 mmol/L vs. 0.85 mmol/L, P = 0.01). Regression analyses models showed a positive correlation of alcohol with serum triglyceride levels, where the unadjusted model conferred a significant increase of 0.354 mmol/L (P = 0.01) in serum triglyceride levels for every unit increase in alcohol. Further, the adjusted model presented an even higher increase in serum triglycerides (0.380 mmol/L, P = 0.008) per unit increase of alcohol.

With the data at hand a conclusion can be made, that the association between alcohol and serum triglycerides in males is independent of age. HDL levels were slightly higher in those who consumed alcohol, however not significant. No association was established between alcohol and HDL-cholesterol in both unadjusted and adjusted models. Levels of LDL-cholesterol were also significantly higher in male alcohol consumers than those who did not consume alcohol (2.73 mmol/L vs. 2.19 mmol/L, P = 0.009). The unadjusted regression model showed a significant association of alcohol with LDL-cholesterol, suggesting an expected increase of 0.536 mmol/L in LDL-cholesterol per unit increase of alcohol. This model was also adjusted by age and conferred an even more significant increase of 0.564 mmol/L in LDL-cholesterol levels attributed to a unit of change in alcohol (P = 0.008). No significant differences were observed in levels of serum urate between male drinkers and non-drinkers, however levels in both drinkers and non-drinkers were relatively high (0.41 mmol/L for drinkers, and 0.38 mmol/L for non-drinkers). No association was established between alcohol and serum urate levels for males in both regression models.
Table 3.15: Summary statistics of male drinkers vs. male non-drinkers presented in mean and standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Drinkers (n = 15)</th>
<th>Non – Drinkers (n = 18)</th>
<th>P – value(^1)</th>
<th>Beta (CI)</th>
<th>P – value(^2)</th>
<th>Beta (CI)</th>
<th>P – value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17.60 ± 1.54</td>
<td>16.88 ± 1.45</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>118.07 ± 8.16</td>
<td>115.47 ± 9.31</td>
<td>0.42</td>
<td>3.344 (-2.963 - 9.652)</td>
<td>0.28</td>
<td>2.608 (-3.899 - 9.115)</td>
<td>0.42</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>61.92 ± 8.07</td>
<td>56 ± 8.97</td>
<td>0.09</td>
<td>4.944 (-1.000 - 10.889)</td>
<td>0.10</td>
<td>4.509 (-1.682 - 10.700)</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>31.52 ± 5.36</td>
<td>26.71 ± 8.18</td>
<td>0.05</td>
<td>4.811 (-0.214 - 9.837)</td>
<td>0.06</td>
<td>5.203 (-0.027 - 10.433)</td>
<td>0.05</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>35.10 ± 3.21</td>
<td>35.13 ± 3.33</td>
<td>0.98</td>
<td>-0.033 (-2.810 - 2.743)</td>
<td>0.98</td>
<td>0.262 (-2.364 - 2.888)</td>
<td>0.83</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.58 ± 0.54</td>
<td>3.86 ± 0.64</td>
<td>0.003</td>
<td>0.719 (0.264 - 1.175)</td>
<td>0.003</td>
<td>0.745 (0.272 - 1.218)</td>
<td>0.003</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.20 ± 0.32</td>
<td>0.85 ± 0.64</td>
<td>0.01</td>
<td>0.354 (0.087 - 0.622)</td>
<td>0.01</td>
<td>0.380 (0.106 - 0.655)</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.30 ± 0.38</td>
<td>1.28 ± 0.37</td>
<td>0.86</td>
<td>0.019 (-0.204 - 0.242)</td>
<td>0.86</td>
<td>0.004 (-0.226 - 0.235)</td>
<td>0.96</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.73 ± 0.46</td>
<td>2.19 ± 0.56</td>
<td>0.009</td>
<td>0.536 (0.142 - 0.930)</td>
<td>0.009</td>
<td>0.564 (0.157 - 0.972)</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.41 ± 0.04</td>
<td>0.38 ± 0.07</td>
<td>0.35</td>
<td>0.022 (-0.025 - 0.070)</td>
<td>0.35</td>
<td>0.029 (-0.018 - 0.077)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

- \(^1\) P – Value of ≤ 0.05 was employed for significant difference between drinkers and non drinkers
- \(^2\) P – Value of ≤ 0.05 was employed to determine significant associations between several variables with alcohol
- Beta-coefficient represents the change in each variable attribute to one unit of change in alcohol
- BP = Blood pressure, BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein.
The same analysis as above was made for females (Table 3.15). Female drinkers were significantly older than those who did not drink (18.78 years vs. 16.21 years, \( P = <0.001 \)). Therefore, these measures were adjusted to account for any confounding effects of age. Systolic blood pressures between female drinkers and non-drinkers were not statistically different and showed no association with alcohol (\( P_{\text{unadjusted}} = 0.62, \ P_{\text{adjusted}} = 0.27 \)). Diastolic pressure however appeared to be higher in female alcohol consumers (68.44 mm/Hg) compared to females who did not drink alcohol (61.00 mm/Hg, \( P = 0.003 \)). An association was also evident between diastolic blood pressure and alcohol (Beta = 6.789 mm Hg, \( P = 0.007 \)). By way of a regression age-adjusted model, findings demonstrated a significant increase of diastolic blood pressure in every unit of increase of alcohol (Beta = 8.43 mmHg, \( P = 0.02 \)). This suggests an age-independent phenomenon, where higher diastolic blood pressure in drinkers was not due to them being significantly older than non-drinkers. No association was established between BMI and alcohol in females in both regression models (\( P_{\text{unadjusted}} = 0.73, \ P_{\text{adjusted}} = 0.59 \)).

Total cholesterol mean estimates showed no significant difference between the two groups (3.82 mmol/L vs. 4.10 mmol/L, \( P = 0.12 \)). The regression model at crude level revealed a decrease in cholesterol levels by 0.285 mmol/L although not significant (\( P_{\text{unadjusted}} = 0.12 \)). When this regression model was adjusted by age, total cholesterol levels for females showed a significant decrease for every unit increase of alcohol (Beta = -0.578 mmol/L, \( P = 0.03 \)). No evidence of a significant difference was observed between the two LDL-C means of female drinkers and non-drinkers (2.08 mmol/L vs. 2.37 mmol/L, \( P = 0.11 \)). No association of LDL-C with alcohol was established in the unadjusted model. The age-adjustment regression model however, revealed that female drinkers had significantly lower LDL-C because they were older than female non-drinkers (Beta = -0.560 mmol/L, \( P = 0.02 \)).

Female drinkers also had higher serum urate levels than female non-drinkers (0.37 mmol/L vs. 0.32 mmol/L, \( P = 0.02 \)). An association was also established between serum urate levels of females and alcohol suggesting that for every unit of increase in alcohol, there is a significant increase of 0.044 mmol/L in serum urate levels (\( P_{\text{unadjusted}} = 0.03 \)). When this measure was adjusted by age, the association evaporated (Beta = 0.40 mmol/L, \( P_{\text{adjusted}} = 0.16 \)). This finding suggests that female drinkers had higher serum urate levels than non-drinkers because they were significantly older, and thus inferring that this was an age-dependent occurrence. No association of alcohol with female serum triglyceride levels and HDL-C was evident.
Table 3.16: Summary statistics of female drinkers vs. female non-drinkers presented in mean and standard deviation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Drinkers (N = 19)</th>
<th>Non-drinkers (n = 28)</th>
<th>P-value¹</th>
<th>Beta (CI)</th>
<th>P-value²</th>
<th>Beta (CI)</th>
<th>P-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.78 ± 1.13</td>
<td>16.21 ± 1.34</td>
<td>&lt;0.001</td>
<td>1.812 (-5.551 – 9.175)</td>
<td>0.62</td>
<td>5.795 (-4.723 – 16.314)</td>
<td>0.27</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.38 ± 14.61</td>
<td>108.00 ± 10.83</td>
<td>0.65</td>
<td>6.789 (1.907 - 11.671)</td>
<td>0.007</td>
<td>8.432 (1.402 - 15.461)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>68.44 ± 8.61</td>
<td>61.00 ± 7.66</td>
<td>0.003</td>
<td>-1.424 (-3.022 – 0.172)</td>
<td>0.08</td>
<td>-0.478 (-2.698 – 1.741)</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.17 ± 4.93</td>
<td>28.68 ± 4.89</td>
<td>0.73</td>
<td>0.492 (-2.447 - 3.431)</td>
<td>0.73</td>
<td>1.142 (-3.101 - 5.386)</td>
<td>0.59</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>34.35 ± 2.11</td>
<td>35.77 ± 2.79</td>
<td>0.80</td>
<td>-0.025 (-0.299 – 0.247)</td>
<td>0.85</td>
<td>0.080 (-0.318 - 0.480)</td>
<td>0.68</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.82 ± 0.68</td>
<td>4.10 ± 0.51</td>
<td>0.12</td>
<td>-0.285 (-0.650 – 0.797)</td>
<td>0.12</td>
<td>-0.578 (-1.101 - (-)0.056)</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.96 ± 0.42</td>
<td>0.99 ± 0.44</td>
<td>0.85</td>
<td>-0.025 (-0.299 – 0.247)</td>
<td>0.85</td>
<td>0.080 (-0.318 - 0.480)</td>
<td>0.68</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.30 ± 0.32</td>
<td>1.28 ± 0.26</td>
<td>0.82</td>
<td>0.020 (-0.159 – 0.200)</td>
<td>0.82</td>
<td>-0.056 (-0.319 - 0.206)</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.08 ± 0.58</td>
<td>2.37 ± 0.54</td>
<td>0.11</td>
<td>-0.284 (-0.632 – 0.638)</td>
<td>0.10</td>
<td>-0.560 (-1.058 - (-)0.062)</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum urate (mmol/L)</td>
<td>0.37 ± 0.06</td>
<td>0.32 ± 0.06</td>
<td>0.02</td>
<td>0.044 (0.004 - 0.084)</td>
<td>0.03</td>
<td>0.040 (-0.017 - 0.099)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

- P-value¹ of ≤ 0.05 was employed for significant difference between drinkers and non-drinkers
- P-value² of ≤ 0.05 was employed to determine significant associations between several variables with alcohol
- Beta-coefficient represents the change in each variable attribute to one unit of change in alcohol
- BP = Blood pressure, BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein
3.4.3.1 Self-reports of problems associated with drinking

Additionally, for the 34 alcohol drinkers, the following is an account of self-reports of problems provided by them that were caused by drinking as portrayed in Figure 3.2. Due to alcohol: 5% had problems with their families, 11% had problems with their friends and 17% were involved in physical fights (n = 6, 3 of which were male and the other 3 were females). 14% of the 34 continued to drink even though they were aware of their problems being caused by their drinking. Furthermore, 5% reported to be under the influence of alcohol in situations where they could have caused an accident or gotten hurt. 14% reported that their drinking and being hung-over often made them negligent of their responsibilities at home, work and caring for children (all females).

Only one of the 34 reported that there have been times where he felt a strong desire for alcohol, a craving for it. 24% (n=8, 6 of which were females) reported that there have been days where they have had more alcohol than they had intended and their drinking continued for more days in a row than they had intended. 11% (all females) had little time for anything else due to too much time spent drinking, and by extension getting over the effects of alcohol. And finally, 29% of drinkers have wanted to quit or cut-down in the past year and the majority were female. A further 29% have tried to quit or cut-down on their drinking and the majority was also female (Refer to Table 3.15 for number of male versus female self-reports).

| Table 3.17 reflect number of problems related to alcohol separated according to sex: Self-reports of controlled study |
|--------------------------------------------------|------------------|------------------|
| Problems with family                            | Males (Total n =15) | Females (Total n = 19) | Total % (out of 34) |
| Problems with friends                            | 0                 | 2                 | 5%                |
| Physical fights                                  | 3                 | 3                 | 17%               |
| Continued drinking knowing alcohol caused problems| 2                 | 3                 | 14%               |
| Accidents                                        | 1                 | 0                 | 5%                |
| Hangover causing negligence of responsibilities   | 0                 | 5                 | 14%               |
| Strong cravings                                  | 1                 | 0                 | 5%                |
| More than intended                               | 2                 | 8                 | 24%               |
| Little time for anything else                    | 0                 | 4                 | 11%               |
| Wanting to quit or cut down in the past year     | 3                 | 7                 | 29%               |
| Tried to quit or cut down                        | 4                 | 6                 | 29%               |
3.4.4 Sugar Consumption

Diet, as an environmental exposure, comes under scrutiny for its correlation with disease, including CVD, obesity and gout. Sugar has been underlined to be a prominent risk factor for metabolic diseases (R. J. Johnson et al., 2009). Sugar consumption has increased exponentially over the past decade, thus an emerging alarm that sugar may be somewhat liable for the increase in prevalence of non-communicable diseases (Johnson, Perez-Pozp et al., 2009; World Health Organization 2003). Several experimental studies have provided data, with much inconsistency as to the impact of sugar on known risk factors for metabolic diseases.

A total of 80 participants completed the food frequency questionnaire (FFQ), which was revised and adapted from Furter (2014). The survey produced records of total sugar intake per day, measured in grams per day, from sugar sweetened (non-alcoholic beverages), sugar - sweetened foods (not including fruit), and also from raw fruit (NaturalSug).

As mentioned earlier two self-administered questionnaires were employed to achieve the data presented in this thesis, the biological questionnaire as well as the food frequency questionnaire as a sole measure of sugar intake. The biological questionnaire however, also included a question by which participants were to self – report on the number of sugar-sweetened beverages they consumed on average per day, as opposed to the food frequency questionnaire where they self-reported on the different types of sugary drinks and foods in grams of total sugar per day. Therefore, the SSB data from both questionnaires were aligned against each other, and plotted on a scatter plot below (Figure 3.3). It was evident that there was a weak positive correlation ($r^2 = 0.30$ respectively), thus inferring that there could possibly be over – reporting as well as under-reporting in the food frequency questionnaire. Acknowledging the premise of over- and under-reporting in the FFQ, a possible explanation could be that the FFQ was a complicated way of obtaining sugar data. After all, the questionnaire required participants to recall on their consumption per day, over the previous month.

Nevertheless, the food frequency questionnaire was designed as the primary measure of sugar in this study; most of the (sugar) analyses used these data. The sugary drink data from the biological questionnaire was employed where necessary.
Figure 3.3: Correlation between sugar intake in grams per day, and frequency per day.

The FFQ data assembled were organized into quartiles according to sugar consumption shown in Table 3.18. The purpose for sorting the data into quartiles was to minimize the effect of extreme and inaccurate reporting. Samples in quartile 1 were low consumers of sugar, and those in quartile four were the highest consumers of sugar.

### 3.4.4.1 Sugar and BMI

For sugar-sweetened beverages (SSB) category (in the FFQ) it was evident that, with the increase in sugar-sweetened beverage consumption, there was an apparent increase in BMI upon comparison of low consumers of sugar in quartile one to high consumers of sugar in quartile four. Low consumers of sugar-sweetened beverages had a mean BMI of 26.69 kg/m², while the highest consumers of SSB had a BMI of 30.60 kg/m². Of note was the p – value; despite the emergence of a non-significant value (0.21), an assumption could be made that maybe with an increased sample size, more statistical power would be achieved, and perhaps establish a significant change in BMI across low to high consumers of sugary drinks.

