Investigating the Association Between Genetic Variants and Response to a One-Year Lifestyle Intervention Targeting Metabolic Syndrome

by

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A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Human Health and Nutritional Sciences

Guelph, Ontario, Canada

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ABSTRACT

INVESTIGATING THE ASSOCIATION BETWEEN GENETIC VARIANTS AND RESPONSE TO A ONE-YEAR LIFESTYLE INTERVENTION TARGETING METABOLIC SYNDROME

Dana E. Lowry
University of Guelph, 2019

Advisor: Dr. David Mutch

Although lifestyle interventions are generally successful for reducing metabolic syndrome (MetS) risk, significant variability in response between people exists. This thesis investigated factors that influence response to a 1-year, personalized, team-based lifestyle intervention targeting individuals with MetS. Using data collected from the Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) program, it was found that reductions in continuous MetS (cMetS) score at 3 months and 1 year were associated with two single nucleotide polymorphisms: rs662799 (A/G) in apolipoprotein A5 (APOA5) and rs1501299 (G/T) in adiponectin (ADIPOQ). Next, predictive models were developed to investigate if baseline variables could predict cMetS score at 1 year in the CHANGE program. Baseline systolic blood pressure and short-term changes in cMetS score were found to be predictive of 1-year cMetS score. Collectively, this thesis demonstrates the power of genetic and bioclinical measurements to unravel the inter-individual variability in response to lifestyle interventions targeting MetS.
ACKNOWLEDGEMENTS

First, thank you to my advisor, Dr. David Mutch. Your guidance and support have made all of my accomplishments possible during these past two years. Thank you for pushing me to strive for excellence, especially in an area that I was unfamiliar with from the beginning. Your overwhelming breadth and depth of knowledge have been inspiring and I am grateful to have grown as a scientist under your mentorship. Thank you for providing me with truly wonderful opportunities that I would not have otherwise had the privilege to experience. Lastly, thank you for always having your door open to chat about science and all things in between!

Thank you to my committee member, Dr. Jess Haines for your insight and expertise throughout my MSc research. Thank you to Dr. Zeny Feng for your patience and guidance. It was an incredible experience to learn from you. Thank you to the entire HHNS faculty and staff!

Thank you to the past and present Mutch lab – I am extremely grateful to have been surrounded by such warm, welcoming, caring and intelligent individuals. I have learned so much from each of you. My experience at the University of Guelph was truly a pleasure because of your wonderful friendship. I cannot thank you enough for your support and laughter, and I cannot want to see what the future holds for each of you!

To my friends near and far, I am forever grateful for your friendships that provided me with so much encouragement and love. Lastly, a massive thank you to my wonderful parents. Your unwavering support throughout all of my endeavors has not gone unnoticed and I could not be luckier to have two caring and loving parents.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin I-Converting Enzyme</td>
</tr>
<tr>
<td>ADBR2</td>
<td>Adrenoceptor β2</td>
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<tr>
<td>ADIPOQ</td>
<td>Adiponectin</td>
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<tr>
<td>APOA5</td>
<td>Apolipoprotein A-V</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>BCAA</td>
<td>Branched Chain Amino Acid</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CHANGE</td>
<td>Canadian Health Advanced by Nutrition and Graded Exercise</td>
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<tr>
<td>cMetS</td>
<td>Continuous Metabolic Syndrome Score</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>D</td>
<td>Deletion Allele</td>
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<tr>
<td>DASH</td>
<td>Dietary Approaches to Stop Hypertension</td>
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<tr>
<td>DPP</td>
<td>Diabetes Prevention Program</td>
</tr>
<tr>
<td>DTC</td>
<td>Direct-to-Consumer</td>
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<tr>
<td>FTO</td>
<td>Fat Mass and Obesity-Associated</td>
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<tr>
<td>GDMI</td>
<td>Global DNA Methylation Index</td>
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<tr>
<td>GNB3</td>
<td>G-protein Subunit β3</td>
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<tr>
<td>GRS</td>
<td>Genetic Risk Score</td>
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<tr>
<td>GWAS</td>
<td>Genome-Wide Association Study</td>
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<tr>
<td>HDL-C</td>
<td>High-Density Lipoprotein Cholesterol</td>
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<tr>
<td>HEI-C</td>
<td>Healthy Eating Index Score – Canadian</td>
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<tr>
<td>HOMA-β</td>
<td>Homeostatic Model Assessment of β-cell Function</td>
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HOMA-IR  Homeostatic Model Assessment of Insulin Resistance
HR      High Responders
HWE     Hardy Weinberg Equilibrium
I       Insertion Allele
IDF     International Diabetes Federation
IFG     Impaired Fasting Glucose
INSIG2  Insulin-Induced Gene 2
IRS1    Insulin Receptor Substrate 1
LDL     Low-Density Lipoprotein
LPL     Lipoprotein Lipase
LR      Low Responders
LTPA    Leisure Time Physical Activity
MC4R    Melanocortin-4 Receptor
MetS    Metabolic Syndrome
NCEP:ATPIII  National Cholesterol Education Program-Third Adult Treatment Panel
NHANES  National Health and Nutrition Examination Survey
PCA     Principal Component Analysis
PI3K    Phosphoinositide-3-Kinase
PPARγ2  Peroxisome Proliferator-Activated Gamma 2
RYGB    Roux-en-Y Gastric Bypass Surgery
SBP     Systolic Blood Pressure
SD      Standard Deviation
SE      Standard Error
SNP     Single Nucleotide Polymorphism
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>SWLM</td>
<td>Successful Weight Loss Maintainers</td>
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<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>uwGRS</td>
<td>Unweighted Genetic Risk Score</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$</td>
<td>Maximum Rate of Oxygen Consumption</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
<tr>
<td>wGRS</td>
<td>Weighted Genetic Risk Score</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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APPENDIX 1: Genetic Risk Score (GRS) Protocol
Chapter 1: Review of the Literature
1.0 Introduction

Type 2 diabetes (T2D) and cardiovascular disease (CVD) are health epidemics increasing globally. The primary risk factors for these diseases (i.e., obesity, fasting glucose, dyslipidemia and hypertension) tend to occur collectively and make up the cardiometabolic disorder known as metabolic syndrome (MetS). Considering each of these components can be managed by modifying dietary and exercise habits, interventions focusing on these two lifestyle factors are often the first approach to prevent and treat MetS. Although lifestyle interventions are widely successful in the general population, there remains a subset of individuals that either do not respond or have adverse responses to these interventions (Bouchard et al. 2012; Fenwick et al. 2018b; Melanson et al. 2013). The variability in response is due to a number of contributing factors, including bioclinical parameters (e.g., waist circumference, fasting glucose, triglyceride levels etc.) and genetics, amongst others. In order to ensure that all individuals with MetS experience improvements during a lifestyle intervention, it is important to investigate whether tailoring/personalizing these interventions to individuals could improve outcomes.

The goals of this review are to: 1) define and discuss MetS, MetS prevalence and risk factors for developing MetS; 2) summarize lifestyle interventions targeting MetS and the factors that affect intervention outcomes; and 3) highlight genetic variations, including single nucleotide polymorphisms and methylation patterns, that have been shown to influence response to lifestyle interventions. Collectively, these sections will highlight the promising discoveries made to-date showing the advantages of personalizing lifestyle interventions for individuals.
1.1 Defining Metabolic Syndrome

Metabolic syndrome encompasses a cluster of risk factors that contribute to the risk for developing T2D and CVD (O'Neill and O'Driscoll 2015). The condition is generally defined as the clinical manifestation of 3 or more of the following 5 risk factors: central obesity (defined using waist circumference [WC]), elevated fasting blood glucose, hypertension, elevated triglycerides (TGs), and reduced high-density lipoprotein cholesterol (HDL-C) (Alberti et al. 2009a). Diagnosing MetS is done by examining whether the five aforementioned risk factors lie above or below defined threshold values. According to a recent meta-analysis, MetS is associated with an increased risk of CVD (relative risk [RR] 2.35; 95% confidence interval [CI]; 2.02 to 2.73), CVD mortality (RR 2.40; 95% CI; 95% CI; 1.87 to 3.08), all-cause mortality (RR 1.58; 95% CI; 1.39 to 1.79; 95% CI; 1.61 to 2.46), myocardial infarction (RR 1.99; 95% CI; 1.8 to 2.85) and stroke (RR 2.27) (Mottillo et al. 2010). In addition, using the NCEP:ATPIII definition, MetS increases incidence of T2D by 5-fold (Ford et al. 2008).

Although the risks associated with MetS are accepted by multiple organizations including the World Health Organization (WHO), the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP:ATPIII) and the International Diabetes Federation (IDF), the threshold values to diagnose the condition differ slightly with each organization (Table 1). In an attempt to unify the criteria, the IDF Task Force on Epidemiology and Prevention, the National Heart, Lung, and Blood Institute, the American Heart Association, the World Health Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity published a joint statement in 2009 defining the clinical diagnostic criteria for MetS

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**Table 1**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Threshold Values</th>
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<tbody>
<tr>
<td>Central Obesity</td>
<td>Defined using waist circumference (WC)</td>
</tr>
<tr>
<td>Elevated glucose</td>
<td>Fasting blood glucose levels</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>Elevated TGs</td>
<td>Triglyceride levels</td>
</tr>
<tr>
<td>Reduced HDL-C</td>
<td>High-density lipoprotein cholesterol (HDL-C)</td>
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(Table 1). The new definition states that central obesity is no longer a pre-requisite for MetS, and that the threshold values for waist circumference should vary with ethnicity and nationality.

1.1.1 Prevalence of Metabolic Syndrome

Despite the aforementioned differences defining MetS, various studies have been conducted to determine its prevalence across different ethnicities, races, ages and sex. Globally, the IDF estimates that 20-25% of the world’s population has MetS (2005). In the United States, an estimated 35% of the adult population has MetS, and nearly 50% of those aged 60 years or older have MetS. Moreover, women and individuals of Hispanic descent have a significantly higher prevalence of MetS compared to men and non-Hispanics whites and African Americans (Aguilar et al. 2015). Although obesity rates remain an upwards trend, Aguilar et al. suggested that between 2007 and 2012, using data from the National Health and Nutrition Examination Survey (NHANES), the prevalence of MetS has remained stable in the US population, and may be declining in women (Aguilar et al. 2015).

The prevalence of MetS in Canada is approximately 19% according to a 2011 study using data from the Canadian Health Measures Survey (Riediger and Clara 2011). Similar to the US, MetS prevalence in Canada increases with age, tends to be more common in women than men, and varies with ethnicity (Anand et al. 2003; Riediger and Clara 2011). In addition, higher levels of education and income were significantly associated with lower levels of MetS within the Canadian population (Riediger and Clara 2011). European prevalence of MetS varies largely based on the geographical region, but is similar overall to Canada when using the common NCEP-ATP III definition for MetS (Kwasny et al. 2018). In Asian populations, the prevalence
of MetS is similar to Canadian and European populations, ranging between 15% in China to 30% in South Korea (Lim et al. 2011; Zhao et al. 2014).

1.1.2 Metabolic Syndrome Assessments, Definitions and Risks

Generally, the five components of MetS are considered as dichotomous variables in relation to defined threshold values (e.g., a person’s waist circumference is either above or below a particular value). The use of dichotomous assessments assumes equal weighting for each component in regard to MetS risk. Furthermore, individuals with borderline threshold values may be erroneously considered to have no cardiometabolic risk. Borderline threshold values likely hold a “hidden” risk that can be better assessed with a continuous, rather than dichotomous, severity score (Wijndaele et al. 2006). These “hidden” risks hold especially true for individuals with borderline values for only 2 or 3 MetS criteria. This limitation prompted several different research groups to develop continuous MetS (cMetS) scores that more accurately depict the severity and risks associated with the five MetS components (Ekelund et al. 2005; Hillier et al. 2006; Soldatovic et al. 2016; Wijndaele et al. 2006). Principal component analysis (PCA) and linear regression (i.e. calculation of Z scores) were the most common approaches used to develop cMetS scores. The cMetS scores are used to gauge the risk of other chronic diseases, namely T2D and CVD. For example, a cMetS score developed by Hillier and colleagues used PCA to demonstrate that each standard deviation (SD) increase in the cMetS score was associated with an increased risk of T2D by 3-fold in men and 5-fold in women, and increased risk of CVD by nearly 2-fold in men and women (Hillier et al. 2006). Indeed, in terms of assessing risk for CVD and T2D, multiple studies have demonstrated increased efficacy with
the use of a cMetS score compared to the more commonly used dichotomous definitions (Janghorbani and Amini 2016; Kang et al. 2012; Mercado et al. 2015).

Table 1. Metabolic syndrome criteria

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<tbody>
<tr>
<td><strong>Insulin resistance</strong></td>
<td>Glucose intolerance OR impaired glucose tolerance OR diabetes OR insulin resistance*</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td><strong>Plus any 2 of the following criteria:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body metric</strong></td>
<td>Waist to hip ratio &gt; 0.9 males and &gt; 0.85 females OR BMI &gt; 30 kg/m²</td>
<td>WC ≥ 102 cm males; ≥ 88 cm females</td>
<td>Increased WC (population and ethnicity-specific)*</td>
<td>Increased WC population and country-specific</td>
</tr>
<tr>
<td></td>
<td><strong>Plus any 2 of the following criteria:</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>Lipids</strong></td>
<td>TGs ≥ 1.7mmol⁻¹, 150mgdl⁻¹ OR HDL-C &gt; 0.9mmol⁻¹, 35 mgdl⁻¹ males; &lt;1.0 mmol⁻¹, &lt;39 mgdl⁻¹ females</td>
<td>TGs ≥ 1.7mmol⁻¹, 150mgdl⁻¹</td>
<td>TGs ≥ 1.7mmol⁻¹, 150mgdl⁻¹ OR TG pharmacotherapy</td>
<td>TGs ≥ 1.7mmol⁻¹, 150mgdl⁻¹ OR TG pharmacotherapy</td>
</tr>
<tr>
<td></td>
<td>HDL-C &lt; 1.03 mmol⁻¹, &lt; 4.0 mgdl⁻¹ males; &lt; 1.29 mmol⁻¹, &lt; 50 mgdl⁻¹ females</td>
<td>HDL-C &lt; 1.03 mmol⁻¹, &lt; 4.0 mgdl⁻¹ males; &lt; 1.29 mmol⁻¹, &lt; 50 mgdl⁻¹ females OR HDL-C pharmacotherapy</td>
<td>HDL-C &lt; 1.03 mmol⁻¹, &lt; 4.0 mgdl⁻¹ males; &lt; 1.29 mmol⁻¹, &lt; 50 mgdl⁻¹ females OR HDL-C pharmacotherapy</td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td>≥ 160/90 mmHg</td>
<td>≥ 130/85 mmHg</td>
<td>≥ 130/85 mmHg OR previous blood pressure pharmacotherapy</td>
<td>≥ 130/85 mmHg OR previous blood pressure pharmacotherapy</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>IFG, IFT or T2D</td>
<td>Fasting plasma glucose ≥ 100 mgdl⁻¹, 5.6mmol⁻¹ OR T2D</td>
<td>Fasting plasma glucose ≥ 100 mgdl⁻¹, 5.6mmol⁻¹ OR T2D</td>
<td>Fasting plasma glucose ≥ 100 mgdl⁻¹, 5.6mmol⁻¹ OR elevated glucose pharmacotherapy</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Microalbuminuria</td>
<td></td>
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</table>

*indicates the criteria must be fulfilled to be diagnosed with MetS; IFG: impaired fasting glycaemia; IGT: impaired glucose tolerance
1.1.3 Risk Factors for Metabolic Syndrome

Risk factors that contribute to the development of MetS include demographics, anthropometric and bioclinical measurements, lifestyle behaviors and genetics. MetS risk varies by ethnicity and sex, where Caucasian males, as well as African American and Hispanic females, are more likely to develop MetS compared to other groups (Moore et al. 2017; Salsberry et al. 2007). Moreover, advanced age, low education level, low socioeconomic status and food insecurity are all significant risk factors for MetS (Moore et al. 2017; Parker et al. 2010; Salsberry et al. 2007). Since elevated fasting glucose is a component of MetS, family history of T2D is another risk factor for MetS (Ghosh et al. 2010; Zhang et al. 2018). Individuals with higher BMI and WC are at increased risk of developing MetS. Furthermore, Okada et al. (2016) demonstrated that even a WC in the upper-normal range poses a significant risk for MetS development. Additionally, Tatsumi et al. (2017) demonstrated that central obesity, characterized by increased visceral adipose tissue, is strongly associated with the risk of MetS development.

