The efficacy of *Nopales* (Opuntia Spp) on Lipoprotein Profile and Oxidative Stress among Moderately Hypercholesterolemic Adults

by

Giselle Adriana Pereira Pignotti

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Graduate Supervisory Committee:

Sonia Vega-López, Chair
Glenn Gaesser
Colleen Keller
Gabriel Shaibi
Karen Sweazea

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ABSTRACT

Background: Evidence about the purported hypoglycemic and hypolipidemic effects of *nopales* (prickly pear cactus pads) is limited. Objective: To evaluate the efficacy of *nopales* for improving cardiometabolic risk factors and oxidative stress, compared to control, in adults with hypercholesterolemia. Design: In a randomized crossover trial, participants were assigned to a 2-wk intervention with 2 cups/day of *nopales* or cucumbers (control), with a 2 to 3-wk washout period. The study included 16 adults (5 male; 46±14 y; BMI = 31.4±5.7 kg/m^2) with moderate hypercholesterolemia (low density lipoprotein cholesterol [LDL-c] = 137±21 mg/dL), but otherwise healthy. Main outcomes measured included: dietary intake (energy, macronutrients and micronutrients), cardiometabolic risk markers (total cholesterol, LDL-c, high density lipoprotein cholesterol [HDL-c], triglycerides, cholesterol distribution in LDL and HDL subfractions, glucose, insulin, homeostasis model assessment, and C-reactive protein), and oxidative stress markers (vitamin C, total antioxidant capacity, oxidized LDL, and LDL susceptibility to oxidation). Effects of treatment, time, or interactions were assessed using repeated measures ANOVA. Results: There was no significant treatment-by-time effect for any dietary composition data, lipid profile, cardiometabolic outcomes, or oxidative stress markers. A significant time effect was observed for energy, which was decreased in both treatments (cucumber, -8.3%; *nopales,* -10.1%; p_{Time}=0.026) mostly due to lower mono and polyunsaturated fatty acids intake (p_{Time}=0.023 and p_{Time}=0.003, respectively). Both treatments significantly increased triglyceride concentrations (cucumber, 14.8%; *nopales,* 15.2%; p_{Time}=0.020). Despite the lack of significant treatment-by-time effects, great individual response variability was observed for all outcomes. After the cucumber

and *nopales* phases, a decrease in LDL-c was observed in 44% and 63% of the participants respectively. On average LDL-c was decreased by 2.0 mg/dL (-1.4%) after the cucumber phase and 3.9 mg/dL (-2.9%) after the *nopales* phase (p_{Time}=0.176). Pro-atherogenic changes in HDL subfractions were observed in both interventions over time, by decreasing the proportion of HDL-c in large HDL (cucumber, -5.1%; *nopales,* -5.9%; p_{Time}=0.021) and increasing the proportion in small HDL (cucumber, 4.1%; *nopales,* 7.9%; p_{Time}=0.002). Conclusions: These data do not support the purported benefits of *nopales* at doses of 2 cups/day for 2-wk on markers of lipoprotein profile, cardiometabolic risk, and oxidative stress in hypercholesterolemic adults.

DEDICATION

I dedicate this dissertation to my family who has shown relentless support throughout the years. I am thankful to my husband Alex for his encouragement, patience, friendship and love, and for always believing in me. To my loving parents, Nelson and Regina, I appreciate your words of encouragement and for giving me your fullest support. To my brother Gustavo and sister Viviane for your guidance and for always making me laugh.

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CHAPTER 1

INTRODUCTION

1.1 Background

Cardiovascular disease (CVD) continues to be the leading cause of death and morbidity among Americans (1). In 2008, one of every three deaths in the United States was attributed to CVD. In that same year, the total cost (direct and indirect) of CVD and stroke was estimated to be $297.7 billion, costing more than any other diagnostic group (2). CVD has a multi-factorial etiology with several potentially modifiable risk factors, and may be prevented with adequate changes in lifestyle. The American Heart Association (AHA) has published recommendations to promote cardiovascular health and prevent CVD that includes seven cardiovascular health behaviors or factors (smoking status, body mass index, dietary intake, physical activity, and levels of blood pressure, blood glucose, and total cholesterol) (3).

Epidemiological studies have shown that risk factor modification can significantly reduce the risk of CVD incidence and mortality. In fact, 44% of the decline for coronary heart disease mortality in the United States from 1980 to 2000 was attributed to reductions in major risk factors (4). Although CVD mortality has declined over the past years, only 8% of US adults had a low risk profile for CVD during 1999-2004 (5). Likewise, the prevalence of meeting all seven CV health behaviors or factors at ideal levels as recommended by the AHA was only 2% in 1988-1994 and 1.2% in 2005-2010. In addition, only one in five adults followed two or more of the five dietary recommendations (consumption of fruits and vegetables ≥ 4.5 cups/d, fish ≥ two 3.5-oz servings/wk, fiber-rich whole grains ≥ three 1-oz–equivalent servings/d, sodium < 1500

mg/d, and sugar-sweetened beverages ≤36 oz/wk), while less than 1% followed four or more components of a healthy diet. Therefore, considerable improvement in CV health behaviors and factors remain to be achieved in order to significantly reduce CVD mortality (6).

The metabolic syndrome (MS) is a cluster of metabolic abnormalities associated with increased risk for diabetes and CVD (7). Its components are abdominal obesity, low high density lipoprotein cholesterol (HDL-c), fasting hyperglycemia, hypertriglyceridemia, and hypertension; three out of five abnormal components qualify a person for the MS (8). The prevalence of MS in the United States was 34% from the combined 1999-2006 National Health and Nutrition Examination Survey (NHANES) data. Mexican Americans presented the highest rates, 37% of men and 43% of women had MS (9). Identifying individuals with the MS may help improve cardiovascular health through a more intensive intervention focusing on lifestyle risk factors (10).

It is well established that dietary quality is an important contributor to chronic disease prevention (11). The intake of fruits and vegetables among most Americans is lower than the recommended five daily servings; in 2007 only 24% of Americans reached the suggested intake (12). Similarly, the average intake of total fiber in 2007-2008 was 15.9 g/day, which is well below the recommended Adequate Intake (25 to 38 g/day or 14 g/1,000 kcal/day) (13). This is of particular concern considering that dietary fiber, especially soluble fiber, is suggested to decrease cholesterol, blunt glycemic response, increase satiety and is associated with lower CVD risk (14).

Elevated low density lipoprotein cholesterol (LDL-c) is associated with atherosclerosis development and increased CVD risk (15). The excessive uptake of

oxidized LDLs by macrophages in the arterial wall gives rise to foam cells and fatty streaks inducing the production of several pro-inflammatory molecules that can result in atherosclerotic lesion (16). Therefore, one of the mechanisms underlying the development and progression of atherosclerosis is increased oxidative stress. Oxidative stress is caused by an imbalance involving the production of reactive oxygen species (ROS) and impaired antioxidant capacity (17). The increased production of ROS results in cellular damage, and particularly affects endothelial cells (17-19). The production of ROS and antioxidant capacity can be modified to some extent by dietary factors (nutrients, phytochemicals and fatty acid composition). Studies have shown that dietary patterns characterized by low saturated fat, total fat, and cholesterol intake; and rich in fruits and vegetables are inversely associated with oxidative stress (20, 21).

A recommended approach to reduce LDL-c is increasing soluble fiber intake (pectin, mucilages, and gums) (22). Incorporating food items that are a good source of fiber could potentially improve other biomarkers of cardiometabolic disease risk. In a recent crossover study, the addition of 102 g of oat bran per day (6 g/day of soluble fiber) for two weeks resulted in greater reduction in total cholesterol (14% vs. 4%), non-HDL cholesterol (16% vs. 3%) and triglycerides (21% vs. 10%) in comparison to a low fiber diet (23).

A less explored approach to using a functional food to improve cardiometabolic disease risk is the consumption of *nopales* (prickly pear cactus pads from the Opuntia species). *Nopales* are vegetables low in fat, high in total fiber and soluble fiber (0.9 g/100 g), and a good source of vitamin A, β-carotene, vitamin C, and phenolic compounds (24, 25). *Nopales* are easily available in Hispanic food markets in the US (25). In a pilot study

conducted by our group, Mexican Americans who reported consuming *nopales* had a mean intake of roughly once per week (3.8 servings/month); 18% of this subgroup reported intake of up to 2.5 servings/week (375 g/week) (unpublished observations).

Because of their fiber content, *nopales* are commonly regarded among Mexicans as a medicinal plant for glycemic and cholesterol control. However, the literature documenting their purported hypocholesterolemic potential is scarce. A study conducted in healthy, obese and diabetic subjects showed that the consumption of 300 grams a day of *nopales* (2.7 g/day of soluble fiber) for 10 days led to significant reductions in total cholesterol (14%) and triglycerides (24%). In addition, a decrease by 22% in glucose levels was observed in diabetic subjects (26). Similar results were observed with the consumption of 250 g a day of prickly pear in hypercholesterolemic or hyperlipidemic individuals (27, 28). Besides the hypocholesterolemic effects, prickly pear has shown to lower oxidative stress as indicated by decreases in isoprostanes (plasma, serum and urinary), malondialdehyde, ratio of reduced to oxidized glutathione (GSH/GSSG) and conjugated dienes lipid hydroperoxide (27, 29). Comparable studies evaluating the effect of *nopales* cactus pads, instead of cactus fruit (prickly pear), on oxidative stress are not available.

1.2 Purpose of Research

In light of the high CVD morbidity and mortality, the promotion of simple interventions to improve serum lipids and decrease oxidative stress are warranted to help prevent chronic diseases. Given the unique composition and potential benefits of *nopales*, their use may be an ideal approach as an adjunct therapy for the reduction in

cardiometabolic risk factors. Therefore, the aim of this study was to evaluate the efficacy of a 2-week intervention with *nopales* pads for the reduction of established CVD risk factors (serum lipids) in comparison to a control food with lower antioxidant and fiber content (cucumber) among adults with moderate hypercholesterolemia in a randomized controlled crossover trial. Because limited data exists on the effects of *nopales* supplementation on other factors associated with cardiometabolic risk, an exploratory assessment of biomarkers of insulin sensitivity, inflammation (high sensitivity C-reactive protein [hsCRP]) and oxidative stress (LDL oxidizability and total antioxidant capacity) was also conducted.

1.3 Hypotheses

1.3.1 Central Hypothesis: *Nopales* intake (2.5 g/day of soluble fiber) versus control will improve the lipid profile in adults with moderate hypercholesterolemia as indicated by total cholesterol, LDL-c, HDL-c, triglycerides, LDL subfractions and HDL subfractions.

Specific Aim 1: To explore the effect of *nopales* intake (2.5 g/day of soluble fiber) on the lipid profile (total cholesterol, LDL-c, HDL-c, triglycerides, LDL subfractions and HDL subfractions) in adults with moderate hypercholesterolemia.

1.3.2 Exploratory Hypothesis 1: *Nopales* intake (2.5 g/day of soluble fiber) versus control will improve cardiometabolic risk factors associated with insulin sensitivity and inflammation in adults with moderate hypercholesterolemia as indicated by fasting glucose, insulin resistance, hsCRP, and blood pressure.

Specific Aim 2: To explore the effect of *nopales* intake on cardiometabolic risk factors (fasting glucose, insulin resistance, hsCRP, and blood pressure) in adults with moderate hypercholesterolemia.

1.3.3 Exploratory Hypothesis 2: *Nopales* intake versus control will improve oxidative stress status in adults with moderate hypercholesterolemia as indicated by LDL oxidation, oxidized LDL, total antioxidant capacity and plasma vitamin C.

Specific Aim 3: To explore whether *nopales* intake affects oxidative stress status (LDL oxidation, oxidized LDL, total antioxidant capacity and vitamin C) in adults with moderate hypercholesterolemia.

CHAPTER 2

LITERATURE REVIEW

2.1 Cardiovascular Disease

It is estimated that one in three American adults over 20 years of age have one or more types of CVD. More than 2 million Americans have a heart attack or stroke each year which over 800,000 die or one death every 39 seconds (2, 30). Despite a 30% decrease in heart disease death rates between 2000 and 2010, CVD continues to be the leading cause of death among Americans, with 24% of all deaths being from CVD in 2010 (1). It was estimated in 2008 that the sum of direct and indirect costs of CVD and stroke was $297.7 billion (2). As the population ages, it is projected that in 2030 40.5% of the US population will have some form of CVD, compared to 37% in 2010. As a consequence, it is estimated that by 2030 direct costs will reach $818 billion and indirect costs $276 billion (31).

Changes in lifestyle may prevent CVD as this is a multi-factorial disease with potentially modifiable risk factors. With the main goal to promote cardiovascular health, and prevent CVD and stroke by 20%, the AHA released the “Strategic Impact Goals for 2020 and Beyond” report. This report comprises a set of health metrics that evaluate seven cardiovascular health behaviors or factors: 1) smoking status; 2) body mass index; 3) healthy diet; 4) physical activity; 5) blood pressure; 6) blood glucose; 7) total cholesterol. Each category is classified into poor, intermediate and ideal. For the healthy diet factor, the report proposes the assessment of dietary quality based on the consumption of 5 dietary components: 1) fruits and vegetables $\geq$ 4.5 cups/d; 2) fish $\geq$ two

3.5-oz servings/wk; 3) fiber-rich whole grains ≥ three 1-oz–equivalent servings/d; 4) sodium < 1500 mg/d; 5) sugar-sweetened beverages ≤ 36 oz/wk). The information based in these metrics will help the AHA monitor changes in cardiovascular risk and focus in areas of greatest concern (3).

According to NHANES data, the prevalence of meeting all seven AHA health metrics at ideal levels was only 2% in 1988-1994 and 1.2% in 2005-2010. In addition, only 20% of adults followed at least two dietary recommendations, while less than 1% followed four or five components of a healthy diet. Meeting a greater number of cardiovascular health metrics was associated with a decrease in mortality. In a fully adjusted model, those who met six or more health metrics had 51% lower risk of all-cause mortality and a 76% lower risk of CVD mortality in comparison to those meeting only one health metric (6). Similar results were observed by Ford et al. using NHANES data (1999-2002). Meeting at least five heath metrics was associated with a 78% reduced risk in all-cause mortality and 88% reduced risk in circulatory system diseases mortality. Those with a healthy eating score classified as ideal had a 49% risk reduction in all-cause mortality in comparison to a poor healthy eating score (32). These epidemiological studies have shown how risk factor modification can significantly reduce all-cause and CVD mortality. In fact, 44% of the decline of coronary heart disease (CHD) mortality in the United States from 1980 to 2000 was attributed to reductions in major risk factors (4). Considerable improvement in CV health behaviors and factors remains to be achieved, and change in dietary factors is one of the components that can significantly reduce CVD mortality.

This chapter will discuss dietary interventions to promote cardiovascular health with an emphasis on fiber and antioxidants from food. First, an overview of lipoprotein metabolism and oxidative stress will be provided to highlight how disturbances in these systems can influence CVD risk.

2.2 Cholesterol Metabolism

Cholesterol is essential for cellular functions such as bile acid synthesis, cell membrane structure, and as a precursor of adrenal steroids, gonadal steroids, and vitamin D. Its structure is composed of one hydroxyl group, four hydrocarbon rings and a branched hydrocarbon chain forming cyclopentano phenanthrene (33, 34). Cholesterol is synthesized in most tissues and can also be acquired through the diet. Cholesterol is carried in lipoprotein particles as cholesteryl ester and free cholesterol (33, 35, 36). Dietary and pharmacological interventions are able to interfere with cholesterol synthesis, catabolism and absorption, consequently impacting circulating cholesterol levels (35).

2.2.1 Cholesterol Synthesis

The cholesterol synthesis or mevalonate pathway starts with Acetyl coenzyme A (Acetyl CoA). The third step of the pathway involves 3-hydroxy-3-methyglutaryl coenzyme A (HMG CoA) reductase, the rate limiting enzyme for cholesterol synthesis that converts HMG CoA into mevalonic acid. Phosphate groups are incorporated into mevalonate at the expense of ATP producing isopentenyl pyrophosphate, when rearranged, results in the formation of dymethylallyl pyrophosphate. The addition of dimethylallyl pyrophosphate and isopentenyl pyrophosphate forms farnesyl

pyrophosphate. The combination of two farnesyl pyrophosphate forms squalene that once oxidized and rearranged produces lanosterol. After 19 reactions lanosterol is converted to cholesterol (34, 37). This process is energetically expensive, therefore cholesterol biosynthesis is tightly regulated. For instance, an increase in dietary cholesterol intake will down regulate hepatic cholesterol synthesis (37).

As the pool of cholesterol in the liver increases, the release of sterol regulatory element binding protein (SREBP) is inhibited. SREBP is involved in the transcription of genes involved in cholesterol metabolism, such as the LDL receptor and cholesterol biosynthesis enzymes, including HMG CoA reductase. Therefore, suppressing the release of SREBP will down-regulate LDL receptor expression and cholesterol biosynthesis. Down-regulation of LDL receptor causes LDL to be retained in plasma (38). At the same time that SREBP stimulates LDL receptor, expression it also enhances lipid synthesis, the balance between these opposing effects will influence circulating cholesterol concentrations (39).

2.2.2 Cholesterol Catabolism

Another pathway contributing to the maintenance of cholesterol homeostasis is the catabolism in the liver of cholesterol into bile acids, which are important emulsifiers involved in the process of lipid digestion (40). The first reaction involved in the classic pathway of bile acid synthesis is the hydroxylation of cholesterol by cholesterol 7α-hydroxylase (CYP7A1) encoded by the gene CYP7A1. Similar to HMG CoA reductase, CYP7A1 is a rate limiting enzyme. Bile acids exert a negative feedback on their own synthesis by inhibiting the expression of CYP7A1, again showing a tight regulation on cholesterol homeostasis. Whether dietary cholesterol inhibits or induces the expression of

CYP7A1 is still under debate, as a large variability in the response is observed depending on the animal model used (40).

Once released from the gallbladder, bile acids enter the enterohepatic circulation and can be either reabsorbed in the intestines or excreted in feces. One of the main mechanisms underlying the LDL-c lowering effect of fibers, plant sterols and some drug therapies is the interruption of the enterohepatic circulation of bile acids by inhibiting absorption. To counterbalance fecal bile acid loss, a higher proportion of cholesterol is converted into bile acids, leading to a decrease in LDL-c concentration (34, 40). Although the intraluminal cholesterol available for absorption is derived from both diet (exogenous) and bile (endogenous), the majority of cholesterol is derived from bile. In fact, the diet is thought to contribute with only 200 to 600 g per day of cholesterol to the intraluminal pool, while 800–1200 g per day of cholesterol is derived from bile (35, 41). Therefore inhibiting bile acid reabsorption can significantly impact hepatic cholesterol homeostasis.

2.2.3 Cholesterol Absorption

Dietary cholesterol is a lipid with a variable proportions of free and esterified cholesterol. Cholesteryl ester (10 to 15% of dietary cholesterol) requires hydrolysis by cholesterol esterase to a non-esterified form, as only free cholesterol is absorbed in the intestinal lumen (41). Due to its hydrophobic nature, free cholesterol needs to be incorporated into bile salt micelles which act as carriers and solubilizers prior to absorption (35, 36). Besides free cholesterol, micelles also carry triglycerides, monoacylglycerides, phospholipids, and fatty acids (36).

Micelle absorption in the proximal jejunum occurs by passive diffusion and protein-mediated processes. The Niemann-Pick C1-like 1 protein (NPC1L1) is the main transporter identified for cholesterol absorption. Other carriers involved in cholesterol absorption include the scavenger receptor B-I, and CD36 (35, 42). NPC1L1 is located at the brush-border membrane of the enterocyte, and its inhibition decreases the absorption of cholesterol and other sterols. In contrast to the NPC1L1 action, two ATP-binding cassette transporters (ABCG5 and ABCG8) are involved in cholesterol and plant sterols efflux back to the intestinal lumen (42). This results in limited cholesterol and phytosterol absorption, causing excretion of unabsorbed sterols (42, 43). Once cholesterol enters the enterocyte, it undergoes a re-esterification process by acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT 2) forming cholesteryl esters. About 70 to 80% of the cholesterol that reaches the lymph is esterified. While non-esterified cholesterol is more likely to be transferred back to the intestinal lumen via ABCG5 and 8, cholestereryl ester is incorporated into nascent chylomicrons and is released into the lymph, indicating that ACAT 2 is involved in an important step of cholesterol absorption. (33, 44).

Dietary factors may reduce cholesterol absorption through different mechanisms. Plant sterols (phytosterols and stanols) compete with cholesterol for limited micellar solubilization and interfere with transporter-mediated processes of cholesterol uptake (35, 45). While dietary fiber can bind to bile acids in the intestines and increase fecal excretion (35). This last mechanism will be further explained later in this chapter. According to the National Cholesterol Education Program Adult Treatment Panel III Report, 2 g/day of plant sterols and 5 to 10 g/day of viscous fiber reduces LDL-c by 6 to

10% and 3 to 5% respectively (46). Therefore dietary interventions are a major component of primary risk prevention of CVD (47).

2.3 Lipoproteins

Lipoproteins are three-dimensional structures that carry lipids and protein in plasma and are fundamental for lipid transport in the circulation. The structure of lipoproteins consists of a hydrophobic core comprised of cholesteryl esters and triglycerides, surrounded by a monolayer of phospholipids, unesterified cholesterol and apolipoproteins (Apo As, Apo Bs, Apo Cs, Apo D and Apo E). Lipoproteins are heterogeneous according to particle size, density, shape, content and function. The major lipoprotein classes according to physical-chemical characteristics are high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), and chylomicrons (33).

Lipoproteins are dynamic in nature and constantly exchange molecules (lipids and apoproteins) among each other altering their physical and chemical properties (33). This remodeling process involves the cholesteryl ester transfer protein (CETP) and the enzyme lecithin cholesterol acyl transferase (LCAT) (48). CETP transfers cholesteryl ester in exchange for triglycerides from HDL to LDL and other Apo B-containing lipoproteins (48, 49). LCAT is involved in the esterification of cholesterol in HDL particles promoting cholesteryl ester migration into the HDL core and further cholesterol efflux from tissues into HDL. This is important to cholesterol transport from the peripheral tissues to the liver (48, 50).

2.3.1 Lipoprotein Metabolism

Chylomicrons are the largest lipoproteins (75 – 1200 nm) and have the lowest density (0.95 g/dL) (33). They are synthesized in the intestine and require Apo B-48 to be assembled and secreted by mucosal cells into the lymph (38). They can also have other apoproteins including C-I, C-II, C-III, A-I, A-II and E (33). Chylomicrons acquire apoproteins, and free and esterified cholesterol mainly from HDL particles. Their main function is to transport dietary fat, mainly triglycerides (90% of its content) to the liver. While in circulation, triglycerides from chylomicrons are hydrolyzed by lipoprotein lipase (LPL) producing chylomicron remnants, a process that is activated by Apo C-II. The chylomicron remnants are quickly removed from circulation via a LDL-like receptor protein in the liver that can recognize Apo E and Apo B (33, 51). The half-life of chylomicrons in healthy subjects is short, approximately 4.8 hours (52).

Endogenous cholesterol is transported from the liver to the cells by Apo B containing lipoproteins (VLDL, IDL, and LDL). First, VLDL is synthesized in the liver and requires Apo B-100 for its assembly and secretion (51). This is a large lipoprotein (30-80 nm) with a low density (0.95 – 1.006 g/dL) (33). Besides one non-exchangeable Apo B-100, VLDL has Apo C-I, II and III and Apo E, mostly acquired from HDL particles. This lipoprotein carries mostly triglycerides (50 to 60%) to extrahepatic tissues and is the main carrier of triglycerides in the fasting state. The triglycerides in its core are converted to free fatty acid and mono/diglyceride as is hydrolyzed by LPL, allowing the uptake of free fatty acids by peripheral tissues (51, 53). This causes VLDL to become denser and smaller, giving rise to IDL (25-35 nm; 1.006 – 1.019 g/dL). This lipoprotein has a short half-life and is removed from circulation when recognized by LDL receptors

in the liver via Apo B and E (33, 51). Impaired clearance of chylomicron remnants may prolong the circulation time of VLDL particles due to a decrease in the availability of lipoprotein lipase (LPL), which hydrolyses triglycerides (53).

When IDL is not taken up by the liver, triglycerides in its core are further hydrolyzed by hepatic lipase (HL) and LPL producing LDL, the major cholesterol carrier in the circulation (51). The residence time of VLDL is approximately 3.6 hours and about 50% is converted into LDL, whereas the residence time of LDL is nearly 3.6 days (52). In comparison to VLDL, LDL has smaller size (18-25 nm) but higher density (1.019 – 1.063 g/dL) (33). Because it is derived from VLDL, its major protein is apo-B-100, its lipid core contains mainly cholesteryl ester and unesterified cholesterol (38). LDL particles are removed from circulation by LDL-receptors in the liver.

Lastly, HDL is the lipoprotein smallest in size (5-12 nm) and highest in density (1.063-1.21 g/dL) (33). Apo A-1 is its main protein (about 60%), in addition to Apo A-II, Apo A-IV, Apo CI-III, and Apo E. This is the only lipoprotein that does not carry Apo B (54). Nascent HDL is generated from Apo-AI secreted in the liver and intestines and is poor in cholesterol, but acquires phospholipids and free cholesterol quickly from extra-hepatic tissues. (49).

HDL is known to have anti-atherogenic actions, the main one being related to reverse cholesterol transport (49). In this process, HDL mediates the efflux of cholesterol from foam cells of extrahepatic tissues back to the plasma and eventually to the liver. This represents a critical step in the prevention or reversal of atherosclerosis (55). ATP binding cassette A1 (ABC1) is a transporter that, in combination with lipid poor apo-A1, promotes the efflux of free cholesterol from macrophages and extra hepatic tissues

forming nascent HDL. Free cholesterol is esterified by lecithin-cholesterol acyltransferase (LCAT), producing mature HDL particles. HDL particles become larger through the uptake of free cholesterol from macrophages and endothelial cells by ABCG1-mediated cholesterol efflux. Cholesteryl ester from the core of HDL can be up-taken by the scavenger receptor B1 (SR-B1) in the liver or transferred to apo-B containing lipoprotein in exchange for triglycerides in a process mediated by CETP (51, 55). Cholesterol in the liver can be recycled, used to produce sterols or excreted from the body in bile (38).

2.3.2 LDL Heterogeneity and Small, Dense LDL Particles

It is well known that an increased LDL-c concentration is highly correlated with CVD (56). However, lipoproteins are heterogeneous according to particle size, density and composition. Therefore, there is increasing attention to the relationship between lipoprotein subfractions and CVD risk (57).

Several approaches can be used to classify LDL particles. When based on their density, they tend to be grouped into large LDL particles (>213 Å), which are less atherogenic, and small, dense LDL particles (<212 Å). For individual subfractions, generally seven major LDL subclasses are identified. LDL-1 is the largest and least dense, and LDL-7 is the smallest and most dense. (58). In addition, the LDL is recognized to have three different phenotypes: phenotype A is characterized by a predominance of large LDL particles; phenotype B is characterized by small LDL particles; and an intermediate phenotype is a mix of patterns A and B (59). Phenotype B is often associated with increased triglycerides and decreased HDL-c concentrations, a condition also known as atherogenic lipoprotein phenotype (60).

