Effect of a Lifestyle and Type 2 Diabetes-Prevention Intervention on Biomarkers of
Oxidative Stress in Obese Prediabetic Latino Youth

by

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ABSTRACT

Background. Effects of lifestyle interventions on early biomarkers of oxidative stress and CVD risk in youth with prediabetes are unknown. Objective. To evaluate the effects of a lifestyle intervention to prevent type 2 diabetes among obese prediabetic Latino adolescents on oxidized lipoproteins. Design: In a quasi-experimental design, 35 adolescents (51.4% male, age 15.5(1.0) y, body mass index (BMI) percentile 98.5(1.2), and glucose 2 hours after an oral glucose tolerance test-OGTT 141.2(12.2) mg/dL) participated in a 12-week intervention that included weekly exercise (three 60 min-sessions) and nutrition education (one 60 min-session). Outcomes measured at baseline and post-intervention were: fasting oxidized LDL and oxidized HDL (oxLDL and oxHDL) as oxidative stress variables; dietary intake of fresh fruit and vegetable (F&V) and fitness (VO₂max) as behavioral variables; weight, BMI, body fat, and waist circumference as anthropometric variables; fasting glucose and insulin, 2hour glucose and insulin after an OGTT, insulin resistance (HOMA-IR), and lipid panel (triglycerides, total cholesterol, VLDL-c, LDL-c, HDL-c, and Non-HDL) as cardiometabolic variables.

Results. Comparing baseline to post-intervention, significant decreases in oxLDL concentration were shown (51.0(14.0) and 48.7(12.8) U/L, p=0.022); however, the intervention did not decrease oxHDL (395.2(94.6) and 416.1(98.4) ng/mL, p=0.944). F&V dietary intake (116.4(97.0) and 165.8(91.0) g/d, p=0.025) and VO₂max (29.7(5.0) and 31.6(4.7) ml*kg⁻¹*min⁻¹, p<0.001) significantly increased. Within-subjects correlations between changes in F&V intake and oxidized lipoproteins, adjusted for VO₂max changes, were non-significant (R=-0.15, p=0.52 for oxLDL; R=0.22, p=0.25 for
oxHDL). Anthropometric variables were significantly reduced (weight -1.3% p=0.042; BMI -2.2% and BMI percentile -0.4%, p=0.001; body fat -6.6% and waist circumference -1.8%, p=0.025). Cardiometabolic variables significantly improved, including reductions in glucose 2hour (-19.3% p<0.001), fasting insulin (-12.9% p=0.008), insulin 2hour (-53.5% p<0.001), and HOMA-IR (-12.5% p=0.015), with 23 participants (66%) that reverted toward a normal glucose tolerance status. Most lipid panel significantly changed (triglycerides -10.2% p=0.032; total cholesterol -5.4% p=0.002; VLDL-c -10.4% p=0.029; HDL-c -3.2% p=0.022; and Non-HDL -5.5% p=0.0007). **Conclusion.** The intervention resulted in differential effects on oxidized lipoproteins and significant improvements in behavioral, anthropometric and cardiometabolic variables, reducing the high metabolic risk of obese prediabetic kids.
DEDICATION

I dedicate this dissertation to my family: my husband Alfonso and my kids Ana Laura and Alfonso, for showing patience, support and solidarity during the previous three and a half years. We sacrificed priceless time, which we could have spent together. Thank you very much!

To my community at Cd. Obregón, Sonora, México, and to all Mexicans and other Latinos deserving that our research efforts being directed to improve their health and life conditions.

To my students, who deserve a well-trained mentor who can offer high-quality education and can challenge their ideas to help them achieve their own dreams.

Finally, I would like to express that this PhD process has been an amazing experience which I had always dreamed of having. I have learned so much in many ways throughout my doctorate. Now I am sure that studying in a foreign country has been one of the most incredible experiences of my life. But above all, I met people so wonderful that I truly believe having met them is the most valuable blessing of this journey.

“Nada te turbe, nada te espante, todo se pasa: Dios no se muda. La paciencia todo lo alcanza; quien a Dios tiene nada le falta; solo Dios basta.”.......Santa Teresa de Jesús.

“Let nothing disturb you, let nothing frighten you, all things are passing away: God never changes. Patience obtains all things, whoever has God lacks nothing; God alone suffices.”.......St. Teresa of Avila.
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CHAPTER 1. INTRODUCTION

1.1 Background

Atherosclerosis is the initial hallmark of cardiovascular diseases (CVD), which are the major source of morbidity and mortality globally.\(^1\) In 2014, the World Health Organization (WHO) reported that 17.5 million people die from CVD yearly. CVD accounted for an estimated of 31% of all deaths, representing the number one cause worldwide.\(^2\) The proportion is similar in the U.S., where CVD causes almost 801,000 deaths (approximately 1 out of 3 deaths) every year.\(^3\)

In regards to morbidity, in the U.S. about 92.1 million American adults have some form of CVD or survived a stroke. Projections are not positive, since 43.9% of the U.S. population by 2030 are expected to have some form of CVD.\(^3\) Regarding economic burdens, an estimated of more than $316 billion represents direct and indirect CVD costs, which includes health care and loss of productivity.\(^4\) Hence, CVD represents the condition with the most expensive health-care cost and to which more deaths are attributed. Therefore, it is essential to take actions against CVD in a preventive manner.

Recommendations from the American Heart Association (AHA) to prevent CVD are mainly focused on reducing risk factors. These are obesity, hypercholesterolemia (mainly elevated low-density lipoprotein cholesterol concentration, LDL-c), hypertension, low high-density lipoprotein cholesterol concentration (HDL-c), and diabetes mellitus (DM).\(^5,6\) The current elevated prevalence of diabetes and obesity are projected to contribute raising the atherosclerosis estimates in the near future.\(^7,8\)
Regarding CVD risk by ethnic group, in the most recent Heart and Stroke Statistics reports from the AHA Latinos showed elevated estimates of cardiometabolic risk factors such as obesity, hypercholesterolemia, high blood pressure, diagnosed DM, and low physical activity (PA) levels, compared to non-Latino white adults.\textsuperscript{4,9,10} Furthermore, in a recent study Latinos had a low-CV risk profile, an ideal metric for preventing cardiometabolic diseases.\textsuperscript{11} Besides, Latinos have elevated genetic susceptibility for type 2 diabetes (T2D) according to some identified single nuclear polymorphisms, compared to other ethnic groups.\textsuperscript{12} The CV risk in Latinos is even greater considering that this group is a minority with low income and low education,\textsuperscript{13} who also have limited access to medical care and consequently lack of health-related information.\textsuperscript{14} Undoubtedly, Latinos are among the most vulnerable groups for CVD.\textsuperscript{15-17}

Combating the risk for CVD entails huge efforts from healthcare systems worldwide, but especially to communities with a high proportion of Latinos, such as U.S.\textsuperscript{18-20} Latinos are the youngest major ethnic group in U.S.,\textsuperscript{21} and in Arizona Latinos comprise 40.8\% of the population.\textsuperscript{22} Being Phoenix the 6\textsuperscript{th} most crowded city across the nation,\textsuperscript{23} the economic burden for the health system in Arizona to combat CVD is even greater than for other states in U.S.\textsuperscript{24}

Despite that evident CVD seldom emerge before adulthood, early atherosclerotic manifestations can be seen during childhood and youth,\textsuperscript{25-27} especially in individuals with diabetes.\textsuperscript{28} These early manifestations represent the main concern for children because during last decades childhood obesity and diabetes have reached epidemic proportions.\textsuperscript{29-31} In this sense, Latino children are at a substantial CV risk because of the elevated
prevalence of obesity, T2D, and cardiometabolic factors,\textsuperscript{32,33} as well as the health-disparities that Latino children face.\textsuperscript{34} According to Dabelea et al.,\textsuperscript{35} in five areas of the U.S., the prevalence of T2D among Latino youth was 0.79 per 1000 youth in 2009, being the greatest increase from 2001 to 2009, compared to other ethnic groups. Additionally, according to the 1999-2014 NHANES data in adolescents from 12-19 y old, Hispanics had greater prevalence of overweight and obesity (22.8\% and 8.8\%), compared to non-Hispanic whites (19.6\% and 6.7\%).\textsuperscript{32} Furthermore, the 2005-2014 NHANES data showed a weighted prevalence of diabetes of 0.76\% and a prevalence of prediabetes of 22.9\%, compared to non-Hispanic whites (0.6\% and 15.1\%, respectively).\textsuperscript{36} Hence, Latino children represent a high-risk group for atherosclerosis and CVD, as Latino adults do, and unfortunately Latino children will probably account for most T2DM and CVD cases as adults in the near future.\textsuperscript{37} Despite this increased cardiometabolic risk in Latino children, most studies in Latinos are targeting adult populations with frank CVD, with just few documented studies at early ages mainly focused on endothelial dysfunction associated with cardiometabolic risk factors.\textsuperscript{38-42}

Atherosclerosis, which initiates CVD, is defined as a chronic, progressive, and multifactorial disease caused by complex and interrelated etiological factors that affect large and medium-sized arteries. It is characterized by the accumulation of fatty substances, cholesterol deposits, inflammatory and chemotactic proteins, cellular waste products, calcium, and fibrin in the intima layer of arteries.\textsuperscript{43} In atherosclerosis lipoprotein metabolism plays a pivotal role, since atherosclerotic lesions are promoted by low-density lipoproteins, and are hindered by high-density lipoproteins.\textsuperscript{44}
A lipoprotein is defined as “a spherical particle that is used for the transfer of lipids and lipophilic substances in the circulation. It is composed of various amounts of phospholipids, cholesterol, and triglycerides as well as apoproteins.” Lipoproteins are complexes of amphipathic proteins with lipids at variable ratios, densities, and sizes. Their main function is to transport water-insoluble lipids between cells and organs through the lymph and blood. Plasma lipoproteins have traditionally been grouped into five major classes, based on their buoyant density: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Enterocytes and hepatocytes package cholesterol and cholesteryl esters into lipoproteins of various sizes and compositions that are further modified in the circulation. Lipoproteins are synthesized and catabolized in three distinct pathways: the chylomicron pathway, the VLDL/LDL/IDL pathway, and the HDL pathway, all of which are metabolically interrelated.

Atherosclerosis starts when LDL particles, especially those small and dense, traverse the endothelium and are retained by proteoglycans in the inner lining of arterial walls. Once LDL particles are retained, oxidative enzymes such as myeloperoxidase (MPO) oxidize them. These oxidized LDL (oxLDL) particles are able to recruit inflammatory cells such as monocytes, which adhere to the surface of the endothelium and transmigrate into the intima layer. At this site, monocytes proliferate, differentiate into macrophages and form foam cells via up taking of lipoproteins, promoting the atherosclerotic lesion through the production of chemotactic proteins, deposition of cholesterol, and the continued recruitment of inflammatory cells. These set of cells perpetuate inflammatory and oxidative responses together with a gradual and slow
thickening of the wall, impeding blood flow in the vessel lumen.\textsuperscript{47,48} The regular blood flow causes physical forces such as shear stress, which disrupts the atherosclerotic lesion and allows its elements to be exposed to circulation.\textsuperscript{49} The atherosclerotic components can travel throughout the arterial system, resulting in thrombosis and compromising oxygen supply in target organs like heart and brain.\textsuperscript{50} This process can lead to multiple fatal consequences such as hemorrhage, rupture, calcification, and thrombosis.\textsuperscript{51}

Regarding the role of HDL in atherosclerosis, its main beneficial effect is attributed to a mechanism called “reverse cholesterol transport”, in which HDL mediates the transfer of cholesterol from LDL, VLDL, and macrophages in the artery wall to be transported and degraded to the liver, and finally excreted into the bile.\textsuperscript{52,53} Experimental assays have shown other beneficial effects for HDL particles, such as impediment of LDL oxidation in endothelial and smooth muscle cell cultures and suppression of the expression of chemotactic factors.\textsuperscript{54} The favorable effects of HDL in atherosclerosis, such as cholesterol efflux, antiinflammation, and antioxidation, are mainly associated with small and dense HDL particles.\textsuperscript{55,56} These small particles are rich in antioxidant enzymes and antioxidant proteins such as paraoxonase-1 (PON-1), apoL-I and apoA-I.\textsuperscript{57} Actually PON-1 in HDL in an experimental assay prevented the accumulation of lipid hydroperoxides on LDL.\textsuperscript{58} However, HDL particles can also be oxidized by prooxidant enzymes such as MPO and by ROS, transforming the favorable HDL particles into dysfunctional oxidized HDL (oxHDL) particles that are unable to induce all the aforementioned benefits.\textsuperscript{59-62}
Besides lipoprotein oxidation, there are certain metabolic conditions that intensify atherosclerosis, such as insulin resistance and hyperglycemia, which are closely related to the progression of T2D. In fact, in diabetic people the risk of coronary atherosclerosis is three- to five-fold higher comparing to non-diabetics, after controlling for other variables. Insulin resistance and hyperglycemia may promote both atherogenesis and advanced plaque progression. Moreover, insulin resistance and hyperglycemia are likely to have additive or synergistic pro-atherogenic effects.

Insulin resistance is clinically defined as “the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population.” The processes by which insulin resistance promote atherosclerosis involve both systemic effects (particularly dyslipidemia, but also hypertension and a pro-inflammatory state), as well as impaired insulin signaling (both down- and up-regulation of insulin receptor signaling). The impairment of insulin receptor-mediated signaling occurs at the level of the intimal cells and includes endothelial cells, vascular smooth muscle cells, and macrophages.

In systemic insulin resistance-induced dyslipidemia, free fatty acids (FFAs) rise at circulation due to the loss of suppressive effects of insulin on lipolysis in adipocytes. FFAs are then transported to the liver, promoting the production of VLDLs. Moreover, lipoprotein lipase action is reduced in insulin resistance, which is an enzyme found in the endothelium of peripheral capillaries that is rate-limiting for the clearance of triglyceride-rich lipoproteins. As a consequence, hypertriglyceridemia occurs in insulin resistance, reflecting elevated VLDL particles which when metabolized, promote atheroma formation. The presence of elevated VLDL particles also affects HDL metabolism.
Triglycerides in VLDL are transferred to HDL through the action of cholesteryl ester transfer protein (CETP), resulting in a triglyceride-enriched HDL particle that is more rapidly cleared from the circulation. This leaves fewer HDL particles to accept cholesterol from the vasculature, then promoting atherogenesis.⁶⁶,⁶⁹

In regards to hyperglycemia, although evidence from human and animal studies is not as strong as that for insulin resistance, there are studies supporting a direct pro-atherogenic role of hyperglycemia at the level of the endothelium. Data suggest that hyperglycemia in the setting of a non-diabetes-mediated hypercholesterolemic background is enough to promote early lesion formation. However, diabetes-induced elevation of atherogenic lipoproteins are required to accelerate progression of advanced plaques in diabetic mice, beyond that normally observed in non-diabetic mice.⁶⁵

Besides, the frequent excess of caloric intake usually present in obesity and T2D particularly affects pancreatic β-cells and endothelial cells, leading to their dysfunction. The elevated glucose and FFA concentrations on pancreatic β-cells inhibit essential cellular elements for first-phase insulin production, suppressing the insulin secretion through processes known as glucotoxicity and lipotoxicity.⁷⁰ Once these processes become irreversible, they cause fatal islet cell injury and acceleration of β-cell loss, with openly impaired action and secretion of insulin.⁷¹,⁷² All the effects of insulin resistance and hyperglycemia on the endothelium perpetuate the vascular complications of T2D.

