The Effects of the Dietary Supplement Cardioflex Q10 on Reducing Cardiovascular Disease Risk Factors in Adults

By

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A Thesis submitted to the Faculty of Graduate Studies of The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

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Abstract

The objective of this study was to determine whether daily supplementation with the dietary supplement Cardioflex for 90 days would improve plasma lipid blood profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A) and other cardiovascular disease risk factors in adults aged 30 – 65 years old. A randomized double-blind placebo-controlled parallel clinical study was conducted with 67 participants who received one daily serving (10g) of Cardioflex (n=33) or 10g of a maltodextrin placebo (n=34). Plasma lipid profile, anthropometric measures (waist circumference, body mass index), cardiac measures (heart rate, heart rate variability (HRV), accelerated plethysmograph age (biological age of arteries), blood pressure), and inflammatory and endothelial function biomarkers (C-reactive protein, interleukin-6, soluble intracellular adhesion molecule-1, soluble vascular cell adhesion molecule-1) were measured at baseline and endpoint of the study. Analysis of covariance was conducted using SPSS. In this study, HRV increased for the Cardioflex group (65.24 $\pm$ 9.44 ms to 68.72 $\pm$ 9.78 ms) while a decrease (69.95 $\pm$ 9.37 ms to 63.68 $\pm$ 9.08 ms) was seen in the placebo group, resulting in an overall 6.8 millisecond higher post-treatment HRV in the Cardioflex group (P=0.04). However, multiple regression analysis showed no association between HRV and heart rate, blood pressure, or APG age. There were no other significant time x group interactions from the Cardioflex treatment in blood lipid or any of the other dependent variables tested. Further research is needed to explore Cardioflex's potential effects on HRV and other cardiovascular disease risk factors.

Acknowledgments

I am deeply grateful for the professional guidance and support from my supervisor Dr. Semone Myrie; I am very indebted to her patience and invaluable advice.

I would like to thank my advisory committee; Dr. Rebecca Mollard, Dr. Miyoung Suh, and Dr. Stephen Cornish for their guidance throughout this process; their ideas and feedback have been invaluable.

I would like to thank Gerry Bohemier, Denise Delay, and Mark Pinder for their help with the clinical phase of the research. Special thanks to Stephanie Jew, for coordinating all the phlebotomists throughout my research.

I also want to acknowledge the funding support from Innotech Nutrition and MITACS. I am very appreciative of a local Winnipeg company supporting research.

Finally, thanks to my colleges, peers, and family members for their support throughout the last 4 years. This journey would have not been possible without all of you.

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

ALT = Alanine aminotransferase

APG = accelerated plethysmograph

AST = Aspartate aminotransferase

BMI = Body mass index

BMR = Basal metabolic rate

CoQ10 = Coenzyme Q10

CRP = C-reactive protein

CVD = Cardiovascular diseases

DPA = Digital Pulsewave analysis

EAR = Estimated average requirement

HDL = High-density lipoprotein

HRV = Heart rate variability

IL-6 = Interleukin-6

LDH = Lactate dehydrogenase

LDL = Low-density lipoprotein

MIS = Multi-ingredient supplement

NEAT = Non exercise activity thermogensis

RDA = Recommended dietary allowance

siCAM = Soluble intracellular adhesion molecule

SvCAM = Soluble vascular cell adhesion molecule

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

Cardiovascular disease (CVD), a general term for conditions affecting the function of the heart and/or blood vessels, has a global prevalence that cannot be ignored, with heart disease and stroke as the leading cause of death worldwide (World Health Organization, 2018). In North America, CVD is also the number one cause of death, with approximately 610,000 people dying from heart disease in the United States (US) every year (Mozaffarian et al., 2015). In Canada, CVD is the leading cause of death and prescription drug use (Heart and Stroke Foundation of Canada, 2017), with Health Canada 2012/2013 statistics stating that about 2.4 million (8.5%) Canadian adults live with heart disease, and about 158,700 adult Canadians receive a new diagnosis annually (Health Canada, 2017). Dyslipidemia, as characterized by elevated levels of serum total cholesterol, low-density lipoprotein (LDL)-cholesterol or triglycerides and/or reduced levels of high-density lipoprotein (HDL)-cholesterol, is an established risk factor for CVD (Orozco-Beltran et al., 2017) and is routinely used by health care practitioners for assessing cardiovascular risk (Niroumand et al., 2015). Between 2009 and 2011, unhealthy elevated total cholesterol levels (above 6.2 mmol/L) were present in 39% of Canadians, according to results from the Canadian Health Measures Survey (Statistics Canada, 2018). Hypertension, another established risk factor for CVD, affects approximately 1 in 4 adult Canadian and is currently ranked as the leading risk factor for death worldwide (DeGuire, Clarke, Rouleau, Roy, & Bushnik, 2019).

Overall, the aforementioned statistics highlight the gravity of the CVD situation in Canada, hence a need for some strategies to combat the morbidity and mortality related to CVD. The Heart and Stroke Foundation of Canada estimates that up to 80% of CVD cases are

preventable by reducing modifiable risk factors such as unhealthy diet, physical inactivity, an unhealthy bodyweight, smoking, stress, and excessive alcohol consumption (Government Of Canada, 2016). However, overall adherence to government guidelines is low and statistical data also shows that many Canadians are not following healthy lifestyle habits to help reduce their risks for CVD. Health Canada statistics show that only 20% of adults achieved the recommended Canadian physical activity guidelines for adults (Statistics Canada, 2013), 76.9% of Canadians aged 15 or older reported consuming alcohol at least once per year (Health Canada, 2016), and that 15.8% of Canadians smoke tobacco regularly (Canada, 2020a). Government assessment of adult Canadians found that 50% of women and 70% of men have energy intakes that exceed their energy needs (Statistics Canada, 2013) and only 28.6% of Canadians consumed the recommended 5 or more servings of fruits and vegetables (Statistics Canada, 2017). Overconsumption of calories and insufficient fruit and vegetable intake can lead to obesity and nutrient deficiencies, both of which are associated with increased prevalence of chronic diseases including heart disease, stroke, cancer, respiratory diseases, diabetes, and obesity (WHO, 2003).

Inadequate micronutrient intakes are prevalent in North American populations (Reider, Chung, Devarshi, Grant, & Hazels Mitmesser, 2020). For example, adult Canadian consumption quantities of magnesium and vitamin D are 34% and 80%, respectively, less than the estimated average requirement (EAR) (Health Canada, 2012). Similarly, US national surveys found that 94.3% of the population does not meet the recommended dietary allowance (RDA) for vitamin D, 88.5% for vitamin E, 52.2% for magnesium, and 38.9% for vitamin C (Wallace, McBurney, & Fulgoni, 2014). Observational data have identified associations between magnesium (Widman, Wester, Stegmayr, & Wirell, 1993), vitamin D (Wang et al., 2008), vitamin E (Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991), and vitamin C (Block, Jensen, Norkus, Hudes, & Crawford, 2008) intake with CVD risk. Although correlation does not equal causation, there are some suggestions that

dietary supplements may have the potential to help improve health by addressing some of the noted micronutrients inadequacy and reduce CVD risk by adding additional nutrients and more concentrated doses of nutrients to people's diets. Results from the 2009 to 2012 National Health and Nutrition Examination Survey (NHANES), data representative of 10,698 US adults, showed that compared to food alone, multi-vitamin-mineral use at any frequency was associated with decreased micronutrient inadequacies and a lower risk of nutrient deficiencies (Blumberg, Frei, Fulgoni, Weaver, & Zeisel, 2017). However, despite observational data suggesting strong correlations between nutrient intake and decreased risk of CVD, most intervention data with nutrient supplements have not supported these relationships (Monsen, 2000). For example, vitamin D intake has been associated with decreased CVD risk (Scragg, 1981) but vitamin D supplementation trials have failed to show clear improvements in blood pressure, insulin sensitivity, or lipid parameters, suggesting that the link between vitamin D deficiency and CVD may be an epiphenomenon (Moats & Rimm, 2007). This review will provide some information about why the discrepancy between the observational and interventional data may exist, although there are no clear answers.

The objective of this review is to evaluate the existing literature and summarize the available information linking assessment of cardiovascular health, strategies to improve cardiovascular health and more specifically, what supplements may reduce CVD risk factors, and why the mixed results from intervention studies and epidemiological studies in the role of nutrient supplements in the prevention of CVD.

CARDIOVASCULAR DISEASE OVERVIEW

CVD is a major cause of mortality worldwide (Lozano et al., 2012). CVD accounted for 24% of all deaths in the United States in 2014 (Writing Group et al., 2016) and 19% of all deaths in Canada in 2018 (Canada, 2020b).

CVD refers to any disease of the heart and/or blood vessels and includes heart diseases, artery diseases, stroke, and hypertension. CVD is usually caused by atherosclerosis, a buildup of plaques in the arteries. Elevated total cholesterol and LDL-cholesterol are involved in the pathogenesis of atherosclerosis (Johnston, Korolenko, Pirro, & Sahebkar, 2017). Although lowering LDL-cholesterol and total cholesterol levels are the primary targets in reducing atherosclerotic CVD (Carey et al., 2010), high levels of triglyceride and low levels of HDL-cholesterol are considered independent risk factors for coronary heart disease (Emerging Risk Factors et al., 2009) and ischemic stroke (O'Donnell et al., 2010). Observational data collected from 2005 to 2008 indicate that approximately 50% of adults in the United States have elevated cholesterol values and approximately 33% have elevated LDL-cholesterol levels (Benjamin et al., 2019). Similarly, data collected from 2012 to 2013 indicate that 38% of adult Canadians have dyslipidemia, as defined by an LDL-cholesterol ≥3.5mmol/L or total cholesterol:HDL-cholesterol ratio ≥5.0 (Canada, 2015). Pharmacologic treatments of dyslipidemia for the prevention of CVD are well accepted, with statins being the most commonly prescribed medication (Fuentes, Pineda, & Venkata, 2018). While statins are routinely used for the prevention and treatment of CVD, there is the desire for new technologies to replace hypolipidemic agents because patients can develop statin intolerance, high-dose statin therapy has been associated with an increased risk of developing type-2 diabetes (Sattar et al., 2010), and statin therapy substantially increases the risk of liver injury (Liang, He, & Zhao, 2018). Indeed, although highly effective, these drugs can cause side effects in some patients.

Preliminary evidence from intervention studies with nutrient supplements has confirmed the hypolipidemic efficacy and safety of certain nutraceuticals (Lan et al., 2015; Li et al., 2014; Mannarino, Ministrini, & Pirro, 2014). Nutritional supplements may be a novel approach for risk reduction and prevention of CVD or used in conjunction with

pharmaceutical treatments to manage conditions for those with CVD. For example, it is known that as blood pressure increases, there is a clinically significant association with the risk of CVD (Liszka, Mainous, King, Everett, & Egan, 2005). Dietary intakes of potassium and magnesium have been inversely associated with blood pressure (Kesteloot & Joossens, 1988), which leads to the hypothesis that hypertensive persons who have low habitual intakes of potassium and magnesium would be responsive to supplementation. This hypothesis has been supported by cohort studies and clinical trials to date (Kass, Weekes, & Carpenter, 2012; Yang et al., 2011).

Given the known prevalence and mortality rate attributed to CVD, many countries have implemented programs and policies to provide tools and strategies to reduce the incidence of initial and recurrent cardiovascular events. For example, The American Heart Association has published position statements defining cardiovascular health and the metrics needed to monitor it over time, from which health care providers and patients can acquire proven strategies and expertise for improving cardiovascular health (Lloyd-Jones et al., 2010). Dietary supplements have not been included in government recommendations to improve cardiovascular because observational studies do not provide clear confirmation in the efficacy of nutrient supplements in the prevention of CVD. Demonstrating efficacy requires replicable clinical studies with well-defined products and experimental designs. The growing consumer interest for dietary supplements will likely provide researchers with the opportunity to perform more ad hoc studies to clarify the exact role and importance of these supplements in the prevention and treatment of CVD. Once a consensus has been made, dietary supplements may start to be implemented as another strategy to help individuals with improving their cardiovascular health.

WHO IS AT RISK FOR CVD?

Multiple modifiable and nonmodifiable risk factors are attributed to causing CVD. Non-modifiable risk factors for CVD include sex, age, and race and/or ethnicity. Women will generally manifest CVD at an older age than in men, as the menopause transition is associated with a worsening CVD risk profile (Agrinier et al., 2010). The risk of developing heart disease and/or stroke increases with age, and people of African and Hispanic heritage are generally at the highest risk for developing heart disease and/or stroke (Leigh, Alvarez, & Rodriguez, 2016).

The greatest modifiable risk factors for developing heart disease and/or stroke include smoking, a diet rich in saturated fat, physical inactivity, stress, and being overweight (Heart and Stroke Foundation of Canada, 2017). Despite overwhelming evidence that healthy lifestyle choices significantly affect short and long-term CVD risks, it seems frustratingly difficult to get individuals to adopt the recommended habits and practices, with organizations such as the American Heart Association estimating that only 5% of individuals follow all of the lifestyle practice recommendations to achieve ideal cardiovascular health (Lloyd-Jones et al., 2010). Physical inactivity is a significant risk factor for coronary heart disease (King et al., 2019), yet fewer than 20% of adolescents perform the recommended 60 minutes of daily physical activity (CDC, 2018). Obesity is established as one of the most important independent risk factors for CVD (Carbone et al., 2019), yet dietary recall data indicates that nearly the entire U.S. population consumes a diet with fewer vegetables, fruits, milk and whole grains than recommended, and a pervasive overconsumption of fats, added sugars and alcoholic beverages (Krebs-Smith, Guenther, Subar, Kirkpatrick, & Dodd, 2010).

There is an abundance of evidence to suggest that diets rich in fruits and vegetables, whole grains, fish, and low-fat dairy products, and diets low in saturated fats and sodium can reduce the risk of developing chronic diseases such as CVD. For example, the Healthy Ageing Longitudinal study in Europe (HALE) assessed the effects of a Mediterranean-type diet (a diet comprised mainly of whole grains, fruits, vegetables, nuts, and olive oil) in elderly men and women, found a 23% lower risk of death from all causes (Knoops et al., 2004). A randomized clinical study by Esposito et al evaluated the effects of a Mediterranean-style of diet on individuals with the traits of metabolic syndrome with the results showing that those on the Mediterranean diet had improved endothelial function, decreased inflammation, and a reduced risk of heart attack and stroke (Esposito et al., 2004). National guidelines, examples as given by The American Heart Association and The Heart and Stroke Foundation of Canada, emphasize the importance of balancing calories as a strategy for maintaining healthy bodyweight and thereby reducing CVD risk (American Heart Association Nutrition et al., 2006; Heart and Stroke Foundation of Canada, 2017). Obesity represents a significant risk factor for CVD because of its association with other risk factors (type 2 diabetes, hypertension, metabolic syndrome) but also because it serves as an independent risk factor (Eckel & Krauss, 1998). Furthermore, the distribution of body fat also serves as an independent risk factor because intra-abdominal fat promotes insulin resistance, elevated triglycerides, hypertension, and low HDL-cholesterol (Despres, 1993). The Look AHEAD (Action for Health and Diabetes) Trial showed that overweight individuals who lost 7% of body weight significantly lowered CVD risk factors (Look et al., 2013).

Overall, daily habits and actions profoundly affect the likelihood of developing CVD. Overwhelming epidemiological evidence shows that cigarette smoking significantly increases the risk of heart disease (Jha et al., 2013) and there is a reasonably extensive body of literature showing that stress and psychological factors may trigger acute cardiac

events and increase the risk of CVD (Mittleman & Mostofsky, 2011). In summary, regular physical activity, proper nutrition, weight management, tobacco avoidance, and stress reduction are all key modalities that lower the risk of CVD. Metrics such as Metabolic Syndrome give individuals and healthcare professional quantifiable tools for determining the likelihood of an individual developing heart disease, diabetes, or stoke. The risk factors that contribute to metabolic syndrome include obesity, increased waist circumference, high blood pressure, low HDL-cholesterol, high triglycerides, and glucose intolerance.

Traits Of Metabolic Syndrome

Metabolic syndrome is a risk assessment tool that can be used to identify individuals at an elevated risk for atherosclerotic CVD and future cardiovascular events (Grundy et al., 2004). Based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) clinical guidelines, an individual is classified as having metabolic syndrome when he/she presents with at least 3 of the 5 identified traits summarized in Table 1, including abdominal obesity, elevated blood pressure, low HDL-cholesterol, high triglycerides, and hyperglycemia (Lorenzo, Williams, Hunt, & Haffner, 2007).

Table 1 - National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) Clinical Guidelines of Metabolic Syndrome*

Risk Factor	**Defining Level**
Abdominal obesity, given as waist circumference*†	
Men	>102 cm (>40 in)
Women	>88 cm (>35 in)

Risk Factor	Defining Level
Triglycerides	≥ 1.7 mmol/L
HDL-cholesterol	
Men	< 1.03mmol/L
Women	< 1.29 mmol/L
Blood pressure†	≥130/≥85 mmHg
Fasting glucose	≥ 6.1 mmol/L

*Information adapted from(Alberti & Zimmet, 1998; Zimmet, Magliano, Matsuzawa, Alberti, & Shaw, 2005)
HDL = high-density lipoprotein; †blood pressure is given as systolic/diastolic

Traits of metabolic syndrome are associated with accelerated atherosclerosis, a greater incidence of coronary heart disease (Ford, 2005; Grundy, 2002), and significantly elevated risk of cardiovascular mortality (Gami et al., 2007). A cohort study that followed 3323 middle-aged healthy adults for the development of new CVD and coronary heart disease over an 8-year period found that metabolic syndrome is associated with an increased risk for CVD and coronary heart disease in both sexes. Risk estimates for CVD and coronary heart disease were 34% and 29% in men, and 16% and 8% in women, respectively (Wilson, D'Agostino, Parise, Sullivan, & Meigs, 2005).

BLOOD LIPID PROFILE

Elevations in levels of total cholesterol and LDL-cholesterol, and reductions in HDL-cholesterol levels are established risk factors for the development and pathogenesis of CVD; primarily atherosclerosis. These observations originally came from epidemiological

studies such as the Framingham heart study (Kannel, Dawber, Friedman, Glennon, & McNamara, 1964). Subsequent research using atherosclerosis imaging consistently show relationships of cholesterol levels to atherosclerosis development and regression (J. G. Robinson et al., 2018). Furthermore, trajectory analysis finds atherosclerotic CVD rates are 3 to 4 times higher in individuals with lifelong high LDL-cholesterol and low HDL-cholesterol levels (Duncan, Vasan, & Xanthakis, 2019).

Consistent evidence from numerous and multiple different types of clinical and genetic studies have established that intermediate-density lipoproteins, low-density lipoproteins, very low-density lipoproteins, and lipoprotein(a) are directly implicated in the development of atherosclerosis and CVD (Goldstein & Brown, 2015). Thus, some clinicians have advocated for measuring lipoprotein(a) in addition to the established lipid barometers of total cholesterol, HDL-cholesterol, and LDL-cholesterol (Di Angelantonio et al., 2009). Genetic and epidemiologic studies have identified lipoprotein(a) as a risk factor for atherosclerosis, coronary heart disease, and stroke; particularly if combined with other lipid and thrombogenic risk factors (Margaglione et al., 1996).