No significant trend was observed in BMI across low consumers of (added sugar), sugar-sweetened foods (SSF) in quartile one, through to the high SSF consumers in quartile four (P = 0.66). The BMI of low and high consumers of fruit, or natural sugar were virtually similar and showed no significant trend across the four quartiles of consumers of natural sugar (P = 0.68).
3.4.4.2 Sugar and Serum Urate

An account for serum urate levels was also tabulated (Table 3.18) to investigate whether there was a trend of change in serum urate concentrations between low and high consumers of the three different categories of sugar.

For consumers of sugary drinks (SSB), no significant trend in serum urate levels was evident across the quartiles (P = 0.79). For sugar-sweetened foods (SSF), although there seemed to be a decrease in the mean of serum urate levels, by way of the ANOVA test, a non-significant trend was evident thus inferring no change in serum urate levels across the SSF quartiles (P = 0.34). The means of serum urate levels in the natural sugar category (sugar from fruits) seemed to be increasing across the four quartiles. However, very much like the SSB and SSF categories, the test of variance demonstrated a non-significant trend of change across the serum urate levels, based on consumption of natural sugar, or sugar from fruits.
Table 3.18 - difference in BMI and serum urate across the four quartiles of sugar intake. Data presented in mean and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SSB (g/day)</strong></td>
<td>29.52 ± 10.96</td>
<td>88.70 ± 23.27</td>
<td>220.25 ± 81.52</td>
<td>638.95 ± 191.49</td>
<td>-</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>26.69 ± 4.47</td>
<td>29.42 ± 5.25</td>
<td>28.90 ± 5.05</td>
<td>30.60 ± 8.17</td>
<td>0.21</td>
</tr>
<tr>
<td>Serum Urate (mmol/L)</td>
<td>0.37 ± 0.06</td>
<td>0.36 ± 0.09</td>
<td>0.37 ± 0.06</td>
<td>0.38 ± 0.05</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>SSF (g/day)</strong></td>
<td>41.55 ± 17.15</td>
<td>89.53 ± 15.33</td>
<td>181.31 ± 37.48</td>
<td>577.69 ± 370.39</td>
<td>-</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>29.81 ± 7.79</td>
<td>29.81 ± 7.79</td>
<td>27.99 ± 5.61</td>
<td>28.10 ± 4.67</td>
<td>0.66</td>
</tr>
<tr>
<td>Serum Urate (mmol/L)</td>
<td>0.39 ± 0.09</td>
<td>0.36 ± 0.06</td>
<td>0.38 ± 0.04</td>
<td>0.34 ± 0.07</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>NaturalSug (g/day)</strong></td>
<td>10.43 ± 7.69</td>
<td>39.68 ± 8.94</td>
<td>73.08 ± 12.08</td>
<td>256.29 ± 167.37</td>
<td>-</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>29.55 ± 8.56</td>
<td>28.58 ± 4.08</td>
<td>27.68 ± 6.39</td>
<td>29.76 ± 3.82</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum Urate (mmol/L)</td>
<td>0.35 ± 0.04</td>
<td>0.37 ± 0.07</td>
<td>0.36 ± 0.10</td>
<td>0.38 ± 0.05</td>
<td>0.17</td>
</tr>
</tbody>
</table>

- SSB = Sugar sweetened drinks (non-alcoholic beverages)
- SSF = Sugar sweetened foods (not including fruit)
- NaturalSug = Natural sugar (from fruits)
- *One-Way ANOVA test (P< 0.05 for significance)
### 3.4.4.3 Sugar and other biochemical parameters.

Table 3.19 Biochemical measures arranged by sugar sweetened beverage (SSB) quartiles

<table>
<thead>
<tr>
<th></th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.90 ± 0.54</td>
<td>3.94 ± 0.67</td>
<td>4.16 ± 0.70</td>
<td>4.25 ± 0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.96 ± 0.36</td>
<td>1.08 ± 0.59</td>
<td>0.97 ± 0.30</td>
<td>0.93 ± 0.34</td>
<td>0.66</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>34.47 ± 3.69</td>
<td>34.16 ± 9.08</td>
<td>34.50 ± 2.32</td>
<td>35.84 ± 1.89</td>
<td>0.17</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.29 ± 0.19</td>
<td>1.21 ± 0.30</td>
<td>1.23 ± 0.15</td>
<td>1.43 ± 0.38</td>
<td>0.27</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.16 ± 0.47</td>
<td>2.24 ± 0.61</td>
<td>2.49 ± 0.59</td>
<td>2.40 ± 0.59</td>
<td>0.57</td>
</tr>
</tbody>
</table>

- HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol
- P value ≤ 0.05 was employed for significance
<table>
<thead>
<tr>
<th></th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.96 ± 0.70</td>
<td>3.95 ± 0.71</td>
<td>4.12 ± 0.68</td>
<td>4.20 ± 0.46</td>
<td>0.39</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.94 ± 0.44</td>
<td>1.04 ± 0.32</td>
<td>0.96 ± 0.40</td>
<td>1.02 ± 0.49</td>
<td>0.93</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>33.63 ± 8.64</td>
<td>33.33 ± 8.82</td>
<td>34.94 ± 3.17</td>
<td>35.16 ± 2.28</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.22 ± 0.25</td>
<td>1.29 ± 0.22</td>
<td>1.35 ± 0.33</td>
<td>1.29 ± 0.31</td>
<td>0.56</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.31 ± 0.57</td>
<td>2.19 ± 0.75</td>
<td>2.33 ± 0.48</td>
<td>2.44 ± 0.50</td>
<td>0.33</td>
</tr>
</tbody>
</table>

- HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol
- P value ≤ 0.05 was employed for significance
<table>
<thead>
<tr>
<th></th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.06 ± 0.61</td>
<td>3.98 ± 0.53</td>
<td>3.91 ± 0.65</td>
<td>4.28 ± 0.73</td>
<td>0.28</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.99 ± 0.41</td>
<td>0.85 ± 0.35</td>
<td>1.05 ± 0.53</td>
<td>1.06 ± 0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>33.21 ± 3.14</td>
<td>34.52 ± 2.63</td>
<td>36.35 ± 3.48</td>
<td>34.90 ± 1.86</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.27 ± 0.24</td>
<td>1.33 ± 0.30</td>
<td>1.31 ± 0.37</td>
<td>1.24 ± 0.22</td>
<td>0.73</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.35 ± 0.56</td>
<td>2.25 ± 0.52</td>
<td>2.11 ± 0.46</td>
<td>2.55 ± 0.68</td>
<td>0.11</td>
</tr>
</tbody>
</table>

- HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol
- P value ≤ 0.05 was employed for significance
Illustrated above (Table 3.19) are the mean estimates of other biochemical traits organized by SSB quartiles. All biochemical traits showed no significant trend across the lowest consumers of sugar sweetened beverages (Quartile 1) and the highest quartile (Quartile 4).

Table 3.20 shows mean estimates of biochemical trait concentrations divided into lowest to highest consumption of sugar-sweetened foods, of which exclude fruit. No significant trends were observed between biochemical traits across Quartile 1 to quartile 4.

Table 3.21 illustrates the mean estimates of biochemical traits stratified by natural sugar from fruits, where quartile one is the lowest consumption of fruits, and the highest consumption of fruits is quartile four. All traits showed no significant trends of change in biochemical traits between low to highest consumption of fruit quartiles.
3.5 Genetics

3.5.1 SLC2A9 and serum urate

Studies have confirmed that 3.70% of the phenotypic variance in serum urate levels of those with European descent is explained by genetic variants within SLC2A9. Furthermore, variants of the SLC2A9 gene have been documented to have a robust correlation with gout in European, New Zealand Māori, and New Zealand Pacific Island sample sets (Kottgen et al., 2013; Hollis-Moffatt et al., 2009). However, very little is known about the association of this locus with serum urate and gout in youth, certainly not adolescents of Pacific Island descent.

Two genetic variants of the SLC2A9 gene that have been reported of late to be associated with serum urate levels, were investigated in this study for evidence of association with serum urate. The rs11942223 variant has been established to strongly influence serum urate levels in Europeans, and also the rs3775948, which has showed a strong association with serum urate levels in Asian samples (Okada et al., 2012).

In consort with inherited genetic variants, dietary factors such as sugar-sweetened beverage and alcohol consumption influence serum urate levels and gout risk including Polynesians (Batt et al., 2014, Choi et al., 2004, Choi and Curhan, 2008, Choi et al., 2008, Rasheed et al., 2013). Thus this section will also test for the interaction of genetic variants of this gene with sugar-sweetened beverages in the Pacific Island teenagers’ sample set.
Table 3.22 illustrates the association analyses of rs11942223 and rs3775948 with serum urate.

<table>
<thead>
<tr>
<th>Genotype Distributions (rs11942223)</th>
<th>Association of serum urate with rs11942223</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td>MAF (C)</td>
</tr>
<tr>
<td>67 (0.850)</td>
<td>0.07</td>
</tr>
<tr>
<td>11 (0.141)</td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype Distributions (rs3775948)</th>
<th>Association of serum urate with rs3775948</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td>MAF (G)</td>
</tr>
<tr>
<td>23 (0.281)</td>
<td>0.449</td>
</tr>
<tr>
<td>41 (0.518)</td>
<td></td>
</tr>
<tr>
<td>15 (0.189)</td>
<td></td>
</tr>
</tbody>
</table>

- Adjusted 1 = Age and sex
- Adjusted 2 = Age, sex and BMI
- Adjusted 3 = Age, sex, BMI, sugar consumption (SSB g/day) and physical activity
The association analysis of the genetic variant rs11942223 with serum urate demonstrated no significant correlation between this particular variant and serum urate levels at unadjusted value (Beta = 0.008, $P = 0.72$). To account for confounders this model was adjusted in attempt to examine further whether there was a direct association between the SLC2A9 rs11942223 and serum urate. The adjusted models all showed no evidence of direct association between this SNP and serum urate (Table 3.22). The association, or lack thereof, of the rs11942223 genetic variant with serum urate established in this study is not consistent with the evidence in literature, which pronounces a direct association of this SNP with urate levels, however, no evidence in literature has pronounced any association of this variant with urate in Pacific people. The genetic variant rs3775948 also showed no association with serum urate levels. Following multiple adjustments for confounding factors, no sign of significant trends were apparent. A limitation of these results could possibly be the small sample size ($n = 80$), hence low statistical power. Perhaps with a greater cohort, we will be able to assess the true relationship between these genetic variants with serum urate to illuminate the relationship of serum urate with these specific variants of the SLC2A9 gene.

Table 3.23: Interaction terms between sugar intake (g/day) and rs11942223 genotype for serum urate (mmol/L)

<table>
<thead>
<tr>
<th>Sugary drink consumption (grams/day) as continuous variable</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta (95% CI)</td>
<td>P value</td>
<td>Beta (95% CI)</td>
</tr>
<tr>
<td>-0.0001 (-0.0005 - 0.0002)</td>
<td>0.40</td>
<td>-0.0007 (-0.0007 - 0.0006)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex and BMI

Table 3.23 exemplifies the gene – environment interaction. This test was carried out to investigate whether rs11942223 had any interaction with sugar consumption (SSB) in grams per day (data from FFQ) for an effect on serum urate levels. Findings demonstrated no significant interaction between rs11942223 and sugar consumption in the unadjusted model (unadjusted $p_{\text{interaction}} = 0.40$) as well as the adjusted model (adjusted $p_{\text{interaction}} = 0.84$).
Table 3.24: Interaction terms between sugary drink consumption (frequency per day) and rs11942223 genotype for serum urate (mmol/L)

<table>
<thead>
<tr>
<th>Sugary drink consumption (frequency per day) as continuous variable</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta (95% CI)</td>
<td>P value</td>
<td>Beta (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>-0.005 (-0.057 – 0.045)</td>
<td>0.81</td>
<td>0.008 (-0.040 – 0.57)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex and BMI

Batt and associates (2014) recently carried out a study by which they set out to test for non-additive interaction between SSB consumption and SLC2A9 genotype. Their interaction data suggested that consumption of simple sugars that originate from SSB had a physiological influence on the SLC2A9-mediated renal uric acid excretion (Batt et al., 2014). Therefore, as mentioned earlier, the biological questionnaire for this study had a question for self-reports of the number of sugar-sweetened beverages consumed per day. Table 3.24 shows the interaction terms between the sugar consumption data from the biological questionnaire in amounts per day, and the rs11942223 genotype for serum urate. Findings show that SSB consumption (frequency per day) had no significant interaction with rs11942223 for an influence on serum urate levels in both the unadjusted (unadjusted $p_{interaction} = 0.81$) and adjusted models (adjusted $p_{interaction} = 0.73$).

Table 3.25: Interaction terms between sugar intake (g/day) and rs3775948 genotype for serum urate (mmol/L)

<table>
<thead>
<tr>
<th>Sugary drink consumption (g/day) as continuous variable</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta (95% CI)</td>
<td>P value</td>
<td>Beta (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>$6.10 \times 10^{-7}$ (-0.000 – 0.000)</td>
<td>0.98</td>
<td>0.00002 (-0.00004 – 0.00008)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex and BMI

Following the same rule of analysis for table 3.23, the analysis was also performed to investigate for any interaction between the second SNP of interest- rs3775948 with sugar intake in grams per day, for serum urate. Results tabulated in table 3.25 showed no significant interaction of this SNP with sugary drink consumption for serum urate, in the unadjusted model (unadjusted $p_{interaction} = 0.98$). Adjustments were also made for common confounders, i.e. age, sex and BMI and the results showed no significant interaction (adjusted $p_{interaction} = 0.51$; Table 3.25).
Interaction terms between SSB (frequency per day) and rs3775948 genotype showed no significant interaction for an influence on serum urate levels. As shown in Table 3.26, the interaction at the crude level showed no significant interaction (unadjusted $p_{interaction} = 0.47$), as well as the adjusted model (adjusted $p_{interaction} = 0.68$).

Overall, the exploratory findings from this section produced no evidence to support any interaction between both sugar data SSB (frequency per day) and sugar consumption (g/day), and the genetic variants of the SLC2A9 gene investigated in this study, to cause influence on serum urate levels.

### 3.5.2 FTO and BMI

A direct association between the function and expression of FTO and obesity-related is poorly understood. Recently, several studies have suggested that IRX3 is likely the new “fat gene” contender. The obesity-associated FTO region has been established to network directly with the promoters of the IRX3 as well as the FTO in the genomes of mice, zebra fish and humans (Smemo et al., 2014), thus suggesting that the causal gene at the FTO locus is IRX3. Smemo and colleagues (2014) findings revealed that IRX3 is a functional long-range marker of obesity-related variants within FTO and signifies a new determining factor of body mass and composition. For the purposes of this section, two variants from the FTO gene were selected (rs9922047 and rs9923233) to investigate for an association with BMI in this particular cohort. As variations within the FTO have been found to be associated with the obesity phenotype in several European populations, this is a first attempt to investigate these two FTO variants for an association with BMI in a Pacific adolescents cohort, with the hope to illuminate the relationship between these variants of FTO with obesity and BMI in a Pacific population.
Table 3.27 illustrates association analysis of FTO genetic variants with BMI

<table>
<thead>
<tr>
<th>Genotype Distributions (rs9922047)</th>
<th>Association of BMI and rs9922047</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>GC</td>
<td>MAF (C)</td>
</tr>
<tr>
<td>CC</td>
<td>CC</td>
</tr>
<tr>
<td>31 (0.397)</td>
<td>35 (0.449)</td>
</tr>
<tr>
<td>CC</td>
<td>CC</td>
</tr>
<tr>
<td>12 (0.153)</td>
<td>0.378</td>
</tr>
</tbody>
</table>

- Adjusted 1 = age and sex
- Adjusted 2 = age, sex and physical activity
- Adjusted three = age, sex, physical activity and sugar consumption
Table 3.27 shows the association analysis of the gene FTO with BMI, and did not provide any significant associations in this model. Even after adjusting for multiple confounders that could possibly affect this test, in the end no demonstration of a significant trend was evident.