The proposed mechanisms by which obesity, insulin resistance, hyperglycemia, and T2D adversely affect the endothelium are oxidative stress and inflammation.⁷³,⁷⁴ Oxidative stress is defined as “an imbalance between oxidants and antioxidants in favor
of the oxidants, potentially leading to damage.\textsuperscript{75} Oxidative stress is a condition in which reactive oxygen species (ROS) and reactive nitrogen species (RNS) are overproduced, as well as other free-radical oxidants. These oxidants are able to promote further oxidation of biological molecules, such as proteins, lipids, and DNA.\textsuperscript{76} The suggested factors triggering oxidative stress are the excessive caloric intake and lack of PA that are present in obesity and T2D. The surplus of nutrients produces a substrate-induced increase in citric-acid cycle activity, generating an excess of mitochondrial NADH (the reduced form of nicotinamide adenine dinucleotide). The excess of NADH yields the consequent overproduction of free radicals, particularly superoxide anion radical, and ROS, leading to an oxidative imbalance. Moreover, in diabetes, the excessive production of oxidants is intensified by the low expression of antioxidant enzymes.\textsuperscript{77,78}

Within the endothelium, the overproduction of superoxide anion radical is accompanied by the higher production of nitric oxide, favoring the generation of peroxynitrite, a potent oxidant that can cause DNA fragmentation and lipid peroxidation, ultimately resulting in acute endothelial dysfunction.\textsuperscript{79} Besides, excessive superoxide anion disrupts the balance between dilation and constriction. With this imbalance, the vasculature suffers severe pro-atherogenic changes, such as vasoconstriction, lipoprotein oxidation, leukocyte adherence, inflammation, and some other diverse abnormalities, which are essential steps in atherosclerosis.\textsuperscript{80,81}

Among all the aforementioned pro-atherogenic changes caused by oxidative stress, the oxidation of lipoproteins is of paramount importance given their role in atherosclerosis. Furthermore, due to their chemical composition lipoproteins are especially susceptible to oxidative stress, leading to increased concentrations of oxLDL.
As reported previously, investigations have proposed oxLDL particles as precursors of atherosclerotic lesions.\(^\text{84-87}\) There are multiple studies in adults in which oxLDL has been associated with CV events\(^\text{86-91}\) and also found as a predictor of these events.\(^\text{92,93}\) In regard to oxHDL, experimental studies have shown it as a dysfunctional particle unable to carry out reverse cholesterol transport and to counteract the oxidation of LDL.\(^\text{94-96}\) Additionally, although fewer investigations than those regarding oxLDL, some studies with cross-sectional data in adults have still shown significant greater oxHDL concentrations in cardiometabolic conditions such as obesity and liver fat, compared to overweight/obese and to normal weight individuals.\(^\text{97,98}\) Despite the fact that both lipoproteins have been found in atherosclerotic lesions in experimental studies, and the independent associations that oxLDL has shown with CVD and oxHDL with some cardiometabolic conditions, research in humans accounting for the synergistic associations of both oxidized lipoproteins with cardiometabolic risk are extremely limited, and studies in youth are even scarcer. Only a research by Marin et al. (2015) has reported levels of both oxidized lipoproteins in overweight/obese youths and obese T2D kids, compared to normal weight control. Therefore, it is crucial to do comprehensive research that accounts for synergistic associations of oxidized lipoproteins with cardiometabolic conditions.

Concerning pediatric population, various cross-sectional studies in children and adolescents have analyzed differences in oxLDL concentration among groups, such as overweight and obese,\(^\text{99-102}\) severely obese,\(^\text{103,104}\) non-alcoholic fatty liver disease (NAFLD),\(^\text{105}\) and diabetics,\(^\text{106-108}\) compared to normal weight group. Most studies have shown significant differences between groups, with greater levels of oxLDL in
metabolically impaired individuals such as NAFLD and diabetics, as well as in overweight/obese kids, when these groups are compared to control normal-weight groups. However, no significant differences in oxLDL between groups have also been documented.\textsuperscript{109}

In contrast, studies investigating oxHDL have been scarce, with three studies in adults: two from the Cardiovascular Risk in Young Finns Study\textsuperscript{98,110} and one with female adults compared to normal weight.\textsuperscript{97} From The Young Finns Study, Kresanov et al. (2013) showed an inverse association between oxHDL and age, insulin, and waist circumference, respectively.\textsuperscript{110} The same research group reported a high oxidation score directly associated with fatty liver in a study published by Kaikkonen et al.\textsuperscript{98} In the study with adult females, obese patients displayed a 6-fold increase in oxHDL compared to normal weight, and the oxHDL/total HDL ratio increased with increasing BMI.\textsuperscript{97} Regarding pediatric population, only one study has compared oxHDL concentrations among normal weight, obese, and obese+T2D adolescents.\textsuperscript{107} In this, oxHDL was 65\% higher in the obese+T2D group, compared to the normal weight group and with the obese-only group. In this latter study, oxHDL was not correlated with insulin resistance (HOMA-IR), while oxLDL and lean body mass were positively associated with oxHDL.\textsuperscript{107}

Concerning interventions to decrease oxidation through lowering oxidative stress levels, these are focused on improving the antioxidant defense system of individuals. The improvement may be reached by either augmenting the endogenous enzymatic antioxidants, mainly by exercise, or increasing the intake of antioxidant nutrients by
promoting a diet rich in fruit and vegetables, or both.\textsuperscript{111,112} Interventions to improve the antioxidant defense system have been documented in the adult population, as well as in youth. In the pediatric population, interventions reducing oxidative stress levels have used dietary modification only,\textsuperscript{113-116} exercise only,\textsuperscript{117,118} lifestyle modifications (diet and exercise),\textsuperscript{119,120} and bariatric surgery.\textsuperscript{121,122} The effects of these interventions have also been explored on different health conditions. Hypercholesterolemia,\textsuperscript{123} non-alcoholic fatty liver disease (NAFLD),\textsuperscript{116,124} overweight/obesity,\textsuperscript{119,120} and severe obesity,\textsuperscript{114,121,122} are some of the conditions in which pediatric interventions have been evaluated. It is worth mentioning that despite the preponderant role that hyperglycemia has on oxidative stress, up to current knowledge no intervention addressing either prediabetics or T2D children have been documented.

With respect to exercise, interventions have resulted in significant decreases in pro-oxidant parameters and increases in enzymatic antioxidant capacity with aerobic and endurance exercise.\textsuperscript{125-128} Regarding dietary modifications, studies have tested antioxidant-rich foods such as increases in fruit and vegetable consumption, supplementation, and caloric restriction, with most research resulting in significant reductions in oxidative stress after the intervention.\textsuperscript{123,129,130} Using both exercise training and dietary modification within the same intervention has seemed to be more effective decreasing oxidative stress than using only exercise or diet, particularly in the youth population.\textsuperscript{119,120,131,132} Moreover, including supplementation with antioxidants on lifestyle interventions has been beneficial decreasing oxidative stress.\textsuperscript{115,116} It has been suggested that additive and/or synergistic effects are present in these comprehensive interventions that include both exercise and antioxidants.
With respect to interventions specifically decreasing oxidized lipoproteins in children and adolescents, oxLDL has been more frequently analyzed in programs using exercise, \(^{118}\) dietary modifications, \(^{124,130}\) supplements, \(^{129}\) lifestyle modifications, \(^{119,120}\) and bariatric surgery. \(^{121}\) Once again, most interventions but not all have demonstrated beneficial results decreasing oxLDL, \(^{120,121,124}\) and findings from some few interventions showed no reductions in oxidized lipoproteins \(^{133}\) or authors needed to divide participants into responders and non-responders to show effects. \(^{130}\) Regarding studies in youth measuring oxHDL have been scant and mainly related to HDL function. Two interventions consisted of measuring the effect of a lifestyle intervention on HDL-cholesterol efflux capacity, \(^{134}\) and the effect of bariatric surgery on HDL function. \(^{122}\) Both interventions resulted in beneficial effects on HDL functions.

Globally, results from most interventions previously mentioned effectively have induced significant decreases in oxidative stress in youth. \(^{117,133,135}\) Nevertheless, some studies have shown no effects on oxidative stress in the pediatric population. \(^{118,121,122}\) It is important mentioning that interventions have differed in its components, making them also different in nature. The differences include characteristics of participants, type of intervention (dietary, exercise, or diet and exercise together), intensity and duration of intervention, inclusion or not of the family within the program, among other elements. All these differences may make studies difficult to compare when addressing the same type of individuals. Moreover, the use of different biomarkers is also an important difference since oxidative stress can cause multiple oxidation reactions and can affect different types of molecules. It must be commented that lipoprotein oxidation as response outcome from an intervention is of paramount importance in the context of oxidative
stress, insulin resistance, hyperglycemia, and atherosclerosis. The proposed roles of oxLDL in the initiation of an atherosclerotic lesion and oxHDL as an impaired molecule unable to counteract the oxidation of LDL, make interventions addressing these molecules being essential.

Collectively, findings from studies reported here indicate that more research needs to be done within the context of interventions reducing early atherosclerotic biomarkers related to oxidative stress. Among all reactions and processes associated with atherosclerosis herein mentioned, lipoprotein oxidation is of fundamental value because of their role in the atherosclerotic lesions. It is imperative to execute research in an early-age population with increased CVD risks, such as those with obesity, insulin resistance, and hyperglycemia. Research is necessary in order to guarantee that exercise training and dietary antioxidants promote beneficial changes in the oxidative environment of young high-risk individuals, thereby reducing levels of oxidative stress in the pediatric population.

Accordingly, up to current knowledge no lifestyle intervention involving the promotion of fruit and vegetable intake and exercise training has been evaluated in obese prediabetic Latino youth, which are at high risk for developing CVD due to obesity, insulin resistance, and as well as the hyperglycemic state, which are metabolic conditions that promote oxidative stress. Even though oxidative stress is one of the mechanisms underlying the gradual development and progression of atherosclerosis and diabetic complications, no studies exist testing the effects of lifestyle interventions on both ox
LDL and oxHDL as early oxidative stress biomarkers of atherosclerosis in obese prediabetic Latino youth.

1.2 Purpose of Research

Considering the high risk for CVD at which obese, insulin resistant and hyperglycemic people are, particularly some ethnic minorities like Latinos, there is an urgent need to promote prevention interventions, especially at childhood. The evaluation of the effects of a T2D-prevention intervention on levels of oxLDL and oxHDL as early biomarkers of atherosclerotic risk represents an ideal approach for investigating how oxidative stress is affected by lifestyle modifications. Therefore, the purpose of this study was to evaluate the effects of a lifestyle and T2D-prevention intervention for obese prediabetic Latino adolescents on levels of oxidized lipoproteins as early biomarkers of oxidative stress.

1.3 Specific Aims and Hypotheses

1.3.1 Specific Aim 1. To analyze the effect of a lifestyle and T2D-prevention intervention for obese prediabetic Latino adolescents on concentrations of oxLDL.

Hypothesis 1. OxLDL concentration will be significantly lower after a lifestyle and T2D-prevention intervention in obese prediabetic Latino adolescents, compared to baseline.
1.3.2 **Specific Aim 2.** To analyze the effect of a lifestyle and T2D-prevention intervention for obese prediabetic Latino adolescents on concentrations of oxHDL.

**Hypothesis 2.** OxHDL concentration will be significantly lower after a lifestyle and T2D-prevention intervention in obese prediabetic Latino adolescents, compared to baseline.

1.3.3 **Specific Aim 3.** To explore how changes in dietary intake of fresh fruit and vegetable (F&V) after a lifestyle and T2D-prevention intervention are significantly correlated with changes in oxLDL and oxHDL in obese prediabetic Latino adolescents, after controlling for changes in cardiorespiratory fitness.

**Hypothesis 3.** Changes in dietary intake of fresh F&V will inversely and independently correlate with changes in oxLDL and oxHDL in obese prediabetic Latino adolescents, after controlling for changes in cardiorespiratory fitness.
CHAPTER 2. LITERATURE REVIEW

2.1 Cardiovascular Risk Estimates in Latinos

Compared to other ethnicities, Latinos are at increased risk for CVD. In the most recent National Surveys, Latinos have shown elevated estimates of cardiometabolic risk factors. For instance, in 2016 Mexican-American adults (the most representative Latino group in the U.S.) had a higher prevalence of obesity, hypercholesterolemia, high blood pressure, as well as low PA levels.\textsuperscript{9,10} Not only physiological and behavioral risk factors are altered in Latinos, but their genetics also predispose them to certain cardiometabolic diseases. Latinos have shown single nuclear polymorphisms associated with T2D, rendering them genetically susceptible to this disease.\textsuperscript{12} Moreover, in the Hispanic Community Health Study/Study of Latinos, a substantial proportion of Latinos had a low-cardiovascular (CV) risk profile, which is an ideal metric for preventing cardiometabolic diseases.\textsuperscript{11} Moreover, healthcare systems worldwide make tremendous efforts fighting CVD, but it seems that these efforts are not enough in communities with a high proportion of Latinos.\textsuperscript{18-20,136}

Regarding children and adolescents, NHANES from 1999-2014 showed that among youth, those aged 12-19 y old had the highest prevalence of overweight (37.4%), and all classes of obesity. With respect to ethnicity, Latino adolescents showed the highest prevalence of overweight (41.8%) and class I and II obesity.\textsuperscript{32} Interestingly, in children from NHANES 1999-2012, mean values of most cardiometabolic variables were
higher as the severity of obesity was greater, with an exception for HDL in which mean values were lower as BMI increased.\textsuperscript{31}

With respect to diabetes, in the 12-19 years group from NHANES 2005-2014, the weighted prevalence of combined T1D and T2D was 0.8\%, and for prediabetes was 17.7\%, with almost one-third undiagnosed. Latino adolescents who were T2D undiagnosed (39.5\% from all diabetic children) and prediabetic (22.9\% from all diabetic children) were higher compared to non-Latino whites.\textsuperscript{36} Besides, in a recent study with individuals living in five regions of the U.S., the prevalence of diabetes significantly increased between years 2001 and 2009.\textsuperscript{35} In 2001, 0.34 per 1000 youth were diagnosed with T2D, while in 2009 the prevalence was 0.46 per 1000 youth. Considering ethnicity, in 2009 the prevalence of T2D was 0.79 per 1000 among Latino youth, with the greatest increase in the prevalence of T2D being from 2001 to 2009, compared to other ethnic groups.\textsuperscript{35}