In addition to cholesterol and lipoprotein fractions, other lipid components such as triglycerides may contribute to the development of CVD. Epidemiological studies have shown elevated triglyceride levels are associated with increased risk for coronary artery disease (Di Angelantonio et al., 2009; Iso et al., 2014). However, in contrast to cholesterol and lipoproteins, triglycerides receive less attention because there is little definite evidence from randomized controlled trials (Tada, Nohara, & Kawashiri, 2018). Furthermore, triglycerides are major structural components of lipoproteins such as chylomicrons, very low-density lipoproteins, and intermediate-density lipoproteins. Correlative studies of triglycerides and CVD implicate LDL-cholesterol, very-low-density lipoprotein and chylomicron in atherosclerosis development, but the accompanying

associations of these lipoproteins and CVD risk obscure any conclusions about direct relationships between CVD and triglycerides (Katcher, Hill, Lanford, Yoo, & Kris-Etherton, 2009). Thus, elevated serum triglyceride levels also reflect increased levels of lipoproteins and may only serve as causal risk factors for atherosclerosis and atherosclerotic CVD. Also, triglycerides are unlikely to directly cause atherosclerosis because triglycerides, unlike cholesterol, can be degraded by most cells in the body. Future randomized intervention trials are needed to determine whether triglyceride-lowering therapy reduces inflammation, atherosclerotic CVD, and mortality in patients with elevated triglyceride levels, independent of changes in cholesterol and lipoproteins.

Lipid-lowering clinical trials tend to be conducted in high-risk populations so that significant differences in treatment effects can be detected over relatively short time intervals. However, the treatment effects achieved in these trials are remarkably high compared with the effects seen in medium-low risk populations (Jennifer G Robinson, 2014). Guidelines rely on these trials for lipid-lowering prevention recommendations, but they provide little guidance for the general public. Future studies are needed with low-medium risk populations given that their blood lipid pattern of development will provide greater guidance in identifying high-risk individuals earlier in life.

Individuals with dyslipidemia are observed to have depressed heart rate variability (HRV) (Alper Kepez, 2015). Attenuation in HRV is associated with higher cardiovascular risk in healthy individuals and patients with established CVD (Tsuji et al., 1996).

HEART RATE VARIABILITY

Heart rate variability (HRV) calculates the temporal variation of time between two consecutive heartbeats. HRV is used to assess cardiac autonomic function. On a standard electrocardiogram (ECG), a heartbeat is displayed as a PQRST complex, with each letter

corresponding to a different part of the heart's action. The "R" of the complex is the area from which the values for HRV analysis are taken. The distance (in milliseconds) between each heartbeat is defined as the "RR interval". HRV is calculated as the standard deviation of all of the RR intervals from a section of recording.

Poor HRV is associated with high blood pressure, heart disease, obesity, and diabetes (Brito Diaz, Aleman Sanchez, & Cabrera de Leon, 2014). Epidemiological studies show reduced HRV is associated with an increased risk for coronary heart disease, CVD mortality, and worse prognosis in patients with heart disease or heart failure (Tsuji et al., 1994). Thus, HRV may play a role in mediating the cardiovascular risk factors on CVD causation and prognosis (Steptoe et al., 2002). A 2013 meta-analysis, including eight studies and 21, 988 participants, found that reduced HRV was associated with a 32–45% increased risk of a first cardiovascular event (Hillebrand et al., 2013).

Correlations between elevated biomarkers of inflammation, such as C-reactive protein (CRP) and interleukin 6 (IL-6), and reduced HRV has been observed in patients with congestive heart failure and coronary heart disease (Aronson, Mittleman, & Burger, 2001; Janszky et al., 2004). Population studies with individuals free of CVD have demonstrated the same association (Aeschbacher et al., 2017). A cohort study with 2064 healthy individuals aged 25-41 years found that inflammatory parameters were strongly associated with decreased HRV (Aeschbacher et al., 2017).

Dietary factors can influence HRV. In several investigations, a correlation was observed between serum magnesium levels and HRV (Almoznino-Sarafian et al., 2009; Bobkowski et al., 2003). Both magnesium deficiency and decreased HRV are common in patients with heart failure (Almoznino-Sarafian et al., 2009). Magnesium citrate supplementation of 300mg/day for 5 weeks significantly increased HRV in patients with systolic heart failure (Almoznino-Sarafian et al., 2009). Decreased intracellular magnesium and decreased HRV

were reported in healthy subjects during chronic sleep deprivation (Takase et al., 2004). Sleep deprivation is associated with increased norepinephrine, and norepinephrine is known to reduce intracellular magnesium concentrations and decrease HRV by inflicting imbalances in sympathetic/parasympathetic activity (Sesay, Tauzin-Fin, Gosse, Ballanger, & Maurette, 2008). Taken collectively, it seems plausible to speculate that magnesium supplementation increases HRV by raising intracellular magnesium concentrations and reducing catecholamine secretion.

The dysregulation of the autonomic nervous system (ANS) has been implicated in the development of hypertension. Lower HRV is associated with higher values of blood pressure, increasing the risk of developing hypertension (Mohamed Faisal Lutfi, 2011).

BLOOD PRESSURE

High blood pressure is one of the traits of metabolic syndrome. It has been reported that approximately one-third of hypertensive patients have metabolic syndrome (Cuspidi et al., 2004). Hypertension is a strong and consistent predictor of the development of CVD (Arima et al., 2009; Flint et al., 2019; Stokes, Kannel, Wolf, D'Agostino, & Cupples, 1989). In 2015, 1 in 4 men and 1 in 5 women had hypertension, with an estimated total of 1.13 billion people worldwide (Oparil et al., 2018). Observational studies have demonstrated graded associations between systolic and diastolic blood pressure and risk level for CVD (Muntner & Whelton, 2017). A meta-analysis using 1 million adults from 61 prospective studies found each 10 mmHg increase in systolic clinical blood pressure increased the risk of CVD mortality by 40% and the risk of ischemic heart disease by 30% (Lewington et al., 2002).

Blood pressure presents the challenge of measurement error and variability. The accurate measurement of blood pressure is essential for the diagnosis and management of

hypertension. A blood pressure reading can change by several points based on the machine used, the location the blood pressure cuff is applied on the arm, how tight the cuff is applied, and which arm is used. Even with strict adherence to guidelines, studies comparing blood pressure measurements taken by different individuals have reported inconsistencies (Sebo, Pechere-Bertschi, Herrmann, Haller, & Bovier, 2014). Thus, standardizing who does the measurements, frequent measurements, and recording the average of multiple measurements should be done to minimize 'white coat' syndrome during clinical blood pressure measurements.

Ambulatory and home blood pressure measurements can help minimize 'white coat' syndrome and labile hypertension. Results from some studies suggest that in-office-clinical blood pressure might not be as effective in predicting cardiovascular events as out-of-office blood pressure measurements (Teramoto et al., 2012). The HONEST (Home Blood Pressure Measurement With Olmesartan Naïve Patients to Establish Standard Target Blood Pressure) study, which included over 20,000 hypertensive patients, found that morning home blood pressure may be superior to clinical blood pressure in predicting future cardiovascular events (Kario et al., 2014). Similarly, in the Ohkubo study (Ohkubo et al., 1998), Finn-HOME study (Niiranen, Hanninen, Johansson, Reunanen, & Jula, 2010), and SHEAF study (Bobrie et al., 2004), home blood pressure was found to be superior to clinical blood pressure for the prediction of future cardiovascular events. Home blood pressure measurements are useful to supplement in-office-clinical blood in the diagnosis of hypertension and as a screening tool for the white-coat effect. Furthermore, measuring blood pressure with other cardiovascular parameters can help improve the generalizability of the data.

Numerous studies have shown that hypertension is associated with endothelial dysfunction, which is involved in the development of atherosclerosis and increases the

risk of CVD (Panza, Quyyumi, Brush, & Epstein, 1990). Pulse wave velocity, a marker of endothelial function, directly and independently predicts an increase in blood pressure and the future development of hypertension (Koivistoinen et al., 2018). Interventions aimed at reducing CVD risk, including antihypertensive therapy, are more effective if they simultaneously improve endothelial function (Modena, Bonetti, Coppi, Bursi, & Rossi, 2002).

ENDOTHELIAL FUNCTION

CVD risk factors, such as hypertension, are associated with the development of endothelial dysfunction (Widlansky, Gokce, Keaney, & Vita, 2003). Endothelial dysfunction, an impaired function of the inner lining of blood vessels characterized by decreased vasodilation, diminished production of nitric oxide and/or an imbalance in endothelium-derived relaxing and contracting factors, contributes significantly to the pathomechanism of atherosclerosis and CVD (Rubanyi, 1993) and is considered an early marker for atherosclerosis (Davignon & Ganz, 2004). In addition, endothelial dysfunction affects lipid profile, C-reactive protein, serum glucose, and blood pressure (Tousoulis, Antoniades, & Stefanadis, 2005).

Endothelial function is readily measurable and is an established barometer of CVD risk (Vita & Keaney, 2002). Evaluation of endothelial function may allow for early identification of atherosclerosis, preceding angiographic or ultrasonic evidence of atherosclerotic plaque. Several invasive and non-invasive techniques are used to evaluate endothelial function. Invasive techniques, which involve infusions of vasoconstrictive agents, are considered the gold standard for evaluating endothelial function since they allow for dose-response relations of endothelial agonists and antagonists and assessment of basal endothelial function through the infusion of nitric oxide synthase inhibitors (Hasdai & Lerman, 1999). In addition, there are several non-invasive techniques with

comparable results and good reproducibility that can be used as screening tests for the early identification of atherosclerosis (Tousoulis et al., 2005). Table 2 below summarizes invasive and non-invasive techniques used to evaluate endothelial function.

Table 2 - Invasive and Non-invasive techniques to evaluate endothelial function*

Technique	Method	Strengths	Limitations
Invasive Intra-arterial infusion	Vasoactive agents are delivered via intra-arterial infusion and the response is measured with ultrasound or strain gauge plethysmography.	Allows for the evaluation of both dose-response relations and basal endothelial function.	This technique is invasive, costly, and carries a risk of coronary ischemia. Therefore, widespread clinical use is limited.
Invasive Venous occlusion plethysmography	Venous occlusion plethysmography measures volume changes during hyperaemia in the forearm provoked by vasoactive agents following forearm occlusion. Forearm endothelial dysfunction is a known marker for cardiovascular complications.	Accurate, reproducible, and drugs can be administered at sub systemic doses.	This technique is invasive and time-consuming.
Non-invasive Flow-mediated dilation	Flow-mediated dilation (FMD) is the measurement of changes in brachial artery diameter in response to shear stress achieved by inflation of a pneumatic cuff. On deflation of the cuff, the percentage change in brachial artery diameter is recorded as the FMD. A lack of vasodilation would	Reproducible, with a coefficient of variation of about 3–4% in short term (2 hour interval) as well as in longer term (>3 week interval) repeated measurements (Lind, Hall, & Johansson, 2002)	The lack of a standardized protocol for measuring FMD precludes the accurate comparison of data between studies.

	suggest endothelial dysfunction.		
Non-invasive Pulse Wave Analysis	Digital Pulsewave Analysis (DPA) evaluates the shape and contour of a pulse wave to assess endothelial function and arterial stiffness.	Transportable, reliable, and inexpensive, offering great clinical and epidemiological potential.	This technique can be influenced by non-endothelial factors.

*Information adapted from (Al-Qaisi, Kharbanda, Mittal, & Donald, 2008)

Considering the relationship between endothelial dysfunction and atherosclerosis, the status of endothelial function may serve as an early marker for CVD. As measures of endothelial function become clinically applicable, this may translate into improved risk assessment for predicting, preventing, and treating CVD. For widespread clinical usage, the ideal technique for measuring endothelial function must be non-invasive, reliable, and reproducible. The pulse wave analysis technique is particularly suitable for clinical usage. Strengths of this technique include simplicity of assessment, the low time commitment for subjects, non-invasiveness, portability, and cost-effectiveness.

DIGITAL PULSE WAVE ANALYSIS

The Digital Pulsewave Analysis (DPA) machine is a non-invasive test that evaluates the shape and contour of a pulse wave to assess endothelial function and arterial stiffness (Emma von Wowern, 2017). The DPA machine obtains information through an infrared light fingertip probe. The fingertip probe detects the changes in the amount of light absorbed by hemoglobin allowing the device to graph the pulse of blood flowing from the heart. The DPA machine converts the changes of transmitted light into a pulse waveform and displays the waveform on the LCD screen. The DPA also displays the accelerated

plethysmograph (APG) waveform to provide an evaluation of arterial stiffness. The DPA machine uses the APG waveform to quantify an APG type, assigning a biological age of the arteries from the elasticity of the aorta. An APG type of A corresponds to an age of 20, B to an age of 30, C to an age of 40, D to an age of 50, E to an age of 60, F to an age of 70 and E to an age of 80.

Assessment of endothelial function evaluates the functional health of the vascular system and arteries (Stoner, Young, & Fryer, 2012). Increased aortic stiffness causes decreased cardiac efficiency, which increases myocardial oxygen consumption and increases the risk of developing a heart attack (Cecelja & Chowienczyk, 2012). The DPA machine has been validated in population studies to detect the early signs of vascular disease. In 2007, the European Society of Hypertension and the European Society of Cardiology added pulse wave analysis measurement to their guideline for the management of arterial hypertension (Brian Peskin, 2010). Digital pulse wave analysis was found to be a stronger predictor of cardiovascular events and all-cause mortality then blood pressure in hemodialysis patients who underwent 48-hour ambulatory monitoring with standard intradialytic (between hemodialysis treatments) intervals and followed-up with after 28.1 ± 11.2 months (Sarafidis et al., 2017).

However, the DPA machine can be easily influenced by non-endothelial factors so a standardized measurement protocol is required for accurate comparison of data between patients. Research demonstrates that when following a standardized protocol, pulse wave analysis is a reliable instrument for endothelial function analysis with good repeatability of intra-observer measurement and high reproducibility of inter-observer measurement (Chang, Chen, & Wang, 2015).

INFLAMMATION AND ENDOTHELIAL FUNCTION BLOOD BIOMARKERS

Chronic inflammation is associated with early-stage endothelial dysfunction screening using the pulse wave analysis method (Castellon & Bogdanova, 2013). Endothelial dysfunction is an integrated marker of the damage to arterial walls. The atherosclerosis that develops is the main cause of cardiovascular morbidity and mortality. Furthermore, Atherosclerosis is a pro-inflammatory state and leads to increased cardiovascular events such as myocardial infarction and stroke. Population studies show that elevations in certain inflammatory markers are correlated with endothelial dysfunction and an increased risk of developing CVD (Jarvisalo, Juonala, & Raitakari, 2006). This is supported by literature showing that inflammatory diseases are associated with increases in CVD and atherosclerosis, as well as endothelial dysfunction (Castellon & Bogdanova, 2016). Many clinical studies have shown strong and consistent relationships between CRP and CVD prediction (Pahwa, Goyal, Bansal, & Jialal, 2020). Overexpression of IL-6 has been linked to the development of several diseases including heart disease, diabetes, rheumatoid arthritis, and cancer (Simpson, Hammacher, Smith, Matthews, & Ward, 1997).

Soluble intercellular and soluble vascular cell adhesion molecules play an important role in the adhesion of leukocytes to the vascular endothelium. Soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) facilities the attachment of inflammatory cells to the vascular endothelial wall. Elevated sVCAM-1 represent biomarkers for inflammation in the endothelium (Ponthieux et al., 2004). Increased expression of sVCAM-1 is observed in many diseases with an inflammatory component (Gearing & Newman, 1993). sVCAM-1 seems to be the most specific at identifying atherosclerosis. Serum levels of sVCAM-1 appear to correlate with the pathogenesis of atherosclerosis and might allow for the detection of early stages of atherosclerosis (Peter, Weirich, Nordt, Ruef, & Bode, 1999). Soluble intracellular adhesion molecule-1 (sICAM-1) regulates the adhesion of leukocytes

to vascular endothelial, a process crucial to atherosclerosis. Abnormal levels of circulating sICAM-1 have been linked to the development of several diseases such as CVD, hypertension, and rheumatoid arthritis, and may serve as a marker of CVD progression (Witkowska & Borawska, 2004).

Atherosclerosis is a disease characterized by low-grade chronic inflammation of the arterial walls that is precipitated by elevated levels of LDL-cholesterol in the blood (Galkina & Ley, 2009). Meta-analyses of long-term prospective studies have reported that the risk of coronary heart disease is about 90% greater in those with elevated CRP (Danesh, Collins, Appleby, & Peto, 1998), and about five times greater in those with elevated sVCAM-1 (Hwang et al., 1997). Circulating concentrations of inflammation and endothelial function blood biomarkers are useful for the prevention and treatment of atherosclerosis and other CVDs.

NUTRITION AND IMPROVING CARDIOVASCULAR HEALTH

Multiple modifiable and nonmodifiable risk factors are attributed to causing CVD. The prevailing view by researchers is that most cases of CVD can be minimized or prevented, for the most part, by improving the modifiable risk factors through lifestyle changes. Health Canada and The American Heart Association state that the main lifestyle changes to reduce the modifiable risk factors for CVD are to not smoke, choose good nutrition (a diet rich in fruits and vegetables and low in saturated fats, sugar, and salt), be physically active, maintain a healthy body weight, reduce stress and limit alcohol consumption (Association, 2016; Public Health Agency of Canada, 2017). While all these modifiable factors are interplayed to exacerbate CVD risk, this review will focus only on the nutrition aspect, as nutritional interventions have proven to be a tangible strategy in preventing and treating CVD. Obesity is established as one of the most important independent risk factors for CVD. The main mechanism through which obesity increases CVD risk involves

changes in hemodynamic parameters (Carbone et al., 2019). Accumulation of fat mass around the abdomen has been recognized as a major cardiometabolic risk factor (Tchernof & Despres, 2013). Visceral fat favors the production of inflammatory cytokines with pro-atherosclerotic properties (Frayn, Karpe, Fielding, Macdonald, & Coppack, 2003).

Large scale epidemiological studies have shown correlations between CVD mortality and BMI (Valavanis, Mougiakakou, Grimaldi, & Nikita, 2010) and the role of a high-fat diet in the pathogenesis of atherosclerosis (Ornish et al., 1998; Shaper, 1972). Following these observations, the first ecologic studies provided further insights on the impact of different lipids on CVD, such as the association of CVD incidence and mortality with saturated fat intake and the potential protective role of omega-3 polyunsaturated fatty acids (Dyerberg & Bang, 1979). From this research, the American Heart Association Nutrition Committee released the first dietary recommendations, stating that "diet may play an important role in the pathogenesis of atherosclerosis and the fat content and the total calories in the diet are probably important factors" (Page, Stare, Corcoran, Pollack, & Wilkinson, 1957). Despite decades of nutritional research illustrating the beneficial effects of nutrition for reducing the risk of chronic diseases, including CVD, unfortunately, the adherence of the general population to recommended dietary patterns that emerged from such research is very low, especially in the subgroups expected to receive the greatest benefits. In 2013, diet-associated risk was responsible for 37% of deaths globally (Mortality & Causes of Death, 2015). Notably, 9 out of the 25 leading global risk factors for disability-adjusted life years were related to inappropriate eating habits such as a low intake of fruits and vegetables, whole grains, omega-3, fiber, and excessive intake of sodium and saturated fat (Mortality & Causes of Death, 2015).

Several reasons may explain the poor adherence to the suggested dietary patterns. First, trends influenced by the food industry promote increased consumption of highly-refined

energy-dense products, instead of unrefined fresh food (Scourboutakos, Semnani-Azad, & L'Abbe, 2013). Second, the recommended diet patterns are more expensive than the standard American diet food patterns (Young, Batch, & Svetkey, 2008). When standardised to 2000 kcal, healthier food-based diet patterns cost an average of $1.54/day more than less healthy options (Rao, Afshin, Singh, & Mozaffarian, 2013). Third, the perceived lack of palatability, availability, and cultural acceptability of this kind of diet (Bertoni et al., 2011). Finding strategies to increase the adherence of the evidence-based recommendations to the population should be considered as important, if not even more important, then the knowledge itself.