It is recognized that small, dense LDL particles are more atherogenic than larger buoyant LDL particles. Small, dense LDL has been associated with numerous chronic diseases. In a case control study, type 2 diabetes has been associated with a twofold increase in the frequency of LDL phenotype B in comparison to healthy controls (61). Similarly, patients with metabolic syndrome showed significantly higher concentrations of small, dense LDL than individuals without this syndrome. Additionally, the concentration of cholesterol in small, dense LDL was directly related to the number of components of metabolic syndrome (62). Results from the Stanford Five-City Project showed significant smaller LDL peak particle diameter in patients with coronary artery disease (CAD, 26.2 ± 1.0 nm) compared with age, gender and ethnicity matched-controls (26.7 ± 0.9 nm) (63). In the Quebec Cardiovascular Study, after 5 years of follow-up, a 2.2-fold increased risk of ischemic heart disease was observed in men with smaller LDL particle size (<256 Å) compared to those with larger LDL particles (64). After 13 years of follow-up, the same study showed that this relationship was attenuated, especially if follow-up was longer than 7 years, indicating that small dense LDL phenotype may be a better predictor in the short term (65).

Alterations in the VLDL-IDL-LDL pathway as a result of diet, chronic disease or genetics will affect the LDL phenotype (66). For instance, insulin resistance or defects in adipose tissue can lead to an increase in free fatty acid levels, causing a greater uptake of free fatty acids by the liver. Consequently, the liver increases triglyceride and VLDL production and decreases Apo B hydrolysis (66, 67). An overproduction of VLDL is generally followed by an overproduction of IDL and LDL (66). As a result of the increased pool of triglyceride-rich VLDL, a greater exchange of triglycerides in VLDL

for cholesteryl ester in LDL takes place, mediated by CETP, producing cholesterol-depleted and triglyceride-enriched LDL. Subsequently, the triglyceride-enriched LDL is hydrolized by hepatic lipase and LPL, producing small, dense LDL particles. This process is generally accompanied by an exchange of cholesteryl ester in the HDL core for triglycerides leading to a triglyceride-enriched but cholesteryl ester-depleted HDL, which is more rapidly up-taken by the liver (51, 66, 67). The decreased removal of postprandial triglyceride-enriched lipoproteins (chylomicrons and chylomicron remnants) further leads to the formation of small, dense LDL because chylomicrons compete with VLDL for LPL, increasing the residence time of VLDL in the circulation (67).

There are several mechanisms that explain the atherogenic properties of small, dense LDL. The LDL receptor has a lower affinity for small, dense LDL compared to larger LDL particles, resulting in longer residence time in plasma for the former particles. In contrast, the small, dense LDL has a greater affinity for the scavenger receptor involved in the formation of foam cells and atherosclerosis (67). Additionally, the prolonged residence time and lower vitamin E content causes small, dense LDL particles to be more susceptible to oxidation and glycation (67, 68). Also, these particles can enter the arterial wall more readily and their high affinity for proteoglycans results in prolonged residence time in the vascular wall (68). Lastly, small dense LDL particles promote endothelial dysfunction by stimulating the production of plasminogen activator inhibitor I (PAI-I) and thromboxane A2 (67, 68).

Although small, dense LDL has been consistently related to CVD risk, most studies have not confirmed this LDL subfraction as an independent risk factor for CVD (69). Krauss et al. has pointed out that studies using the gradient gel electrophoresis

method have been more consistent in showing the benefits of assessing LDL subfractions, even after adjusting for other covariates (70). Nonetheless, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has included increased small, dense LDL as an emerging CVD risk factor (71).

2.3.3 HDL Heterogeneity

Having increased levels of large HDL particles may be an indicator of efficient reverse cholesterol transport due to the ability to carry more cholesterol in their core. Studies have shown an inverse relationship between low levels of large HDL particles and CVD risk (72-74). In the Framingham Offspring Study, patients with CHD had significantly lower concentrations of very large HDL and higher levels of very small HDL when compared to all healthy controls or low-HDL-c matched controls. In addition, the amount very large HDL had the strongest inverse association with CHD showing that for each mg/dl of very large HDL increase there was significant decrease in coronary heart disease (CHD) odds ratio by 26% (72). Similarly, among patients with low HDL-cholesterol concentrations as their primary lipid disorder in the Veterans Affair HDL Intervention trial (VA-HIT), having low levels of large HDL particles was a better predictor of CAD risk than HDL cholesterol concentrations (73). Recent results from a long follow-up study have demonstrated that both large and small HDL particles were inversely related to CHD risk; however a greater CHD risk reduction was associated with large HDL (74).

Besides the large HDL particles, some studies have acknowledged that small HDL particles may be inversely related to CHD risk while other studies have found no significant differences between HDL subfractions and CVD risk (75-77). Another study

using the VA-HIT follow-up data observed that the small HDL subclass, which constituted the majority of the total HDL particles in the sample studied, was associated with lower CHD risk (76). In the Caerphilly Study, total HDL-c and small HDL-c, but not large HDL-c, were inversely associated with CHD risk incidence. However, total HDL-c alone was a better predictor than both subfractions (75). Results from the Women's Health Study showed that women with CVD had significantly smaller HDL particles. However, HDL subfractions (large, medium or small) did not confer additional predictability for CVD. The best predictor of CVD was the total/HDL cholesterol ratio (77).

Some of the conflicting results described above might be explained by the fact that the multiple biological activities of HDL may be related to specific subfractions of HDL, as recently reviewed by Camont et al. (2011). Anti-oxidative activity has been linked to small, dense HDL that showed an increased ability to protect LDL from oxidative damage. It is suggested that the composition of small HDL has diminished sphingomyelin and free cholesterol. This confers increased fluidity to the lipid surface of the small HDL, facilitating the incorporation of oxidized lipids. The anti-inflammatory activity of HDL is also associated with small HDL, which seems to be related to differences in phospholipids composition. Phospholipids with variable fatty acid chain length and unsaturation differ in their ability to inhibit inflammation. Some of the effects includes inhibition of monocyte activation and adhesion to the endothelium and inhibition of adhesion molecule expression in endothelial cells. Both large and small HDL particles are associated with antithrombotic activity. Large HDL is involved with inhibition of platelet aggregation and small HDL presents an anticoagulant activity. In

addition, the vasodilatory activity is associated with large HDL which attenuates the production of thromboxane A2, a vasoconstrictor. Finally, cholesterol efflux from macrophages can be mediated by ABCA1 and is related to small, dense HDL, which also activates LCAT. The large HDL promotes cholesterol efflux from macrophages thorough ABCG1 and SR-B1 (78).

2.4 Oxidative Stress

Oxidative stress has been implicated as the underlying cause in the development and progression of atherosclerosis (79). The process of oxidative stress takes place when the generation of ROS exceeds the protective capability of the antioxidants defense. This process can be induced by hyperglycemia, free fatty acids and insulin resistance. ROS are highly reactive due to their atomic structure containing one or more unpaired electrons such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and peroxynitrite ($OONO^-$). The increased production of ROS results in protein, lipid and DNA damage, and particularly affects endothelial cells (19). The antioxidant defense is composed of non-enzymatic compounds, such as vitamins (A, C, and E) and minerals (copper, zinc, and selenium), and enzymatic mechanisms like superoxide dismutase (SOD), catalase and glutathione peroxidase (18, 19).

ROS have important biological functions by assisting the immune system, mediating cell signaling and playing a critical role in apoptosis (80). However, substantial evidence shows that excess ROS formation is associated with chronic diseases such as diabetes and CVD. Davi et al., have reported significantly higher oxidative stress in patients with Type 1 and Type 2 diabetes by approximately twofold in comparison to age-

matched controls (81). In a case-controlled study, Monnier et al., observed a higher production of 8-iso prostaglandin F2, a marker of oxidative stress, in patients with diabetes compared to healthy controls, as well as a significant relationship between glucose fluctuations and oxidative stress activation ($r=0.86$, $p>0.001$) (82). In addition, markers of oxidative stress are higher in subjects with CHD, and might be employed to evaluate the status of chronic heart failure (HF) (83). According to Nagayoshi et al., in a study that evaluated oxidative stress in subjects with and without CHD, increased levels of urinary 8-hydroxy-2′-deoxyguanosine, and in vivo oxidative DNA damage were associated with the severity of HF and the progression of atherosclerosis (84).

2.4.1 Reactive Oxygen Species Sources

Enzymes from numerous cells have the ability to constantly produce low levels of ROS. These enzymes, including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase and uncoupled nitric oxidase synthase (NOS), are tightly regulated under normal conditions. Under certain disease conditions, such as atherosclerosis, diabetes, and hypertension, excessive superoxide production takes place (85-87).

NADPH oxidase is an important source of superoxide in endothelial and smooth muscle cells. This enzyme helps regulate intracellular signaling cascades. However, the over-stimulation of NADPH oxidase by angiotensin II, thrombin, platelet-derived growth factor, TNF-α and sheer stress causes an increase in superoxide production (85, 86). The xanthine oxidase is present in circulation and endothelial cells, producing superoxide and hydrogen peroxide stimulated by cytokines (87). This enzyme seems to be a major contributor to oxidative stress in ischemia and reperfusion injuries (85). Another source

of superoxide is the mitochondrial transport chain. The increase in the electron donors flux into the electron transport chain can be stimulated in endothelial cells by angiotensin II, oxidized LDL, protein kinase C, and high glucose and free fatty acids levels. Once the voltage across the mitochondrial membrane reaches the critical threshold, the electron transfer is blocked causing the electron to be donated to an oxygen molecule, thus producing superoxide (85, 87). Lastly, increased NOS is involved in ROS production in endothelial cells. Nitric oxide (NO) is an endothelium-derived relaxing factor with anti-atherosclerotic properties. NO has the capability to inhibit platelet aggregation, proliferation of smooth muscle cells, and the expression of adhesion molecules. These processes are involved in the development and progression of atherosclerosis. Increased NOS may indicate a limited capacity of the enzyme to produce NO (87). Under certain conditions, NO can be converted to various reactive nitrogen species, such as peroxynitrite. The uncoupling of NO is associated with atherosclerosis, hypercholesterolemia, diabetes and hypertension (86).

2.4.2 Antioxidant Sources

Antioxidants are molecules or systems that can delay or inhibit a biological target against oxidative damage. The antioxidant defense system is composed of enzymatic and non-enzymatic mechanisms that work in synergy with each other to counteract free radicals (18, 88, 89). Mechanisms of action include catalytic removal of ROS, binding of proteins to pro-oxidant metal ions, protection against protein damage, and reduction of free radicals by electron donors (88, 90).

The major enzymes involved in the antioxidant defense system are SOD, catalase and glutathione peroxidase. SOD converts superoxide into hydrogen peroxide, reducing

the chance of peroxynitrite formation from the reaction with nitric oxide. This enzyme utilizes transition metals such as copper, zinc and manganese, as cofactors in its active sites. Subsequently, catalase converts hydrogen peroxide produced by SOD into water and oxygen, preventing the formation of hydroxyl radical. Glutathione peroxidase (GSH-Px), which is selenium dependent, also removes hydrogen peroxide by coupling its reduction with the oxidation of GSH (reduced form of glutathione) to GSSG (oxidized form of glutathione). Glutathione is important for the regeneration of vitamins C and E (18, 88, 90, 91).

The human diet has several compounds known for their antioxidant properties such as ascorbate (vitamin C), tocopherols (vitamin E), carotenoids, and polyphenols (88). Minerals, such as selenium, copper, zinc, and manganese act as cofactors for antioxidant enzymes (90). Ascorbic acid is a water-soluble vitamin found in fruits and vegetables, especially citrus fruits, peppers, kiwi, cherries, melons, tomatoes, leafy greens, broccoli, cauliflower, brussels sprouts, and kale. Ascorbic acid is able to scavenge oxygen and chelate pro-oxidant metal ions such as Fe^{2+} and Cu^{2+}. When ascorbic acid is oxidized by ROS it is transformed into dehydro-ascorbate, which can be recycled back into ascorbic acid by the enzyme dehydro-ascorbate reductase (88). Ascorbic acid is also capable of regenerating tocopherol from the tocopheroxyl radical generated in LDL particles from inhibiting lipid peroxidation (92). Tocopherols are lipid soluble vitamins found in vegetable oils (soybean, sunflower, olive oil), wheat germ, nuts, and some green leafy vegetables (88). The most active form of vitamin E is α-tocopherol. Its main function is to inhibit lipid peroxidation by scavenging peroxyl radicals and preventing further reactions (88, 93). Carotenoids are lipid soluble vitamins present in fruits and

vegetables, such as carrots, tomatoes, citrus fruits, spinach and corn (88). Their antioxidant properties are related to the ability to scavenge radicals and quench singlet oxygen atoms (94). Polyphenols (flavonoids and phenolic acids) are a group of compounds known for their water soluble antioxidant properties. Over 8,000 different polyphenolic compounds have been described and are found in plant foods and their products, such as chocolate, green tea, berry and wine (91, 95). The antioxidant effects of polyphenols are related to free radical scavenging activities, through which free radicals are converted to a relatively harmless form, and to the chelation of pro-oxidant metal ions (96).

In summary, the most common non-enzymatic antioxidant mechanisms are: (1) free radical scavenging activity; (2) chelation of pro-oxidant metal ions such as Fe^{2+} and Cu^{2+}, preventing Fenton reactions; (3) quenching singlet oxygen; (4) breaking the free radical chain reaction; and (5) reducing local oxygen concentrations (97). In addition, there is evidence of indirect antioxidant effects. One example is that polyphenols can up-regulate endogenous antioxidant enzymes such as SOD, catalase and glutathione peroxidase. Another mechanism is the modulation of cell signaling of inflammatory pathways such as the down-regulation of nuclear factor-kappa B (NF-κB), which will inhibit several cytokines. Finally, changes in chromatin remodeling through the activation of histone deacetylase will inhibit the expression of pro-inflammatory genes (98, 99).

There is an increasing interest in the Total Antioxidant Capacity (TAC) which accounts for the cumulative action of all antioxidants available in plasma and body fluids. This measurement provides information on the antioxidant capacity of known and unknown compounds and their synergistic effects. Different methods have been

developed to measure the TAC, including total radical-trapping antioxidant parameters (TRAP), oxygen radical absorbance capacity (ORAC), trolox equivalent antioxidant capacity (TEAC), and ferric reducing ability of plasma (FRAP) (100).

2.4.3 Oxidized LDL

Oxidative stress causes damage to several biological components including lipids, protein and DNA. Of particular importance is the lipid oxidation that occurs in LDL, producing oxidized LDL (OxLDL). Lipid peroxidation has been shown to initiate and accelerate the atherosclerotic process (17, 101, 102). Increased OxLDL concentrations are associated with several chronic diseases such as CVD (101, 103), type 2 diabetes (104, 105), and metabolic syndrome (106).

In a cross-sectional study OxLDL was positively correlated with acute coronary syndrome severity. In a sequence of highest to lowest OxLDL concentrations were individuals with acute myocardial infarction, followed by unstable angina pectoris, stable angina pectoris, and healthy control (101). Similarly, Weinbrenner et al. observed that individuals with stable CHD had 67% higher concentrations of OxLDL compared to sex-matched healthy controls (103). In diabetic patients, OxLDL was positively related to diabetes duration. An OxLDL concentration of those with prolonged diabetes was almost two times higher compared to newly diagnosed patients, even with LDL-c maintained at a desirable level (105). Results from the Coronary Artery Risk Development in Young Adults (CARDIA), a population-based 15-year follow-up study, showed that increased OxLDL was significantly associated with incidence of Metabolic Syndrome. Individuals in the upper quintile of OxLDL were 3.5 times more likely to develop Metabolic Syndrome than individuals in the first quintile. When evaluating each Metabolic

Syndrome components individually, central obesity, hyperglycemia, and hypertriglyceridemia were associated with increased OxLDL (106).

The process of LDL oxidation is progressive and causes the formation of mildly to extensively OxLDL (107). Monocytes migrate into the subendothelial space where they can differentiate into macrophages that are able to incorporate lipoproteins. OxLDLs are readily taken up by macrophages due to their increased affinity to receptors such as the scavenger receptor A1 (SR-A1), CD36, and the lectin-like oxidized low-density lipoprotein receptor (LOX-1) (16, 108). This leads to the accumulation of cholesterol in the macrophages, which eventually results in foam cell formation. Fatty streaks are formed by the accumulation of foam cells in the subendothelial space, leading to endothelial inflammatory response and atherosclerotic lesion development (16). OxLDL contributes to the formation of a fibrous cap over the fatty streak and stimulation of the fibrous cap rupture (109). In addition, OxLDL induces toxic effects in all stages of atherosclerosis including endothelial cell apoptosis, increased expression of adhesion molecules, impairment of eNOS activation, and increased formation of platelet clots (107, 109). For those reasons, OxLDL is more pro-atherogenic than native unmodified LDL. Therefore, strategies to decrease LDL oxidation may prevent the development and progression of atherosclerosis (109, 110).

2.5 Dietary Interventions and Cardiovascular Disease Risk

Dietary quality contributes to chronic disease prevention and diet is an important modifiable risk factor (3, 11). The Dietary Guidelines for Americans (111) and the AHA (47) recommend increased intakes of fruits, vegetables and whole grains. However, the intake of fruits and vegetables among most Americans is lower than the recommended

five daily servings. For example, in 2007 only 24% of Americans reached the suggested intake (12). Similarly, the mean intake of total fiber in 2007-2008 was of 15.9 g/day, well below the recommended Adequate Intake (25 to 38 g/day or 14 g/1,000 kcal/day) (13). The health benefits of fruits and vegetables can be partially explained by the presence of antioxidant vitamins, phytochemicals and fiber. Therefore, the impact of dietary interventions on cardiovascular outcomes with an emphasis on naturally occurring dietary fibers and antioxidants will be further discussed.

2.5.1 Dietary Fiber

Dietary fibers are oligosaccharides, polysaccharides and lignin that are resistant to digestion and absorption in human's small intestine (112). The main sources of dietary fiber in the human diet are fruits, vegetables, cereal products, and legumes. Dietary fiber can be categorized according to its chemical and physical properties, particularly water solubility. Soluble fiber, also known as viscous fiber, causes gel formation and fermentation. Examples of this type of fiber include pectin, β-glucans, gums, and mucilages. Lignins, cellulose, and hemicellulose are examples of insoluble/nonviscous fiber (113).

Observational studies have shown an association between fiber intake and lower CVD incidence and mortality (114-117). In a population-based cohort study in which older (>65 years of age) men and women free of chronic disease at baseline were followed for almost 9 years, cereal fiber intake was associated with a 21% lower risk of incident CVD comparing the highest with the lowest quintile intake. However the same relationship was not observed with fiber provided by fruits and vegetables (114). Similar results were observed for mortality risk by the National Institutes of Health–American

Association of Retired Persons Diet and Health Study, during a 9 year follow-up. Dietary fiber intake was significantly associated with a lower risk of all-cause mortality, showing a 22% risk reduction for men and women in the highest fiber intake quintile compared to the lowest quintile. In addition, higher dietary fiber intake was associated with lower cardiovascular mortality risk by 24% in men and 34% in women. However, when evaluating specific sources of fiber, only grains were consistently associated with lower risk of total and cause-specific mortality (115). Bellavia et al. evaluated the dose-response relationship between fruit and vegetable intake and all-cause mortality during a 13-year follow-up study. Not consuming fruits and vegetables was associated with a 53% higher mortality rate than consuming five or more servings a day. Those consuming at least one fruit a day lived 19 months longer than those not consuming fruits, but consuming more than one fruit a day was not associated with additional survival. Whereas individuals consuming three vegetables a day lived 32 months longer than individuals not consuming vegetables (116). A meta-analysis of eight cohort studies ranging from 8 to 19 years of follow-up evaluated the relationship between fiber intake in healthy participants and first hemorrhagic or ischemic stroke. The results indicated that increasing dietary fiber intake by 7g/day is associated with a 7% risk reduction of primary stroke. However, not enough scientific evidence was available to clarify if a specific food source and/or type of fiber is more beneficial for stroke prevention (117).

Observational studies have also assessed the relationship between CVD risk factors and dietary fiber intake (118-121). In a prospective cohort study following pre-menopausal women, fiber consumption of at least 22 g/day was associated with lower total cholesterol (-8%) and LDL cholesterol (-8%) levels, but not triglycerides and HDL-c

(121). Another cohort study with postmenopausal women showed that higher intakes of cereal fiber was associated with a smaller declines in minimum coronary artery diameter, a measure of progression of CAD. Other sources of fiber did not present beneficial effects (119). A baseline assessment from the PREDIMED trial (Prevención con Dieta Mediterránea) of men and women at high cardiovascular risk showed a modest but significant association between intima-media thickness (IMT) and total dietary fiber intake (p=0.03). However, the only specific food group inversely associated with IMT was fruit intake (p=0.007) (120). In a study including participants free of heart disease, the progression of atherosclerosis evaluated according to IMT was inversely associated with the intake of total fiber and viscous fiber, but not nonviscous fiber. Pectin, a type of viscous fiber which is mainly present in fruits and vegetables, presented the strongest inverse association (118). Nonetheless, it is important to notice that a major limitation of observational studies is the reliance on dietary self-report; additionally they do not provide information on causality.

Randomized controlled trials (RCTs) can provide valuable information regarding the effect of different types of fiber on CVD risk factors. According to a meta-analysis of RCTs conducted by Brown et al. (1999), soluble fiber showed to be more effective at reducing cardiovascular risk factors. An increase in soluble fiber intake by 3 g/day is associated with a 5 mg/dL decrease in total cholesterol and LDL-c (122).Although a small change, this could reflect a 4% reduction of coronary heart disease incidence. In addition, similar effects were observed from the different types of soluble fiber (oat, psyllium, and pectin) (122). Two types of soluble fiber, psyllium and β-glucan, have been approved for health claims related to CVD by the Food and Drug Administration (FDA)

(123). The assumption is that consuming four servings a day of foods rich in β-glucan (1.78 g/serving) and psyllium (0.75 g/serving) reduces CVD risk. A randomized crossover study including 68 hyperlipidemic adults tested the effects of a diet high in psyllium and β-glucan (8 g/day) in comparison to a control low fat diet on serum lipid risk factors. Modest improvements in total cholesterol (-2%, p=0.001) and triglycerides (-5%, p=0.001), but not LDL-c (-1%, p=0.064) were observed in the high fiber diet compared to control (123). In 367 hypercholesterolemic individuals the consumption of oat β-glucan cereal significantly lowered LDL-c from 4.7% to 6.5%. A higher molecular weight (MW) oat β-glucan was more efficient at reducing LDL-c than lower MW due to its increased viscosity (124). Barley is another source of β-glucan that has been studied for its effects on blood lipids. In a 10-week RCT, barley β-glucan of high and low MW (3 g/d and 5 g/d) were administered to 155 hypercholesterolemic individuals. LDL-c was reduced by 9% with 3 g/day of both high and low MW barley β-glucan, and by 13% to 15% with 5 g/day of low and high MW barley β-glucan respectively (125). The long-term (26 weeks) effect of psyllium husk fiber as an adjunct diet therapy to the AHA step 1 diet was evaluated in 248 individuals with hypercholesterolemia. In comparison to control, the psyllium group presented 4.7% lower total cholesterol and 6.7% lower LDL-c after 26 weeks of intervention (126). As noted by Brown et al., simultaneous changes in the diet associated with the displacement of other nutrients (i.e. saturated fat, cholesterol) by fiber rich sources can confound the relation between fiber intake and blood lipids concentrations (122).

The mechanisms by which soluble fiber can reduce blood cholesterol levels are related to some of its characteristics such as viscosity and fermentability. The

hypocholesterolemic effect of soluble fiber is primarily due to its ability to disrupt the enterohepatic circulation of bile salts and cholesterol by binding them in the intestinal lumen, which results in reduced absorption and consequently increased fecal excretion. In response, the liver up-regulates bile acid synthesis through the uptake of free cholesterol from the circulation. In turn, this causes a concomitant decrease in LDL-c concentrations (127-129). Another mechanism of action of soluble fiber is caused by the reduction in insulin secretion due to delay in gastric emptying and decreased absorption of glucose and other macronutrients. This leads to decreased insulin response as a consequence of lower postprandial glucose concentrations. The lower insulin response results in the inhibition of HMG-CoA reductase and consequently decreased hepatic cholesterol synthesis (128, 130). Lastly, soluble fiber undergoes bacterial fermentation in the colon, producing short chain fatty acids (SCFA) particularly acetate, propionate and butyrate. The ratio of propionate to acetate may affect lipid metabolism. Propionate has been shown to reduce hepatic cholesterol synthesis by inhibiting HMG-CoA reductase and by reducing acetate availability and incorporation into serum lipids (128, 129).

2.5.2 Dietary Antioxidants

Adequate fruit and vegetable intake is associated with a lower risk of several chronic diseases including CVD and type 2 diabetes (131-133). In part, this may be due to the antioxidant properties of several compounds commonly found in fruits and vegetables, including carotenoids, vitamins A, C, E, and polyphenols (87, 134). It has been demonstrated that the consumption of antioxidant-rich fruits and vegetables increases overall antioxidant status (135). The process by which the mobilization of

antioxidants counterbalance excessive ROS formation is fundamental to avoid redox balance pro-oxidant conditions (19). As previously explained, antioxidants work in synergy with each other and against ROS through different mechanisms such as inhibiting lipid peroxidation and hydroperoxide formation, and scavenging free radicals (18).

A report by Miller, showed that a diet high in fruits and vegetables and low in fat caused a decline in oxidative stress and increased antioxidant capacity measured by isoprostanes and oxygen radical absorbing capacity (ORAC) respectively (136). Similarly, in a study that evaluated the Dietary Approaches to Stop Hypertension (DASH) diet, which is rich in fruits and vegetables, consumption of the diet for four weeks was associated with a 23% increase in the antioxidant capacity (FRAP) among obese hypertensive patients. However, the same association was not observed in lean normotensive participants (137). Whereas in a crossover intervention including 33 healthy adults, food selection based on high antioxidant content for two weeks increased plasma α-tocopherol, but not plasma TAC. Nonetheless, improvements were observed in CRP levels and liver function (alanine aminotransferase, gamma-glutamyltranspeptidase, and alkaline phosphatase) (138).

Polyphenols are one of the main dietary antioxidants with a total intake estimated to be ten times higher than that of vitamin C (139). Flavonoids are the most common group of plant polyphenols with an estimate daily total intake of 1 g/day. Commonly consumed flavonoid-rich foods includes cocoa, tea, highly colored fruits and vegetables and its derivate (i.e. grapes, berry, red wine) (140). In a large prospective study including older men and women, intakes of flavonoid in the highest quintile were associated with a

22% lower CVD mortality risk. Individual flavonoids, such as anthocyanidins, flavan-3-ols, flavones, flavonols, and proanthocyanidins, were also associated with lower CVD mortality risk (141). Similar results were observed in the Women's Health study with 16 years of follow-up. Flavanones and anthocyanidins were inversely associated with CHD and CVD mortality, but not stroke mortality. When analyzing specific foods, apples, pears and red wine were associated with lower CHD and CVD mortality. Whereas grapefruit intake was associated only with reduced CHD mortality, and chocolate and strawberries to reduced CVD mortality (142).