Other elevated estimates of cardiometabolic risk factors in Latino adolescents are a high prevalence of physical inactivity, with 20.3\% of girls and 12.1\% of boys reporting more inactivity, and 41.3\% of Latinos who were less likely to meet the current aerobic physical activity (PA) guidelines. In regards to high blood pressure, from 2003 to 2013 the death rate and the number of deaths increased 1.7\% and 75.5\% respectively, in Latino children.\textsuperscript{9} In NHANES from 1999-2008, among U.S. adolescents aged 12 to 19 y the overall prevalence was 14\% for prehypertension/ hypertension, 22\% for borderline-high/high low-density lipoprotein cholesterol, and 6\% for low high-density lipoprotein
cholesterol (<35 mg/dL), being Latinos the most contributing group to these high estimates.\textsuperscript{137}

Therefore, Latino children are in substantial cardiometabolic risk because of the elevated prevalence of obesity, T2D, hypertension, and other associated conditions,\textsuperscript{31-33} as well as the disparities in health-care that this ethnic group displays.\textsuperscript{34} These estimates make Latino adolescents an ethnic minority group with a high genetic, behavioral, and social risk for CVD. Due to these estimates, current Latino children and adolescents will probably account for most T2D and CVD cases as adults,\textsuperscript{37} with the consequent clinical and economic burden.\textsuperscript{138}

2.2 Defining Oxidative Stress

Oxidative stress results from an imbalance between oxidant production and antioxidant activity in cells and plasma. Thus, if any increased production of oxidants is present together with any antioxidant loss or dysfunction, oxidative stress is present.\textsuperscript{139} Oxidants are substances that can undergo repetitive rounds of oxidation and reduction, and eventually yield to an increased production of superoxide anion radicals and secondary oxidants such as reactive oxygen species (ROS) or reactive nitrogen species (RNS).\textsuperscript{75,140} Within balance, ROS are primordial for physiological functions, including gene expression, cellular growth, infection defense, and modulation of endothelial function.\textsuperscript{126} However, overproduction of ROS and/or diminished antioxidant capacity can lead to dysfunctional oxidative stress. Besides ROS, free radicals (any chemical species
containing one or more unpaired electrons) and several no radical species are important oxidants as well.¹⁴¹

ROS and RNS have been implicated in atherogenesis within the vessel wall.⁵¹,⁷⁴ These oxidants can mainly originate from both cellular and extracellular sources, as well as from enzymatic and non-enzymatic pathways. Oxidant sources of physiological relevance are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidases, nitric oxide synthase (NOS), MPO, lipoxygenases, mitochondrial respiration, transition metals, and some others.⁷⁶ MPO is of special importance since it can generate a series of secondary oxidation products with the ability to give rise to oxidized biomolecules, including oxLDL and oxHDL.¹⁴²

2.3 Insulin Resistance and Oxidative Stress as Common Pathogenic Factors for T2D and CVD

One of the common conditions present in T2D and CVD is insulin resistance.¹⁴³ Insulin resistance is an early condition which can lead to glucose dysregulation, impaired glucose tolerance (prediabetes), and finally to diabetes.¹⁴⁴ Clinically, insulin resistance is defined as “the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population.”¹⁶⁷ Insulin resistance is associated with an excess of caloric intake, lack of PA and obesity. In the insulin resistant state, compensatory hyperinsulinemia by pancreatic β-cells maintains normal levels of blood glucose. However, when β-cells are no longer
able to increase insulin production and compensate for insulin resistance, prediabetes appears. Persistence of all these conditions leads to frank diabetes. Moreover, obesity, insulin resistance, prediabetes, and overt diabetes, have been associated with an increased risk for CVD.\textsuperscript{145,146}

The ”common soil” hypothesis postulates that T2DM and CVD also share common genetic and environmental antecedents.\textsuperscript{147} Evidence points towards oxidative stress generation as the common persistent pathogenic factor mediating T2DM and CVD, ultimately favoring atherosclerotic complications.\textsuperscript{148} The proposed mechanism relates oxidative stress with insulin resistance derived from excess in caloric intake and lack of PA. This surplus produces a substrate-induced increase in citric-acid cycle activity, generating an excess of mitochondrial NADH. The excess of NADH yields the consequent overproduction of free radicals, particularly superoxide anion radical, and ROS, leading to the implications of oxidative imbalance. In this context, insulin resistance may be seen as a compensatory mechanism protecting cells against additional insulin-stimulated caloric uptake. Moreover, in diabetes, the excessive production of oxidants is intensified by the low expression of antioxidant enzymes.\textsuperscript{77,78}

The excess of caloric intake particularly affects pancreatic $\beta$-cells and endothelial cells, which leads to oxidative stress-related dysfunction. This effect is related to facilitated diffusion as the mechanism of these cells for glucose uptake since insulin-regulated glucose transport is not present.\textsuperscript{149} Thus, when overfeeding, $\beta$-cells and endothelial cells cannot decrease the input of nutrients through insulin resistance, allowing intracellular concentration of ROS to increase further. Specifically, $\beta$-cells are
low in free-radical sequestering enzymes, making them susceptible to ROS, with the ultimate damage in insulin secretion.$^{150,151}$

Studies suggest that elevated glucose and free fatty acid concentrations on β-cells induce mitochondrial production of ROS,$^{152}$ inhibit essential cellular elements for first-phase insulin production and suppress the insulin secretion through processes known as glucotoxicity and lipotoxicity.$^{70}$ These processes are reversible at early stages; however, repeated exposure to hyperglycemia and high concentrations of free fatty acids can lead to irreversible β-cell dysfunction, causing fatal islet cell injury and accelerating β-cell loss. At this stage, both action and secretion of insulin is frankly impaired, accelerating the progression to T2D.$^{71,72}$ Investigations have shown that oxidative stress induced by hyperglycemia is associated with the progression of diabetic complications, such as microvascular disease (diabetic retinopathy, nephropathy, and peripheral neuropathy), and macrovascular disease.$^{76}$

### 2.4 Oxidative Stress and Endothelial Dysfunction

Overproduction of mitochondrial superoxide anion radical through oxidative stress in the microvasculature is a subjacent characteristic of endothelial dysfunction. Increased oxidative stress in vulnerable tissues such as endothelium is exacerbated via activation of NADPH oxidase by the high activity of local renin-angiotensin systems.$^{153,154}$
In vitro, arteries from normal-glycemic animals exhibited attenuated endothelium-dependent relaxation after been exposed to exogenous hyperglycemia.\textsuperscript{155} Furthermore, superoxide production was increased under hyperglycemic conditions in endothelial cells.\textsuperscript{156} In vivo and in cross-sectional studies with both diabetic and non-diabetic subjects, hyperglycemia also induced endothelial dysfunction.\textsuperscript{157} From all these studies it has been proposed that the hyperglycemic-overproduction of superoxide anion radical was the key component in the activation of pathways involved in the pathophysiology of endothelial dysfunction.

Another source of ROS in vascular cells is the overproduction of superoxide and hydrogen peroxide by endothelial nitric oxide synthase (eNOS), a pathway known as NOS uncoupling.\textsuperscript{158} The overproduction of superoxide anion radical is accompanied by a higher production of nitric oxide, favoring the generation of peroxynitrite, a potent oxidant that can cause DNA fragmentation and lipid peroxidation, ultimately resulting in acute endothelial dysfunction.\textsuperscript{79} Under all mentioned processes, excessive superoxide anion disrupts the balance between dilation and constriction. With this imbalance, the vasculature is prone to vasoconstriction, leading to lipoprotein oxidation, leukocyte adherence, inflammation, and some other diverse abnormalities, which are essential steps in atherosclerosis.\textsuperscript{80,81}

2.5 Atherosclerosis

Atherosclerosis is defined as a chronic, progressive, and multifactorial disease caused by complex and interrelated etiological factors that affect large and medium-sized arteries. It is characterized by the accumulation of fatty substances, cholesterol deposits,
inflammatory and chemotactic proteins, cellular waste products, calcium, and fibrin in the intima layer of arteries.\textsuperscript{43}

High plasma LDL-c concentration has been the proposed factor that contributes to the initiation and progression of atherosclerosis.\textsuperscript{159-161} Interventions attempting to decrease LDL-c concentrations have shown reductions in CV events.\textsuperscript{162-164} In contrast, high plasma HDL-c concentrations have been traditionally associated with lower risk for atherosclerosis.\textsuperscript{165-167} However, studies focusing on HDL-c concentration have been controversial, since some studies have shown increased CVD risk at higher levels of HDL-c.\textsuperscript{168,169} Moreover, there have been interventions showing improvements on cardiometabolic, oxidative stress and inflammatory biomarkers despite that HDL-c levels decreased.\textsuperscript{170-172} Furthermore, other interventions that raised HDL-c did not have beneficial reductions in atherosclerosis.\textsuperscript{173} Current research is proposing indicators different than LDL-c and HDL-c concentration, such as lipoprotein particle size and number,\textsuperscript{174} as well as lipoprotein function,\textsuperscript{175} as better biomarkers associated with CVD.\textsuperscript{176-180} In this sense, evidence is pointing toward oxidized lipoprotein levels and lipoprotein dysfunction as measurement of oxidative stress, one of the main mechanisms associated with atherosclerosis.\textsuperscript{82,98,181,182}

Three main hypotheses are proposed to explain how atherosclerosis begins: response to injury, response to retention, and oxidative modification hypotheses.\textsuperscript{51} Among these hypotheses, the oxidative modification hypothesis represents the most convincing one; however, some authors agree that these hypotheses are all-inclusive.\textsuperscript{51,183} The oxidative modification hypothesis describes how oxidative stress promotes
atherosclerosis. Within this hypothesis, the proposed essential mechanism explaining the role that ROS play in diabetes-induced endothelial dysfunction is the oxidation of lipoproteins, which are key elements inducing atherosclerosis.184,185

2.6 Role of Lipoproteins in Atherosclerosis

A lipoprotein is defined as “a spherical particle that is used for the transfer of lipids and lipophilic substances in the circulation. It is composed of various amounts of phospholipids, cholesterol, and triglycerides as well as apoproteins.”45 Proteins and lipids in lipoproteins are at variable proportion, densities, and sizes. Main function of a lipoprotein is to transport water-insoluble lipids among cells and organs through the aqueous milieu (lymph and blood). There are traditionally five major classes of plasma lipoproteins, based on their buoyant density: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).44 Cholesterol and cholesteryl esters are packaged by enterocytes and hepatocytes into lipoproteins of different sizes and compositions that are further modified in the circulation. Lipoproteins are synthesized and catabolized in three distinct pathways: the chylomicron pathway, the VLDL/LDL/IDL pathway, and the HDL pathway, all of which are metabolically interrelated.46

2.6.1 LDL and Oxidized LDL

According to the oxidative modification hypothesis, oxLDL plays a preponderant role in atherosclerosis initiation. The process starts when LDL particles (especially those
small and dense) traverse the sub-endothelial space of arterial sites that are susceptible to injury. During their trajectory, LDL particles are modified by oxidant enzymes, from which MPO has been mainly linked to this oxidation.\textsuperscript{101,142,186} Consequently, apolipoprotein and lipids within the LDL particles are oxidized,\textsuperscript{187} rendering them susceptible to macrophage uptake via scavenger receptor pathways.\textsuperscript{188} This results in the conversion of macrophages into cholesterol ester-laden foam cells\textsuperscript{189} that induce the synthesis of chemotactic proteins in smooth muscle and endothelial cells.\textsuperscript{190,191} As foam cells and chemotactic proteins accumulate, inflammatory cells are recruited.\textsuperscript{54,192} Recruitment of inflammatory cells may result in continued macrophage internalization, LDL oxidation, and proliferation of smooth muscle cells.\textsuperscript{193} At this step, the atherosclerotic lesion begins and becomes the platform for catalytic extension of the lesion and the full-scale spectrum of atherosclerosis.

2.6.2 HDL and Oxidized HDL

Regarding the role of HDL in atherosclerosis, its main beneficial effect is attributed to a mechanism called “reverse cholesterol transport,” in which HDL mediates the transfer of cholesterol from LDL, VLDL, and macrophages in the artery wall\textsuperscript{52,53} to be transported and degraded to the liver, and finally excreted into the bile. Originally, the concentration of cholesterol in HDL (HDL-c) was considered as the main biomarker associated with reverse cholesterol transport.\textsuperscript{194,195} However, recent findings suggest that HDL-c may not fully associate with CVD risk.\textsuperscript{196}

Evidence showing that low HDL-c is not a strong predictor of CVD events comes from diverse epidemiological studies that have showed no association between HDL-c
and CVD events. Randomized control trials (RCT) using pharmacological increases of HDL-c have failed in reducing CVD risk. Drugs such as torcetrapib and dalcetrapib (cholesterol ester transfer protein inhibitors)\textsuperscript{173,199,200} and niacin,\textsuperscript{201} which raised HDL-c, have shown no reductions in CVD events or atherosclerosis. This evidence has directed the light toward HDL function as a better indicator of CVD risk.

Multiple beneficial functions have been associated with HDL particles, such as antioxidant, anti-inflammatory, antithrombotic, promotion of NO production, and protection from apoptosis.\textsuperscript{202} HDL particles have shown protection against LDL-induced cytotoxicity and LDL oxidation on endothelial cells.\textsuperscript{203,204} Experimental assays showed that HDL impaired the oxidation of LDL in endothelial and smooth muscle cell cultures, also impairing the expression of chemotactic factors.\textsuperscript{54} The favorable effects of HDL in atherosclerosis, such as cholesterol efflux, antiinflammation, and antioxidation, were mainly associated with small, dense HDL particles.\textsuperscript{55,56} These small particles are rich in antioxidant enzymes and antioxidant proteins such as paraoxonase-1 (PON-1), apoL-I and apoA-I.\textsuperscript{57} PON-1 in HDL has been associated with the prevention of lipid hydroperoxide accumulation on LDL.\textsuperscript{58} Furthermore, when HDL from normal subjects was tested on cultures of human aortic endothelial and smooth muscle cells, this lipoprotein prevented the oxidation of LDL and the induction of monocyte chemoattractant protein-1 (MCP-1).\textsuperscript{205} Besides, normal HDL containing the antioxidant enzymes platelet-activating factor acetylhydrolase and PON-1 prevented the formation of LDL-derived oxidized phospholipids.\textsuperscript{58,206} LDL from mice genetically susceptible to fatty streak lesion formation showed resistance to oxidation after being incubated with apoA-I.
Moreover, humans who were infused with apoA-I and human artery wall cells treated with this apoprotein showed resistance of LDL to cell-mediated oxidation.\textsuperscript{207}

Conversely, when HDL is modified, it loses its function of removing cholesterol and becomes dysfunctional, exhibiting proatherogenic and proinflammatory properties.\textsuperscript{59,60,96} Particularly, HDL is vulnerable to modification by oxidants such as those generated by MPO, producing oxHDL.\textsuperscript{61,208} Experimental studies exploring HDL dysfunction showed that impaired effects in reverse transport were associated with apoA-I oxidation by MPO. This apoA-I oxidation induced proliferation and migration of vascular smooth muscle cells, which represent one of the preliminary steps in atherosclerotic lesions.\textsuperscript{62}

Likewise, when HDL from subjects with an acute phase reaction was used to inhibit the oxidation of LDL, it did not show any antioxidant activity, neither inhibition of monocyte chemotactic activity. This finding was associated with reduced activities of HDL-enzymes, which resulted in higher cell-mediated LDL oxidation and increased MCP-1 by the modified HDL during the acute phase reaction.\textsuperscript{209} Additionally, HDL obtained from coronary artery disease (CAD) normolipidemic patients was unsuccessful inhibiting LDL oxidation and MCP-1 generation. Conversely, HDL from sex-matched control participants inhibited these processes.\textsuperscript{210} Same results were obtained with HDL from patients with CHD, showing failed inhibition of LDL-induced MCP-1 production.\textsuperscript{211} Moreover, HDL from T2D subjects showed diminished protective properties in endothelium, which suggested that diabetes mellitus is a condition that can
induce HDL dysfunction. Collectively, findings indicated that HDL from subjects with CVD and T2D was somehow defective preventing LDL oxidation.