New research shows that intervention strategies based on single nutrients appear to not be enough to prevent the onset and the progression of CVD (Heidemann et al., 2008). There is growing evidence that single nutrients have limited magnitude when compared with whole foods or more complex dietary interventions on the prevention and progression of chronic diseases (Heidemann et al., 2008). Similar to single nutrient-based strategies, most intervention studies using single nutrient supplements have not shown favourable results in reducing CVD risk, even when using dosages at or above the EAR values (Monsen, 2000). Since many of the common ingredients used in nutrient supplements act on different physiological mechanisms, many researchers speculate whether certain combinations of these ingredients may have a synergistic effect on the overall efficacy of the supplement (Jagim et al., 2016). Synergy occurs from the potentiation of pharmacokinetics, such that one ingredient enhances the therapeutic effect of another active ingredient by modulating its absorption, distribution, metabolism, and excretion. For example, the combination of Salvia miltiorrhiza (Danshen) and Pueraria lobata (Gegen) was characterized as synergistic in the anti-inflammation assay when taken together, highlighting the applicability of multi-herb formulas to treat disease (Wing-Shing Cheung et al., 2012). Multi-ingredient supplements may be a way to improve

health and reduce CVD risk factors independent of dietary adherence. However, the body of literature and quality of studies examining the efficacy of multi-ingredient supplementation is preliminary at best.

DIETARY SUPPLEMENTS AND THE RISK FOR DEVELOPING CVD

The efficacy of dietary supplements in preventing and treating CVD has been deeply studied (Moyer & Force, 2014), but there is generally a lack of randomized trials and the understanding of their role is complicated by the fact that people consuming dietary supplements generally have a healthier diet and lifestyle. The lack of consensuses on their efficacy is further complicated by all the different forms of nutrient supplements available. For example, magnesium supplements can come in the form of magnesium citrate, magnesium oxide, magnesium chloride, magnesium lactate, magnesium malate, magnesium taurate, magnesium L-threonate, magnesium sulfate, magnesium glycinate, or magnesium orotate; all having different percent composition of magnesium per magnesium oxide compound and different bioavailability. Thus, comparing different studies often isn't an 'apples to apples' comparison.

Multi-vitamin-mineral supplementation has been advocated to reduce cardiovascular events (Holmquist, Larsson, Wolk, & de Faire, 2003); vitamin D intake has been associated with reduced occurrence of coronary artery disease and heart failure (Wang et al., 2008); coenzyme q10 deficiency has been associated with myocardial dysfunction and hypertension (Marcoff & Thompson, 2007). Supplementing with amino acids involved in the synthesize of collagen may help circumvent negative changes in cardiac physiology such as endothelial dysfunction and increased arterial stiffness (Wong, Mohamed, &

Niedzwiecki, 2015). However, not all intervention studies have supported these findings and there is still a lack of consensus (Marinangeli, Jones, Kassis, & Eskin, 2010).

The growing popularity of dietary supplements will provide researchers with the opportunity to perform more ad hoc studies to clarify their role and the exact importance of each one in the prevention and management of CVD and CVD risk factors.

MULTI-INGREDIENTS VERSE SINGLE INGREDIENT SUPPLEMENTS

In the last 10 years, multi-ingredients supplements (MIS) have increased in popularity. MIS products typically contain a blend of ingredients such as amino acids, herbal botanicals, vitamins, and minerals. Many of the ingredients used in MIS formulations act on different physiological pathways which may give certain combinations of these ingredients a synergistic effect (Jagim et al., 2016). However, the current body of literature and quality of studies examining the efficacy of MIS supplementation is very preliminary.

The small number of studies done on MIS and CVD risk have shown mixed results, although mostly positive. Total cholesterol and LDL-cholesterol decreased significantly when participants were given a MIS supplement containing red yeast rice, bioflavonoids, polycosanol, omega-3 fatty acids, resveratrol, coenzyme Q10, folic acid, vitamin B3, B6, B12, and black pepper extract (Hobbs, Caso, McMahon, & Nymark, 2014). Participants saw a significant reduction in total cholesterol, LDL-cholesterol, triglycerides, heart rate, and diastolic blood pressure when given a proprietary MIS (Houston, 2016). No improvements in body composition, blood lipid profile, or CRP were seen when overweight men and women were given MIS containing caffeine, conjugated linoleic acid, green tea, and branched-chain amino acids taken for 8 weeks (Ormsbee et al., 2014). A reduction in homocysteine and LDL-cholesterol oxidation rates were noted at the 12-

week midpoint and persisted throughout the 24-week study when healthy individuals were given a 24-ingredient multivitamin/mineral MIS (Earnest, Cooper, Marks, & Mitchell, 2002).

Given the potential benefits of MIS demonstrated in the literature to date, further investigations are warranted and needed not only to determine the efficiency of MIS, but also the safety since anecdotally we know that many people consume these products consistently for many years.

L-Lysine, L-Proline, L-Threonine

The literature demonstrates that amino acids can play a significant role in cardiovascular health. For example, supplementation with the amino acid L-citrulline has been shown to have beneficial effects on the cardiovascular system by increasing nitric oxide levels in the blood (Schwedhelm et al., 2008). Diminished bioavailability of nitric oxide contributes to the development of CVD and multiple CVD risk factors including hypertension, atherosclerosis, insulin resistance, and type-2 diabetes (Brandes, Fleming, & Busse, 2005; Hayashi et al., 1991). However, the body of literature on L-lysine, L-Proline, and L-Threonine is limited. Indeed, there are only a few published studies looking at the supplemental effects of L-lysine, L-Proline, and L-Threonine on CVD risk.

In vitro studies reported the potential of L-proline supplementation to reduce inflammation and scavenge free radicals (Kaul, Sharma, & Mehta, 2008). L-Proline supplementation prevented the inflammatory effects of lipopolysaccharide when administered into the cerebral cortex and cerebellum of young Wistar rats (Andrade et al., 2018). L-threonine supplementation has been shown to lower blood pressure in patients with CVD (Tuttle, Milton, Packard, Shuler, & Short, 2012). In an animal study administration of L-threonine reduced the concentrations of total cholesterol, triglycerides, and LDL-cholesterol when Pekin ducks were given 1.4 g/kg of L-threonine for

21 days (Jiang et al., 2017). Information is particularly scarce regarding the supplemental effects of L-lysine on CVD risk factors. Ivanov et al looked at the anti-atherogenic effects of a mixture of ascorbic acid, L-lysine, L-proline, L-arginine, L-cysteine, and green tea phenolics. The researchers found that the MIS has the potential to block the development of atherosclerosis by inhibiting the atherogenic responses of vascular smooth muscle cells to pathologic stimuli (Ivanov, Roomi, Kalinovsky, Niedzwiecki, & Rath, 2007).

In summary, research shows the potential of L-proline to reduce inflammation, L-threonine has been shown to lower blood pressure in patients with CVD, and a MIS containing L-lysine may have the potential to block the development of atherosclerosis. Further investigations with L-lysine, L-proline, and L-threonine are needed to provide a better understanding of how these amino acids affect cardiovascular health.

L-GLUTAMINE

Several clinical trials have revealed that glutamine supplementation has cardioprotective effects when given to patients with coronary heart disease (Khogali, Pringle, Weryk, & Rennie, 2002; Lomivorotov et al., 2011). The administration of glutamine resulted in a significantly lower median systemic vascular resistance index in ischemic heart disease patients following cardiopulmonary bypass (Lomivorotov et al., 2011). A population study that followed 74 082 US females and 42 303 US men found dietary glutamine was inversely related to the risk of mortality, particularly CVD mortality, independent of other dietary and lifestyle factors (Ma et al., 2018). In addition to the potential role in cardiovascular health, glutamine metabolism is closely related to malignancy of tumour cells, and glutamine supplementation has been implicated in the detection, monitoring, and treatment of cancer (Hensley, Wasti, & DeBerardinis, 2013). Further investigations with L-glutamine are needed to provide a better understanding of how this amino acid affects cardiovascular health.

Vitamin C (Ascorbic Acid)

Vitamin C is an antioxidant that may help reduce the risk of CVD (Knekt et al., 2004). Numerous epidemiologic studies suggest that vitamin C lowers the incidence of CVD and CVD mortality (Moser & Chun, 2016). The literature suggests that blood plasma levels of vitamin C are inversely correlated to CVD and CVD risk factors such as hypertension (Enstrom, Kanim, & Klein, 1992), (Block et al., 2008). Hypertensive subjects are found to have significantly lower levels of blood plasma vitamin C compared to normotensive subjects (Ness, Chee, & Elliott, 1997). The observed difference in blood pressure estimated from an increased 50 μmol/L difference in plasma ascorbic acid is -3.6 to -17.8 for systolic blood pressure and -2.6 to - 9.4 for diastolic blood pressure, respectively (Ness et al., 1997). Similarly, a depletion–repletion study on vitamin C found an inverse correlation of blood plasma vitamin C levels to blood pressure (Block et al., 2001). One month of vitamin C depletion was followed by 1-month repletion with 117 mg/day. Persons in the bottom fourth of the plasma ascorbic acid distribution had >7 mmHg higher diastolic blood pressure than did those in the top (Block et al., 2001). The antihypertensive effects of vitamin C are supported by multiple other studies. A meta-analysis including 29 clinical trials found that an average supplemental vitamin C dose of 500 mg per day reduced systolic blood pressure by 4.85 ± 1.21 mmHg and diastolic blood pressure by 1.67 ± 0.72 mmHg in hypertensive patients (Juraschek, Guallar, Appel, & Miller, 2012).

Vitamin C supplementation also appears to improve endothelial function. A systemic review of 44 clinical trials showed a significant positive effect of vitamin C on endothelial function, with stronger effects in those at higher CVD risk (Ashor, Lara, Mathers, & Siervo, 2014). Overall, current research suggests that vitamin C deficiency is associated with a higher risk of CVD and that vitamin C may improve endothelial function and blood pressure, especially in individuals with low plasma vitamin C levels.

POTASSIUM (POTASSIUM GLUCONTAE)

Clinical studies have shown that increasing potassium intake can reduce the risk of CVD. A meta-analysis that included 11 studies and 247,510 participants found that higher dietary potassium intake, through a combination of diet and supplementation, was associated with lower rates of stroke, coronary heart disease, and other CVD (D'Elia, Barba, Cappuccio, & Strazzullo, 2011). Observational studies find that higher dietary potassium intakes lower blood pressure in a dose-responsive manner in both hypertensive and non-hypertensive patients (Lanham-New, Lambert, & Frassetto, 2012). A cohort study with 12, 267 US adults found that those who consumed a diet with a higher potassium-to-sodium ratio had a significantly decreased risk of hypertension (Yang et al., 2011) and that the potassium-induced reductions in blood pressure lower the incidence of stroke, coronary heart disease, myocardial infarction, and other CVD (M. C. Houston, 2011). Specifically, a 1000mg/day higher potassium intake was associated with lower all-cause mortality risk of 0.80 (95% CI, 0.67-0.94) (Yang et al., 2011).

In summary, these findings indicate that higher potassium intake is associated with decreased risk of hypertension, CVD and all-cause mortality.

MAGNESIUM (MAGNESIUM ASCORBATE)

Magnesium serves as a cofactor in more than 300 enzymatic reactions in the human body, including those responsible for regulating blood pressure, glycaemic control, and lipid peroxidation. Low blood serum levels of magnesium have been associated with an increased risk for several chronic diseases including diabetes, hypertension, coronary heart disease, and osteoporosis (Kass et al., 2012). A greater intake of magnesium is associated with a lower coronary artery calcification score (Hruby et al., 2014) and improved endothelial function in patients with coronary artery disease (Shechter et al.,

2000). A meta-analysis including more than 400,000 adults from different cohorts found a 14% protection against the risk of CVD mortality in individuals with the highest dietary intake of magnesium (Fang et al., 2016). Magnesium also appears to play a role in regulating blood pressure. Clinical trials show an inverse relationship between dietary magnesium intake and blood pressure (Swaminathan, 2003). For example, a meta-analysis of 11 studies by Qu et al revealed an association between dietary magnesium intake and reduced CVD events (P=0.024) (Qu et al., 2013).

Although the amount of literature on magnesium and CVD risk is extensive, most of these studies are epidemiological studies. The number of intervention studies on magnesium and CVD risk is sparse in comparison. However, the intervention studies that have been done do show promising antihypertensive results for magnesium supplements. A meta-analysis that included 22 clinical trials found that daily magnesium supplementation at a mean dose of 410mg/day was associated with a decrease in systolic blood pressure of 3.4 ± 2 mmHg and diastolic blood pressure of 2.5 ± 1 mmHg (Kass et al., 2012). Another limitation of the intervention studies that have been done is that magnesium supplements are only studied in isolation, not in combination with other ingredients. Compliance can also be an issue, a study looking at atrial fibrillation prevention with oral magnesium supplementation reported only a 75% compliance of participants in the treatment group (Lutsey et al., 2018).

In summary, magnesium intake is associated with reduced risk of CVD, but more intervention studies are needed to clarify the role of magnesium supplementation on CVD risk and treatment.

COENZYME Q10 (UBIQUINOL)

Coenzyme Q10 (CoQ10) is an antioxidant and free radical scavenger that reduces oxidative stress, regenerates vitamins, reduces oxidation of LDL-cholesterol, and is involved in mitochondrial oxidative phosphorylation (Langsjoen & Langsjoen, 1999). A large number of clinical trials support the antihypertensive effects of CoQ10 supplements. A meta-analysis of three randomized, placebo-controlled, clinical trials found that daily supplementation of 100 mg of CoQ10 caused an average decrease in systolic blood pressure of 11 mmHg (95% CI 8, 14) and an average decrease in diastolic blood pressure of 7 mmHg (95% CI 5, 8) after 4 weeks of treatment (Ho, Bellusci, & Wright, 2009). Another meta-analysis of 12 CoQ10 clinical trials (362 patients) found that the average systolic blood pressure in the treatment group decreased from 167.7 mmHg to 151.1 mmHg ($P<0.001$) and that the average diastolic blood pressure decreased from 103.0 mmHg to 94.8 mmHg ($P<0.001$) (Rosenfeldt et al., 2007).

CoQ10 exists in the body in either its oxidized form, ubiquinone, or in its reduced form, ubiquinol. The oxidized form (ubiquinone) needs to be transformed into the reduced form (ubiquinol) to be used by the body. Research shows the intestinal absorption and efficacy of supplemental CoQ10 is vastly improved by using the ubiquinol form (Ankola, Viswanad, Bhardwaj, Ramarao, & Kumar, 2007). Results from a double-blind, randomized, crossover trial with two 2-week intervention phases and a 2-week washout between crossovers show that ubiquinol supplementation (200mg/day) significantly increased plasma CoQ10 from 1.3 to 3.4 µmol/L ($p < 0.05$), whereas the ubiquinone supplement did not significantly increased plasma CoQ10 levels (Zhang, Liu, Chen, & Oliver Chen, 2018).

In summary, CoQ10 supplementation is associated with antihypertensive effects, with the ubiquinol form showing the best bioavailability.

FOLATE (FOLIC ACID)

Folate regulates the metabolic process of lowering blood serum homocysteine levels, which prevents oxidative stress and atherosclerosis (Guthikonda & Haynes, 2006). A meta-analysis of 39,420 patients found an association between low dietary folate intake and an increased risk of stroke (Zeng, Xu, Xu, Wang, & Wang, 2015). Numerous epidemiological studies have reported an inverse correlation between dietary folate and blood plasma folate status with homocysteinemia, a known risk factor for CVD (Verhoef et al., 1996; Zeng et al., 2015). These correlations have also been observed in atherosclerotic patients and linked to the incidence of cardiovascular disorders (Pancharuniti et al., 1994; K. Robinson et al., 1995).

Several intervention studies have shown the benefits of folate supplementation on endothelial function. Folate supplementation improved flow-mediated endothelium-dependent vasodilation in hyperhomocysteinemia and coronary atherosclerosis patients (Bellamy et al., 1999) and improved serotonin-induced endothelium-dependent vasodilation in hypercholesterolemia patients (Chambers et al., 2000). In the study done by Bellamy et al, folate supplementation (5mg/day for six weeks) improved endothelium-dependent flow-mediated glyceryl trinitrate-mediated brachial artery dilatation (P=0.015) and reduced homocysteine (8.7±2.5 vs. 12.1±3.6 µmol/L) (Bellamy et al., 1999). Another study using a MIS of folate, vitamin B6, and vitamin B12 saw reduced progression of carotid plaque in premature atherosclerosis and hyperhomocysteinemia patients (Peterson & Spence, 1998). 38 participants (18 men and 20 women, mean age 57.9 ± 11. 7) were given 2.5mg folic acid, 25mg vitamin B6, and 250 µg vitamin B12 daily. The rate of carotid plaque progression before MIS treatment was 0.31 ± 0.39 cm^2/year; after MIS treatment it was -0.05 ± 0.25 cm^2/year (P=0·0002) (Peterson & Spence, 1998).

In summary, these studies show reduced homocysteine and improved endothelial function from folate supplementation, suggesting a role for folate supplementation in the prevention and treatment of CVD.

SELENIUM (SELENOMETHIONINE)

The antioxidant activity of selenium has to been shown to decrease lipid peroxidation and help prevent CVD and other chronic diseases (Vural, Kabaca, Firat, & Degirmencioglu, 2017). A meta-analysis including 25 observational studies found that the blood concentrations of selenium were inversely associated with coronary heart disease risk (Flores-Mateo, Navas-Acien, Pastor-Barriuso, & Guallar, 2006). A 50% increase in selenium intake was associated with a 24% reduction in risk for developing coronary heart disease. The authors based these findings on the fact that low selenium levels increase platelet aggregability and vasoconstriction, which increases the risk of atherosclerosis. Selenium may also prevent metal-induced oxidative damage from toxic metals that have been implicated in atherogenesis, such as mercury, cadmium and arsenic, by forming inactive complexes with the metals (Nayab Batool Rizvi, 2014).

A randomized, placebo-controlled study that gave daily selenium supplements containing 100, 200, or 300 mcg or a placebo to 474 healthy adults aged 60 to 74 years found that all groups who received the selenium supplements had lowered levels of total cholesterol and LDL-cholesterol levels (Rayman, Stranges, Griffin, Pastor-Barriuso, & Guallar, 2011). The adjusted change in total cholesterol difference compared with the placebo group was -0.22 mmol/L (95% CI, -0.42 to -0.03 mmol/L, P = 0.02) for 100 mcg of selenium per day, -0.25 mmol/L (95% CI, -0.44 to -0.07 mmol/L, P = 0.008) for 200 mcg of selenium per day, and -0.07 mmol/L (95%CI, -0.26 to 0.12 mmol/L, P = 0.46) for 300 mcg of selenium per day. The 300 mcg/day group also had significantly increased HDL-cholesterol levels (P=0.01) (Rayman et al., 2011).

In summary, selenium intake is inversely associated with coronary heart disease risk, and an intervention study done by Raymen et al suggests that selenium supplementation improves plasma lipid profile in healthy adults aged 60 to 74 years.

VITAMIN D (CHOLECALCIFEROL)

Vitamin D has received attention for its potential role in preventing CVD. Vitamin D receptors are located on arterial cell walls and Vitamin D metabolites regulate cardiovascular function by regulating inflammation, thrombosis, and the renin–angiotensin-aldosterone system (Gouni-Berthold, Krone, & Berthold, 2009).