Randomized controlled trials have been conducted in order to test the effects of specific flavonoid-rich foods on oxidative stress and other CVD risk factors. Baba et al. randomly assigned 25 individuals to a either placebo or 26g of cocoa per day for twelve weeks. The intake of cocoa powder increased the lag time of conjugated dienes production indicating a lower susceptibility to LDL oxidation in comparison to the control group. However, oxidized LDL was not significantly reduced after the intervention (143). In a randomized crossover study, 23 individuals were fed two diets (four weeks each) with similar macronutrient composition, fiber and caffeine intake. One of the diets was supplemented with cocoa powder and dark chocolate (CPDC diet). Antioxidant capacity, measured by ORAC, increased by 4% and LDL oxidation lag time increased by 8% in the CPDC diet (144). In another randomized crossover trial, the acute consumption of dark chocolate bar, liquid cocoa and a cocoa-free placebo was evaluated in 45 overweight adults. Flow-mediated dilation (FMD) was improved by 4.3% after dark chocolate bar consumption and by 5.7% after sugar-free cocoa consumption (145).

In vitro studies with tea, a rich source of the flavonoids catechins, have shown to inhibit LDL oxidation and platelet aggregation (146). In vivo, Hirano-Ohmori et al. measured serum malondialdehyde-modified LDL (MDA-LDL) and 8-epi-prostaglandin-F2α (8-epi-PGF2α), measures of oxidative stress, after a consumption of 7 cups a day of green tea for two weeks in 22 healthy males non-smokers. At the end of the green tea period the MDA-LDL concentrations were significantly decreased. However, the 8-epi-PGF2α was not significantly altered after the intervention (147). This is consistent with a crossover study that evaluated the effects of green tea, black tea, and hot water with caffeine (1000ml/day) for seven days each on urinary F2-isoprostane excretion, a measure of lipid peroxidation. No change in isoprostanes was observed after the green or black tea intervention (148). In another study, green tea consumption by 14 healthy individuals showed an acute effect by decreasing FMD by 3.7% (p=0.02), however no changes were observed for inflammatory markers (CRP, IL-6) or antioxidant capacity (TAC) (149).

Grapes are a good source of flavonoids, such as catechin, quercetin, and anthocyanins. Therefore Stein et al. evaluated the intake of grape juice for 14 days in adults with coronary artery disease. A significant improvement in FMD and a reduction in LDL susceptibility to oxidation were observed after the grape juice period even after adjustments for lipid lowering and antioxidant therapies (150). Another study compared the effects of grape juice and α-tocopherol for two weeks on markers of oxidative stress in healthy individuals. Both interventions had similar effects and significantly increased ORAC and LDL lag time to oxidation. However, neither α-tocopherol nor grape juice intake significantly changed urinary F_2-isoprostane concentrations after two weeks of

intervention (151). In contrast, in a double-blind crossover trial older adults consuming one cup of either tart cherry juice or placebo for 14 days observed significantly lower F_2-isoprostanes after the cherry juice phase in comparison to placebo (152). Pignatelli at al. randomly assigned healthy individuals to drink red or white wine with the same alcohol percentage for 15 days. A significant decrease in urinary PGF2α was observed in both wine groups; however, it was more pronounced in the red wine group. Plasma polyphenols was higher in the red wine group. In addition, at the end of the intervention an inverse correlation between excretion of PGF2α and polyphenols was observed (153). A randomized cross-over study, Estruch et al. assigned healthy men to consume wine or gin for 28 days. The wine group presented a significant reduction in plasma SOD activity and MDA levels in comparison to the gin group. In addition, there was a significant decrease in the lag time of LDL oxidation and in oxidized LDL concentrations (154).

A meta-analysis of randomized controlled trial data showed that the consumption of some flavonoid-rich foods was associated with lower cardiovascular risk factors. Chocolate and cocoa were associated with improved FMD acutely (4%), and systolic (-5.9 mm Hg) and diastolic (-3.3 mm Hg) BP after chronic intake. On the other hand, black tea intake caused an acute increase in systolic and diastolic BP independent of caffeine content. Chronic green tea consumption was associated with reduced LDL-c concentrations (-8.9 mg/dL). In addition, the authors emphasized that there is limited data from high quality intervention trials to assess potential efficacy of other flavonoid-rich sources such as grapes and wine on CVD risk factors; therefore more studies are warranted (155).

2.5.3 Effects of *Nopales* (*Opuntia spp*) and Prickly Pear Intake

Nopales are the prickly pear cactus pads from the *Opuntia* species. They are a traditional and popular food in Mexico with a total yearly production of 600,000 tons (25). They are low in fat and high in total (2.0 g/100 g) and soluble fiber (0.9 g/100 g). Moreover, they are a good source of vitamin A (443 IU/100 g), β-carotene (242 mg/100 g), vitamin C (5.3 mg/100 g), and phenolic compounds (8-9 mg/100 g) (24, 25). According to a study evaluating the availability of culturally specific fruits and vegetables in Chicago, approximately 23% of grocery stores carry cactus pads (156). Prickly pear cactus pads are commonly used as a medicinal plant for glycemic and cholesterol control among Mexicans. When evaluating the use of complementary and alternative medicine among diabetic patients of different ethnicities, 33% of Hispanics reported the use of prickly pear cactus as an adjunct therapy for diabetes (157).

The literature documenting *nopales'* hypocholesterolemic effects is scarce. Most published studies have focused on cactus fruit (prickly pears) (27-29) rather than the more commonly consumed pads (*nopales*). In two separate studies with hypercholesterolemic or hyperlipidemic individuals, prickly pear intake (250g/day) lowered total and LDL-c by about 10% (27, 28). The effects were greater with a longer supplementation period (eight weeks vs. four weeks). In addition, in hyperlipidemic subjects the consumption of prickly pears led to an 11% decrease in both fasting blood glucose and insulin (28). The only study evaluating the effects of actual cactus pads (*nopales*) on lipids and glycemia is a 1983 study from Mexico that included 8 healthy, 14 obese, and 7 diabetic subjects from 26 to 65 years of age who consumed broiled *nopales* (300 g/day for ten days) before the three main meals. In this study there was a significant

reduction in total cholesterol by 14%, triglycerides by 24% relative to baseline in all subjects, and fasting glucose by 22% in diabetic subjects (26). Another study evaluated the postprandial glucose response in subjects with type 2 diabetes after the consumption of three types of Mexican breakfasts with and without *nopales*. The incremental area under the curve for glucose was reduced by 20 to 48% in all breakfasts containing *nopales* compared to the same breakfast without *nopales* (158).

Some studies evaluating the effects of cactus fruit have also investigated its effects on oxidative stress. In a study with young individuals suffering from familial heterozygous isolated hypercholesterolemia, consumption of prickly pears for four weeks was associated with lower oxidative stress as measured by an 8%, 6% and 9% decrease in plasma, serum and urinary isoprostanes, respectively ($p<0.05$) (27). In a shorter study (two weeks) with healthy adults, prickly pear intake (250g) significantly improved markers of oxidative stress relative to vitamin C supplementation (75 mg), resulting in lower malondialdehyde (-74%), F2-isoprostanes (-29%), ratio of reduced to oxidized glutathione (GSH/GSSG; 49%), and conjugated dienes lipid hydroperoxide (-40%) (29). Studies evaluating the effect of *nopales* cactus pads on oxidative stress are not available.

More recent studies incorporated *nopales* into the diet as a supplement and showed disparate effects on markers of CVD. Guevara-Cruz et al. evaluated the intake of a drink containing dehydrated *nopales*, chia seeds, oats and soybean protein in participants with metabolic syndrome. Triglyceride levels were reduced by 15% and CRP levels by 18% in the intervention group; however, no changes were observed for total cholesterol, HDL-c, LDL-c, glucose and insulin (159). In a crossover study, healthy participants consumed tortillas made with ground *nopales* and bars with prickly pear fruit

jam twice a day for three weeks each. Increased levels of trolox-equivalent antioxidant capacity (TEAC; 11%), polyphenols (8%), and vitamin C (25%) were observed after the *nopales* tortillas phase. While only vitamin C (25%) was increased after the prickly pear bar phase. After the *nopales* tortilla phase and prickly pear phase decreases in malondialdehyde (MDA; 25% and 12%), glucose (18% and 13%) and LDL-c (17% and 7%) were observed respectively. The consumption of *nopales* tortilla also showed reductions in total cholesterol (9%) and triglycerides (10%) (160).

Despite the purported benefits, little is known about potential risks associated with consumption of prickly pear cactus. There is documentation of adverse effects among patients with type 2 diabetes taking prescription medication concomitantly with prickly pear cactus. According to a study by Bush et al., out of 804 diabetic patients, 15% used herbal medicine. Potential adverse interactions were observed in 40% of the herbal medicine users. Eight cases were of hypoglycemia caused by *nopales* intake (161). A case of a 58-year-old male with type 2 diabetes treated with metformin and glipizde have been described. The patient reported consuming prickly pear cactus daily for two months and four cases of hypoglycemia were observed during that time, with blood glucose reading ranging from 49 to 69 mg/dL (162).

CHAPTER 3

METHODOLOGY

3.1 Study Design

In this randomized crossover trial, participants were randomly assigned to supplementation for two weeks with 2 cups/day of *nopales* (280 g; intervention) or cucumbers (266 g; control), divided into two 1-cup daily doses for each of their two main meals. After a two- to three-week washout period, participants received the alternative treatment for an additional two weeks (**Figure 1**). Participants were asked to refrain from making any additional changes to their usual diet throughout the duration of the entire study. The study was approved by Arizona State University's Institutional Review Board (Appendix A).

3.2 Participant Selection

The sample was comprised of 27 adults (18 - 70 y) with moderate hypercholesterolemia (LDL-c ≥ 120 mg/dL). Participants were excluded as follows: (a) use of hypolipidemic medications; (b) regular physical activity (≥ 30 min /day for ≥ 5 days/week), (c) presence of known chronic diseases (e.g., diabetes, CVD, cancer, hepatitis, inflammatory conditions, gastrointestinal disorders); (d) consumption of > 4 servings/day of fruits and vegetables; (e) following a restrictive diet (e.g., carbohydrate restriction, veganism) or having any condition likely to require specialized dietary modifications; (f) use of supplements (antioxidants, fiber and botanicals); (g) latex allergy; (h) fear of needles; (i) breastfeeding, pregnancy, or intent to become pregnant; (j)

unwillingness to comply with study protocol; or (k) participation in other research studies. Subjects interested in participating in the study were pre-screened by a questionnaire to identify those that met the inclusion criteria (Appendix B).

Potential participants who met the inclusion criteria signed a screening consent form (Appendix C) in which they agreed to be involved in the screening process. They attended a screening blood draw after a 12-hour fast to verify the presence of moderate hypercholesterolemia (LDL-c ≥ 120 mg/dL). The entire protocol was explained to individuals who met all inclusion criteria and participants provided written consent for the full study prior to enrollment (Appendix D).

Figure 2 shows the Consort flow diagram for the present study. A total of 277 subjects were assessed for eligibility of which 75 were eligible and agreed to be screened for moderate hypercholesterolemia. Of those, 30 participants were eligible for the study, of which three individuals decided not to participate and 27 participants provided written consent to participate. After providing informed consent, but prior to baseline data collection, four participants were no longer interested in the study and did not participate in any study procedures. Twenty three participants started the intervention and 16 completed the study. The reasons for dropping out included dislike of the study food (*nopales*, n=3), lack of time (n=1), lost to follow up (n=2), and accident non-related to the study (n=1).

Figure 1. Study Flow Chart

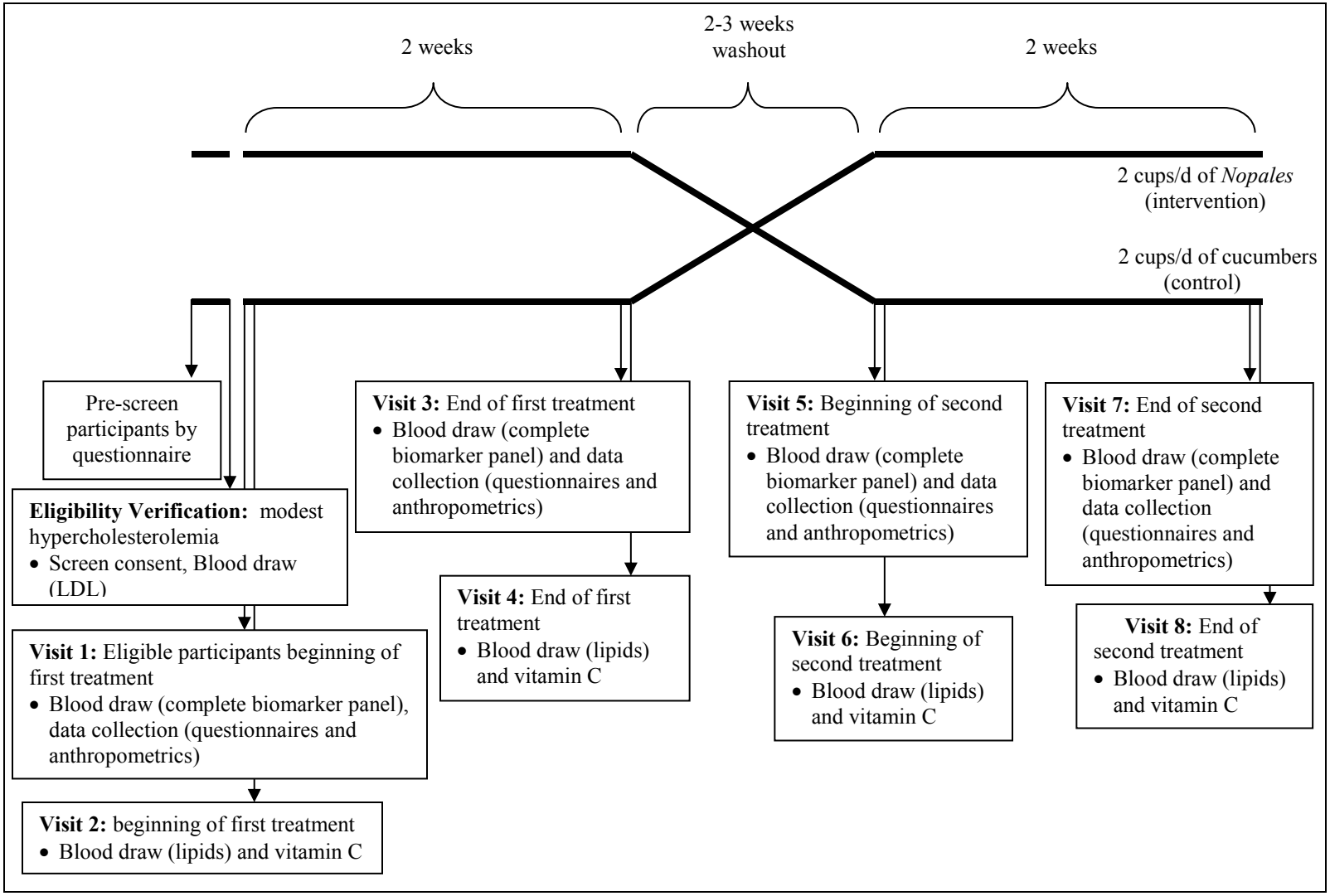
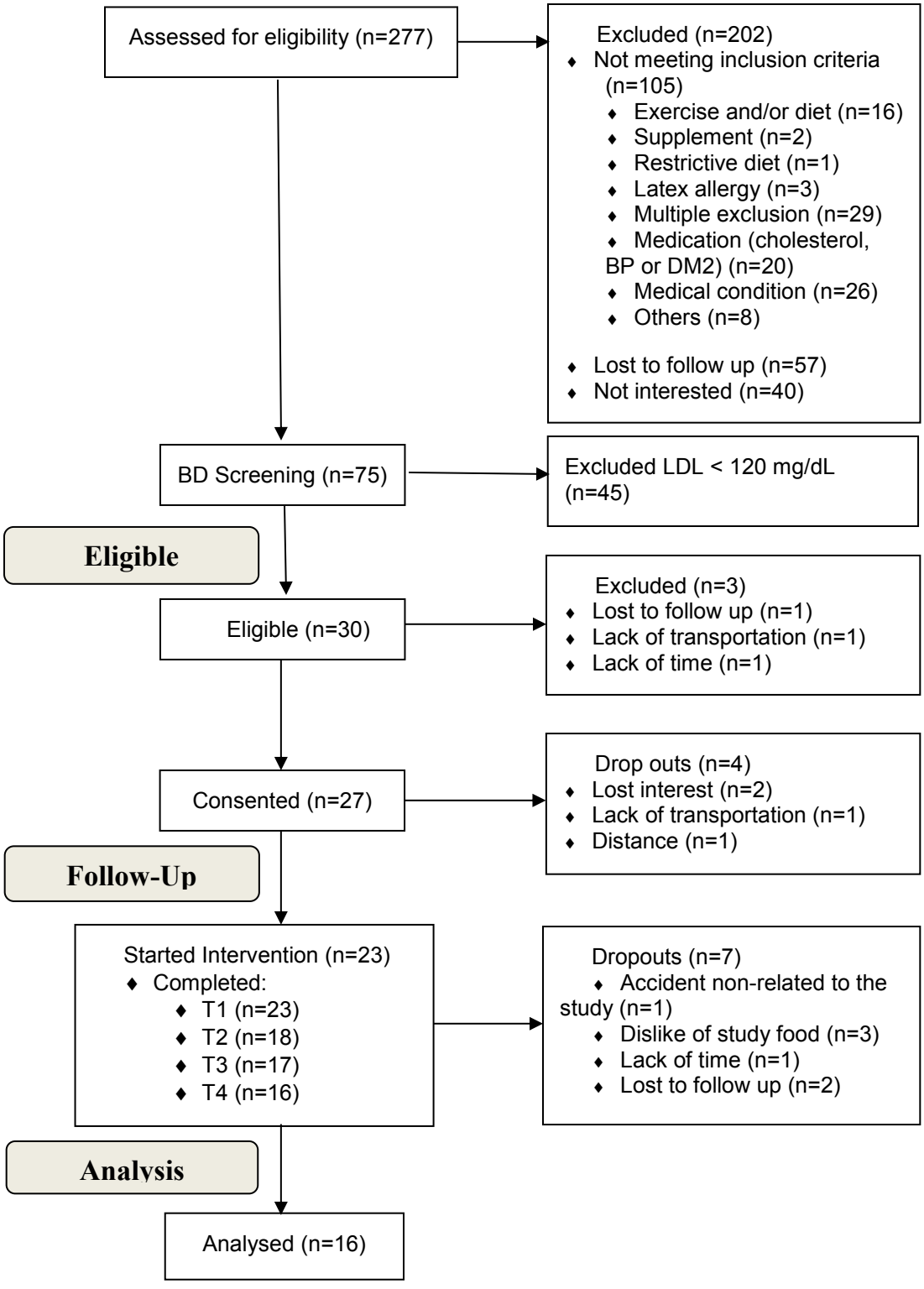


Figure 2. *Nopales* Study CONSORT 2013 Flow Diagram (08-09-13)



3.3 Test Foods

The test foods (*nopales* and cucumbers) were purchased from local groceries stores (Ranch Market and Food City). Fresh cucumbers were given to participants along with measuring cups and instructions. Cucumbers were distributed once a week since they last about 7 to 10 days. Participants were advised to: (1) keep the cucumbers in the refrigerator; (2) wash, peel, and cut into 1 inch cubes before measuring 1-cup (approximately 130 g); (3) season and prepare according to personal preferences. The *nopales* were purchased already cleaned (thorns removed) and chopped, and were cooked in the Nutrition Metabolic Kitchen according to the following standard procedure. After thorough rinsing under cold running water, the *nopales* were cooked in boiling water for ten minutes and placed in ice to cool down. *Nopales* were packed in bags containing 140g each (equivalent to 1 cup) and then refrigerated before being distributed to the participants. *Nopales* were distributed once a week (14 bags/week) and participants were instructed to place them in the refrigerator or freezer until needed for consumption. Recipes for preparing the study foods in diverse ways were distributed to improve adherence. Participants were instructed to consume half of the daily test food contents with each of their two main meals. The selected dose (280 g/d) is equivalent to two cups of cooked *nopales*. Cucumber (2 cups/day or 266 g/day) was chosen for the control intervention because although it is similar to *Nopales* in weight and volume, it has a lower content of soluble fiber (0.2 g/100 g vs. 0.9 g/100 g) and antioxidant micronutrients (**Table 1**) (24).

To monitor compliance and whether participants maintained their usual dietary habits, participants were asked to complete a 3-day food record (Appendix E) prior to the

beginning and during the last week of each intervention phase. In addition, they were asked to keep a tally of servings of test foods per day (Appendix F). The 3-day food records were checked carefully for completeness and the data were analyzed using Nutrition Data System for Research (NDSR) software (University of Minnesota, Minneapolis, MN) (24). Dietary variables of interest were estimates of total energy intake, percentage of energy provided by macronutrients (carbohydrate, fat and protein, and different types of carbohydrate and fat), and antioxidant micronutrients (carotenoids, vitamins A, C and E) consumed by the participants.

Table 1. Nutritional characteristics of test foods

	***Nopales* (cooked)**		**Cucumber (raw)**	
	100 g	**280 g**	**100 g**	**266 g**
Energy (kcal)	15	42	15	40
Carbohydrates (g)	3.28	9.18	3.63	9.66
Fat (g)	0.05	0.14	0.11	0.29
Protein (g)	1.4	3.92	0.65	1.73
Total Fiber (g)	2.0	5.6	1.1	2.9
Soluble fiber (g)	0.9	2.5	0.2	0.5
Vitamin A (IU)	443	1240	105	279
Vitamin C (mg)	5.3	14.8	2.8	7.5
β-carotene (μg)	242	678	45	120
α-carotene (μg)	47	132	11	29

Source: NDSR, 2012

Before and after each intervention phase a likert-type scale survey was administered to assess gastrointestinal symptoms (abdominal pain, increased bowel movements, bloating, flatulence, fullness and increased liquids; Appendix G). In addition, after each intervention, a likert-type scale survey was used to evaluate the acceptability and satisfaction of test foods (appearance, consistency/texture, flavor and overall satisfaction; Appendix H).

3.4 Blood Collection

Fasting blood samples were collected at the beginning and end of each dietary intervention period on two separate days at least one day apart to account for day-to-day variability in plasma lipid concentrations. On both days, participants were asked to fast for 10-12 hours prior to their arrival to the Nutrition Laboratory at Arizona State University. On the first blood collection day for each timepoint, fasting blood was collected from the antecubital vein into evacuated tubes as follows: one serum separating tube (7 ml), two EDTA-containing tubes (10 ml each), one heparin-containing evacuated tubes (4 ml), and one EDTA and glycolysis inhibitor (potassium oxalate and sodium fluoride) tube (2ml). On the second blood collection day blood was collected into one EDTA-containing evacuated tube (7 ml). Blood was centrifuged at 1,100 x g at 4°C for 20 minutes, and serum/plasma was separated, aliquoted, and stored at -70°C for future analysis. For vitamin C measurement, fresh EDTA plasma was mixed with an equal volume of 10% trichloroacetic acid before the supernatant was frozen. All samples were analyzed at once upon sample collection completion.

3.5 Measurements

3.5.1 Socio-demographic Questionnaire

At baseline after signing the consent form, participants answered a questionnaire about socio-demographic characteristics. The information collected included: gender, age, ethnicity, marital status, employment status, household income, education, and smoking status (Appendix I).

3.5.2 Anthropometric Measurements

Body weight was measured in kilograms using a calibrated scale and height was measured in centimeters using a wall-mounted stadiometer. Waist circumference was measured in centimeters using a flexible tape measure at the midpoint between the lowest rib and the iliac crest. Blood pressure was taken from the non-dominant arm (unless contraindicated) following a five minute rest using an Omron IntelliSense HEM-907XL automated blood pressure monitor (Omron Healthcare, Inc., Bannockburn, IL). All measurements were done in triplicate. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). A Tanita body composition analyzer (model TBF-300A, Tanita Corporation, Tokyo, Japan) was used to measured percent body fat.

3.5.3 Cardiometabolic Risk Biomarkers

A complete lipid panel (total cholesterol, HDL-c, LDL-c, and triglycerides) and glucose were measured in plasma using an automated chemistry analyzer (Cobas C111; Roche Diagnostics, Indianapolis, IN) using colorimetric enzymatic reagents. Glucose was measured in plasma that had potassium oxalate and sodium fluoride added as glycolysis

inhibitors. Plasma hsCRP was measured with a turbidimetric enzymatic assay using an automated chemistry analyzer (Cobas C111; Roche Diagnostics, Indianapolis, IN).

The Lipoprint/Lipoware system (Quantimetrix Co., Redondo Beach, CA) was used to measure the cholesterol distribution in LDL and HDL subfractions and mean LDL particle size (163). Lipoprotein particles were separated from a plasma sample by nongradient polyacrylamide tube gel electrophoresis following the manufacturer instructions. The amount of cholesterol present in each of the different lipoprotein bands was quantified by densitometric analysis using the Lipoware computer software provided as part of the Lipoprint system. This analysis resulted in the identification of seven LDL subfractions and ten HDL subfractions. The relative percentage of total cholesterol in large and small LDL particles was calculated by adding the proportion of cholesterol in fractions LDL_1 plus LDL_2, and LDL_3 through LDL_7, respectively. Similarly, the relative percentage of HDL-cholesterol in large (HDL_1 through HDL_3), intermediate (HDL_4 through HDL_6), and small HDL (HDL_7 through HDL10) particles was calculated. In addition, LDL phenotype patterns were classified as LDL phenotypes A (predominantly large, buoyant LDL), B (predominantly small, dense LDL) or intermediate.

Insulin was measured in serum using the ultra-sensitive human insulin radioimmunoassay kit (Millipore, Billerica, MA). Insulin sensitivity was assessed by calculating the homeostatic model assessment (HOMA) score (164):

$$\text{HOMA} = \text{glucose (mmol/L)} \times \text{insulin (}\mu\text{U/ml)}/22.5$$

LDL susceptibility to oxidation was measured by monitoring the formation of conjugated dienes catalyzed by incubating with Cu^{2+} after the isolation of LDL from plasma using density gradient ultracentrifugation, as previously described (165). First, sample density was adjusted to 1.21 g/mL by adding potassium bromide. The adjusted samples were transferred to an ultracentrifuge tube (Quick-Seal Polyallomer tube, Beckman, Brea, CA). Samples were carefully overlayered with a 1.006 g/mL density solution. Samples were centrifuged for 3 hours at 80,000 rpm and 15°C using an Optima MAX-XP ultracentrifuge (Beckman, Brea, CA) with a fixed angle MLN 80 rotor (Beckman, Brea, CA). The LDL layer was removed and transferred to desalting columns (Econo-Pac 10DG columns, BioRad, Hercules, CA) for EDTA removal. The protein content of the LDL fractions was measured using the Modified Lowry Protein Assay Kit (Thermo Scientific, Waltham, MA), after which volume was adjusted with a phosphate buffered saline (PBS) solution to achieve 100 µg protein/1000 µl. The samples were incubated with 2.5 µM of copper sulfate at 37°C and the kinetics of conjugated dienes formation was assessed by continuous absorbance monitoring at 234 nm at 2-min intervals for 7 hours (165). The following measurements were acquired from the oxidation curve output: (1) lag time (min), the intercept of lines drawn through the linear portion of the propagation phase and the lag phase; and (2) oxidation rate (nmol diene x mg LDL protein x min-1), the slope of the linear portion of the propagation phase).