The impelling cause behind the vivid increasing prevalence of obesity over the past years has been attributed to the vicissitudes in lifestyle, of which include an increase in energy intake and the absence of physical activity (Bouchard, 2008, Hill, 2006, Papas et al., 2007). Furthermore, the increase in physical activity levels has also been shown to have an association with reduced metabolic risk and body corpulence (Ekelund et al., 2007). Genetic predisposition and lifestyle factors alike have been established to increase susceptibility to obesity, and the scientific discoveries presented by genome-wide association studies have made way for the thriving recognition of a great quantity of obesogenic loci (Frayling et al., 2007, Loos et al., 2008, Pichler et al., 2013, Scuteri et al., 2007, Thorleifsson et al., 2009, Willer et al., 2009). One of the most immense investigations of a gene x physical activity interaction in obesity is for the FTO locus (Andreasen et al., 2008, Rampersaud et al., 2008). Li and colleagues (2010) in their findings conveyed a significant interaction between their SNPs of interest and physical activity for an effect on BMI/obesity, and thus showed that being physically active can modify the genetic tendency to obesity.

Therefore, to investigate the association between the FTO SNP’s of interest and physical activity for an altering-effect on BMI, an interaction analysis was carried out by including the FTO (SNP) – Physical activity interaction term in an additive model, also adjusting for sex and age (refer to Tables 3.28 and 3.29).

Table 3.28: Interaction terms between physical activity and rs9922047 genotype for BMI (kg/m²)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Coefficient (95% CI)</td>
<td>P</td>
<td>β Coefficient (95% CI)</td>
</tr>
<tr>
<td>1.134 (-4.432 - 6.702)</td>
<td>0.68</td>
<td>0.994 (-4.589 - 6.577)</td>
</tr>
</tbody>
</table>

* Adjusted for age and sex

Table 3.28 represents the interaction analysis between physical activity and the rs9922047 SNP for an effect on BMI. The results reflected no significant interaction between physical activity and rs9922047 for an effect on BMI levels.
Table 3.29: Interaction terms between physical activity and rs9923233 genotype for BMI (kg/m²)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Coefficient</td>
<td>P</td>
<td>β Coefficient (95% CI)</td>
</tr>
<tr>
<td>-0.542 ( -4.932 - 6.018)</td>
<td>0.84</td>
<td>-0.438 ( -5.100 – 5.978)</td>
</tr>
</tbody>
</table>

*Adjusted for age and sex

The same analysis was carried out for the second SNP of interest rs9923233 as shown above in Table 3.29. No evidence of a gene-environment interaction manifested from this testing. That is, the FTO genetic variant rs9923233 showed no significant interaction with physical activity for an effect on BMI levels.

From these results, a conclusion can be drawn that the two genetic variants of the FTO gene that were tested in this study, exhibited no significant interactions with physical activity for an influence on BMI levels.
CHAPTER 4: DISCUSSION AND CONCLUSION
4.1 Baseline findings

Participants (n = 80) were of Pacific Island descent, aged between 15 and 20 years with a mean of 17.4 years (SD 1.67), with a higher proportion of females (58.75%) than males (41.25%). Baseline characteristics of the cohort showed biochemical measures (refer to Table 3.1) were well within reference ranges. Separated by sex, males were statistically taller (176.17 cm vs. 166.09 cm, P = <0.01) and heavier (89.44 kg vs. 79.69 kg, P = 0.02) compared to females. The results showed the total mean estimate for BMI was 28.89 kg/m².

It has been established that ethnicity is a confounding factor for the developed international BMI standards (Gallagher et al., 2000). Several studies have revised a Māori-specific BMI standard to determine differences in body composition in European and Polynesian people living in New Zealand, where the Polynesian population was a pooled sample of Māori and Pacific Island subjects (Rush et al., 1997, Swinburn, 1998, Swinburn et al., 1999). The findings from these studies showed that there was a higher percentage of body fat and fat-mass in Polynesians, but at any given BMI, NZ Europeans had higher fat mass than Polynesians. Furthermore, upon comparison, an increase of 3 – 5 kg/m² in BMI was associated with higher fat mass but only evident in NZ Polynesians and not NZ Europeans, even with the two populations having the same level of fatness (Swinburn, 1998, Swinburn et al., 1999). Therefore, a proposal was made by the authors that there ought to be an increase of the normal standard ranges by two units when defining Polynesian BMI categories (healthy, overweight or obese) (Swinburn et al., 1999). In 2003, these recommendations were incorporated into the National Cardiovascular guidelines (NZGG, 2003), and were later reverted back to the standard international cut-off points to comply with the existing international practice. Therefore, although there were no significant differences between the BMI of males and females, the relative BMI for both sexes as well as the mean estimated BMI of the cohort as a whole translates into a classification category of overweight, by employment of the international BMI cut-off points. Males had significantly higher systolic blood pressure than females (116.64 mm Hg vs. 109.36 mm Hg, P = 0.005), and to the contrary females had higher diastolic pressure than males (Males = 59.00 mm Hg vs. Females = 63.91 mm Hg, P = 0.001).

In New Zealand, gout is estimated to occur in 3.24% of Europeans, 6.06% of Māori and 7.63% of Pacific people over the age of 20 years old (Winnard et al., 2012). Sex-related urate differences are well documented, with males having considerably higher urate levels than
females (Zhu et al., 2011). Consistent with the established knowledge on sex differences, the mean estimate for serum urate in males was higher than that of females. (0.39 mmol/L vs. 0.34 mmol/L, P = 0.02).

4.2 Comparison with Grant and associates’ findings

This section will discuss the comparisons made between results from this study and the findings established by Grant and associates (2008) in both community samples of Pacific Island teenagers. One of Grant’s objectives aimed to examine the components of the metabolic syndrome in their sample. The metabolic syndrome is a disorder that results from the growing incidence of obesity (Eckel et al., 2005). In 2005, the International Diabetes Federation produced a new set of criteria and released a consensus for identifying the metabolic syndrome. Their definition required the existence of abdominal obesity as well as any of the following factors: elevated triglycerides, low high density lipoprotein cholesterol (HDL-C), elevated blood pressure or raised fasting plasma glucose (Alberti et al., 2005). However, the definition employed by Grant and associates to diagnose the metabolic syndrome in their cohort was adapted from Cruz and associates (Cruz et al., 2004) which was based on having three of the following risk factors; abdominal obesity, low high-density lipoprotein cholesterol (HDL-C), hypertriglyceridemia, high blood pressure and/or glucose intolerance. The definition by Cruz was also used to identify components of the metabolic syndrome in this present study.

The present study found components of the metabolic syndrome were higher in individuals with higher BMI, or in the overweight and obese categories (Figure 4.1). This is consistent with the findings established in Grant et al., (Figure 4.2) and also other studies (Gutin et al., 2004, Weber et al., 2014) that also found that with the increase in BMI category (from healthy weight to overweight/obesity), the number of metabolic risk factors increased. The study by Grant et al (2008) showed waist circumference to be the most common component of the metabolic syndrome present in their cohort, followed by low HDL-C, high blood pressure and elevated triglyceride levels (Table 3.4). Consistent with the previous study, high waist circumference, an indicator of abdominal obesity was also the most common component of the metabolic syndrome in this study. Increased body composition measures including BMI and abdominal obesity (waist circumference) have been previously reported to be associated with an increased
risk of heart disease as well as its associated risk factors (Despres et al., 2008, Shai et al., 2006, Wannamethee et al., 2005, World Health Organization, 2000, Yusuf et al., 2005).

In this 2013 - 2014 community sample of Pacific Island teenagers, 6% of the cohort met the full criteria for metabolic syndrome compared to the 7% reported previously by Grant and associates (2008).

Figure 4.1 shows percentage of each category and the number of MS components (Present study)

Figure 4.2 shows percentage of each category and the number of MS components (Grant et al., 2008)
Figures 3.2 and 3.3 present the number of risk factors for the metabolic syndrome in the two cohorts. Figure 3.2 illustrates the findings from this study, replicating that of Figure 3.3, which reflects findings from Grant and associates’ study. It was evident that those with healthy BMI had fewer numbers of risk factors for the metabolic syndrome. The above figures show that the components of the metabolic syndrome were predominantly detected in individuals that were obese, for both studies. Therefore the findings from this study, together with Grant’s, translate into the higher the BMI, the greater the number of metabolic syndrome components, thus the higher the risk of cardiovascular disease and diabetes. It is worth to note a report by the Framingham Heart Study established a similar finding that reported overweight and obese individuals who did not have the metabolic syndrome were at low risk of diabetes, and those with the metabolic syndrome were at a significantly higher risk of diabetes “regardless of BMI status” (Meigs et al., 2006).

It has almost been ten years since Grant’s study took place. An interesting find upon comparison was the levels of alcohol consumption and smoking. Over the course of approximately 8 years (2006 – 2014), the number of smokers decreased from Grant’s 15 smokers (total = 18.75%; 13.75% females, 5% males) to this study’s 4 self-reports (total = 5%; 3.75% females, and 1.25% males) of smoking. This is consistent with the reports that have revealed the rates of smoking in New Zealand declining since 1970’s (Ministry of Health, 2006b, Ministry of Health, 2010), and the same could be said about the trends of smoking in Pacific people aged 15 years old and older living in New Zealand. Between the years of 1990 – 2012/2013, the prevalence of smoking declined from 38% to 25% in Pacific peoples when compared to the general European population of New Zealand whose prevalence of smoking in the years 2012/2013 was 15% (Ministry of Health, 2013, Tala Pasifika, 2010). A further decrease in Pacific smokers was observed in 2013, dropping to 23% compared to 15% for all New Zealanders (Scoop Media, 2013, Statistics NZ, 2013a).

Despite the overal decline in tobacco use in New Zealand, the disparity in the prevalence of smoking amid lower and higher socioeconomic status, and between different ethnic groups has intensified (Ministry of Health, 2009). Dating back to between 2009 and 2012, compared to the general European population of New Zealand, Māori and Pacific peoples smoking rates were significantly higher. The high smoking rates in some of these populations were also, like this study, more prevalent in females; the smoking prevalence for European New Zealanders was 16% in males and 17% in females, the Māori population in New Zealand, smoking prevalence
was also higher in women (44%) than men (38%), and finally the statistics for Pacific people showed a trend in the opposite direction where males had higher smoking prevalence (28%) than females (25%) (Ministry of Health, 2009, Ministry of Health, 2012b). While current statistics on Pacific adults dictate the habitual use of tobacco in more Pacific men than women (Salmond et al., 2006), the patterns of smoking in Pacific adolescents indicate that Pacific girls are more likely to be smokers than males and this was reflected in Grant’s study who reported 15 self reports of smoking, 11 of which were female (13.75% of the total cohort). Furthermore, while smoking rates dropped in 8 years, this study also found that of the 4 self-reports of smoking (5%), three were females (3.75% of the total cohort). These findings confirmed that youth females smoked more than males; the more recent study by Teevale and colleagues (2013) found that Pacific youth girls were known to be smoking at virtually twice the degree (15.4%) of Pacific youth boys (8.5%).

There is limited knowledge as to why Pacific young people smoke. However, studies have shown that the main risk factors for smoking in adolescents are when parent (s) or the networks around them smoke (Scragg et al., 2010, Teevale et al., 2013). While there are no known interventions put in place by the Pacific Trust Otago following Grant’s study, a plausible explanation as to the drop in smoking rates among teenagers in this region (Dunedin), could be tied to the already recognized public health interventions set in place, which reflect the tackling of the high smoking rates in New Zealand; an issue that is nationally of high priority. Confronting the issue includes the Smoke-free Environments Amendment Act (The Parliament of New Zealand, 2003) which bans smoking at, and in public locations including indoor areas of licensed grounds, workplaces and schools. Additionally is the incessant rise in taxes of tobacco products, and the accepted legislation of adding warning labels on cigarette packets. These are added onto the law of restricting the selling of cigarettes to minors (under 18 years of age).

Despite the laws put in place by the government to decrease the rates of smoking in both adults and teenagers alike, there still seems to be some lack of law enforcement where illegal, under-aged purchasing of cigarettes is occurring frequently at small local stores (dairies) (Teevale et al., 2013). Thus the need to further support the protocols that stress limiting the access of youth to cigarettes by penalizing vendors who continue to sell cigarettes to adolescents younger than 18 years of age, and further increasing the cost of cigarettes (Wong et al., 2007). The increase in the pricing of cigarettes has been proved effective in the attempts of quitting smoking among young Pacific people (Glover et al., 2010).
The numbers for alcohol consumption took an opposite turn compared to smoking. An apparent increase was evident in the numbers of alcohol drinkers upon comparison of the two studies. Grant reported 17.50\% (\(n = 14\)) of their cohort to be drinkers with a higher proportion of male drinkers (11.25\%, \(n = 11\)) than females (3.75\%, \(n = 3\)). Additional to the increase in total number of drinkers in this study (42.50\%, \(n = 34\)), this study established a higher percentage of female drinkers (23.75\%, \(n = 19\)) than male drinkers (18.75\%, \(n = 15\)). This increase in alcohol consumption raises the need for the Pacific Trust Otago to put an intervention in place that would tackle the high rate of alcohol consumption in Dunedin teenagers, as alcohol has been reported and known to have negative effects on human behaviour and also it’s popularity to be a risk factor for certain disorders (as discussed more in detail, later in the Alcohol section of this chapter).

## 4.3 Important Clinical Measure

### 4.3.1 HbA1c and diabetes

Diabetes mellitus is the most common endocrinological disorder, characterized by metabolic risk factors and enduring complications (Pickup, 1997), with a growing number of 135 million in 1995 to a predicted 380 million in 2025, according to a meta-analysis and review by Singh and colleagues (Singh et al., 2009). The most recent report by the Ministry of Health (2014) with national rates of diabetes reflected that diabetes was highest amongst Pacific (9.5\%) and Māori (7.6\%) people when compared to the general European population of New Zealand (4.9\%).

In this present study, the measure for diabetes used was HbA1c - a modified form of haemoglobin. The measure of HbA1c is a globally employed marker of chronic glycaemia, showing the average blood glucose levels over a two to three month period of time (American Diabetes Association, 2010). Research has shown that increasing levels of HbA1c is prognostic for development of T2D later in life (Mook-Kanamori et al., 2014) thus implying the higher a person’s HbA1c, the greater the risk of diabetes, and by extension diabetes-related complications.