Lifestyle factors including smoking, alcohol consumption, Western dietary pattern and lack of physical activity are all major contributors to the development of MetS. For example, a dose dependent response exists between smoking and MetS (Sun et al. 2012). Additionally, individuals who are light alcohol consumers (0.1-5g/d) have a lower risk of MetS compared to non-drinkers and heavy-drinkers (Sun et al. 2014). A recent meta-analysis reported that diets similar to the Mediterranean diet are protective, whereas the Western diet significantly contributes to the development of MetS (Rodriguez-Monforte et al. 2017). Other dietary patterns that increase risk of MetS include a diet low in dairy and a diet high in salt (Baudrand et al. 2014; Kim and Je 2016). Two meta-analyses investigated the effect of programmed exercise and
leisure-time-physical-activity, and reported inverse dose-dependent responses between physical activity and MetS risk (Ostman et al. 2017; Zhang et al. 2017).

It is well documented that individuals with a particular genetic makeup may be more susceptible to develop MetS than others (Brown and Walker 2016). Family and twin studies among various populations revealed that the heritability of MetS ranges between 20-35% (Gurka et al. 2014), whereas heritability for its individual components can vary between 15-60% (Bellia et al. 2009; Carmelli et al. 1994; Khan et al. 2015; Lin et al. 2005; Watanabe et al. 1999). This strong genetic component prompted researchers to conduct genome-wide-association studies (GWAS) and next-generation sequencing studies to investigate the associations between genetic variants and individual MetS components (as well as MetS as a whole). In terms of obesity, blood lipids and hypertension, studies have reproducibly reported associations with variants in 56, 157 and 90 loci, respectively (Stancakova and Laakso 2014). Other studies have consistently identified approximately 20 gene variants associated with MetS as a whole (Brown and Walker 2016). Given the obvious complexity of MetS and the severe consequences of the disease, it is imperative to better understand the influence of each risk factor to determine effective personalized interventions for the prevention and treatment of MetS.

1.2 Lifestyle Interventions Targeting Metabolic Syndrome

Although the individual components of MetS may be treated pharmaceutically, lifestyle interventions are the first strategy used to mitigate and reverse MetS (Perez-Martinez et al. 2017). Changes in lifestyle habits (i.e. diet, exercise) and behaviors are the primary factors that reproducibly and sustainably improve MetS as a whole, as well as its individual components (Bassi et al. 2014). Lifestyle interventions encourage changes in an individual’s daily habits,
with the goal of adopting and maintaining beneficial behaviors over time. However, the term “lifestyle intervention” is vague and can be comprised of any number of dietary regimens, exercise programs and/or counselling sessions. These interventions can range from strictly structured programs to brief e-mail or telephone check-ups, and consequently lead to highly variable outcomes (Bassi et al. 2014). As outlined in the following subsections, the efficacy of the program tends to increase when participants are engaged in more structured interventions that facilitate in depth and meaningful interactions with their healthcare providers.

1.2.1 Dietary Interventions Targeting Metabolic Syndrome

Various dietary interventions have been proposed and implemented for MetS patients, including hypocaloric, low-fat, high-fat, high-protein, the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets (Bassi et al. 2014; Garcia et al. 2016; Perez-Martinez et al. 2017). These diets typically aim to reduce body mass index (BMI) and improve diet quality. Recently, an international panel proposed the DASH, Mediterranean and the new Nordic diet as preferential dietary interventions to manage MetS because of their abundance in whole grains, vegetables and unsaturated fats (Perez-Martinez et al. 2017). It is important to note that these three recommended diets advocate general dietary patterns that consist of consuming foods with high quality, rather than advising calorically restricted and quantitative dietary approaches. There exists very strong evidence that a Mediterranean diet improves MetS components even in the absence of a caloric restriction (Mozaffarian 2016; Perez-Martinez et al. 2017).

Although strong evidence supports the Mediterranean, DASH and Nordic dietary patterns for long-term MetS management and prevention, other studies have shown that overall
adherence to any dietary intervention is more impactful on short-term weight loss and improvements in MetS than a specific dietary program (Dansinger et al. 2005; Sacks et al. 2009). Results from Dansinger et al. (2005) showed that four different diets – a low-carbohydrate diet, a balanced diet (40-30-30 % of calories from carbohydrates, fat and protein), a calorically-restricted diet, and a vegetarian and fat-restricted diet all resulted in comparable weight loss and improvements in LDL:HDL ratio in overweight and obese patients with MetS. Similarly, Sacks et al. (2009) randomly assigned four diets of varying carbohydrate, fat and protein content, and concluded that clinically relevant weight loss, as well as improvements in lipid and insulin levels, was achieved regardless of macronutrient emphasis, but were influenced by participant adherence levels.

1.2.2 Exercise Interventions Targeting Metabolic Syndrome

Exercise regimes can be just as varied as dietary interventions in structure, intensity and frequency. However, physical activity is well documented to prevent and manage chronic diseases, especially those with the cardiometabolic dysfunctions characterizing MetS (Warburton et al. 2006). Interventions focusing on increased exercise in MetS patients have shown significant reductions in MetS risk, lower BMI and improvements in individual components such as WC, fasting glucose, triglycerides and HDL-C (Ostman et al. 2017). This subsection will highlight the ongoing debate regarding the optimal form of exercise for lifestyle interventions targeting MetS.

Aerobic exercise interventions generally show consistent decreases in MetS prevalence, risk and individual MetS components (Earnest et al. 2014; Kemmler et al. 2009; Pattyn et al. 2013). A randomized control trial by Bateman et al. (2011) demonstrated that 8 months of
resistance training in MetS patients did not change their cMetS scores, but that aerobic training alone (and in combination with resistance training) significantly reduced their cMetS scores. In contrast, other studies have shown significant reductions in MetS risk, prevalence and metabolic factors with the implementation of resistance training (Conceicao et al. 2013; Misra et al. 2008; Tomeleri et al. 2018). In addition, Bakker et al. (2017) demonstrated that less than 1 hour of resistance training, regardless of aerobic exercise, further decreases the risk of MetS. Definitive evidence regarding the exact type of exercise best suited for MetS is currently lacking and remains inconsistent in the literature.

Despite these mixed findings, a recent meta-analysis concluded that there are no differences in body composition measures (BMI, body mass, total fat mass and WC), cardiovascular outcome measures (VO2max, blood pressure) or metabolic outcome measures (fasting blood glucose, TGs, HDL-C) when comparing aerobic versus combined aerobic and resistance training in MetS exercise interventions. Nevertheless, this meta-analysis indicated that VO2max (a common marker of cardiorespiratory fitness), systolic blood pressure and fasting blood glucose are more greatly reduced with high intensity aerobic exercise compared to a combined aerobic and resistance training regimen (Ostman et al. 2017). As such, the intensity of exercise training programs may be more influential than the type of exercise program itself. Along these lines, Tjonna et al. (2008) demonstrated in a randomized clinical trial that high intensity aerobic interval training compared to continuous moderate aerobic exercise significantly improved aerobic capacity, insulin signaling in fat and skeletal muscle, and reduced blood glucose and lipogenesis in adipose tissue.
The above studies demonstrate benefits from structured physical activity programs; however, unregulated physical activity that accumulates throughout the day, termed leisure-time-physical-activity (LTPA), can also significantly reduce prevalence of MetS and improve individual MetS components. Numerous studies, including two meta-analyses, suggest a strong dose-dependent relationship between increased LTPA, especially moderate-vigorous LTPA (Ilanne-Parikka et al. 2010), and reduction in MetS risk (He et al. 2014; Zhang et al. 2017).

### 1.2.3 Combined Lifestyle Interventions Targeting Metabolic Syndrome

Given the clear evidence that dietary changes and increased physical activity prevent and alleviate MetS risk, most lifestyle interventions use a combined approach as they tend to be more efficacious than either approach alone (Jacobs et al. 2009; Zhang et al. 2017). Objectives of more recent combined lifestyle interventions are increasingly focused on the sustainability of the program and how to maintain long-term behavioral changes. Adherence to dietary and exercise regimens, both during and after a lifestyle intervention, is an important determinant for the success of the intervention and continuous motivation is a key factor that drives an individual’s adherence to these lifestyle interventions (Burgess et al. 2017). The following subsections will highlight the various approaches taken to improve intervention adherence and increase motivation including: team-based approaches, group sessions, individualized dietary and/or exercise programs, behavioral treatment strategies (i.e., motivational interviewing) and technology (e.g., pedometers, text message/email reminders, etc).
1.2.3.1 Structured vs. Unstructured Lifestyle Interventions

The majority of the literature to date suggests that structured and directed lifestyle interventions have greater success at reducing MetS prevalence and improving MetS components compared to single session, independent interventions. In two separate studies, Bo et al. (2007) and Pettman et al. (2009) randomized MetS patients into two groups consisting of: 1) an intervention group that received multiple counselling/educational sessions, and 2) a control group that received a single session of standardized healthy lifestyle guidelines. In both studies, MetS prevalence and clinical MetS criteria were significantly reduced in the intervention groups compared to the control groups after 1 year and 16 weeks, respectively. Similar results were seen in a trial that randomized individuals into multiple sessions versus single session counseling groups after achieving a 5% weight loss (Munakata et al. 2011). MetS patients that have multiple counselling sessions over the course of an intervention were found to be more likely to continue the habits learned during the intervention. In the randomized trial by den Boer et al. (2013), MetS prevalence was reduced and maintained 4 years post-intervention in individuals who received periodic counselling sessions throughout the trial compared to individuals who received one initial counselling session (i.e., the control group). However, similar to dietary regimens, Busnello et al. (2011) demonstrated that individuals with MetS who were strongly motivated to adhere to the lifestyle intervention and make positive behavioral changes were the greatest responders (BMI and WC reductions) to the intervention, regardless of whether they were in the intervention or control group.
1.2.3.2 Group-based vs. Individual-based Lifestyle Interventions

As discussed above, maintaining contact with healthcare professionals throughout a lifestyle intervention is beneficial, but it is unclear whether group or private counselling sessions are most effective. Few studies have directly compared group and individual counselling in a MetS lifestyle intervention. Results from Saboya et al. (2017) showed that when lifestyle interventions with identical program material are implemented in either a group or an individual setting, both led to significant decreases in BMI and WC compared to standard care. However, the group interventions were significantly more effective at reducing BMI and WC compared to the individual counselling. Additionally, a MetS lifestyle intervention conducted entirely by telephone, whether in group conference calls or individual calls, significantly reduced BMI and WC from baseline (Weinstock et al. 2013). However, approximately 20% more individuals in the group intervention compared to the individual intervention reported clinically relevant weight loss, i.e., >5% loss of body weight.

Group interventions may be more effective than individual interventions because encouragement from peers and increased accountability can provide additional motivation, i.e., external motivators. A community-wide study by Tran et al. (2017b) based their program on social cognitive theory to motivate and encourage their MetS participants to adopt and maintain healthy lifestyle behaviors. The intervention communities combined group dietary and lifestyle counselling/education sessions with consistent walking groups led by local walk leaders. By the end of the 6-month intervention, the intervention communities showed significant increases in HDL-C and decreases in BMI, WC, weight, waist-to-hip ratio and mean number of MetS components (Tran et al. 2017b). Furthermore, the intervention groups showed significant
improvements in walking time, the amount of moderate-intensity physical activity and total activity, and significant reductions in sitting time relative to the control group (Tran et al. 2017a). A follow-up was not done for this study, although a post-intervention analysis to investigate whether these behaviors were sustained due to a strong group environment would have been interesting. A study by Jane et al. (2017) noted that when individuals participating in a MetS clinical trial had access to an online Facebook community detailing a lifestyle intervention, they lost significantly more weight (>4.8% loss in body weight) than the group that received information on a pamphlet. These results suggest that social media and other technology may be appropriate tools to assist in community building to motivate improved adherence to lifestyle interventions.

1.2.3.3 In-Person vs. Technological Interface for Lifestyle Interventions

Holding in-person counselling/educational sessions is time-consuming and expensive. Therefore, researchers have investigated whether face-to-face meetings could be replaced by technology-interfaced meetings, such as telephone calls, emails or text messages. Ma et al. (2013) compared the efficacy of a coach-led, group intervention with a self-directed DVD intervention, and found that both approaches significantly reduced BMI, WC and fasting plasma glucose in individuals with MetS over 3 months. These results suggest that personal contact may serve as an accessory to technology-interfaced interventions in MetS lifestyle interventions, instead of vice versa.

Many studies targeting improvements in MetS have implemented lifestyle interventions that use telephone-based motivational counselling or web-based intervention instructions rather than face-to-face interactions (Chen et al. 2013; Svetkey et al. 2008; Tate et al. 2006). A study
conducted in Taiwanese women showed significant improvements in WC, fasting blood glucose and mean number of MetS components after a lifestyle intervention run solely through an internet platform (Patrick et al. 2009). Another study showed an experimental group (consisting of weekly motivational phone interviews) had significant improvements in HDL-C, WC, and MetS scores when compared to a brief group (single intervention session) and a control group (standard care) (Lin et al. 2016). In contrast, Svetkey et al. (2008) reported that MetS patients randomized to a weight-loss maintenance program regained more weight in the monthly interactive web-based maintenance program compared to the monthly personal contact program. Given the mixed evidence that currently exists in the literature, lifestyle interventions implementing technology within a personal contact lifestyle intervention may be the most successful and cost-effective protocols for managing MetS and encouraging sustained behavioral changes.

1.2.3.4 Generalized vs. Personalized Lifestyle Interventions

Multiple studies have demonstrated significant improvements in MetS components and lifestyle habits through personalized MetS interventions. Bo et al. (2007) conducted a randomized clinical trial to investigate the benefits of individualized dietary advice and goal setting in MetS patients. This intervention group was compared to a control group that received one-time general lifestyle advice by a general practitioner. After 1 year, participants in the intervention group showed significant improvements in all MetS components, except for fasting glucose, compared to the control group. The intervention group increased their hours of physical activity and significantly improved their diet quality by reducing consumption of total and saturated fat and increasing consumption of protein and fiber (Bo et al. 2007). Another study
compared a personalized intervention to a generalized intervention in Japanese workers and demonstrated significant decreases in WC and BMI in individuals participating in the personalized lifestyle intervention compared to the general intervention (Watanabe et al. 2017).

Recently, Jeejeebhoy et al. (2017) reported that 19% of individuals who participated in a supervised and personalized team-based Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) lifestyle intervention program reversed their MetS after 1 year. Additionally, at 1 year, participants in this program showed significant increases in their diet quality (assessed using the healthy-eating index score - HEI-C), VO$_{2\text{max}}$, and HDL-C and decreased their systolic and diastolic blood pressure, TGs, WC and cMetS scores. Since the intervention was supervised (i.e., maintained regular personal contact during the 1 year intervention), the success of the intervention cannot be definitively attributed to the personalization of the intervention, and may instead be attributed to the intensive structure and practitioner interaction. Lastly, a recent systematic review of lifestyle interventions targeting South Asian migrants in various Western countries including Norway, USA, and Scotland, reported that only those interventions that were personalized to the patients’ particular culture were successful (Martin et al. 2018).