Epidemiological studies show an inverse relationship between blood serum vitamin D concentrations and CVD risk, as well as the prevalence of established cardiovascular risk factors such as hypertension, diabetes, obesity, glucose intolerance, and hypertriglyceridemia (Holick, 2007; Judd, Nanes, Ziegler, Wilson, & Tangpricha, 2008). Observational studies show the prevalence of chronic heart failure is greater in cohorts with low vitamin D status (Zittermann et al., 2003). Vitamin D deficiency may increase CVD risk by causing the development of hypertension and a disrupted immune system that promotes inflammation, vascular dysfunction, and insulin resistance (Kempker, Tangpricha, Ziegler, & Martin, 2012; Motiwala & Wang, 2012). The NHANES III study measured serum blood vitamin D in approximately 13,000 US adults and found vitamin D status was inversely associated with established CVD risk factors such as hypertension (Scragg, Sowers, & Bell, 2007). Systolic BP was 3.0 ± 0.7 mmHg lower (P = 0.0004) and diastolic BP was 1.6 ± 0.6 mmHg lower (P = 0.011) for participants in the highest quintile of vitamin D status compared with the lowest quintile (Scragg et al., 2007).

Intervention studies support these findings. High dose vitamin D supplements improved endothelial function in patients with a history of stroke (Witham, Dove, Sugden, Doney, & Struthers, 2012). Flow-mediated dilatation was significantly higher in the Vitamin D treatment group at 8 weeks (6.9% vs 3.7%, P = 0.007) (Witham et al., 2012). Vitamin D supplementation (2800 IU/day for 12 weeks) increased HDL-cholesterol in 81 healthy postmenopausal women with secondary hyperparathyroidism (Bislev et al., 2018). Vitamin D supplementation of 1,333IU for 3 months lowered C-reactive protein levels, induced relaxation of vascular smooth muscle cells, and decreased rennin production by the kidneys in healthy adults (Timms et al., 2002).

In summary, epidemiological studies show an inverse relationship between blood serum vitamin D concentrations and CVD risk. Intervention studies suggest vitamin D supplementation improves endothelial function, increases HDL-cholesterol, and lower CRP. The results of these studies suggest that vitamin D supplementation may lower CVD risk.

VITAMIN E (D-ALPHA TOCOPHERYL ACETATE)

Vitamin E is an antioxidant known to play a vital role in fertility, neurologic function, and disease prevention including atherosclerosis, fatty liver disease, cancer, and neurodegenerative diseases (Sugamura & Keaney, 2011). An inverse association between vitamin E intake and CVD risk has been demonstrated in several observational studies (Rimm et al., 1993; Stampfer et al., 1993). In a study done by Stephens et al, treatment with vitamin E reduced the risk of myocardial infarction in patients with coronary atherosclerosis (Stephens et al., 1996). In this double-blind, placebo-controlled study vitamin E treatment (800IU daily for first 546 patients; 400 IU daily for remaining 489) significantly reduced the risk of cardiovascular death and non-fatal myocardial infarction

(41 vs 64 events; relative risk 0.53 [95% Cl, 0.34-0.83]; P=0.005) in patients with angiographically proven coronary atherosclerosis (Stephens et al., 1996).

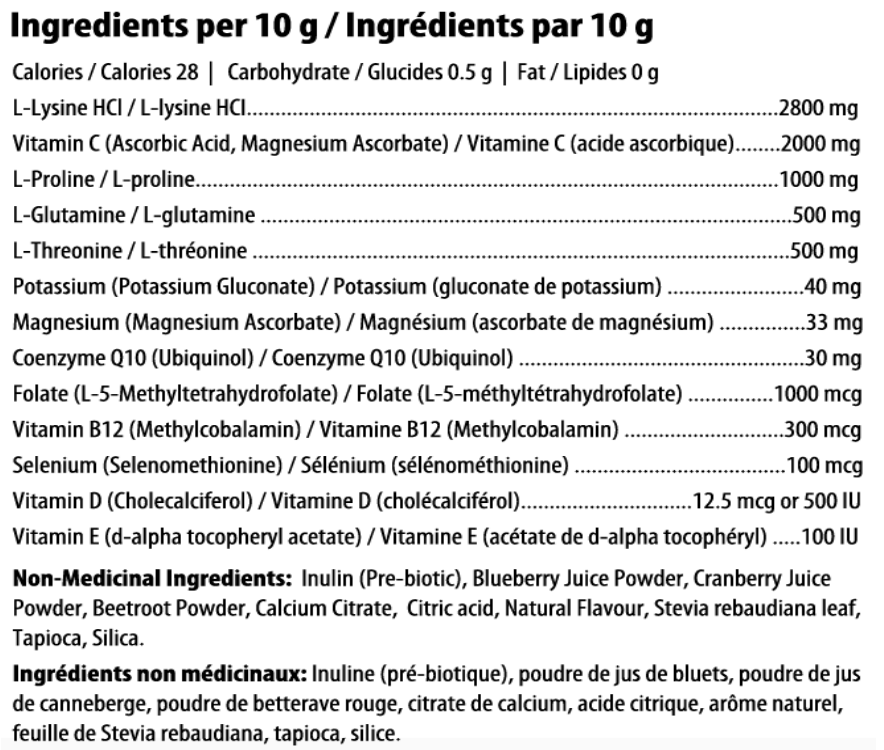
A meta-analysis that included 39,910 healthy US males found that men who consumed more than 60 IU of vitamin E daily, through a combination of diet and supplementation, had a significantly lower risk of coronary heart disease (P=0.003) (Rimm et al., 1993). The relative risk for men consuming more than 60 IU per day of vitamin E was 0.64 (%95CL, 0.49 to 0.83) as compared with those consuming less than 7.5 IU per day (Rimm et al., 1993). Similarly, a meta-analysis done with 87,245 healthy US females found that the women who consumed vitamin E supplements continuously for more than 2 years had a significantly lower risk of coronary heart disease (P<0.001) (Stampfer et al., 1993). After adjustment for age and smoking status, the relative risk for those in the highest fifth quintile of intake was 0.66 (95% CL, 0.50 to 0.87), as compared with those in the lowest fifth (Stampfer et al., 1993).
Altogether, these studies show that vitamin E intake is associated with reduced risk of coronary heart disease and suggest that vitamin E supplementation reduces the risk of cardiovascular death in patients with coronary atherosclerosis.

CARDIOFLEX

Amino acids, vitamins, minerals, and CoQ10 were all identified as nutraceuticals that may be beneficial in preventing and treating CVD. Evidence shows that amino acids have beneficial effects on the cardiovascular system by increasing nitric oxide levels in the blood, vitamins with antioxidant properties can improve endothelial function and reduce homocysteine, minerals may lower coronary artery calcification and CoQ10 supplementation is shown to have antihypertensive effects. Cardioflex Q10 is a cardiovascular health MIS designed to maintain and support cardiovascular health. As shown in Figure 1, Cardioflex contains 13 main ingredients, including 4,700 mg of amino

acids (L-lysine, L-proline, L-glutamine, L-threonine), vitamins (vitamins A, C, E, folate, vitamin B12), minerals (potassium, magnesium, selenium) and CoQ10. Cardioflex has been sold in the commercial market for 16 years, and its increasing year-over-sales performance suggests continued consumer interest and market growth (InnotechNutrition.com).

Based on the review of the individual nutrients discussed above, each of the individual ingredients in Cardioflex has shown positive results in improving health and reducing CVD risk factors. Many of the nutrients used in Cardioflex act on different physiological mechanisms, which may have a synergistic effect on the overall efficacy of the supplement. For example, Yerum et al confirmed the synergistic effect of the combination of vitamin C and vitamin E against the oxidation of delipidized human serum combined with phosphatidylcholine liposomes in an in vitro biological model system (Yeum, Beretta, Krinsky, Russell, & Aldini, 2009). However, these results have never been validated with an intervention study. The preliminary research and growing market for dietary supplements give justification to perform more ad hoc studies to clarify the exact role of MIS nutrient supplements in CVD risk and treatment.



Ingredients per 10 g / Ingrédients par 10 g

Calories / Calories 28 | Carbohydrate / Glucides 0.5 g | Fat / Lipides 0 g

L-Lysine HCl / L-lysine HCl.....2800 mg
Vitamin C (Ascorbic Acid, Magnesium Ascorbate) / Vitamine C (acide ascorbique).....2000 mg
L-Proline / L-proline.....1000 mg
L-Glutamine / L-glutamine .....500 mg
L-Threonine / L-thréonine .....500 mg
Potassium (Potassium Gluconate) / Potassium (gluconate de potassium) .....40 mg
Magnesium (Magnesium Ascorbate) / Magnésium (ascorbate de magnésium) .....33 mg
Coenzyme Q10 (Ubiquinol) / Coenzyme Q10 (Ubiquinol) .....30 mg
Folate (L-5-Methyltetrahydrofolate) / Folate (L-5-méthyltétrahydrofolate) .....1000 mcg
Vitamin B12 (Methylcobalamin) / Vitamine B12 (Methylcobalamin) .....300 mcg
Selenium (Selenomethionine) / Sélénium (sélénométhionine) .....100 mcg
Vitamin D (Cholecalciferol) / Vitamine D (cholécalciférol).....12.5 mcg or 500 IU
Vitamin E (d-alpha tocopheryl acetate) / Vitamine E (acétate de d-alpha tocophéryl) .....100 IU

Non-Medicinal Ingredients: Inulin (Pre-biotic), Blueberry Juice Powder, Cranberry Juice Powder, Beetroot Powder, Calcium Citrate, Citric acid, Natural Flavour, Stevia rebaudiana leaf, Tapioca, Silica.

Ingrédients non médicinaux: Inuline (pré-biotique), poudre de jus de bluets, poudre de jus de canneberge, poudre de betterave rouge, citrate de calcium, acide citrique, arôme naturel, feuille de Stevia rebaudiana, tapioca, silice.

Figure 1 – Cardioflex supplement facts panel

CONCLUSION

In this literature review, we reviewed what is known about CVD and the potential role of dietary supplements to reduce CVD risk. The prevalence of supplement use has increased dramatically over the past 20 years (Elizabeth D Kantor, 2016). The North America dietary supplements market size was estimated at USD 37.37 billion in 2016 and is expected to reach USD 68.22 billion by 2025 (Grand View Research, 2017). Rising concerns regarding CVD and other chronic diseases are expected to increase consumer spending on dietary supplements. Supplements are purchased without medical prescriptions, causing consumers to look to manufacturers and retailers of supplements for health information; as a result, the information can be skewed toward commercial interests (Stephanie Y. Crawford, 2005).

The effects of nutrient intake on CVD and CVD risk factors have been studied for a long time. Although strong associations have been observed, epidemiological studies do not provide clear confirmation in the efficacy of nutrient supplements in the prevention of CVD. Demonstrating efficacy requires replicable clinical studies with well-defined products and experimental designs. In addition, combining multiple nutrients into MIS may cause the formula to have synergistic or antagonistic properties.

Given the potential benefits of nutrient supplements and the growing market for dietary supplements, further investigations are warranted and needed. Not only to determine the efficiency of MIS, but also the safety, since anecdotally we know that people consume these products consistently for many years.

Chapter 2: Study Rationale, Objective, and Hypothesis

RATIONALE

CVD is the leading cause of death worldwide (Mc Namara, Alzubaidi, & Jackson, 2019). CVD accounted for 24% of all deaths in the United States in 2014 (Benjamin et al., 2019) and 19% of all deaths in Canada in 2018 (Canada, 2020b). The prevailing view by cardiovascular researchers is that most cases of CVD can be minimized and/or prevented by good nutrition, choosing not to smoke, regular physical activity, a healthy body weight, stress reduction, and limited alcohol consumption. Despite these recommendations, adherence to government health guidelines is low and a lot of patients at high risk for developing CVD do not make the lifestyle changes recommended. Given the prevalence of CVD worldwide, it is critical to explore new strategies aimed at reducing CVD prevalence and mortality.

Dietary supplements may be a way to reduce CVD prevalence and risk. The sparse and inconsistent epidemiologic data relating dietary supplement use to the risk of CVD can be partially explained by the differences in the supplement given, genotypic differences in individuals, and baseline intake of nutrients. For example, a case-control study in Sweden, where fruits and vegetable consumptions are low, showed an inverse association between intake of multi-vitamin-mineral supplement and the incidence of myocardial infarction (Holmquist et al., 2003). Generally speaking, study participants who report taking dietary supplements generally have a healthier lifestyle; they report eating more nutritious food, less likely to smoke or drink alcohol, and exercise more (Chen et al., 2019). It is plausible to hypothesize that individuals who aren't following government health guidelines will benefit more from dietary supplements more than individuals following healthier lifestyles.

In particular, antioxidant supplements are a promising area of research in the prevention and treatment of CVD. Observational data have identified associations between intake of carotenoids, folic acid, and vitamin E and CVD risk (Lichtenstein, 2009). Research shows that antioxidants have the ability to inhibit the oxidation of cholesterol and improve endothelial function (Malekmohammad, Sewell, & Rafieian-Kopaei, 2019). High antioxidant intake has been shown to prevent atherosclerosis (Nunez-Cordoba & Martinez-Gonzalez, 2011).

Despite biological plausibility about the effects of antioxidants to reduce CVD risks as suggested with some observational studies, data derived from most nutrient supplement trials have been disappointing (Monsen, 2000). The discrepancies between observational and interventional data may be due to confounding diet and lifestyle patterns and unaccounted for genotypic variations. It may also be that specific nutrients need to be

given together to translate into significant changes. Specific nutrients working in concert may produce a health benefit greater than the sum of the individual parts. For example, human studies have shown convincingly the dose-dependent enhancing effects of ascorbic acid (vitamin C) on iron absorption (Lynch & Cook, 1980). Whether it is because they enhance each other's absorption or because they have more potent physiological effects when taken together, pairing nutrients that have a synergistic effect may lead to more favourable outcomes. It may be that combinations of nutrient supplements need to be given together to translate into significant changes.

Although direct comparisons between multi-ingredient supplements and single ingredients have never been done, a substantial body of evidence suggests that multi-ingredient formulations greatly increase the efficacy of the product (Harty et al., 2018). For example, there is research to suggest that multi-ingredient supplements can improve lipid blood profiles in as little as 30 days (Hobbs et al., 2014). However, the body of literature evaluating dietary supplements to reduce CVD prevalence and prognosis is minimal. The view by most health care practitioners and government agencies is that currently there is insufficient data to recommend the routine use of nutrient supplements to prevent or treat CVD. It is only in the last decade that dietary supplement databases with open access for the public have existed in the USA (Coates, 2016). Although a great deal of progress has been made in recent years, much remains to be done to ensure that dietary supplements are safe, efficacious, and reasonable in cost so that they contribute positively to the public's health. Given the potential benefit of these products, more research needs to be done.

Cardioflex is a multi-ingredient supplement comprised of amino acids (L-lysine, L-proline, L-glutamine, L-threonine), vitamins (vitamins A, C, E, folate, vitamin B12), minerals (potassium, magnesium, selenium) and CoQ10. The individual ingredients in Cardioflex

have shown or been hypothesized to show, positive results in reducing CVD and CVD risk factors. For example, the literature demonstrates that amino acids can play a significant role in cardiovascular health. L-threonine supplementation has been shown to lower blood pressure in patients with CVD (Tuttle et al., 2012). High antioxidant intake has been shown to prevent atherosclerosis (Nunez-Cordoba & Martinez-Gonzalez, 2011). Low blood serum levels of magnesium have been associated with an increased risk for several chronic diseases including diabetes, hypertension, coronary heart disease, and osteoporosis (Kass et al., 2012). Multiple randomized, placebo-controlled, clinical trials show that daily supplementation of CoQ10 can cause significant antihypertensive effects in as little as 4 weeks of treatment (Ho et al., 2009).

This clinical trial will help clarify the role of multi-ingredient supplements containing amino acids, vitamins, and minerals in the prevention and management of CVD and CVD risk factors.

RESEARCH OBJECTIVES

The overall objective of this study is to determine whether daily supplementation with the dietary supplement Cardioflex for 90 days will reduce risk factor biomarkers for CVD in adults aged 30–65 years old.

The primary objective is to determine whether daily supplementation with the dietary supplement Cardioflex will improve their plasma lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A).

The secondary objectives are to determine if Cardioflex supplementation will improve anthropometric measures (waist circumference, body mass index), cardiac measures (heart rate, heart rate variability, accelerated plethysmography (APG age, which measures

the biological age of arteries), blood pressure), and inflammatory and endothelial function biomarkers (C-reactive protein, IL-6, sICAM-1, sVCAM-1,) and assess the safety of the supplement by measuring serum levels of liver and kidney enzymes (alanine transaminase; ALT, aspartate transaminase; AST, lactate dehydrogenase; LHD, blood urea nitrogen; BUN, and creatinine). To quantify confounding variables, participants were asked to record everything they ate and drank in a food log and wore a pedometer to track total steps on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study.

HYPOTHESES

- Participants who consume one serving (10 g) of Cardioflex per day, the label recommended dosage, will have improved lipid profile, as reflected by lower levels of total cholesterol, LDL-cholesterol, triglycerides, and lipoprotein A, and higher HDL-cholesterol than participants who consumed the placebo.
- Participants who consume one serving of Cardioflex per day will have improved cardiac health, as measured by lower blood pressure, higher HRV, lower APG age, and lower levels of inflammation biomarkers (C-reactive protein, IL-6, sICAM-1, and sVCAM-1) than participants who consumed the placebo.
- No significant changes will be seen in anthropometric measures (waist circumference, body mass index) or liver and kidney functions biomarkers (ALT, AST, LHD, BUN or creatinine) between the Cardioflex and the placebo group.

CHAPTER 3: MATERIALS AND METHODS

STUDY DESIGN

This was a randomized, double-blind, placebo-controlled, parallel design study where participants were assigned to receive one serving (10g) of Cardioflex or 10g of a

maltodextrin placebo daily for 90 days and independent of any other diet or exercise interventions. The study was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), located at the University of Manitoba Ft. Garry Campus. The study was registered on clinicaltrial.gov with ID number NCT03826914 and approved by the University of Manitoba Joint-Faculty Research Ethics Board (JFREB) with protocol number J2018:010 (HS21609).

The current lack of accepted guideline recommendations for nutrient supplements is explained by the lack of clinical evidence showing nutrient therapy's effectiveness and safety. Hence, a placebo-controlled randomized clinical trial is warranted. Randomly assigning the intervention eliminates the influence of unknown confounding variables or bias that could lead to an incorrect measurement of treatment effect. Randomization eliminates confounding baseline variables, thus eliminating the possibility that the observed effects are because one treatment group had a more advantageous starting point. The placebo control allows participants, investigators, and study staff to be blinded. The advantage of a placebo-controlled trial over an observational study is the ability to demonstrate causality.

Participants were given the treatments as individual stick packs so there was no variance in the daily dosage consumed. Participants were told to mix the supplement with water and consume it before eating breakfast daily. The group supplementing with Cardioflex ingested one serving (10g) of Cardioflex daily, whereas the placebo group ingested 10g of maltodextrin placebo. We chose this as our placebo supplement because it was likely to have minimal effect on CVD health biomarkers. A log was given to the participants to record their supplement intake. Compliance with the supplement protocol was assessed by measuring blood levels of CoQ10 and providing participants with more treatment packages than the total number of days of the study and asking the participants to return

any unused stick packs to the supplement coordinator at the end of the study. We chose blood levels of CoQ10 as our main indicator of compliance because it is not readily found in food and previous research found a significant increase in plasma CoQ10 concentrations when participants were given Coq10 supplements daily (Niklowitz, Sonnenschein, Janetzky, Andler, & Menke, 2007). Thus, it is anticipated that a participant's CoQ10 blood values should increase with Cardioflex supplementation and would indicate compliance with taking the supplement. In addition, participants were given a randomized number of stick packs between 93 and 100, giving every participant at least a few stick packs to return at the end of the clinical trial. Participants were not told the number of stick packs they were given.