2.7 Myeloperoxidase-related Oxidation of Lipoproteins

MPO is a heme protein derived from leukocytes that is mostly found in neutrophils, monocytes, and macrophages. This enzyme uses $\text{H}_2\text{O}_2$ and chlorine anion to produce hypochlorous acid (HOCl) and further reactive oxidants, free radical species, and oxidant chlorinated molecules such as 3-chlorotyrosine, chlorohydrins, alpha-chloro fatty acid aldehydes, and tyrosyl radicals. Tyrosyl radicals have taken part in secondary oxidative reactions like the oxidation of LDL. Similarly, some other series of ancillary oxidation products by MPO like hydroxyl-amino acids, alpha-hydroxy and $\alpha,\beta$-unsaturated aldehydes can finally convert to the chemically well-characterized advanced glycation end product, NE-(carboxymethyl)lysine. There is immunohistochemical evidence of the presence of MPO/$\text{H}_2\text{O}_2$/halide system in human atherosclerotic lesions, in which colocalization of MPO and hypochlorite-modified proteins were found. All these molecules have the ability to generate oxidized biomolecules such as oxLDL and oxHDL.

Elevated concentrations of chlorotyrosine and other oxidative byproducts were found in LDL obtained from human atherosclerotic lesions. Evidence suggested that MPO-generated hypochlorous acid was probably the initially responsible oxidant for both lipids and apo-B within LDL particles. Yang et al. (1999) showed that oxidation of
LDL by MPO in vitro produced selective modification of apoB-100.\textsuperscript{223} Similarly, Hazell et al. (1994) reported that oxidation of LDL by hypochlorite caused aggregation mediated by oxidation of lysine residues of apoB, without involving lipid peroxidation.\textsuperscript{224} However, another research group (Heller et al., 2000) found that p-hydroxyphenyl-acetaldehyde, an aldehyde generated by MPO, modified phospholipid amino groups of LDL in the human atherosclerotic intima.\textsuperscript{225} Together all data suggest that both modifications of lipids and apoB contributed to the production of oxLDL, which finally convert macrophages into foam cells.\textsuperscript{226}

MPO-immunoreactive macrophages were found in human atherosclerotic plaques, within arteries and plaque rupture sites.\textsuperscript{227} Additionally, when activated MPO-containing macrophages were added to endothelial cells, cells were detached and dead. Likewise, hypochlorous acid added to endothelial cells induced apoptosis and release of tissue factor in a dose-dependent manner.\textsuperscript{228} Similarly, Steffen et al. (2006) found that cytotoxicity of MPO/nitrite-oxLDL toward endothelial cells was due to oxygenated cholesterols (oxysterols) formed during oxidation of LDL, specifically by a high 7 beta-hydroxycholesterol to 7-ketocholesterol ratio.\textsuperscript{229} Data from the aforementioned studies suggests that MPO and metabolites derived from its activity play an important role in the activation and subsequent apoptosis of macrophages within arteries and endothelial cells.

Regarding HDL oxidation by MPO, when this enzyme is released in circulation and endothelial lesions, it binds to HDL at the helix 8-region of apoA-I. This binding is linked to the selective oxidative modification of apoA-I by the MPO-produced oxidants, showing increased concentrations of chlorotyrosine and nitrotyrosine.\textsuperscript{230} These
compounds are suggestive of protein contact with reactive chlorinating and nitrating oxidants produced by MPO.\textsuperscript{231,232} The oxidation of apoA-I is supposed to lead to functional impairment of HDL within the arterial wall, impeding the reverse cholesterol transport and promoting the initiation and development of plaque by these oxHDL particles.\textsuperscript{222}

In a clinical investigation, subjects with greater concentrations of chlorotyrosine and nitrotyrosine within apoA-I had 16-fold, and 6-fold increased probabilities of having CVD, respectively.\textsuperscript{231} Increased levels of these oxidants within apoA-I are strongly associated with cholesterol efflux impairment.\textsuperscript{233} Moreover, elevated MPO-modification of apoA-I is linked to impairment of binding HDL to lecithin:cholesterol acyltransferase (LCAT), which is the enzyme responsible for converting free cholesterol into cholesteryl ester within HDL.\textsuperscript{234} Some other functional impairments of HDL due to the exposure to MPO or its oxidant products are a loss of anti-apoptotic and anti-inflammatory activities, specifically the diminishment of scavenger receptor–B1 (SR-B1) binding activity, as well as activation of nuclear factor-kB and expression of adhesion molecules.\textsuperscript{61}

\textbf{2.8 Cross-sectional Studies Associating Oxidized Lipoproteins with CVD-related Outcomes}

Several studies have associated oxidized lipoproteins with CVD risk factors and/or outcomes in youth. In regard to oxLDL, in ages as early as 3-5 years oxLDL levels (10.2±0.7 mg/mL) and lipid peroxides (6.8±0.5 mmol/mg protein) were significantly
higher in obese kids compared to control normal weight (7.2±0.7 mg/mL, and 4.2±0.2 mmol/mg protein, respectively).\textsuperscript{235} Similarly, Kelly et al. (2010) analyzed oxLDL levels in normal weight and overweight/obese children from 6-18 y old. OxLDL levels were significantly elevated in overweight/obese children (69.1±14.1 U/L), compared to normal weight (56.6±12.4 U/L), and this biomarker was significantly correlated with adiposity and insulin resistance, independent of body fatness.\textsuperscript{100} Same research group reported oxLDL in extreme pediatric obesity, normal weight, and overweight/obese children from 13.5±2.5 y old. Authors indicated that oxLDL increased significantly across BMI groups, concluding that extreme pediatric obesity was associated with increased concentrations of oxidative stress and inflammation.\textsuperscript{103} Likewise, in a previous study from our research group, Ryder et al. (2013) determined whether insulin resistance and abdominal adiposity were associated with elevated oxLDL in 123 Latino adolescents (16.3±2.5 y old). Levels of oxLDL were significantly different between groups (38.8±10.5 in lean vs. 44.7±13.9 U/L in overweight/obese adolescents), and waist circumference and insulin sensitivity were significant predictors of oxLDL.\textsuperscript{99}

With respect to relationships between oxLDL and CVD outcomes like carotid intima-media thickness (C-IMT) and flow-mediated dilation (FMD), obese children showed significantly higher levels of oxLDL (901.8±1128.9 ng/mL) than healthy children (279.5±270.5 ng/mL), with no significant correlation between oxLDL and carotid IMT.\textsuperscript{236} In another study, Jarvisalo et al. (2004) examined the correlates of nitrate-mediated dilatation (NMD) including brachial artery endothelial function, oxLDL, and C-IMT levels, in 142 children (age 8-17 y): 87 healthy, 41 with diabetes and 14 with familial hypercholesterolemia. NMD inversely correlated with oxLDL (r=-0.18,
p=0.045), and reduced endothelial function, increased oxidative stress, and preclinical carotid atherosclerosis were independent determinants of impaired NMD in this group. Likewise, when metabolites of oxLDL such as MDA and conjugated diene (CDE) were analyzed from children of 16-18 y old with parents with premature myocardial infarction (MI), an increased susceptibility of LDL oxidation was found.

Moreover, oxidative stress levels have been analyzed in a study performed by Stringer et al. (2009) in youth 12-15 y old from 3 groups: T2DM; age-, gender-, and BMI–matched (obese); and unmatched normal weight controls. Levels of oxLDL were significantly similar in T2D and obese, compared to normal weight control. Besides, Dasari et al. (2016) studied adolescents 13- to 21 y old with habitually low PA classified as healthy weight, healthy obese, or obese with T2D. OxLDL was higher (p<0.05) in T2DM (70.3±5.0 U/L) and healthy obese (58.1±3.8) than healthy weight (48.4±2) and positively correlated with mean amplitude of glycemic excursions (MAGE, r = 0.77).

Other cross-sectional studies have been carried out using oxHDL as a biomarker of oxidative stress, although most of them are from the adult population. However, in a study with pediatric individuals by Marin et al. (2015), children aged 11-18 y were grouped as normal weight, obese, and obese+T2D. Biomarkers of oxidative stress were oxHDL, oxLDL, and MPO. OxHDL was 65% higher in the T2D group, whereas oxLDL was 23% and 56% higher in obese and in T2D, respectively, compared to normal weight. MPO was 88% elevated in T2D, compared to normal weight.

Two studies from a longitudinal research regarding The Cardiovascular Risk in Young Finns Study showed significant findings with respect to oxHDL in adults.
Kaikkonen et al. (2016) determined levels of conjugated dienes in isolated HDL (oxHDL lipids) and LDL (oxLDL lipids) and their associations with future fatty liver in middle-aged participants with normal liver (n=1286) and fatty liver (n=288). Subjects with elevated oxLDL lipids (odds ratio (OR)=1.27 for 1-SD change in oxLDL lipids, p=0.011), and with high oxidation score (oxLDL lipids + oxLDL protein, OR=1.34, p=0.012), had an elevated risk for fatty liver. The strongest direct association was seen with a high oxLDL lipids/oxHDL lipids ratio (OR=1.49, p=0.001).\textsuperscript{98} Likewise, in the same cohort, the associations of oxHDL lipids with atherosclerosis risk factors in 1395 Finnish adults aged 24–39 y (54.9% women) suggested that advanced age was associated with lower oxHDL lipids levels. In men, lower oxHDL lipids were associated with elevated insulin levels, and in women with higher waist circumference and smoking. By contrast, higher CRP concentrations and alcohol intake were associated with higher oxHDL lipid levels.\textsuperscript{110}

OxHDL levels were also analyzed in human obese adult females with metabolic syndrome grouped by BMI (17–25 and 30–40 kg/m\textsuperscript{2}), without overt CVD. Participants with a BMI greater than 30 kg/m\textsuperscript{2} displayed higher levels of oxHDL and isoprostane (p<0.05), compared to BMI lower than 25 kg/m\textsuperscript{2}. Higher levels of oxHDL were associated with greater concentrations of angiotensin II and the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) (p<0.05). Authors concluded that oxHDL represented a unique biomarker profile in obese females with metabolic syndrome at risk for developing CVD.\textsuperscript{97}
As it can be observed in the aforementioned cross-sectional studies, research has shown a trend with higher levels of oxidized lipoproteins in overweight and obesity compared to normal weight, but also some studies have shown no significant differences between groups. Therefore, no consensus exists yet. Controversy in findings arises when chronic conditions are taken into account, such as prediabetes and T2D. Therefore, more research needs to be done to determine the relationships between lipoproteins and oxidative stress in metabolic conditions at early stages.

2.9 Preventing Oxidative Stress through Diet and Physical Activity

The antioxidant defense system of an individual, which consists of the endogenous antioxidant enzymes and dietary antioxidants, can compensate oxidative stress. Numerous studies support the hypothesis that exercise-training and dietary intake of antioxidants alleviate oxidative stress.\textsuperscript{111,112,239} With respect to the endogenous enzymatic component enhanced by exercise, effects of interventions have been associated with a significant reduction in pro-oxidant parameters and an increase in enzymatic antioxidant capacity, regardless of intensity, volume, type of exercise, and studied population.\textsuperscript{125,126} Nevertheless, moderate to high-intensity exercise has proven to stimulate the endogenous antioxidant defense systems.\textsuperscript{127,128}

Regarding dietary antioxidant nutrients, diets rich in fruit and vegetables, nuts, legumes, and whole grains, have reported exerting beneficial effects reducing CVD risk.\textsuperscript{240-244} Bioactive compounds such as soluble fiber, vitamins (vitamin A, vitamin E,
and vitamin C), phytochemicals (carotenoids, polyphenols, etc.), and minerals found in these foods act as antioxidants. The most likely proposed mechanism by which dietary antioxidant components reduce CVD has been decreasing ROS and their oxidative by-products. The antioxidants found in fruit and vegetables are associated with the scavenging of free radicals related to atherosclerosis, and with decreasing LDL susceptibility to oxidation. Furthermore, lipid-soluble vitamins and mono- and poly-unsaturated fatty acids in grains, as well as protein, phytosterols, soluble and insoluble fibers in legumes, are all related to lowering-cholesterol effects, thus reducing CVD risk. With respect to whole grains, besides the antioxidant effects of its bioactive components, its reduced glycemic response has been proposed to decrease ROS generation. All these dietary antioxidants are especially important in at-risk population like obese and diabetics, in who oxidative stress is more likely to occur and antioxidant mechanisms are diminished. Multiple dietary antioxidants have been used for diabetics to ameliorate CVD complications. Compounds such as α-tocopherol, α-lipoate, and ascorbic acid supplementation have shown to be beneficial.

2.10 Behavioral Interventions Intended to Decrease Oxidative Stress

2.10.1 Exercise Interventions

Although acute exercise induces an acute increase in ROS, exercise is one of the tested strategies that suggest long-term improvements of the endogenous enzymatic antioxidant capacity. In children and adolescents as well as adults, chronic exercise
induces beneficial adaptations of the endogenous enzymatic antioxidant defense. Several studies in youth have shown positive effects on endogenous enzymatic activity, most of them exclusively applying aerobic and endurance exercise. With respect to regularly exercised population, oxidative stress levels after 23 weeks of swimming were evaluated in boys and girls of 10-11 y old, in which increased levels of reduced glutathione/oxidized glutathione ratio (GSH/GSSG) were observed. Similarly, long-term exercise in team handball enhanced superoxide dismutase (SOD) activity; while aerobic capacity was positively associated with catalase activity, and negatively associated with hydroperoxide levels in participants of 16-19 y old.