Despite the success of these personalized interventions, Pettman et al. (2009) showed that providing individuals with general advice on dietary and physical activity habits, in conjunction with a supervised group-based program, is sufficient to significantly reduce BMI, WC, systolic and diastolic blood pressure, total cholesterol and LDL-C. The current evidence suggests that interventions with personalized aspects can improve MetS components and change lifestyle
behaviors, but it is difficult to definitively attribute the success of the lifestyle interventions solely to the personalization.

1.3 Factors Influencing a Person’s Response to Lifestyle Interventions

Although lifestyle interventions combining diet and exercise modifications successfully reduce MetS prevalence and improve individual MetS components in the general population, there exists high inter-individual response to these interventions. This high variability is not unique to lifestyle interventions targeting MetS, as it has also been demonstrated in programs that focus on weight loss, insulin resistance and aerobic fitness/exercise (Bouchard et al. 2012). People that successfully respond to the lifestyle interventions are largely considered to be “responders”, those that are unresponsive are considered to be “non-responders” and those that experience a negative response to the intervention are often termed “adverse-responders”.

1.3.1 Evidence of Varying Responses from Dietary and Exercise Interventions

A study by Bouchard et al. (2012) explored the notion of differential responses to exercise interventions in a retrospective meta-analysis including six studies. The study defined “adverse response” as a response that is \( \geq 2 \) technical errors (TEs) in the adverse direction. Across the six studies, the prevalence of adverse responders was approximately 8.3% for fasting insulin, 13.3% for HDL-C, 10.3% TGs and 12.2% for systolic blood pressure. The study also determined that despite the heterogeneous exercise programs and study populations, the change in \( VO_{2max} \) was not correlated to the adverse responses.

Varying metabolic responses to different foods have also been well documented in some dietary interventions. A paper by Zeevi et al. (2015) investigated the inter-individual
postprandial glucose response to different foods in an 800-person cohort. The authors developed an algorithm using the postprandial glucose response, bioclinical measurements and microbiome data to predict an individuals’ response in a dietary intervention. This study showed that postprandial glucose responses were highly variable across individuals, even when consuming standardized meals, and that a personalized dietary intervention using the developed algorithm reduced post-meal glucose response. Another recent study categorized participants into responders and non-responders, where responders demonstrated a ≥ 10% reduction in homeostatic model assessment of insulin resistance (HOMA-IR) and non-responders demonstrated a <10% reduction in HOMA-IR (McMorrow et al. 2018). The authors noted responders (which represented 40% of the study population) had significantly greater baseline insulin, HOMA-IR, HOMA-β, LDL and total cholesterol compared to non-responders. Additionally, the baseline HOMA-IR and LDL:HDL cholesterol ratio predicted response to the intervention. This suggests that individuals who are responders to a lifestyle intervention may be more severely afflicted at the start of the intervention compared to non-responders.

1.3.2 Baseline Bioclinical Measurements Influence Response to Lifestyle Interventions

Results from multiple studies have found correlations between baseline bioclinical measurements and response to various interventions targeting weight loss and/or metabolic risk. Uysal et al. (2014) reported that baseline MetS risk factors, including BMI, WC, HOMA-IR, systolic blood pressure and diastolic blood pressure, are inversely correlated with weight loss in obese adolescents during a 1 year lifestyle intervention. In addition, a lifestyle intervention that used the change in systolic blood pressure over 24 weeks as a proxy for response to the year-long intervention noted that “responders” (≥ 4mmHg reduction in systolic blood pressure) had
significantly lower diastolic blood pressure, WC and increased VO$_{2\text{max}}$ by the end of the intervention compared to non-responders (Stuckey et al. 2015). Lastly, a study that investigated the persistence of MetS in Roux-en-Y gastric bypass surgery (RYGB) patients found that baseline TGs, HbA1c and T2D were significant predictors of MetS resolution 1 year post-surgery (Martini et al. 2015). These studies highlight the strong associations between baseline bioclinical measurements and response to interventions, as well as the potential to use baseline measurements to predict individual responses to these interventions.

1.3.3 Genetic Influences on Lifestyle Intervention Responses

Individual response to lifestyle interventions may be partly due to inter-individual differences in genetic make-up. The two most studied genetic variations include single nucleotide polymorphisms (SNPs) and epigenetic markers, namely global and tissue specific DNA methylation. The next section will provide a brief overview on both the SNPs and epigenetic markers associated with MetS as a whole and with the individual MetS components in the context of a lifestyle intervention.

1.3.3.1 SNPs

Lifestyle interventions targeting MetS generally focus on SNPs associated with the individual MetS components rather than MetS as a whole, thus the following section will discuss SNPs that have been associated with changes in individual MetS components in response to lifestyle interventions.
1.3.3.1.1 SNPs Associated with Body Weight

Several SNPs are associated with changes in central obesity, characterized by waist circumference (WC) and BMI, in the context of lifestyle interventions. Although the SNP rs9939609 (A/T) from the fat mass and obesity-associated (FTO) gene is one of the most studied SNPs in obesity research, results remain inconclusive regarding its role as a modifier of response to lifestyle interventions. Some studies have demonstrated that A allele carriers (AA + AT) experience different responses to lifestyle interventions compared to TT homozygotes. In a year-long weight loss and maintenance program for severely obese participants, Woehning et al. (2013) demonstrated that individuals with the AA genotype were less likely to lose additional weight and more likely to regain weight in the weight maintenance period compared to T allele carriers (AT + TT). In contrast, a 3-year Mediterranean diet program found that AA carriers experienced the lowest body weight gain compared to TT homozygotes (Razquin et al. 2010). Another trial comparing four different weight loss diets demonstrated that A-allele carriers who specifically consumed a hypo-caloric and high-protein diet reported fewer cravings and decreased appetite compared to the TT-homozygotes, although no changes in BMI or WC were observed (Huang et al. 2014). Despite the reported differences in these studies, a recent meta-analysis examining the effects of rs9939609 and body weight in response to lifestyle interventions indicated no differences in BMI, WC or weight loss according to FTO genotype (Livingstone et al. 2016).

Another well-studied gene associated with central obesity is the melanocortin-4 receptor (MC4R), which is involved in satiety and appetite control. Lifestyle interventions have reported differences in weight change and maintenance in both adults and children according to several
SNPs in the \( MC4R \) gene (Pan et al. 2013; Reinehr et al. 2009). In the Diabetes Prevention Program (DPP), over 3000 individuals were randomized to one of three programs: a lifestyle intervention (low fat hypocaloric diet and exercise program), a metformin group or a placebo control group. Although several SNPs in the \( MC4R \) gene were associated with weight loss, only one SNP (rs1943218; C/T) was associated with 6-month and 2-year weight change after controlling for treatment assignment (Pan et al. 2013). In contrast, while a lifestyle intervention in obese children observed no genotype influence on weight loss, children carrying an \( MC4R \) SNP that decreases MC4R activity were unable to maintain weight loss to the same extent as children without this mutation (Reinehr et al. 2009).

Lastly, the gene encoding the peroxisome proliferator-activated gamma 2 (\( PPAR\gamma 2 \)) nuclear hormone receptor, which is highly expressed in adipose tissue and involved in fat metabolism and adipogenesis, has been associated with changes in fat mass and weight loss in lifestyle interventions (Matsuo et al. 2009; Vogels et al. 2005). A weight-loss study in middle-aged Japanese women observed associations between six \( PPAR\gamma 2 \) SNPs and weight loss across a 14-week calorie-restricted diet intervention (Matsuo et al. 2009). Another calorie-restricted diet intervention demonstrated a significant association between rs1801282 (C/G) in \( PPAR\gamma 2 \), in which CC carriers lost more body weight and body fat during the intervention and maintained more weight loss after the intervention compared to G allele carriers (GG + GC). In addition, two lifestyle interventions combining dietary and exercise regimes reported interactions between dietary fat intake, weight loss and rs1801282 genotype (Garaulet et al. 2011; Lindi et al. 2002).

Collectively, these examples suggest that specific genetic variants may modify a person’s body weight response to a lifestyle intervention. Consequently, \textit{a priori} knowledge of these
SNPs may help to predict whether a person’s body weight will change in response to a particular intervention or not.

1.3.3.1.2 SNPs Associated with Dyslipidemia

Although the heritability of blood TG and HDL-C levels is estimated to range between 60-80%, few lifestyle interventions have studied the associations between SNPs and changes in these lipid levels (O’Connell et al. 1988; Souren et al. 2007). Apolipoprotein A-V (APOA5) is an important regulator of TG levels by influencing interactions between circulating lipoproteins and lipoprotein lipase and the hepatic LDL-receptors (Su et al. 2018). Lifestyle interventions targeting dyslipidemia report an association between TG and HDL-C level changes with APOA5 genotype (Jang et al. 2010; Zhang et al. 2012). A study in ~300 Korean men showed that individuals carrying the TT-genotype in the rs662799 (T/C) SNP in APOA5 showed greater improvements in TG and HDL-C levels after incorporating legumes, increased vegetable intake and regular walking into their daily lives (Jang et al. 2010). In a 2-year weight-loss diet intervention, Zhang et al. (2012) reported diet-genotype interactions between rs964184 (C/G) and fat intake. Individuals carrying the G-allele in the low-fat group had greater reductions in total cholesterol and LDL-C levels, whereas G-allele carriers in the high-fat group had greater increases in HDL-C levels. (Jang et al. 2010; Zhang et al. 2012). Another important apolipoprotein, apoliprotein E (APOE), is associated with lipid levels and MetS (Eichner et al. 2002; Kristiansson et al. 2012), and the three existing APOE isoforms are differentially associated with TG and HDL-C levels (Povel et al. 2011).

More recently, the transcription factor 7 like 2 gene (TCF7L2), normally investigated in relation to glucose homeostasis, was shown to influence postprandial lipid metabolism (Perez-
Martinez et al. 2012). A Mediterranean diet intervention reported differences in lipid profiles according to genotype (rs7903146; C/T) and diet adherence. When adherence to the diet was low, TT carriers showed increased TGs, total cholesterol and LDL-C, but this interaction disappeared in individuals with high adherence (Corella et al. 2013). Numerous other SNPs have been identified that influence a person’s blood lipid response to lifestyle interventions. Huggins et al. (2013) evaluated 82 SNPs in over 3500 participants from lifestyle interventions and identified variants in CETP, LIPC, FADS2, APOB and PGS1 that modulated changes in both HDL-C and TG levels in lifestyle interventions. Evidently, there is a strong genetic influence that contributes to the differential responses in lipid parameters observed in lifestyle interventions; however, it is imperative that more studies are conducted in this area to reproduce these encouraging findings.

1.3.3.1.3 SNPs Associated with Glucose Homeostasis

Several GWAS have identified loci that are associated with glycemic traits and T2D, confirming the strong genetic contribution to insulin resistance (Brown and Walker 2016; Dupuis et al. 2010). Despite the number of genetic variants identified through these past studies, only a few have been examined within the context of a lifestyle intervention. SNPs in TCF7L2 are associated with increased risk of T2D, likely due to the enzyme’s role in insulin secretion and sensitivity (Katsoulis et al. 2018; Tong et al. 2009). In a lifestyle intervention, Florez et al. (2006) reported differences in baseline insulin secretion and sensitivity according to TCF7L2 genotype, but noted these differences were mitigated by a lifestyle intervention consisting of a low-fat, low-calorie diet, exercise intervention, and diet and exercise counselling sessions. This
suggests that the influence of *TCF7L2* risk alleles on insulin secretion/sensitivity may be overcome by lifestyle interventions.

Phosphoinositide-3-kinase (*PI3K*) and insulin receptor substrate 1 (*IRS1*) have important roles in the insulin signalling pathway, and have been shown to influence glucose and insulin levels (Rung et al. 2009). In the Finnish Diabetes Prevention study, the *PI3K* genotype influenced a person’s change in glucose to a 2-hr oral glucose tolerance test for individuals enrolled in the intervention group. In contrast, no differences in T2D risk or glucose responses were found for SNPs in *IRS1* (Laukkanen et al. 2004). In the POUNDS LOST trial, Qi et al. (2011) reported differences in HOMA-IR after a diet-induced weight loss intervention according to *IRS1* genotype and carbohydrate/fat dietary ratio. Another study by Huang et al. (2016) investigated a genetic risk score (GRS) of 31 variants related to glucose homeostasis in the same POUNDS LOST trial cohort. The authors reported an interaction between the GRS, measures of insulin resistance, and dietary protein intake. Additionally, several studies have identified other SNPs that influence T2D risk, as well as glucose and insulin levels, in lifestyle interventions, including *LIPC, ADRA2B* and *ENPP1* (Moore et al. 2009; Siitonen et al. 2004; Todorova et al. 2004). Overall, numerous SNPs have been associated with changes in glucose tolerance, which may provide a partial explanation for the wide-range of responses in insulin and glucose levels reported in lifestyle interventions.

### 1.3.3.1.4 SNPs Associated with Hypertension

Variability in blood pressure is estimated to be 30-60% heritable and more than 60 SNPs have been found to influence blood pressure (Ehret et al. 2016; Shih and O'Connor 2008). Among the identified variants, SNPs in the angiotensin I-converting enzyme (*ACE*) gene are the
most studied genetic variant. As part of the renin-angiotensin-aldosterone system, *ACE* is an important regulator of blood pressure, especially in blood pressure response to salt intake (Ge et al. 2007). In a study by Poch et al. (2001) individuals participated in a low-salt intake and a high-salt intake crossover study. The results indicated that individuals exhibited different responses to salt intake according to their *ACE* (rs4646994; I/D) genotype. Specifically, individuals with the insertion allele (I) were more likely to be salt sensitive, i.e., experience a 5-10% increase in systolic blood pressure in response to dietary salt intake, and show increased blood pressure with high salt intake, compared to individuals with the deletion allele (D). Another study demonstrated that individuals with the I/I genotype had significantly higher systolic blood pressure while consuming a high-salt diet compared to carriers of the deletion (D) allele (Giner et al. 2000).

In the context of exercise interventions, the role of the *ACE* genotype on blood pressure responses is more controversial. One exercise trial that investigated rs4646994 and blood pressure response after aerobic exercise in hypertensive men found carriers of the D allele to have lower systolic blood pressure post-exercise compared to I allele carriers (Blanchard et al. 2006), whereas another trial in hypertensive patients reported lower systolic blood pressure in carriers of the I allele following a walking regime compared to D allele carriers (Goessler et al. 2015). Additionally, two lifestyle interventions with similar protocols reported opposing results, in which individuals with the I/I genotype demonstrated greater reductions in diastolic blood pressure post-intervention (Kim 2009), while the other study failed to find a genotype effect (Mota et al. 2013). Collectively, these studies provide some intriguing evidence that specific genetic variants may modify a person’s blood pressure response to lifestyle interventions.
1.3.3.2 Epigenetics

Epigenetic studies that investigate DNA methylation patterns before and after lifestyle interventions highlight an emerging area of genetics that may be used to better understand differential responses. Milagro et al. (2011) examined global and gene specific methylation patterns in high (HR; ≥5% weight loss) and low (LR; <5% weight loss) responders in an 8-week diet-induced weight loss intervention. At baseline, HRs and LRs differed in methylation patterns (±20%) in over 1000 CpG sites, and some of these methylation patterns were correlated with anthropometric changes such as fat mass, BMI and waist circumference. Following the dietary intervention, only 15 CpG sites were differentially (±20%) methylated between HRs and LRs, with HRs exhibiting 8-fold more changes in DNA methylation compared to LRs. Another study comparing high- (>1.1 decrease in BMI-standard deviation score) and low-responders (<0.4 BMI-standard deviation score) in overweight adolescents identified 5 DNA regions (AQP9, DUSP22, HIPK3, TNNT1 and TNNI3) that were differentially methylated in relation to the degree of weight loss response during a 10-week lifestyle intervention (Moleres et al. 2013). Moreover, a calculated methylation score based on these 5 DNA regions was associated with significant changes in weight, BMI-standard deviation score and body fat mass loss during the intervention. In another study, Huang et al. (2015) demonstrated that similar methylation patterns exist between successful weight loss maintainers (SWLM) and normal weight individuals, but that the methylation patterns significantly differed between SWLM and obese individuals.