Oxidized LDL was measured in EDTA plasma using a commercially available ELISA kit (Mercodia AB, Uppsala Sweden) based on direct sandwich technique using the same specific murine monoclonal antibody mAb-4E6 as in the assay described by Holvoet et al (166).

Total antioxidant capacity (TAC) was measured in serum using a colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI) based on the generation of ABTS radical cation from the interaction between metmyoglobin and hydrogen peroxide, and was measured at 750 nm (167).

Vitamin C was measured according to the method described by Omaye et al. (168). Briefly, standards were made with 5% trichloroacetic acid (TCA) ranging from 0 to 20 µg/ml. Samples and standards were incubated for 3 hours at 37°C after the addition of 100µl of 2,4-Dinitrophenylhydrazine/thiourea/copper (DTC) solution. Ice cold 65% sulfuric acid was added and samples were incubated at room temperature for 30 minutes. Samples and standards were measured at 520 nm spectrophotometrically. Vitamin C concentration was calculated using a linear regression equation.

3.6 Sample Size Calculation

A power analysis was performed to determine the sample size necessary to detect significant changes in total cholesterol. Using data from Frati-Munati (26), it was determined that to detect a minimum difference of 31 mg/dl change in total cholesterol (at a 0.05 significance level and power > 0.80) with a within-person standard deviation of 40 mg/dL, a total of 16 subjects are needed (Appendix J). With an expected 20% dropout rate, target recruitment was 20 subjects. Additional participants were enrolled to ensure study completion by 16 participants.

3.7 Statistical Analyses

Untransformed data is reported as mean values ± standard deviation (mean ± SD). Mean values of both blood draws for lipids were used for statistical analysis. Prior to the analysis, normality of the outcome measurements was assessed. Baseline comparison between genders and test food satisfaction were analysed using an independent samples t-test for normally distributed variables or a Mann-Whitney Test for non-normally distributed variables. For the other outcomes measured, a multivariate general linear model for repeated measures was used to determine significant treatment, time, or interactions effects. Non-normally distributed data were log-transformed or squared root transformed. A p value ≤ 0.05 was considered statistically significant. All analyses were conducted using SPSS (version 21, Chicago, IL).

CHAPTER 4

RESULTS

4.1 Participant Characteristics

The sociodemographic characteristics of the study participants are presented in **Table 2**. A total of 16 participants completed the study. Participants' mean age was 46.5 ± 13.9 years. A majority of participants were female (68.8%). Participants identified themselves as Hispanic (43.8%), white (37.5%), or black (6.3%). About 44% of participants were single, 25% were married, and 12.5% were living together. Seventy five percent of participants were currently working, and about one-third of the participants reported a monthly household income of $3,000 or more (31%) and 44% had a monthly income below $2,000. The majority of participants had a college degree or higher (56.3%) or some college education (31.3%). None of the study participants reported smoking (data not shown).

Table 3 shows participants' physiologic characteristics at baseline. Participants' mean BMI was 31.4 ± 5.7 kg/m^2 and their mean percent body fat was 37.2 ± 9.0%. According the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III criteria (7), mean waist circumference was within desirable girth for men (94.8 ± 6.4; desired ≤ 102 cm) but not for women (105.0 ± 12.6 cm; desired ≤ 88 cm). Mean blood pressures were within the recommended levels (SBP = 123.3 ± 10.9 mm Hg; desired < 130 mm Hg, and DBP = 79.2 ± 10.8 mm Hg; desired < 85 mm Hg). Fasting glucose (99.2 ± 10.7 mg/dL, desired < 100 mg/dL) and total cholesterol (199.3 ± 24.6 mg/dl; desired < 200 mg/dL) were very close to the upper limit for recommended

concentrations. Per study design, LDL-c concentrations were elevated (137.1 ± 20.5 mg/dL, desired < 100mg/dL). HDL-c concentrations were slightly above minimum desirable values for women (50.9 ± 13.6 mg/dL; desired > 50 mg/dL) but not for men (33.1 ± 7.1 mg/dL; desired > 40 mg/dL, p=0.022). In addition, participants had triglyceride concentrations that were slightly above recommended levels (151.9 ± 84.9 mg/dL; desired<150 mg/dL).

Figure 3 shows the distribution of LDL subclass phenotype at baseline. Although participants had moderately high LDL-c concentrations, 44% of them were classified as having a LDL pattern A (the less atherogenic profile), 33% were classified as having an intermediate phenotype, and 25% presented the more atherogenic pattern B phenotype, which is characterized by having more of the small, dense LDL particles.

4.2 Dietary Intake

Dietary composition data before and during the consumption of both study foods is shown in **Table 4.** No significant treatment-by-time effect was observed for any of the dietary composition data. A significant time effect was observed for intakes of energy (p_{Time}=0.026), total fat (p_{Time}=0.003), monounsaturated fatty acid (MUFA; p_{Time}=0.023), polyunsaturated fatty acid (PUFA; p_{Time}=0.003) and vitamin E (p_{Time}=0.026). Energy intake was not significantly different at pre-phases (cucumber, 1747 ± 565 kcal; *nopales*, 1829 ± 600 kcal) and was decreased by 8.3% and 10.1% during the cucumber and *nopales* phases, respectively. Fat intake was the major contributor to the decrease in energy intake. During the cucumber and *nopales* phases, fat intake was decreased by 16.2% and 14.4% respectively. The decrease in fat intake was mainly due to reduced

consumption of monounsaturated (cucumber, -13.2%; *nopales* -18.9%) and polyunsaturated fatty acids (cucumber, -13.7%; *nopales* -23.2%). Lastly, vitamin E intake was decreased by 35% in the cucumber phase and by 29% in the *nopales* phase. While no significant changes were observed in total and soluble fiber intake in both treatment phases.

When evaluating dietary intake by food group (**Table 5**), a significant treatment-by-time effect was observed for the intake of the nuts/avocado group (p_{Int}=0.044). The intake of this food group was reduced in both treatments, but the reduction was greater in the *nopales* phase because at the beginning of this phase intake was greater than at the beginning of the cucumber phase. In addition, a significant time effect was observed for intake of vegetables, legumes, and refined grains. The intakes of vegetables were increased during both phases (p_{Time}=0.003), while the consumption of legumes (p_{Time}=0.002) and refined grains (p_{Time}=0.029) were decreased. A significant treatment effect was observed for intakes of fruits and starchy vegetables that were higher during the cucumber phase compared to *nopales* phase (fruit, p_{Treat}=0.028; starchy vegetables, p_{Treat}=0.001).

Prior to each intervention phase, participants were given a compliance calendar to keep a tally of the cups of test foods consumed. Compliance data were obtained for 75% of the participants. Among those participants, mean compliance was 92 ± 8% for the cucumber phase and 94 ± 6% for the *nopales* phase and did not significantly differ between the two groups (p=0.555). The compliance of participants who did not return the calendars was verified by the inclusion of test foods in the 3-day food record.

Table 2. Descriptive characteristics of study participants.

Variable	All participants (n = 16)
Age (years; mean ± SD)	46.5 ± 13.9
Gender (%, n)	
Male	31.2 (5)
Female	68.8 (11)
Ethnicity (%, n)	
White	37.5 (6)
Black	6.3 (1)
Hispanic	43.8 (7)
Other	12.5 (2)
Marital Status (%, n)	
Single	43.8 (7)
Married	25.0 (4)
Living together	12.5 (2)
Separated	6.3 (1)
Divorced	6.3 (1)
Widowed	6.3 (1)
Currently working (%, n)	
Yes	75 (12)
No	25 (4)
Monthly Family Income (%, n)	
$0 - $1000	12.5 (2)
$1001 - $2000	31.3 (5)
$2001 - $3000	6.3 (1)
$3001 - $4000	18.8 (3)
> $4000	12.5 (2)
Refused / Not sure	18.8 (3)
Education (%, n)	
Completed middle school	6.3 (1)
Completed high school	6.3 (1)
Some college	31.3 (5)
College graduate or higher	56.3 (9)

Table 3. Physiologic characteristics of study participants at baseline.[a]

Variable	All participants (n=16)	Men (n=5)	Women (n=11)	p Value
BMI (kg/m^2) [b]	31.4 ± 5.7	28.2 ± 3.2	32.9 ± 6.4	0.070
Body fat (%)	37.2 ± 9.0	26.5 ± 3.9	42.1 ± 6.3	**0.000**
Waist circumference (cm) [c]	101.8 ± 12.0	94.8 ± 7.1	105.0 ± 13.2	0.126
Systolic blood pressure (mm Hg)	123.3 ± 10.9	123.1 ± 12.1	123.4 ± 11.4	0.968
Diastolic blood pressure (mm Hg) [c]	79.2 ± 10.8	80.6 ± 14.1	78.6 ± 10.3	0.913
Fasting lipids (mg/dL)				
Total cholesterol	199.3 ± 24.6	208.9 ± 29.7	194.9 ± 23.5	0.326
LDL-cholesterol [b]	137.1 ± 20.5	145.1 ± 17.4	133.4 ± 22.4	0.321
HDL-cholesterol [b]	45.3 ± 14.6	33.1 ± 8.0	50.9 ± 14.3	**0.022**
Triglycerides	151.9 ± 84.9	216.0 ± 104	122.8 ± 64.5	**0.044**
LDL-c/HDL-c	3.4 ± 0.4	4.6 ± 1.5	2.9 ± 1.0	**0.014**
Glucose (mg/dL)	99.2 ± 10.7	95.2 ± 4.9	101.0 ± 12.8	0.211
Insulin (uU/mL)	19.1 ± 2.2	17.6 ± 7.8	19.7 ± 9.4	0.669
HOMA	4.8 ± 0.7	4.2 ± 2.0	5.1 ± 2.9	0.536
hsCRP (mg/dL) [b]	5.5 ± 5.8	2.0 ± 0.9	7.2 ± 6.7	**0.030**

[a] Data shown as mean ± SD. Mean values for men and women were analysed using an independent samples t-test for normally distributed variables or a Mann-Whitney Test for non-normally distributed variables.
[b] Abbreviations BMI – body mass index; HDL – high density lipoprotein; LDL – low density lipoprotein; HOMA - homeostasis model assessment ; hsCRP – high sensitivity C-reactive protein.
[c] non-normally distributed.

Figure 3. LDL subclass phenotype at baseline

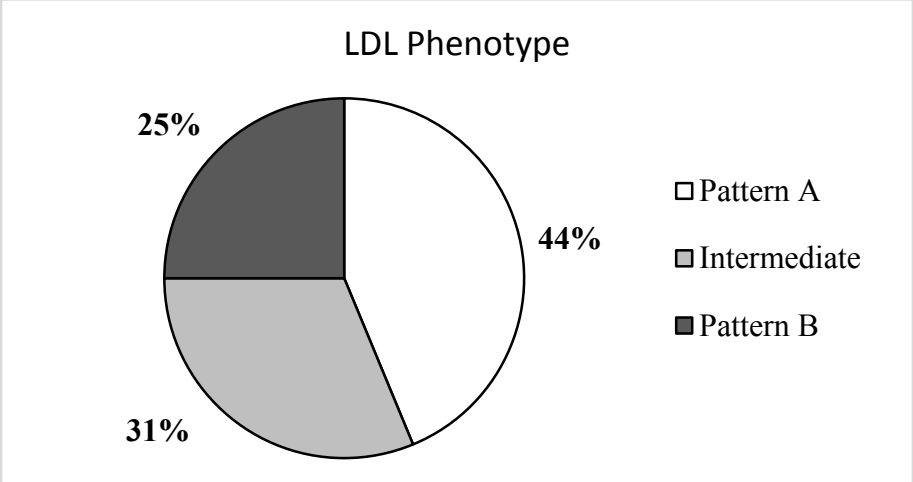


Table 4. Nutrient intake before and after each treatment.[a]

	Cucumber Phase		*Nopales* Phase		p-value		
	Pre	Post	Pre	Post	Treat [b]	Time	Int [b]
Total grams per day	2287 ± 1070	2350 ± 1086	2538 ± 951	2131 ± 947	0.933	0.394	0.167
Energy intake (kcal/d)	1747 ± 565	1602 ± 588	1829 ± 600	1645 ± 526	0.467	**0.026**	0.825
Energy from carbohydrate (%)	47.3 ± 6.4	49.9 ± 8.9	44.3 ± 7.8	44.5 ± 6.8	**0.012**	0.237	0.307
Carbohydrates (g)	210.2 ± 69.9	203.9 ± 76.5	203.6 ± 70.4	186.5 ± 65.9	0.331	0.196	0.649
Energy from protein (%)	17.2 ± 3.3	16.9 ± 2.7	17.5 ± 3.8	18.9 ± 5.5	0.245	0.359	0.329
Protein (g)	73.1 ± 23.9	67.2 ± 23.6	75.9 ± 18.6	76.5 ± 25.1	0.269	0.431	0.262
Energy from fat (%)	35.6 ± 7.4	32.9 ± 7.8	36.4 ± 6.7	35.1 ± 5.4	0.351	0.169	0.421
Fat (g)	72.6 ± 30.6	60.7 ± 30.2	76.6 ± 31.8	65.6 ± 24.1	0.331	**0.003**	0.588
Energy from SFA (%)[b]	11.0 ± 2.4	10.2 ± 3.1	11.3 ± 3.0	11.4 ± 3.3	0.356	0.478	0.320
SFA (g) [c]	22.3 ± 10.1	20.9 ± 12.5	25.1 ± 12.4	22.3 ± 12.6	0.240	0.062	0.459
Energy from MUFA (%)[b]	13.2 ± 4.0	12.2 ± 3.8	13.4 ± 3.4	12.7 ± 3.2	0.579	0.373	0.721
MUFA (g) [c]	27.3 ± 14.3	23.7 ± 12.9	28.1 ± 12.2	22.8 ± 7.7	0.458	**0.023**	0.650
Energy from PUFA (&)[b]	8.3 ± 2.4	7.5 ± 2.4	8.6 ± 2.0	8.1 ± 2.5	0.476	0.263	0.831
PUFA (g) [c]	16.8 ± 6.0	14.5 ± 8.6	18.5 ± 7.0	14.2 ± 5.7	0.459	**0.003**	0.880
Cholesterol (mg/d)	249 ± 147	269 ± 175	284 ± 163	287 ± 123	0.568	0.650	0.632
Total sugars (g/d)	79.0 ± 34.0	86.9 ± 48.7	83.0 ± 45.0	70.0 ± 34.7	0.344	0.531	0.260

[a] n = 15. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Abbreviations: SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; Treat – treatment; Int –interaction.
[c] Log-transformed prior to analysis.

Table 4. Nutrient intake before and after each treatment.[a] (continued)

	Cucumber Phase		*es* Phase		p-value		
	Pre	Post	Pre	Post	Treat [b]	Time	Int [b]
Total fiber (g/day)[c]	21.5 ± 10.5	18.6 ± 6.7	20.4 ± 8.8	19.2 ± 5.1	0.744	0.149	0.679
Total fiber (g/1000kcal/day)	12.2 ± 3.6	12.1 ± 3.4	12.0 ± 3.9	12.5 ± 3.6	0.974	0.708	0.628
Soluble fiber (g/d)[c]	6.5 ± 3.0	5.8 ± 1.9	6.6 ± 2.9	6.6 ± 1.6	0.279	0.604	0.555
Soluble fiber (g/1000kcal/day)	3.7 ± 1.3	3.7 ± 1.0	3.9 ± 1.5	4.3 ± 1.3	0.250	0.331	0.466
Insoluble fiber (g/d)[c]	15.1 ± 7.9	12.8 ± 5.0	13.6 ± 6.0	12.5 ± 3.7	0.891	0.117	0.752
Insoluble fiber (g/1000kcal/day)	8.5 ± 2.7	8.3 ± 2.6	8.0 ± 2.6	8.1 ± 2.4	0.571	0.949	0.699
Vitamin C (m/d)[c]	99 ± 62	102 ± 49	108 ± 91	98 ± 81	0.498	0.730	0.177
Vitamin E (mg/d)[c]	11.1 ± 11.1	7.2 ± 4.0	9.7 ± 4.1	6.9 ± 3.2	0.756	**0.001**	0.752
Vitamin A (µg/d)[c]	970 ± 490	940 ± 414	1275 ± 988	952 ± 375	0.120	0.636	0.735
α-carotene (µg/d)[c]	486 ± 538	425 ± 741	480 ± 558	591 ± 724	**0.009**	0.893	0.176
β-carotene (µg/d)[c]	3756 ± 2658	3108 ± 2238	5027 ± 5258	3239 ± 2183	0.210	0.362	0.912

[a] n = 15. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Abbreviations: SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; Treat – treatment; Int –interaction.
[c] Log-transformed prior to analysis.

Table 5. Intake of selected food groups before and after each treatment.[a]

Food Group (serving/day)	Cucumber Phase		*Nopales* Phase		p-value		
	Pre	Post	Pre	Post	Treat[b]	Time	Int[b]
Vegetable [c]	3.21 ± 3.70	5.91 ± 1.72	4.29 ± 3.40	5.37 ± 1.82	0.646	**0.003**	0.221
Fruit [c]	2.42 ± 2.49	1.47 ± 1.77	1.19 ± 1.62	0.83 ± 0.94	**0.028**	0.171	0.252
Legumes [c]	0.38 ± 0.37	0.08 ± 0.26	0.36 ± 0.34	0.20 ± 0.37	0.581	**0.002**	0.29
Starchy vegetables [c]	0.25 ± 0.30	0.90 ± 1.22	0.16 ± 0.29	0.20 ± 0.35	**0.001**	0.052	0.207
Whole Grain	1.33 ± 1.24	1.62 ± 1.31	1.16 ± 1.14	1.23 ± 1.20	0.299	0.446	0.531
Refined Grain [c]	4.28 ± 2.64	3.04 ± 2.39	3.74 ± 1.52	3.34 ± 2.38	0.899	**0.029**	0.436
Eggs [c]	0.54 ± 0.68	0.71 ± 0.76	0.68 ± 0.59	0.71 ± 0.80	0.619	0.554	0.359
Nuts & Avocado [c]	0.40 ± 0.57	0.16 ± 0.18	1.34 ± 1.54	0.35 ± 0.63	0.057	**0.001**	**0.044**
Margarine [c]	0.66 ± 1.13	0.69 ± 1.22	0.78 ± 1.27	0.56 ± 1.04	0.751	0.679	0.516
Vegetable oil [c]	1.39 ± 1.61	0.71 ± 1.81	1.58 ± 2.20	1.52 ± 1.95	0.226	0.118	0.207
Butter/shortening [c]	0.30 ± 0.35	0.49 ± 1.16	0.53 ± 0.72	0.96 ± 1.22	0.171	0.308	0.281
Salad Dressing [c]	0.42 ± 0.51	0.48 ± 0.66	0.66 ± 0.56	0.38 ± 0.58	0.503	0.168	0.113
Sugar and sweets [c]	1.42 ± 1.61	1.42 ± 1.93	1.65 ± 1.72	1.29 ± 2.70	0.780	0.320	0.864
Sugar-sweetened drinks [c]	0.35 ± 0.60	0.55 ± 0.90	0.38 ± 0.51	0.35 ± 0.63	0.861	0.963	0.153

[a] n = 15. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Abbreviations: Treat – treatment; Int –interaction.
[c] square-root-transformed prior to analysis.

4.3 Cardiometabolic Risk Outcomes

The intervention effects on cardiometabolic disease risk factors are shown in **Table 6**. No significant treatment-by-time effects were observed for the cardiometabolic outcomes measured. Weight, BMI and percent body fat remained constant throughout the study time. Comparable responses after the consumption of cucumber and *nopales* were observed. There was a non-significant trend for WC decrease after the consumption of both study foods (cucumber, -0.4%; *nopales*, -1.4%; p_{Time}=0.053). There was also a non-significant trend for a systolic blood pressure reduction after the consumption of cucumbers (-2.1%) and *nopales* (-2.6%, p_{Time}=0.070). Consumption of both study foods resulted in a significant increase in triglycerides (cucumber, 14.8%; *nopales,* 15.2%; p_{Time}=0.020). LDL-c at pre-phase was not significantly correlated with LDL-c change in both treatments (cucumber, r^2=0.235 and p=0.381; *nopales*, r^2=-0.283 and p=0.298). Results for cardiometabolic risk outcomes did not change when excluding 3 participants from the analysis that did not have LDL-c over 120 mg/dL at time point 1 (appendix K).

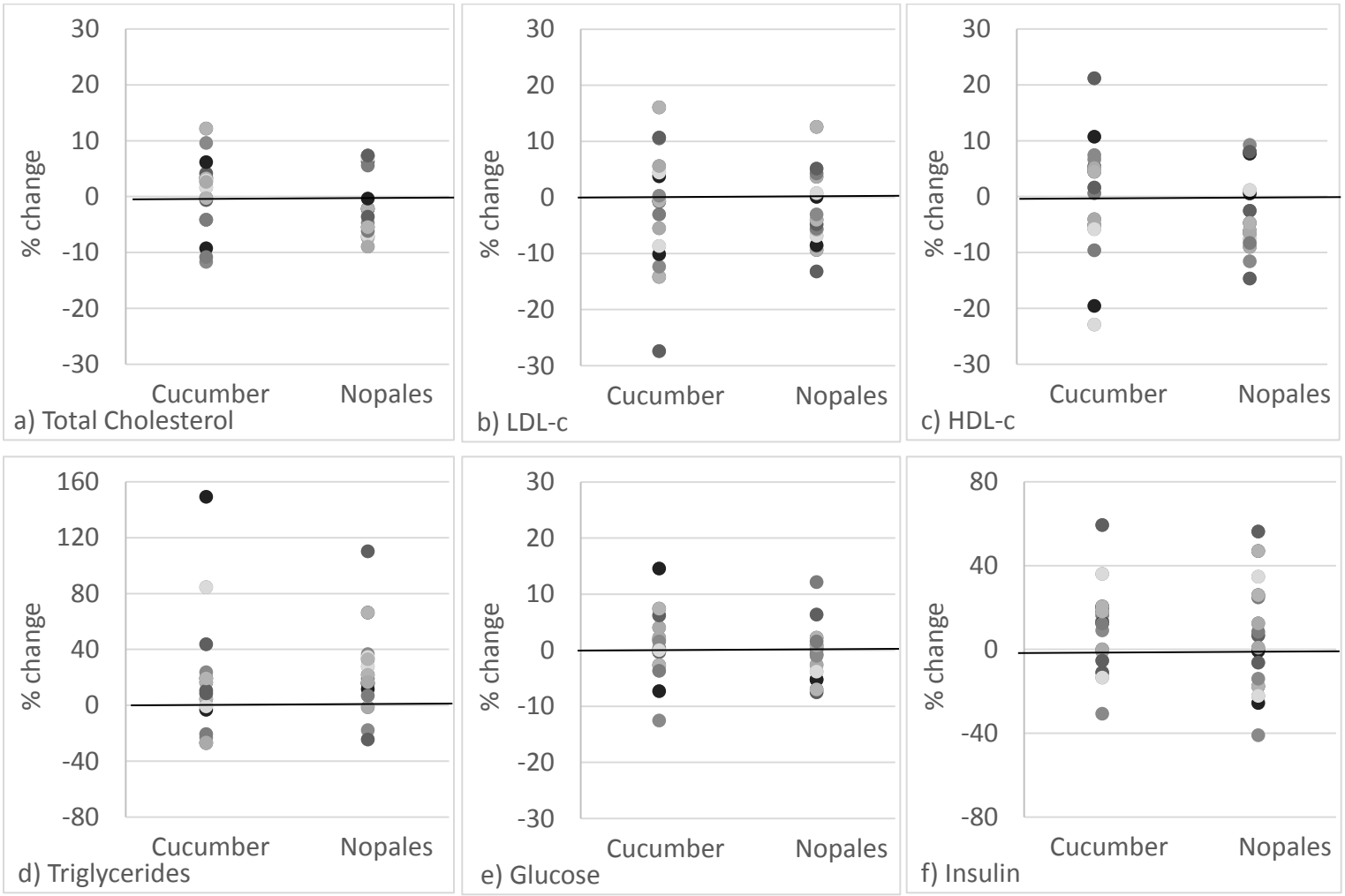
Figure 4 denotes individual changes, relative to pre-treatment values after the consumption of both test foods for the major cardiometabolic outcomes. Great individual response variability was observed for all outcomes. For the primary outcome LDL-c, a decrease was observed in 7 (44%) and 10 (63%) participants after the cucumber and *nopales* phases respectively. Whereas not significant, the average decrease in LDL-c over time was 2.0 mg/dL (-1.4%) after the cucumber intake and 3.9 mg/dL (-2.9%) after the *nopales* intake (p_{Time}=0.176).

Table 6. Effect of test food intake on cardiometabolic disease risk factors[a]

	Cucumber Phase			*Nopales* Phase			p-value		
	Pre	Post	% Change	Pre	Post	% Change	Treat[b]	Time	Int[b]
Weight (kg)	84.2 ± 13.3	84.5 ± 13.5	0.3	84.1 ± 13.1	83.9 ± 13.1	-0.2	0.237	0.637	0.155
BMI (kg/m^2) [b]	31.5 ± 5.9	31.6 ± 5.9	0.3	31.4 ± 5.8	31.3 ± 5.8	-0.3	0.182	0.920	0.212
Body Fat (%)	36.8 ± 9.6	36.8 ± 9.0	0	36.7 ± 9.1	36.9 ± 9.2	0.5	0.959	0.832	0.663
WC (cm) [b]	102.4 ± 11.9	102.0 ± 11.8	-0.4	102.7 ± 12.3	101.3 ± 11.6	-1.4	0.702	0.053	0.438
BP (mm Hg)									
Systolic [c]	121.7 ± 11.9	119.1 ± 11.7	-2.1	121.7 ± 11.3	118.5 ± 11.2	-2.6	0.852	0.070	0.818
Diastolic [c]	78.7 ± 10.0	77.7 ± 8.6	-1.3	78.2 ± 10.8	78.9 ± 10.2	0.9	0.796	0.950	0.459
Lipids (mg/dL)									
Total cholesterol	200.0 ± 26.9	201.0 ± 32.3	0.5	199.2 ± 26.5	196.5 ± 26.6	-1.4	0.440	0.745	0.319
HDL-c [b]	45.4 ± 14.0	45.8 ± 15.8	0.9	46.0 ± 15.9	44.4 ± 14.6	-3.5	0.687	0.403	0.242
LDL-c [b]	138.5 ± 22.3	136.5 ± 29.4	-1.4	135.9 ± 21.5	132.0 ± 20.1	-2.9	0.341	0.176	0.680
Triglycerides [c]	150.2 ± 87.4	172.5 ± 108.3	14.8	164.6 ± 124.4	189.7 ± 117.1	15.2	0.090	**0.020**	0.435
LDL-c/HDL-c	3.39 ± 1.38	3.41 ± 1.72	0.6	3.36 ± 1.43	3.33 ± 1.37	-0.9	0.437	0.956	0.759
Glucose (mg/dL)	97.9 ± 11.4	99.3 ± 8.1	1.4	98.3 ± 10.1	97.4 ± 11.3	-0.9	0.399	0.822	0.416
Insulin (uU/mL)	19.6 ± 9.3	21.3 ± 10.0	8.7	19.8 ± 9.2	20.4 ± 9.2	3.0	0.709	0.387	0.245
HOMA [b]	4.9 ± 2.7	5.3 ± 2.6	8.2	4.9 ± 2.1	5.0 ± 2.5	2.0	0.516	0.469	0.268
hsCRP (mg/dL) [b, c]	5.5 ± 6.0	4.7 ± 4.7	-14.5	4.8 ± 5.0	4.8 ± 4.8	0.0	0.431	0.787	0.685

[a] n = 16. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA;
[b] Abbreviations: BMI – body mass index; WC – waist circumferences; BP – blood pressure; HDL-c – high density lipoprotein cholesterol; LDL-c – low density lipoprotein cholesterol; HOMA - homeostasis model assessment ; hsCRP – high sensitivity C-reactive protein; Treat – treatment; Int –interaction;
[c] Log-transformed prior to analysis.