None of the participants in this study reported having diabetes. 6.5\% of the 77 available samples for testing were indicative of pre-diabetes with a mean estimate of 42.00 mmol/mol or 6.0\% by the old units. The known clinical cut-off provided by the NZSSD for pre-diabetes is between 41
– 49 mmol/M. However, literature has indicated that multiple studies have debated the generally accepted clinical HbA1c cut-off to demarcate prediabetes (Ackermann et al., 2011, Bennett et al., 2007, Buell et al., 2007, Cheng et al., 2011, Choi et al., 2011, Heianza et al., 2011). This debate appears to be lingering still with at least three differing cut-offs (42.10 mmol/mol, 38.8 mmol/mol and 36.36 mmol/mol) autonomously recommended by professional establishments (American Diabetes Association, 2010, Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003, International Expert Committee, 2009). Furthermore, a study in Qatar found an increased risk of T2D when the cut-off for HbA1c was lowered to 5.7% (39 mmol/mol) (Mook-Kanamori et al., 2014). Therefore, given the ongoing debates as to which cut-off point is necessary, this study included the sample with an HbA1c level of 40 mmol/mol (5.8%) in the pre-diabetes samples.

Evidence from this study reflected the most common factors out of range in pre-diabetes samples were serum urate and high BMI. This finding, at baseline in our pre-diabetic samples is comparable with a previous study on middle-aged adults who did not have a history of diabetes. Their results showed that participants with elevated levels of HbA1c had higher BMI and adverse lipid profiles (Selvin et al., 2010). In addition, from this present study, at baseline, the prediabetic samples were on average 16.4 years (SD 1.82) of age with a mean BMI of 29.69 kg/m². One participant in the pre-diabetes samples in particular showed interesting results. At the age of 15 years, this female participant had the highest recorded HbA1c level (45 mmol/mol), a serum urate level of 0.42 mmol/L and a BMI of 38.75 kg/m² Earlier research has shown Māori and Pacific people with T2D are much younger, more likely to be obese and have poorer glycaemic control (Tomlin et al., 2006).

Furthermore, it was also observed that serum urate levels were out of range in pre-diabetic samples. However, by way of a simple linear regression model, results from this present study (Table 3.8) did not show any significant association of serum urate with HbA1c, even after adjusting for confounding factors ($P_{\text{unadjusted}} = 0.38$, $P_{\text{adjusted}} = 0.58$). These findings were conflicting with findings from a previous study of European adults, (Choi and Ford, 2008) which found serum urate levels increased with increasing HbA1c. In addition, another report also found a statistically significant positive correlation ($r = 0.092$) between serum urate and HbA1c, thus suggesting an increase in serum urate with an increase of HbA1c (Gill et al., 2013). The increase in serum urate as HbA1c increases could be justified by the mechanisms that evoke a link between increased serum urate production and hyperinsulinaemia.
Choi and Ford (2008) also found that in their sample, pre-diabetic individuals were at a higher risk of hyperuricaemia and gout, particularly in women. Of note in this study was the highest HbA1c level belonged to a female of 15 years of age, a BMI indicative of extreme obesity (38.75 kg/m²), and a serum urate level of 0.42 mmol/L which corresponds to the findings by Choi and Ford.

4.3.2 The effects of BMI on lipid concentrations

This study was not able to obtain any significant associations between BMI and other clinical traits such as total cholesterol, HDL-C, LDL-C and HbA1c (Table 3.9). Significant associations however were established with serum urate (P = 0.01) and triglycerides (P = 0.01).

4.3.2.1 BMI and Serum urate

Elevated levels of serum urate have been widely studied and known to co-exist with the components of the metabolic syndrome, of which includes obesity (Costa et al., 2002, Klein et al., 1973, Oyama et al., 2006, Schmidt et al., 1996). The vicissitudes in serum urate have been credited to a gradual decline in the clearance of urate at the stages of adolescence (Munan et al., 1977, Stapleton et al., 1978). Thus, it has been hypothesized that high renal retention could be the causal mechanism leading to hyperuricaemia (Garbagnati, 1996).

Consistent with the literature (Gao et al., 2010, Ishizaka et al., 2010, Lyngdoh et al., 2012a, Lyngdoh et al., 2012b), this study found a strong positive correlation between BMI and serum urate (Table 3.9). The regression models from this study provide evidence supporting the association of BMI and serum urate levels at both the unadjusted and adjusted models; thus suggesting that a high BMI has a mediating effect on raising serum urate at the crude level and also the adjustment models for age and sex, and the combined adjustment (2) with physical activity: ($\beta_{\text{unadjusted}} = 0.002 \text{ mmol/L}, P = 0.05$, $\beta_{\text{adjusted1}} = 0.02 \text{ mmol/L}, P = 0.02$, $\beta_{\text{adjusted2}} = 0.03 \text{ mmol/L}, P = 0.01$).

The association between BMI and serum urate from this study is in line with other studies (Lyngdoh et al., 2012b, Oikonen et al., 2012) that have employed a widely-used and now popular method: the Mendelian randomisation method. (C. Reactive Protein Coronary Heart Disease Genetics Collaboration et al., 2011, Casas et al., 2005, De Silva et al., 2011, Fall et al.,
2013, Holmes et al., 2014a, Holmes et al., 2014b, Klerk et al., 2002, Lim et al., 2014, Pichler et al., 2013, Voight et al., 2012, Welsh et al., 2010). Lyngdoh et al., and (2012b) and Oikonen et al., (2012) in their studies provided evidence of a causative role of adiposity on elevating serum urate levels, but not the reverse. Lyngdoh and associates (2012b) achieved their results by employment of genetic variants of the FTO, MC4R and TMEM18 genes as instruments to elucidate the influence of adiposity on serum urate levels. The causal positive correlation was not entirely unforeseen and results attuned to the premise that hyperinsulinaemia, a result of overweight and obesity, increases renal proximal tubular reabsorption of serum urate, and as a consequence, elevating levels of serum urate (Facchini et al., 1991). These findings further support the existing evidence that suggests a decrease in weight results in a decline in serum urate levels, whereas the increase in weight consequently results in the increase in serum urate; thus conferring a causal role of adiposity on the fluxes of serum urate levels (Gao et al., 2010, Ishizaka et al., 2010, Zhu et al., 2010).

4.3.2.1.1 Summary

In summary, the underlying mechanisms as to the relationship between urate and different measures of adiposity and obesity, has driven a lingering ambiguous debate regarding causation (Masuo et al., 2003, Oyama et al., 2006, Winnard et al., 2012). While the association between BMI with serum urate are confirmed in this study and other studies alike, and may reflect a causal relationship of BMI to urate, the absolute chain of causality remains indistinct. This is a reflection of the intricacy surrounding the causal rapport between different markers of adiposity including BMI, and urate. It is also the possibility of some overlooked factors that influence both BMI and serum urate that may lead to their association. For example, these discoveries may stem from factors (principally diet) that possibly mediate the simultaneous variations in BMI and serum urate. The associations of BMI with other factors are discussed further in this chapter.

4.3.2.2 BMI and Triglycerides

Several lipid and lipoprotein abnormalities have been examined and noted in individuals with obesity. These include elevated levels of cholesterol, apolipoprotein B, triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol. Of these markers, the more consistent and well-established changes have been observed in triglyceride levels and HDL-cholesterol. Studies have demonstrated different markers of adiposity to have strong correlations with lipid
abnormalities. Other studies have concluded a strong association between central obesity (rather than obesity in general) and lipid/lipoprotein abnormalities (Seidell et al., 1991, Terry et al., 1989, Walton et al., 1995), while at least one other report has reported that body fat distribution may be a significant determinant of the varying levels of triglycerides and HDL-cholesterol in males and females (Freedman et al., 1990).

This study found an increase of 0.027 mmol/L in triglycerides attributed to one unit of increase in BMI irrespective of age and sex. When physical activity was included as a potential confounder, an even higher increase in triglycerides was exposed; thus suggesting an increase of 0.208 mmol/L in triglycerides attributed to every one-unit increase in BMI.

Consistent with the literature, this study’s findings support the already established association of BMI with triglycerides. High levels of triglycerides, or hypertriglyceridemia is a common lipid abnormality that has been reported to be a risk factor for the onset of T2D and premature cardiovascular disease (Brunzell et al., 1976, Hopkins et al., 2003). Furthermore, the Framingham study and epidemiological studies have found a correlation between obesity and increased coronary heart disease-related events (Anderson et al., 1987, Bertias et al., 2003, Newman et al., 1990).

The manifestation of hypertriglyceridemia in this particular cohort could be attributed to the high BMI (overweight category), and with being overweight comes insulin resistance, thus resulting in elevating levels of triglycerides. Subramanian and Chait (2012) in their study revealed that hypertriglyceridemia transpires in combination with low HDL-C levels, a finding not reflected in this study, and is often linked with the increased risk of cardiovascular disease. The supposed mechanisms leading up to cardiac events include insulin resistance that consequently results in high delivery of free fatty acids to the liver as a result of increased peripheral lipolysis (Subramanian and Chait, 2012), hypertension and diabetes (Usitupa et al., 1984), and increased atherosclerosis in association with dyslipidaemia (Kannel et al., 1967). The influence of dyslipidaemia on cardiovascular events is attributed highly to the increased levels of triglycerides (Jousilahti et al., 1996).

The pathophysiology of hypertriglyceridemia includes metabolic alterations in obesity. Adipose tissue from different sites varies significantly in its metabolism and consequences. Approximately 80% of body fat is accounted for by subcutaneous adipose tissue, and when this fat is in excess, it results in peripheral obesity (Wajchenberg, 2000). Excess subcutaneous fat has
not been shown to make a considerable contribution to elevating triglyceride levels, nor does it infer an increased risk in metabolic disease (Wajchenberg, 2000). However, omental adipose tissue (responsible for intra-abdominal adiposity) in excess is exclusive in numerous facets. Visceral adiposity (or intra-abdominal fat) is greatly vascular and drains directly into the portal vein; furthermore, the aptitude of insulin to suppress lipolysis and re-esterify free fatty-acids (FFA) is considerably reduced in visceral adipocytes (Zierath et al., 1998). The association of visceral adiposity with elevated metabolic and cardiovascular risk is believed to be secondary to high rates of lipolysis from the intra-abdominal depots (Despres and Lemieux, 2006, Kissebah et al., 1982). The surplus of intra-abdominal fat has been associated with fat in the liver, cardiac and skeletal muscles – all of which contribute to insulin resistance, a major risk factor for diabetes and ischemic heart disease, that significantly influences lipoprotein metabolism and is associated with elevated levels of triglycerides.

Impairment to the biological action of insulin at the cellular level (insulin resistance) is thought to be a fundamental and perhaps, the primary underlying metabolic dysfunction in the development of the characteristic dyslipidaemia seen in visceral obesity. The increase in intra-abdominal fat deposition (together with aging) plays a role in the development of insulin resistance (Seidell et al., 1988). The impaired ability of the fat cell to sufficiently store excess triglycerides is a possible initial step in the underlying hypertriglyceridemia of insulin resistance (Ginsberg, 2000).

4.3.2.2.1 Summary

Findings from this study agrees with the common belief that increasing BMI consequently leads to higher levels of triglycerides; and the impact of obesity on insulin resistance is well established to lead to increased levels of triglycerides. This raises the importance in lowering BMI, especially when the consequences of hypertriglyceridemia entail a predisposition to the development of premature atherosclerotic cardiovascular disease and further, to pancreatitis when triglyceride levels are strikingly high (Subramanian and Chait 2011). Furthermore, these results are a reflection of the growing recognition of the significance of triglyceride levels as an independent risk factor for cardiovascular disease and the need for interventions and clinical trials to assess whether lowering triglyceride levels will in fact reduce disease risk.
4.3.2.3 BMI and Blood Pressure

Elevated systolic and diastolic pressures among adolescents results in distress to their health and consequently causing additional complications later in life (Goon et al., 2013, Jafar et al., 2005). The correlation between blood pressure and high body weight in adolescents has already been established, although, an increase in hypertension has also been observed in non-obese adolescents (Davis et al., 2005a, He et al., 2000, Sorof et al., 2004). A positive correlation was established between BMI and systolic (Beta 0.454, P = 0.03) and diastolic pressures (Beta 0.514, P = 0.02) (Table 3.10).

Findings from this study corroborate the results from previous studies that have reported a positive association between BMI and blood pressure (Czernichow et al., 2002, Wilsgaard et al., 2000). A recent large cross-sectional study investigating trends in adolescent obesity, and the association between BMI and blood pressure found the BMI in adolescents was significantly associated with SBP and DBP in both males and females. A crucial finding from this study was that the positive correlation between BMI and blood pressure was observed in both healthy (normal) weight and overweight groups (Chorin et al., 2015). In a study conducted among Tangkhul Naga Tribal males of Northeast India, correlation analyses between BMI and blood pressure demonstrated positive significant associations. Their findings demonstrated, like this study, the mean systolic and diastolic pressures increased with increasing BMI levels (Mungreiphy et al., 2011). Furthermore, a significant association was also observed in a study conducted among Punjabi females of Delhi (Kapoor, 2000), and in other reported studies (Gupta et al., 1995). While other studies further complicate the relationship between BMI and blood pressure by suggesting a threshold effect below which no correlation between variables is observed (Bunker, 1995), the sample size is relatively small compared to most studies (n = 299 females, 500 males), thus producing inadequate statistical power to distinguish a true relationship.

Additionally, studies with larger samples investigating this relationship seldom examine the association of blood pressure with BMI across the different spans of BMI figures. Therefore the unresolved question remains as to whether there is a linear relationship across the entire BMI range. Dating back, a study in South Africa found no apparent association between blood pressure and BMI for women in unindustrialised areas, even when BMI values were reflective of obesity (i.e., 30 kg/m²) (Walker et al., 1990). To the contrary, Chorin and colleagues (2015) in
their most recent study oppose the suggestions by these authors. Their data, derived from a cohort of more than 700,000 adolescents of both sexes demonstrated a “near-linear” association between BMI and blood pressure, without any threshold effect. Thus concluded, their results sturdily imply that one key factor in determining high blood pressure in adolescents is BMI. This, in a way is reflected by this study’s results; where the cohort at BMI baseline is at an overweight category, and by way of a regression model, elevated levels of systolic and diastolic blood pressures are attributed to every unit increase of BMI.

The underlying pathophysiological mechanism between the change in BMI and blood pressure is still obscure. Several authors with children and adolescent samples have concentrated on several main pathophysiological mechanisms: instabilities in autonomic function (Reaven et al., 1996, Scherrer et al., 1994), the possibility that weight gain stimulates sympathetic activation, and also the likely involvement of leptin (Masuo et al., 2000), insulin resistance (Landsberg, 1992), and defects in vascular structure and function (Rocchini, 2002a, Rocchini, 2002b, Swinburn et al., 1999). Furthermore, possible important factors in linking body weight and high blood pressure may be attributed to the activation of the renin-angiotensin system, and in addition the physical compression of the kidney (Hall et al., 2000).