Considering overweight/obese children, a randomized trial with 112 participants 7-11 y old compared to a no-exercise control group tested two different doses of exercise. Although reduced fatness and improved fitness were observed after the intervention, no effect of exercise was found on 8-isoprostane levels as a biomarker of oxidative stress. Similar findings were observed by Kelly et al. (2007), who studied 19 overweight children randomly assigned to an aerobic exercise training or sedentary control group for 8 weeks, and found no change in 8-isoprostane levels.

With respect to oxLDL in exercise interventions, in a study carried out by Youssef et al. (2015), aerobic training for 12 weeks suppressed exercise-induced lipid peroxidation and inflammation in overweight/obese adolescent girls of 14-19 y old, compared to non-trained overweight/obese and the normal weight trained group. In contrast, Tjønna et al. (2009) studied two groups of overweight adolescents of 14.0±0.3 y randomized to either aerobic interval training or a multi-treatment approach. Authors found no significant changes in oxLDL in any group after 3 months of intervention.
2.10.2 Dietary and Supplement Interventions

Regarding dietary antioxidants as a way to reduce oxidative stress in youth, studies have tested antioxidant-rich foods, supplementation, and caloric restriction. Among interventions using foods rich in antioxidants, the effect of 500 mL/day of pure (100%) mandarin juice (Citrus clementine) on biomarkers of oxidative stress in normal weight hypercholesterolemic children (8-12 y old) was tested during 28 days. After the intervention, plasma antioxidants vitamin E and C and intra-erythrocyte glutathione levels were significantly increased. Although antibodies to oxLDL remained unchanged, levels of MDA and carbonyl groups were significantly decreased, suggesting reductions in oxidative stress. Furthermore, the response to a commercial antioxidant supplement (a gummy-type candy) made from dried fruit, vegetable extracts, and added antioxidants, consumed twice a day for 21 days, was tested in healthy children aged 5-10 y, compared to placebo. Levels of breath pentane and urine 8-hydroxydeoxyguanosine, MDA, nitrites, and 8-isoprostane were not significantly different between groups, neither baseline or after intervention.

With respect to supplementation, zinc was offered in a placebo-controlled crossover trial among 60 obese Iranian children (aged 6–10 y old). Participants were randomized into one of two groups; one group received 20 mg of elemental zinc and the other group received placebo, on a regular daily basis for 8 weeks. After a 4-week washout period, the groups were crossed over. In both groups and irrespective of the order of receiving zinc and placebo, significant decreases were found for Apo B/ApoA-I
ratio, oxLDL, leptin and MDA, total and LDL-c after receiving zinc, without significant change after receiving placebo.  

In regard to pediatric nonalcoholic fatty liver disease, a dietary intervention intended to decrease oxidative stress was evaluated using low-fructose diet versus low-fat diet in 10 children with NFLD (13.3±0.65 y) randomized to a 6-month pilot study. After the intervention, oxLDL was significantly decreased in the low-fructose group, but there was no change in the low-fat group.  

With respect to dietary caloric restriction, research with overweight/obese participants aged 7 to 15 y old aimed to analyze the impact of a 10-week diet-restricted intervention on oxidative status after weight loss (WL). After the intervention, subjects were dichotomized at the median of BMI-SDS change, as high (HR) and low responders (LR). Baseline serum TAC values in HR subjects did significantly predict the reduction in urinary F2 isoprostane after the program. Notably, changes in dietary TAC were associated with a decrease in body weight after the intervention in the HR group. In another study carried out by the same research group, obese children with mean age of 11 years followed a 10-week weight loss program, in which they were also dichotomized as HR and LR after the intervention. The intervention consisted of moderate energy-restricted diet, nutritional education, and family involvement. After the intervention, oxLDL significantly decreased in the HR group, and a positive correlation between changes in oxLDL and BMI-SDS was found. Similarly, in severely obese prepubertal children with mean age of 9.2±1.5 y compared to healthy normal weight subjects, a 6-mo dietary restriction-weight loss program normalized oxidant status, with significant
increases in lag phase and decreases in MDA. However, oxidative stress biomarkers returned to baseline levels after another 6 months of free diet.\textsuperscript{114}

\subsection*{2.10.3 Lifestyle-modification Interventions}

Lifestyle interventions intended to diminish oxidative stress generally include the synergistic effects of exercise training and dietary modification. Roberts et al. (2007) examined overweight children (age 8–17) before and after a lifestyle modification intervention that consisted on a high-fiber, low-fat diet provided ad libitum and daily exercise (2–2.5 h) in a 2-week residential program. Significant reductions were noted in 8-isoprostane, MPO, markers of endothelial dysfunction (sICAM-1, sE-selectin, CRP, MMP-9, and cellular MCP-1), as well as in superoxide and hydrogen peroxide in cultured, serum-stimulated human aortic endothelial cells.\textsuperscript{131} Likewise in a study performed by Li (2017), the effect of an exercise and dietary restriction intervention on oxidative stress was evaluated after 4 weeks in obese male adolescents aged 15.5±2.1 y old. The activities of the antioxidant SOD and glutathione peroxidase (GPx) enzymes were significantly increased, and protein carbonyls concentrations (PC) were reduced.\textsuperscript{132}

Additionally, Kelishadi et al. (2008) applied a lifestyle intervention regarding aerobic PA with moderate to vigorous intensity 3 days/week and dietary advice for 6 weeks, in obese children aged 12-18 y. After the intervention, oxidative stress biomarkers such as oxLDL and MDA significantly decreased and showed an inverse association with changes in mean FMD and a direct association with changes in mean C-IMT.\textsuperscript{119}

Furthermore, in a pilot study our research group reported significantly decreased concentrations of oxLDL and sE-selectin, compared to baseline, in 15 obese Latino
adolescents with a mean age of 15.0±1.0 y after a 12-week community-based lifestyle intervention focused on healthy diet and three 60-min sessions per week of PA.\textsuperscript{120}

Similarly, lifestyle interventions have been applied using additional supplementation with antioxidants. A lifestyle modification program by Murer et al. (2014) with diet and exercise included daily antioxidants (vitamin E, 400 IU; vitamin C, 500 mg; selenium, 50 mg) in overweight/obese children and adolescents (age 12.76 ±1.5 y) in a 4-month fashion, where participants were randomly assigned to supplementation or placebo. There was a significant treatment effect of supplementation on antioxidant status measured by α-tocopherol, ascorbic acid, selenium, and oxidative stress measured by 8-isoprostane.\textsuperscript{115} Moreover, an intervention by D’Adamo et al. (2013) in obese prepubertal children aged 8.3±1.6 y with NAFLD has proven to be effective decreasing isoprostane as a biomarker of oxidative stress, as well as other cardiometabolic markers. The program consisted of a 6-month lifestyle intervention combined with vitamin E supplementation (600 mg/day), compared to age and sex-matched obese peers who underwent lifestyle intervention only.\textsuperscript{116}

Changes in oxidative stress biomarkers have also been analyzed after other types of interventions, like bariatric surgery. In extreme obese adolescents with age lower than 19 years who underwent Roux-en-Y gastric bypass (RYGB) or the vertical sleeve gastrectomy (VSG), Kelly et al. (2016) reported significant reductions after 12 months for interleukin-6 (IL-6), oxLDL and leptin, whereas adiponectin was significantly increased. Changes in these biomarkers did not differ by type of surgery.\textsuperscript{121}
Interventions focused on HDL dysfunction are limited. Regarding adults, the effects of a lifestyle intervention on the inflammatory/anti-inflammatory properties of HDL were examined in obese men (n = 22). Participants were allocated to a 3-week residential program and provided with a high-fiber, low-fat diet ad libitum, and daily aerobic exercise. Lipid hydroperoxides and the HDL inflammatory/anti-inflammatory indexes were analyzed pre- and post-intervention, The HDL indexes were defined as the ability of serum-HDL to alter LDL-induced monocyte chemotactic activity (MCA) in a human artery wall co-culture. Findings showed significant decreases in lipid hydroperoxides, as well as in the HDL inflammatory index, with significant increases in HDL-anti-inflammatory index. Conversely, the effect of aerobic exercise for 6 months on lipid peroxide transport function of HDL was analyzed in a randomized controlled trial with sedentary women aged 43–63 y, compared to a control group. Authors reported that levels of oxHDL significantly increased 5% in the exercise group and decreased 2% in the control group. Additionally, the ratio of oxHDL/HDL-c increased by 5% in the exercise group and decreased by 1.5% in the control group, while CETP and adiponectin concentrations remained unchanged.

In the pediatric population, two interventions are documented with HDL function as an outcome, while up to our knowledge no intervention has analyzed oxHDL levels. Wesnigk et al. (2016) reported the impact of a lifestyle intervention on HDL-mediated eNOS activation and HDL-reverse cholesterol transport in obese adolescents of 15±1 y old, who were randomized to a 10-month intervention group (restricted diet and exercise) or to a usual care group (UC). Anti-atherosclerotic HDL function and endothelial function improved, related to an increase in HDL-mediated eNOS-Ser1177
phosphorylation and to significant improvements in reverse cholesterol transport.\textsuperscript{134} Besides, adolescent males with severe obesity and mean age 17.4±1.6 y were studied at baseline and 1 year after VSG bariatric surgery, compared to a lean group. The hypothesis was that atherogenic HDL profile (HDL subspecies and HDL function) would improve with metabolic surgery. Results indicated a reduction of 30% in HDL lipid peroxidation potential, significant increases in large apoE-rich HDL subspecies, 12% increased cholesterol efflux capacity, and 25% increased HDL anti-oxidative capacity.\textsuperscript{122}

Findings respect to the effect of lifestyle interventions on youth regarding oxLDL and oxHDL are not conclusive. Most of the research has been done in overweight/obese population, but the effects of interventions on other metabolically ill population like prediabetics have not been analyzed. Moreover, no intervention has been done considering both oxLDL and oxHDL lipoproteins, which are specific early biomarkers of oxidative stress, as well as of atherosclerosis. This gap between the effects of interventions on oxidized lipoproteins and its application on young population at high-risk needs to be reduced in order to prevent CVD at early stages.
CHAPTER 3. RESEARCH DESIGN AND METHODS

3.1 Parent Study: Every Little Step Counts

This study is a secondary analysis from a recently completed randomized controlled trial (RCT) designed for preventing diabetes in youth: The Every Little Step Counts Study (ELSC). This parent study was a T2D prevention intervention for obese Latino adolescents, with the theoretical model in Figure 1 guiding the study. The ELSC study comprised 160 obese Latino adolescents aged 14–16 y to test the efficacy of a culturally-grounded, community-based lifestyle education intervention to improve insulin sensitivity and weight-specific quality of life in the short-term (3-month) and long-term (12-month). All participants (intervention and control) underwent testing at baseline, 3-months (post-intervention), 6-months (post-booster), and 12-months (9-months post-intervention). Control youth were offered a modified version of the intervention upon completion.

Briefly, adolescents at baseline were randomized to either the intensive lifestyle intervention or a control group. The intervention group consisted of one session weekly of nutrition education and three sessions weekly of exercise, during 12 weeks (3 months from baseline). During this period, the control group received health information materials and monthly phone calls, emails, or texts, according to their preference. The first follow-up was 3 months after the end of the intervention (6 months from baseline), in which the intervention group completed three monthly booster sessions following the intensive intervention period. These sessions were designed to support ongoing healthy lifestyle behaviors and address any challenges encountered in maintaining a healthy
lifestyle. During this first follow-up the control group continued having monthly phone calls, emails, and texts. The second follow-up was 9 months after the end of the intervention (12 months from baseline), in which both the intervention group and the control group had the same monthly phone calls, emails, or texts, to maintain contact, encourage participation, and avoid attrition during this period. The study design for the parent study is shown in Figure 2.

The Institutional Review Board at Arizona State University approved the study protocol and all study-related documents (Appendix A). The study was registered at www.clinicaltrials.gov (Clinicaltrials.gov Identifier: NCT01236794). Written parental consent (Appendix B) and child assent (Appendix C) were obtained by study staff prior to any data collection procedures.

Figure 1. Theoretical model of the ELSC parent study.272
3.2 Recruitment

Participants were recruited through health organizations and centers partnering with ASU. Other organizations with the potential to recruit participants were community centers, churches, markets, etc., with predominantly Latino attendees. Study staff posted flyers including contact information from ASU, and also advertised in magazines, newspapers, and Internet media targeting the Latino population. Recruitment materials were in English and Spanish (Appendix D).

Youth and parents/guardians interested in the study contacted study staff and were informed about the intervention. Kids were asked to have an initial meeting for evaluation of eligibility, recruitment, and screening at the ASU Nutrition Laboratory.
During this initial meeting, participants were again informed about the lifestyle intervention, procedures, potential risks and benefits, and about the fact that they were free to withdraw at any time from the study. They were also informed that non-participation would not affect them in any way and that confidentiality would be maintained throughout the study using an ID number in each material and test to identify each participant. After being informed about the entire study protocol, participants and parents willing to participate in the study provided written informed consent and were subjected to the screening evaluation. The evaluation was in fasting conditions (minimum of 10 hours) and participants were asked not to modify their regular lifestyle behaviors.

3.3 Eligibility and Screening

Inclusion criteria were: a) age 14-16 y, b) being obese, measured through BMI percentile greater or equal to 95th for age and gender according to CDC growth charts, or BMI greater or equal to 30 kg/m², and c) from Latino origin (parent-reported). For the secondary analysis in this dissertation, an extra inclusion criterion was: d) having increased risk for diabetes using an expanded definition of prediabetes according to the American Diabetes Association (ADA) criteria of either fasting glucose levels greater or equal to 100 mg/dL and/or a expanded definition of 2 hour glucose levels greater or equal to 120 mg/dL after a 75 g dose of an oral glucose tolerance test (OGTT). This last inclusion criterion ensured that any participant with prediabetes during the initial screening session was automatically assigned to the intervention group. This was justified given the rapid progression of T2D in youth with prediabetes and the ethical implications of randomizing a prediabetic kid to a control group.
The exclusion criteria were: a) chronic or acute metabolic diseases, b) prior or current pregnancy, c) use of any medication that could influence carbohydrate metabolism, d) diagnosis of T1DM or T2DM, and e) participation in any other research program or activities related with health-improvements within previous 6 months. Participants detected with any health-related condition through the recruitment stage were referred to our partner clinic or their family doctor.

3.4 Study Design

Youths with prediabetes at the parent study were not randomized and were assigned to the lifestyle intervention, being the study participants for this dissertation. Thus, this secondary data analysis followed a quasi-experimental design with pre- (baseline) and post-intervention (3-months) measurements.

3.5 Sample Size and Effect Size

A convenience sample from the parent study was used in the secondary analysis to include only kids with prediabetes. The total sample size that made up the current secondary analysis was 35 participants. With this sample size and based on oxLDL as outcome for one sample t-test using a significance level of 0.05, with power =0.95, the calculated effect size was d=0.5675, which is considered a medium effect.