Figure 4. Percent change of major biomarkers for each intervention phase



n=16. Individual percent change according to treatment for a) Total Cholesterol; b) Low density-lipoprotein cholesterol (LDL-c); c) high-density lipoprotein cholesterol (HDL-c); d) Triglycerides; e) Glucose; f) Insulin

4.4 Lipoprotein subfractions

The LDL particle size and cholesterol distribution in LDL and HDL subfractions are shown in **Table 7**. No significant treatment-by-time effect was observed for the distribution of cholesterol among any lipoprotein subfractions. Additionally, no significant treatment or time effects were observed in LDL size and the proportion of cholesterol in small LDL. The distribution of cholesterol in large LDL did not significantly change over time; however, it was modestly, but significantly higher during the cucumber phase (p_{Treat}=0.037). No treatment effects were observed in the distribution of HDL cholesterol in HDL subfractions; however, similar pro-atherogenic changes were observed in both interventions over time. After the consumption of both study foods, the proportion of HDL cholesterol significantly decreased in large HDL (p_{Time}=0.021) and significantly increased in small HDL (p_{Time}=0.002).

4.5 Oxidative Stress

There were no significant treatment, time, or interactive effects for plasma, vitamin C, TAC, oxidized LDL and LDL oxidation (lag time and oxidation rate; **Table 8**). A time effect trend was observed for TAC (p_{Time}=0.057), in which TAC increased 1.7% after the cucumber phase and 6.8% after the *nopales* phase. The observed mean values for oxidative stress markers were within the reference range for vitamin C (0.5 – 2.0 mg/dL)(168), TAC (1.0 - 2.3 mM)(169), and oxidized LDL (26 - 117 U/L)(166).

Table 7. Effect of diet intervention on lipoprotein subfractions.[a]

	Cucumber Phase			*Nopales* Phase			p-value		
	Pre	Post	% Change	Pre	Post	% Change	Treat [b]	Time	Int [b]
LDL peak diameter (Å)	267.5 ± 4.9	267.0 ± 5.4	-0.2	267.3 ± 5.3	266.5 ± 5.9	-0.3	0.323	0.175	0.665
% cholesterol in large LDL [b, c]	32.3 ± 4.0	31.6 ± 5.0	-2.0	30.8 ± 5.7	30.0 ± 5.9	-2.8	**0.037**	0.267	0.981
% cholesterol in small LDL [b, c]	4.1 ± 4.5	4.4 ± 5.1	8.0	3.8 ± 4.0	4.4 ± 5.1	16.8	0.573	0.679	0.117
% HDL-c in large HDL [b]	24.3 ± 12.4	23.1 ± 13.7	-5.1	23.7 ± 13.1	22.4 ± 12.0	-5.9	0.481	**0.021**	0.897
% HDL-c in intermediate HDL [b]	53.8 ± 5.1	54.2 ± 5.3	0.7	54.2 ± 5.6	53.8 ± 4.9	-0.6	0.980	0.962	0.581
% HDL-c in small HDL [b]	21.7 ± 8.9	22.6 ± 9.9	4.1	21.9 ± 8.5	23.6 ± 8.9	7.9	0.455	**0.002**	0.358

[a] n = 16. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Abbreviations: HDL – high density lipoprotein; HDL-c – high density lipoprotein cholesterol; LDL – low density lipoprotein; Treat – treatment; Int –interaction.
[c] Log-transformed prior to analysis.

Table 8. Effect of the diet interventions on oxidative stress markers.[a]

	Cucumber Phase			*Nopales* Phase			p-value		
	Pre	Post	% Change	Pre	Post	% Change	Treat[b]	Time	Int[b]
Vitamin C (mg/dL)	1.17 ± 0.43	1.20 ± 0.42	2.6	1.13 ± 0.47	1.14 ± 0.45	0.9	0.099	0.634	0.744
TAC (mM)[c]	1.15 ± 0.41	1.17 ± 0.31	1.7	1.18 ± 0.36	1.26 ± 0.32	6.8	0.284	0.057	0.410
Oxidized LDL (U/L)	83.2 ± 22.7	81.5 ± 21.3	-2.0	81.0 ± 17.6	80.9 ± 18.2	-0.1	0.600	0.546	0.592
LDL oxidation[b, d]									
Lag time (min)[d]	204.4 ± 39.1	194.1 ± 23.1	-5.0	206.9 ± 37.7	211.3 ± 49.9	2.1	0.472	0.624	0.258
Oxidation Rate[c, e]	11.03 ± 2.7	11.04 ± 2.1	0.1	11.21 ± 2.3	11.24 ± 1.9	0.3	0.695	0.961	0.983

[a] n = 16. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Abbreviations: TAC – total antioxidant capacity ; LDL – low density lipoprotein; G – group; Treat – treatment; Int –interaction.
[c] Log-transformed prior to analysis.
[d] n=14.
[e] (nmol diene / mg LDL protein x min).

4.6 Side Effects and Test Food Outcomes

The participants responded to a likert-type scale about gastrointestinal symptoms before and after each intervention phase to evaluate possible side effects of the test foods (**Table 9**). There was no significant treatment-by-time effect for gastrointestinal symptoms. No side effects were observed for abdominal pain, increased bowel movements, bloating, flatulence, fullness and increased liquids after the consumption of the test foods. The most common responses were "none" or "mild" for all categories.

Table 9. Gastrointestinal symptoms by intervention and time point.[a,b]

	Cucumber Phase		*Nopales* Phase		p-value		
	Pre	Post	Pre	Post	Treat	Time	Int
Abdominal pain	0.1 ± 0.3	0.2 ± 0.6	0.2 ± 0.8	0.2 ± 0.4	0.791	0.791	0.423
Increased bowel movements	0.2 ± 0.5	0.9 ± 0.8	0.3 ± 0.6	0.6 ± 0.8	0.549	**0.016**	0.312
Bloating	0.7 ± 0.9	0.2 ± 0.4	0.2 ± 0.6	0.6 ± 0.6	0.633	0.697	0.054
Flatulence	0.7 ± 0.9	0.6 ± 0.7	0.4 ±0.7	1.0 ± 1.2	0.544	0.119	0.145
Fullness	0.6 ± 0.7	0.9 ± 1.0	0.6 ± 0.7	1.4 ± 1.1	0.168	**0.006**	0.120
Increased liquids	0.4 ± 0.8	0.4 ± 0.9	1.0 ± 1.2	0.9 ± 1.4	0.059	0.879	0.753

[a] n = 16. Data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA.
[b] Likert-type scale: 0 = none; 1 = mild; 2 = moderate; 3 = high; 4 = severe.

Participants also responded to a satisfaction and acceptability survey about the test foods, and the results are presented in **Table 10**. The cucumbers received significantly higher rates than the *nopales* for all acceptability and satisfaction components: appearance, consistency, flavor and overall satisfaction. Overall, cucumbers

were considered “good” and *nopales* “satisfactory”. Changes in the main outcome LDL-c were not associated with satisfaction of test foods (appendix K).

Table 10. Acceptability and Satisfaction of test foods.[a,b]

	Cucumber Phase	*Nopales* Phase	p-value
	Post	Post	
Appearance	3.8 ± 0.4	2.9 ± 0.9	**0.005**
Consistency	3.8 ± 0.4	2.4 ± 1.1	**0.003**
Flavor	3.6 ± 0.6	2.5 ± 1.0	**0.002**
Overall Satisfaction	3.7 ± 0.6	2.8 ± 0.9	**0.002**

[a] n = 16. Data shown as mean ± SD. Mean values for post cucumber and *nopales* phases were analysed using Mann-Whitney Test due to non-normally distributed variables.
[b] Likert-type scale: 0 = unsatisfactory; 1 = needs improvement; 2 = satisfactory; 3 = good; 4 = excellent.

CHAPTER 5

DISCUSSION

Nopales have been used as complementary and alternative medicine for glycemic and cholesterol control by Mexicans and Native Americans, but evidence regarding their cardiometabolic health benefits is scarce. To date, human studies assessing the effects of *nopales* pads have been limited to one study that evaluated their effects on fasting lipids and glucose levels (26), and one study that evaluated postprandial glycemia (158). Additional studies have used other cactus-based products: cactus pads incorporated into tortillas (160), dehydrated cactus pads (156, 159, 170), and prickly pear fruit (27, 29, 171, 172). The purported benefits of *nopales* are also based on animal studies using isolated pectin from cactus pads (173-175), and dehydrated cactus pads (176). Given the paucity of evidence regarding the effects of *nopales* intake, the present randomized crossover trial was designed to evaluate the effects of *nopales* intake compared to cucumbers on cardiometabolic risk factors in hyperlipidemic adults. This is the first controlled study specifically designed to testing the effects of *nopales* in its most commonly consumed form (boiled) on cardiometabolic risk factors. Furthermore, this study adds to previous work by including measurements of the distribution of cholesterol among lipoprotein subfractions and biomarkers of oxidative stress as potential mechanisms involved in the purported effects of *nopales* intake.

5.1 Cardiometabolic risk factors: lipids and lipoproteins

Limited evidence is available on the hypocholesterolemic effects of *nopales* from controlled trials. The present study did not show significant improvements on plasma lipids after the consumption of *nopales* compared to cucumbers. The only study that evaluated the effects of *nopales* on markers of cardiovascular risk was conducted by Frati-Munari et al. (26) showed in a quasi-experimental design with 8 non-obese, 14 obese, and 7 diabetic hypercholesterolemic adult participants (28-65 years of age) that *nopales* intake resulted in a 31 mg/dL decrease in total cholesterol ($p<0.001$). This decrease was more pronounced in obese and diabetic participants compared to non-obese. In comparison to our study, Frati-Munari et al. (26) included participants with higher baseline total cholesterol (220 ± 52 mg/dL) and LDL-c (164 ± 43 mg/dL). In addition, significant weight loss (1.5 kg) was observed only in the obese and diabetic groups, which were the same groups that experienced greater improvements in lipids. It is possible that weight loss contributed to some of the changes observed. In contrast, participants from the present study remained weight-stable throughout the entire study. The dose utilized in our study (280 g/day for 14 days) was similar to the Frati-Munari study (300 g/day for 10 days). However, in the present study *nopales* were provided boiled, instead of broiled.

One of the proposed bioactive compounds from *nopales* is dietary fiber. The current study was designed to provide an additional 2.5 g/day of soluble fiber. Boiling *nopales* has been shown not to interfere with soluble fiber content, although the viscosity and gel consistency may be compromised (177). According to a meta-analysis conducted by Brown et al. (22), increasing soluble fiber intake (oat β-glucan, psyllium, and pectin)

by 3 g/day is associated with a 5 mg/dL decrease in both total cholesterol and LDL-c. Although not statistically significant, we observed that after the *nopales* phase plasma total cholesterol and LDL-c concentrations were 4.5 mg/dL lower than after the cucumber phase. Nevertheless, we observed large individual response variability in LDL-c concentrations to both treatments. It has been proposed that the heterogeneity in the lipid response to dietary interventions is related to genetic polymorphisms, including variations in the genes encoding apoproteins, CETP, LCAT, LDL receptor, and cholesterol 7α-hydroxylase (178, 179).

Due to the scarcity of studies with cactus pads, studies evaluating prickly pear fruit will also be discussed. It is important to note that the nutritional composition of prickly pears (fruit) and *nopales* (pads) is not the same, and that the effects may not be comparable. Budinsky et al. (27) evaluated the effects of 250 g of prickly pear pulp consumption for four weeks compared to a healthy diet in hypercholesterolemic individuals. Prickly pear pulp intake led to a reduction in total cholesterol (-22.5 mg/dL, $p<0.01$) and LDL-c (-16.5 mg/dL, $p<0.04$), while triglycerides and HDL-c did not change with the intervention. In contrast to the current study, Budinsky et al. (27) included participants with a higher baseline LDL-c (226 ± 26 mg/dL), and provided the entire diet to participants keeping energy and macronutrients constant throughout the study. In the present study participants had mild hypercholesterolemia (LDL-c = 137 ± 20 mg/dL) and although they were asked to maintain their habitual diet, they may have displaced other fiber rich foods when adding the test foods. In a parallel study that included 68 women from 20 to 55 years of age with metabolic syndrome, the effects of dehydrated cactus pads capsules three times a day after meals for 6 weeks on lipids was evaluated. In

agreement with our study, no significant changes in total cholesterol, LDL-c, HDL-c and triglycerides were observed (170).

From studies with guinea pigs, the main proposed mechanism for the hypocholesterolemic effect of prickly pears or prickly pear pectin is associated with a decrease in intestinal cholesterol absorption coupled with an increase in the expression of Apo B/E receptors in the liver (171, 173, 175). This would result in increased bile acid excretion and the interruption of the enterohepatic circulation of cholesterol and bile acids (173, 175). Similarly, in individuals with familial hypercholesterolemia consumption of 250 g/day prickly pear for six weeks, a 16% decrease in LDL-c was accompanied by a 27% increase in LDL uptake by the liver (171).

Lipoproteins are heterogeneous according to particle size, density and composition. Therefore, assessing the distribution of cholesterol among different lipoprotein subfractions may provide information on anti- or pro-atherogenic effects of dietary interventions beyond traditional lipids measures (57). Few studies have evaluated the effects of soluble fiber on lipoprotein remodeling. The addition of 3 g of β-glucagon from barley to a National Cholesterol Education Step I diet (low fat, low cholesterol diet) in mildly hypercholesterolemic subjects led to a 14% lower LDL-c concentration. However, the amount of cholesterol in large LDL particles were also lower compared to control, while no change was observed for small LDL particles (180). In contrast, Davy et al. (181) showed that the addition of oat cereal for twelve weeks not only reduced LDL-c (-2.5%), but also the concentrations of small, dense LDL-c (-17.3%) and the number of LDL particles (-5%) compared to wheat cereal. In another study, a one month diet therapy that included 7 g of psyllium and 2 g of plant sterols significantly reduced LDL-c

concentration and increased LDL peak size in hypercholesterolemic individuals. This resulted in the lower prevalence of the more proatherogenic LDL pattern B from 27 to 18% (182).

Studies have shown that small, dense LDL particles are strongly associated with increased triglycerides concentration (63, 183). An increased pool of triglyceride-rich and cholesterol-poor VLDL leads to the production of LDL with fewer lipids in its core, resulting in smaller, denser particles. Furthermore, LDL particle size is reduced as particles become triglyceride-enriched and cholesterol-depleted by the action of hepatic lipase and CETP producing small, dense LDL particles. (51, 66, 67). In the present study, triglycerides concentration increased after the consumption of both test foods (p_{Time}=0.020) and tended to be higher after the *nopales* phase (p_{Treat}=0.090). However, no differences were observed for LDL size and LDL lipoprotein subfractions over time or between treatments. In contrast, pro-atherogenic changes in HDL subfractions were observed after the consumption of both study foods by decreasing the proportion HDL cholesterol in large HDL and increasing the proportion in small HDL. Elevations in triglycerides have been observed when dietary fat is replaced by carbohydrates (184-186). The suggested mechanism for carbohydrate-induced triglyceride elevation is by reducing triglycerides clearance rather than increasing its production (187). However, it is unlikely that this was the underlying cause for an increase in triglycerides as two cups of the test food offered only adds 10 g of carbohydrates. In addition, according to participants' reported food intake, the contribution of each macronutrient to total energy intake was not altered throughout the study. Conversely, the displacement of other food sources when incorporating the test foods to the habitual diet cannot be ignored. The

dietary assessment has shown a decrease in the overall consumption of monounsaturated and polyunsaturated fatty acids with the addition of both test foods. Studies have reported that decreasing the consumption of unsaturated fatty acids increases triglycerides levels (188-190).

5.2 Cardiometabolic risk factors: glucose and insulin

The most studied health outcome of prickly pear consumption has been on its hypoglycemic effects (26, 28, 158, 191). In the present study, the consumption of *nopales*, relative to cucumber intake, did not result in significant effects on fasting glucose, insulin or HOMA. In contrast, other studies have documented a reduction in fasting glucose concentrations after the consumption of *nopales* or *nopales* products. In a study by Frati-Munari et al. (26) the consumption of *nopales* decreased glucose concentrations by 63.4 ± 27.1 mg/dL ($p<0.001$) among participants with diabetes. The decrease observed in non-diabetic individuals, albeit statistically significant, was of a much lower magnitude (3.5 ± 1.5 mg/dL) (26). Similarly, in a crossover study including 28 healthy adults, the consumption of 200 g of tortillas made with *nopales* for three weeks decreased glucose concentrations by 17 mg/dL (17.5%) (160).

In the postprandial period, the effects of *nopales* on postprandial glycemia have been investigated in individuals with type 2 diabetes. The consumption of different breakfast menus containing *nopales,* compared to the same breakfast without *nopales,* resulted in a decrease in the incremental area under the curve for the glycemic response. The extent of the reduction differed from 20 to 48% depending on the breakfast preparation (chilaquiles, burritos, or quesadillas), suggesting that changes are also related

to the macronutrient content and other components of the mixed meal (158). In a parallel study with 29 participants the supplementation with 200 mg of capsules containing cactus pads and fruit extract in pre-diabetic individuals have shown to decrease glucose postprandially. However, after 16 weeks of supplementation no significant changes were observed in fasting glucose, insulin, lipids and CRP compared to the placebo (191). There are two hypothesized mechanisms underlying the hypoglycemic effects of *nopales.* The first mechanism is related to lower postprandial sugar absorption due to the soluble fiber content of the *nopales*. The second mechanism is related to improved cellular sensitivity to insulin due to the presence of bioactive components. However, the exact mechanisms remain unclear (25).

5.3 Oxidative stress

It has been suggested that oxidative stress is involved in the development and progression of chronic diseases, such as diabetes and atherosclerosis. Few studies have examined the effects of prickly pear fruit on oxidative stress outcomes; however, to date the effects of *nopales* pads on markers of oxidative stress have not been investigated. In the current study, measurements of oxidized LDL, LDL susceptibility to oxidation, vitamin C and total antioxidant capacity were used as markers of oxidative stress. Emphasis was given to lipid peroxidation markers due to its role on the atherosclerotic process (17, 101, 102). Our study provided no support that the consumption of *nopales* decreased oxidative stress.

The lack of improvement in markers of oxidative stress in the present study may be explained by a combination of factors. First, *nopales* did not significantly reduce

substrates for oxidation such as LDL-c or small, dense LDL-c concentrations. The development of oxidized LDL is dependent not only on ROS formation and antioxidant capacity but also on substrate concentration availability, more specifically of lipids (136). In fact, it has been shown that oxidized LDL is positively associated with LDL-c concentrations and inversely associated with HDL-c concentrations (192-194). LDL size also influences LDL susceptibility to oxidation. Several studies have shown an inverse association between LDL size and oxidized LDL concentrations (193, 195, 196). Small, dense LDL particles have a lower affinity for LDL receptors, prolonging its residence time in the circulation and increasing its susceptibility to oxidation and glycation (67, 68). Therefore, the lack of significant changes in LDL-c concentrations and LDL size may partially explain the absence of improvements in lipid peroxidation markers.

Second, some phytochemicals present in other functional foods may not be present in the *nopales* pads. It has been previously reported that the consumption of prickly pear fruit decreases urinary and plasma isoprostanes in individuals with familial hypercholesterolemia. The effects observed were more pronounced in females than males and isoprostanes were positively related to LDL-c concentrations (27). Similarly, Tesorieri et al. (29) observed in healthy adults significant decreases in isoprostanes, malondialdehyde and conjugated dienes lipid hydroperoxide after prickly pear fruit intake for two weeks compared to vitamin C. Interestingly, plasma vitamins C and E were significantly increased in both groups; however, oxidative markers were only decreased with prickly pear fruit intake. This would suggest that prickly pear fruit has other antioxidants or bioactive components that prevented oxidative damage. The authors suggested that the presence of betalains could be responsible for the positive findings

(29). The same group observed a postprandial increase in the distribution of betalain pigments in red blood cells after prickly pear fruit intake, conferring protection to the cells (172). Unlike the fruit, *nopales* pads used in the present study do not contain betalains.

Finally, it is also possible that thermal treatment (i.e. boiling) may have attenuated bioactive components thus decreasing the antioxidant activity potential of *nopales*. Ramirez-Moreno et al. (177) evaluated the effects of boiling cactus pads (*Opuntia spp* of two different cultivars) for 20 minutes on nutritional composition and antioxidant capacity. Boiling caused losses mostly of soluble compounds, such as vitamin C and soluble sugars (glucose and fructose), with retention values between 49 to 67%. The retention of β-carotene varied from 54 to 84% depending on the cultivar. Another study evaluating the thermal treatment effects on antioxidant activity of cactus pads identified three types of carotenoids, lutein, β-carotene, and α-cryptoxanthin. The thermal treatment actually increased the extractability of carotenoids, improving their bioavailability. Despite the fact that the phenolic compounds were decreased, the antioxidant activity was significantly increased. This suggests that carotenoids are the main known antioxidant compounds in cactus pads (197). This is in agreement with another study evaluating the effects of boiling on vegetables, such as carrots and broccoli, where an increase in TAC was observed. The authors suggested that the increase in antioxidant activity is due to the matrix softening and increased extractability of the compounds (198). Therefore, the modifications related to boiling could potentially lead to positive or negative changes in the metabolic effects after *nopales* ingestion.

5.4 Dietary Intake

Feeding studies can help establish causal relationships between diet and disease risk factors due to its controlled nature (199). In the present study participants received the test foods weekly and were advised to maintain their habitual diet. Dietary assessment before and during each intervention was performed to evaluate if other changes besides the inclusion of the test foods were made to the diet. A significant decrease in energy intake was observed during both treatment phases. This change is probably related to the increased satiety as the increased intake of low energy-dense foods can displace high energy-dense foods. Despite the fact that the contribution of each macronutrient to the total energy intake did not change throughout the treatments, a significant decrease in the total amount of fat was observed with the introduction of both test foods. While this may appear to be a beneficial shift, the decrease in total fat was mostly due to reductions in mono and polyunsaturated fatty acids provided by nuts and avocados. Previous studies have shown that increased intake of unsaturated fatty acids have a major impact on lipids by decreasing the ratio of total to HDL cholesterol and decreasing triglycerides (188-190).

We observed no significant change in total, soluble or insoluble fiber intakes with the addition of the test foods to the participant's habitual diet. Participants were encouraged to not change their diet when adding the test foods. If that would hold true, an increase of 2.9 g and 5.6 g of total fiber and of 0.5 g and 2.5 g of soluble fiber would be expected in the cucumber and *nopales* phases respectively. However, a non-significant decrease in fiber intake was observed during both treatment phases. When adjusting for calorie intake, it was noted that fiber intake remained the same throughout the study. The

3-day food records were checked to confirm the consumption of 2 cups/day of test foods, which were described. The analysis of dietary intake by food group showed that vegetables intake was increased on average by 2.7 servings/day during the cucumber phase. Since cucumbers are not a rich source of fiber, it did not reflect an increased fiber intake. Conversely, during the *nopales* phase, vegetable intake was increased on average by 1 serving/day, suggesting that participants displaced other vegetables when adding *nopales*. Since this was a new food for several participants, it is possible that a greater shift took place in order to incorporate *nopales* into their habitual diet.

The displacement of nutrients due to the addition of functional foods has not been extensively studied. This process may blunt the actual functional food metabolic responses or in some cases overestimate its effect size. For instance, the extent of soy protein health benefits has been contested as recent trials showed only a modest cholesterol-lowering effect of isoflavone supplementation compared to older trials. Jenkins et al. (200) has shown that the intrinsic cholesterol-lowering effects of soy protein contributed to a 4.3% LDL-c reduction while the displacement from the diet of saturated fat and cholesterol-rich foods contributed a 3.6 to 6% LDL-c reduction. Thus, extrinsic effects must also be considered when evaluating the effects of functional foods on metabolic responses.

5.5 Limitations and Strengths

There are some limitations to our study. It is possible that our study may have lacked statistical power to detect changes in outcomes of interest. The sample size calculation estimated that at least 16 subjects would be necessary for this study to have

enough statistical power for detecting changes in total cholesterol. Recruitment was increased in order to assure that the minimum sample size was achieved. The sample size calculation was based on Frati-Munari et al. (26) study, the only study available about humans using *nopales*. It is possible that the effect size based on that study may have been overestimated because of its quasi-experimental design, it included participants with higher total- and LDL-c concentrations, and participants lost weight during the study. When using actual LDL-c changes from the present study to conduct a power analysis, the suggested sample size for having a statistical power (at a 0.05 significance level and power > 0.80) was 42 participants. The attrition rate (41%) was higher than anticipated (20%). In part, this was attributed to the participants dropping out of the study before starting the actual intervention. However, since some participants dropped out because of their dislike of *nopales*, implementing a taste test during the recruitment phase may have helped exclude participants that disliked the test food. Although at least two weeks are necessary in order to observe lipoprotein metabolism changes (201), it is possible that greater effects would have been observed if the test food intake phases had been longer. A longer intervention time would have also provided information regarding the sustainability of adding *nopales* to a habitual diet. We relied on self-report data to estimate dietary intake using 3-day food records. This method has fewer problems with omission of foods compared to other methods since it does not rely on memory (202). However, estimating portion sizes can be a source of error and underestimation of energy intake has been reported (202). Improved dietary adherence and avoidance of nutrients displacement may have been achieved by providing the complete diet to participants.

Although this approach would control participant's energy and nutrients intake, it would also lose its applicability to free-living individuals and such studies are highly costly.

The present study has a number of strengths worth discussing. The randomized crossover design allows each participant to serve as their own control, minimizing individual variation. In order to reduce treatment effect variability and the interference of external factors, participants were recruited based on the presence of moderately high LDL-c and were otherwise healthy. Study inclusion criteria were stringent. Participants were non-smoking, sedentary, with low habitual fruit and vegetable consumption and not taking drugs or supplements known to interfere with the study's outcomes. Another strength from this study is that the participants' body weight was maintained during the study, minimizing the effects of weight loss on the measured outcomes. Finally, extra care was taken to measure the lipids to provide accurate values. The LDL-c concentration was directly measured in plasma rather than using the Friedewald formula (203), which is known to underestimate LDL-c in the presence of increased triglyceride concentrations. Also, all lipids were measured in two separate days at the beginning and end of each phase to decrease the effect of day-to-day variability by about 3% (204).

5.6 Future research

The present study was conducted to evaluate the short-term effect of *nopales* consumption on cardiometabolic risk factors under free-living condition. Future research evaluating *nopales* should include a larger sample size and opt for a tightly controlled study design under metabolic conditions. In this approach it is possible to monitor participants more closely, assuring adherence to the diet and consistency throughout the

study. This would allow a more accurate assessment of *nopales'* intrinsic effects by ruling out the extrinsic effects of food displacement. In addition, a longer study duration of at least four weeks may be necessary to show greater changes in the lipoprotein profile.