A personalized 12-month weight loss intervention combined SNP and epigenetic profiles to investigate the association between allelic variations, global DNA methylation index (GDMI) and weight loss (Pirini et al. 2018). The study gave specific dietary recommendations based on
the participants’ genetic profiles related to five energy balance and lipid metabolism genes: 
INSIG2 insulin-induced gene 2 (INSIG2); MC4R; adrenocceptor β2 (ADBR2); APOA5; and G-protein subunit β3 (GNB3). The study reported an inverse correlation between weight loss and GDMI, and a significant association between INSIG2 genotype and GDMI. Although the study was unable to explain the associations between GDMI, INSIG2 and weight loss, future studies should consider incorporating methylation patterns with allelic variations to identify new interactions that may modulate outcomes to lifestyle interventions.

1.4 Predictive Modeling for Lifestyle Intervention Outcomes

The high interest to identify factors that contribute to inter-individual responses to lifestyle interventions has naturally led to the field of predictive modeling. Several researchers have attempted to develop a statistical model to predict the development of MetS based on factors including bioclinical measurements, metabolites and genetics. Typically, studies use follow-up observational approaches in which baseline characteristics are used to predict the development of MetS, or MetS risk at the end of an intervention. Zhang et al. (2015) designed a statistical model based on 11 routine biomarkers, including specific blood and inflammatory markers (i.e., white blood cell count and lymphocyte count), to predict MetS incidence after a 5-year follow-up period in 15000 Han Chinese. Another study attempted to associate baseline bioclinical “phenotypes”, e.g., high TGs/low HDL-C, in adolescents with 10-year MetS development in adulthood (Hosseinpanah et al. 2015). However, the authors found that body weight was a better indicator than baseline bioclinical “phenotypes” for predicting MetS development. One study, conducted in ~7500 non-obese individuals, combined clinical data, age, sex, BMI, smoking history, alcohol consumption history, and exercise with or without the allelic
variations of 10 SNPs into a predictive model for MetS development and reported more accurate and sensitive results with the addition of the SNPs (Choe et al. 2018).

Recently, blood metabolites have also been assessed for their use as predictors of MetS. For example, Lee et al. (2015) identified baseline branched chain amino acid (BCAA) levels as a significant predictor of insulin resistance and MetS at the two-year follow-up point in a small cohort of Korean children. Lastly, a recent study used a systems metabolomics approach and combined 80 variables, including dietary records, bioclinical data and metabolite measurements, into a predictive algorithm to identify MetS after a 5-year follow-up period (Pujos-Guillot et al. 2017). The study showed that a model using metabolic markers, combined with bioclinical data, resulted in the most sensitive model and was specific to the MetS component.

Although studies establishing statistical models for the development of MetS are useful for the prevention of MetS, predictive models targeting the outcome response of MetS lifestyle interventions would improve treatment and management of MetS. There remains a gap in the literature regarding predictors that can classify study participants into responders, non-responders and adverse responders, which would assist practitioners in pre-emptively determining whether a patient would benefit from a specific lifestyle intervention, and in turn greatly increase clinical efficiency at managing MetS.

1.5 Conclusions

Overall, strong evidence supports the value of lifestyle interventions for the management and treatment of MetS in the general population. However, there is also an abundance of literature demonstrating that people experience a wide range of responses to these interventions. Current research highlights baseline bioclinical measurements and genetic variations as two
important factors that contribute to these varying responses. In terms of genetic variations, there is a growing body of evidence suggesting that SNPs play an important role modifying a person’s response to interventions for MetS. In light of this evidence, it is probable that personalizing lifestyle interventions that take genetic information into account may foster greater success for individuals with MetS.
CHAPTER 2: Rationale, Research Objectives and Hypotheses
2.0 Rationale

It is well recognized that MetS greatly increases the risk of T2D and CVD, both of which are leading causes of global mortality. Lifestyle interventions are the first line of prevention and management to combat MetS. Despite the evidence outlining the success of lifestyle interventions, people respond differently to these diet and exercise programs. While most people with MetS respond favourably to lifestyle interventions, some have no response or even show an adverse response. The etiology of MetS and its components is complex and the heterogeneity in responses may be attributable to a variety of factors. These factors are important to identify in order to design more effective personalized lifestyle interventions. By studying the factors that affect an individual’s response to a lifestyle intervention, healthcare practitioners may prescribe diet and exercise programs that are more likely to elicit successful outcomes for their patients.

2.1 Research Objectives

The overall objective of this thesis was to investigate the factors that influence the response to a personalized, team-based lifestyle intervention targeting individuals with MetS.

Specific objectives of this thesis were:

1. To examine SNPs previously found to be associated with MetS on changes in cMetS score, as well as individual MetS components, during a 1-year lifestyle intervention.

2. Develop a genetic risk score (GRS) to examine its impact on changes in cMetS score.
3. To determine which variables (i.e., genetics, diet, aerobic fitness, and bioclinical measurements) could be used to develop a model to predict improvements in cMetS score after 1 year of the Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) program.

2.2 Hypotheses

Given the thesis objectives outlined above, I hypothesized that:

1. Genetic variants would moderate improvements in cMetS score in response to the CHANGE lifestyle intervention.

2. Individuals who carry no risk variants (i.e. a GRS of zero) would show a greater reduction in cMetS score than individuals carrying risk alleles.

3. Individuals with a more metabolically unhealthy profile at baseline will have a greater response to the CHANGE program, and this baseline profile may be used to predict an individual’s future success to this lifestyle intervention.
CHAPTER 3: Variants in APOA5 And ADIPOQ Moderate Improvements in Metabolic Syndrome During a One-Year Lifestyle Intervention

Presented as published in:

3.0 Abstract

**Background:** Metabolic syndrome (MetS) comprises a cluster of risk factors including central obesity, hypertension, dyslipidemia and impaired glucose homeostasis. Lifestyle interventions that promote improvements in diet quality and physical activity represent a first line of therapy for MetS. However, varying responses to lifestyle interventions are well-documented and may be partially explained by underlying genetic differences. The aim of this study was to investigate if variants in genes previously associated with MetS influence the magnitude of change in MetS risk during a 1-year lifestyle intervention.

**Methods:** The present study used data collected from the Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) study cohort (n=159 men and women) to investigate the effect of 17 candidate single nucleotide polymorphisms (SNPs) on response to a 1-year lifestyle intervention. Associations between SNPs and the continuous MetS (cMetS) score, as well as individual MetS components, were examined.

**Results:** Reductions in cMetS score at both 3 months and 1 year were significantly associated with 2 variants: rs662799 (A/G) in apolipoprotein A5 (*APOA5*) and rs1501299 (G/T) in adiponectin (*ADIPOQ*). Individuals carrying a minor T allele in rs1501299 experienced a greater reduction in cMetS score at both 3 months and 1 year, whereas major allele AA homozygotes in rs662799 experienced greater reductions in cMetS score during the intervention. No associations were identified between the aforementioned SNPs and individual components of MetS. Both unweighted and weighted genetic risk scores (GRS) using these 2 SNPs revealed that individuals carrying none of the risk alleles experienced significantly greater reductions in cMetS score after 1 year.
Conclusions: The findings from the current study suggest that individuals with certain genotypes may benefit more from a lifestyle intervention for MetS and that specific variants, either independently or as part of a GRS, could be used as a nutrigenetic tool to tailor the intervention to reduce risk of MetS.
3.1 Introduction

Metabolic syndrome (MetS) encompasses a cluster of factors that contribute to the risk for developing type 2 diabetes (T2D) and cardiovascular disease (CVD) (Brown and Walker 2016). The prevalence of MetS is estimated to be as high as 40% in some populations, particularly in developed countries and older generations (Neill et al. 2015). Although nuances exist regarding the definitions for MetS proposed by various organizations, the condition is generally defined as the clinical manifestation of three or more of the following five risk factors: central obesity (defined using waist circumference), elevated fasting blood glucose, hypertension, elevated triglycerides and reduced high-density lipoprotein cholesterol (HDL-C) (Alberti et al. 2009b). Diagnosing MetS is generally done by examining whether the five aforementioned risk factors lie above or below defined threshold values. However, the use of dichotomous assessments is not without criticism, which prompted the development of a continuous MetS (cMetS) score (Hillier et al. 2006). Specifically, the cMetS score was developed by Hillier and colleagues as a practical method to evaluate MetS risk using principal component analysis. These authors demonstrated that each standard deviation increase in the cMetS score was associated with increased risk of T2D and CVD.

Initial treatment for MetS typically includes a lifestyle intervention comprising changes in diet and physical activity, which has been shown to reduce both the severity of MetS and dependency on pharmacological therapy (Pérez-Martínez et al. 2018). While lifestyle interventions for MetS are generally successful, a wide range of responses are observed between people, with some individuals showing great improvements while others show little-to-no
improvement (Bouchard et al. 2012; Fenwick et al. 2018b; Wagh and Stone 2004). These variable responses to lifestyle interventions may stem, in part, from underlying genetic differences (Fenwick et al. 2018a). Indeed, hundreds of single nucleotide polymorphisms (SNPs) have been associated with MetS as a whole, as well as with the individual MetS components (Brown and Walker 2016). Moreover, many of these SNPs have also been reported to influence a person's response to lifestyle interventions (Fenwick et al. 2018b). Consequently, it stands to reason that SNPs may influence the degree of improvement experienced by individuals undergoing lifestyle modifications for MetS (Horne et al. 2018).

The Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) feasibility study evaluated a year-long tailored lifestyle intervention for individuals with MetS (Jeejeebhoy et al. 2017). We previously reported that the CHANGE study resulted in a 19% reversal of MetS (Jeejeebhoy et al. 2017); however, it is unknown if genetic variants moderate improvements in MetS risk. The primary objective of the present study was to examine the influence of SNPs previously found to be associated with MetS on changes in cMetS score, as well as individual MetS components, during a 1-year lifestyle intervention. A secondary objective was to develop un-weighted (uw) and weighted (w) genetic risk scores (GRS) to examine their impact on changes in cMetS score. We hypothesized that genetic variants would moderate improvements in cMetS score in response to a lifestyle intervention. We anticipate that our findings will help to further personalize lifestyle interventions that aim to reduce MetS risk.
3.2 Methods

*CHANGE Feasibility Study Overview*

The data used for the current analyses corresponds to the subset of individuals enrolled in the CHANGE feasibility study who provided a DNA sample (*Figure 1*). For a thorough description of the CHANGE feasibility study, as well as inclusion and exclusion criteria, please see Jeejeebhoy et al. (2017). Briefly, the CHANGE feasibility study was a prospective, longitudinal before-after demonstration study carried out at three primary healthcare clinics across Canada, recruiting individuals with MetS between 2012 and 2014. The primary objective of the CHANGE study was to reverse MetS and improve its components by the end of the intervention period. This year-long personalized lifestyle intervention used a team-based approach comprising a primary care physician, a kinesiologist and a dietitian. The goal of the intervention was to create sustainable behavioural changes within the cohort by adhering to an individual diet plan broadly based on the Mediterranean diet principles and a combination of weekly aerobic, resistance and flexibility exercises (Klein et al. 2017; Royall et al. 2014). Diet quality was assessed using the Canadian Healthy Eating Index (HEI-C), which provides a score ranging from 0-100 based on a person’s adherence to *Canada’s Food Guide* recommendations (Garriguet 2009). Dieticians met with participants to create individualised dietary plans based on a care map that integrated evidence-based dietary advice with principles from behaviour change models (Royall et al. 2014). Participants also met with kinesiologists to develop individualised fitness plans that included supervised and unsupervised activities to improve aerobic, resistance and flexibility training (Klein et al. 2017). Aerobic capacity was evaluated using maximal oxygen consumption (VO₂max) (Ebbeling et al. 1991). All participants attended weekly visits
(over the first three months) and monthly visits (over the final nine months) with a dietitian and kinesiologist, and were followed by a primary physician quarterly. Standard blood clinical measurements were completed at baseline, 3-months and 12-months to monitor common biomarkers of metabolic health (e.g., lipids, glucose, etc.). The cMetS score was calculated by combining the weighted effects of waist circumference, triglycerides, blood glucose and systolic blood pressure using the approach developed by Hillier and colleagues (Hillier et al. 2006).

Written and oral informed consent was obtained from all eligible patients before inclusion. This protocol was approved by a Research Ethics Board of the participating clinics and affiliated universities.

**DNA Extraction and Genotyping**

Blood samples were drawn at baseline, 3 months and 12 months and stored at −80 °C until analysis. DNA was extracted from whole blood using the Qiagen PAXgene Blood DNA kit, according to manufacturer instructions (Qiagen, Toronto, Ontario, Canada). DNA quantity was determined using a NanoDrop 2000c (Fisher Scientific, Waltham, Mass., USA) and quality was visually assessed on a 1% agarose gel. DNA samples were collected from each participant at baseline and 12 months; therefore the sample with the highest quality was used for genotyping.

**SNP selection**

SNPs of interest were selected based on an extensive search of the existing literature. The search criteria included SNPs that have been previously associated with MetS in genome-wide association studies, lifestyle intervention studies, and/or meta-analyses. Only SNPs with a minor allele frequency >10% according to the 1000 Genomes Project
(http://www.internationalgenome.org/; accessed 09/2016) were selected. A panel of 17 SNPs corresponding to 12 genes was chosen for the present analysis (Table 1) (Brown and Walker 2016; de Luis et al. 2017; Emamian et al. 2015; Fenwick et al. 2018b; Gaulton et al. 2008; Hou et al. 2017a; Hou et al. 2017b; Paththinige et al. 2017; Petersen et al. 2010; Tanaka et al. 2009; Wenquan et al. 2011; Xi et al. 2012).