4.3.2.3.1 Summary

The association between blood pressure and BMI established in this study does not reflect causality. But with the ever-so growing incidence of childhood and adolescent overweight and obesity, these findings provide important information that pertains to the Pacific community of Dunedin. Further research is needed to describe the relationship between body habitus and cardiovascular risk factors and also the effectiveness of interventions already in place and that need to be put in place in order to prevent the development of high blood pressure in the burgeoning Pacific young. This is also valuable information provided to the Pacific Trust Otago, that the stabilisation and maintaining of a healthy BMI may well function to avoid increases in blood pressure, and by extension serve as an aid in reducing the prevalence of hypertension.
4.4 Lifestyle Factors

4.4.1 Physical activity

4.4.1.1 Physical activity & BMI

Physical inactivity is one of the chief problems in public health of the 21st century (Blair, 2009). In addition to physical activity being a crucial factor in maintaining a healthy lifestyle, it also plays a role in the prevention of non-communicable diseases (World Health Organization, 2010). The potential role of physical activity in playing a protective role against high body weight and excess build up of adipose tissue has resulted in an abundance of studies establishing the association between physical activity and excessive adiposity; the nexus between physical inactivity and obesity. (McManus and Mellecker, 2012).

Self-reports of exercise habits from this study showed 42.50% of this cohort were moderately active, with the remaining 57.5% highly active. The most sobering observation attained from this section was the baseline BMI levels between the two groups – moderately active and highly active. The student t-test revealed a significant difference in BMI levels between the two groups (Table 3.11) where individuals who were highly active had a significantly lower BMI than those who were moderately active (30.68 kg/m² vs. 27.56 kg/m², P = 0.02). To further dissect the relationship between BMI and physical activity, by way of a linear regression model, findings from this study showed a significant association between physical activity and BMI (Table 3.10), where at the unadjusted model established a significant lowering of 1.700 kg/m² in BMI attributed a change in physical activity from moderately to highly active. The prevailing ruling from these tests (Table 3.10) support the importance of partaking in physical activity in lowering and maintaining healthy BMI. These findings are in line with the wealth of evidence that is available on the health benefits of sufficient physical activity, or more so, the ill-health resulting from insufficient physical activity (Dumith et al., 2012, Ekelund et al., 2007, Hallal et al., 2012a, Hallal et al., 2012b, James et al., 1997, Lee et al., 2012, McManus and Mellecker, 2012, Nelson, 2000, Reis et al., 2014, Utter et al., 2003, Williams, 2008, World Health Organization, 2003b).
4.4.1.1 Summary

In summary, the findings from this study reflect the importance of physical activity in maintaining a healthy body weight. A significant difference in BMI was evident between those who were moderately active and highly active, where individuals who were highly active had significantly lower BMI status than those who were moderately active. Moreover, the positive correlation of physical activity with BMI further supports the already prevailing knowledge, suggesting that there is a decrease in BMI with every unit increase of physical activity.

These findings will be crucial for the Pacific Trust Otago in tailoring interventions, such as to promote physical activity in maintaining healthy body weight. As the candidate has been actively working with the Pacific Trust Otago, it is worthwhile to mention that the Pacific Trust Otago promotes regular physical exercise. Sports tournaments are held three times a year at the Edgar Centre, hosted by the Pacific Trust Otago. The tournaments are open to all Pacific Island communities and church groups, but are particularly set up to encourage regular physical activity for the Pacific youth in Dunedin. Additionally, weekly exercise classes are held three days a week. These classes include Zumba, boxing and circuit training to cater for everyone at any level that suits them.

4.4.1.2 Physical activity & HbA1c

While no association was established between BMI and HbA1c in this study, the health-damaging effects of obesity have been discovered for several comorbidities, of which include cardiovascular disease, T2D and associated risk factors such as lipids and blood pressure (Prospective Studies et al., 2009).

Physical activity plays a crucial role in the managing of type 1 and T2D, and also in the deterrence of diabetes complications (Chimen et al., 2012). Other studies have showed that physical activity is a keystone in diabetes management, conjoint with dietary and pharmacological interventions (American Diabetes, 2011, Colberg et al., 2010). Physical activity plays a role in the acute reduction of blood glucose levels and the increasing of insulin sensitivity in both those with (Lehmann et al., 1997, Wallberg-Henriksson et al., 1982, Yki-Jarvinen et al., 1984), and without (Mikines et al., 1988, Schmitz et al., 2002) diabetes. One of the other
documented possible health benefits of physical activity is improved metabolic control, with lowering of glycosylated haemoglobin (Beraki et al., 2014).

Studies have shown the benefits of physical activity in lowering HbA1c. Michaliszyn and Faulkner (2010) in their study of 16 adolescents with type 1 diabetes reported the importance of both the intensity and amount of physical activity in the improvement of glucose control. Their findings showed significant decreases in HbA1c levels with increased time spent in moderate, and moderate to vigorous physical activity (Michaliszyn and Faulkner, 2010). This was also consistent with other studies that also established a correlation between HbA1c and physical activity; most of which utilised a questionnaire approach to assess the association between physical activity and HbA1c (Bernardini et al., 2004, Herbst et al., 2006, Miculis et al., 2010, Robertson et al., 2009, Schweiger et al., 2010, Williams et al., 2011). Herbst and colleagues (2006), in a large German cross-sectional questionnaire study on 19,143 patients found glycosylated haemoglobin (HbA1c) levels to be higher in those who engaged less in regular physical activity. Further, preceding meta-analyses have also found significant reduction of HbA1c by an estimated 0.6% with physical activity (Boule et al., 2001, Boule et al., 2003, Snowling and Hopkins, 2006, Thomas et al., 2006). The associations of physical activity and HbA1c previously reported have been on participants or patients that were already diabetes-confirmed; these associations could therefore be accredited to high insulin sensitivity and better uptake of glucose in skeletal muscle, mirrored by the low need for insulin and lower HbA1c levels (Beraki et al., 2014). While HbA1c-lowering effects of physical activity have been successfully established by several studies, other studies (Aman et al., 2009, Edmunds et al., 2010, Sarnblad et al., 2005, Wallberg-Henriksson et al., 1982) including this current study failed to replicate these findings. The linear regression model (Table 3.12) did not find any association between HbA1c and physical activity at the crude level (P = 0.39), the age and sex-adjusted model (P = 0.47) and also the combined model that included age, sex and BMI (P = 0.28). Possible explanations to the lack of significant association between physical activity and HbA1c in this study would be the small sample size. Furthermore, the employment of different methods, particularly scales, of assessing physical activity may explain the conflicting results.

4.4.1.2.1 Summary

In summary, while this study found no significant association, the already documented association between physical activity and the decreased occurrence of long-term complications
The limitations aforementioned may have led to this study’s failure to produce the association between physical activity and HbA1c. Nonetheless, the prevailing view from literature reflects the importance and high recommendations of frequent engagement in physical activity in lowering both BMI and HbA1c levels; thus prevention of both obesity and risk of diabetes. These findings will again be beneficial for the Pacific Trust Otago, in that these results will be informative, and further stress the need for interventions to be put in place.

4.4.2 Alcohol Consumption

Findings from this study demonstrated a significant associations of alcohol with increased BMI, increased diastolic pressure, increased triglycerides, and increased serum urate levels (Table 3.13); all of which have been well established risk factors for diabetes, heart disease, and in the case of elevated serum urate levels – hyperuricaemia – may be indicative of an increased risk of gout.

4.4.2.1 General association of alcohol consumption and BMI

This study reports a difference, trending towards significance (P = 0.09) in BMI between teenagers who consumed alcohol (30.21 kg/m² = obese) and non-alcohol consumers (27.91 kg/m² = overweight), with evidence of a significant association of alcohol consumption with increase BMI. This is consistent with the already reported findings of the positive correlation of alcohol consumption and BMI. A large cross-sectional study showed that those who drank more had considerably higher BMI values than those who drank less (Arif and Rohrer, 2005). Another study in the United States of America sought to investigate association between drinking patterns and BMI found immense independent correlations between alcohol drinking patterns and BMI. The authors reported significant increases (P = <0.001) of BMI in both males and females, where the increase in BMI in males increased from 26.5% to 27.5%, and an increase from 25.1% to 25.9% in females, attributed to an increase in quantity (1 drink per drinking day to 4 drinks per drinking day), rather than frequency (number of drinking days in the past year) (Breslow and Smothers, 2005). This is also consistent with our sex-stratified analyses for alcohol and BMI (Tables 3.14 and Table 3.15).

The associations between BMI and alcohol consumption reported in the literature, conjoint with
the positive association established from this study, are for numerous reasons biologically reasonable. From what is known, alcohol, a source of dietary consumption high in calories, may well kindle food intake (Caton et al., 2004, Yeomans et al., 2003). Additionally, it is the second most energy-dense, and least gratifying macronutrient consumed (Caton et al., 2004, Prentice, 1995, Yeomans et al., 2003). Several studies have also alluded to the possibility of (consumed) liquids failing to initiate and activate physiologic satiety mechanisms (Almiron-Roig et al., 2003, Almiron-Roig and Drewnowski, 2003, DiMeglio and Mattes, 2000). However, this relationship is multifaceted as it encompasses other contributing aspects such as the time of consumption and the social setting involved (Almiron-Roig et al., 2003). Conclusively, to the biological possibilities of the association and effects of alcohol on BMI, Zurlo et al., (1990) also reported the reducing-effects of alcohol on fat oxidation, and thus the likelihood of weight gain as it also favors fat storage (Zurlo et al., 1990).

4.4.2.1.1 Summary

The findings from this section pose a question based on the current results; is alcohol consumption causing or contributing to obesity in Pacific adolescents in Dunedin? It appears likely, provided the wealth of (biological) evidence that assert alcohol contributes to weight gain. This information would be highly informative and helpful to Pacific Trust Otago in tailoring interventions and strive to provide health promotion policies to the Pacific adolescent, and wider Pacific community regarding the modifications in lifestyle. In particular, an alcohol intervention.

4.4.2.2 Alcohol and cardiovascular risk

The overall association between alcohol intake and diastolic pressure (Table 3.13) inferred a significant increase in diastolic pressure by 5.868 mm Hg per unit increase of alcohol, irrespective of age and sex (P_{adjusted} = 0.01). However, the sex-specific association tests only demonstrated a significant association of alcohol consumption with diastolic pressure in females, suggesting an increase of 6.789 mm Hg in DBP per unit increase of alcohol (P = 0.007). When age corrected, a further increase (Beta = 8.43 mm Hg, P_{age-adjusted} = 0.02) was evident. Although there was a significant difference in age between female drinkers and non-drinkers (18.80 vs. 16.20, respectively, P = <0.01), the age-adjusted model suggested that the association between alcohol and diastolic pressure in females was an age-independent phenomenon, thus implying that high diastolic pressures in female drinkers in this particular cohort was not because they
were older than their non-alcohol-drinking counterparts. This association is conflicting with an earlier study that found no association of alcohol on diastolic blood pressure in women (Maheswaran et al., 1991).

There is a wealth of evidence, both from observational and epidemiological studies that have revealed an empiric association between alcohol consumption and the increased risk of hypertension in both males and females. (Beulens et al., 2007, Criqui et al., 1981, Djousse and Gaziano, 2007, Fuchs et al., 2001, Klatsky et al., 1986, Klatsky et al., 1977, Malinski et al., 2004, Potter and Beevers, 1984). These studies have demonstrated both linear and non-linear correlations, suggesting either a U-shaped, J-shaped or a threshold by which alcohol consumption elevates blood pressure. However, most of the data available in the literature has drawn conclusions as to this relationship from older populations and middle-aged individuals; minimal data are available among young adults and similarly, adolescents (Gillman et al., 1995). The mechanism by which alcohol increases blood pressure remains unclear. From what is known, the link between alcohol consumption and high blood pressure is not through the continuity of structural modifications, but rather through hormonal, neural or other modifiable physiological mechanisms. For example, some studies have suggested that possible mechanisms include alcoholic stimulation of the sympathetic nervous system and the renin-angiotensin-aldosterone system, increasing effects of alcohol on plasma cortisol levels through magnesium loss into the urine, the inhibition of nitric oxide, the increase in intracellular calcium in smooth muscle, facilitated by alterations in electrolyte transport and modification of insulin resistance (Beilin et al., 1996, Klatsky, 1996, Randin et al., 1995, Tomson and Lip, 2006, Yamada et al., 2004)

Lipid levels in the Pacific (or Pacific groups) are affected by adverse changes in lifestyle and diet, further adding to the increased risk of heart disease (Hodge et al., 1997, Nestel and Zimmet, 1981, Stanhope et al., 1981, Taylor et al., 1992). There seems to be an inadequacy in the number of studies in New Zealand that focus on the risk factors, particularly lipids, associated with cardiovascular disease and diabetes in New Zealand adolescents. This study reports alcohol consumption to be significantly associated with an increase in lipids; triglycerides, serum urate, and increased diastolic pressure in the overall cohort (together with the aforementioned increased BMI; Table 3.13), all of which are established risk factors for cardiovascular disease and diabetes. The Tokelau migrant study conducted in the 1970s reported that Tokelauans who migrated to New Zealand (n = 1158) had much higher total energy intake as a result of increased
consumption of carbohydrates, protein, cholesterol and alcohol, when compared to their Tokelauan counterparts – non-migrants who remained in Tokelau (n = 765); further supporting the aforementioned concept that the effects of modernization (both of which include diet and lifestyle) may influence lipid levels in Pacific people. The association of high triglycerides – hypertriglyceridemia - and cardiovascular disease has been long-standing (Austin et al., 1998, Sarwar et al., 2007). The overall association of alcohol and increased triglycerides in this study infers an increase of 0.380 mmol/L in triglycerides per unit increase of alcohol, independent of age and sex (Table 3.13).

The prevailing biological mechanism that accounts for the elevated levels of triglycerides upon consumption of alcohol appears to be elevated VLDL formation in the liver. Alcohol consumption intensifies the synthesis of (triglycerides) and triglyceride-rich lipoproteins in the liver. (Frohlich, 1996, Hannuksela et al., 2004, Steinberg et al., 1991). Moreover, the lipolysis in fatty tissue is generated by alcohol, thus leading to a greater supply of fatty acids to the liver (Barona and Lieber, 1979). The influence of alcohol on the catabolism of triglyceride-rich particles remains obscure. However, while alcohol may influence triglyceride levels to rise, and by extension induce hypertriglyceridemia, there is insufficient evidence regarding high alcohol consumption in hypertriglyceridemic patients.

The association of alcohol with increased triglycerides in this study is consistent with findings from the literature. A study in the Netherlands showed an association of alcohol with serum triglycerides in their study sample of 300 subjects (Bessembinders et al., 2011). The authors found triglyceride levels were significantly higher in those who consumed more alcohol (P < 0.001). Stanhope et al., (1981) also reported that within their study, upon comparison of alcohol consumers and abstainers, consumers of alcohol had higher triglycerides than non-drinkers. While their study made comparisons between Tokelauan migrants to New Zealand and Tokelauans remaining in Tokelau, this observation was only observed in the latter. Similarly, controlled trials evaluating the effects of alcohol intake on serum levels in regular drinkers showed a more or less decrease (0.22 and 0.26 mmol/L, respectively) in fasting triglycerides when regular drinkers and those associated with binge drinking cut down their consumption of alcohol. Thus suggesting that the amount of alcohol, rather than drinking patterns, is related to the elevating levels of triglycerides (Rakic et al., 1998). Further, an investigation on the effects of alcohol consumption in patients with mild hypertriglyceridemia showed that two alcoholic beverages after six hours increased triglyceride levels by 3% in those with hypertriglyceridemia,
and 53% in those without. The study however concluded that alcohol intake individually is not a significant predictor of plasma triglycerides in hypertriglyceridemic patients (Pownall et al., 1999).