A decrease in energy intake was observed with the addition of both test foods to the diet. Vegetables are low energy density foods and a good source of fiber which may have caused an increase in satiety levels and a consequent shift in eating patterns. It would be interesting to evaluate changes in hormones that are related to satiety. For example, ghrelin is known to stimulate appetite, whereas other hormones, such as leptin, cholescystokinin (CCK), and peptide YY (PYY), are known to inhibit appetite. These biomarkers could help assess the satiating effects of foods provided as well as clarify food intake regulation (205).

People spend most of their day in a postprandial state causing transient elevations in glucose and lipids that can lead to oxidative stress, inflammatory response and endothelial dysfunction. Studies have shown that increased postprandial triglycerides are associated with incident cardiovascular events and cardiovascular mortality (206-208). Previous studies only evaluated the effects of *nopales* and dehydrated prickly pear on postprandial glycemia (158, 191). Therefore, future studies should investigate the effects of isocaloric meals with and without *nopales* on postprandial lipemia.

CHAPTER 6

CONCLUSION

Research assessing strategies to decrease CVD risk are in growing need as CVD continues to be the leading cause of morbidity and mortality among Americans. The present study was designed to evaluate the effects of adding *nopales* to a participant's habitual diet on markers of cardiometabolic disease risk. This test food was selected based on its nutritional composition and purported hypocholesterolemic effects. Previous studies have shown that prickly pear pads and fruits can help reduce fasting and postprandial glucose, as well as fasting LDL-c. We elected prickly pear pads in its most common preparation (boiled) as this would better reflect how this vegetable is normally consumed, and results would be more applicable to the general population. The primary aim of the study was to evaluate the effects of *nopales* on lipoprotein metabolism as literature is scarce in this topic. To our knowledge, this is the first study to examine the effects of *nopales* on lipoprotein subfractions. In addition, two exploratory analyses were conducted in order to evaluate the effects of *nopales* on cardiometabolic risk factors and oxidative stress.

This study did not confirm the main hypothesis that *nopales* intake compared to control would improve the lipid profile of adults with moderate hypercholesterolemia. No significant treatment-by-time effect was observed for total cholesterol, LDL-c, HDL-c, triglycerides or any of the lipoprotein subfractions. In addition, the other cardiometabolic risk factors (glucose, insulin, HOMA, blood pressure, and CRP) and oxidative stress

(total antioxidant capacity, plasma vitamin C, oxidized LDL, and LDL oxidation) were not improved in the *nopales* phase compared to control. Reasons for the lack of the effect of *nopales* intake on the outcomes measured may be related to the study small sample size, treatment duration, lack of compliance to treatment, and the displacement of other foods. The latter is an area that deserves further attention as shifts in eating patterns due to the addition of function foods may have detrimental effects on health. More research is needed to better understand the effects of food displacement and the best strategies to introduce a new food to an individual's habitual diet. Incorporating functional foods into a diet is appealing because one simple change may be able to mitigate disease risk and promote health. However, by modifying only one aspect of the diet modest improvements should be expected. Greater effects can be achieved by modifying the overall dietary pattern but long term compliance may be a challenge.

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APPENDIX A

IRB APPROVAL

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Sonia Vega-Lopez 425 North
From:	Carol Johnston, Chair Bioscience Full Board
Date:	12/21/2012
Committee Action:	Approval
IRB Action Date	12/21/2012
Approval Date	12/21/2012
IRB Protocol #	1211008560
Study Title	The Efficacy of Nopales (Opuntia Spp) on Lipoprotein Profile and Oxidative Stress among Moderately Hypercholesterolemic Adults
Expiration Date	12/20/2013

The above-referenced protocol has been APPROVED following Full Board Review by the Institutional Review Board.

This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date noted above. Please allow sufficient time for continued approval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Bioscience Full Board immediately. If necessary a member of the Committee will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Bioscience Full Board. The new procedure is not to be initiated until the IRB approval has been given.

APPENDIX B

SCREENING FORM

SCREENING FORM

Screening ID#: _____ Date of Phone Call: _____ Recruiter: _____

Recruiter: Obtain verbal consent to ask eligibility criteria questions by reading and asking the following:

In order to determine whether you qualify or not for the study I need to ask a few questions about you and some general health information. This will take about 15 minutes. Can I ask these questions at this time?

YES ☐ NO ☐

If YES, continue asking eligibility verification questions.
If NO, inform participant that you cannot proceed and thank him/her for their time. **(STOP)**

(Do not read) Participant's gender:

MALE ☐ FEMALE ☐

ELIGIBILITY VERIFICATION

How old are you? _____

(Do not read) Is age between 18 and 70 years? YES ☐ NO ☐

Do you live in the Phoenix area? YES ☐ N NO ☐

If the answer to any of these questions is NO, read: **(STOP)**

At this point you do not qualify for this study. Thank you very much for your time.

If the answer to all of these questions is YES, continue.

Are you afraid of needles or blood drawing? YES ☐ NO ☐
Do you faint when you have your blood drawn? YES ☐ NO ☐
Is it usually hard for medical personnel to draw your blood? YES ☐ NO ☐

If participant is a woman <50 y old please ask:

Are you Pregnant? YES ☐ NO ☐
Breastfeeding? YES ☐ NO ☐
Are you planning to become pregnant in the next 3 months? YES ☐ NO ☐

Are you following any of the following diets?
Vegan YES ☐ NO ☐
Very low carbohydrate YES ☐ NO ☐
Atkins YES ☐ NO ☐
Are you following any other specific diet? What type? _____

(Do not read) Is this a restrictive diet? YES ☐ NO ☐
Recruiter: consider any extreme diet or any diet that restricts a major food group, except for regular vegetarian diets, as a restrictive diet.

Do you eat 4 or more servings/day of fruits and vegetables YES ☐ NO ☐

Do you take supplements such as antioxidants, fiber or botanicals? YES ☐ NO ☐
Do you exercise for 30 minutes or more a day, 5 times or more per week? YES ☐ NO ☐
Are you allergic to LATEX, *nopales*, or cucumber? YES ☐ NO ☐
Has a doctor or health care provider ever told you that you have any of the following:
Heart disease? Diabetes? High blood pressure?
Kidney disease? Liver disease? Cancer ?
Hepatitis? Thyroid disease? HIV? Arthritis?
YES ☐ NO ☐

Are you taking any of the following medications?
Cholesterol-lowering
Blood pressure
Diabetes or blood sugar control YES ☐ NO ☐

Has a doctor or health care provider ever told you that you have any other medical condition I did not mention?
YES ☐ NO ☐

Are you enrolled in any other research study anywhere? YES ☐ NO ☐

If the answer to any of these questions is YES, read: (STOP)

At this point you do not qualify for this study. Thank you very much for your time.

Is patient eligible for screening?	**YES ☐**	**NO ☐ (STOP)**

If YES, continue explaining the study procedures.

Do you know your LDL cholesterol level? YES ☐ NO ☐ NOT SURE ☐

If yes, what is it? _____

Will you be able to maintain your current diet and physical activity for a consecutive 7 weeks?
YES ☐ NO ☐ NOT SURE ☐

Prior to starting the study we will ask you to attend a preliminary screening visit to verify you have moderately high cholesterol. If you qualify for the study, you will be asked to provide written informed consent for all study-related procedures. You will be randomly assigned to supplementation for 16 days with 2 cups/day of *nopales* or 2 cups/day of cucumbers. After 2-3 weeks of no intervention, you will receive the alternative treatment for an additional 16 days. We would then ask you to come for two study visits before and after each dietary intervention for a total of 8 visits, in which we will measure your height, weight, waist, blood pressure, and we will draw your blood. You will have to fast for 12 hours before your study visits. Before each visit we will ask you to keep record of your food intake for three days and to bring that information with you at the time of your appointment. Are you willing to participate in this study?
☐ **YES** ☐ **NO**

If YES, continue explaining the screening procedures.

As part of this research study we will ask you to come to our ASU study site for a preliminary visit to explain the study to you, ask you to sign a screening procedures consent form and we will collect your blood draw (about 5 ml or 1 teaspoon) to check your blood cholesterol. You will need to fast for 12 hours prior to your study visit. This means you that you should not eat or drink

anything but water starting 12 hours before your appointment. This appointment will be scheduled early in the morning so that you can come before you have breakfast. We will give you a light snack after the blood draw.

Which of the following would you prefer?

☐ **A**. Meet first to sign the screening consent form and subsequently schedule your formal screening visit.
Recruiter: Fill out A. and B. in the box below.

☐ **B.** Consent now to fast prior to attending the screening visit at which I will sign the screening consent form before any other procedure takes place.
Recruiter: Only fill out B. in the box below.

Have you donated blood in the past 4 weeks? YES ☐ NO ☐

If YES, when?

_____

Recruiter: Schedule screening visit at least 4 weeks after the blood donation date.

Where did you hear about this study? _____

A. Meeting to sign screening consent form date and time: _____

B. Screening visit date and time:
_____

Recruiter: Read the following:

Thank you for your time.

LDL ELIGIBILITY

Screening LDL-Cholesterol: _____

Is LDL-C ≥ 130 mg/dL? **YES** ☐ **NO (Ineligible participant)** ☐

Is LDL-C ≥ 190 mg/dL? **YES** ☐ **NO** ☐

If YES, refer to PCP for care. Enroll only if participant refuses medication use or if based on prior use participant is intolerant to hypolipidemic medications.

If NO, participant is eligible to participate. **Study ID:**
_____

APPENDIX C

CONSENT FORM SCREENING

ARIZONA STATE UNIVERSITY
PREADMISSION SCREENING INFORMED CONSENT FORM

The Effect Of *Nopales* (*Opuntia Spp*) On Lipoprotein Profile And Oxidative Stress Among Moderately Hypercholesterolemic Adults

Principal Investigator: Sonia Vega-López

Co-Investigator: Giselle Pereira Pignotti

INTRODUCTION

The purpose of this form is to record the consent of those who agree to be involved in the screening process to assess your eligibility to participate in this research study. If you are found to be eligible you will be asked to sign a separate informed consent for the actual research study.

RESEARCHERS

Dr. Sonia Vega-López, Assistant Professor, School of Nutrition and Health Promotion at Arizona State University and her co-investigators have invited your participation in a research study.

STUDY PURPOSE

The overall purpose of the research is to determine the effects of eating *nopales* pads (cactus pads) and cucumber on factors that increase the risk for developing heart disease and diabetes among adults with moderately elevated blood cholesterol. The purpose of the screening process is to find out if you are eligible to be part of the study.

DESCRIPTION OF SCREENING PROCESS

You have participated in some pre-screening procedures by answering a questionnaire about your health, medications and lifestyle either using a web-based survey (Survey Monkey) or via a telephone interview. Based on your responses, you may qualify for the study if your blood cholesterol is slightly high. You have now been asked to participate in a preliminary blood draw screening to assess your blood cholesterol. If you have given verbal consent to come to the laboratory in a fasted state (no food for 12 hours; nothing but water to drink for the past 12 hours) for the blood draw screening, this blood draw will take place today. If you have not given verbal consent to fast for this blood draw, or if you are not fasting, your screening visit will be scheduled once you sign this consent form.

You will have 5 ml (about 1 teaspoon) of blood drawn from the vein in your arm to verify the presence of moderately high blood cholesterol (LDL- ["bad"] cholesterol ≥ 120 mg/dL). The results of the LDL-cholesterol test will be made available to you. If your LDL-cholesterol is high (≥ 190 mg/dL) you will be advised to contact your primary care provider for proper medical care for high cholesterol. In this case, you will only be admitted to the study if after consulting with your primary care provider you do not want to take cholesterol lowering medications, or if in the past you have had side effects to those medications.

If you agree to participate in the screening procedures to learn if you qualify for the study, the screening procedures will last for up to 1 hour and will involve a fasting blood draw at the ASU Downtown campus. Approximately 120 people from the Phoenix area will be screened to participate in this study.

RISKS

If you decide to participate in this screening visit, there is a small risk that you will feel uncomfortable from hunger, dizzy, or lightheaded due to fasting. You may face a risk of bruising and discomfort, dizziness, and fainting associated with blood drawing. However, this risk is small. The research team will minimize these risks by using trained personnel to draw your blood and by giving you a snack after the blood draw.

BENEFITS

There are no direct benefits from participating in this screening process. The results of this screening procedure may or may not qualify you to be admitted into the research study. The results of the LDL-cholesterol test will be made available to you.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. Your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Vega-López will code all the data and blood samples so that they do not contain any information that could identify you. All confidential information will be kept in a locked filing cabinet in Dr. Vega-López' office or in a password-protected computer, and will only be available to members of the research team. All samples and study materials will be destroyed 10 years after the study has been completed or upon your withdrawal from the study. At this point, blood samples will be discarded and study-related documents will be shredded.

WITHDRAWAL PRIVILEGE

Taking part in this research study is totally your choice. It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. You can decide to stop taking part in this study at any time for any reason.

COSTS AND COMPENSATION

The researchers want your decision about participating in the screening process to be absolutely voluntary. There will be no monetary compensation for participating in the screening process. You will be offered a copy of the LDL-cholesterol exam.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Vega-López, 500 N 3rd ST, Phoenix, AZ 85004; (602) 827-2268; sonia.vega.lopez@asu.edu .

If you have questions about your rights as a subject/participant in this research, or is you feel you have been placed at risk, you can contact the Chair of the Humans Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at (480) 965-6788.

This form explains the nature, demands, benefits and any risks of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study

_____	_____	_____
Subject's Signature	Printed Name	Date

_____	_____
Contact phone number	E-mail

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX D

CONSENT FORM FULL STUDY

ARIZONA STATE UNIVERSITY
FINAL STUDY INFORMED CONSENT FORM
The Effect of *Nopales* (*Opuntia Spp*) on Lipoprotein Profile and Oxidative Stress among Moderately Hypercholesterolemic Adults

Principal investigator: Sonia Vega-López
Co-investigator: Giselle Pereira Pignotti

INTRODUCTION

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study. You have participated in some screening procedures as outlined in the screening consent form. You have now been invited to participate in the study, since you are in reasonable health and you qualify for the research study.

RESEARCHERS

Dr. Sonia Vega-López, Assistant Professor, School of Nutrition and Health Promotion at Arizona State University and her co-investigators have invited your participation in a research study.

STUDY PURPOSE

The purpose of the research is to determine the effects of eating *nopales* pads (cactus pads) and cucumber on factors that increase the risk for developing heart disease and diabetes among adults with moderately high blood cholesterol (LDL- ["bad"] cholesterol ≥ 120 mg/dL) but otherwise healthy.

DESCRIPTION OF RESEARCH STUDY

If you decide to participate in this research study, it will involve a commitment of approximately seven weeks in which there will be two 16-day test phases. During these test phases you will be provided with either one of the test foods: *nopales* or cucumbers, and you will be asked to consume 2 cups/day of the test food (divided into two 1-cup daily doses with each of two main meals). The order in which you consume the test foods will be assigned to you randomly (by chance, like a toss coin). There will be a 2 to 3 week break in between the two diet phases. All study procedures described below will be done for research purposes only.

All test foods (*nopales* or cucumbers) will be provided to you by our study personnel. You will be asked to attend the study site twice per week to pick up the study during each intervention phase. The test foods (*nopales* and cucumber) will be purchased from a local produce distributor. The *nopales* will be cooked in our metabolic kitchen, and will be packed in sealable plastic bags containing the daily amount you will have to consume (2 cups each). You will receive 3 to 4 bags every time you come to the study site to get your study foods. When you are consuming cucumbers, you will get them whole in enough amount to provide the daily dose (2 cups), and you will be given detailed instructions for rinsing, chopping and measuring the appropriate amount of cucumbers (using a study provided measuring cup) for daily consumption. You will have to keep all study foods (*nopales* or cucumbers) refrigerated and will have to refrain from sharing these foods with others. You will also have to maintain your usual level of physical activity and usual diet throughout the research study period and to keep tally of servings of test foods per day.

We will ask you to fill a 3-day food record form in which you will record everything that you eat and drink for three days (two weekdays and one weekend) before and during the last week of each testing phase. You will be asked to complete this questionnaire and bring it to the

next study visit. Our study personnel will be available to assist you if you have any questions or need help filling out the questionnaire.

All testing will be completed during 2 separate days (A and B) at the beginning (days 1 and 2) and 2 separate days at the end (days 15 and 16) of each intervention phase, for a total of 8 test visits. All testing visits will involve a blood draw. We need to draw your blood on 2 separate days so that we can average your blood lipid values (cholesterol and other fats) as they normally vary on a day-to-day basis.

You will need to fast for 12 hours prior to your study visits. This means you that you should not eat or drink anything but water starting 12 hours before your appointment. This appointment will be scheduled early in the morning so that you can come before you have breakfast. We will give you a light snack after each blood draw.

Test day A - You will be asked to use the restroom to empty your bladder after which measurements of height, weight and waist circumference will be taken 3 times. You will be asked to sit down for a few minutes after which we will measure your blood pressure 3 times.

We will collect a blood sample to measure your blood lipids (cholesterol and other fats), sugar, indicators of risk for heart disease and diabetes, indicators of how cholesterol and sugar are processed in your body, and indicators of inflammation, response to oxidation, and dietary quality. The total amount of blood that we will draw will be 38 ml (about 3 tablespoons).

We will store some of the blood we collect (15 ml or about 1 tablespoon) for the future measurement of additional markers of diabetes and heart disease risk, inflammation, response to oxidation, and dietary quality. You will be given the option to decide whether you want us to store your blood for future use. If you agree to have your blood stored for future use, you give us permission to share this blood with other investigators without notifying you. No genetic analysis will be performed on any blood collected.

During the first visit we will also measure your body composition by estimating the relative amounts of fat and lean tissue using an FDA-approved bone density measurement machine (Dual-Energy X-Ray Absorptiometry or DEXA). For this, you will lie face up in the DEXA bed for 7 minutes while the DEXA arm passes over your entire body. If you are a woman, we will ask you to provide a urine sample to conduct a pregnancy test in our laboratory prior to the DEXA measurement. A negative test is required before the DEXA can take place. If the pregnancy test shows a positive result, you will no longer be able to continue in the study, and will ask you to go to your regular health care provider for appropriate follow-up procedures.

Test day B – We will collect an additional blood sample (7 ml, about ½ tablespoon) to measure your blood lipids and account for how they vary on a day-to-day basis, and vitamin C.

You will be asked to complete a survey with the help of an interviewer to ask information about any gastrointestinal discomfort, the acceptability of the test foods, and your satisfaction with the study. On the first survey you will also be asked to give information about your sociodemographic characteristics.

If you agree to be part of the study, then your participation will last for approximately 7 weeks and will consist of 8 test visits to the Downtown campus of ASU, each lasting about 60 min, and no more than 2 hours, plus biweekly visits to pick up your study foods during the test phases. Each test visit will include a fasting blood draw. Approximately 60 people from the Phoenix area will be participating in this study.

RISKS

If you decide to participate in this study, then you may face a risk of bruising and discomfort, dizziness and fainting associated with blood drawing. However, this risk is small. There is also a small risk that you will feel uncomfortable from hunger, dizzy, or lightheaded due to fasting. The research team will minimize these risks by using trained personnel to draw your blood and by giving you a snack after the blood draw.

You might experience mild discomfort during blood pressure testing as the cuff inflates. However, the risk is small, and discomfort will go away after the cuff is deflated.

For the body composition measurement, you will be exposed to minimal radiation (1-4 microSieverts) that is within an acceptable range as provided by the US FDA. The amount of radiation (1-4 microSieverts) that you would be exposed to is quite minimal. For example, you would receive radiation exposure of approximately 30 to 40 microSieverts during a typical chest x-ray. If you are a woman, you will be asked to provide a urine sample to conduct a pregnancy test before the DEXA to avoid fetus exposure to radiation. If a pregnancy test is positive, you will no longer be able to take part of the study. There is a small risk that the pregnancy test will be negative when you are in fact pregnant (false negative; tests are 99% accurate), which could lead to radiation exposure to a fetus.

There is no known risk of consuming two cups per day of *nopales* (cactus pads) or cucumber. There is a small possibility of having an allergic reaction to the test foods. Other possible side effects include nausea and headaches. It is possible that you may experience increased stool volume and more frequent bowel movements associated with increased fiber intake.

It is possible that study foods may spoil if they are not kept refrigerated. As with most vegetables, there is a small risk of food poisoning if the test foods are not refrigerated, although this risk is smaller than that of foods becoming spoiled. To minimize this risk, we will ask you to keep the test foods refrigerated, to pick up the test foods twice per week (so that you only have a small amount of foods at once), and to pick up the test foods closer to the time when you will be going home to ensure you can place them in the refrigerator promptly.

As with any research, there is some possibility that you may be subject to risks that have not yet been identified. There are no feasible alternative procedures available for this study. You can ask your doctor for a health exam.

BENEFITS

There are no direct benefits from participating in this study. However, knowledge may be gained from your participation, which may benefit the health of others. You will be offered a copy of select results (e.g. serum lipids, blood pressure, weight status) from the beginning and end of treatment assessments.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Vega-López will code all the data and blood samples so that they do not contain any information that could identify you. All confidential information will be kept in a locked filing cabinet in Dr. Vega-López' office or in a password-protected computer, and will only be available

to members of the research team. All samples and study materials will be destroyed 10 years after the study has been completed or upon your withdrawal from the study. Blood samples will be discarded and study-related documents will be shredded.

WITHDRAWAL PRIVILEGE

Taking part in this research study is totally your choice. It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. You can decide to stop taking part in this study at any time for any reason.

COSTS AND COMPENSATION

The researchers want your decision about participating in the study to be absolutely voluntary. Yet they recognize that your participation may pose some inconveniences due to the time needed to complete the research activities and because we will draw a blood sample from you in each visit. In order to compensate for your time and discomfort, you will receive a total of $100 in gift cards, a set of ear phones, and a pen drive for completing the entire study.

You will receive compensation as follows:

Visit 1 A - $15

Visit 1 B - $10

Visit 2 A - $15

Visit 2 B - $10 and one set of ear phones

Visit 3 A - $15

Visit 3 B - $10

Visit 4 A - $15

Visit 4 B - $10 and one pen drive.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Vega-López, 500 N 3rd ST, Phoenix, AZ 85004; (602) 827-2268; sonia.vega.lopez@asu.edu .

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Humans Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at (480) 965-6788.

This form explains the nature, demands, benefits and any risks of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study

_____	_____	_____
Subject's Signature	Printed Name	Date

_____	_____
Contact phone number	E-mail

Your initials here indicate whether you give us permission to store 15 ml (1 tablespoon) of your blood for future use for the measurement of additional markers of diabetes and heart disease risk, inflammation, response to oxidation, and dietary quality.

I ☐ DO consent to have my blood stored for future analyses.

I ☐ DO NOT consent to have my blood stored for future analyses. _____

Subject's initials

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX E

FOOD RECORD FORM

FOOD RECORD FORM

A food record form is used to record the amounts and types of foods you eat and drink each day. Please record your food intake for **three random days** (e.g. Tuesday, Thursday, Sunday, etc.), including one weekend day. In recording the foods/beverages you consume each day, try to be as precise as possible with the amounts and descriptions of each food/beverage consumed.

Attached you will find a blank food record form. Below are instructions for completing the form.

- **Record everything, forget nothing.** Do not forget to write down everything that you eat and drink, including foods consumed for both meals and snacks.
- **Include condiments and oils used for cooking.**
- **Be accurate with food descriptions.** Write down clear descriptions of the food or beverage that you consume. In addition, it is important to mention how the food was prepared. For example: baked chicken, toasted wheat bread, boiled carrots.
- **Record the amount of food/beverage consumed.** You can use household measures such as cups, tablespoons, teaspoons, etc., or weight and volume measures such as ounces, pounds, grams, etc.
- **Record everything immediately after eating.** Carry the food record with you everywhere so that you don't forget to write down anything you've eaten.
- **Ask the assistance of the person who prepared the food.** The person who prepares your meals or snacks, if it is not yourself, may have better idea of what was in the food you ate than you do.
- **Include all supplements.** Include vitamins, minerals, Tums, Fibercon, etc.
- Use more than one form per day if needed.

Example of a Food Record:

Day of the week: Monday

Place and Time	Amount	Portion Size	Description of Foods and Beverages	Brand
Home 9:00 am	2	Large	Eggs, scrambled	N/A
Home 9:00 am	1	teaspoon	Canola oil	Crisco
Home 9:00 am	1	cup	whole milk	Hood
Home 9:00 am	1	Large	wheat bagel	Lenders
Home 9:00 am	2	slices	baked ham	Boar's Head
Home 9:00 am	1	ounce	Cheddar cheese	Stella

FOOD RECORD FORM

Day of the week _____ Date _____

Place and Time	Amount	Portion Size	Description of Foods and Beverages	Brand

APPENDIX F

CALENDAR – EXPERIMENTAL FOODS

CALENDAR – EXPERIMENTAL FOODS

Experimental Food: ☐ Cactus Pads ☐ Cucumber

Place an "X" on the calendar for each day you ate 1 cup of the experimental food. If you forget to eat the experimental food, do not mark "X" in the calendar.

JUL

SUN	MON	TUE	WED	THUR	FRI	SAT
	1 ☐ ☐	2 ☐ ☐	3 ☐ ☐	4 ☐ ☐	5 ☐ ☐	6 ☐ ☐
7 ☐ ☐	8 ☐ ☐	9 ☐ ☐	10 ☐ ☐	11 ☐ ☐	12 ☐ ☐	13 ☐ ☐
14 ☐ ☐	15 ☐ ☐	16 ☐ ☐	17 ☐ ☐	18 ☐ ☐	19 ☐ ☐	20 ☐ ☐
21 ☐ ☐	22 ☐ ☐	23 ☐ ☐	24 ☐ ☐	25 ☐ ☐	26 ☐ ☐	27 ☐ ☐
28 ☐ ☐	29 ☐ ☐	30 ☐ ☐	31 ☐ ☐			

AUG

SUN	MON	TUE	WED	THUR	FRI	SAT
				1 ☐ ☐	2 ☐ ☐	3 ☐ ☐
4 ☐ ☐	5 ☐ ☐	6 ☐ ☐	7 ☐ ☐	8 ☐ ☐	9 ☐ ☐	10 ☐ ☐
11 ☐ ☐	12 ☐ ☐	13 ☐ ☐	14 ☐ ☐	15 ☐ ☐	16 ☐ ☐	17 ☐ ☐
18 ☐ ☐	19 ☐ ☐	20 ☐ ☐	21 ☐ ☐	22 ☐ ☐	23 ☐ ☐	24 ☐ ☐
25 ☐ ☐	26 ☐ ☐	27 ☐ ☐	28 ☐ ☐	29 ☐ ☐	30 ☐ ☐	31 ☐ ☐

APPENDIX G

GASTROINTESTINAL SYMPTOMS SURVEY

GASTROINTESTINAL SYMPTOMS SURVEY

Test food: ☐ *Nopales* ☐ Cucumber

☐ Pre ☐ Post

Did you experience in the last 2 weeks:

	None	Mild	Moderate	High	Severe
1. Abdominal pain	☐	☐	☐	☐	☐
2. Increased bowel movements	☐	☐	☐	☐	☐
3. Bloating	☐	☐	☐	☐	☐
4. Flatulence	☐	☐	☐	☐	☐
5. Fullness	☐	☐	☐	☐	☐
6. Increased liquids	☐	☐	☐	☐	☐

APPENDIX H

ACCEPTABILITY AND SATISFACTION OF TEST FOOD SURVEY

ACCEPTABILITY AND SATISFACTION OF TEST FOOD SURVEY

Test food: ☐ *Nopales* ☐ Cucumber

	Excellent	Good	Satisfactory	Needs Improvement	Unsatisfactory
1. Appearance	☐	☐	☐	☐	☐
2. Consistency / Texture	☐	☐	☐	☐	☐
3. Flavor	☐	☐	☐	☐	☐
4. Overall Satisfaction	☐	☐	☐	☐	☐

APPENDIX I

SOCIO-DEMOGRAPHIC QUESTIONNAIRE

INTERVIEW INFORMATION

Study ID _____ Date _____

1) Interviewer name: _____

SOCIODEMOGRAPHIC CHARACTERISTICS/ PERSONAL INFORMATION

2) Gender: ☐$_1$ Male ☐$_2$Female

3) How old are you? _____

4) Do you identify yourself as any of the following?
(Interviewer, please read the options)

☐$_1$ White
☐$_2$ Black
☐$_3$ Hispanic or Latino
☐$_4$ Native American
☐$_{77}$ Other (please specify):_____
☐$_{99}$ Refuse

5) What is your current marital status? (Interviewer, please read all options; make sure single means "never been married")

☐$_1$ Single/No partner
☐$_2$ Married
☐$_3$ Living together (not married)
☐$_4$ Separated
☐$_5$ Divorced
☐$_6$ Widowed
☐$_{77}$ Other, please specify: _____
☐$_{99}$ Refused

6) Are you currently working?