**Table 2: Selected candidate SNPs associated with individual MetS components.**

MAF, minor allele frequency. MAF = Minor allele frequency

<table>
<thead>
<tr>
<th>MetS Component</th>
<th>Gene</th>
<th>SNP</th>
<th>Major/Minor Allele</th>
<th>MAF Based on 1000 Genomes</th>
<th>MAF in CHANGE study participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure</td>
<td><em>ATP2B1</em></td>
<td>rs17249754</td>
<td>G/A</td>
<td>0.2095</td>
<td>0.1572</td>
</tr>
<tr>
<td></td>
<td><em>ACE</em></td>
<td>rs4343</td>
<td>A/G</td>
<td>0.3568</td>
<td>0.4840</td>
</tr>
<tr>
<td>Fasting Blood Glucose</td>
<td><em>GLUT2</em></td>
<td>rs5400</td>
<td>G/A</td>
<td>0.2153</td>
<td>0.1604</td>
</tr>
<tr>
<td></td>
<td><em>TCF7L2</em></td>
<td>rs12255372</td>
<td>G/T</td>
<td>0.2139</td>
<td>0.2830</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs7903146</td>
<td>C/T</td>
<td>0.2278</td>
<td>0.3050</td>
</tr>
<tr>
<td></td>
<td><em>ADIPOQ</em></td>
<td>rs1501299</td>
<td>G/T</td>
<td>0.3003</td>
<td>0.2642</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td><em>FADS1</em></td>
<td>rs174537</td>
<td>G/T</td>
<td>0.3029</td>
<td>0.3584</td>
</tr>
<tr>
<td></td>
<td><em>CETP</em></td>
<td>rs1800775</td>
<td>A/C</td>
<td>0.4535</td>
<td>0.4748</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs247617</td>
<td>C/A</td>
<td>0.2680</td>
<td>0.2893</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs5882</td>
<td>A/G</td>
<td>0.4661</td>
<td>0.2987</td>
</tr>
<tr>
<td></td>
<td><em>APOC3</em></td>
<td>rs2854117</td>
<td>C/T</td>
<td>0.4992</td>
<td>0.2642</td>
</tr>
<tr>
<td>SNP</td>
<td>Gene</td>
<td>SNP ID</td>
<td>Allele 1</td>
<td>Allele 2</td>
<td>Frequency 1</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>APOA5</td>
<td>rs662799</td>
<td>A/G</td>
<td>0.1629</td>
<td>0.0912</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs964184</td>
<td>C/G</td>
<td>0.2222</td>
<td>0.1792</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs670</td>
<td>C/T</td>
<td>0.1885</td>
<td>0.1950</td>
<td></td>
</tr>
<tr>
<td>APOA1</td>
<td>rs12970134</td>
<td>G/A</td>
<td>0.2075</td>
<td>0.2421</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs17782313</td>
<td>T/C</td>
<td>0.2400</td>
<td>0.3073</td>
<td></td>
</tr>
<tr>
<td>FTO</td>
<td>rs9939609</td>
<td>T/A</td>
<td>0.3401</td>
<td>0.4308</td>
<td></td>
</tr>
</tbody>
</table>

**SNP Analysis**

All DNA samples were diluted to a concentration of 20 ng/µl. Genotyping was performed at The Centre for Applied Genomics (The Hospital for Sick Children, Toronto, Canada) using the Sequenom MassARRAY platform, which is based on detection through MALDI-TOF MS (Mass Array, Sequenom, San Diego, CA). Positive and negative controls, made up of a Yoruban HapMap trio and water samples, respectively, were used for quality control. Twelve DNA samples were randomly selected for replication and 100% concordance was achieved. Hardy-Weinberg equilibrium (HWE) was evaluated for all SNPs using a $\chi^2$-square test.

**Genetic Risk Score**

The SNPs used to build un-weighted (uw) and weighted (w) GRSs were identified based on associations between individual SNPs and change in cMetS score. An uwGRS ranging from 0-2 was built, where a participant's GRS was calculated by summing the number of risk alleles.
for two SNPs: rs662799 (G) and rs1501299 (G). A GRS=0 corresponds to a participant carrying no risk alleles, GRS=1 corresponds to a participant carrying one risk allele, and a GRS=2 corresponds to a participant carrying both risk alleles. The wGRS was built by taking into account the effect size ($\beta$/standard error) of each SNP, as revealed from the individual additive models for rs662799 and rs1501299.

**Statistical Analyses**

Linear regressions were used to investigate associations between SNPs and cMetS score, as well as between SNPs and individual MetS components. Models accounted for the following covariates: age, sex, BMI, ethnicity, change in HEI-C, participating site and baseline medication. Medication was treated as a dichotomous variable (Y/N), where participants using any medication related to a MetS component were considered “Y”. BMI was not included as a covariate when investigating cMetS score and waist circumference. We constructed both dominant (MM vs. Mm+mm) and additive (MM vs. Mm vs. mm) genetic models for all regression analyses. The term “minor allele carrier” is used throughout the manuscript to refer to participants who are either heterozygous or minor homozygous carriers for a given SNP. The change in cMetS score at 3 months and 12 months was calculated as “cMetS score at 3-months – cMetS score at baseline” and “cMetS score at 12-months – cMetS score at baseline”, respectively. Since we chose to not account for multiple testing, we only considered associations that were statistically significant at both time points, and in both dominant and additive models, to reduce risk of reporting false positives.

Prior to analyses, data was assessed for normality using a Shapiro-Wilk test. A Friedman’s repeated measures test was used when analysing continuous data in study
participants at baseline, 3-months and 12-months. A χ-square test was used to compare
categorical data at baseline, 3-months and 12-months. All data is presented as mean ± standard
error. Linear regressions were performed with JMP 13 Statistical Software (SAS Institute, Cary,
NC, USA). All other statistical tests were performed using GraphPad 6 Prism (GraphPad
Software, Inc., CA, USA). A P<0.05 was considered statistically significant.

3.3 Results

Characterization of the CHANGE cohort

Participants who did not meet our criteria for MetS at baseline, or were missing either a
DNA sample or 12-month cMetS data, were excluded from the present analysis (Figure 1). The
final sample size for the present analyses was 159 (77 males and 82 females). The mean age of
participants was 60.7 ± 0.73 years (median of 62; range: 18-75). The majority of the study
population was Caucasian (n=131, 82.4%), while the remainder of the cohort comprised of 16
(10%) individuals of European/Sub-Saharan African/Mediterranean/Arab ethnicity, 10 (6.3%)
individuals of Asian/South Central American ethnicity and 2 (1.3%) individuals of unknown
ethnicity.
Baseline, 3-month and 12-month characteristics for the 159 participants are presented in Table 2. Consistent with the original CHANGE feasibility study, in this subset of patients both the HEI-C and estimated VO\textsubscript{2max} improved significantly by 3 months and these improvements were maintained at 12 months (\(P<0.0001\) for both). The cMetS score decreased at 3 months and the reduction was maintained at 12 months (\(P<0.0001\)). Individual MetS components significantly improved across the duration of the study in our subset of participants. Blood lipid profiles were improved at 3 months and maintained at 12 months. Specifically, triglyceride levels were reduced (\(P=0.0006\)) and HDL-C levels were increased (\(P<0.0001\)). These improvements aligned with trends in other blood lipid markers, i.e., a reduction in LDL-C (\(P=0.07\)) and an increase in APOA1 (\(P=0.07\)). Waist circumference decreased significantly after
3 months ($P<0.0001$), which reflected reductions in BMI. Both systolic and diastolic blood pressure were reduced after 3 months (both $P<0.0001$) and these reductions persisted at 12-months. Fasting blood glucose ($P=0.3$) was the only MetS component that did not change during the intervention.

**Table 3: Participants characteristics at baseline, 3 months and 12 months.**

Values denote mean ± SE. Continuous data was analyzed using a Friedman’s repeated measures test, while categorical data was compared using a chi-square test. Values within a row that have a different superscript letter are statistically different from one another ($P<0.05$). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HEI-C, Healthy Eating Index-Canadian; LDL-C, low-density lipoprotein cholesterol; VO$_{2\text{max}}$, maximal oxygen consumption.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline (n=159)</th>
<th>3 months (n=148)</th>
<th>12 months (n=159)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (years)</td>
<td>60.7 ± 0.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>82 (51.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI, (kg/m$^2$)</td>
<td>31.0 ± 0.27$^a$</td>
<td>30.4 ± 0.27$^b$</td>
<td>30.1 ± 0.29$^b$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Current smoker, no. (%)</td>
<td>14 (8.8)</td>
<td>13 (8.8)</td>
<td>12 (7.55)</td>
<td>0.9</td>
</tr>
<tr>
<td>HEI-C</td>
<td>58.2 ± 1.2$^a$</td>
<td>68.4 ± 1.0$^b$</td>
<td>68.6 ± 1.1$^b$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg/min)</td>
<td>32.2 ± 0.57$^a$</td>
<td>34.9 ± 0.57$^b$</td>
<td>35.2 ± 0.55$^b$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>LDL-C, (mmol/L)</td>
<td>2.54 ± 0.08</td>
<td>2.49 ± 0.09</td>
<td>2.49 ± 0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>APOA1, (mmol/L)*</td>
<td>1.43 ± 0.02</td>
<td>Not measured</td>
<td>1.46 ± 0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>cMetS score</td>
<td>2.36 ± 0.08$^a$</td>
<td>1.79 ± 0.08$^b$</td>
<td>1.94 ± 0.09$^b$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td><strong>Metabolic syndrome criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Elevated blood pressure or using pharmacotherapy, no. (%)</td>
<td>144 (90.6)</td>
<td>135 (84.9)</td>
<td>131 (82.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>Systolic blood pressure, (mmHg)</td>
<td>133.9 ± 1.2$^a$</td>
<td>126.6 ± 1.1$^b$</td>
<td>130.2 ± 1.1$^c$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Diastolic blood pressure, (mmHg)</td>
<td>79.9 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.7 ± 0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.1 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Received pharmacotherapy for elevated blood pressure, no. (%)</td>
<td>128 (80.5)</td>
<td>117 (79.1)</td>
<td>127(79.9)</td>
<td>0.95</td>
</tr>
<tr>
<td>2. Elevated fasting blood glucose or using pharmacotherapy, no. (%)</td>
<td>135 (84.9)</td>
<td>122 (76.7)</td>
<td>125 (78.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>6.39 ± 0.09</td>
<td>6.26 ± 0.09</td>
<td>6.44 ± 0.11</td>
<td>0.3</td>
</tr>
<tr>
<td>Received pharmacotherapy for elevated blood glucose levels, no. (%)</td>
<td>71 (44.7)</td>
<td>65 (43.9)</td>
<td>70 (44.0)</td>
<td>0.99</td>
</tr>
<tr>
<td>3. Elevated triglycerides or using pharmacotherapy, no. (%)</td>
<td>98 (61.6)</td>
<td>78 (49.1)</td>
<td>83 (52.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>Triglyceride level (mmol/L)</td>
<td>2.22 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.88 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0006</td>
</tr>
<tr>
<td>Pharmacotherapy for dyslipidemia, no. (%)</td>
<td>6 (3.7)</td>
<td>6 (4.1)</td>
<td>6 (3.7)</td>
<td>0.99</td>
</tr>
<tr>
<td>4. Reduced HDL-C, no. (%)</td>
<td>79 (50.0)</td>
<td>83 (52.2)</td>
<td>60 (37.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.19 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5. Large waist circumference, no. (%)</td>
<td>149 (93.7)</td>
<td>135 (84.9)</td>
<td>129 (81.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>106.1 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.4 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.1 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*<sup>APOA1</sup> was not measured at 3 months, therefore a Wilcoxon repeated measures test was used.

<sup>1</sup> Metabolic syndrome criteria defined as follows: blood pressure ≥ 130/85 mm Hg or receiving pharmacotherapy; fasting blood glucose ≥ 5.6 mmol/L or receiving pharmacotherapy; triglyceride level ≥ 1.7 mmol/L or receiving pharmacotherapy; male patients with an HDL-C level < 1.0 mmol/L or female patients with an HDL-C level < 1.3 mmol/L; waist circumference as determined by prespecified technique (Europid, white, sub-Saharan African, Mediterranean, middle eastern [Arab] patients ≥ 94 cm for men, 80 cm for women; Asian and South Central American patients ≥ 90 cm for men, 80 cm for women; white
SNPs influence the magnitude of change in cMetS score

Seventeen candidate SNPs previously reported in the literature to be associated with individual MetS components were analysed in the present investigation. All SNPs but one (rs17782313) were in Hardy-Weinburg equilibrium. No significant associations were observed between the 17 candidate SNPs and the cMetS score at baseline (data not shown). However, examining the change in cMetS score at both 3 and 12 months revealed several statistically significant and consistent associations with various SNPs (Table 3). In particular, rs662799 (A/G) in APOA5 and rs1501299 (G/T) in ADIPOQ were associated with change in cMetS score at both 3-months and 12-months. Furthermore, these associations were significant in both dominant and additive genetic models. Individuals homozygous for the major A allele for rs662799 experienced greater reductions in cMetS score compared to AG+GG carriers at both time points (Figure 2A). In contrast, individuals carrying a minor T allele in rs1501299 (GT+TT) experienced greater reductions in cMetS score at both time points (Figures 2B). Significant associations were observed between other SNPs and the change in cMetS score; however, these associations were not consistent across time points and/or models. Therefore these SNPs were not considered in the construction of GRSs.
Data obtained using a dominant genetic model is presented for rs662799 in APOA5 (a) and rs1501299 in ADIPOQ (b). All linear regressions were adjusted for age, sex, ethnicity, HEI-C, participating site and baseline medication. A 2-way ANOVA was used to examine the main effects of Genotype and Time, as well as Genotype x Time interaction. 3-months n=148; 12-months n=159.

Table 4: Associations between candidate SNPs and change in cMetS score at 3 and 12 months

Data corresponding to dominant and additive genetic models for all linear regressions are presented. Models accounted for the following covariates: HEI-C, BMI, age, ethnicity, sex, recruitment site, and baseline medication. Statistically significant associations are highlighted with bold font (P<0.05).

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>3-month change in cMetS score (n=148)</th>
<th>12-month change in cMetS score (n=159)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Additive Model</td>
<td>Dominant Model</td>
<td>Additive Model</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>ATP2B1</td>
<td>rs17249754</td>
<td>p=0.99, β=0.01</td>
<td>p=0.67, β=0.04</td>
</tr>
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<td>ACE</td>
<td>rs4343</td>
<td>p=0.81, β=0.10</td>
<td>p=0.46, β=0.06</td>
</tr>
<tr>
<td>GLUT2</td>
<td>rs5400</td>
<td>p=0.86, β=0.01</td>
<td>p=0.85, β=0.02</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs12255372</td>
<td>p=0.05, β=0.17</td>
<td>p=0.07, β=0.15</td>
</tr>
<tr>
<td></td>
<td>rs7903146</td>
<td>p=0.10, β=0.14</td>
<td>p=0.21, β=0.11</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP</td>
<td>p-value 1</td>
<td>β-value 1</td>
</tr>
<tr>
<td>--------</td>
<td>----------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>rs1501299</td>
<td>p=0.02, β=0.2</td>
<td>p=0.05, β=0.16</td>
</tr>
<tr>
<td>FADS1</td>
<td>rs174537</td>
<td>p=0.76, β=0.03</td>
<td>p=0.14, β=0.12</td>
</tr>
<tr>
<td>CETP</td>
<td>rs1800775</td>
<td>p=0.83, β=0.02</td>
<td>p=0.55, β=0.05</td>
</tr>
<tr>
<td></td>
<td>rs247617</td>
<td>p=0.96, β=0.01</td>
<td>p=0.87, β=0.01</td>
</tr>
<tr>
<td></td>
<td>rs5882</td>
<td>p=0.8, β=0.02</td>
<td>p=0.77, β=0.02</td>
</tr>
<tr>
<td>APOC3</td>
<td>rs2854117</td>
<td>p=0.06, β=0.16</td>
<td>p=0.19, β=0.11</td>
</tr>
<tr>
<td>APOA5</td>
<td>rs662799</td>
<td>p=0.02, β=0.19</td>
<td>p=0.03, β=0.18</td>
</tr>
<tr>
<td></td>
<td>rs964184</td>
<td>p=0.23, β=0.1</td>
<td>p=0.27, β=0.09</td>
</tr>
<tr>
<td>APOA1</td>
<td>rs670</td>
<td>p=0.21, β=0.1</td>
<td>p=0.05, β=0.16</td>
</tr>
<tr>
<td>MC4R</td>
<td>rs12970134</td>
<td>p=0.98, β=0.01</td>
<td>p=0.96, β=0.01</td>
</tr>
<tr>
<td></td>
<td>rs17782313</td>
<td>p=0.84, β=0.02</td>
<td>p=0.80, β=0.02</td>
</tr>
<tr>
<td>FTO</td>
<td>rs9939609</td>
<td>p=0.83, β=0.02</td>
<td>p=0.5, β=0.06</td>
</tr>
</tbody>
</table>

Genetic risk score and change in cMetS score

We established GRSs corresponding to the two aforementioned SNPs: rs662799 and rs1501299. These two SNPs were associated with a change in cMetS score at both 3-months and 12-months using both dominant and additive genetic models. At baseline, no associations were identified between the uwGRS (or wGRS) and cMetS score (data not shown). In contrast, the 12-month change in cMetS score was significantly associated with uwGRS ($P=0.0006$; Figure 3), with a similar trend seen at 3-months ($P=0.005$; data not shown). Individuals with an uwGRS of 0 (i.e., carrying no risk alleles) showed greater reductions in their cMetS score compared to individuals with at least 1 risk allele (0 vs. 1, $P=0.05$; 0 vs. 2, $P=0.004$). Although we did not
detect statistical differences between individuals carrying 1 or 2 risk alleles, it appears that the change in cMetS score is reduced as the number of risk alleles increases. The wGRS showed similar results, with significant associations seen with the 12-month change in cMetS (P=0.003) and the 3-month change in cMetS (P=0.002).