In contrast, other studies have found no association between alcohol and triglycerides (Marques-Vidal et al., 1995, Nanchahal et al., 2000). There is an inadequate amount of studies that have studied the effects of alcohol on triglyceride levels in adolescents, not only in New Zealand but globally. Nonetheless, there is existing evidence of testing for association of alcohol on triglycerides in females and males, in separate studies. For example, a study in the UK on women found no association between alcohol consumption with triglycerides (Nanchahal et al., 2000), which is in agreement with the lack of association in females produced in this study. To the contrary, another study in British women found moderately lower triglycerides in females with a daily intake of 17.74 milliliters of alcohol (Razay et al., 1992). Furthermore, findings from a study on Japanese Americans in Seattle and Japanese men in Japan reported an association of alcohol consumption with a significant increase in triglyceride levels in native Japanese men. A negative association was observed in male Japanese in America. Different alcohol consumption levels may have influenced the difference in direction of association; native Japanese males were moderate to heavy drinkers, while male Japanese in America were light to moderate drinkers. (Namekata et al., 1997).

Findings from Namekata et al. (1997) were consistent with results from this study, which showed a significant association of alcohol and elevated triglycerides in males (Page-adjusted = 0.008). The model reflected an age independent phenomena, inferring an increase of 0.380 mmol/L in triglycerides for every unit increase of alcohol. This finding was not apparent in females; in fact, no association was established in females.

It is worth to mention that alcohol consumption data was achieved through a questionnaire that asked generally whether participants drank alcohol; where the answer was assessed on a yes or no basis, without the indication of frequency and/or amount as these were assessed differently. For example participants were asked to recall whether they have had an alcohol beverage in the past year, how many drinks they were to consume on a working day, likewise in the weekends, and also to recall binge drinking in the previous year. However, numbers in these analyses were very small, and hence these findings should be interpreted with caution.
4.4.2.3 Alcohol consumption and hyperuricaemia and gout

There was a positive association between alcohol intake and serum urate levels in this cohort of Pacific adolescents (Table 3.13) in the unstratified model. The association at the crude level established in this study between alcohol consumption and serum urate levels (Beta = 0.044 mmol/L, P = 0.03) may be compared to the study carried out by Evans in the early 1960’s on Polynesians. The authors observed an increase in serum urate levels with reported alcohol intake in males across all age ranges, that were of Polynesian descent (Evans et al., 1968). Moreover, the authors also observed a higher prevalence of gout in people who consumed more alcohol. The relationship reported by Evans and colleagues, together with the findings from this study corroborate with findings from a more recent study that found a positive association between alcohol intake and risk of gout in a larger cohort of Pacific Islanders (Rasheed et al., 2013), and also in a cohort of European subjects (Choi et al., 2004).

One of the proposed mechanisms by which alcohol influences urate is through lactic acid. Lactic acid production is increased by ethanol thus influencing renal handling of urate by impairing the kidneys, resulting in renal under-excretion, thus elevating serum urate levels (Fam, 2005, Schlesinger, 2005). Additionally, habitual alcohol consumption can also increase serum urate levels by increasing the degradation of adenosine triphosphate (ATP) to adenosine monophosphate (a precursor for urate) resulting in an increase production of purine and, thus, increased conversion to urate (Faller and Fox, 1982, Puig and Fox, 1984).

4.4.2.3.1 Summary

The findings from this study confirm the association of alcohol consumption and serum urate levels, as already established in literature. However, the association from this study must be interpreted with caution. Potential confounders may not have been accounted for in these tests and also the sample size is concerning in terms of statistical power. Therefore, these tests must be repeated on a larger representative sample of Pacific adolescents.
4.4.3 Sugar Consumption

Diet records are considered to be the “gold standard” as they offer a comprehensive account of the type and amount of food and beverages consumed over a specified amount of time (Gibson, 2005). For the purposes of this study, there were two separate questions to collect sugar consumption. The first was the simple-recall question, “how many sugary drinks do you normally drink per day? And secondly, the more comprehensive questions in the FFQ that required memory recall of SSB over the previous month. FFQs are designed to measure habitual diet and are frequently used in epidemiological studies to examine for association between dietary habits and different health outcomes (Gibson, 2005, Willett, 1987). The availability of FFQs formulated to solely measure SSB consumption are scarce.

A linear regression (Figure 3.4) showed a weak positive Pearson correlation between sugar intake in grams per day (FFQ) and the number of drinks consumed per day ($R^2 = 0.298$). While a positive correlation is expected between the daily number of drinks and sugar intake, they are only weakly associated, varying widely. A possible explanation may be the differences in sugar content of drinks and other dietary sources of sugar. The wide variations and weak correlation suggest that these factors/variables are sources of dietary information, while not completely independent, including both in an analysis may strengthen a model as number of drinks is a poor proxy for net sugar intake, a weakness of several existing studies.

It is possible that participants found at least one of the methods more effective. But from what is known several explanations may account for the weak correlation observed here. Sugar is firstly a nutrient difficult to measure, and it is also normally under-reported. Errors of exclusion are very common. This is seen across all dietary assessment methods (Livingstone and Black, 2003). Therefore, participants may have deliberately under-reported in either the simple question or the FFQ. Furthermore, Willet (1998) and Gibson (2005) in their studies expressed that long dietary questionnaires may increase boredom and fatigue, consequently impairing concentration and reducing the accuracy of estimates from the FFQ. So the premise of under-reporting, and/or over-reporting ought to be acknowledged here. For future, perhaps a feedback from the participants as to which is more effective would solve this.

The data discussed in the sugar sections that follow make reference to the data from the FFQ. Primarily because it was designed as a measure for SSB for this project.
4.4.3.1 Sugar and BMI

The sugar-sweetened beverage (SSB) data attained from the FFQ was divided into quartiles: 1 being the low consumer of SSB and 4 being the highest (Table 3.17). Although not significant, it was evident that with the increase of SSB consumption, there was an apparent increase in BMI from quartile 1 (26.69 kg/m\(^2\)) to quartile 4 (30.60 kg/m\(^2\)). The role of SSBs in the development of overweight and obesity has been given much attention. Several studies, advocacy groups, and professional groups have pronounced the fundamental role of SSB consumption in the development of overweight and obesity, and merits special attention as policies are established to prevent, manage and reduce the rates of overweight and obesity (Ebbeling et al., 2002, Mello et al., 2006).

A systematic review and meta-analysis of randomised controlled studies and cohorts that reported on the correlations between free sugars and body weight in both children and adults recently emerged (Te Morenga et al., 2013). The authors found that in adults, a decrease in weight (0.80 kg, 95% C.I. -1.21, -0.39) was associated with decreased sugar consumption, while an increase in sugar consumption was associated with a significant weight gain in adults (0.75 kg 95% C.I. 0.30, 1.19). Similarly, results from the cohort studies also showed significant associations between sugar consumption and weight consequences.

To the contrary, Sievenpiper (2012) found dissimilar results, showing no effect of sugars on weight gain. The study found elevated doses of fructose to cause weight gain [0.53 kg (95% C.I. 0.26, 0.79)], but this was only where fructose was supplemented to provide excess energy (hyper caloric trials). Isocaloric trials by contrast showed no effect between fructose and body weight [-0.14 kg (95% C.I. -0.37, 0.10)] (Sievenpiper et al., 2012). The findings from this study concluded that fructose caused no increase in body weight when substituted for other carbohydrates in diets with calories alike. The dissimilarities in results between these studies may be attributed to the fact that Sievenpiper et al’s (2012) study was funded by the sugar industry; thus it is notable that their conclusions suggested excess intake was responsible for weight gain, rather than any negative effects of fructose as established by Te Morenga et al (2013). Hence, results from Sievenpiper et al.’s study on their analysis of feeding trials ought to be interpreted with caution. Furthermore, already critiqued by (Merriman et al., 2014) the 2012 meta-analysis of studies(Wang et al., 2012) examining the effects of fructose in isocalori diets
was flawed and compromised by the inclusion of rather large studies that subjugated the weighting of the meta-analysis carried out.

The quartiles analysis from this study show that an increase in SSB consumption may lead to increase in BMI. The most important finding to be considered here is sugar consumption. Perhaps the Pacific Trust Otago will use this data, in conjoint with the existing knowledge of the negative effects of sugar, and therefore strongly discourage the consumption of SSB in all gatherings, Pacific.

4.4.3.2 Sugar and serum urate

It has been established that SSBs are a significant source of calories for adolescents, contributing at least 301 kilocalories per day, or 13% of total daily energy (Wang et al., 2008). Two epidemiological studies on adults showed that high consumption of sugary drinks was associated with elevated levels of serum urate (Choi et al., 2008, Gao et al., 2007). While SSB consumption has been reported to be associated with obesity in adolescents (Malik et al., 2006) (a finding not replicated by this study), it is still unknown how SSB consumption is independently associated with levels of serum urate in adolescents.

This study investigated whether there was a trend of change in serum urate concentrations between high and low consumers of three different categories of sugar: SSB, sugar-sweetened foods (SSF) and natural sugar from fruits. Findings demonstrated no significant trends in serum urate levels across the quartiles for all three categories of sugar (SSB, \( P = 0.79 \), SSF, \( P = 0.34 \) and sugar from fruits, \( P = 0.17 \)). Although not significant, it is worthwhile to note the apparent trend of increase in serum urate levels in the natural sugar category (quartile 1 = 0.35 mmol/L, quartile 4 = 0.38 mmol/L, \( P = 0.17 \)). Several studies have proposed the positive effects of some fruits in managing urate. For example, a study by Buchter-Weisbrodt and Schober (1998) showed the consumption of apples to be beneficial as it contains malic acid, which dissolves urate, and thus is an important source of relief for gout sufferers (Buchter-Weisbrodt and Schöber, 1998). Furthermore, a more recent study reported the beneficial effects of tart cherry consumption on inflammation (Kuehl et al., 2010). This present study seemed to have an opposite effect, suggesting an increase in serum urate with fruit consumption.

In this short section, the findings suggest that fruit consumption may be correlated with elevated levels of serum urate. Therefore, the knowledge of the sugar content of fruits is of importance
today. Furthermore, this needs to be repeated on a larger scale with more statistical analysis, as sugar results were not significant.
4.5 Genetics

4.5.1 SLC2A9

The SLC2A9 gene is an important locus due to its involvement in urate re-absorption (Caulfield et al., 2008, Doring et al., 2008, Vitart et al., 2008) and can explain 3.5% variation in serum urate concentration among Europeans (Kottgen et al., 2013). With its extremely large effect on serum urate, the risk allele of this locus increases the gout risk by greater than 50% in Europeans (Hollis-Moffatt et al., 2009, Kottgen et al., 2013). The findings from this study did not successfully establish an association with the genetic variants of interest of the SLC2A9 gene. In testing for a direct association of the rs11942223 and rs3775948 genetic variants with serum urate, no association was established. The rarity of the rs11942223 variant, together with the small sample size may account for the preclusion of any association being established. The findings from this study are conflicting with studies that have found robust associations of these genetic variants in European and Asian populations (Hollis-Moffatt et al., 2009, Okada et al., 2012).

Batt and associates (2014) produced evidence for non-additive interaction between the genotypes of SLC2A9 and the consumption of sugar-sweetened beverages (SSB) and a role in gout development in three populations; Māori, Pacific and Europeans. Their initial observations revealed the increase in gout risk by SSB consumption which corroborated other studies that revealed positive associations between high consumption of fructose-sweetened beverages and increased urate, and increased risk of gout incidence in both males and females (Choi and Curhan, 2008, Choi et al., 2010, Choi et al., 2008). Findings from Batt showed that the OR for four drinks per day was 6.89 (P = 0.045) for Europeans, 5.19 (P = 0.010) for Māori and 2.84 (P = 0.043) for Pacific Islanders. Interaction analyses demonstrated a significant interaction term in the collective (P_{interaction} = 0.01), but not when the data was meta-analyzed (P_{interaction} = 0.99). Furthermore, they also found a trend towards significant evidence for interaction between SLC2A9 genotype (rs6449173) and SSB consumption in determining serum urate levels (P_{interaction} = 0.062). Overall, epidemiological evidence of non-additive interaction between sugary sweetened beverages (SSB) consumption and a urate-associated variant of SLC2A9, generates a hypothesis that physiologically SSB derived fructose can influence the SLC2A9-mediated excretion of urate by interfering with the ability of SLC2A9 to transport urate (Batt et
al., 2014). In an attempt to replicate these findings, this study carried out an interaction analysis for any evidence of interaction between the SLC2A9 genotypes with SSB for an influence on serum urate levels. (Tables 3.22 – 3.24). No significant association term was established by this study.

A limitation of these results is the relatively small sample size (n = 80). Perhaps with a greater cohort, we will be able to assess the true relationship between these genetic variants with serum urate to illuminate the relationship of serum urate with these specific variants of the SLC2A9 gene.

**4.5.2 FTO**

This study set out to examine the association of two FTO genetic variants rs9922047 and rs9923233 with BMI in a cohort of Pacific teenagers living in New Zealand. To the candidate’s understanding, only two studies have examined associations of the FTO variants with BMI in Pacific populations. A study carried out in 2007 investigated three FTO genetic variants (rs1421085, rs17817449 and rs9939609) for association with BMI. The authors found, that in their population sample of 516 Oceanic subjects, no association between the three variants of FTO and BMI was established (Ohashi et al., 2007). The second study on a Pacific population is the more recent study carried out on 1089 Samoans. The authors investigated 51 genetic variants of the FTO gene for association with BMI and also found no significant associations (Karns et al., 2012). Consistent with the findings from both studies on Pacific populations, this present study also found no significant associations of the two genetic variants of interest with BMI.

Genetic epidemiological studies have resolutely affirmed that, as well as lifestyle factors, genetic factors also play influence in the development of obesity (Maes et al., 1997). Recent studies have suggested that being physically active may alter the genetic susceptibility to obesity, with the genetic burden being more extreme in physically inactive persons, as opposed to those who are physically active (Andreasen et al., 2008, Franks et al., 2008, Sonestedt et al., 2009). The most widely studied example of a gene x physical activity interaction in obesity is for the FTO gene (Andreasen et al., 2008, Rampersaud et al., 2008). A recent study carried out by Kilpelainen et al., (2011) meta-analyzed 45 studies of adults (n = 218,166) and nine studies of children and adolescents (n = 19,268) successfully confirmed that physical activity attenuates the influence of FTO variation on BMI and obesity. The authors found that the association of the FTO risk allele
with the odds of obesity was reduced by approximately 30% in physically active adults. No interaction was established between the FTO variant and physical activity on BMI in children and adolescents in their study. Although a possible explanation would be that the sample size of the children and adolescent cohort was 11 times smaller than in the meta-analysis of adults.

This present study also tested for association and non-additive interaction between FTO genotypes of interest and physical activity in determining BMI. No significant interaction between physical activity and the genetic variants of FTO in determining BMI was evident. Identifying interactions between genetic variants and environmental/lifestyle factors is very challenging. This is because it is essential that these type of studies are conducted on large magnitude cohorts (Smith and Day, 1984). Furthermore, interaction studies are made further complicated by the strain of accurately measuring lifestyle exposures, which decreases statistical power and requires large sample sizes to counterbalance this effect (Wong et al., 2003). Therefore, the findings from the interaction testing in this section, or lack of interaction thereof, is not surprising provided the sample size was quite trivial compared to existing reports.
Conclusion

Overall, the findings from this study are reflective of the current status of Pacific adolescent health in Dunedin. Most importantly is that this study provides evidence of significant correlations between alcohol and increased BMI, blood pressure and several biochemical traits. Because these are significant and well established risk factors for metabolic diseases such as heart disease and diabetes, this study provides information crucial to the Pacific Trust Otago for targeted interventions to improve the long-term metabolic health of young Pacific people in Dunedin.