☐$_1$ Yes ☐$_2$ No, *go to question # 8*

7) Which of the following best describes your current employment status?

☐$_1$ Working full-time, 35 hours per week or more
☐$_2$ Working part-time, less than 35 hours per week
Skip to question 9

8) Which of the following best describes your current employment status?

(Interviewer please read all options and check only one box.)

☐3 Unemployed or laid off and looking for work
☐4 Unemployed and not looking for work
☐5 Homemaker
☐6 In school
☐7 Retired
☐8 Disabled, not able to work
☐9 Other? (Please specify):_____

9) Including money from all salaries/work, government assistance and (if applicable) unemployment, what is the total amount of money your **household** receives **PER MONTH**?

(Interviewer, if participant is not sure read all the options; make sure answer includes food stamps, alimony, and foster care)

☐1 $0-1000
☐2 $1001-2000
☐3 $2001-3000
☐4 $3001-4000
☐5 >$4000
☐77 Other amount, (specify) _____
☐88 DK
☐99 Refused

10) Last grade you completed in school

☐1 Less than 6th grade
☐2 Completed elementary school (6th grade)
☐3 Completed middle school (9th grade)
☐4 Completed high school (12th grade)
☐5 Some college
☐6 College graduate or higher
☐77 Other, (specify) _____
☐88 DK
☐99 Refused

11) Have you ever smoked cigarettes?

☐1 Yes ☐2 No, *go to question # 14*

12) Do you currently smoke cigarettes?

☐1 Yes ☐2 No, *go to question # 14*

13) How many cigarettes do you smoke per day? _____

Please indicate if any of your family members have been diagnosed with the following:

Chronic diseases	Family members			
	[1]Both parents	[2]Father/mother	[3]Sibling(s)	[4]Children
14) Diabetes				
15) Heart disease				
16) Cancer				
17) High cholesterol				
18) High blood pressure				

DIETARY HABITS

19) Are you usually the main person cooking the meals in your home?

☐$_1$ Yes
☐$_2$ No, Who does it? _____
☐$_3$ Share cooking with another person in household. Who? _____
☐$_{99}$ Refused

How often do you eat each of the main meals during a typical week?

Meals	During a typical week, how many times do you eat…?	Where do you usually eat…?	Reason for skipping…
20) Breakfast			
21) Lunch			
22) Dinner			

23) Do you eat foods OR snacks between your meals?

☐$_1$ Yes,
☐$_2$ No
☐$_{99}$ Refused

24) How often do you eat foods OR snacks between your meals? # times_____
☐$_1$ daily

☐$_2$ weekly
☐$_3$ monthly
☐$_4$ yearly
☐$_5$ never
☐$_{99}$ Refused

25) What type of foods do you usually eat between your meals?

_____

_____

26) How often do you eat snacks in place of a main meal (such as breakfast, lunch and dinner)?

☐$_1$ Never
☐$_2$ Once a week
☐$_3$ Twice a week
☐$_4$ 3 to 4 times per week
☐$_5$ 5 or 6 times per week
☐$_6$ 7 or more times per week
☐$_{99}$ Refused

PHYSICAL ACTIVITY

27) Select the option that best describes your on-the-job activities during the past year:

☐$_1$ I have no job or regular work

☐$_2$ I spent most of the day sitting or standing. When I was at work, I did such things as writing, typing, talking on the telephone, assembling small parts, or operating a machine that takes very little exertion or strength. If I drove a car or truck while at work, I did not lift or carry anything for more than a few minutes each day.

☐$_3$ I spent most of the day walking or using my hands and arms in work that required moderate exertion. When I was at work, I did such things as delivering mail, patrolling on guard duty, doing mechanical work on automobiles or other large machines, house painting, or operating a machine that requires some moderate-activity work of me. If I drove a truck or lift, my job required me to lift and carry things frequently.

☐$_4$ I spent most of the day lifting or carrying heavy objects or moving most of my body in some other way. When I was at work, I did such things as stacking cargo or inventory, handling parts or materials, or doing work like that of a carpenter who builds structures or a gardener who does most of the work without machines.

☐$_5$ I spent most of the day doing hard physical labor. When I was at work, I did such things as digging or chopping with heavy tools or carrying heavy loads (bricks, for example) to the place where they were to be used. If I drove a truck or operated equipment, my job also required me to do hard physical work most of the day with only short breaks.

28) Select the option that best describes your leisure-time activities during the past year:

☐1 Most of my leisure time was spent without very much physical activity. I mostly did things like watching television, reading, or playing cards. If I did anything else, it was likely to be light chores around the house or yard or some easy-going game like bowling or catch. Only occasionally, no more than once or twice a month, did I do anything more vigorous, like jogging, playing tennis, or active gardening.

☐2 Weekdays, when I got home from work, I did few active things, but most weekends I was able to get outdoors for some light exercise—going for walks, playing a round of golf (without motorized carts), or doing some active chores around the house.

☐3 Three times per week, on average, I engaged in some moderate activity, such as brisk walking or slow jogging, swimming, or riding a bike for 15–20 minutes or more, or I spent 45 minutes to an hour or more doing moderately difficult chores, such as raking or washing windows, mowing the lawn or vacuuming, or playing games such a doubles tennis or basketball.

☐4 During my leisure time over the past year, I engaged in a regular program of physical fitness involving some kind of heavy physical activity at least three times per week. Examples of heavy physical activity are jogging, running, or riding fast on a bicycle for 30 minutes or more; heavy gardening or other chores for an hour or more; active games or sports such as handball or tennis for an hour or more; or a regular program involving calisthenics and jogging or the equivalent for 30 minute or more.

☐5 Over the past year, I engaged in a regular program of physical fitness along the lines described in the last paragraph (I), but I did it almost daily—five or more times per week.

END OF INTERVIEW

Interviewer: Read…
Thank you so much for your time.

APPENDIX J

POWER ANALYSIS AND EFFECT SIZE

1. Power Analysis based on Frati-Munari (1983) study

[1] *-- Tuesday, October 15, 2013 -- 11:55:09*
t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.7750000**
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
Output:	Noncentrality parameter δ	=	3.1000000
	Critical t	=	2.1314495
	Df	=	15
	Total sample size	=	**16**
	Actual power	=	0.8255337

2. Power analysis based on the current study effect size

[1] *-- Tuesday, October 15, 2013 -- 10:34:56*
t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.4465102**
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
Output:	Noncentrality parameter δ	=	2.8937168
	Critical t	=	2.0195410
	Df	=	41
	Total sample size	=	**42**
	Actual power	=	0.8065830

[2] *-- Tuesday, October 15, 2013 -- 10:35:00*
t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.4465102**
	α err prob	=	0.05
	Power (1-β err prob)	=	0.85
Output:	Noncentrality parameter δ	=	3.0611197
	Critical t	=	2.0128956
	Df	=	46
	Total sample size	=	**47**
	Actual power	=	0.8500538

[3] -- *Tuesday, October 15, 2013 -- 10:35:04*

t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.4465102**
	α err prob	=	0.05
	Power (1-β err prob)	=	**0.90**
Output:	Noncentrality parameter δ	=	3.3114083
	Critical t	=	2.0048793
	Df	=	54
	Total sample size	=	55
	Actual power	=	0.9018217

[4] -- *Tuesday, October 15, 2013 -- 10:35:07*

t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.4465102**
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
Output:	Noncentrality parameter δ	=	3.6820174
	Critical t	=	1.9960084
	Df	=	67
	Total sample size	=	**68**
	Actual power	=	0.9524195

[5] -- *Tuesday, October 15, 2013 -- 10:35:10*

t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	**0.4465102**
	α err prob	=	0.05
	Power (1-β err prob)	=	0.99
Output:	Noncentrality parameter δ	=	4.3520431
	Critical t	=	1.9855234
	Df	=	94
	Total sample size	=	**95**
	Actual power	=	0.9905428

APPENDIX K

ADDITIONAL RESULTS

Table 11. Effect of test food intake on cardiometabolic disease risk factors[a]

	Cucumber Phase			*Nopales* Phase			p-value		
	Pre	Post	% Change	Pre	Post	% Change	Treat [b]	Time	Int [b]
Weight (kg)	84.1 ± 12.9	84.6 ± 13.2	0.6	84.2 ± 12.8	84.1 ± 12.8	-0.1	0.671	0.366	0.155
BMI (kg/m^2) [b]	30.9 ± 5.8	31.0 ± 5.8	0.3	30.9 ± 5.7	30.8 ± 5.7	-0.3	0.604	0.419	0.213
Body Fat (%)	36.0 ± 9.4	36.1 ± 9.0	0.3	36.0 ± 9.1	36.3 ± 9.2	0.8	0.861	0.552	0.804
WC (cm) [b]	103.0 ± 11.8	102.6 ± 11.9	-0.4	103.3 ± 12.1	102.0 ± 11.4	-1.3	0.767	0.102	0.551
BP (mm Hg)									
Systolic [c]	122.0 ± 13.1	120.0 ± 12.3	-1.6	121.7 ± 12.3	119.3 ± 12.1	-2.0	0.772	0.225	0.870
Diastolic [c]	78.5 ± 10.8	77.2 ± 9.6	-1.7	78.3 ± 11.9	79.2 ± 11.1	1.1	0.742	0.847	0.568
Lipids (mg/dL)									
Total cholesterol	206.6 ± 25.3	208.4 ± 29.9	0.9	204.4 ± 26.5	203.2 ± 24.7	-0.6	0.372	0.909	0.463
HDL-c [b]	44.2 ± 14.6	44.1 ± 16.4	-0.1	43.4 ± 15.8	42.4 ± 14.7	-2.3	0.258	0.542	0.665
LDL-c [b]	145.3 ± 18.7	143.2 ± 27.9	-1.4	141.5 ± 19.9	137.7 ± 18.4	-2.7	0.310	0.253	0.767
Triglycerides [c]	155.7 ± 94.3	187.9 ± 114.0	20.7	177.3 ± 134.5	201.2 ± 124.8	13.5	0.138	**0.022**	0.984
LDL-c/HDL-c	3.67 ± 1.39	3.73 ± 1.77	1.6	3.68 ± 1.40	3.62 ± 1.35	-1.6	0.586	0.941	0.514
Glucose (mg/dL)	96.2 ± 8.7	98.9 ± 7.7	2.8	97.1 ± 7.4	95.7 ± 9.3	-1.4	0.164	0.649	0.227
Insulin (uU/mL)	20.0 ± 8.6	21.6 ± 10.6	8.0	19.8 ± 8.0	21.6 ± 9.8	9.1	0.609	0.080	0.389
HOMA [b]	4.5 ± 2.1	5.3 ± 2.8	17.8	4.8 ± 2.0	5.2 ± 2.6	8.3	0.856	0.053	0.282
hsCRP (mg/dL) [b, c]	6.3 ± 6.4	5.3 ± 4.9	-15.9	5.6 ± 5.2	5.7 ± 5.0	1.8	0.763	0.970	0.741

[a] n = 13. Untransformed data shown as mean ± SD. Effects of treatment, time, or interactions were assessed using a repeated measures ANOVA;
[b] Abbreviations: BMI – body mass index; WC – waist circumferences; BP – blood pressure; HDL-c – high density lipoprotein cholesterol; LDL-c – low density lipoprotein cholesterol; HOMA - homeostasis model assessment ; hsCRP – high sensitivity C-reactive protein; Treat – treatment; Int – interaction; [c] Log-transformed prior to analysis.

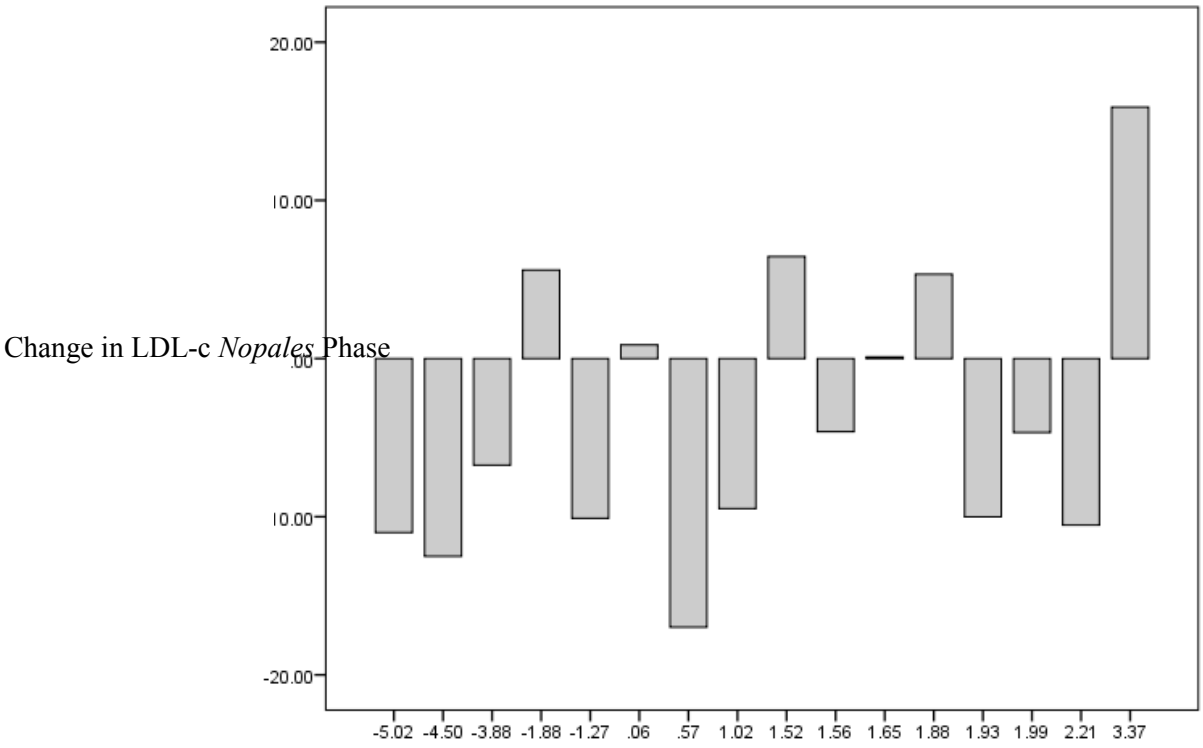
Table 12. Correlation of changes in dietary factors and changes in fating plasma lipids during *nopales* phase

Change in Dietary intake	Change in total cholesterol		Change in LDL		Change in HDL		Change in TG	
	r^2	*p*-value	r^2	*p*-value	r^2	*p*-value	r^2	*p*-value
Energy	-0.098	0.718	0.184	0.498	0.057	0.835	-0.222	0.408
SFA	-0.248	0.353	-0.248	0.353	0.065	0.811	0.018	0.946
MUFA	0.151	0.576	0.075	0.782	0.548	**0.028**	0.000	0.999
PUFA	-0.118	0.663	-0.049	0.857	0.307	0.247	-0.242	0.366
Soluble Fiber	0.286	0.282	0.405	0.120	-0.088	0.746	0.052	0.848

Table 13. Correlation of changes in LDL-c, LDL baseline values, gastrointestinal symptoms and acceptability of test foods.

Dietary intake	Change in LDL Cucumber		Change in LDL *Nopales*	
	r^2	*p*-value	r^2	*p*-value
Pre phase LDL	0.235	0.381	-0.283	0.298
Increased bowel movements	-0.190	0.481	0.258	0.334
Fullness	0.125	0.644	0.321	0.226
Overall test food acceptability	0.048	0.859	-0.044	0.872

Figure 5. Changes in LDL-c ranked by changes in soluble fiber intake during *nopales* phase



APPENDIX L

DATA SHEETS

ID	Gender	Age (y)	Ethnicity	Marital Status	Employment Status	Monthly Income	Education
1	Female	55	White	Single	Working	refused	college or higher
3	Male	29	White	Single	Working	$0-$1000	college or higher
4	Female	29	Hispanic	Single	Working	$1001-$2000	college or higher
5	Male	32	White	Married	Working	$3001-$4000	college or higher
6	Female	40	Hispanic	Single	Not working	refused	some college
9	Female	62	White	Divorced	Working	$1001-$2000	college or higher
10	Female	42	White /Asian	Married	Working	$1001-$2000	college or higher
11	Female	46	Hispanic	Widowed	Working	$1001-$2000	some college
12	Female	68	Hispanic	Married	Working	$3001-$4000	college or higher
14	Female	64	White	Single	Not working	$1001-$2000	some college
16	Female	48	Hispanic	Separated	Working	$0-$1000	completed high school
20	Female	48	Black	Married	Working	>$4000	college or higher
21	Female	54	Asian	Living together	Not working	Do not know	completed middle school
23	Male	66	White	Living together	Working	$2001-$3000	some college
25	Male	36	Hispanic	Single	Working	$3001-$4000	college or higher
26	Male	26	Hispanic	Single	Not working	>$4000	some college

ID	Pre Cucumber					
	Weight (kg)	**BMI (kg/m^2)**	**Body Fat (%)**	**WC (cm)**	**SBP (mm Hg)**	**DBP (mm Hg)**
1	68.7	25.1	30.7	80.3	105.0	64.0
3	98.4	32.1	33.4	97.2	122.7	78.0
4	98.5	40.2	48.2	110.5	116.0	88.0
5	81.4	25.1	21.9	94.6	121.0	74.3
6	82	30.7	43.6	110.6	116.0	77.7
9	67.7	26.4	39.5	99.0	127.3	75.0
10	107.1	40.6	48.6	118.5	141.0	104.7
11	99.8	41.0	50.7	124.5	99.3	69.7
12	91.5	35.9	45.9	104.9	126.3	77.0
14	92.9	37.6	41	116.0	144.3	75.0
16	65.3	26.6	35.1	94.3	119.0	72.3
20	82.9	32.4	43.7	101.2	122.3	78.7
21	64.1	26.3	27.6	83.1	119.3	74.0
23	76.8	24.7	24.6	96.6	108.0	71.0
25	87.6	27.7	23.9	103.5	129.7	87.7
26	82.7	31.6	31.2	103.3	130.7	92.7

BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure

ID	Post Cucumber					
	Weight (kg)	**BMI (kg/m^2)**	**Body Fat (%)**	**WC (cm)**	**SBP (mm Hg)**	**DBP (mm Hg)**
1	67.4	24.5	30.0	78.4	109.33	65.3
3	99.1	32.4	33.2	96.8	123.00	77.7
4	98.6	40.0	47.2	107.3	106.00	80.3
5	81.2	25.1	22.2	96.1	117.67	69.3
6	83.8	31.4	43.9	110.6	110.33	77.7
9	66.9	26.0	37.8	96.9	128.00	73.0
10	107.8	40.8	47.3	118.6	145.67	98.0
11	100.6	41.3	50.6	123.7	94.67	71.7
12	90.5	35.5	43.8	107.2	114.00	76.0
14	91.8	37.2	41.6	114.5	128.33	76.0
16	66.0	26.9	35.7	94.3	124.67	81.3
20	82.7	32.3	43.4	104.7	115.33	73.0
21	64.0	26.3	28.3	83.6	125.33	75.3
23	78.6	25.3	30.5	97.9	112.33	69.0
25	89.3	28.2	22.5	99.3	125.33	88.3
26	84.1	32.1	30.8	101.8	126.00	91.3

BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure

ID	Pre *Nopales*					
	Weight (kg)	**BMI (kg/m^2)**	**Body Fat (%)**	**WC (cm)**	**SBP (mm Hg)**	**DBP (mm Hg)**
1	67.8	24.7	33.3	78.3	110.0	63.3
3	96.6	31.5	32.5	96.6	116.3	80.3
4	97.2	39.6	47.6	112.1	116.7	83.3
5	83.5	25.8	23.2	98.0	118.0	62.0
6	85.7	32.1	43.7	111.2	113.0	80.0
9	67.4	26.2	37.9	100.2	121.3	74.3
10	107.2	40.6	47.8	119.8	150.0	104.3
11	99.7	41.0	51.1	122.2	114.0	78.0
12	89.0	34.9	43.9	105.1	119.3	74.0
14	91.5	37.0	40.2	116.7	133.3	75.3
16	65.3	26.6	35.1	94.2	120.0	75.0
20	82.4	32.2	44.1	108.6	113.0	74.3
21	63.6	26.1	28.3	82.3	129.3	76.7
23	76.5	24.6	24.7	97.0	110.7	73.7
25	89.1	28.1	23.8	100.3	121.3	77.7
26	82.4	31.5	30.4	100.1	141.0	99.3

BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure

ID	Post *Nopales*					
	Weight (kg)	**BMI (kg/m^2)**	**Body Fat (%)**	**WC (cm)**	**SBP (mm Hg)**	**DBP (mm Hg)**
1	67.8	24.7	32.5	79.43	106.7	66.7
3	97.3	31.8	32.8	95.53	123.0	78.3
4	97.0	39.4	47.0	111.33	115.0	85.0
5	81.5	25.2	23.7	98.27	116.0	71.7
6	86.3	32.1	45.0	102.33	106.3	74.0
9	67.4	26.2	37.5	101.00	126.0	72.7
10	107.2	40.6	47.0	118.60	134.7	97.0
11	98.4	40.4	51.8	125.30	95.7	71.0
12	88.3	34.7	44.1	101.50	108.7	76.3
14	92.1	37.3	43.5	110.77	120.7	67.0
16	65.4	26.6	34.9	94.77	112.7	73.3
20	82.9	32.4	43.1	100.00	129.0	84.3
21	63.5	26.1	27.9	82.23	121.3	73.0
23	76.3	24.5	24.6	96.67	117.0	82.0
25	88.4	27.9	24.3	100.17	124.3	90.0
26	82.6	31.6	30.6	103.33	139.3	101.0

BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood pressure

ID	Pre Cucumber							
	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	TG (mg/dL)	Glucose (mg/dL)	Insulin (uU/mL)	HOMA	CRP (mg/dL)
1	197.3	139.2	64.5	43.9	87.0	7.3	1.6	0.9
3	229.8	167.0	24.8	267.1	102.5	26.3	6.7	4.1
4	163.0	103.6	40.3	186.3	126.5	37.6	11.8	4.2
5	169.8	116.4	48.5	90.8	97.0	19.2	4.6	0.3
6	169.0	120.5	24.0	193.4	103.0	23.5	6.0	11.4
9	240.5	181.7	61.8	70.7	90.0	9.6	2.1	3.6
10	211.8	150.0	42.5	199.2	111.0	28.8	7.9	6.1
11	209.8	147.1	55.5	112.0	106.0	13.0	3.4	9.7
12	169.8	112.4	48.0	104.3	103.5	18.8	4.8	1.9
14	187.3	128.7	52.3	78.2	95.0	16.7	3.9	19.7
16	210.5	151.2	35.0	215.3	88.5	18.6	4.1	2.1
20	216.8	147.5	64.3	53.6	88.5	25.1	5.5	17.2
21	181.8	111.4	63.8	87.8	85.5	11.1	2.4	0.2
23	208.0	149.8	36.5	164.0	107.0	9.2	2.4	0.8
25	251.8	161.9	30.8	367.8	87.0	13.1	2.8	2.9
26	183.3	128.4	33.8	168.0	88.0	36.1	7.9	2.8

TC – total cholesterol; LDL-c – low density lipoprotein cholesterol; HDL-c – high density lipoprotein cholesterol; TG – triglycerides; HOMA – homeostasis model assessment; CRP – c-reactive protein

ID	Post Cucumber							
	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	TG (mg/dL)	Glucose (mg/dL)	Insulin (uU/mL)	HOMA	CRP (mg/dL)
1	204.0	139.6	68.0	63.1	89.5	6.5	1.4	1.1
3	257.8	193.8	23.5	311.0	102.0	29.8	7.5	3.4
4	144.0	89.0	43.3	143.2	113.5	26.1	7.3	3.0
5	154.0	84.5	39.0	226.3	88.5	23.1	5.0	1.2
6	171.8	108.3	18.5	356.7	102.5	32.0	8.1	5.0
9	230.5	171.7	59.3	51.5	94.0	9.6	2.2	2.3
10	203.0	148.8	42.8	158.0	111.5	33.5	9.2	7.3
11	218.5	146.2	67.3	123.6	105.5	20.7	5.4	10.8
12	175.3	117.4	50.3	108.9	100.5	22.7	5.6	2.0
14	205.3	142.2	55.8	96.7	97.0	18.2	4.4	14.6
16	223.5	167.3	38.8	208.2	110.5	20.9	5.7	2.4
20	223.0	155.8	60.5	53.1	88.5	21.7	4.8	14.8
21	186.5	115.6	67.0	64.3	90.0	11.1	2.5	0.2
23	185.5	131.3	33.0	195.7	101.5	10.3	2.6	1.4
25	250.3	147.8	31.3	399.2	97.0	12.4	3.0	2.8
26	182.8	124.5	35.3	200.0	97.5	42.6	10.3	2.4

TC – total cholesterol; LDL-c – low density lipoprotein cholesterol; HDL-c – high density lipoprotein cholesterol; TG – triglycerides; HOMA – homeostasis model assessment; CRP – c-reactive protein