3.6 Lifestyle and T2D-prevention Intervention

The 12-week lifestyle intervention was held at the YMCA as a partner institution. The lifestyle program curriculum was a culturally-grounded, community-based
intervention with community input into its development, design and implementation, and using “real-world” strategies for reaching vulnerable and underserved youth.\textsuperscript{272} The intervention consisted of the encouragement of social support and self-efficacy constructs within the Social Cognitive Theory (SCT).\textsuperscript{273} This theory considers that individual, social and environmental factors interact in a reciprocal manner to influence behavior. SCT constructs such as increasing self-efficacy, observational learning, goal setting, self-monitoring, and fostering social support, were integrated into each intervention session.

Moreover, the overall program was based on an Expanded Eco-developmental model (Figure 3) and included multiple eco-developmental levels that influence individual health behaviors and health outcomes during the critical life period of adolescence. The levels of influence included in the intervention were social (family and friends), community, culture, and policy level factors.\textsuperscript{272}

The focus of the intervention was on obesity- and T2D-risk factors and health complications, healthy eating, family roles and responsibilities, PA and sedentary behaviors, and emotional well-being. The intervention included nutrition education and PA modules during 12 weeks and facilitators were partners from St. Vincent de Paul (nutrition module) and YMCA (PA module). Nutrition education consisted of one weekly session (60 min/session) delivered in a group setting by bilingual/bicultural certified “promotoras” (community health workers) from St. Vincent de Paul. The nutrition education sessions required the mandatory participation of at least one parent (or an adult relative) per kid. This module initiated with a discussion about their baseline lipid panel and glucose measurements found during screening. In the next sessions, healthy lifestyle choices were informed and behavioral strategies were implemented to improve health and
reducing T2D risk. Among topics were health awareness, roles and responsibilities, benefits of PA and exercise, reducing calories from sugar and fat, nutrition fact labels, the plate method, benefits from breakfast, reducing fast foods, and promoting healthy snacks. Participants practiced behavior change strategies such as goal setting, self-monitoring, decision-making, and positive self-imaging, which were strategies established in the curriculum (Table 1).

![Figure 3. Conceptual framework of the ELSC parent study.](image)

The PA module consisted of three sessions per week (60 min/session) and included individual and group activities concerning structured aerobic and resistance exercise, and unstructured games. The focus of this module was on motor skill acquisition, exercise confidence, and developing a fitness base. A certified instructor
from YMCA conducted the PA sessions. Aerobic activities included running, spinning and cardio kickboxing, among others. Resistance activities included circuit training using age and size appropriate equipment. Some other activities were team sports, games, and activities promoting social support among youth. Heart rate monitoring and rate of perceived exertion were used to monitor and document exercise intensity throughout the program. Heart rate was monitored on a weekly basis with a target of 150 beats per minute. Participants were asked to complete at least 75% attendance from the 12-week lifestyle intervention.

3.7 Outcome Measurements

Outcomes were measured at baseline and immediately after the intervention completion. Parents were asked to complete a family history questionnaire. Fasting blood samples were collected in order to measure lipid panel and biomarkers of oxidative stress. An oral glucose tolerance test (OGTT) was conducted to evaluate glucose and insulin concentrations, as well a HOMA-IR. Dietary intake of fresh F&V through a FFQ, as well as estimated VO₂ peak by a cardiorespiratory fitness test (CRF) were measured as behavior variables.

3.7.1 Family History Questionnaire

Parents were asked to complete a family history questionnaire (Appendix E).²⁷⁷ Outcomes obtained from this were age, sex, country of family origin, country of birth, and family history of T2D, which were used to describe the sample.
Table 1. Overview of lifestyle education sessions in the ELSC parent study.

<table>
<thead>
<tr>
<th>Session 1</th>
<th>Introduction</th>
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<tbody>
<tr>
<td>Session 2</td>
<td>Understanding your health status</td>
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<tr>
<td>Session 3</td>
<td>Healthy meal planning</td>
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<tr>
<td>Session 4</td>
<td>Reducing sugar and fat intake</td>
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<td>Session 5</td>
<td>Increasing fiber intake</td>
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<td>Session 6</td>
<td>Eating breakfast</td>
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<td>Session 7</td>
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<td>Session 9</td>
<td>Importance of physical activity</td>
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<td>Session 10</td>
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<tr>
<td>Session 11</td>
<td>Enhancing self-esteem</td>
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<tr>
<td>Session 12</td>
<td>Sustaining a healthy and balanced lifestyle</td>
</tr>
</tbody>
</table>

3.7.2 Food Frequency Questionnaire

Dietary intake of fresh fruit and vegetables (F&V) was assessed using the Block Food Screener for Ages 2-17y version 2007 (Appendix F). This 41-item food questionnaire was adapted from the Block Kids 2004 FFQ, an 80-item questionnaire designed to assess food and nutrient intake in children ages 2-17 years. The food list for this screener was created using data from the NHANES 2001-2002 and 2003-2004, identifying the most important sources, appropriate portion sizes and nutrient
composition of each of the following food groups: whole grains, fruits, vegetables, potatoes, dairy, protein foods (meat, poultry, fish and legumes), high fat foods, and sweetened foods with added sugar. This screener has been used to evaluate dietary intake of kids from diverse ages and ethnicities, although it appears more useful for adolescents. Recently, Hunsberger et al. examined the relative validity of this screener compared to three 24-h dietary recalls served as the reference, and no systematic difference between the two instruments were found for vegetable, dairy and meat/fish/poultry fat consumption. According to this validation study, this questionnaire is a useful dietary assessment instrument for the nutrients and food groups it was designed to assess in children age 10-17 years.

The current version of this screener asks about food eaten "last week" in individual portion sizes (servings/day), with servings computed to g/d. The intake of fresh F&V as a composite variable was the main outcome, although individual foods were also reported. This screener took about 10-12 minutes to be self-completed by the participants.

3.7.3 Anthropometry and Blood Pressure

Anthropometrics and sitting blood pressure were measured at baseline and after the intervention. Weight was recorded to the nearest 0.1 kg using a balance beam medical scale, and height and waist to the nearest 0.1 cm using a stadiometer and a tape measure. BMI was calculated as weight divided by height squared (kg/m²), and BMI percentiles were calculated based upon the Centers for Disease Control and Prevention 2000 growth charts (CDC, Atlanta, GA., USA). Sitting blood pressure was measured using an
appropriately-sized cuff on the right arm after the participant had rested quietly for 5 min using an Omron IntelliSense HEM-907XL automated blood pressure monitor (Omron Healthcare, Inc., Bannockburn, IL). All measurements were done in triplicate and averaged. Body composition was estimated by bioelectrical impedance analysis using a Tanita Scale (TBF-300A, Tanita Corp of America, Arlington Heights, Illinois).

3.7.4 Cardiorespiratory Fitness Test

Participants completed a single stage treadmill walking test, a submaximal aerobic fitness test, that estimated VO$_2$max. Summarizing, resting heart rate (HR) from the participant was recorded and after been familiarized with equipment, the participant warmed-up on the treadmill selecting a comfortable speed at 0% grade that brings the HR to 50-70% of the HR max (recommended walking speed is from 3.4 to 4 mph). At the 4-min marker, the grade was increased by 5%, without changing the speed, and participant walked another 4 min. The steady-state HR (SS HR) was recorded from the average of the final 30 sec of the last two minutes at the 5% grade. Finally, the participant was asked to slow the speed and decrease the grade for 1-2 min cooldown (HR below 100 bpm). VO$_2$max was calculated by the Ebbeling equation:

\[
\text{VO}_2\text{max (ml·kg}^{-1}·\text{min}^{-1}) = 15.1 +21.8 \text{ (speed in mph)} - 0.327 \text{ (SS HR in bpm)} - 0.263 \text{ (speed x age in years)} + 0.00504 \text{ (SS HR in bpm x age in years)} + 5.98 \text{ (gender; female = 0, male = 1)}.
\]
3.7.5 Cardiometabolic Risk Biomarkers: Lipid Panel and OGGT

Fasting-state blood samples (with at least 10 h overnight fast) were drawn at baseline and after intervention (within 24-48 hours of the last intervention session) from the antecubital vein to be collected into evacuated tubes as follows: one serum separating tube (10 mL), one EDTA-containing tube (4 mL), and one EDTA and glycolysis inhibitor (potassium oxalate and sodium fluoride) tube (4 mL). Blood was centrifuged at 3000 rpm at 5°C for 15 minutes, and serum/plasma was separated, aliquoted, and stored at -70°C for future analysis. The serum lipid panel was conducted by Sonora Quest Laboratory (Phoenix, AZ) and included total cholesterol, HDL-c, triglycerides, LDL-c (calculated), and non-HDL cholesterol (calculated). Total cholesterol and triglycerides were measured by photometric and HDL-c by homogenous enzyme immunoassay, using the corresponding colorimetric enzymatic reagents. Non-HDL-c was calculated as total cholesterol (TC) minus HDL-c. LDL-c was calculated using the Friedewald equation. VLDL-c was estimated dividing the triglyceride value (mg/dL) by 5, according to Wilson et al.

An OGGT was conducted in accordance with ADA. Summarizing, blood samples were drawn in potassium oxalate and sodium fluoride EDTA tubes (4 mL) for glucose measurements and in EDTA tubes (4 mL) for insulin measurements at fasting conditions (-15 and -5 minutes before the dextrose solution, averaged). After that, participants consumed the dextrose solution (1.75 g/kg, with a maximum of 75 g) and venous samples were drawn 120 min after, to measure 2hour glucose and 2hour insulin. Fasting plasma glucose (FPG) and 2hour glucose were analyzed by the glucose oxidase method using the automated chemistry analyzer Cobas C111 (Roche Diagnostics,
Indianapolis, IN). Fasting insulin and 2hour insulin were measured by ELISA (ALPCO Diagnostics, Windham, NH).

Homeostasis model assessment of insulin resistance (HOMA-IR)\textsuperscript{291} was calculated using the following equation:

\[
\text{HOMA} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (\(\mu U/mL\))} / 22.
\]

3.7.6 Oxidative Stress Biomarkers: oxLDL and oxHDL

Oxidative stress biomarkers were measured in serum at baseline and after intervention using commercially available ELISA kits through sandwich enzyme immunoassay techniques. OxLDL was measured through a kit from Mercodia AB (Uppsala, Sweden) with the same specific murine monoclonal antibody mAb-4E6 as in the assay described by Holvoet et al.\textsuperscript{292} Mercodia Oxidized LDL ELISA is a solid phase two-site enzyme immunoassay that is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. OxHDL was measured with an antibody specific for oxHDL with a kit from MyBioSource, Inc. (San Diego, CA.). This assay employs the quantitative sandwich enzyme immunoassay technique using antibodies specific for Ox-HDL. The corresponding procedures were conducted according to each kit.

3.8 Statistical Analyses

Untransformed outcome variables were expressed as mean (standard deviation, SD). For skewed variables, inverse or natural log transformations were calculated to meet the normality assumption. Variables that did not meet this assumption after
transformation (BMI percentile and dietary intake of apples, bananas and oranges) were analyzed by non-parametric Wilcoxon test. Changes in outcome measures for each participant were calculated subtracting baseline (pre) values from post values (post – pre), divided by baseline, and expressed as a percent. Changes were reported as mean (SD).

For Specific Aims 1 and 2, differences between pre- and post-intervention outcomes were evaluated using Paired samples t-tests for unadjusted models. For Specific Aim 3, within-subjects correlations were examined between changes in oxidative stress biomarkers and changes in fresh F&V dietary intake, after adjustment for changes in cardiorespiratory fitness. For this latter statistical analysis, the linear mixed models with maximum likelihood estimates were used to compute the correlation matrix and within-subject correlation coefficients, similar to partial correlation estimated by Bland and Altman (1995). All statistical analyses were performed using a p-value lower than 0.05 (p<0.05) as significant, with SPSS 22.0 (IBM, Armonk, NY, USA) and SAS 9.4 (SAS Institute, Cary, NC) softwares.
CHAPTER 4. RESULTS

4.1 Socio-demographic Characteristics

The sociodemographic characteristics of the study participants at baseline are shown in Table 2. A total of 35 Latino adolescents, mean age 15.5 (1.0) years and 51.4% male, participated in this intervention. A majority of participants identified themselves as Mexican (88.6%), and 77.1% were born in the U.S. Regarding family history of diabetes, 43% had either mother and/or father history of T2D, and none of the participants had a diabetic sibling.

4.2 Anthropometric Characteristics

Participant anthropometric and physiologic characteristics at baseline and after the intervention are shown in Table 3. By design, BMI was greater than the 95th percentile at baseline, with a mean BMI percentile of 98.5 (1.2) and BMI of 35.0 (4.9) kg/m². After the intervention, most anthropometric variables significantly decreased, such as weight (-1.3% change, p=0.042), BMI and BMI percentile (-2.2% and -0.4% change, p=0.001, respectively), body fat (-6.6% change, p=0.025), and waist circumference (-1.8% change, p=0.025). Conversely, height (0.5% change, p<0.001) and VO₂ max (7.0% change, p<0.001) significantly increased after the intervention. Regarding VO₂ max, 29 out of 32 participants who completed both baseline and after intervention tests increased their VO₂ max (90%). Systolic and diastolic blood pressure were comparable without significant change after the intervention (p>0.05).
<table>
<thead>
<tr>
<th>Variable</th>
<th>All participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>15.5 (1.0)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (51.4)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (48.6)</td>
</tr>
<tr>
<td>Country of family origin, n (%)</td>
<td></td>
</tr>
<tr>
<td>Mexican</td>
<td>31 (88.6)</td>
</tr>
<tr>
<td>Central American</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td>South American</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Born in U.S., n (%)</td>
<td>27 (77.1)</td>
</tr>
<tr>
<td>Family history of T2DM, n (%)</td>
<td>15 (43)</td>
</tr>
</tbody>
</table>
Table 3. Anthropometric and physiologic characteristics at baseline and post-intervention.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Post</th>
<th>% Change</th>
<th>p value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>97.4 (16.7)</td>
<td>96.2 (17.3)</td>
<td>-1.3 (3.3)</td>
<td>0.042</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.6 (8.2)</td>
<td>167.4 (8.6)</td>
<td>0.5 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>35.0 (4.9)</td>
<td>34.3 (4.9)</td>
<td>-2.2 (2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>98.5 (1.2)</td>
<td>98.1 (1.9)</td>
<td>-0.4 (0.8)</td>
<td>0.001\textsuperscript{c}</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>46.0 (7.9)</td>
<td>42.8 (6.8)</td>
<td>-6.6 (8.8)</td>
<td>0.025</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.2 (10.4)</td>
<td>108.1 (10.9)</td>
<td>-1.8 (4.6)</td>
<td>0.025</td>
</tr>
<tr>
<td>VO\textsubscript{2} max\textsuperscript{d}</td>
<td>29.7 (5.0)</td>
<td>31.6 (4.7)</td>
<td>7.0 (8.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>127.1 (16.5)</td>
<td>126.1 (10.9)</td>
<td>0.3 (11.5)</td>
<td>0.696</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73.7 (9.3)</td>
<td>72.2 (7.0)</td>
<td>-1.3 (9.4)</td>
<td>0.204</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data are shown as raw data, mean (SD).