The significant associations of alcohol with increases in BMI, serum urate and triglycerides, as well as diastolic blood pressure are well established risk factors for metabolic diseases. Another crucial finding from this study is the association of physical activity with BMI; where a lowering of BMI was attributed to a change in physical activity from moderate to highly active. The Pacific Trust Otago already have several physical activity and exercise programmes set up to encourage both adults and adolescents to partake in high physical activity. However, while this cohort’s baseline BMI classes them in an overweight category, the findings in the physical activity section are of significance to further emphasize the importance of physical activity as it lowers BMI and by extension, reduce the risk of metabolic diseases.

This research, in it’s whole scope, could impact Pacific health by identifying pathways that could be targeted in treating or preventing metabolic disorders at an early stage (before adulthood), and possibly interventions targeted to specific Pacific groups. Furthermore, provided the limited evidence on the health of Pacific adolescents, this study makes a significant contribution to literature.

The main limitations of this study are its sample size and the inaccuracies of self-reporting. Like most epidemiological studies, for statistical power and representative findings, the sample size ought to be on a rather large scale. Genetics studies, alike. For example, if the gene-environment interaction portion of this study was carried out on a larger scale, the findings could demonstrate interaction of SSB with causal genetic risk factors that would contribute to the evidence base for public health interventions for reducing consumption of SSB in Pacific people. By improving the sample size, this could possibly allow for more meaningful correlations in the whole study.
Another limitation of this study was the different forms of alcohol investigated in the questionnaire. For the purposes of this study, the investigations were particular to whether the participants consumed alcohol or not. The type of alcohol was not considered. This poses as a possible limitation due to the established association between alcohol and cardiovascular risk, and the type or form of alcohol is a significant confounder (Barefoot et al., 2002, Frost et al., 2005) and also the different patterns of drinking, and the context by which it is drunk (Grønbaek, 2007) (some drink during meals everyday, or once a week at parties etc).
Future Directions

This study provides important information regarding the metabolic health of Pacific Island adolescents in New Zealand. Furthermore, the data produced will be crucial for guiding current and future interventions.

However, further investigations are needed in the following directions:

- The metabolic health study on Pacific adolescents should be investigated in a larger data set.
- Follow-up the study on the same participants over a period of 2-5 years allowing for another assessment of health status.
  - This step will be crucial in documenting the change of health status, if any.
  - It will also allow for assessment of interventions put in place by the Pacific Trust Otago, whether these have been effective or not.
- Additional factors that should be measured include:
  - Fasting random glucose levels.
  - Oral glucose tolerance tests need to be carried out in pre-diabetic samples.
    - A second test for confirmation of good measure.
  - Type of alcohol consumed by participants need to be considered.
    - Different alcohols have different sugar content and this would be necessary to measure to eliminate any possible confounding.
- Future genetics studies on larger Pacific cohorts need to be carried out.
  - Pacific-specific genome wide association studies for biochemical measures.
  - Gene and environment interactions are crucial to document the responses of genotypes to environmental variations. While this study provided no evidence of such interactions, these genetic variants should be tested again in a larger sample for possible gene-environment interaction with sugary drink and physical activity to test the possible influence of non-additive interaction on the risk of elevating serum urate levels, and BMI.
Appendices
INFORMATION SHEET FOR TEENAGED PARTICIPANTS

Thank you for being interested in this project. Please read this information sheet carefully before deciding whether or not to take part. If you decide to take part we thank you. If you decide not to take part there we thank you for thinking about it.

Why are we doing this Project?
The first aim of this study is to measure physical health in Dunedin Pacific teenagers. Very little is known about things that increase the chance of poor health among Pacific teenagers. This is a concern because more and more people are being diagnosed with type 2 diabetes and other related conditions such as gout, kidney disease and obesity. The results will give us new knowledge to use for improving the health of future generations, and also the Pacific Trust Otago can use this information to improve services that cater for the Pacific Island Community.

The second aim of this study is to measure the mental health and wellbeing of Pacific teenagers living in Dunedin. We are interested in your views of wellbeing and things that might influence your wellbeing. We hope that the findings from this part of the study will help us understand what is important for young people's wellbeing and how we can best provide support.

Do I have to take part?
Your participation is voluntary. We would like for you to consent to participate in this research, as we believe that you can make an important contribution to research. If you do not wish to participate, just say so and we will go no further. If you choose not to take part there would be no disadvantage for you.

What will Participants be asked to do?
If you agree to take part in this project, you will be asked to donate a blood sample (and a urine sample). This will require you to visit the Human Nutrition Department, University of Otago where you will spend about an hour. The Pacific Trust Otago team will provide all transport to and from the Human Nutrition Department if required.

During your visit to the Human Nutrition Department you will be asked to fill out questionnaires about personal information about yourself. This questionnaire will take approximately 40 minutes to complete. If there are questions you do not want to answer, then you may skip those questions.

Your blood and urine samples will be sent to the Biochemistry Department, University of Otago for testing of biochemical markers of physical health and from which DNA will be prepared. The DNA allows us to test your DNA for genes that play a role in physical health – we will use this information to study the way genes and the way you live work together in physical health. We will also use this information to ‘smooth out’ the genetic differences between people – this will improve the results of our study. If you wish lotu (prayer) to be said before disposal of your by-products of making the DNA (by standard Medical Waste procedures) please indicate this on the Consent form.

Having a blood sample taken may hurt a little, and some people get a small bruise where the needle is poked in. Sometimes, the needle hole can become infected but this hardly ever happens. Please let us know if you might faint when blood is taken, or when you see blood. This will help us be ready and take the sample when you’re lying down.
Your time and contribution towards this project is highly valued. As a small token of our appreciation, you will receive a $20 Warehouse gift voucher as a thank you for taking part.

**What Information will be Collected and What Use will be made of it?**
The information collected will be safely stored in such a way that only those mentioned below, and technical staff in their laboratories, will be able to gain access to it. Information obtained as a result of the research will be kept for at least 5 years in secure storage. Any personal information held on you such as contact details, biological samples and information obtained from questionnaires may be destroyed at the end of the research even though the results from the research will, in most cases, be kept for much longer or possibly indefinitely.

Your samples will be stored within Dr Merriman’s laboratory, which is locked after 5pm. Using a unique study code personal information will be stored on a dedicated computer server located within the Department of Biochemistry and protected by University of Otago data security processes. The information will be accessible by password only to defined users (including Pacific Trust Otago) determined by Dr Merriman. Paperwork will be stored within Dr Merriman’s laboratory.

The results of this project will be published. Your information will be analyzed with the entire group and you will not be identified.

We will provide you with a copy of your blood results and an explanation of what they mean. We will also provide you and the Dunedin Pacific community with a summary of the findings from the study. This summary will be about the group as a whole and not about you as an individual.

We might also need to check your medical records. On the Consent Form you will be given the options to consent or not to this.

**Can you change your Mind and Withdraw from the Project?**
You may withdraw from the project at any time and without any disadvantage to yourself of any kind, just contact any of the researchers listed below.

**What if you have any Questions?**
If you have any questions about our project, either now or in the future, please feel free to contact:

Jarrod Moors (Student Researcher)  A/Prof Tony Merriman (Supervisor)
Department of Biochemistry  Department of Biochemistry
University Number: (03) 479-5798  University Number: (03) 479-5798
Email: [jarrod.moors@otago.ac.nz]  Email: [tony.merriman@otago.ac.nz]

Dr Mele Taumoepae  Finau Taungapeau/Eric Nabalagi
Department of Psychology  Pacific Trust Otago
University Number: (03) 479-4029  Telephone Number: (03) 455-1722
Email: [mele@psy.otago.ac.nz]

This project has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the human Ethics Committee Administrator (ph. 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the income.
THE EVALUATION OF PHYSICAL HEALTH IN A POPULATION OF PACIFIC ISLAND
TEENAGERS RESIDING IN NEW ZEALAND (DUNEDIN).

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that: -

1. I have read the Information Sheet for this study and have had details of the study explained to me

2. My participation in the project is entirely voluntary;

3. I am free to withdraw from the project at any time without any disadvantage;

4. I as the participant will remain anonymous;

5. Personal identifying information will be destroyed at the completion of the project but any raw data on which the results of the project depend on will be retained in secure storage for at least five years;

6. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity;

7. I would like my information: (Circle your preference)
   a. Returned to me
   b. Returned to my family
   c. Both a and b
   d. Other (please specify) ..........................................................

8. At the end of the study, I consent to any remaining sample being disposed using:
   ☐ Standard disposal methods, OR;
   ☐ Disposed with appropriate karakia (prayer)
9. I will receive at $20 Warehouse gift voucher as a token on appreciation for participating in this project.

10. I consent for my medical records to be attained from my General Practitioner (GP)

   ☐ Yes
   
   GPs Name ____________________________
   Address _______________________________________
   Phone ________________________________

   ☐ No

I agree to take part in this project.

................................................................. ............................................
(Signature of participant) (Date)

In my opinion the participant has given willing and informed consent.

Investigator's signature.................................................... Date: .........................

(NAME AND ROLE IN BLOCK CAPITALS)..........................................................

This project has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the human Ethics Committee Administrator (ph. 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the income.
Appendix C

Pacific Island Youth Study
THE PHYSICAL HEALTH OF DUNEDIN PACIFIC ISLAND TEENAGERS

Date: ___/___/_____

Recruitment site: __________________________

Name: _____________________________________________

NHI: _______________

DOB: _______________

Address: ___________________________________________

_________________________________________

Email: _____________________________________________

Ph. Home: ________________

Ph. Work: ________________

Ph. Mobile: ________________

GP Name: ___________________________________________

GP address: 

______________________________

______________________________

______________________________

GP Phone: ________________
1. Age .................................. Years

2. Weight ............................. Kg

3. Height .............................. cm

4. BMI ................................. Kg/m²

5. Waist ............................... cm

6. Hip ................................. cm

7. Blood pressure ................. mmHg

8. Blood glucose ...................... mmol/L
9. *Because this is a genetics study we need to accurately understand your genetic origin - the best way to do this is from the ethnic origin of your grandparents.* Please fill in the following boxes for each of your grandparents. If you do not know their origin please indicate this with a question mark. Tick as many circles as you need within each box.

10. If applicable, who are your iwi? __________________________________________
Participant Questionnaire

Baseline information
1. Current age ________________ years
2. Gender MALE / FEMALE
3. At this time are you: (Please circle all that apply)
   a. In paid employment?
   b. Self-employed?
   c. Working full time?
   d. Working part time?
   e. In physical/manual employment?
   f. In non-physical employment?
   g. A homemaker?
   h. A student? (Specify)
      □ High School
      □ University
   i. Unemployed?
   j. Not working because of ill health/disability?
   k. Other ________________________________

4. How many years of education have you completed?
   Please circle the number of years at school, college, university etc.
   1 2 3 4 5 6 7 8 9 10 11 (5th form) 12 13 (7th form) University

Has your doctor told you that you have any of the following:
5. Type 2 diabetes YES / NO
   How treated? DIET / EXERCISE / MEDICATION
   When diagnosed?
   Have you had your glucose measured? YES / NO
   Diabetes during pregnancy YES / NO
6. Gout YES/NO
   Does anyone in your family have gout? YES / NO
   If so, who?
7. High blood pressure YES / NO
   How treated?
   Have you had your blood pressure done? YES / NO
8. Lipid problems YES / NO
   Have you had your lipids measured? YES / NO
9. Heart problems YES / NO
   a. Angina? YES / NO
   b. Heart failure? YES / NO
c. Pacemaker/stent/bypass? YES / NO
d. Heart attack? YES / NO
e. Stroke? YES / NO
f. Rheumatic Heart disease? YES / NO

10. Kidney problems YES / NO
   Specify:

11. Have you ever had Rheumatic Fever? YES / NO

12. Have you been diagnosed with cancer?

13. Other diseases/conditions

14. Are you taking any regular medication? Yes / No
   Specify .................................................................

15. Have you been immobilized/had total bed rest for at least 1 month in
   the last year? Yes / No
   Specify .................................................................

16. GIRLS ONLY: Have you started menstruating? Yes / No
   At what age did your periods start? ...........Years and .......... months
Physical Activity: Please circle all that applies in the attached activity sheet

Now, some questions about the other things you do in your spare time.
In the last 12 months

1. How long do you usually spend per day at a computer?
   On weekdays?
   On weekend days?

2. How long per day do you usually watch television other than videos/DVDs?
   On weekdays?
   On weekend days?

3. How long per day do you usually watch videos or DVDs?
   On weekdays?
   On weekend days?

4. How much time per day do you usually spend playing on an X-box, playstation or other game machine (including hand-held games)?
   On weekdays?
   On weekend days?

5. How much time per day do you usually spend reading?
   On weekdays?
   On weekend days?

6. How much time do you usually spend talking on the telephone (cellphone or landline)?
   On weekdays?
   On weekend days?

7. How much time per day do you usually spend doing homework?
   On weekdays?
   On weekend days?

8. How much time per day do you usually spend socialising or hanging out with friends?
   On weekdays?
   On weekend days?
9. What other sorts of things do you do in your spare time (e.g. listening to music, artwork, playing board or card games, playing music)?

Please describe:

10. How much time per day do you usually spend doing the other activities (listed in question above)

   On weekdays?

   On weekend days?

11. Do you have a cellphone?

   If yes: - How many texts do you usually send per month?

**Diet**

1. How many sugar-sweetened drinks (including fruit juice), but not including diet drinks, do you normally drink per day?

   Can or large glass: (Please circle the number that applies)

   0  1  2  3  4  5  more than 5

2. How many pieces of whole fresh fruit do you usually eat per day? (please circle the number that applies)

   0  1  2  3  4  5  more than 5

3. How many times do you normally eat seafood in a week? ________

**Smoking**

Do you Smoke? Yes/No/Ex-Smoker

If yes, continue with this question, if not move on to the next question.

1. How old were you when you started smoking?

2. How old when you began to smoke regularly (more than 1 a day)?

3. In the last year have you smoked daily for a month or more?

4. How many cigarettes do you smoke per day?

5. What brand do/did you smoke?

6. Have you ever considered quitting smoking?
Alcohol
Do you drink alcohol?  Yes/No
If yes, continue with this question, if not move on to the next question.

When I use the term “drink” I mean a glass of wine, a can or bottle of beer, a ‘shot’ or ‘nip’ of hard liquor either alone or in a mixed drink.