ID	Pre *Nopales*							
	TC (mg/dL)	**LDL-c (mg/dL)**	**HDL-c (mg/dL)**	**TG (mg/dL)**	**Glucose (mg/dL)**	**Insulin (uU/mL)**	**HOMA**	**CRP (mg/dL)**
1	187.8	130.3	59.3	45.1	86.5	8.1	1.7	0.8
3	228.0	164.9	23.8	253.7	100.0	22.0	5.4	2.8
4	165.3	106.5	47.5	159.1	126.0	27.5	8.6	2.3
5	189.5	140.9	45.8	87.1	96.0	16.2	3.8	0.8
6	163.8	113.5	21.3	252.3	101.0	24.3	6.1	8.7
9	253.8	189.6	64.0	82.6	95.0	11.6	2.7	2.2
10	193.5	144.5	40.5	133.1	99.0	33.4	8.2	7.3
11	194.0	126.4	58.0	129.7	113.0	14.5	4.0	10.1
12	175.8	113.3	53.3	95.7	95.0	22.7	5.3	2.1
14	214.5	147.1	56.8	121.6	97.5	24.6	5.9	12.6
16	213.5	154.3	35.8	215.1	106.0	21.5	5.6	3.2
20	214.3	143.7	66.0	54.3	86.5	28.5	6.1	17.7
21	189.5	115.6	71.8	73.5	90.0	9.4	2.1	0.2
23	170.0	129.2	33.3	163.6	96.0	8.4	2.0	1.6
25	242.3	126.1	28.0	559.4	88.5	15.9	3.5	3.2
26	192.5	128.7	31.8	207.9	97.0	28.4	6.8	2.0

TC – total cholesterol; LDL-c – low density lipoprotein cholesterol; HDL-c – high density lipoprotein cholesterol; TG – triglycerides; HOMA – homeostasis model assessment; CRP – c-reactive protein

ID	Post *Nopales*							
	TC (mg/dL)	**LDL-c (mg/dL)**	**HDL-c (mg/dL)**	**TG (mg/dL)**	**Glucose (mg/dL)**	**Insulin (uU/mL)**	**HOMA**	**CRP (mg/dL)**
1	199.5	135.9	57.8	94.9	85.5	8.7	1.8	1.6
3	244.8	155.4	22.3	421.9	100.5	32.3	8.0	2.6
4	157.5	96.4	42.0	217.3	126.0	16.2	5.0	1.5
5	185.3	134.2	46.0	100.6	88.5	16.1	3.5	0.6
6	175.0	113.6	21.5	321.9	102.0	32.7	8.2	12.2
9	235.3	179.0	58.3	81.3	90.0	11.7	2.6	2.2
10	204.3	149.9	43.8	158.4	116.5	36.1	10.4	6.1
11	208.3	142.3	49.5	150.2	103.5	22.6	5.8	7.6
12	172.0	114.2	50.0	116.4	97.5	18.7	4.5	2.2
14	202.8	137.0	62.0	99.8	96.0	21.1	5.0	13.7
16	212.8	149.6	38.5	239.9	98.0	16.0	3.9	1.5
20	199.3	131.2	61.0	73.1	81.0	22.2	4.4	12.7
21	172.5	111.0	67.0	85.6	91.0	10.6	2.4	0.2
23	159.5	118.2	30.5	174.7	98.0	10.4	2.5	0.5
25	233.5	132.6	30.3	422.4	96.5	14.9	3.6	10.3
26	182.0	111.8	30.3	276.3	88.0	35.8	7.8	2.1

TC – total cholesterol; LDL-c – low density lipoprotein cholesterol; HDL-c – high density lipoprotein cholesterol; TG – triglycerides; HOMA – homeostasis model assessment; CRP – c-reactive protein

ID	Pre Cucumber						
	LDL size (Å)	**% C small LDL**	**% C large LDL**	**LDL pattern**	**% HDL-c small HDL**	**% HDL-c interm HDL**	**% HDL-c large HDL**
1	270.0	2.7	35.4	A	11.2	41.8	47.0
3	263.0	9.0	31.9	B	36.6	58.0	2.3
4	267.0	2.7	31.4	intermed.	22.5	62.2	15.3
5	273.0	0.0	34.0	A	15.6	50.0	34.4
6	265.0	4.4	34.6	intermed.	25.3	59.3	15.4
9	272.0	0.0	39.3	A	18.3	55.5	26.3
10	267.0	3.2	33.4	intermed.	25.7	51.7	22.6
11	267.0	2.9	39.1	intermed.	21.6	58.7	19.6
12	271.0	0.0	28.7	A	16.2	54.6	29.2
14	273.0	0.5	33.4	A	11.9	54.1	34.0
16	262.0	9.3	29.8	B	33.3	48.4	18.3
20	274.0	0.0	31.1	A	9.0	49.0	42.0
21	272.0	0.0	30.0	A	10.9	50.0	39.1
23	261.0	11.4	28.5	B	23.7	56.4	19.9
25	258.0	13.4	23.0	B	32.9	57.6	9.4
26	265.0	6.2	33.2	intermed.	32.3	53.6	14.1

LDL – low density lipoprotein; C – cholesterol; HDL-c – high density lipoprotein cholesterol

ID	Post Cucumber						
	LDL size (Å)	**% C small LDL**	**% C large LDL**	**LDL pattern**	**% HDL-c small HDL**	**% HDL-c interm HDL**	**% HDL-c large HDL**
1	270.0	1.2	36.8	A	8.9	42.8	48.3
3	259.0	13.1	27.3	B	35.5	64.5	0.0
4	270.0	1.2	30.6	A	23.4	56.4	20.2
5	270.0	0.0	30.8	A	14.7	49.4	35.9
6	265.0	3.5	28.7	intermed.	26.6	61.6	11.8
9	273.0	0.0	39.5	A	18.7	53.9	27.4
10	266.0	4.7	33.1	intermed.	26.9	51.7	21.4
11	267.0	3.4	37.5	intermed.	27.5	54.2	18.3
12	269.0	1.2	33.6	A	15.6	52.5	31.9
14	272.0	0.6	34.2	A	12.1	55.2	32.7
16	263.0	9.8	34.0	B	38.0	48.7	13.3
20	273.0	0.0	35.2	A	9.5	51.5	39.0
21	274.0	0.0	31.8	A	9.1	52.9	37.5
23	259.0	13.0	24.5	B	29.9	53.6	16.6
25	257.0	13.2	19.8	B	34.3	60.2	2.9
26	265.0	6.2	28.9	B	30.4	57.5	12.0

LDL – low density lipoprotein; C – cholesterol; HDL-c – high density lipoprotein cholesterol

ID	Pre *Nopales*						
	LDL size (Å)	**% C small LDL**	**% C large LDL**	**LDL pattern**	**% HDL-c small HDL**	**% HDL-c interm HDL**	**% HDL-c large HDL**
1	269.0	2.7	35.2	A	11.5	48.6	40.0
3	262.0	8.3	27.7	B	28.4	64.0	5.6
4	268.0	2.3	33.4	intermed.	23.3	62.3	14.4
5	271.0	0.0	36.7	A	20.8	46.8	32.3
6	264.0	5.5	32.1	B	29.3	58.8	11.9
9	270.0	1.2	39.0	A	21.4	50.4	28.2
10	268.0	1.8	35.8	A	26.3	52.9	20.8
11	267.0	3.0	33.2	intermed.	23.4	57.6	19.0
12	272.0	0.0	29.8	A	13.2	54.4	32.4
14	271.0	1.0	31.9	A	15.1	53.7	31.2
16	264.0	7.9	30.7	B	34.3	52.5	13.1
20	274.0	0.0	30.3	A	8.9	45.5	45.6
21	274.0	0.0	26.7	A	8.9	48.9	42.3
23	265.0	6.7	29.6	B	20.1	52.7	27.2
25	253.0	13.7	14.2	B	33.6	62.1	2.5
26	264.0	6.7	26.9	B	31.4	55.3	13.3

LDL – low density lipoprotein; C – cholesterol; HDL-c – high density lipoprotein cholesterol

ID	Post *Nopales*						
	LDL size (Å)	**% C small LDL**	**% C large LDL**	**LDL pattern**	**% HDL-c small HDL**	**% HDL-c interm HDL**	**% HDL-c large HDL**
1	269.0	2.1	34.2	A	14.2	47.4	38.4
3	256.0	16.3	23.8	B	34.3	60.2	3.1
4	269.0	1.3	31.4	A	22.8	61.8	15.4
5	272.0	0.0	35.7	A	20.8	46.1	33.1
6	262.0	9.0	26.2	B	31.3	58.4	10.2
9	270.0	1.0	43.9	A	22.9	53.1	24.0
10	267.0	3.0	35.9	intermed.	27.5	52.6	19.9
11	266.0	3.4	30.9	intermed.	26.3	58.7	15.0
12	270.0	0.0	28.8	A	15.7	52.9	31.4
14	273.0	0.0	34.1	A	14.6	56.6	28.9
16	262.0	9.2	26.4	B	35.5	47.5	17.0
20	273.0	0.0	31.5	A	11.9	50.9	37.2
21	274.0	0.0	22.8	A	9.2	47.9	42.1
23	260.0	12.0	25.7	B	20.6	55.0	24.4
25	256.0	14.9	20.9	B	33.5	58.2	8.2
26	265.0	5.6	27.3	B	36.5	54.2	9.3

LDL – low density lipoprotein; C – cholesterol; HDL-c – high density lipoprotein cholesterol

ID	Pre Cucumber				
	Plasma Vitamin C (mg/dL)	**Total Antioxidant Capacity (mM)**	**Oxidized LDL (U/L)**	**Lag time (min)**	**Oxidation Rate (mmol diene/mg LDL ptn * min)**
1	1.96	0.81	61.20	224.00	13.25
3	1.17	1.06	103.12	162.00	11.05
4	0.71	0.87	60.54		
5	1.38	0.86	72.12	229.00	10.50
6	1.20	1.02	85.93	208.00	8.95
9	1.58	1.05	83.74	259.00	12.35
10	0.59	0.91	105.51	198.00	9.95
11	0.70	0.69	88.29	175.00	14.55
12	1.17	0.88	61.94	234.00	5.50
14	1.95	0.91	62.15	189.00	7.65
16	1.22	1.79	122.77	174.00	14.55
20	0.97	1.10	71.02	174.00	12.80
21	1.28	1.82	49.88	292.00	10.25
23	1.05	1.48	98.75	185.00	13.95
25	1.22	2.06	124.67	158.00	9.15
26	0.49	1.18	79.25		

LDL – low density lipoprotein; ptn - protein

ID	Post Cucumber				
	Plasma Vitamin C (mg/dL)	Total Antioxidant Capacity (mM)	Oxidized LDL (U/L)	Lag time (min)	Oxidation Rate (mmol diene/mg LDL ptn * min)
1	2.10	0.96	66.96	222.00	12.75
3	0.88	0.95	119.02	157.00	11.20
4	0.84	1.09	48.76	287.00	9.30
5	0.85	0.81	58.50	174.00	8.25
6	1.26	1.11	89.65	201.00	11.85
9	1.71	1.24	82.22	228.00	9.65
10	0.59	1.19	93.20	186.00	11.50
11	1.45	0.71	88.33	163.00	15.65
12	1.55	0.96	58.62	239.00	8.40
14	1.72	1.00	72.62	189.00	9.25
16	1.25	1.57	114.68	185.00	13.90
20	0.95	1.12	76.58	197.00	8.80
21	1.30	1.43	54.50	192.00	10.95
23	1.20	1.37	91.14	192.00	12.00
25	1.02	1.99	109.87	192.00	10.40
26	0.66	1.16	79.58	339.00	12.25

LDL – low density lipoprotein; ptn - protein

ID	Pre *Nopales*				
	Plasma Vitamin C (mg/dL)	**Total Antioxidant Capacity (mM)**	**Oxidized LDL (U/L)**	**Lag time (min)**	**Oxidation Rate (mmol diene/mg LDL ptn * min)**
1	1.85	0.99	67.84	205.00	9.25
3	0.86	1.15	100.21	314.00	9.05
4	0.79	0.96	55.58	214.00	15.70
5	0.78	1.02	85.79	186.00	10.75
6	1.09	0.93	79.15	226.00	10.80
9	1.68	0.83	92.14	235.00	10.30
10	0.62	1.13	90.68	202.00	12.35
11	0.92	0.65	65.60	184.00	12.30
12	1.34	0.81	62.48	209.00	6.75
14	1.94	0.81	73.85	167.00	10.80
16	1.27	1.60	117.00	164.00	15.10
20	0.83	1.36	78.25	198.00	11.35
21	1.41	1.76	52.22	230.00	10.90
23	1.44	1.68	88.48	180.00	15.50
25	1.11	1.73	99.22	196.00	11.80
26	0.18	1.40	87.67	237.00	10.85

LDL – low density lipoprotein; ptn - protein

ID	Post *Nopales*				
	Plasma Vitamin C (mg/dL)	**Total Antioxidant Capacity (mM)**	**Oxidized LDL (U/L)**	**Lag time (min)**	**Oxidation Rate (mmol diene/mg LDL ptn * min)**
1	2.26	1.20	61.13	222.00	10.10
3	0.91	1.55	103.26	349.00	10.55
4	0.48	0.90	53.48	205.00	11.60
5	1.07	1.34	88.78	184.00	12.35
6	1.42	0.94	84.07	215.00	9.00
9	1.38	0.88	100.84	229.00	14.80
10	0.67	0.95	92.07	184.00	12.65
11	1.30	1.07	77.67	135.00	12.35
12	1.32	0.93	65.03	255.00	7.45
14	1.54	0.99	74.28	188.00	10.45
16	0.86	1.34	110.79	177.00	12.25
20	0.77	1.43	63.12	232.00	10.95
21	1.48	1.73	55.29	220.00	9.60
23	1.05	1.60	84.60	183.00	13.60
25	1.17	1.87	103.48	185.00	11.25
26	0.58	1.39	77.73	312.00	12.50

LDL – low density lipoprotein; ptn - protein

ID	Pre Cucumber								
	Total (g)	**Energy (kcal)**	**Fat (g)**	**CHO (g)**	**Ptn (g)**	**SFA (g)**	**MUFA (g)**	**PUFA (g)**	**Cholest (mg)**
1	1358	1076	51.2	104.5	57.7	12.5	20.1	14.7	131.4
3	2431	2550	122.1	308.7	72.4	34.3	58.7	18.5	358.5
4	1791	1348	55.3	164.3	53.0	19.7	18.3	12.0	160.5
5	965	1384	51.2	185.8	56.3	17.3	17.5	12.9	67.6
6	1443	1381	69.5	134.6	58.2	19.0	24.1	20.2	344.1
9	3313	2181	106.9	245.0	81.9	29.9	46.9	22.4	148.2
10	1161	1261	58.2	146.8	42.8	17.7	18.1	17.7	84.4
11									
12	2551	1287	55.0	157.9	51.3	14.9	24.6	9.8	139.3
14	1494	1774	74.5	196.5	84.3	21.0	26.1	20.7	368.4
16	3488	1434	38.4	197.9	80.3	10.3	13.5	10.4	279.1
20	2012	1798	67.7	235.1	71.0	22.6	20.2	19.5	273.0
21	2237	1584	49.6	226.5	63.3	21.9	15.2	7.1	262.8
23	3124	1860	45.5	283.8	92.5	12.1	16.7	13.6	131.2
25	1997	2105	106.6	199.4	93.4	33.1	41.4	23.3	611.1
26	4954	3179	136.7	366.7	138.7	47.8	48.3	29.2	373.5

CHO – carbohydrate; Ptn – protein; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; Cholest – cholesterol

ID	Post Cucumber								
	Total (g)	Energy (kcal)	Fat (g)	CHO (g)	Ptn (g)	SFA (g)	MUFA (g)	PUFA (g)	Cholest (mg)
1	1592	1421	61.0	149.2	63.2	20.1	24.4	11.9	199.8
3	1953	2062	82.6	260.1	82.9	25.0	23.2	27.6	234.9
4	2225	1575	52.6	223.7	60.0	19.4	19.2	8.4	204.8
5	918	1116	33.4	173.1	37.0	8.8	13.5	8.5	33.7
6	1856	911	46.6	93.1	36.7	14.6	20.8	6.7	368.8
9	2830	1479	60.6	185.7	64.2	15.3	21.9	18.6	250.7
10	1079	1286	52.1	146.8	61.3	18.7	18.6	9.9	266.0
11	2276	2491	127.4	167.5	166.3	53.0	46.3	16.0	681.7
12	2749	1034	36.4	151.0	33.4	7.8	14.0	11.0	123.7
14	1380	1208	52.4	135.1	51.6	18.9	19.4	8.5	453.5
16	4369	1305	29.7	193.0	70.8	10.4	10.3	4.6	236.0
20	2903	1944	54.5	290.7	88.9	14.7	19.1	16.2	151.5
21	1946	1308	38.6	180.1	63.1	13.1	14.1	7.5	145.3
23	3540	2307	69.1	362.0	77.7	18.8	19.1	25.1	130.1
25	1573	1920	91.3	180.2	97.4	31.6	35.9	15.6	648.7
26	4343	3152	150.6	335.3	119.4	44.6	59.3	35.5	590.5

CHO – carbohydrate; Ptn – protein; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; Cholest - cholesterol

ID	Pre *Nopales*								
	Total (g)	**Energy (kcal)**	**Fat (g)**	**CHO (g)**	**Ptn (g)**	**SFA (g)**	**MUFA (g)**	**PUFA (g)**	**Cholest (mg)**
1	1938	1437	65.1	137.2	86.9	16.8	22.9	20.5	245.2
3	1685	2452	134.0	215.6	98.3	49.9	54.0	21.2	618.4
4	2195	1561	70.0	166.4	70.5	19.6	25.4	18.6	259.7
5	4317	2066	88.1	246.0	89.0	29.0	31.4	20.7	242.4
6	1934	1292	39.6	137.4	41.0	12.9	16.3	7.4	84.4
9	2751	2202	113.3	213.6	80.8	34.9	46.2	23.2	326.5
10	1923	930	37.9	100.9	46.4	12.8	12.8	7.6	320.5
11	1741	2282	100.2	171.7	88.8	39.9	30.1	22.1	377.7
12	2632	1125	48.4	120.8	52.0	12.8	21.4	9.4	308.0
14	1187	1924	71.2	259.6	68.7	21.0	22.8	22.9	189.5
16	3159	1237	31.7	179.7	64.6	8.7	9.9	10.0	157.4
20	2506	1918	58.2	295.8	76.4	18.5	18.1	16.1	179.2
21	1840	1655	73.5	161.7	90.2	21.7	24.6	20.5	443.2
23	3740	2423	96.2	328.2	83.6	24.5	37.9	28.6	56.8
25	1985	2018	96.7	187.8	103.8	34.7	35.9	16.9	596.0
26	4289	3191	125.6	303.5	86.6	44.5	39.5	30.6	226.2

CHO – carbohydrate; Ptn – protein; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; Cholest - cholesterol

ID	Post *Nopales*								
	Total (g)	**Energy (kcal)**	**Fat (g)**	**CHO (g)**	**Ptn (g)**	**SFA (g)**	**MUFA (g)**	**PUFA (g)**	**Cholest (mg)**
1	1778	1148	48.1	81.4	101.9	11.9	21.9	9.9	323.6
3	1800	2499	112.2	253.8	119.8	48.2	41.3	12.8	456.7
4	2699	1507	56.1	184.8	60.9	19.3	17.1	14.1	355.8
5	845	1172	45.4	150.9	44.7	12.3	19.2	10.4	70.9
6	1687	1135	57.8	128.6	35.7	15.8	27.8	10.3	79.3
9	2477	2430	111.1	263.2	107.9	55.7	29.0	16.2	526.1
10	740	776	32.8	76.0	45.8	12.0	11.4	5.5	320.8
11	2836	1659	54.6	220.8	81.2	21.4	16.1	11.0	371.9
12	2665	1203	48.7	140.4	50.1	12.8	18.9	13.0	350.9
14	1412	1986	93.0	207.8	86.5	28.9	35.3	21.9	234.2
16	4396	1294	45.4	160.2	66.9	12.6	17.6	11.2	223.8
20	2139	2098	61.5	299.7	93.5	18.7	17.0	21.5	226.6
21	2131	1748	52.7	235.7	85.8	19.9	18.9	8.6	321.1
23	3024	1880	83.4	203.4	80.1	23.8	27.3	27.1	178.6
25	2918	2206	67.1	243.8	90.7	22.3	23.5	16.0	297.2
26	1266	1586	69.5	167.3	77.0	21.9	23.2	18.8	340.5

CHO – carbohydrate; Ptn – protein; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; Cholest - cholesterol

ID	Pre Cucumber								
	Total Sugars (g)	**Total Fiber (g)**	**Sol. Fiber (g)**	**Insol. Fiber (g)**	**Vit E (mg)**	**Vit C (mg)**	**Vit A (µg)**	**α-carotene (µg)**	**β-carotene (µg)**
1	47.0	17.0	6.0	11.0	5.3	60.1	639.5	39.2	2531.4
3	141.6	35.4	10.1	25.4	16.7	254.8	2100.1	1488.3	10097.5
4	66.6	10.9	3.5	7.4	4.4	20.7	294.9	23.5	256.4
5	62.9	20.2	4.6	15.6	5.2	68.7	917.3	1023.3	2573.2
6	31.7	13.7	5.2	8.5	8.5	25.2	451.9	85.7	1602.0
9	127.1	34.9	9.2	25.8	29.9	150.7	961.3	424.4	3312.1
10	55.1	14.7	4.1	10.6	5.6	107.0	540.0	371.2	2238.5
11									
12	65.6	22.6	7.9	14.7	5.2	99.0	547.1	338.2	2477.8
14	112.3	13.4	3.0	10.3	8.2	105.6	1731.5	1339.7	8586.0
16	101.9	13.7	4.5	9.2	5.8	183.5	938.3	202.7	3680.0
20	99.1	13.7	4.6	9.2	8.6	92.7	1146.4	65.1	4939.9
21	24.6	22.2	9.5	12.9	4.5	53.1	1300.6	1401.8	5378.6
23	101.5	28.0	5.4	22.7	9.1	95.0	729.8	186.3	1647.7
25	74.0	15.6	6.1	9.6	6.6	53.3	968.9	261.9	2327.7
26	74.0	46.9	13.6	33.1	43.4	127.7	1290.5	44.9	4701.9

Sol. – soluble; Insol. – insoluble; vit – vitamin;

ID	Post Cucumber								
	Total Sugars (g)	**Total Fiber (g)**	**Sol. Fiber (g)**	**Insol. Fiber (g)**	**Vit E (mg)**	**Vit C (mg)**	**Vit A (µg)**	**α-carotene (µg)**	**β-carotene (µg)**
1	59.8	20.6	6.1	14.5	8.8	66.4	879.9	43.4	3276.8
3	124.5	24.1	6.5	17.3	11.4	138.0	627.9	150.8	1268.8
4	114.4	16.0	4.5	11.5	5.0	67.3	806.6	55.0	1599.0
5	55.7	16.1	5.1	11.0	6.2	53.9	590.2	75.5	570.0
6	30.2	14.1	5.4	8.7	3.9	51.7	328.3	60.9	639.8
9	80.9	24.0	5.5	18.5	15.7	111.2	958.9	374.5	2791.5
10	40.5	12.8	3.7	9.0	4.6	72.2	853.2	50.8	2892.3
11	55.2	16.0	6.0	9.9	5.4	74.1	2035.7	2875.0	8133.1
12	74.9	17.4	4.7	12.8	4.6	205.7	912.3	413.8	4264.5
14	94.2	8.1	2.7	5.4	4.2	109.1	913.3	53.9	2671.2
16	99.4	16.0	4.6	11.2	4.3	141.6	1046.6	732.6	4087.6
20	165.4	18.6	7.6	11.0	4.1	99.7	668.8	125.3	1879.8
21	9.9	18.0	6.1	11.9	6.2	108.9	1526.6	1373.0	7655.7
23	190.8	35.8	11.0	24.8	12.3	190.1	1281.9	282.1	4549.3
25	64.6	12.4	5.0	7.4	3.8	31.0	1104.5	41.5	2435.9
26	98.6	27.8	8.2	19.5	14.6	115.9	519.9	102.7	1026.5

Sol. – soluble; Insol. – insoluble; vit – vitamin;

ID	Pre *Nopales*								
	Total Sugars (g)	**Total Fiber (g)**	**Sol. Fiber (g)**	**Insol. Fiber (g)**	**Vit E (mg)**	**Vit C (mg)**	**Vit A (µg)**	**α-carotene (µg)**	**β-carotene (µg)**
1	73.7	22.9	7.9	15.2	9.7	167.3	689.8	218.9	2350.6
3	98.8	12.9	4.3	8.5	9.9	24.4	1283.9	757.0	3379.9
4	33.5	17.2	5.6	11.6	7.1	21.7	217.5	60.9	351.9
5	101.8	22.2	7.2	15.0	17.0	191.9	4186.8	736.9	21660.9
6	40.0	16.6	6.0	10.5	4.9	47.2	561.8	650.4	1823.5
9	91.0	26.0	7.2	18.8	16.3	97.3	1396.4	688.2	5467.3
10	26.0	10.4	2.5	7.8	3.1	44.2	422.4	13.0	1091.0
11	75.6	10.3	3.4	6.9	7.3	37.3	758.6	33.5	1929.0
12	28.2	17.9	6.6	11.0	7.6	86.6	997.9	245.2	4615.6
14	126.8	15.0	6.7	7.9	8.3	65.5	1962.4	71.4	6999.9
16	85.7	16.4	4.6	10.9	5.8	131.0	792.4	636.3	3635.4
20	167.9	35.0	12.5	21.8	9.4	164.7	793.1	281.2	3103.8
21	34.5	20.9	5.8	15.1	12.4	197.3	2384.0	2234.9	11127.5
23	142.5	43.9	13.8	29.7	16.3	365.3	1986.0	848.0	7912.9
25	69.5	16.5	5.6	10.7	9.3	55.0	604.2	31.0	1314.8
26	126.4	22.1	5.3	16.5	11.0	42.5	1367.3	177.7	3674.5

Sol. – soluble; Insol. – insoluble; vit – vitamin;

ID	Post *Nopales*								
	Total Sugars (g)	**Total Fiber (g)**	**Sol. Fiber (g)**	**Insol. Fiber (g)**	**Vit E (mg)**	**Vit C (mg)**	**Vit A (µg)**	**α-carotene (µg)**	**β-carotene (µg)**
1	42.9	17.5	6.0	11.5	11.6	106.2	848.5	146.5	2656.6
3	113.4	17.5	5.4	11.8	5.4	25.3	584.8	183.9	722.1
4	64.3	21.3	7.6	13.7	6.2	37.5	635.5	206.7	1178.7
5	60.3	11.2	3.4	7.8	5.2	61.7	546.1	383.5	1229.4
6	49.1	19.2	7.6	11.6	8.6	283.5	674.5	361.6	2657.1
9	123.5	29.6	9.4	20.4	14.8	64.2	1163.6	374.2	3161.8
10	15.9	13.5	4.4	9.1	2.2	22.7	1637.6	3022.7	7169.6
11	109.8	18.5	6.8	11.7	4.0	263.8	573.8	216.7	1234.3
12	52.9	18.4	6.7	11.7	5.6	86.4	1099.8	320.4	4664.3
14	147.2	13.9	5.4	8.3	5.0	70.1	1229.2	780.3	2323.8
16	55.2	19.0	6.6	12.1	4.7	171.7	1276.8	1339.9	5887.3
20	92.1	21.3	8.0	13.4	4.4	41.9	780.7	917.3	2721.0
21	45.4	24.2	7.4	16.9	6.8	51.3	1408.4	240.6	6900.8
23	56.2	29.0	8.8	20.2	10.7	160.6	1499.5	364.9	5998.2
25	72.4	17.8	7.1	10.6	7.7	78.3	788.9	450.6	2201.3
26	59.6	15.8	5.9	9.9	8.1	38.3	498.9	149.9	1124.7

Sol. – soluble; Insol. – insoluble; vit – vitamin