\textsuperscript{b} Two-sided paired samples t-test.

\textsuperscript{c} Non-parametric Wilcoxon test.

\textsuperscript{d} n=32
4.3 Cardiometabolic Risk Biomarkers: Lipid Panel and OGTT Measurements

CVD risk biomarkers at baseline and after intervention are shown in Table 4. At baseline participants had fasting glucose levels of 85.6 (7.9) mg/dL, and per study design participants had prediabetes as measured by 2-hour glucose levels after the OGTT (141.2 (12.2) mg/dL). After the intervention mean changes for fasting and 2-hour glucose levels were -0.3% (p=0.655) and -19.3% (p<0.001), respectively. Fasting insulin, 2-hour insulin and HOMA-IR were significantly reduced by -12.9% (p=0.008), -53.5% (p<0.001), and -12.5% (p=0.015), respectively. After the intervention, 23 participants (66%) reverted the IGT status toward normal glucose tolerance (NGT) according to the criteria of the Standards of Medical Care in Diabetes (2017) by the ADA. Concerning serum lipids, most variables were decreased after the intervention. Significant changes were found in triglycerides (-10.2% change, p=0.032), total cholesterol (-5.4% change, p=0.002), VLDL-c (-10.4% change, p=0.029), HDL-c (-3.2% change, p=0.022), and Non-HDL (-5.5% change, p=0.0007). Although LDL-c also decreased after the intervention, it did not show statistical significance (-2.5% change, p=0.119).

According to the criteria of the Expert Panel on Integrated Guidelines for CVD in Children and Adolescents, mean triglycerides at baseline were considered high (151.2 mg/dL), and the levels after intervention (129.3 mg/dL) reached borderline category of 90-129 mg/dL. Total cholesterol both at baseline (155 mg/dL) and after intervention (145.8 mg/dL) were at acceptable levels of <170 mg/dL. In case of VLDL-c there are no guidelines for this lipoprotein, although this showed the greatest decreased according to the mean % of change (-10.4%, p=0.029). Regarding LDL-c, values at baseline and after intervention (85.5 and 82.3 mg/dL, respectively) were at the acceptable level of <110
mg/dL. In contrast, HDL-c at baseline was low (39.3 mg/dL) and even decreased after the intervention (37.7 mg/dL) keeping the low category of <40 mg/dL. Non-HDL levels both at baseline (115.7 mg/dL) and after intervention (108.2 mg/dL) were also at the acceptable level of < 120 mg/dL.

Table 4. Cardiometabolic risk biomarkers at baseline and post-intervention.\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Post</th>
<th>% Change</th>
<th>p value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>85.6 (7.9)</td>
<td>85.1 (7.2)</td>
<td>-0.3 (8.2)</td>
<td>0.655</td>
</tr>
<tr>
<td>2hour Glucose (mg/dL)</td>
<td>141.2 (12.2)</td>
<td>113.7 (30.5)</td>
<td>-19.3 (21.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting Insulin (µU/mL)</td>
<td>33.5 (40.3)</td>
<td>27.6 (35.1)</td>
<td>-12.9 (32.6)</td>
<td>0.008(^c)</td>
</tr>
<tr>
<td>2hour Insulin (µU/mL)</td>
<td>388.1 (164.9)</td>
<td>175.1 (139.6)</td>
<td>-53.5 (32.8)</td>
<td>&lt;0.001(^d)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>8.1 (9.9)</td>
<td>6.5 (8.3)</td>
<td>-12.5 (34.3)</td>
<td>0.015(^c)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>151.2 (74.1)</td>
<td>129.3 (68.2)</td>
<td>-10.2 (27.6)</td>
<td>0.032(^d)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>155.0 (32.6)</td>
<td>145.8 (29.6)</td>
<td>-5.4 (9.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>VLDL-c (mg/dL)</td>
<td>30.3 (14.8)</td>
<td>25.9 (13.6)</td>
<td>-10.4 (27.5)</td>
<td>0.029(^d)</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>85.5 (24.6)</td>
<td>82.3 (21.7)</td>
<td>-2.5 (12.4)</td>
<td>0.119</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>39.3 (7.9)</td>
<td>37.7 (6.9)</td>
<td>-3.2 (10.1)</td>
<td>0.022</td>
</tr>
<tr>
<td>Non-HDL (mg/dL)</td>
<td>115.7 (32.2)</td>
<td>108.2 (28.0)</td>
<td>-5.5 (11.7)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

\(^a\) Data are shown as raw data, mean (SD).

\(^b\) Two-sided paired samples t-test.

\(^c\) P value using inverse-transformed data.

\(^d\) P value using log-transformed data.
4.4 Oxidative Stress Biomarkers: Oxidized Lipoproteins

Biomarkers of oxidative stress are shown in Table 5. According to the proposed hypothesis in Specific Aim 1, mean oxLDL was significantly decreased after the intervention (51.0 (14.0) U/L at baseline, and 48.7 (12.8) U/L after the intervention), with -3.5% of mean change (p=0.022). Conversely, results from oxHDL were contrary to the proposed hypothesis in Specific Aim 2, since oxHDL did not decrease after the intervention (p=0.944). In fact, mean oxHDL increased (395.2 (94.6) ng/mL at baseline, and 416.1 (98.4) ng/mL after intervention), with mean change of 8.3%. A decrease in oxLDL was observed in 20 participants (57%), and contrary to the hypothesis in Specific Aim 2, an increase in oxHDL was observed in 20 participants (57%).

Table 5. Oxidative stress biomarkers at baseline and post-intervention.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Post</th>
<th>% Change</th>
<th>p value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>OxLDL (U/L)</td>
<td>51.0 (14.0)</td>
<td>48.7 (12.8)</td>
<td>-3.5 (11.5)</td>
<td>0.022\textsuperscript{c}</td>
</tr>
<tr>
<td>OxLDL/LDL-c</td>
<td>0.603 (.07)</td>
<td>0.599 (0.1)</td>
<td>-0.4 (11.1)</td>
<td>0.356</td>
</tr>
<tr>
<td>OxHDL (ng/mL)</td>
<td>395.2 (94.6)</td>
<td>416.1 (98.4)</td>
<td>8.3 (23.3)</td>
<td>0.944</td>
</tr>
<tr>
<td>OxHDL/HDL-c</td>
<td>10.5 (3.6)</td>
<td>11.5 (3.9)</td>
<td>13.3 (28.9)</td>
<td>0.009\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data are shown as raw data, mean (SD).

\textsuperscript{b} Left-sided paired samples t-test.

\textsuperscript{c} P value using log-transformed data.
4.5 Dietary Intake of Fresh Fruit and Vegetables

Mean dietary intake of individual foods constituting our main outcome of fresh F&V (g/d), are shown in Table 6. For all the individual foods, mean intakes were non-significantly increased after the intervention (all with p>0.05). Concerning our main outcome, mean dietary intake of fresh F&V were significantly increased (116.4 (97.0) g/d at baseline and 165.8 (91.0) g/d after the intervention, p=0.025), with 19 participants (54%) reporting increases after the intervention.

Table 6. Dietary intake of fresh fruit and vegetables at baseline and post-intervention.\(^a\)

<table>
<thead>
<tr>
<th>Food</th>
<th>Baseline</th>
<th>Post</th>
<th>% Change</th>
<th>(p) value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple, bananas, and oranges (g/d)</td>
<td>57.7 (59.8)</td>
<td>73.6 (58.1)</td>
<td>34.5 (108.6)</td>
<td>0.233(^c)</td>
</tr>
<tr>
<td>Any other fruit (g/d)</td>
<td>26.8 (32.1)</td>
<td>35.1 (38.1)</td>
<td>53.0 (215.7)</td>
<td>0.058(^d)</td>
</tr>
<tr>
<td>Lettuce salad (g/d)</td>
<td>14.4 (19.8)</td>
<td>25.1 (23.4)</td>
<td>37.3 (169.2)</td>
<td>0.481(^d)</td>
</tr>
<tr>
<td>Tomatoes (g/d)</td>
<td>3.3 (6.4)</td>
<td>6.2 (11.9)</td>
<td>104.2 (367.7)</td>
<td>0.426(^d)</td>
</tr>
<tr>
<td>Any other vegetable (g/d)</td>
<td>14.2 (18.2)</td>
<td>25.9 (22.6)</td>
<td>71.3 (204.9)</td>
<td>0.170(^d)</td>
</tr>
<tr>
<td>Total fresh F&amp;V (g/d)</td>
<td>116.4 (97.0)</td>
<td>165.8 (91.0)</td>
<td>186.9 (409.8)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

\(^a\) Data are shown as raw data, mean (SD).

\(^b\) Right-sided paired samples t-test.

\(^c\) Non-parametric Wilcoxon test.

\(^d\) \(P\) value using log-transformed data.
4.6 Correlations Between Oxidative Stress-related Biomarkers

The correlation coefficients with repeated observations were computed in within-subject designs, using changes in oxLDL and changes in oxHDL as dependent variables respectively. Each model used changes in fresh F&V intake as the dependent variable, and each was controlled for changes in VO$_2$max. Table 7 shows the R coefficient for each correlation model, it can be noted that correlations were weak and no significant, with R coefficients for the correlation between oxLDL and Fresh F&V intake of R= -0.15 (p=0.52), and for oxHDL and Fresh F&V intake of R= 0.22 (p=0.25).

Table 7. Within-subjects correlation coefficients between changes in oxidative stress biomarkers and changes in fruit and vegetable intake.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Covariate</th>
<th>Correlation coefficient (R)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized LDL</td>
<td>Fresh F&amp;V intake</td>
<td>VO$_2$max</td>
<td>-0.15</td>
<td>0.52</td>
</tr>
<tr>
<td>Oxidized HDL</td>
<td>Fresh F&amp;V intake</td>
<td>VO$_2$max</td>
<td>0.22</td>
<td>0.25</td>
</tr>
</tbody>
</table>
CHAPTER 5. DISCUSSION

Research regarding oxidative stress and CVD has been done in experimental models with cells, tissues, and animals.\(^68,156,190,222,296\) In cross-sectional studies the majority of information comes from the adult population.\(^85,91,297\) Efforts to reduce oxidative stress and CVD complications through dietary, supplement and/or exercise interventions have focused on adults\(^298-301\) and pediatric population such as overweight and obese.\(^113,114,119,120\) Few dietary and/or exercise interventions have been implemented among obese youth at high risk for CVD, such as children with liver steatosis,\(^116\) NAFLD,\(^302,303\) hypercholesterolemia,\(^123\) and metabolic syndrome,\(^304,305\) but children with prediabetes and or T2DM, up to current knowledge, have not been addressed. Practical lifestyle interventions that can be adapted and extended to daily life in prediabetic and T2D children, and focused on early oxidative stress biomarkers such as both oxLDL and oxHDL in a comprehensive manner and beyond current cardiometabolic biomarkers, up to current knowledge, have not been published yet.

Given the paucity of evidence regarding the effect of preventive interventions on lipoprotein oxidation in youth at high risk for cardiometabolic conditions, the present study was designed to evaluate the effects of a comprehensive T2D prevention intervention for obese prediabetic Latino adolescents on levels of oxidized lipoproteins as early biomarkers of oxidative stress. This is the first study specifically designed to test the effect of diet and PA modification on both oxLDL and oxHDL levels. Furthermore, this study adds to previous work by including measurements of behavioral variables such as changes in fresh F&V intake, as well as CRF (VO\(_2\)max), as potential correlates in the
decreases of oxidized lipoproteins. The present study will be compared to interventions focused on oxidative-stress biomarkers using lifestyle modification in children. Interventions using variables different than oxLDL and oxHDL will also be discussed, since interventions are mainly focused on metabolites related to the oxidative process itself, such as isoprostane, MDA, etc. Some studies consider metabolites more directly associated with the atherosclerotic process, such as oxLDL, and even fewer have analyzed oxHDL.

5.1 Anthropometric Outcomes and Oxidative Stress Biomarkers

Anthropometric variables are worth to report in interventions attempted to decrease cardiometabolic risk in children, even though their primary aim was not focused on anthropometry. It is important to analyze changes in CVD within the context of changes in anthropometry, to consider the contribution of the latter to the former. Moreover, evidence has been controversial showing CV health improvements when variables such as weight, waist circumference, BMI, and body fat are or are not decreased in obese children.\(^{306-308}\)

The present study showed significant reductions in weight, BMI, total body fat, and waist circumference after the intervention \((p<0.05)\), with significant decreases in oxLDL \((p=0.022)\). Several lifestyle interventions have reported similar findings that are in agreement with the current study regarding decreases in oxLDL. Kelishadi et al. (2008)\(^{119}\) evaluated the effect of a 6-week lifestyle program among obese children, age 12 to 18 y. This intervention was different in duration (6 weeks) than the present study, although the PA and dietary curriculum, as well as family participation, were similar. PA
sessions were 60 min, 3 days/week, and dietary advice was based on the optimized mixed diet, promoting the use of unrefined carbohydrate, dietary fiber from high-fiber whole grains, high intake of F&V, and restriction of hydrogenated fat. Similar to the current study, results from Kelishadi et al. showed that BMI, waist circumference, and percent body fat were significantly decreased (p<0.05), together with significant decreases in oxLDL (p=0.02).

In a study by Li et al. (2017), oxidative stress biomarkers were evaluated with exercise coupled with dietary restriction through a 4-week program in 20 male obese adolescents. After the program, weight, BMI, lean body mass, body fat mass and fat mass ratio were significantly decreased (all p<0.05). Significant decreases in protein carbonyls, total glutathione, and total nitrites were observed, while activities of the antioxidant enzymes superoxide dismutase SOD and glutathione peroxidase were significantly increased. Although the same oxidative indicators were not measured, the present intervention also resulted in significant decreases in oxLDL. The study performed by Li et al. differed from the present study in terms of both PA and diet. Li et al. tested exercise training three times/day and 6 days/week during 4 weeks, with diets formulated by a dietician. Conversely, the current intervention had a longer duration (12 weeks) and lower intensity, and diet modification consisted of nutrition education. Despite the difference in the characteristics of the intervention, both studies showed decreases in anthropometry and oxidative biomarkers.

Some studies with intentional weight loss as part of the design have been reported. In a study carried out by Mohn et al. (2005) with severely obese Caucasian children (n=18; mean age 9.2±1.54 y; BMI >2 SD), a dietary restriction-weight loss
program was investigated in comparison with healthy Caucasian subjects matched for sex, age, and pubertal stage. Oxidant status normalized after 6 months in the intervention group (p=0.003), with similar levels than the control normal weight group. Changes in oxidant status were accompanied by decreases in BMI, waist-to-hip ratio, and fat mass (all p<0.05), but returned to baseline levels together with fatness indexes after 6 months of free diet. Even though the intervention from Mohn et al.\textsuperscript{114} differed from the current intervention in terms of the provided personalized hypocaloric diet and duration (6 months), the main difference was the inclusion of a control group. In the present intervention it was ethically inappropriate to include a control group with prediabetic children since this condition is critical enough to automatically direct them to a preventive intervention.