Participants were instructed to follow the same diet and maintain the same level of physical activity throughout the clinical trial. To access dietary intake and physical activity, participants were asked to record everything they ate and drank in a food log and wore a pedometer to track total steps on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study. A 90-day study length was chosen because previously published data saw significant changes in triglycerides, LDL-cholesterol, HDL-cholesterol, and total cholesterol when participants were given a multi-ingredient supplement for 90 days (Lombardo et al., 2013). A sample size calculation was completed using previously published data (Hobbs et al., 2014) to ensure enough participants were recruited. We calculated that we would need 60 subjects to reach statistical significance. With an expected attrition rate of 20%, we determined that 72 participants needed to be recruited.

To determine the effects of Cardioflex on CVD risk, the dependent variables tested included anthropometrics (weight, height, waist circumference), cardiovascular parameters (blood pressure, heart rate, HRV, APG age), dietary intake, plasma lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A), and

inflammatory and endothelial function biomarkers (C-reactive protein, IL-6, sICAM-1, sVCAM-1). Anthropometrics were included because large scale epidemiological studies have shown correlations between CVD mortality and BMI (Valavanis et al., 2010) and increased waist circumference is recognized as a major cardiometabolic risk factor (Tchernof & Despres, 2013). Cardiovascular parameters carry valuable information for the prediction of long-term and near-term CVD risk. Blood pressure is a consistent risk factor for the development of atherosclerosis (Lu, Cassis, & Daugherty, 2007). A higher resting heart rate is linked with greater CVD risk (Fox et al., 2007). Reduced HRV is associated with an increased risk for coronary heart disease and CVD mortality (Liao et al., 1997). Arterial elasticity is an established risk factor to predict future CVD events (Glasser et al., 1997). Lipid profile is recognized as an established risk factor in the development and progression of CVD (Graham, Cooney, Bradley, Dudina, & Reiner, 2012; Jellinger et al., 2012). Inflammation and endothelial function play a significant role in developing atherosclerosis, and risk of future cardiovascular events (Jarvisalo et al., 2006).

PARTICIPANTS

Recruitment of study participants employed several strategies including email, flyers, and in-person presentations. The study eligibility criteria were adult men and women between the age of 30-65 years old, perform less than 150 minutes of moderate or vigorous physical activity per week, have not used prescription cholesterol or blood pressure medication in the last 3 months, be willing to stop taking any dietary supplements during the study period of 90 days, not pregnant nor planning on getting pregnant during the study, and an APG type of D, E, F or G and/or an HDL-cholesterol to total cholesterol ratio of $\leq$24 percent.

Participants were stratified (male/female) and randomized (block size = 5) to the study treatments using a computer-generated randomization (www.randomizer.org) numbering system.

SAMPLE SIZE

A sample size calculation was completed using a standard sample size in equation 1 to determine the number of participants needed for the study. With α = .05 and β = .90 and a 12% mean decrease of cholesterol using previously published data (Hobbs et al., 2014) in the supplementation group, we calculated that we would need 60 subjects to reach statistical significance. With an expected attrition rate of 20%, we determined that 72 participants needed to be recruited.

$$n = \frac{2\left(Z_{\alpha} + Z_{1-\beta}\right)^2 \sigma^2}{\Delta^2}$$

Equation 1 - Estimated sample size equation

$Z_{\alpha} = contasnt\ t\ othe\ accepted\ error, Z_{1-\beta} = constant\ according\ to\ power,$

$\sigma = standard\ deviation,\ \Delta =$ effect size

Overall, 90 adults were screened for eligibility, with 75 adults being deemed eligible. At the time of starting the study 69 adults chose to participant, and 6 eligible adults chose not to participate for personal reasons not related to the study.

STUDY PROCEDURES AND ASSESSMENTS

BLOOD COLLECTION

Blood was drawn at 3 time points in the study – the assessment for eligibility for entry into the study, baseline, and end of the 90-day supplement period. All blood samples

were collected in the morning after at least 8 hours overnight fast. The participants reported to the RCFFN at the University of Manitoba Ft. Garry Campus. Blood samples were taken by venipuncture from an antecubital vein into serum tubes by a certified phlebotomist. For eligibility assessment, one 5mL serum tube was withdrawn. For clinical trial baseline and endpoint measurements, two 10mL serum tubes, and one 5mL serum tube, totaling 25mL of blood was withdrawn from each participant. Samples were centrifuged at 4100g for 10 minutes. The plasma was allocated into Eppendorf tubes and frozen at -86 ° C for later analysis.

Cardiac Measurements

Participants were required to sit for a minimum of 5 minutes rest at the beginning of each assessment session. Blood pressure was measured at the brachial artery of the left arm, using an automatic sphygmomanometer (Series 10, Omron). The blood pressure of each participant was taken three times, and the average was recorded. Accelerated plethysmograph (APG) type, HR, and HRV were assessed using a Meridian digital pulse-wave analyzer (DPA) machine (Long Life Cardio LLC, USA). HRV was measured using the standard deviation of all of the RR intervals (the distance between each heartbeat) after 5 minutes of recording.

ANTHROPOMETRICS

All anthropometric measurements were collected with minimal clothing, without shoes and all metals removed from the body. Height in cm was measured using a standard stadiometer, body mass was measured using a digital scale (7562EF, Taylor Precision Products, USA). BMI calculation was performed by dividing weight in kg by height in meters squared (kg/m^2). Waist circumference was measured at the level of the iliac crest with the participants in a standing position.

Dietary Intake Records and Analysis

Detailed dietary data and total steps were collected from the participants on 3 occasions; that is, data was collected on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study using a 1-day food record method to determine participants' intake of all food, and drink, and a pedometer to track daily total steps. Participants recorded all details of food and drink type, method of preparation, and estimated portions. A 1-day food record was chosen to minimize respondent burden. Food and activity records were collected from participants during the final assessment session.

Basal metabolic rate (BMR) was calculated by Equation 2 for input into Goldberg's energy cut-off equation. The Goldberg's energy cut-off equation (Black, 2000) with a perceived activity level of 1.5 and 95% confidence interval was used to remove outliers who provided diet records of poor validity. Three outliers were removed from the dietary analysis; one in the Cardioflex group and two in the placebo group.

Men: BMR = 88.362 + (13.397 x weight in kg) + (4.799 x height in cm) - (5.677 x age in years)

Women: BMR = 447.593 + (9.247 x weight in kg) + (3.098 x height in cm) - (4.330 x age in years)

Equation 2 – Basal metabolic rate calculation*
*Information adapted from (Harris & Benedict, 1918)

Dietary records were analysed for differences in energy intake, protein, carbohydrate, fat, and micronutrients among treatments. Dietary analysis was completed by inputting all food record data into Food Processor Nutrition Analysis Software V11.7 (ESHA Research, USA).

BLOOD ANALYSIS

Serum concentrations of total cholesterol, triglycerides, HDL-cholesterol, BUN, creatinine, AST, ALT, and LDH were measured using automated enzymatic methods on a Vitros-350 chemistry analyzer. LDL-cholesterol levels were estimated using the Friedewald formula: LDL-cholesterol = Total cholesterol - HDL-cholesterol - triglycerides/2.2 (Rifai N, 2006).

CRP, IL-6, SiCAM, and SvCAM were measured using Meso Scale Discovery V-PLEX assays (MSD, Rockville, Md., USA). 25 μL of sample, calibrator, or control was added to each well of a 96-well plate. The calibrator and control were provided by Meso Scale Discovery. The plate was sealed and incubated at room temperature for 2 hours on a plate shaker set to 400 rpm. Next, 25 μL of detection antibody was added to each well. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm. Finally, 150 μL of read buffer was added to each plate and the plates were read on the Meso Scale Discovery QuikPlex SQ 120 instrument. All blood analysis was done in duplicates, with the average of the two values being recorded.

Lipoprotein A was measured using ELISA assay (Human lipoprotein A No.ab212165, Abcam Trading Company, Ltd, China). 50 μL of sample or standard and 50 μL of antibody were added to each well of a 96-well plate. The plate was incubated at room temperature for 1 hour on a plate shaker set to 400 rpm. Next, 100 µL of TMB substrate was added to each well, followed by incubating the plate for 10 minutes on a plate shaker set to 400 rpm. Finally, 100 µL of stop solution was added to each well and the plate was read with a microplate reader set to 450 nm (autoreader EL311; Bio-tek instruments). All blood analysis was done in duplicates, with the average of the two values being recorded.

Coenyzme Q10 was measured using ELISA assay (Human CoQ10 No. CSB- E14081h, Cusabio® Biotech Co., Ltd, China). 50µl of sample or standard and 50 of HRP-conjugate

was added to each well of a 96 well plate. The plate incubated for 40 minutes at 37°C. Next, 90 µl of TMB substrate was added to each well. The plate incubated for 20 minutes at 37°C. Finally, 50µl of stop solution was added to each well, and the plate was read with a microplate reader set to 450 nm (autoreader EL311; Bio-tek instruments). The blood analysis was done in duplicates, with the average of the two values being recorded.

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics (v.25). Significance was determined using the treatment effect between groups. The significance value was set at $p \leq 0.05$. An independent t-test showed that there were no statistical differences between the starting points of the two groups ($P>0.05$), indicating that the two groups were indistinguishable from each other.

The primary analysis was conducted using the post-treatment biomarker value, comparing the two groups, and controlling for their baseline values by using analysis of covariance. Analysis of covariance examines the differences in the mean values of the dependent variable that is related to the effect of the controlled independent variable while taking into account the influence of the uncontrolled variables. In this study, the dependent variable was the biomarkers being tested, the controlled independent variable was the treatment given and the uncontrolled variables were the baseline differences between participants. Analysis of covariance makes the assumption that the data is normally distributed in equal variances. To check the assumption of normal distribution, a Shapiro–Wilk test was performed. To check the assumption of equal variance, a Levene's test was performed.

Results are given as mean $\pm$ standard deviation (SD). Outliers were determined as values greater or less than the mean $\pm$ 3*SD (Jones, 2019). If outliers were present, they were

removed, and the results were recalculated. For each parameter, the percent change was calculated by equation 3.

$$\%\ Change = \frac{Final\ Value - Starting\ Value}{Starting\ Value}\ x\ 100$$

Equation 3: Percent change calculation

CHAPTER 4: RESULTS

PARTICIPANT DEMOGRAPHICS

Overall, as shown in Figure 2, 67 participants completed the study. Two participants (one in the Cardioflex treatment and one in the placebo treatment) did not complete the study due to personal reasons not related to the clinical trial.

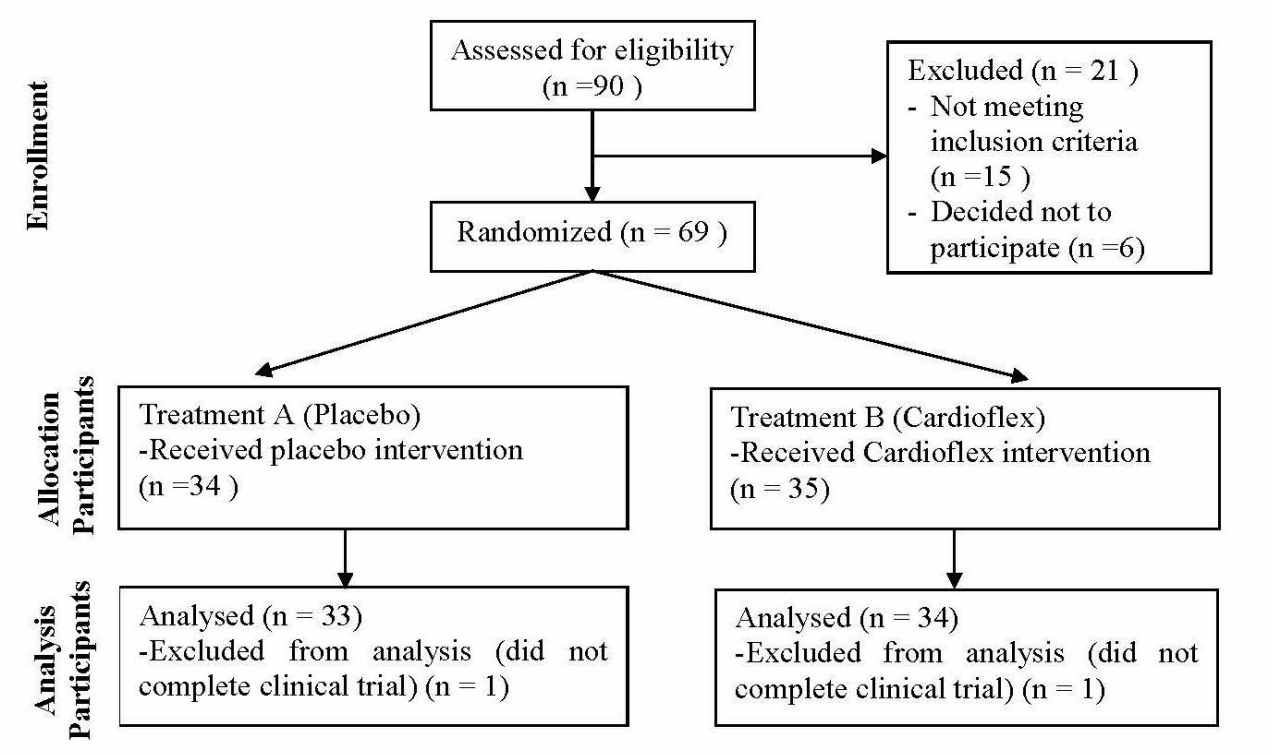


Figure 2 - Participant flow diagram

Table 3 summarizes the endpoint demographics of the 67 participants who completed the clinical trial. The mean age of the total population was 48.3 $\pm$ 8.8 years, and BMI was 30.1 $\pm$ 5.9 kg/m^2. There were no significant differences between groups using an independent t-test (P>0.05). All participants completed their assigned dosages. No adverse events were reported, although a few participants complained of the supplement tasting too sweet. The majority (>50%) of participants wrote in their food log that they were highly satisfied with the supplements and many stated that they would recommend the supplement.

Table 3 - Demographics of participants who completed the study

	Sex	Age (years)	BMI (kg/m$^{2)}$	Waist Men (cm)	Waist Female (cm)
Placebo	18 M, 15F	48.9 $\pm$ 8.5	30.2 $\pm$ 5.4	98.4 $\pm$ 13.3	96.5 $\pm$ 17.5
Cardioflex	22 M, 12F	47.7 $\pm$ 9.2	29.9 $\pm$ 4.7	100.6 $\pm$ 12.9	96.9 $\pm$ 13.0
Overall	40 M, 27F	48.3 $\pm$ 8.8	30.1 $\pm$ 5.9	99.5 $\pm$ 13.7	96.7 $\pm$ 14.0

Values expressed as means ± SD.
BMI = Body Mass Index; M= male; F= female.

Body Mass Index & Waist Circumference

Table 4 shows that Cardioflex had no impact on BMI or waist circumference during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study period, BMI remained stable for the Cardioflex group (+0.2%) while a small, non-significant decrease (-1.9%) was seen for the placebo group.

Table 4 – Body mass index and waist circumference of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)	Normal	High Risk
Start BMI (kg/m^2)	30.77 ± 5.65	29.84 ± 4.23			18.5 to 24.9	> 30
Final BMI (kg/m^2)	30.18 ± 5.35	29.90 ± 4.73				
Percent Change	-1.9%	+0.2%				
Difference Between Treatments			0.272 (-2.23, 2.78)	-0.606 (-1.53, 0.31)		
Significance			P=0.829	P=0.192		
Start Waist Men (cm)	103.00 ± 13.43	106.57 ± 13.51			< 94	> 102
Final Waist Men (cm)	98.39 ± 13.33	100.57 ± 12.85				
Percent Change	-4.48%	-5.63%				
Difference Between Treatments			-2.172 (-10.55, 6.21)	1.144 (-1.35, 3.64)		
Significance			P=0.603	P=0.359		
Start Waist Female (cm)	101.00 ± 17.25	98.62 ± 11.01			<80	>89
Final Waist Female (cm)	96.46 ± 17.54	96.88 ± 13.00				
Percent Change	-4.50%	-1.76%				
Difference Between Treatments			-0.420 (-12.74, 11.90)	-2.780 (-7.43, 1.87)		
Significance			P=0.945	P=0.229		

Values are expressed as means ± SD. Adjusted and unadjusted post-treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data was measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for

90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups endpoints, and controlling for their baseline values.
BMI = body mass index

CARDIOVASCULAR PARAMETERS

BLOOD PRESSURE

Table 5 shows that Cardioflex had no impact on blood pressure during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study period, systolic and diastolic blood pressure remained stable for the Cardioflex group (-1.1%, -0.3%) while a small, non-significant decrease (-3.2%, -2.2%) was seen for the placebo group.

Table 5 - Blood pressure of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)	Normal	High Risk
Start Systolic BP (mmHg)	127.54± 15.14	128.62 ± 19.01			<120	>140
Final Systolic BP (mmHg)	123.45 ± 14.29	127.18 ± 16.16				
Percent Change	-3.2%	-1.1%				
Difference Between Treatments			-3.722 (-11.17, 3.73)	-3.005 (-7.94, 1.93)		
Significance			P=0.322	P=0.229		
Start Diastolic BP (mmHg)	85.39 ± 9.91	84.12 ± 13.36			<80	>90

Final Diastolic BP (mmHg)	83.55 ± 9.07	83.88 ± 8.68				
Percent Change	-2.2%	-0.3%				
Difference Between Treatments			-0.337 (-4.67, 4.00)	-1.036 (-4.03, 1.96)		
Significance			P=0.877	P=0.492		

Values are expressed as means ± SD. Adjusted and unadjusted post-treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The dietary data were measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at the analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
BP = blood pressure

HEART RATE AND HEART RATE VARIABILITY

Table 6 shows that Cardioflex had no impact on heart rate, but it did have a significant impact on HRV (p=0.04) during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study period, HRV increased for the Cardioflex group (+5.3%) while a decrease (-9.0%) was seen with the placebo group. After adjusting for baseline starting values, the Cardioflex treatment group had a 6.8 higher post-treatment average HRV than the placebo. However, multiple regression analysis showed no associations between HRV and heart rate, blood pressure, or APG age.

Table 6 - Heart rate and heart rate variability of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment difference (Placebo – Cardioflex)
Start HR (beats/min)	69.97 ± 9.56	64.85 ± 10.00		

Final HR (beats/min)	67.70 ± 11.63	63.85 ± 8.99		
Percent Change	-3.2%	-1.5%		
Difference Between Treatments			3.844 (-1.22, 8.91)	0.348 (-3.48, 4.18)
Significance			P=0.134	P=0.856
Start HRV	69.95 ± 9.37	65.24 ± 9.44		
Final HRV	63.68 ± 9.08	68.72 ± 9.78		
Percent Change	-9.0%	+5.3%		
Difference Between Treatments			-5.045 (-9.65, -0.44)	-6.772 (-11.20, -2.24)
Significance			P=0.032*	P=0.04*

Values are expressed as means ± SD. Adjusted and unadjusted post-treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data was measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
*Indicates a significant main effect for group, where the Cardioflex treatment group had a higher heart rate variability than the placebo group.
HR = heart rate
HRV = heart rate variability

ARTERIAL AGE

Table 7 shows that Cardioflex had no impact on APG age during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study period, APG age remained stable for the Cardioflex group (-1.4%) while a small, non-significant decrease (-3.1%) was seen for the placebo group.