1. In the past year, how many weeks out of 52, have you had any wine, beer or other drink containing alcohol?

2. In a typical week when you had something to drink, how many drinks would you have, in total, from Monday to Thursday, on work days?
   SUM Total drinks Monday - Thursday (4 days) ____________

3. And how many drinks, in total, would you usually have from Friday through to Sunday, on weekends?
   SUM Total drinks Friday - Sunday (3 days) ____________

4. In the past year, how many times did you have five or more drinks in one sitting or occasion?
   ____________

5. What about last night? Did you have 5 or more drinks in the last night?

In the past year, has drinking caused you to have any of the following problems?
Please use code: [0= No, 1= Maybe, 2=Yes]

6. Problems with your family?

7. Problems with your friends?

8. Problems with people at work or where you study?

9. While drinking, have you gotten into physical fights?

10. Have you had a traffic accident when you were under the influence of alcohol?

11. Have you continued to drink once you knew drinking was causing you any of these problems?

12. In the past year, have you been under the influence of alcohol in situations where you could have caused an accident or gotten hurt, for example whilst driving, riding a bike, operating machinery, or anything else?

13. Did drinking or being hung-over often make you neglect your responsibilities at work, at home, or caring for children?

14. In the past year, has your drinking caused you to be arrested by the Police for disturbing the peace, or for driving while under the influence of alcohol?

15. Have you felt a strong desire for alcohol, a craving for it?

16. Have there been many days when you had a lot more to drink than you meant to when you began, or your drinking continued for more days in a row than you intended?
17. In the past year, has there been a period when you spent so much time drinking or getting over the effects of alcohol that you had little time for anything else?

18. Has your drinking caused you to give-up or greatly reduce any important activities IN ORDER to drink, such as sports, work or socializing with friends or family?

19. Have you been tolerant to alcohol, that is, you needed to drink a lot more to get an effect or found that you could no longer get the same effect from the amount you used to drink?

20. In the past year have you wanted to quit or cut-down on your drinking?

21. Have you tried to quit or cut-down on your drinking?

FRACTURE

Have you ever broken any bones (fractures)? Yes / No

Please detail below the following factors:
Bone broken, age, how it happened, height of fall/ speed involved

..............................................................................................................

.............................................................................................................................

.............................................................................................................................

.............................................................................................................................

.............................................................................................................................

...............................................................................................................................

.............................................................................................................................

.............................................................................................................................

.............................................................................................................................

.............................................................................................................................

.............................................................................................................................
Sunshine Exposure

In the past 12 months, how many times did you have red OR painful sunburn that lasted a day or more? [SUNBURN]

0 1 2 3 4 5 or more times

For the following questions, think about what you do when you are outside during daylight savings months (October – March, inclusive) on a sunny day:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>How often do you wear SUNSCREEN?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you wear a SHIRT WITH SLEEVES that cover your shoulders?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you wear clothing that covers your lower legs?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you wear a HAT?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you wear a HAT that shades the face, neck and ears (e.g. broad-brimmed, legionnaires or bucket style)?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you stay in the SHADE or UNDER A SUN UMBRELLA?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How often do you wear SUNGLASSES?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

How often do you spend time in the sun in order to get a tan?

How would you describe your natural, untanned skin at the END OF WINTER? Choose one option:

<table>
<thead>
<tr>
<th>Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very fair</td>
</tr>
<tr>
<td>Fair</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>Olive</td>
</tr>
<tr>
<td>Dark</td>
</tr>
<tr>
<td>Very dark</td>
</tr>
<tr>
<td>Black</td>
</tr>
</tbody>
</table>

Have you used a sunbed (i.e., tanning bed) in the past year?

YES NO
If yes, how often on average? (please circle)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>Twice a week</td>
<td>Once a week</td>
<td>Once every 2 weeks</td>
<td>About once per month</td>
<td>Once every 2 to 3 months</td>
<td>Less often than once every 2 to 3 months</td>
<td>Other <em>(please specify)</em></td>
</tr>
</tbody>
</table>

*If Other, please specify: _____________________________________________

**INSTRUCTIONS**: Listed below is a series of statements that represent possible feelings that individuals might have about themselves. For the following questions, indicate the degree of your agreement or disagreement by check the appropriate response.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree 1</th>
<th>Disagree 2</th>
<th>Neutral 3</th>
<th>Agree 4</th>
<th>Strongly Agree 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I rarely overindulge in anything.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I often crave excitement.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I have little difficulty resisting temptations.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>I wouldn’t enjoy vacationing in Las Vegas.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>When I am having my favorite foods, I tend to eat too much.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I have sometimes done things just for ‘kicks’ or ‘thrills.’</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I seldom give in to my impulses.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I tend to avoid movies that are shocking or scary.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I sometimes eat myself sick.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>I love the excitement of roller coasters.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Sometimes I do things on impulse that I later regret.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I’m attracted to bright colors and flashy styles.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>I am always able to keep my feelings under control.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>I like being part of the crowd at sporting events.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>I have trouble resisting my cravings.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>I have a leisurely style in work and play.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>I have never literally jumped for joy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>When I do things, I do them vigorously.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>I have sometimes experienced intense joy or ecstasy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>My work is likely to be slow but steady.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Sometimes I bubble with happiness.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>I’m not as quick and lively as other people.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>I rarely use words like “fantastic!” or “sensational!” to describe my experiences.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>I usually seem to be in a hurry.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Only if the participant has gout:

1. Age at first gout attack
   ___________________ Years

2. Number of gout attacks in the past year
   ___________________

3. Is there tophus as evidenced by clinical examination? YES/NO

4. Has the participant ever had diuretic treatment? YES/NO
   If so, did this induce gout? YES/NO

5. What treatment has the participant had for gout in the past?
   - Allopurinol YES / NO
   - Steroid YES / NO
   - Anti-inflammatories YES / NO
   - Probenecid YES / NO
   - Colchicine YES / NO
   - Other YES / NO

6. Were there any side effects from these treatments? (Specify drug)
   _______________________________________________________________

7. Do certain foods/drink trigger your gout? YES / NO
   If so, please list them: ___________________________________________

8. Does seafood trigger your gout? YES / NO

9. Does alcohol trigger your gout? YES / NO

10. Urate (at diagnosis) (Date: ___/___/____) ____________ mmol/L
Appendix D

Food Frequency Questionnaire

We would like to learn more about Pacific Island teenagers’ eating patterns in New Zealand.

How can you help?
- Please tell us about YOU (not someone else in your household)
- Please be honest
- Answer each question as best as you can.
- Tick or fill in **ONE answer** for **EACH question**.

Fa’afetai lava (thank you very much) for helping us with this important project!
Talofa lava, I am Sione and over the past month I usually drank water 4 times a day.

Sione writes:
Over the last month, on average, how often do you drink water?
☐ never (go to next question)
4 times a day
☐ times a week
☐ times a month
How much do you usually have?
1 cup OR ___ ml OR ___ litre

PLEASE NOTE: Each item has 2 questions:
• “how often”
• “how much”.
These photos may help you estimate **how much** you usually drink:
Think about your usual drinking pattern over the past month…

1. On average, how often do you drink fruit drink (eg. Golden Circle, Thextons, Ribena)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

How much do you usually drink each time?

___ cup OR

___ ml OR

___ litre

2. On average, how often do you drink 100% fruit juice (no added sugar) (eg. Just Juice, Fresh-Up)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

How much do you usually drink each time?

___ cup OR

___ ml OR

___ litre

3. On average, how often do you drink low-calorie cordial (eg. Thriftee, Vitafresh Low Calorie)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

How much do you usually drink each time?

___ cup OR

___ ml OR

___ litre
4. On average, **how often** do you drink regular **cordial** (eg. Raro, Refresh, Vitafresh)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

How do you **prepare** the cordial?

☐ strong (less water)

☐ following packet instructions (1 packet = 1 litre)

☐ weak (more water)

**How much** do you usually drink each time?  _____ cup  OR  _____ ml  OR  _____ litre

5. On average, **how often** do you drink **diet soft drink** (eg. Coke Zero, Diet lemonade)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

**How much** do you usually drink each time?  _____ cup  OR  _____ ml  OR  _____ litre

6. On average, **how often** do you drink **regular soft drink** (eg. Coke, Lemonade)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

**How much** do you usually drink each time?  _____ cup  OR  _____ ml  OR
7. On average, **how often** do you drink **sports drink** (eg. Gatorade, Powerade)?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**How much** do you usually drink **each time**?  
___ cup OR 
___ ml OR 
___ litre

8. On average, **how often** do you drink **energy drink** (eg. V, Red Bull, Mother)?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**How much** do you usually drink **each time**?  
___ cup OR 
___ ml OR 
___ litre

9. On average, **how often** do you drink **flavoured milk** (eg. Primo, Calci Yum, bought milkshake)?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**How much** do you usually drink **each time**?  
___ cup OR 
___ ml OR 
___ litre
Think about your *usual* drinking pattern *over the past month*…

**PLEASE NOTE:** For the following questions:

- 1 nip = 30ml
- 1 standard wine glass = 100ml
- 1 large wine glass = 150ml
- 1 pint or handle = 400ml
- 1 wine bottle = 750ml
- 1 jug = 1000ml

This photos may help you estimate **how much** you usually drink:

10. On average, **how often** do you drink beer, lager or cider?  
☐ never (go to next question)  
☐ times a day  
☐ times a week  
☐ times a month  

**How much** do you usually drink **each time**?  
____ ml OR  
____ litre

11. On average, **how often** do you drink wine (red, white or sparkling)?  
☐ never (go to next question)  
☐ times a day
How much do you usually drink each time?  
☐ standard glass (100ml) OR  
☐ large wine glass (150ml) OR  
☐ wine bottle (750ml)

12. On average, how often do you drink port, sherry, vermouth or liqueurs?  
☐ never (go to next question)  
☐ times a day  
☐ times a week  
☐ times a month

How much do you usually drink each time?  
☐ small sherry glass (60ml) OR  
☐ standard wine glass (100ml)  
☐ large wine glass (150ml)

13. On average, how often do you drink straight spirits (no mixer) (eg. gin, whisky, vodka, rum)?  
☐ never (go to next question)  
☐ times a day  
☐ times a week  
☐ times a month

How much do you usually drink each time?  
☐ nip (30ml)  
☐ double nip (60ml)

14. On average, how often do you drink spirits with mixer (eg. RTDs, gin and tonic, rum and Coke)?  
☐ never (go to next question)  
☐ times a day  
☐ times a week  
☐ times a month
How much do you usually drink each time?  

____ spirit glass (150ml) with one nip  
____ spirit glass (150ml) with two nips OR  
____ tall glass (200ml) with one nip OR  
____ tall glass (200ml) with two nips OR  
____ one bottle (330ml)
**PLEASE NOTE:** For the following questions:

- 1 teaspoon = 5ml
- 1 heaped teaspoon = 14ml
- 1 big spoon = 15ml

15. On average, **how often** do you add **sugar or honey** to your tea or coffee?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

**How much** do you usually add **each time**?  ____ teaspoon  ____ big spoon  ____ ml

16. On average, **how often** do you add **sugar or honey** to your Milo/hot water or other drinks?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

**How much** do you usually add **each time**?  ____ teaspoon  ____ big spoon  ____ ml

17. On average, **how often** do you add **Milo, powder drinking chocolate or other milk mix** (e.g. Nesquik, Pams, Hansells) **to your drinks**?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

**How much** do you usually add **each time**?  ____ teaspoon
18. On average, how often do you eat jam, honey, syrup, chutney or Nutella on your bread?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

How much do you usually eat each time? (Please circle)

19. On average, how often do you put tomato sauce on your food?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

How much do you usually add each time? _____ teaspoon

20. On average, how often do you eat dried fruit (eg. raisins, sultanas, prunes)?
☐ never (go to next question)
☐ times a day
21. On average, how often do you eat canned fruit, stewed or baked fruit or frozen fruit?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

How much do you usually eat each time?   ____ cup
                                          ____ handful=1/2 cup

22. On average, how often do you eat fresh raw fruit (eg. apple, banana, orange, pear, grapes)?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

How much do you usually eat each time?   ____ whole piece of fruit
                                          ____ cup

23. On average, how often do you eat yoghurt, dairy food, milk pudding, mousse or custard?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

How much do you usually eat each time?   ____ pottle
                                          ____ cup
24. On average, **how often** do you eat ice cream, ice blocks, jelly, frozen dairy dessert or frozen yoghurt?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**How much** do you usually eat **each time**? (please circle)

Photo A       Photo B       Photo C       OR______ ice block

25. On average, **how often** do you put sugar, jam, honey, syrup or sweet sauce on other foods like pancakes or ice cream?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**How much** do you usually add **each time**?  ___ teaspoon  ___ dessert spoon  ___ ml

26. On average, **how often** do you eat breakfast cereals?

- ☐ never (go to next question)
- ☐ times a day
- ☐ times a week
- ☐ times a month

**Circle** the type of cereal you eat **most** often:

- Weetbix
- Cornflakes
- Ricies
- Coco pops
- Nutra-grain
- Porridge
- Other:______________________ OR ____ weetbix

**How much** do you usually eat each time? (please circle)

Photo A       Photo B       Photo C
27. On average, **how often** do you eat muesli bars, cereal bars or nuts bars?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

*How much* do you usually eat **each time**?

☐ bar

☐ g

28. On average, **how often** do you eat chocolate biscuits (eg. Tim Tam, Toffee Pop) or cream-filled sweet biscuits?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

*How much* do you usually eat **each time**?

☐ biscuit

29. On average, **how often** do you eat other sweet biscuits (eg. wine biscuits, gingernuts)?

☐ never (go to next question)

☐ times a day

☐ times a week

☐ times a month

*How much* do you usually eat **each time**?

☐ small biscuit (eg.Round wine)

☐ small double biscuit (eg. Cameo cream)

☐ large cookie

30. On average, **how often** do you eat sweet buns, iced buns, doughnuts or pastries?
☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

**How much** do you usually eat each time?  
☐ doughnut
☐ bun
☐ pastry

---

31. On average, **how often** do you eat cake, sponge, muffins or baked pudding?

☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

**How much** do you usually eat each time? (please circle)

Photo A  Photo B  Photo C  OR  ☐ g

32. On average, **how often** do you eat lollies (eg. jet planes, mints, toffees, liquorice) ?

☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

**How much** do you usually eat each time?  
☐ lollies
☐ family pack

---

33. On average, **how often** do you eat chocolate or chocolate bars (eg. Moro, Crunchie)?

☐ never (go to next question)
☐ times a day
☐ times a week
☐ times a month

**How much** do you usually eat each time?  
☐ square
If you have been diagnosed with high blood pressure, high cholesterol, heart disease or angina, diabetes or gout has your diet changed since diagnosis? ☐ Yes ☐ No

How has it changed? (tick as many that apply)

☐ I eat less  ☐ I eat more

☐ I eat less sugar  ☐ I eat more sugar

☐ I eat less fat  ☐ I eat more fat

☐ I eat less fruit  ☐ I eat more fruit

☐ I choose diet drinks  ☐ I choose fruit juice

Other: ____________________________________________

Have you lost weight since diagnosis? ☐ Yes ☐ No

THE END…please check every page to see if you have answered every question

Fa’afetai tele lava (thank you very much) for helping us with this important project!
References


Barry, D. & Petry, N. M. 2009. Associations between body mass index and substance use disorders differ by gender: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Addict Behav*, 34, 51-60.


Garrod, A. B. 1876. *A treatise on gout and rheumatic gout (rheumatoid arthritis)*, Longmans, Green.


Ministry of Health 2012a. The Health of New Zealand Adults 2011/12: Key findings of the New Zealand Health Survey. Wellington.


Sport and Recreation New Zealand 2008. Recreation and Physical Activity Participation Among New Zealand Adults: Key Results of the 2007/08 Active NZ Survey. Wellington: SPARC.