![Graph showing the unweighted (uw) genetic risk score (uwGRS) is associated with change in cMetS score at 12 months.

The uwGRS was calculated by summing the number of risk alleles in the 2 SNPs (rs662799 and rs1501299) that were independently associated with change in cMetS score, where 0 corresponds to individuals carrying no risk alleles, 1 corresponds to individuals carrying one risk allele, and so on. Bars sharing letters are not significantly different from one another (p <0.05). The number of individuals in each group: GRS = 0 (n = 56), GRS = 1 (n = 87), GRS = 2 (n = 16).

3.4 Discussion

The present study examined whether genetic variants influence the magnitude of the reduction in cMetS score during a year-long lifestyle intervention in a subset of participants from the CHANGE feasibility study. The primary findings of this investigation were: 1) two SNPs were consistently associated with a change in cMetS score at both 3 months and 12 months (rs662799 (A/G) in APOA5 and rs1501299 (G/T) in ADIPOQ), and 2) participants carrying none
of the risk alleles in the two aforementioned genes (GRS=0) showed the greatest reduction in cMetS score during the intervention. Together, this study identified novel lifestyle-gene interactions and showed that individuals carrying neither risk alleles in APOA5 and ADIPOQ may experience an improved response to a lifestyle intervention for MetS.

Although not the primary objective of this genetic-based study, the improvements in biochemical measures and dietary behaviours observed within our subset of individuals mirror the findings from the entire CHANGE study cohort, as previously reported (Jeejeebhoy et al. 2017). The decrease in cMetS score, increase in HEI-C and VO_{2max}, as well as improvements in all MetS components except fasting blood glucose, highlight the effectiveness of the lifestyle intervention used in the CHANGE feasibility study. The current investigation revealed that the magnitude of the reduction in cMetS score in response to the lifestyle intervention is moderated by common SNPs in two genes.

APOA-V is an important regulator of circulating triglyceride levels through two primary mechanisms. First, circulating APOA-V can interact with lipoprotein lipase (LPL) on the luminal side of endothelial cells to promote the hydrolysis of triglyceride-rich lipoproteins. Second, APOA-V, can bind the hepatic LDL-receptor to encourage the clearance of lipoprotein remnants (Guardiola et al. 2010). Previous reports have shown that MetS prevalence and the risk of MetS is increased in individuals carrying the minor G allele in rs662799 (also known as -1131T>C), a SNP located in the upstream promoter region of the APOA5 gene (Ong et al. 2011; Sarwar et al. 2010; Xu et al. 2013; Zhang et al. 2011). Specifically, people carrying the minor allele have been shown to have elevated triglyceride levels and, in some instances, reduced HDL-C levels in both healthy and unhealthy individuals (Lin et al. 2017; Sarwar et al. 2010).
In the present study, minor allele carriers showed smaller reductions in cMetS score in response to the lifestyle intervention compared to major allele homozygotes. These findings align with previous reports investigating the modifying effect of this SNP on triglyceride levels during lifestyle and/or dietary interventions. For example, a 3-year trial in which Korean individuals with impaired fasting glucose (IFG) or newly diagnosed T2D replaced all refined white rice with whole grains, barley or legumes and increased vegetable intake, showed that those carrying the minor allele had lower plasma APOA-V levels and higher triglyceride levels at both baseline and following the intervention compared to individuals carrying the major allele (Kim et al. 2014). Additionally, a 3-month intervention consisting of diet quality improvements and regular walking in individuals with hypertriglyceridemia showed greater improvements in major allele carriers for both APOA-V and triglyceride levels compared to minor allele carriers, despite a similar degree of compliance (Jang et al. 2010). In contrast, the aforementioned genotype effect in response to a lifestyle intervention was not observed in a Japanese cohort (Yamasaki et al. 2015). Collectively, these studies suggest a potential moderating effect for rs662799 on blood triglyceride levels in response to longer-term lifestyle interventions.

Interestingly, previous reports suggest that the frequency of the minor "risk" allele is more common in the Asian population compared to those of European descent (Kim et al. 2014). This suggests that the moderating effect of this SNP in response to a lifestyle intervention should be investigated more closely in distinct subgroups of the general population.

Adiponectin is a well-known adipokine encoded by the *ADIPOQ* gene. Circulating adiponectin levels have been associated with improvements in both MetS and insulin resistance, and genetic variants in *ADIPOQ* have been shown to influence circulating levels (Menzaghi et al. 2007). For example, individuals carrying the minor T allele in rs1501299 were reported to
have higher adiponectin levels in both Asian and European populations (Gonzalez-Sanchez et al. 2005; Li et al. 2015). The present study demonstrated that rs1501299 genotype is associated with reductions in cMetS score. Specifically, individuals carrying the minor T allele (GT+TT) showed a greater response to the lifestyle intervention compared to those homozygous for the major G allele. This is intriguing given evidence suggests that individuals homozygous for the G allele are at greater metabolic risk than T-allele carriers. For example, a recent meta-analysis of Chinese Han populations reported a greater G allele frequency in individuals with MetS (Gao et al. 2013). Further, individuals homozygous for the GG genotype showed impaired glucose tolerance in Spanish subjects (Gonzalez-Sanchez et al. 2005). We did not identify any associations between rs1501299 and fasting glucose levels at baseline or during the intervention; therefore, we are unable to hypothesize why the change in cMetS score is influenced by rs1501299 genotype.

Shin et al. (2006) examined the association between rs1501299 and circulating adiponectin and insulin resistance in response to a 12-week weight loss intervention. In contrast to our findings, these authors showed that GG homozygotes showed significant improvements in insulin sensitivity and increases in adiponectin following the weight-loss intervention, with little-to-no change seen in carriers of the T allele. It is unclear why our results, which show that T allele carriers experienced greater improvements in metabolic health in response to the lifestyle intervention, do not align with those reported by Shin et al. (2006). Measuring plasma adiponectin levels may have provided some insight to help understand this apparent discrepancy; however, differences in the lifestyle intervention (i.e., caloric deficit vs. improvement in diet quality), length of time of the intervention (3 months vs 12 months), and population make-up
should be acknowledged. Thus further investigations of this particular SNP as a moderator of response to lifestyle interventions are necessary.

In addition to individual SNP associations, genetic risk scores (both uwGRS and wGRS) provided strong correlation with the lifestyle intervention outcomes. The significantly greater reduction in cMetS score seen in individuals carrying no risk alleles (GRS=0) compared to individuals with at least 1 risk allele highlights the need to consider overall risk scores rather than individual SNPs. To the best of our knowledge, this is one of the first studies to examine GRSs in the context of a lifestyle intervention for MetS. A recent study by San-Cristobal et al. (2017) used a GRS comprised of 14 SNPs to explore associations between Mediterranean Diet adherence, blood biomarkers, and genetic background in volunteers of the Food4Me study. Individuals were categorized into “low” and “high” GRS groups, with those having a low GRS experiencing a greater reduction in total cholesterol levels after 6-months compared to those with a high GRS. The SNPs used to create a GRS in this past study are different than those used in the present study; however, the results align to suggest that individuals with a greater number of “risk” alleles would benefit from more tailored strategies to improve health outcomes. Conversely, an unfavourable genetic profile may predict an adverse response to what would be normally expected from healthy eating and exercise training.

The present study has several limitations and strengths to be considered. Primary limitations include the relatively small sample size (n=159), minor differences in diet and exercise alterations between individuals due to personalized advice (as opposed to a standardized lifestyle intervention), lack of information regarding alcohol intake, and the fact that all subjects had MetS at recruitment (i.e., no control group) may have limited our ability to detect certain
gene associations (e.g., rs670 and HDL-C). Future studies to validate the genetic findings on subsequent MetS intervention populations are warranted. However, a major strength of the CHANGE study was the duration of the intervention and data collection at multiple time points. Furthermore, the use of three diverse participating sites across Canada means our results are generalizable, at minimum across North America. Moreover, having a physician, dietitian and kinesiologist monitor each participant’s progress at regular intervals during the study, and make adjustments if necessary, was optimal to minimize risk of non-compliance. Finally, we were overly conservative in regard to the SNPs used in our GRS analyses. Specifically, both SNPs were significantly associated with changes in cMetS at both 3- and 12-months in both dominant and additive genetic models. This conservative approach reinforces the significant findings uncovered with our uwGRS and wGRS models.

In conclusion, the present study demonstrated that specific genetic variants, both alone and when combined into a GRS, influence the magnitude of change in cMetS score in response to a lifestyle intervention. These results reinforce the potential value of assessing genetic risk to better tailor health management and goal-setting during an intervention. Moreover, knowledge of gene-lifestyle interactions may contribute to the development and/or refinement of nutrigenetic advice for healthcare practitioners and direct-to-consumer genetic companies alike.

### 3.5 Acknowledgements

Metabolic Syndrome Canada is a not-for-profit charitable organization that partially supported this work.
3.6 Disclosure Statement

Metabolic Syndrome Canada is a not-for-profit charitable organization that funded the current study. Rupinder Dhaliwal was paid for her work on the study by Queen’s University from this grant. Rupinder Dhaliwal became an employee of Metabolic Syndrome Canada after the completion of study enrolment. Doug Klein received a grant as a participating site for patient enrolment and data collection from Metabolic Syndrome Canada. Paula Brauer, Dawna Royall, David M Mutch and Angelo Tremblay received grants for program development from Metabolic Syndrome Canada. Khursheed Jeejeebhoy is on the board of directors for Metabolic Syndrome Canada and will be involved in discussions about fundraising for this non-profit organization.
**Chapter 4:** Predicting 1-Year Response to a Personalized Lifestyle Intervention for Canadians with Metabolic Syndrome


*Manuscript in preparation*
4.0 Abstract

Metabolic syndrome (MetS) comprises a cluster of risk factors that includes central obesity, hypertension, dyslipidemia and impaired glucose homeostasis. Although lifestyle interventions reduce MetS risk, not everyone responds to the same extent. The primary objective of the present study was to identify baseline variables (i.e., genetics, diet scores, aerobic fitness, and bioclinical measurements) that could predict 1-year changes in cMetS score in individuals participating in the Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) program. Predictive models were trained using data from 157 participants, and then tested on an independent subset of 30 individuals. First, a linear mixed-effect model revealed that age, medication use, fasting glucose, triglycerides, HDL-C, waist circumference, systolic blood pressure and fibre intake were significantly associated with cMetS score across all time points. Next, we used multiple linear regression to develop a predictive model using 12-month cMetS score as the outcome variable (Model 1). Model 1 was 86% accurate for predicting cMetS score (r=0.69). As a secondary outcome (Model 2), we also examined if a person’s categorical response at 12-months could be predicted (i.e. positive responder, negative responder, adverse responder). A confusion matrix showed Model 2 to be 72% concordant between predicted and observed responses. Short-term change in cMetS score and baseline systolic blood pressure were consistent predictive factors in both models. These predictive models need to be further tested in independent cohorts, but provide a potentially promising tool for improved health management of individuals with MetS.
4.1 Introduction

The estimated prevalence of metabolic syndrome (MetS) in the general Canadian adult population is 19.1% (approximately 1 in 5 people), with the prevalence significantly greater in older adults (Riediger and Clara 2011). MetS comprises a collection of conditions that increases a person’s risk for type 2 diabetes (T2D) and cardiovascular disease (CVD) (Brown and Walker 2016). MetS is commonly defined as the clinical manifestation of 3 of the following 5 conditions: central obesity (waist circumference), elevated fasting blood glucose, hypertension, elevated triglycerides and reduced high-density lipoprotein cholesterol (HDL-C). It has recently been proposed that the use of a continuous MetS (cMetS) score is a more practical method to evaluate a person’s MetS risk (Hillier et al. 2006).

Lifestyle interventions combining diet and exercise regimens are widely used to prevent and manage MetS (Perez-Martinez et al. 2017). Although these interventions are generally successful for alleviating MetS risk, not everyone responds to the same extent (Bouchard et al. 2012; Uysal et al. 2014). The basis for different responses is multifactorial, and can be attributed to inter-individual variations in genetics, diet quality, and clinical markers, amongst others (Fenwick et al. 2018b; McMorrow et al. 2018). Given that numerous factors influence a person’s response to an intervention, a model to predict outcome would by tremendously valuable for both patients and healthcare practitioners alike. Evidence from past studies that predicted changes in various cardiometabolic outcomes provide encouragement to explore the development of a model that can predict changes in MetS risk in response to a lifestyle intervention (Hosseinpanah et al. 2015; Lee et al. 2015; Vilar-Gomez et al. 2016; Zhang et al. 2015). For example, Vilar-Gomez et al. (2016) proposed a non-invasive scoring system to
predict the resolution of non-alcoholic steatohepatitis after a 1-year diet and exercise intervention that precluded the need to collect liver biopsies. Thus, developing a model to predict changes in MetS risk in response to a lifestyle intervention has the potential to improve patient care.

The Canadian Health Advanced by Nutrition and Graded Exercise (CHANGE) feasibility study evaluated a 1-year personalized lifestyle intervention for individuals with MetS (Jeejeebhoy et al. 2017). We previously reported a 19% reversal of MetS in the CHANGE cohort (Jeejeebhoy et al. 2017), and showed that reductions in cMetS score at 3 months and 1 year are associated with genetic variants in APOA5 (rs662799) and ADIPOQ (rs1501299) (Lowry et al. 2018), improved aerobic fitness (VO$_{2\text{max}}$) (unpublished results), and greater healthy eating index (HEI-C) diet scores (Brauer et al. 2019). The primary objective of this study was to develop and evaluate a model using baseline diet quality, aerobic fitness, and bioclinical measurements, as well as genetic information, to predict change in cMetS score after 1-year of the CHANGE program.

4.2 Methods

The present study used data from a subset of individuals enrolled in the CHANGE feasibility study. For a thorough description of the feasibility study, including inclusion and exclusion criterion, please refer to Jeejeebhoy et al. (2017). Briefly, the CHANGE program involved supervised and personalized fitness programs and individualized diet plans with the goal of reversing MetS and improving its components. Diet quality was assessed using the Healthy Eating Index (HEI-C) (Garriguet 2009) and aerobic capacity was measured using maximal oxygen consumption (VO$_{2\text{max}}$) (Ebbeling et al. 1991). Standard bioclinical measurements were completed at baseline, 3- and 12-months to monitor common markers of
metabolic health (e.g., lipids, glucose, etc.). The cMetS score was calculated using the equation developed by Hillier et al. (2006). A weighted genetic risk score (wGRS) was calculated using two SNPs *APOA5* (rs662799) and *ADIPOQ* (rs1501299), as previously described (Lowry et al. 2018).

**Predictor Model Development**

*Step 1:* The cohort was randomly divided into training (n=157) and test (n=29) sets, where the test set was used to assess model performance. A $\chi^2$ test was used to compare baseline categorical data, and a one-way ANOVA was used to compare baseline continuous data, between the total cohort (n=186), training set and test sets.