Table 7 - Accelerated plethysmograph age of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Start APG Age	46.36 ± 15.14	44.68 ± 16.74		
Final APG Age	44.90 ± 14.24	44.06 ± 15.96		
Percent Change	-3.1%	-1.4%		
Difference Between Treatments			0.850 (-6.54, 8.24)	0.031 (-6.37, 6.44)
Significance			P=0.819	P=0.992

Values are expressed as means ± SD. Adjusted and unadjusted post-treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data was measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
APG = accelerated plethysmograph, an assessment of arterial elasticity

DIETARY INTAKE

Table 8 shows that there were no differences in body weight, basal metabolic rate or total steps, but there was a significant difference in daily caloric intake (P=0.007) during the study period as measured at two time-points: beginning (day 1) and end of the study (day 90). Throughout the study, daily caloric intake increased for the Cardioflex group (+25.8%) while a decrease was seen with the placebo group (-14.4%). After adjusting for baseline values, the Cardioflex treatment group consumed a daily average of 724 more kilocalories than the placebo group.

Table 8 - Daily dietary intake, body weight, basal metabolic rate and total steps of participants before and after 90 days of Cardioflex or placebo supplementation

	Cardioflex (n=33)		Placebo (n=31)			
Parameter	Start	Final	Start	Final	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Calories (kcal)	2270 ± 657 Q_2 = 2269 Q_{3-1}=1035	2856 ± 1157 Q_2 = 2641 Q_{3-1}=989	2633 ± 1032 Q_2 = 2497 Q_{3-1}=654	2255 ± 724 Q_2 = 2217 Q_{3-1}=839	-601.2 (-79.6, -1122.7) P=0.025*	-724.0 (-209.8, -1238.2) P=0.007*
Percent Change	+25.8%		-14.4%			
Body Weight (kg)	89.9 ± 17.0 Q_2 = 88 Q_{3-1}= 14	89.2 ± 17.4 Q_2 = 87 Q_{3-1}= 15	89.8 ± 20.4 Q_2 = 88 Q_{3-1}= 22	89.2 ± 20.4 Q_2 = 89 Q_{3-1}= 23	0.060 (-10.1, 10.2) P=0.991	0.163 (-0.8,1.1) P=0.739
Percent Change	-0.8%		-0.8%			
Basal Metabolic Rate (kcal)	1840 ± 259 Q_2 = 1804 Q_{3-1}= 282	1762 ± 260 Q_2 = 1779 Q_{3-1}= 326	1805 ± 301 Q_2 = 1803 Q_{3-1}= 353	1745 ± 344 Q_2 = 1689 Q_{3-1}= 365	-16.3 (-179.0, 146.5) P=0.842	17.2 (-62.9, 97.4) P=0.668
Percent Change	-4.2%		-3.3%			
Total Steps	6721 ± 3489 Q_2 = 6280 Q_{3-1}=3246	9501 ± 4858 Q_2 = 9073 Q_{3-1}=4098	7040 ± 4058 Q_2 = 6190 Q_{3-1}=6200	9164 ± 4913 Q_2 = 7949 Q_{3-1}=5124	-336.7 (-2955.4, 2282.1) P=0.798	-552.6 (-2810.2, 1705.1) P=0.626
Percent Change	+41.4%		+30.1%			

Values are expressed as means ± SD. Adjusted and unadjusted endpoint differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 33) or placebo (n = 31) supplementation group. The data was measured at 2 time-points: beginning (day 1) and end of the study (day 90). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.

*Indicates a significant main effect where the Cardioflex treatment group had a higher calorie intake than the placebo group.
Q_2 = median
Q_{3-1}= interquartile range

MACRONUTRIENT INTAKE

Table 9 shows that there were no differences in protein, carbohydrate, fiber or sugar consumption, but there was a significant difference in fat (P=0.005) and saturated fat (P=0.017) consumption during the study period as measured at two time-points: beginning (day 1) and end of the study (day 90). Throughout the study, the Cardioflex group increased their fat (+42.1%) and saturated fat (+37.5%) while a decrease was seen with the placebo group (-9.4% and -3.4%, respectively). After adjusting for baseline values, the Cardioflex treatment group consumed a daily average of 40.1 grams more fat and 11.0 grams more saturated fat than the placebo group.

Table 9 - Dietary macronutrient intake of participants before and after 90 days of Cardioflex or placebo supplementation

	Cardioflex (n=33)		Placebo (n=31)			
Parameter	Start	Final	Start	Final	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Protein (g)	100.1 ± 48.4 Q_2 = 87 Q_{3-1}=58	121.3 ± 46.2 Q_2= 120 Q_{3-1}=61	109.1 ± 43.0 Q_2= 100 Q_{3-1}=48	104.0 ± 51.9 Q_2= 89 Q_{3-1}=42	-17.241 (-43.5, 9.0) P=0.194	-20.454 (-45.6, 4.7) P=0.108
Percent Change	+21.2%		-4.7%			
Carbohydrate (g)	255.0 ± 86.3 Q_2 = 231 Q_{3-1}=109	292.0 ± 185.9 Q_2 = 277 Q_{3-1}=105	314.1 ± 184.5 Q_2= 290 Q_{3-1}=102	243.6 ± 77.1 Q_2= 249 Q_{3-1}=74	-48.398 (-125.7, 28.9) P=0.215	-62.916 (-140.3, 14.5) P=0.109
Percent Change	+14.5%		-22.4%			

Fiber (g)	26.7 ± 11.3 Q_2 = 27 Q_{3-1}=19	26.7 ± 14.9 Q_2 = 22 Q_{3-1}=12	35.2 ± 37.6 Q_2= 24 Q_{3-1}=18	25.9 ± 12.5 Q_2= 23 Q_{3-1}=14	-0.715 (-8.1, 6.7) P=0.847	-1.164 (-8.7, 6.3) P=0.757
Percent Change	0%		-26.4%			
Sugar (g)	97.0 ± 45.7 Q_2 = 93 Q_{3-1}=36	123.1 ± 137.1 Q_2 = 103 Q_{3-1}=80	115.5 ± 70.2 Q_2= 96 Q_{3-1}=98	98.8 ± 48.5 Q_2= 89 Q_{3-1}=54	-24.310 (-80.2, 31.6) P=0.387	-36.181 (-89.5, 17.1) P=0.179
Percent Change	+26.9%		-14.5%			
Fat (g)	93.8 ± 38.5 Q_2 = 95 Q_{3-1}=43	133.3 ± 59.2 Q_2 = 123 Q_{3-1}=69	105.0 ± 39.9 Q_2= 94 Q_{3-1}=41	95.1 ± 38.7 Q_2= 95 Q_{3-1}=41	-38.242 (-65.3, -11.2) P=0.006*	-40.051 (-67.4, 12.7) P=0.005*
Percent Change	+42.1%		-9.4%			
Saturated Fat (g)	30.4 ± 13.4 Q_2 = 29 Q_{3-1}=16	41.8 ± 19.4 Q_2 = 41 Q_{3-1}=21	32.3 ± 14.1 Q_2= 27 Q_{3-1}=13	31.2 ± 13.3 Q_2= 30 Q_{3-1}=13	-10.629 (-19.6, -1.7) P=0.021*	-10.968 (-19.9, -2.0) P=0.017*
Percent Change	+37.5%		-3.4%			

Values are expressed as means ± SD. Adjusted and unadjusted endpoint differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 33) or placebo (n = 31) supplementation group. The data was measured at 2 time-points: beginning (day 1) and end of the study (day 90). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.

*Indicates a significant main effect where the Cardioflex treatment group had higher fat and higher saturated fat intakes than the placebo group.

Q_2 = median

Q_{3-1}= interquartile range

MICRONUTRIENT INTAKE

Table 10 shows that there were no differences in micronutrient intake during the study period as measured at two time-points: beginning (day 1) and end of the study (day 90). Throughout the study, the micronutrient content remained relatively stable for the

placebo group while a non-significant decrease in vitamin D (-35.2%), increase in vitamin E (+32%) and increase in selenium (+25.2%) was seen in the Cardioflex group

Table 10- Dietary micronutrient intake of participants before and after 90 days of Cardioflex or placebo supplementation

	Cardioflex (n=33)		Placebo (n=31)			
Parameter	Start	Final	Start	Final	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Vitamin C (mg)	106.0 ± 86.9 Q_2 = 82 Q_{3-1}=95	96.7 ± 138.7 Q_2 = 43 Q_{3-1}=79	96.2 ± 89.6 Q_2 = 56 Q_{3-1}=89	103.1 ± 77.7 Q_2 = 100 Q_{3-1}=104	6.419 (-54.4, 67.3) P=0.833	10.329 (-48.2, 68.8) P=0.725
Percent Change	-8.8%		+7.2%			
Vitamin D (IU)	132.8 ± 113.4 Q_2 = 102 Q_{3-1}=141	86.0 ± 83.9 Q_2 = 70 Q_{3-1}=69	140.1 ± 101.9 Q_2 = 103 Q_{3-1}=101	120.1 ± 108.8 Q_2 = 118 Q_{3-1}=142	34.011 (-17.8, 85.8) P=0.194	34.2 (-18.1, 82.6) P=0.205
Percent Change	-35.2%		-14.3%			
Vitamin E (mg)	7.5 ± 9.1 Q_2 = 5 Q_{3-1}=4	9.9 ± 18.8 Q_2 = 5 Q_{3-1}=9	6.2 ± 5.1 Q_2 = 4 Q_{3-1}=5	5.8 ± 7.4 Q_2 = 4 Q_{3-1}=5	-4.151 (-11.9, 3.6) P=0.288	-4.206 (-12.1, 3.6) P=0.288
Percent Change	+32%		-6.5%			
Magnesium (mg)	218.3 ± 91.6 Q_2 = 221 Q_{3-1}=68	221.4 ± 137.8 Q_2 = 196 Q_{3-1}=136	233.3 ± 114.4 Q_2 = 214 Q_{3-1}=132	198.0 ± 116.6 Q_2 = 162 Q_{3-1}=111	-23.403 (-92.1, 45.3) P=0.497	-26.223 (-94.9, 42.5) P=0.447
Percent Change	+1.4%		-11.3%			
Selenium (mcg)	79.1 ± 51.9 Q_2 = 63 Q_{3-1}=75	99.0 ± 74.9 Q_2 = 97 Q_{3-1}=82	89.1± 53.9 Q_2 = 70 Q_{3-1}=59	85.0 ± 85.8 Q_2 = 66 Q_{3-1}=62	-13.994 (-57.1, 29.1) P=0.518	-14.412 (-58.1, 29.3) P=0.511

Percent Change	+25.2%	-4.6%	

Values are expressed as means ± SD. Adjusted and unadjusted endpoint differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 33) or placebo (n = 31) supplementation group. The data was measured at 2 time-points: beginning (day 1) and end of the study (day 90). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
Q_2 = median
Q_{3-1}= interquartile range

BLOOD LIPID BIOMARKERS

Table 11 shows that Cardioflex had no impact on total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides or lipoprotein A during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). However, a significant decrease in total cholesterol was seen in the placebo group (P=0.042). After adjusting for baseline starting values, the placebo group had a 0.43mmol/L lower post-treatment total cholesterol than the Cardioflex treatment.

Figure 3 visually shows that the confidence intervals are smaller for the adjusted post treatment total cholesterol values of participants, as compared to the unadjusted values.

Table 11- Blood lipid profiles of participants before and after 90 days of Cardioflex or placebo supplementation

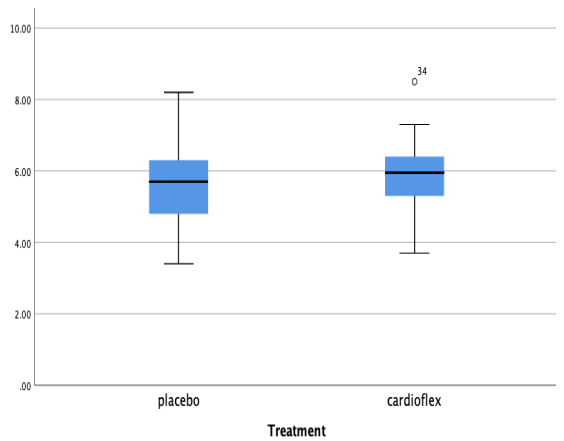
Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)	Normal	High Risk
Start Cholesterol (mmol/L)	6.61 ± 1.39	6.48 ± 1.30			< 5.1	>6.2

Final Cholesterol (mmol/L)	5.57 ± 1.12	5.87 ± 1.00				
Percent Change	-18.7%	-15.0%				
Difference Between Treatments			-0.308 (-0.828, 0.213)	-0.43 (-0.846,-0.015)		
Significance			P=0.242	P=0.042*		
Start HDL (mmol/L)	1.76 ± 0.41	1.77 ± 0.45			≥1.6	< 1.0 (men) <1.3 (women)
Final HDL (mmol/L)	1.40 ± 0.32	1.46 ± 0.41				
Percent Change	-20.5%	-17.5%				
Difference Between Treatments			-0.059 (-0.242, 0.124)	-0.069 (-0.203, 0.066)		
Significance			P=0.524	P=0.312		
Start LDL (mmol/L)	4.53 ± 1.27	4.39 ± 1.33			≤ 2.6	>4.1
Final LDL (mmol/L)	3.89 ± 0.99	4.07 ± 0.93				
Percent Change	-14.1%	-7.3%				
Difference Between Treatments			-0.182 (-0.654, 0.289)	-0.283 (-0.646, 0.080)		
Significance			P=0.443	P=0.124		
Start Lipoprotein A (mg/dL)	36.66 ± 29.87	40.12 ± 28.34			<30	>50
Final Lipoprotein A (mg/dL)	61.88 ± 34.15	57.62 ± 34.31				
Percent Change	+ 68.8%	+43.6%				
Difference Between Treatments			4.259 (-13.13, 21.65)	4.532 (-12.83, 21.90)		
Significance			P=0.626	P=0.604		

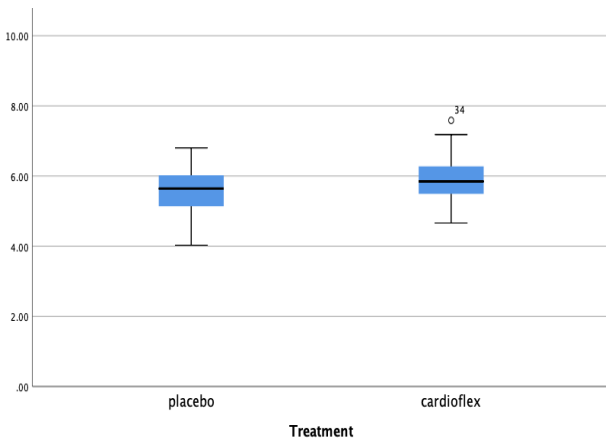
Start Triglycerides (mmol/L)	1.60 ± 0.70	1.96 ± 1.05			≤ 1.7	> 2.2
Final Triglycerides (mmol/L)	1.41 ± 0.63	1.72 ± 0.96				
Percent Change	-11.9%	-12.2%				
Difference Between Treatments			-0.304 (-0.709, 0.102)	-0.027 (-0.332, 0.278)		
Significance			P=0.139	P=0.861		

Values are expressed as means ± SD. Adjusted and unadjusted post treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data were measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
*Indicates a significant main effect for group, where the placebo group had lower total cholesterol than the Cardioflex treatment group.
HDL = high-density lipoprotein cholesterol
LDL = low-density lipoprotein cholesterol

Figure 3 - Unadjusted and adjusted post treatment total cholesterol box plots



Unadjusted Post Treatment Total Cholesterol



Adjusted Post Treatment Total Cholesterol

Adjusted values were calculated by using analysis of covariance, controlling for baseline values.

INFLAMMATION AND ENDOTHELIAL BLOOD BIOMARKERS

Table 12 shows that Cardioflex had no impact on inflammation or endothelial blood biomarkers during the study period as measured at two-time points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study period, non-significant increases in IL-6 and non-significant decreases in CRP, sVCAM, and sICAM were seen in both groups.

Table 12 - Inflammation and endothelial blood biomarkers of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Start IL-6 (mg/L)	0.82 ± 0.56	0.64 ± 0.30		
Final IL-6 (mg/L)	1.02 ± 0.48	1.18 ± 0.97		
Percent Change	+24.4%	+84.4%		
Difference Between Treatments			-0.161 (-0.55, 0.22)	-0.231 (-0.61, 0.15)
Significance			P=0.407	P=0.230
Start CRP (mg/L)	13.58 ± 14.36	10.76 ± 12.08		
Final CRP (mg/L)	6.00 ± 4.75	7.93 ± 8.48		
Percent Change	-55.8%	-26.3%		

Difference Between Treatments			-1.925 (-5.39, 1.54)	-2.349 (-5.52, 0.824)
Significance			P=0.271	P=0.144
Start sICAM-1 (mg/L)	1.89 ± 0.43	1.77 ± 0.43		
Final sICAM-1 (mg/L)	1.34 ± 0.48	1.28 ± 0.40		
Percent Change	-29.1%	-27.7%		
Difference Between Treatments			0.063 (-0.16, 0.28)	0.025 (-0.19, 0.24)
Significance			P=0.564	P=0.810
Start sVCAM-1 (mg/L)	2.04 ± 0.46	1.82 ± 0.42		
Final sVCAM-1 (mg/L)	1.46 ± 0.51	1.34 ± 0.36		
Percent Change	-28.4%	-26.4%		
Difference Between Treatments			0.121 (-0.10, 0.34)	0.100 (-0.13, 0.33)
Significance			P=0.276	P=0.329

Values are expressed as means ± SD. Adjusted and unadjusted post treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data was measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
IL-6 = Interleukin-6
CRP = c-reactive protein
sICAM = soluble intercellular adhesion molecule
sVCAM = soluble vascular adhesion molecule

COMPLIANCE & SAFETY

KIDNEY AND LIVER BLOOD BIOMARKERS

Kidney and liver blood biomarkers were tested to confirm the safety of the supplement.

Table 13 shows that Cardioflex had no impact on kidney or liver blood biomarkers during

the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91). Throughout the study, non-significant decreases in urea, creatinine, AST, ALT, and LDH were seen in both groups.

Table 13 - Kidney and liver blood biomarkers of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Start Urea (mmol/L)	6.99 ± 1.55	7.29 ± 1.86		
Final Urea (mmol/L)	6.01 ± 1.60	6.45 ± 1.34		
Percent Change	-14.0%	-11.5%		
Difference Between Treatments			-0.433 (-1.16, 0.30)	-0.339 (-1.00, 0.33)
Significance			P=0.241	P=0.314
Start Creatinine (mmol/L)	86.15 ± 21.59	89.63 ± 23.53		
Final Creatinine (mmol/L)	73.94 ± 18.36	73.00 ± 12.09		
Percent Change	-14.2%	-18.6%		
Difference Between Treatments			0.937 (-6.75, 8.62)	0.400 (-6.19, 6.99)
Significance			P=0.808	P=0.904
Start AST (mmol/L)	33.57 ± 11.94	39.61 ± 13.31		
Final AST (mmol/L)	26.48 ± 7.24	29.81± 8.47		
Percent Change	-21.1%	-24.7%		

Difference Between Treatments			-3.329 (-7.31, 0.65)	-1.255 (-5.03, 2.52)
Significance			P=0.099	P=0.508
Start ALT (mmol/L)	37.44 ± 15.82	43.00 ± 16.78		
Final ALT (mmol/L)	32.45 ± 10.99	35.18 ± 9.91		
Percent Change	-13.3%	-18.2%		
Difference Between Treatments			-2.730 (-7.95, 2.49)	-0.156 (-4.19, 3.88)
Significance			P=0.300	P=0.938
Start LDH (mmol/L)	552.12 ± 92.10	567.48 ± 95.56		
Final LDH (mmol/L)	480.53 ± 63.69	487.36 ± 65.85		
Percent Change	-13.0%	-14.1%		
Difference Between Treatments			-6.832 (-38.96, 25.29)	-5.848 (-36.97, 25.27)
Significance			P=0.672	P=0.708

Values are expressed as means ± SD. Adjusted and unadjusted post treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The data was measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
AST = aspartate aminotransferase
ALT = alanine aminotransferase
LDH = lactate dehydrogenase

PLASMA COENZYME Q10

Plasma CoQ10 values were tested to confirm compliance with the treatment. Table 14 shows that Cardioflex treatment had a close to a significant impact on plasma CoQ10 (P=0.062) during the study period as measured at two time-points: baseline (day 0) and after supplementing with Cardioflex or placebo for 90 consecutive days (day 91).

Throughout the study, the Cardioflex group saw a non-significant increase in plasma CoQ10 (+215.9%).

Table 14 - Plasma coenzyme q10 values of participants before and after 90 days of Cardioflex or placebo supplementation

Parameter	Placebo (n=33)	Cardioflex (n=34)	Unadjusted Post Treatment Difference (Placebo – Cardioflex)	Adjusted Post Treatment Difference (Placebo – Cardioflex)
Start CoQ10 (ng/mL)	16.82 ± 12.38	15.35 ± 8.08		
Final CoQ10 (ng/mL)	23.43 ± 32.55	48.49 ± 79.82		
Percent Change	+39.3%	+215.9%		
Difference Between Treatments			-25.064 (-55.83, 5.70)	-30.147 (-62.06, 1.77)
Significance			P=0.108	P=0.064

Values are expressed as means ± SD. Adjusted and unadjusted post treatment biomarker differences are given with 95% confidence intervals. Participants were randomized to either a Cardioflex (n = 34) or placebo (n = 33) supplementation group. The dietary data were measured at 2 time-points: baseline (day 0) and after taking the assigned supplement for 90 days (day 91). Data was analyzed by looking at analysis of covariance, comparing the two groups' endpoints, and controlling for their baseline values.
CoQ10 = Coenzyme Q10

CHAPTER 5: DISCUSSION

The objective of this study was to determine whether daily supplementation with the dietary supplement Cardioflex for 90 days would improve plasma lipid blood profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A) and other CVD risk factors in adults aged 30 – 65 years old. Overall, 67 participants (33 in Cardioflex treatment, 34 in placebo) completed the clinical trial.

Compliance with the study treatments was excellent with all participants completing their assigned dosages. Participants were given a randomized number of stick packs between 93 and 100, giving every participant at least three stick packs to return at the end of the clinical trial. Participants were not told the number of stick packs they were given. All participants returned the correct number of stick packs or explained why stick packs were missing, such as reconstituting a stick pack and spilling the beverage before consumption. To further confirm compliance, plasma CoQ10 values were tested at baseline and the end of the study. After adjusting for baseline values, the Cardioflex treatment group saw a 215.9% mean increase in plasma CoQ10 levels and trended towards significance (P=0.064). Previous research shows intestinal absorption of CoQ10 is highly variable between individuals, explaining the large standard deviation in the Cardioflex treatment group (Vitetta et al., 2018). Niklowitz et al (2007) reported a 303% increase in plasma Coq10 concentrations when participants were given 3 mg/kg body weight/day for 28 days (Niklowitz et al., 2007). Considering the CoQ10 dosage in Cardioflex is 30 mg, approximately half the dosage used in the study done by Niklowitz et al, the plasma CoQ10 increased observed in our study suggests compliance with the supplement.

Overall, the only significant difference found in the current study was that the Cardioflex treatment had a significant impact on heart rate variability (P=0.04), with the Cardioflex treatment group having a 6.8 higher post-treatment average than the placebo group. However, multiple regression analysis showed that there was no association between HRV and heart rate, blood pressure, or APG age. There were no other significant time x group interactions from the Cardioflex treatment in blood lipids or any of the other dependent variables tested.

BMI & WAIST CIRCUMFERENCE

BMI and waist circumference did not differ from baseline to the end of the study period, nor between the placebo and Cardioflex treatment, even though the daily caloric intake increased for the Cardioflex group (+25.8%) while a decrease was seen with the placebo group (-14.4%). At the end of the study the average BMI for the Cardioflex group was 31.0 $\pm$ 6.2 kg/m2, and 30.8$\pm$ 5.7 kg/m2 for the placebo group, thus both groups were defined as obese. At the end of the study, the average waist circumference for men in the Cardioflex group was 100.57 $\pm$ 12.85 cm and 96.88 $\pm$ 13.00 cm for females in the Cardioflex group. The final average waist circumference for men in the placebo group was 98.39 $\pm$ 13.33 cm, and 96.46 $\pm$ 17.54 cm for females in the placebo group. Thus, both men and females in both groups had above normal waist circumferences.

This outcome follows the study hypothesis. However, it is interesting to note that although there was a significant increase in calorie consumption in the Cardioflex treatment group, no changes in BMI were observed. It may be that the Cardioflex supplement increased the metabolic rate of participants or the participants in the Cardioflex treatment group subconsciously increased their non exercise activity thermogenesis (NEAT). Research shows a large variability of NEAT energy expenditure among individuals, independent of differences in body size, with differences varying as much as 100-800 kcal/day in subjects (Ravussin, Lillioja, Anderson, Christin, & Bogardus, 1986).

While obesity parameters such as BMI and waist circumference can be used as important independent risk factors for CVD, the similarity in the values between the groups throughout the study indicates that Cardioflex had no impact on body mass index or waist circumference.

CARDIOVASCULAR PARAMETERS

In this study, the cardiovascular parameters that were assessed were blood pressure, heart rate, HRV, and accelerated plethysmograph age. Blood pressure, heart rate, HRV, and arterial elasticity provide valuable information for the prediction of both long-term and near-term CVD risk. Blood pressure is a consistent risk factor for the development of atherosclerosis (Lu et al., 2007). Higher resting heart rate is linked with greater CVD risk and all-cause mortality (Fox et al., 2007). Reduced HRV is associated with an increased risk for the incident of coronary heart disease and CVD mortality (Liao et al., 1997), and arterial elasticity is an established risk factor to predict future CVD events (Glasser et al., 1997).

In the current study, blood pressure, arterial age, and heart rate did not differ from baseline to the end of the study, nor between the placebo and Cardioflex treatment ($P>0.05$). At the end of the study, the average systolic and diastolic blood pressure for the Cardioflex group was 127.18 ± 16.16 mmHg and 83.88 ± 8.68 mmHg, respectively. The average final systolic and diastolic blood pressure for the placebo group was 123.45 ± 14.29 mmHg and 83.55 ± 9.07 mmHg, respectively. Thus, both groups had slightly above normal blood pressure (120/80 mmHg). The average final heart rate in the Cardioflex group was 63.85 ± 8.99 beats/minute, and 67.70 ± 11.63 beats/minute in the placebo group. The average final arterial age in the Cardioflex group was 44.06 ± 15.96, and 44.90 ± 14.24 in the placebo group.

The Cardioflex treatment had a significant impact on HRV ($P=0.04$). At the end of the study, the Cardioflex treatment group had an average HRV of 68.72 ± 9.78 ms, and the placebo group had an average final HRV of 63.68 ± 9.08 ms. After controlling for baseline values, the Cardioflex treatment had a 6.8 millisecond higher post-treatment average HRV

than the placebo. Studies have shown that HRV can be influenced by various factors, including dietary factors. A correlation between serum magnesium levels and HRV has been observed in several investigations (Almoznino-Sarafian et al., 2009; Bobkowski et al., 2003). The same associations have been seen with antioxidant intake and HRV. Peeters et al observed that vitamin C supplementation stimulated the vagus nerve, activating the sympathetic portion of the autonomic nervous system (Peeters et al., 2005). Pomportes et al observed that the ingestion of a multi-vitamin-mineral supplement improves autonomic nervous system function (Pomportes, Davranche, Brisswalter, Hays, & Brisswalter, 2014). Given that the Cardioflex supplement contains vitamins and minerals shown to improve HRV, and that there were no significant differences between dietary micronutrient intake between treatment groups, it is plausible to hypothesize that the Cardioflex supplement may have had beneficial effects on the autonomic nervous system, causing the HRV improvements seen in the Cardioflex group. However, further investigation would be required to make any concrete conclusions because there was no association between HRV and any of the other cardiovascular parameters. Both human and animal data report inverse correlations between HRV and heart rate (Kazmi et al., 2016), supporting the concept that HRV is dependent on heart rate. Similarly, studies have demonstrated that the relationship between HRV and blood pressure is present in hypotensive and non-hypertensive patients (Lucini, Mela, Malliani, & Pagani, 2002), and that reduced HRV is associated with increase arterial stiffness (Jaiswal et al., 2013). The literature suggests that changes in HRV would also show correlations with other cardiovascular parameters (Brito Diaz et al., 2014).

In summary, the HRV improvements seen in the Cardioflex treatment group support the study hypothesis, but further investigation is required to make any conclusions because there were no significant time x group interactions in any of the other cardiovascular parameters tested.

DIETARY INTAKE

In this study, after adjusting for baseline values, results showed that the Cardioflex group consumed an average of 724 more calories per day then the placebo group (P=0.007). At the end of the study, the daily calorie consumption in the Cardioflex group was 2856 ± 1157 kcal, with men consuming 3158 ± 1295 kcal and females consuming 2284 ± 501 kcal, respectively. At the end of the study, the daily calorie consumption in the placebo group was 2255 ± 724 kcal, with men consuming 2510 ± 808 kcal and females consuming 1936 ± 456 kcal, respectively. At the end of the study, the interquartile range in the Cardioflex group was 989 kcal, and 839 kcal in the placebo group, respectively. Previously published research by Beaton et al. (1979) found that the mean daily energy intake was 2,639 kcal/day for men and 1,793 kcal/day for women, giving similar caloric intake variability to our study and suggesting validity of the diet records (Beaton et al., 1979)

Studies such as the Global Burden of Disease study cited diet as a major factor behind the rise in hypertension, type-2 diabetes, obesity, and other CVD risk factors (Lim et al., 2012). The pathogenesis of obesity is influenced by the balance between calories consumed and energy expenditure (Schwartz et al., 2017). Some research shows that vitamin B12 (Bernard, Nakonezny, & Kashner, 1998) and magnesium (Institute of Medicine (IOM), 1997) deficiencies can cause decreases in appetite, making it possible that the Cardioflex treatment group had preliminary deficiencies and the supplement increased appetite by restoring the deficiencies. Nonetheless, the higher daily calorie intake for the Cardioflex group means worse outcomes for CVD factors. Given that the CVD risk factor values were not significantly different between the groups, it is plausible to say that the Cardioflex group was at increased risk, and the higher daily calorie consumption may have masked any significant changes that would have been otherwise

been observed from the Cardioflex treatment. Intervention studies show that moderate calorie restriction significantly reduces CVD risk factors. A study by Kraus et al. (2019) found that an 11.9% reduction in calorie intake caused a reduction of all measured CVD risk factors including LDL-cholesterol, total cholesterol to HDL-cholesterol ratio, systolic and diastolic blood pressures, C-reactive protein, insulin sensitivity index, and metabolic syndrome score (Kraus et al., 2019). These findings suggest a substantial cardiovascular health advantage by practicing moderate calorie restriction.

Furthermore, after adjusting for baseline values, the Cardioflex treatment group consumed an average of 40.1 grams more fat (P=0.005) and 11.0 grams more saturated fat (P=0.017) than the placebo. Ecological studies such as the Seven Countries Study of Cardiovascular Diseases show that CVD incidence and mortality, and higher serum total cholesterol levels are associated with the dietary saturated fat intake (Keys et al., 1986). The higher fat and saturated fat intake in the Cardioflex group means worsen outcomes for CVD factors, especially blood lipid profile. Therefore it is possible to hypothesize that the Cardioflex treatment may have shown improvements in blood lipid profile and other CVD risk factors if diets were controlled to be isocaloric and isolipid, given that a reduction in CVD risk is demonstrated when saturated fat intake is reduced (Skeaff & Miller, 2009).

In summary, the difference in calories, fat, and saturated fat intake between groups may have caused a cardiovascular disadvantage to the Cardioflex treatment group, overshadowing the effects of the supplement.

BLOOD LIPID BIOMARKERS

Lipid profile is recognized as an established risk factor in the development and progression of CVD (Graham et al., 2012; Jellinger et al., 2012). In the current study,

Cardioflex treatment had no impact on lipid profiles (P>0.05). At the end of the study, the total cholesterol in the Cardioflex group was 5.87 $\pm$ 1.00 mmol/L, and 5.57 $\pm$ 1.12 mmol/L in the placebo group, respectively. The final HDL-cholesterol value in the Cardioflex group was 1.46 $\pm$ 0.41 mmol/L, and 1.40 $\pm$ 0.32 mmol/L in the placebo group, respectively. The final LDL-cholesterol value in the Cardioflex group was 4.07 $\pm$ 0.93 mmol/L and 3.89 $\pm$ 0.99 mmol/L in the placebo group, respectively. The final triglyceride values in the Cardioflex group were 1.72 $\pm$ 0.96 mmol/L and 1.41 $\pm$ 0.63 mmol/L in the placebo group, respectively. The final Lipoprotein A values in the Cardioflex group were 57.62 $\pm$ 34.31 mg/dL and 61.88 $\pm$ 34.15 mg/dL in the placebo group, respectively.

Our results are contrary to the results of Hobbs et al (2014) which reported that supplementing with a MIS significantly lowered total cholesterol and LDL-cholesterol (Hobbs et al., 2014). The MIS used in the Hobbs et al study contained herbal nutraceuticals, vitamins, and omega 3 fatty acids. Thus, giving it a very different ingredient profile then Cardioflex. Different ingredients act on different physiological pathways which may cause MIS combinations to have synergistic or antagonistic effects. Therefore, it may be required that different ingredient combinations, and/or different ingredient dosages be changed in Cardioflex to see lipid biomarkers changed. Furthermore, the study done by Hobbs et al (2014) used an open-label uncontrolled study design. A pragmatic uncontrolled study design focuses on the generalizability of results to real-world settings, possibly eliminating the placebo effect or subconscious dietary changes observed in this study. An open-label or cross over study design may have negated the variability between diets observed in our study.

In summary, the lack of improvements in blood lipid profile seen in the Cardioflex treatment group does not support the study hypothesis. Further investigation is required because the significant difference in calorie, fat and saturated fat intake between groups

may have overshadowed any of the significant time x group interactions that would have otherwise been seen in the blood lipid parameters tested.

INFLAMMATION AND ENDOTHELIAL BLOOD BIOMARKERS

The inflammatory cascade and its failure to resolve is involved in the development of atherosclerotic plaque (Wainstein et al., 2017). Elevated levels of inflammatory blood biomarkers are associated with an increased risk of CVD, even in the absence of hyperlipidemia (Ridker, 2003). Plasma levels of sVCAM (Semaan et al., 2000) and sICAM (Mrowka & Sieberth, 1994) are associated with the development and prognosis of atherosclerosis.

In this study, the inflammation parameters assessed were CRP and IL-6. The endothelial function parameters assessed were sVCAM-1 and sICAM-1. Inflammation blood parameters and endothelial function blood parameters did not differ from baseline to the end of the study, nor between the placebo and Cardioflex treatment ($P>0.05$). At the end of the study, the IL-6 values in the Cardioflex group were 1.18 ± 0.97 mg/L, and 1.02 ± 0.48 mg/L in the placebo group, respectively. The final CRP values in the Cardioflex group were 7.93 ± 8.48 mg/L, and 6.00 ± 4.75 mg/L in the placebo group, respectively. The final sICAM-1 values in the Cardioflex group were 1.28 ± 0.40 mg/L, and 1.34 ± 0.48 mg/L in the placebo group, respectively. The final sVCAM-1 values in the Cardioflex group were 1.34 ± 0.36 mg/L and 1.46 ± 0.51 mg/L, respectively.

Despite large biological plausibility, Cardioflex had no impact on inflammation blood biomarkers. Previous studies show that nutritional supplements can provide positive effects in alleviating inflammatory status. Supplemental vitamin C (500 mg twice daily)

reduced CRP and IL-6 in hypertensive patients (Ellulu, Rahmat, Patimah, Khaza'ai, & Abed, 2015). A meta-analysis of 12 clinical trials showed a significant reduction in CRP in vitamin E treated individuals (Saboori, Shab-Bidar, Speakman, Yousefi Rad, & Djafarian, 2015). Vitamin D supplementation inhibited lipopolysaccharide induced IL-6 and tumor necrosis factor-alpha (TNF-α) production in human monocytes (Zhang et al., 2012). Enteral and saline glutamine treatment has been shown to decrease IL-6 production in vivo and in vitro (Coeffier et al., 2001). Supplementation with selenium and CoQ10 reduced cardiovascular mortality and increased cardiac function in elderly persons (Alehagen, Alexander, Aaseth, & Larsson, 2019). Interestingly, in our study, no significant changes in inflammation blood biomarkers were seen, despite Cardioflex containing all the aforementioned individual ingredients cited above.

It may be that the dosage of certain ingredients in Cardioflex need to be increased to see a significant effect. Devaraj et al (2008) found a significant decrease in CRP when subjects took a combination of alpha-tocopherol (800 mg/day) and gamma-tocopherol (800 mg/day) for six weeks (Devaraj, Leonard, Traber, & Jialal, 2008). However, no changes were seen in the groups taking only one of the two supplements, suggesting the need for higher dosages of certain nutraceuticals to observe significant effects. Furthermore, a potentially important difference between studies in this field is differential in starting point, i.e., the baseline levels of biomarkers, and the room for improvement. Participants in our study were not screened for concentrations of inflammatory markers to determine eligibility. Rather, they were enrolled based on poor arterial elasticity and/or a low HDL-cholesterol to total cholesterol ratio. This resulted in enrolling a large number of participants who had relatively low levels of inflammatory biomarkers, leaving little room for improvement. When Azadbakht et al looked at markers of inflammation in the study cited previously (Ellulu et al., 2015), starting IL-6 values were 2.20 ±0 .75 mg/L or about triple the baseline values in our study. Similarly, in an investigation of omega-3

supplementation on inflammatory markers, Trøseid et al enrolled subjects with mean baseline IL-6 concentrations between 1.5 and 1.6 mg/L, approximately 2-fold those in our study (Troseid, Arnesen, Hjerkinn, & Seljeflot, 2009).

It may also be that a longer intervention (greater than 90 days) or a larger sample size is required to translate into significant changes. If the sample size equation is recalculated with $\alpha = .05$, $\beta = .90$, and the 15% mean decrease of total cholesterol Cardioflex treatment results obtained from this study, the estimated sample size of significant changes to 107 participants, almost double the previously calculated number of 60 participants. If the sample size equation is recalculated using the HRV treatment results obtained from this study, the estimated sample size of significant changes to 166 participants.

SAFETY

While there are many potential benefits of dietary supplements, concerns of possible hepatotoxicity and nephrotoxicity are valid. Previous studies show that not all dietary supplements are as safe as many consumers believe. For example, the occurrence of supplement-related liver toxicity ranges from 2% to 16% of all identified cases of hepatoxicity (Navarro et al., 2014). Alternative medicine induced nephrotoxicity accounts for approximately 7% of all medication-related toxicities (Leape et al., 1991). These numbers are likely under-reported because most relevant data on supplement induced hepatoxicity and nephrotoxicity comes from voluntary individual case reports, and it is often impossible to prove a definitive cause-and-effect relationship. The findings from intervention studies such as this will provide regulatory authorities with the information necessary to guide the development of safer products, and the removal of unsafe products from the market.