*Step 2:* A linear mixed effect model was used to identify which of the 29 bioclinical, dietary, exercise and genetic variables were significantly (p<0.05) associated with cMetS score. The mixed effects included fixed effects for the associated baseline variables and the random effect accounts for the subject-specific effect. Variables with a p<0.05 were included in the predictor model development.

*Step 3:* Two different models were considered: 1) 12-month cMetS score predictor model, and 2) 12-month categorical response predictor model.

**Model 1: 12-month cMetS score predictor**

Model 1 was developed to achieve the primary objective of the study: to develop a model to predict a person’s cMetS score at 12-months using baseline data (Model 1). The model included the significant (p<0.05) variables identified in Step 2 in a multiple linear regression.
model with 12-month cMetS score as the outcome variable. Predictor model accuracy was calculated for both the training and test sets, where accuracy was defined as being within one standard deviation (SD) of the measured 12-month cMetS score. The accuracy of the model was also assessed using the Pearson’s correlation coefficient.

**Model 2: Categorical Response Predictor**

The secondary objective of the present study was to develop a categorical model that predicts a person’s response at 12-months using baseline data (Model 2). First, response was categorized into “positive responders”, “non-responders”, and “adverse responders” based on the change in cMetS score during the CHANGE intervention (i.e., baseline cMetS score – 12-month cMetS score). The standard error (SE) in cMetS scores at 12-months was 0.06 and this was used to establish response bins, where “positive responders” experienced a 12-month change in cMetS score greater than +0.06, non-responders had a change in cMetS score ranging from +0.06 to -0.06, and adverse responders experienced a change in cMetS score less than -0.06. The model included the significant (p<0.05) variables identified in Step 2 in a multiple linear regression model with “response bin” as the outcome variable. Model 2 accuracy was examined using a confusion matrix to determine the percentage of individuals who were categorized into the correct bins.

**Statistical Software**

Regression model analyses were performed using JMP 14 Statistical Software (SAS Institute, Car, NC, USA). All other statistical tests were run using GraphPad Prism 7 (GraphPad Software Inc., CA, USA).
4.3 Results

**Participant Characteristics**

The CHANGE cohort was divided into a training (n=157) and a test set (n=29) to develop and then validate predictive models. Baseline cohort characteristics for the total cohort (n=186), training set, and test set were similar in all aspects except for ethnicity (p=0.004), HEI-C score (p=0.04) and waist circumference (p=0.04) (Table 1).

**Table 5: Characteristics of participants at baseline in the overall, training and test sets**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (n=186)</th>
<th>Training set (n=157)</th>
<th>Test set (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (years)*</td>
<td>60.6 ± 0.64</td>
<td>60.8 ± 0.73</td>
<td>59.6 ± 1.3</td>
<td>0.51</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>98 (52.6)</td>
<td>82 (52.2)</td>
<td>16 (55.2)</td>
<td>0.96</td>
</tr>
<tr>
<td>Caucasian, no. (%)</td>
<td>143 (76.9)</td>
<td>129 (82.2)</td>
<td>14 (48.3)</td>
<td><strong>0.0004</strong></td>
</tr>
<tr>
<td>BMI, (kg/m^2)</td>
<td>31.3 ± 0.26</td>
<td>31.1 ± 0.28</td>
<td>32.6 ± 0.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Current smoker, no. (%)</td>
<td>17 (9.14)</td>
<td>14 (8.92)</td>
<td>3 (10.3)</td>
<td>0.97</td>
</tr>
<tr>
<td>HEI-C</td>
<td>59.1 ± 1.1^ab</td>
<td>58 ±1.1^a</td>
<td>65.3 ± 2.5^b</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>VO2max (ml/kg/min)*</td>
<td>32.3 ± 0.49</td>
<td>32 ± 0.54</td>
<td>33.6 ± 1.2</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL-C, (mmol/L)*</td>
<td>2.63 ± 0.08</td>
<td>2.53 ± 0.08</td>
<td>3.15 ± 0.26</td>
<td>0.12</td>
</tr>
<tr>
<td>cMetS score</td>
<td>2.45 ± 0.08</td>
<td>2.37 ± 0.08</td>
<td>2.86 ± 0.25</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Metabolic syndrome criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Elevated blood pressure or</td>
<td>170 (91.4)</td>
<td>143 (91.1)</td>
<td>27 (93.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>using pharmacotherapy, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
<td>p-value</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Systolic blood pressure, (mmHg)</td>
<td>134.2 ± 1.1</td>
<td>133.9 ± 1.2</td>
<td>135.7 ± 2.6</td>
<td>0.84</td>
</tr>
<tr>
<td>Diastolic blood pressure, (mmHg)</td>
<td>80.1 ± 0.65</td>
<td>79.8 ± 0.73</td>
<td>81.6 ± 1.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Received pharmacotherapy for elevated blood pressure, no. (%)</td>
<td>146 (78.5)</td>
<td>128 (81.5)</td>
<td>18 (62.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>2. Elevated fasting blood glucose or using pharmacotherapy, no. (%)</td>
<td>153 (82.3)</td>
<td>133 (84.7)</td>
<td>20 (70)</td>
<td>0.13</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)*</td>
<td>6.45 ± 1.0</td>
<td>6.41 ± 0.09</td>
<td>6.69 ± 0.36</td>
<td>0.98</td>
</tr>
<tr>
<td>Received pharmacotherapy for elevated blood glucose levels, no. (%)</td>
<td>82 (44.1)</td>
<td>70 (44.6)</td>
<td>12 (41.4)</td>
<td>0.95</td>
</tr>
<tr>
<td>3. Elevated triglycerides or using pharmacotherapy, no. (%)</td>
<td>115 (61.8)</td>
<td>96 (61.1)</td>
<td>19 (65.5)</td>
<td>0.91</td>
</tr>
<tr>
<td>Triglyceride level (mmol/L)*</td>
<td>2.13 ± 0.09</td>
<td>2.12 ± 0.1</td>
<td>2.21 ± 0.18</td>
<td>0.67</td>
</tr>
<tr>
<td>Pharmacotherapy for dyslipidemia, no. (%)</td>
<td>6 (3.22)</td>
<td>6 (3.82)</td>
<td>0 (0)</td>
<td>0.56</td>
</tr>
<tr>
<td>4. Reduced HDL-C, no. (%)</td>
<td>93 (50)</td>
<td>78 (50)</td>
<td>15 (51.7)</td>
<td>0.99</td>
</tr>
<tr>
<td>HDL-C (mmol/L)*</td>
<td>1.2 ± 0.02</td>
<td>1.19 ± 0.02</td>
<td>1.26 ± .05</td>
<td>0.39</td>
</tr>
<tr>
<td>5. Large waist circumference, no. (%)</td>
<td>176 (94.6)</td>
<td>148 (94.3)</td>
<td>28 (96.6)</td>
<td>0.88</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>106.9 ± 0.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>106.2 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.9 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis was used for non-normally distributed data; <sup>1</sup> <sup>χ²</sup> tests were used for categorical variables; otherwise all variables were analyzed by one-way ANOVA

<sup>1</sup> Metabolic syndrome criteria defined as follows: blood pressure ≥ 130/85 mm Hg or receiving pharmacotherapy; fasting blood glucose ≥ 5.6 mmol/L or receiving pharmacotherapy; triglyceride level ≥ 1.7 mmol/L or receiving pharmacotherapy; male patients with an HDL-C level < 1.0 mmol/L or female patients with an HDL-C level <1.3 mmol/L; waist circumference as determined be prespecified technique (Europid, white, sub-Saharan African, Mediterranean, middle eastern [Arab] patients ≥ 94 cm for men, 80 cm for women; Asian and South Central American patients ≥ 90 cm for men, 80 cm for women; white American and Canadian patients ≥ 102 cm for men, 88 cm for women.)
**Identifying Predictor Variables**

We first fitted a mixed effects model and regression to determine which of the 29 dietary, exercise, bioclinical and genetic variables were significantly associated with the cMetS score. The results revealed significant associations between cMetS score and age, as well as baseline dietary fibre intake, waist circumference, fasting glucose, triglycerides, systolic blood pressure (SBP), HDL-C, LDL-C, and baseline medication usage (Table 2).

**Table 6: Results from the linear mixed effects model.**

The weighted genetic risk score (wGRS) was calculated based on associations between SNPs *APOA5* (rs662799) and *ADIPOQ* (rs1501299). See Lowry et al. (2018) for more detailed information about the wGRS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.0030</td>
<td>0.0017*</td>
</tr>
<tr>
<td>Sex</td>
<td>0.0368</td>
<td>0.1234</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.0148</td>
<td>0.1231</td>
</tr>
<tr>
<td><strong>Bioclinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.0026</td>
<td>0.3529</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.0510</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>0.4711</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.4913</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.0283</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.0006</td>
<td>0.467</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>-0.1196</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>LDL-C (mmol/L)</strong></td>
<td>0.0165</td>
<td>0.0181*</td>
</tr>
<tr>
<td><strong>Baseline medications (yes/no)</strong></td>
<td>-0.0418</td>
<td>0.0283*</td>
</tr>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VO2max (ml/kg/min)</strong></td>
<td>0.0012</td>
<td>0.5117</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HEI-C</strong></td>
<td>-0.0010</td>
<td>0.0854</td>
</tr>
<tr>
<td><strong>Total calories (kcal/d)</strong></td>
<td>0.0001</td>
<td>0.5398</td>
</tr>
<tr>
<td><strong>Total protein (g/d)</strong></td>
<td>0.0001</td>
<td>0.9151</td>
</tr>
<tr>
<td><strong>Total carbohydrates (g/d)</strong></td>
<td>-0.0002</td>
<td>0.4431</td>
</tr>
<tr>
<td><strong>Total fat (g/d)</strong></td>
<td>-0.0002</td>
<td>0.8229</td>
</tr>
<tr>
<td><strong>Saturated fat (g/d)</strong></td>
<td>-0.0002</td>
<td>0.8814</td>
</tr>
<tr>
<td><strong>Trans fat (g/d)</strong></td>
<td>0.0014</td>
<td>0.8938</td>
</tr>
<tr>
<td><strong>MUFA (g/d)</strong></td>
<td>0.0001</td>
<td>0.9547</td>
</tr>
<tr>
<td><strong>PUFA (g/d)</strong></td>
<td>-0.0004</td>
<td>0.8805</td>
</tr>
<tr>
<td><strong>Omega 6 (g/d)</strong></td>
<td>-0.0009</td>
<td>0.7218</td>
</tr>
<tr>
<td><strong>Omega 3 (g/d)</strong></td>
<td>-0.0061</td>
<td>0.2869</td>
</tr>
<tr>
<td><strong>Sugar (g/d)</strong></td>
<td>-0.0001</td>
<td>0.7236</td>
</tr>
<tr>
<td><strong>Fibre (g/d)</strong></td>
<td>0.0016</td>
<td>0.0309*</td>
</tr>
<tr>
<td><strong>Sodium (mg/d)</strong></td>
<td>0.0001</td>
<td>0.8287</td>
</tr>
<tr>
<td><strong>Genetic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>wGRS</strong></td>
<td>-0.0023</td>
<td>0.5394</td>
</tr>
</tbody>
</table>
**Model Derivation and Development**

Only the significant variables listed in Table 2 were used to develop two predictive models: Model 1 predicted the continuous cMetS score at 12 months, while Model 2 predicted the categorical response at 12 months. For both models, the baseline variables mentioned above, as well the 3-month change in cMetS score (i.e., 3-month $\Delta$cMetS score, calculated as: baseline cMetS score – 3-month cMetS score), were included in the linear regression models to identify which of these variables were significantly associated with 12-month cMetS score. The 3-month $\Delta$cMetS score was included in regression models to examine if a short-term change in cMetS score was a significant predictor of a person’s cMetS score at 12-months compared to baseline variables. Variables that were significantly associated with 12-month cMetS score were used to develop predictive models, and their regression coefficients are displayed in Table 3.

For Model 1, the predicted 12-month cMetS score was calculated using the following formula: $12\text{-month cMetS} = -9.217 + 0.538 \text{ (baseline fasting glucose)} + 0.447 \text{ (baseline triglycerides)} + 0.052 \text{ (baseline waist circumference)} + 0.012 \text{ (baseline systolic blood pressure)} - 0.487 \text{ (3-month } \Delta \text{cMetS score)}$.

For Model 2, study participants in the training set were first categorized into 3 different response bins based on their change in cMetS score after 1-year: positive responders (Bin 1), non-responders (Bin 0), and adverse responders (Bin -1). The baseline variables identified by linear regression (Table 3), were used to calculate the probabilities that an individual be categorized into each response bin, i.e., Bin 1, Bin 0, or Bin -1. The predicted response bin was calculated in Model 2 using the following formulas: Probability of Bin 0 (non-responder) = $5.5844 - 0.0417 \text{ (baseline systolic blood pressure)} - 0.1440 \text{ (baseline fibre intake)} - 0.7459 \text{ (3-}$
month ΔcMetS score); Probability of Bin -1 (adverse responder) = 7.7833 – 0.0646 (baseline systolic blood pressure) + 0.0109 (baseline fibre intake) – 1.2355 (3-month ΔcMetS score);

Table 7: Associations between predictors and outcome variables in the training set for Model 1 and Model 2.

The regression coefficients (β) were used to calculate the model formulas. Model 2 (12-month Intervention response) was estimated by calculating the probabilities each individual would be categorized into one of three bins: Bin 1 (positive responders), Bin 0 (non-responders) and Bin -1 (adverse responders).

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1: 12-month cMetS score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline blood glucose (mmol/L)</td>
<td>0.5380</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Baseline triglycerides (mmol/L)</td>
<td>0.4472</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Baseline systolic blood pressure (mmHg)</td>
<td>0.0119</td>
<td>0.009*</td>
</tr>
<tr>
<td>Baseline waist circumference (cm)</td>
<td>0.0521</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>3-month ΔcMetS score</td>
<td>-0.4869</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Intercept</td>
<td>-9.2173</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Model 2: 12-month Intervention Response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Probability of Bin 0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline systolic blood pressure (mmHg)</td>
<td>-0.0417</td>
<td>0.193</td>
</tr>
<tr>
<td>Baseline fibre intake (g/d)</td>
<td>-0.1440</td>
<td>0.03*</td>
</tr>
<tr>
<td>3-month ΔcMetS score</td>
<td>-0.7459</td>
<td>0.221</td>
</tr>
<tr>
<td>Intercept</td>
<td>5.5844</td>
<td>0.194</td>
</tr>
<tr>
<td><strong>Probability of Bin -1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline systolic blood pressure (mmHg)</td>
<td>-0.0646</td>
<td>0.0006*</td>
</tr>
</tbody>
</table>
Baseline fibre intake (g/d) | 0.0109 | 0.584  
3-month Δ cMetS score | -1.2355 | 0.001*  
Intercept | 7.7883 | 0.002*  

Model Performance

Model 1 had an accuracy rate of 92.5% (135/146; r = 0.79) in the training set and 86.2% (25/29; r = 0.69) in the test set; indicating good performance of the model to predict a person’s cMetS score at 12 months. To calculate accuracy for Model 1 in the training set, 11 individuals were excluded because they did not have a 3-month ΔcMetS score. Predicting response bin (Model 2) performed less well, with only 21 of 29 individuals (~72%) showing concordance between predicted bin and observed bin. The observed bin frequencies in the test set were: 21 positive responders, 4 non-responders and 4 adverse responders; whereas the predictive model categorized 25 individuals as positive responders and 4 individuals as adverse responders.

4.4 Discussion

The present study evaluated whether a model using baseline diet quality, aerobic fitness, and bioclinical measurements, as well as genetic information, could predict change in cMetS score after 1-year of the CHANGE program. Of the two models, Model 1 (12-month cMetS score prediction) more accurately predicted individual response to the lifestyle intervention with an 86% accuracy rate, whereas Model 2 showed only a 72% concordance rate between observed and predicted response bins. Interestingly, baseline systolic blood pressure (SBP) and the 3 month ΔcMetS score were identified as significant predictors in both models. Together, our
findings demonstrate the potential value of using baseline characteristics and short-term changes in cMetS score to predict individuals more likely to respond positively to a lifestyle intervention.