In our study, kidney function blood tests (urea, creatinine, LDH) and liver function blood tests (AST, ALT) were performed to access the safety of the Cardioflex supplement. No significant changes were seen between treatment groups or time x group interactions, suggesting safety of the Cardioflex supplement at a dosage of 1 serving/day for 90 consecutive days. The final urea values in the Cardioflex group were 6.45 $\pm$ 1.34, and 6.01 $\pm$ 1.60 in the placebo group, respectively. The final creatinine values in the Cardioflex group were 73.00 $\pm$ 12.09, and 73.94 $\pm$ 18.36, respectively. The final LDH values in the Cardioflex group were 487.36 $\pm$ 65.85, and 480.53 $\pm$ 63.69 for the placebo group, respectively. The final AST values in the Cardioflex group were 29.81$\pm$ 8.47, and 26.48 $\pm$ 7.24 in the placebo group, respectively. The final ALT values in the Cardioflex group were 35.18 $\pm$ 9.91, and 32.45 $\pm$ 10.99 in the placebo group, respectively.

Table 15 summarizes the maximum dosage allowed by Health Canada for each ingredient in Cardioflex (Health Canada, 2018). Although It may be that a larger dosage (>1 serving/day) is required to translate into significant changes, increasing the dosage to 2 servings/day would exceed the maximum dosage safety limit set by Health Canada for L-Lysine, Vitamin C, and folate. Overconsumption of nutrient supplements can lead to nausea, diarrhea, stomach cramps, hair loss, gastrointestinal upset, fatigue and mild never damage. The results of this study suggesting safety of the Cardioflex supplement at a dosage of 1 serving/day cannot be used to assume that dosages of >1 serving/day are safe.

Table 15 – Maximum Dosages allowed by Health Canada for each Ingredient in Cardioflex*

Ingredient	Dosage in 1 serving of Cardioflex	Dosage in 2 servings of Cardioflex	Maximum dosage allowed by Health Canada
L-Lysine	2800 mg	5600 mg^	3000 mg
Vitamin C	2000 mg	4000 mg^	2000 mg
L-Proline	1000 mg	2000 mg	N/A

L-Glutamine	500 mg	1000 mg	9000 mg
L-threonine	500 mg	1000 mg	N/A
Vitamin E (d-alpha tocopheryl acetate)	67 mg	134 mg	1000 mg
Potassium Gluconate	40 mg	80 mg	200 mg
Magnesium Ascorbate	33 mg	66 mg	500 mg
Ubiquinol CoQ10	30 mg	60 mg	300 mg
Folate (L-5-Methyltetradhydrofolate)	1000 mcg	2000 mcg^	1000 mcg
Vitamin B12 (Methylcobalamin)	300 mcg	600 mcg	1000 mcg
Selenium (Selenomethionine	100 mcg	200 mcg	200 mcg
Vitamin D (Cholecalciferol)	12.5 mcg	25 mcg	25 mcg

*Information extracted from Health Canada compendium of monographs (Health Canada, 2018)

^Dosage exceeds maximum amount allowed by Health Canada

In summary, kidney and liver function blood biomarkers did not differ from baseline to the end of the study, nor between the placebo and Cardioflex treatment, suggesting the safety of the Cardioflex supplement at a dosage of 1 serving/day for 90 consecutive days.

LIMITATIONS AND STRENGTHS

This study has many strengths associated with it. Participants were stratified (male/female) and randomized (block size = 5) to the study treatments, preventing selection bias. An independent t-test showed that there were no statistical differences between the starting points of the two groups, indicating that the two groups were indistinguishable from each other. The study was double-blinded so neither the participants nor the researchers knew which treatment the participants were given, preventing differential treatment between groups or the differential assessment of outcomes. Cardiovascular parameter and anthropometric measurements were done by the same study staff and all measurement protocols were standardized, ensuring

consistency with all data measurements. Compliance was excellent and no adverse events were reported. The study had a 97% attrition rate and the majority of participants were highly satisfied with the supplement.

However, this study also has some limitations associated with it. First, the sample size was small and may not have been statistically powered to demonstrate any effect of the Cardioflex supplement. If the sample size equation is recalculated with the results obtained from this study, the estimated sample size of significant changes to 107 participants, almost double the previously calculated number of 60 participants. Second, participants only recorded foods and beverages consumed over a single day, rather than over a specific time period, such as 3 to 4 days. Day-to-day variations in food intake and errors in food reporting and quantification can cause variations in food records, reducing the precision and validity of the data if only one day is recorded. Third, dietary intakes of participants showed a significant difference in calories, fat, and saturated fat intakes between groups. A crossover study design would have negated the variability between diets since participants would be given both interventions. Thus, any subconscious food changes that occur during the treatment period would be the same in both interventions. Finally, including only baseline and endpoint measurements reduced the reliability of the measurements. In addition, including ambulatory or at-home measurements for blood pressure, heart rate and HRV would eliminate the 'white coat' syndrome that can occur in clinical settings and provide a more accurate measurement of the dynamic behaviour of these parameters.

CONCLUSION & FUTURE DIRECTIONS

The objective of this study was to determine whether daily supplementation with the dietary supplement Cardioflex for 90 days would reduce risk factor biomarkers for CVD in adults aged 30-65. The hypothesis that the Cardioflex supplement would improve plasma

lipid profile and other CVD risk factors more so than a placebo supplement was not proved correct. Although the Cardioflex group saw a significant increase in HRV compared to the placebo group, further investigation would be required to make any conclusions because there was no association between HRV and any of the other parameters. Kidney function blood tests (urea, creatinine, LDH) and liver function blood tests (AST, ALT) were performed to access the safety of the Cardioflex supplement. No significant changes were seen between treatment groups or time x group interactions, suggesting the safety of the Cardioflex supplement at a dosage of 1 serving/day for 90 consecutive days.

It may be that a longer intervention (greater than 90 days), and/or a larger sample size is required to translate into significant changes. Future research should employ a larger treatment dosage, evaluate a larger cohort of individuals, include a midpoint blood draw and ambulatory or at-home blood pressure, heart rate and HRV measurements, and employ a different study design. A titration dosing protocol would allow researchers to determine the appropriate dosage needed to see significant changes. A crossover study design would increase the sample size by 2 and help eliminate the genotypic variations between individuals. Since the washout period is unknown, a washout period of at least one month should be used since previously published intervention trials investigating blood lipid profiles have used washout periods of two weeks (Lee et al., 2017). Thus, a one-month washout period would give an extra 2-week buffer time to ensure the time period between treatments is sufficient. A crossover design would also increase the sample size by 2. Furthermore, adding a midpoint blood draw and including ambulatory or at home measurements for blood pressure, heart rate and HRV would provide more accurate outcome measurements.

In summary, the hypothesis that the Cardioflex supplement would improve plasma lipid profile and other CVD risk factors more so than a placebo supplement was not proved correct. Future research should employ a larger treatment dosage, evaluate a larger

cohort of individuals, include a midpoint blood draw and ambulatory or at-home blood cardiovascular parameter measurements, and employ a different study design.

APPENDIX

CONSENT FORM

University of Manitoba

Faculty of Agriculture and Food Sciences

Food and Human Nutritional Sciences

Title: The effect of the dietary supplement Cardioflex on reducing cardiovascular disease risk factors in adults

Investigators: Semone Myrie, RD, PhD (XXX@uamanitoba.ca; (XXX) XXX-XXXX) and Trevor Kouritzin, MSc candidate (XXX@MyUmanitoba.ca; (XXX) XXX-XXXX)

This study is being conducted by Trevor Kouritzin, a Masters student in the Department of Food and Human Nutritional Sciences as part of his thesis, under the supervision of Dr. Semone Myrie, RD, PhD.

This is a clinical trial. The study will be registered with ClinicalTrials.gov. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Sponsors: Mitacs Accelerate Grant Program (Government of Canada research program), and Innotech Nutrition Solutions, Winnipeg, MB (Winnipeg-based company that manufactures the dietary supplement Cardioflex).

Disclosure: Trevor Kouritzin has worked for Innotech Nutrition Solutions in the past, and as part of the Mitacs grant program is required to complete an internship period with Innotech Nutrition Solutions during his MSc program. Precautions will be taken to reduce

the potential for bias during the study. For example, the study treatments will be blinded to the researchers throughout the study.

Introduction

You are being asked to participate in a research study. Please take your time to carefully review this Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or (if applicable) your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand**.**

Purpose of Study

The purpose of this study is to determine whether 90 days of supplementation with the dietary supplement Cardioflex can reduce cardiovascular risk factors in healthy adults.

Study procedures

Pre-screening:

If you would like to take part in this study, you will be required to first complete a pre-screening questionnaire to provide us with some information about your health and to make sure you meet the eligibility criteria to participate in this study. The pre-screening questionnaire will ask questions about your general health, medical history, use of supplements and current level of physical activity.

Also, as part of the screening criteria, a Meridian digital pulse wave analyzer (DPA) machine will be used to measure the health of your arteries. The machine uses a technique known as accelerated plethysmogram (APG) waveform to measure the stiffness of your arterial walls (the "biological age" of your arteries). The pulse wave analyzer machine is non-invasive. The study coordinator will place a small clip device over your index finger, which shines infrared LED light through your fingertip and obtains pulse wave information based on the light interaction with the blood cells in your finger. The DPA machine converts the changes of transmitted light into an electrocardiogram waveform (measure of arterial stiffness) and pulse rate.

If you meet the first round of preliminary study requirements, then you will need to meet with a phlebotomist to get blood drawn to ensure you also meet the lipid blood marker requirements. If you also meet the preliminary lipid blood marker requirements, then you meet all the inclusion criteria requirements and you are allowed to participate in the study.

Study:

The study will be 90 days, and you will be randomized to receive a study treatment, which means you will be randomly assigned to receive either the experiment or the placebo group. The study will use a parallel design therefore you will only receive one treatment throughout the study. You do not get to choose which group you are in; the treatments are

randomly assigned by the principal investigator using a randomization program. The study will use a double-blinded design, which means you will not be told which treatment group you are assigned until after the study is complete. The investigators in the study will also not know your assigned group until after the study is complete. Participants in the experimental group will be given 1 serving (10g) of Cardioflex each day for the study duration. Participants in the control group will be given a flavored 10g placebo that looks and tastes similar to Cardioflex. You will be instructed to consume the beverage in 500 mL of water upon waking each morning, prior to eating or drinking anything else. The study coordinator will review this in detail with you. You will be instructed to maintain your normal dietary and exercise habits over the course of the study.

The ingredients in Cardioflex are:

Ingredients per 10 g / Ingrédients par 10 g

Calories / Calories 28 | Carbohydrate / Glucides 0.5 g | Fat / Lipides 0 g

L-Lysine HCl / L-lysine HCl	2800 mg
Vitamin C (Ascorbic Acid, Magnesium Ascorbate) / Vitamine C (acide ascorbique)	2000 mg
L-Proline / L-proline	1000 mg
L-Glutamine / L-glutamine	500 mg
L-Threonine / L-thréonine	500 mg
Potassium (Potassium Gluconate) / Potassium (gluconate de potassium)	40 mg
Magnesium (Magnesium Ascorbate) / Magnésium (ascorbate de magnésium)	33 mg
Coenzyme Q10 (Ubiquinol) / Coenzyme Q10 (Ubiquinol)	30 mg
Folate (L-5-Methyltetrahydrofolate) / Folate (L-5-méthyltétrahydrofolate)	1000 mcg
Vitamin B12 (Methylcobalamin) / Vitamine B12 (Methylcobalamin)	300 mcg
Selenium (Selenomethionine) / Sélénium (sélénométhionine)	100 mcg
Vitamin D (Cholecalciferol) / Vitamine D (cholécalciférol)	12.5 mcg or 500 IU
Vitamin E (d-alpha tocopheryl acetate) / Vitamine E (acétate de d-alpha tocophéryl)	100 IU

Non-Medicinal Ingredients: Inulin (Pre-biotic), Blueberry Juice Powder, Cranberry Juice Powder, Beetroot Powder, Calcium Citrate, Citric acid, Natural Flavour, Stevia rebaudiana leaf, Tapioca, Silica.

Ingrédients non médicinaux: Inuline (pré-biotique), poudre de jus de bluets, poudre de jus de canneberge, poudre de betterave rouge, citrate de calcium, acide citrique, arôme naturel, feuille de Stevia rebaudiana, tapioca, silice.

Any changes in your health status at any point during the study needs to be reported immediately to the study investigators.

Should you choose to participate, you will be asked to complete 2 testing sessions [baseline (i.e. day 0 or day 1) and day 90] during the 90 days study period. All testing sessions will occur at the Richard Centre for Functional Foods and Nutraceuticals (RCFFN), located in the Smart park on the Fort Gary campus at the University of Manitoba.

Each testing session will require approximately 30-40 minutes of your time. Each testing session will include measurement of your blood pressure (about 10-15 minutes), your arterial status using the DPA machine (about 5 minutes), anthropometric measurements (about 5 minutes) and collection of blood samples (about 5-10 minutes). For blood collection, you will need to fast (no food or beverage, except water) for a minimum of 8 hours before your appointment. Approximately 25 ml of blood will be taken. Blood will be used to measure various biomarkers, including blood lipid profile, kidney and liver functions, inflammatory and endothelial functions – see specific biomarkers below.

Overall, health markers that will be tested in this study include your waist circumference, body weight, height, atherosclerosis risk factors (blood pressure, heart rate, arterial stiffness, total blood lipid profile (total cholesterol, high density lipoprotein (HDL cholesterol), low density lipoprotein (LDL cholesterol), triglycerides), lipoprotein A, and inflammatory and endothelial function biomarkers (C-reactive protein (CRP), Interleukin-6 (IL-6), soluble intercellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1).

Kidney and liver functions will be assessed to measure the safety of the supplement. These assessments include testing for alanine aminotransferase (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), and Blood Urea Nitrogen (BUN), creatinine). Blood coenzyme Q10 levels will also be tested as a measure of treatment compliance.

To monitor your progress during the study, you will be required to complete the treatment feedback sheets, dietary food records and wear a pedometer at certain times throughout the study. First, you will be given a 90-day compliance sheet document to be used to record what time of the day you took the supplement and if you experienced any side-effects such as gastrointestinal distress. Second, you will be required to complete a 3-day food record at day 1, day 45 and day 90 of the study to help us assess if any major changes in your diet/dietary habits during the study. Finally, you will be required to wear a pedometer that tracks total steps and exercise intensity on week 1, week 6 and week 13 (the final week of the study) to allow us to assess your level of physical activity during the study.

Risks and Discomforts

There are no known serious side effects for any of the procedures proposed in this study. However, as with any clinical trial, there might be as yet unknown or unforeseen risks of taking part.

Cardioflex supplement: No adverse effects have been reported. The active ingredients in Cardioflex are within acceptable levels known to be safe and effective and in accordance with Health Canada licensing guidelines. Health Canada has issued the product a natural health product (NHP) number and the supplement has already been available for purchase to the commercial market.

Blood draw: Blood sampling may have some rare risks, like placing a needle into a vein which may contribute to infection, perforation or penetration of the needle through the vein, and bleeding, pain, or bruising at the site. As part of the process for blood sampling, to help minimize risks for infection, the phlebotomist will start by performing his/her hand hygiene (wash with soap and water) and wearing clean from you. Next, the phlebotomist will disinfect the site on you will be used for blood collection using 70% isopropyl alcohol swab. After blood is drawn and the needle removed from the vein, the phlebotomist will apply gentle pressure to the puncture site using clean gauge or cotton ball to stop bleeding, and a bandage is applied. A cream called EMLA can be applied to the puncture site to numb the skin to and help reduce infection. Infection risk is

also minimized by the use of prepackaged sterilized equipment, and all needles are disposed after a single use.

There are no known risks associated with the blood pressure measurement and measuring arterial stiffness using the Digital pulse wave analyzer (DPA) machine.

Benefits

You may not directly benefit from participation in this research; however, this study will contribute to a better understanding of the effects of the dietary supplement Cardioflex on reducing cardiovascular disease risk factors. All the procedures that will be performed as part of this study are provided at no cost to you. You will receive test results when they become available. You will receive your test results based on your preference of either electronic format or as a hard copy available from the study coordinator.

Confidentiality

Medical / research records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. All records will be kept in a locked secure area and only those persons identified as requiring access to your records will have opportunity to review or copy your medical / research records. Information gathered in this research study may be published or presented in public forums; however, your name and other identifying information will not be used or revealed. Personal information such as your name, address, telephone number and/or any other identifying information will be protected. If the results of the study are published, your identity will remain confidential. No information revealing any personal information such as your name, address or telephone number will be made publicly available.

The University of Manitoba may review records related to the study for quality assurance purposes.

All records such as questionnaires will be kept in a locked cabinet, in a secure area at the RCFFN and only those persons identified as researchers on this study will have access to these records. Study biological samples will be stored in locked freezers at the RCFFN or the Duff Roblin Building, University of Manitoba. Your samples will not be used for any additional analyses, nor stored for any longer than 5 years after the completion of the study, nor shared with any other groups, other than is indicated in the protocol, without your specific consent.

Remuneration and Feedback

You will receive up to a maximum of $50 remuneration at the completion of this study. You will receive $10 for showing up to the pre-screening phase, $20 for showing up to the first testing session and $20 for returning the study pedometer and showing up to the final testing session.

All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment that you will receive.

Feedback about the research results will be provided to you as an electronic document or a paper copy through regular mail (see dissemination section below).

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Should you wish to withdraw your participation from the study please inform the study coordinators so that your file can be officially close. If the study staff feels that it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study staff.

Investigator:	Dr. Semone Myrie XXX@umanitoba.ca	Tel No.	XXX-XXX-XXXX
Co-investigator	Trevor Kouritzin XXX@MyUmanitoba.ca	Tel No.	XXX-XXX-XXXX

This research has been approved by the Joint-Faculty Research Ethics Board at the University of Manitoba. If you have any concerns or complaints about this project you may contact any of the above-named persons or the Human Ethics Coordinator XXX-XXX-XXXX, or e-mail XXX@umanitoba.ca.

A copy of this consent form has been given to you to keep for your records and reference.

Dissemination

The results of the study will be written up in Trevor Kouritzin's master's thesis and may be published in recognized scientific journals and presented to public groups such as at scientific meetings and seminars. Additionally, all study participants will receive their individual results along with the mean value obtained from the whole study population and a summary of findings. However, participants will not be able to have access to the individual results of other study participants.

Please indicate below how you would like to receive your results and a summary of the study findings:

Email:

Ground mail (provide mailing address):

Medical Care for Injury Related to the Study

In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should immediately go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form nor releasing the investigator or the sponsor from their legal and professional responsibilities.

I am aware that there are risks associated with placing a needle into a vein for blood sampling, including risk of bleeding, pain or bruising at the site, and possible infection.
Yes ☐ No ☐

Consent

1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the clinical trial (research study) described above.
2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose and duration of the trial, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.
3. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.
4. By signing and dating this document, I am aware that none of my legal rights are being waived.

Participant signature:_____ Date: _____

Printed name of above:_____

I confirm that I have explained the purpose, duration etc. of this study, as well as any potential risks and benefits, to the participant whose name and signature appears above. I confirm that I believe that the participant has understood and has knowingly given their consent to participate by his/her personally dated signature.

Research staff signature: _____ Date: _____

Printed name of above: _____ Study role: _____

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