Baseline SBP has been found to discriminate “responders” and “non-responders” in previous lifestyle interventions (Sosner et al. 2016; Stuckey et al. 2015). Sosner et al. (2016) reported that individuals with high baseline blood pressures experienced reductions in SBP >2mmHg (responders) during a lifestyle intervention compared to non-responders. Furthermore, a higher proportion of these responders were diagnosed with MetS at baseline, reinforcing the connection between SBP and MetS. Another study used changes in SBP to characterize responders and non-responders in a 52-week lifestyle intervention (Stuckey et al. 2015). Similar to the previous study, this study also found that responders had higher baseline blood pressures, as well as greater improvements in diastolic blood pressure, waist circumference and VO\textsubscript{2}\text{max}, compared to non-responders. Moreover, participants who experienced a SBP reduction greater than 4 mmHg within the first 24 weeks showed significantly greater improvements by the end of the intervention; suggesting that short-term changes in SBP may be a useful predictor for longer-term changes. Collectively, these past studies, in addition to our findings, highlight the importance of targeting improvements in blood pressure as a means to reduce MetS risk. Strong evidence shows that reductions in sodium intake are associated with significant reductions in blood pressure (Mozaffarian et al. 2014). As such, lifestyle interventions addressing diet quality should prioritize reductions in salt intake to maximize improvements in blood pressure and, ultimately, MetS risk.

Short-term changes in bioclinical measures are generally associated with longer-term outcomes in lifestyle interventions, as illustrated above for SBP. Indeed, early improvements in
a person's overall metabolic health and health behaviours are predictive of long-term intervention success (Eaglehouse et al. 2017; Maruthur et al. 2013; Unick et al. 2015). Results from the Diabetes Prevention Program revealed that early changes in fasting blood glucose, Hb1Ac and achieving physical activity goals were all predictors of long-term success (Eaglehouse et al. 2017; Maruthur et al. 2013). Our results show that short-term change in cMetS score is able to predict long-term change in MetS risk. This finding highlights a potential advantage of using cMetS scores to monitor MetS risk in patients rather than the current dichotomous approach using established cut-off values. For example, an individual may experience improvements in MetS components during an intervention that does not change their clinical assessment of MetS risk, i.e., values remain above established cut-offs despite showing improvement. However, expressing these same changes with a cMetS score would reflect these improvements, which would foster improved patient compliance and motivation during an intervention, and assist healthcare practitioners monitor intervention efficacy.

Our linear regression models identified baseline fibre intake to be predictive of 12-month categorical bin response (Model 2), but the variable was only significantly associated with the probability of being a non-responder (i.e., Bin 0, Table 3). Despite this finding, baseline fibre intake was not significantly different (p=0.52) across response bins (data not shown). A recent meta-analysis identified an inverse relationship between dietary fibre intake and MetS risk (Wei et al. 2018); however, we were unable to find any previous report showing a relationship between dietary fibre intake and MetS risk during a lifestyle intervention. Thus, our findings require independent confirmation.
Strengths and Limitations

Limitations of the CHANGE feasibility study include the relatively small sample size and lack of a standardized lifestyle intervention (due to personalization of specific dietary and exercise programs). From a modelling perspective, there is no convention for establishing response bins. We used standard error in the 1-year cMetS score to classify individuals as positive responders, non-responders, and adverse responders. However, developing predictive models using larger sample sizes will enable alternate binning strategies (e.g., standard deviations or confidence intervals) to be investigated. This may generate predictive models that are more sensitive to distinguish subtle inter-individual differences in changes in cMetS scores. However, major strengths of the CHANGE program include the duration of the study, data collection at multiple time points, and the multiple study sites across Canada.

4.5 Conclusion

We demonstrated the potential value of establishing models to predict a person’s change in cMetS score after 1-year of a lifestyle intervention. In particular, SBP and short-term changes in cMetS scores are two parameters that may be valuable for healthcare practitioners monitoring the efficacy of a lifestyle intervention for MetS. Although these predictive models need to be tested further in larger and independent samples, our findings suggest a potentially promising new tool to improve the health management of individuals with MetS.
Chapter 5: Integrative Discussion
5.0 Summary of Findings

The overall aim of this thesis was to investigate factors that influence response to a personalized, team-based lifestyle intervention targeting MetS, and then develop a model to predict individual response to the intervention. Chapter 3 explored whether genetic variants in candidate SNPs modulate response to a lifestyle intervention. Two SNPs, rs662799 (APOA5) and rs1501299 (ADIPOQ), independently and combined in a weighted GRS (wGRS), were associated with changes in cMetS score. Specifically, individuals with the AA genotype in rs662799, individuals carrying a minor T allele in rs1501299 or those with a larger wGRS, experienced greater reductions in their cMetS score during the intervention. In light of these findings, the objective for Chapter 4 was to develop a model to predict individual responses to a lifestyle intervention using genetic data and baseline dietary, exercise and bioclinical variables. The predictive models identified baseline fasting glucose, triglycerides, systolic blood pressure, waist circumference, dietary fibre intake and 3-month change in cMetS score as significant predictors of either 12 month cMetS score or intervention response. Although the wGRS was significantly associated with changes in cMetS score during the intervention, the wGRS was not identified as a significant predictor of 12-month cMetS score or intervention response. Overall, this thesis demonstrated that studying genetic and bioclinical factors will help to elucidate inter-individual variability in response to lifestyle interventions targeting MetS.

5.1 Multifactorial Nature of MetS

The inter-individual variability in response to lifestyle interventions is an underlying theme throughout this thesis. As illustrated in Figure 4, the CHANGE program was no exception to this phenomenon.
The 12-month change in cMetS score (12-month cMetS score – baseline cMetS score) is shown for each individual in the CHANGE program. N=186 from Chapter 3.

The CHANGE program incorporated behavior change models in the frequent and individual dietetic counselling sessions to develop a personalized dietary regimen that encouraged behavior changes that would translate into reduced MetS risk. Participants also met frequently with a kinesiologist who supervised a tailored exercise program for each individual participant. Despite the integration of evidence-based approaches, including supervision and frequent personalized counselling, a number of people did not reduce their MetS risk by the end of the intervention. The variability in response highlights the multifactorial nature of MetS that may be influenced by a myriad of underlying biological factors in addition to motivation and adherence.

This thesis, along with other studies outlined in Chapter 1, revealed that genetics can influence individual response to lifestyle interventions. Based on these results, individuals with particular genotypes may fare better than others in the CHANGE program for MetS. However, genetic variations represent a small piece of a much larger puzzle relating to a person’s overall metabolic response to diet and exercise. It is becoming increasingly clear that individual variations in response to nutrition and exercise cannot be explained by one factor alone (e.g. SNPs), but rather by a combination of variables that also encompasses epigenomics,
metabolomics and microbiomics (de Toro-Martin et al. 2017; Ferguson et al. 2016; Laddu and Hauser 2019).

The multifactorial nature of common chronic diseases, including MetS, is changing how researchers approach the concept of “precision nutrition”. Precision nutrition aims to combine an individual’s genetic, metabolomic and environmental exposure profile (i.e. dietary habits, food behaviors, physical activity, the epigenome, the genome, the microbiota and the metabolome) to tailor nutritional recommendations based on these individual and dynamic parameters (Wang and Hu 2018). New study designs incorporating multiple factors will enhance our understanding of the underlying disease mechanisms and our ability to manage and treat chronic diseases like MetS. For example, the Maastricht study is a 10,000-person epidemiological trial that is monitoring participant health status by analyzing genetic, metabolomic, advanced phenotype (i.e. body composition), lifestyle factors (including dietary and physical activity) and classical bioclinical measurements. Analyses from this kind of large integrative study will provide new insights into the pathology and progression of chronic diseases that will ultimately improve intervention outcome. In another example, Zeevi et al. (2015) demonstrated that the integration of bioclinical, metabolomics and microbiomic measurements can be used to predict individual post-prandial glucose response more successfully than the common practice of carbohydrate counting. The results support the concept of precision nutrition in which personalized diets, based on numerous biological factors, can be used to successfully optimize an individual’s response to certain foods.

Overall, the results from this thesis provide evidence that genetics are an important factor in MetS risk and intervention response. However, the high variability in response to a
personalized lifestyle intervention in combination with the multifactorial nature of MetS highlights the necessity to conduct larger integrative studies to comprehensively characterise individual response to diet.

5.2 Importance of Replication

An interesting finding from this thesis was the apparently contrasting results between Chapters 3 and 4. Specifically, the wGRS was significantly associated with changes in cMetS score at 12-months (Chapter 3); however, the wGRS was not found to be predictive of the 12-month cMetS score (Chapter 4). These findings illustrate the importance of distinguishing association from prediction in the area of genetics and lifestyle interventions. Essentially, this thesis highlights that associations between SNPs and clinical outcomes may not translate easily into predictions of clinical outcomes.

There exists a large number of SNPs identified by GWAS and candidate gene studies that are strongly associated with individual components of MetS, but few studies have actually replicated these findings in validation studies. Our findings were (to some extent) not replicated in the predictive study, and so based on this evidence the SNPs associated with cMetS score cannot be used as a predictive measure in the CHANGE program. This is not to say that further analyses aren’t necessary; more so that additional studies are warranted to examine if the wGRS association reported in this thesis can be replicated. This is particularly notable given the small sample size of the CHANGE cohort used in this thesis. More generally, this same conservative approach needs to be considered with all SNP associations reported in the literature to avoid developing direct-to-consumer (DTC) products that may be based on inconclusive data. Many of the findings used to support DTC products have not been robustly validated, and may be
premature to provide to consumers who are not well-versed in the caveats of gene-association studies.

Once gene associations have been robustly replicated, the next step should be exploring these associations in a dietary intervention. Only two studies have exemplified the concept of replicating and validating genetic associations in the context of lifestyle interventions (Coletta et al. 2018; Gardner et al. 2018). These studies showed that although SNPs have been associated with bioclinical features and diet-gene interactions have been reported, the actual implementation of the DNA-based dietary advice does not result in meaningful differences in study outcomes. A large study (n=609) randomized obese individuals into either healthy low-fat diet group or healthy low-carbohydrate diet groups, and then determined if individuals were aligned or misaligned with their diet based on a panel of 3 SNPs (rs1801282 in PPARG, rs1042714 in ADRB2 and rs1799883 in FABP2). The results indicated no differences in weight loss or other bioclinical parameters between aligned or misaligned dietary groups after 1-year of the dietary intervention (Gardner et al. 2018). This subsequent validation step shows that although the gene associations may have been replicated, dietary recommendations based on these SNPs does not necessarily alter outcomes. Similarly, another small study (n=63) aligned obese women with a hypo-caloric diet based on a very similar panel of SNPs (rs1799883 in FABP2, rs1801282 in PPARG2, rs4994C3 in ADRB3, rs1042713 and rs1042714 in ADRB2). Again, the study found no differences in outcome between individuals aligned or misaligned to their genetically matched diet (Coletta et al. 2018). Collectively, these two studies suggest that using these SNPs to personalize diets does not influence the magnitude of weight loss or other bioclinical changes, despite the multitude of prior association studies that suggested otherwise. However, because many factors can influence intervention outcome, including compliance, knowledge of genetic
profile, delivery method and level of understanding of SNP information, it is impossible to definitively conclude that these SNPs do not influence intervention outcomes. Taken together, these findings, along with those from this thesis, suggest that SNPs are not solely responsible for the variable responses to lifestyle interventions. Other factors, including SNPs not selected for analyses, undoubtedly contribute to lifestyle intervention response.

5.3 Future Directions

Evidently, many areas of research remain untapped regarding lifestyle interventions targeting MetS. Future studies need to expand beyond genetics and incorporate epigenetics, metabolomics, and microbiomics to create a comprehensive picture of disease and identify key biomarkers that characterize MetS and predict response to different intervention strategies. Thus far there are few nutritional interventions that have incorporated multiple ‘-omics’ approaches, but evidence from recent studies, including that of Zeevi et al. (2015), suggest great merit in such integration (McMorrow et al. 2018; Moleres et al. 2013). Additionally, association studies that identify relationships between SNPs and clinical outcomes or diet-gene interactions need to be corroborated in large cohort studies. When these findings are validated, then genetic-based dietary recommendations can be made with greater accuracy and confidence. Future studies need to continue validating SNP associations through replication and dietary interventions to ensure information used in DTC products is sound and based on high-quality evidence.

5.4 Conclusions

This thesis discussed the genetic components that are associated with the inter-individual variability in response to a lifestyle intervention targeting MetS. In particular, SNPs in the
APOA5 and ADIPOQ genes independently and additively modulated individual response to the CHANGE program, but were not considered predictors of cMetS score or intervention response. Future studies investigating the relationship between SNPs and MetS in personalized lifestyle interventions should consider: 1) integrating epigenetics, metabolomics, and microbiomics, and 2) validating SNP associations through tailored dietary interventions. Taken together, this thesis identified new SNP associations with MetS and a novel approach to predict response to a lifestyle intervention targeting MetS.
REFERENCES


Appendix

Genetic Risk Score (GRS) Protocol

This protocol explains how to create a weighted GRS i.e., a GRS that takes into account the effect size ($\beta$/standard error) of 2 or more SNPs.

This protocol is designed for JMP 14 (SAS Institute, Cary, NC, USA).

Equation: \[ GRS = w_1SNP_1 + w_2SNP_2 + \ldots + w_nSNP_n \]

Where: \[ w_1 = \frac{\beta_1}{SE_1} \]

 SNP\(_1\) = 0 or 1 or 2; i.e. these numbers correspond to the number of “risk” alleles, where 0 = no risk alleles, 1 = one risk allele, 2 = two risk alleles

1. Insert a new column (i.e., herein referred to as the "genotype column") and create a formula to recode the additive model of SNP\(_1\) from letters to numbers. This allows for the number of “risk alleles” to be counted. For ex. if the risk allele is G, and the non-risk allele is T, then code genotypes as follows: GG=2, GT=1, TT=0. (See Figure 1).

2. Make certain that the genotype column is set as a continuous variable.

3. Use the fit model to perform a linear regression using the genotype column you created. Add the genotype column and any necessary covariates to the box entitled "Construct Model Effects". The outcome of interest is added to the "Y" box. (See stars in Figure 2)

4. The results of the linear regression will give an output that is titled “Parameter Estimates”. Record the “Std Error” for this first SNP (i.e., SNP\(_1\)). This is SE\(_1\). (See circle in Figure 3)
5. Right click anywhere on the table just produced, go to “Columns”, and select “Std Beta”.

   The “Std Beta” is the standardized beta for SNP₁. This is β₁. (See Figure 4 & circle in Figure 5).

6. Repeat steps 1-5 for every SNP that will be added into the GRS.

7. Create a new column in JMP (entitled “GRS”) and create a formula in which the equation indicated above has the actual values for SE and β for each SNP. (See Figure 6). When inputting the SNP₁ into the formula use the column created in Step 1 as SNP₁ in the formula, and so on for SNP₂,...,SNPₙ. The output of the equation corresponds to the GRS for each individual.
Figure 1: Step 1; screenshot of the formula for the new column.

Figure 2: Step 3; screenshot of the set up for the linear regression.
Figure 3: Step 4; screenshot of the output with the Std Error.

Figure 4: Step 5; screenshot of the right click and selection of Std Beta column.
Figure 5: Step 5; screenshot of the output with the Std Beta column.

Figure 6: Step 7; screenshot of the formula created using the equation $GRS = w_1SNP_1 + w_2SNP_2$; where $w_1 = \beta_1/SE_1$