In another study, Morell-Azanza et al. (2016)\textsuperscript{130} tested a 10-week weight loss program including a moderate energy-restricted diet, nutrition education, and family involvement in 40 obese children with mean age 11 y. Energy restriction varied among participants between 10\% and 40\% of total energy expenditure. After intervention participants were dichotomized at the median of BMI-SDS change as high (HR) and low responders (LR). OxLDL significantly decreased only in the HR group (p=0.028), however weight, the percentage of fat mass, waist circumference, and waist-to-hip ratio significantly decreased in both groups (p<0.05). By contrast, mean significant decreases in anthropometric outcomes and oxLDL were observed during the present intervention, without the need to divide the sample by the degree of BMI reduction.
Together, findings from the aforementioned trials and the present intervention allow inferring that decreases in oxidative stress biomarkers were coupled with weight loss and changes in body fat. Obese children have elevated levels of oxLDL than normal weight children, and higher oxLDL levels have been significantly associated with elevated BMI, body fat, visceral fat, and waist circumference. It has been proposed that adipose tissue up-regulates the overproduction of ROS due to the excessive caloric accumulation. These associations and the interventions reducing both fat mass and oxidative markers suggest that a greater fat mass determines a higher degree of oxidative stress, and that reducing adipose depots may result in decreases in oxidative stress. Therefore decreases in adipose tissue through weight loss might ameliorate the overproduction of oxidative stress biomarkers, as shown by the lower levels of oxLDL after this intervention.

Concerning randomized trials using only exercise, findings have been non-conclusive. Contrary to previous studies here discussed, no effect on isoprostane levels was found in a randomized trial using exercise with two different doses in 112 overweight/obese children 7-11 y old. Lack of effect on isoprostane levels was observed despite reduced fatness (BMI, waist circumference, percent body fat, and visceral adipose tissue) and improved fitness (peak oxygen consumption VO$_2$). Likewise, Youseff et al. (2015) aimed to determine whether aerobic training could reduce lipid peroxidation and inflammation. Classified as normal weight or overweight/obese, 39 adolescent girls (14-19 y old) were randomly assigned to either a non-trained or trained group in a 12-week multivariate aerobic program. In normal weight girls, training prevented exercise-induced lipid peroxidation (p>0.05). Paradoxically, levels of oxLDL
were comparable in overweight/obese girls in trained and non-trained (p>0.05). These results suggested that the effect of exercise was only observed in normal weight girls.

Furthermore, no significant increases in oxLDL levels were reported after two treatments in a study carried out by Tjona et al. (2009), in which 54 overweight/obese adolescent girls (age, 14.0±0.3 y) participated. This intervention was a randomized trial to either aerobic interval training- AIT (twice a week for 3 months) or to a multidisciplinary approach- MTG (exercise, dietary and psychological advice, twice a month for 12 months). AIT was favorable compared to MTG in decreasing BMI, percent fat and mean arterial blood pressure (p<0.05). However, no significant increases in oxLDL levels after three months were reported for both treatments. Comparing both studies, the AIT program by Tjonna et al. and the present study had the same duration (3 months), although the present study found significant decreases in oxLDL and increases in oxHDL.

Likewise, Kelly et al. (2007) reported that in the absence of weight loss, exercise did not improve oxidative stress in 19 overweight children who were randomly assigned to an aerobic program or sedentary control group for 8 weeks. Although exercise improved VO\textsubscript{2}max, there were no differences between the intervention and the control group regarding changes in body weight or body composition, as well as for isoprostane levels (p>0.05). The study suggested that without weight loss, exercise might have little to no effect on oxidative stress. Contrary to the results from Kelly et al, in the present study both changes in anthropometric and oxidative biomarkers were observed.

In addition to aforementioned interventions, other studies have reported significant changes in oxidative stress biomarkers without changes in anthropometry,
which denoted that the former changes are not necessarily conditioned by changes in the anthropometric variables. In a prior pilot study, Ryder et al. (2014)\textsuperscript{120} examined the effects of the same intervention used in the present study in obese Latino youth (n=15, BMI percentile= 96.3±1.1%, 15.0±1.0 y). Relative to baseline data, the intervention resulted in lower oxLDL (- 21.8%, \(p= 0.001\)). When overall responsiveness to change was examined, oxLDL was reduced to 93.3%, with no significant changes in weight, waist circumference, and BMI. Findings differed from the present study in regards to anthropometric changes, in which the pilot study did not show changes, while the present study showed significant reductions in most anthropometric variables. It is important to mention that this pilot study included obese youth, while the current study included youth with prediabetes, a condition that represents hyperglycemia derived from a fail in the compensatory mechanism of insulin secretion. This difference in the severity of the condition may influence the difference observed in anthropometric changes comparing both studies.

Findings from exercise training and PA promotion programs enable to suggest that exercise may induce different but related mechanisms to decrease oxidative stress, since some studies have shown lowering of oxidative stress biomarkers together with weight loss, and others despite no changes in weight and body fat. Results from the present study with changes in oxidized LDL as well as changes in anthropometry allow proposing that weight loss and exercise probably acted synergistically such that some of the beneficial effects of exercise might actually be exerted through decreases in body fat and/or changes in adipocyte function.\textsuperscript{135} It is documented that exercise reduces the secretion of inflammatory and oxidative molecules from adipocytes, as well as promotes
antioxidant enzymes action.\textsuperscript{125,127} Moreover, exercise can make the oxidative metabolism more efficient through improvements in cardiorespiratory fitness, which may lower the overproduction of ROS.\textsuperscript{312} Although the present intervention did not independently evaluate exercise, it was observed that oxLDL levels, weight, and body fatness decreased, and VO\textsubscript{2} peak increased, after the exercise training and the nutrition education.

5.2 Cardiometabolic Outcomes and Oxidative Stress Biomarkers

The present intervention was successful in decreasing most of the cardiometabolic risk outcomes. Significant decreases were observed in 2hour glucose, fasting insulin, 2hour insulin, and HOMA-IR. The effect on 2hour glucose after an OGTT, which is an indicator of the effectiveness of the intervention in preventing T2D, allowed reverting the prediabetic status in 66\% of the sample. Besides, significant decreases were found in most of the lipid panel, with significant mean changes in triglycerides, total cholesterol, VLDL-c, HDL-c, and non-HDL-c (all \(p<0.05\)).

Concerning overweight/obese children, several studies have reported similar cardiometabolic improvements such as those shown in the present study. For instance, dietary modification is one of the common strategies to improve the oxidative status of individuals, and although not comparable to the nutrition education used in this intervention, both strategies have been useful improving cardiometabolic variables. For instance, the dietary modification was tested by Rendo-Urteaga et al. (2014)\textsuperscript{113} to investigate the potential health effects of dietary antioxidants consumed in mixed diets by measuring dietary and serum TAC, and urinary F2-isoprostane levels. The study evaluated the oxidative status of 44 overweight/obese children (mean age 11.5 y) after a
10-week weight loss program that consisted of prescribed fixed full-day meal diet, according to the individualized basal metabolic rate and PA levels. After the intervention, participants were dichotomized at the median of BMI-SDS change, as high (HR) and low responders (LR). Significant reductions were observed in fasting serum glucose, total cholesterol, insulin levels, and HOMA-IR in the HR group (p<0.05), as well as in anthropometric variables (p <0.001). However, opposite to the proposed hypothesis by Rendo-Urteaga et al., dietary TAC was reduced in both groups after intervention, with greater reductions in the HR group, and no significant associations between oxidative status markers and changes in adiposity and biochemical parameters.

Concerning exercise, in a study performed by et al. (2005) the effect of 12 weeks of aerobic exercise training (3 d/wk for 12 weeks, with 40 minutes each session) was examined in 19 overweight/obese girls (age 13.1±1.8 y; BMI 26.8±3.9 kg/m²). CRF and insulin sensitivity improved (p<0.05), without changes in body weight or percent body fat (p>0.05). The present intervention was different than Nassis et al. in anthropometric effects, but it was similar in improving insulin, as well as increasing VO₂max (7.0%, p<0.001), albeit using different indicators.

Similar findings were reported in the intervention by Tjonna et al. (2009) aforementioned, where AIT induced a more favorable regulation of blood glucose and insulin than the MTG. After 3 months, greater decreases in 2-hour glucose and insulin were observed in AIT compared to MTG. Overall, cardiometabolic changes demonstrated that aerobic training had a more robust influence on CV risk factors, compared to usual care. Participants that performed aerobic training had more robust physiological improvements than those participating in the MGT program by Tjonna. However, Tjonna
et al.\textsuperscript{133} reported no changes in oxLDL levels for both the AIT and the MGT programs, while in the present study significant decreases in oxLDL were found.

Bariatric surgery is among effective treatments for severely obese adolescents. Kelly et al. (2016)\textsuperscript{121} evaluated postsurgical changes in serum biomarkers in two cohorts of adolescents with severe obesity that had undergone elective bariatric surgery. Cardiometabolic outcomes such as glucose, insulin, and HOMA-IR were significantly decreased after 6 and 12 months (all with $p<0.005$, except for glucose in cohort 1 at 6 months with $p=0.233$). OxLDL significantly decreased after 12 months ($p=0.03$). These results were consistent with those obtained in the present intervention, in which levels of oxLDL and cardiometabolic variables were significantly decreased. The improvement on these biomarkers indicated that adolescents undergoing any of these interventions would potentially experience a meaningful decrease in cardiometabolic outcomes related to T2D and CVD risk.

Improvements in early oxidative stress biomarkers such as oxLDL in the participants of this study were coupled with decreases in cardiometabolic variables such as insulin resistance and 2hour glucose, as well as triglycerides and VLDL-c. It has been proposed that the excess of caloric intake and lack of PA in obesity leads to the concomitant accumulation of ROS, which are suggested to induce inflammation,\textsuperscript{314} insulin resistance, impaired insulin secretion,\textsuperscript{315} endothelial complications,\textsuperscript{316} and other metabolic disorders.\textsuperscript{143} Oxidative stress and systemic inflammation are closely related pathophysiological processes that are tightly linked with one another,\textsuperscript{317} since ROS can initiate the intracellular signaling cascades that enhance proinflammatory gene expression, and in turn inflammatory cells release ROS that lead to excessive oxidative
Besides, ROS can induce systemic effects known as glucotoxicity and lipotoxicity, which also lead to impairment in insulin action and secretion. Additionally, within the endothelium and under the oxidative modification hypothesis, the proposed initial step by which ROS induce the synthesis of chemotactic proteins and the recruitment of inflammatory cells is the oxidation of LDL. Findings from the present lifestyle intervention may suggest that the exercise training and the dietary modification here promoted led to decreases in ROS that resulted in lowering of oxLDL, with the consequent improvements in insulin resistance and hyperglycemia, as well as in lipids. These findings have important physiologic implications since prediabetic youth progress to T2D more rapidly than adults. Moreover, although not measured here, the suggested changes in oxidative species might also decrease inflammation, with the concomitant improvements in CV risk. Together, all findings lead to the recognition that the present intervention is an effective strategy to reduce oxidative and cardiometabolic biomarkers and to prevent the rapid progression of prediabetic youth toward T2D, thus decreasing CV risk.

5.3 Outcomes Related to HDL Oxidation and Function

Studies concerning oxHDL in humans are scant, with most of the evidence coming from in vitro experimental studies from cells and tissues. This information, although it cannot totally be extrapolated to human studies, is useful when discussing HDL function and oxidation in the human population.

In vitro experimental studies with oxHDL and subspecies generated through oxidation are controversial since the evidence is divided, with some studies indicating
pro-atherogenic properties while others are showing anti-atherogenic properties. Respect to the pro-atherogenic properties, Matsunaga et al. (2003)\textsuperscript{322} determined that oxHDL activated nuclear factor-kB (NF-kB) during treatment of cultured human umbilical vein endothelial cells (HUVECs) with oxHDL. Activation of NF-kB was proposed to be via binding to oxLDL receptor-1 (LOX-1) on the cell surface followed by a significant dose-dependent increase in intracellular ROS production, perpetuating the oxidative environment. NF-kB is suggested to be a vital signaling factor for negative vascular events such as endothelial apoptosis, ROS generation, and inflammatory responses.\textsuperscript{325}

Moreover, Soumyarani et al. (2014)\textsuperscript{323} quantified markers of oxidative stress, inflammation, and cytotoxicity in monocytes after treatment with oxHDL and oxLDL with the same range of MDA. Findings showed that similar to oxLDL (which was a positive control), oxHDL significantly induced oxidative stress, cytotoxicity, and release of TNF-alpha and MMP-9 in monocytes/macrophages, but with lower intensity than oxLDL. TNF-alpha is a well-recognized marker of systemic inflammation,\textsuperscript{326} and MMP-9 is an enzyme that regulates pathological remodeling processes that involve inflammation and fibrosis in CVD.\textsuperscript{327} With respect to cellular apoptosis, in a study performed by Yao et al. (2017)\textsuperscript{324} oxHDL ingested by macrophages caused intracellular lipid accumulation and induced macrophage apoptosis with concomitant activation of the endoplasmic reticulum stress pathway, which resulted in increased inflammation.

Besides, Persegol et al. (2006)\textsuperscript{328} compared the ability of HDL from 16 T2D patients and 13 controls to suppress the inhibition of vasodilation induced by oxLDL using rabbit aorta rings. HDL from control subjects significantly reduced the inhibitory effect of oxLDL on vasodilatation, whereas HDL from T2D patients had no effect. HDL
triglyceride content was significantly higher in T2D patients than in control subjects. Similarly, Yao et al. reported that HDL from patients with metabolic syndrome induced macrophage apoptosis and oxidative stress. These studies showed that the ability of HDL to counteract the inhibition of endothelium-dependent vasorelaxation induced by oxLDL was impaired in metabolic syndrome and T2D patients, suggesting that HDL are less atheroprotective in metabolically impaired patients than in control subjects.

Conversely to the evidence of pro-atherogenic properties, there are also experimental studies proposing that oxHDL has anti-atherogenic properties. A study carried out by Girona et al. (1997) reported the effects of oxHDL subfractions 2 and 3 (oxHDL$_2$ and oxHDL$_3$) compared to oxLDL and some products of oxidation (hydroperoxides and aldehydes), on the secretion of TNF-alpha from THP-1 human monocytes-derived macrophages in vitro. Inhibition of TNF-alpha by oxHDL$_2$ (p<0.05), oxHDL$_3$ (p<0.05) and oxLDL (p<0.05) from THP-1 macrophages was observed in the presence and absence of lipopolysaccharide. This inhibition was opposite of that reported by Soumyarani et al. (2014). Taking into account the differential effects showed on TNF-alpha, more research needs to be done to clarify the role of oxHDL in the inhibition or induction of secretion of inflammatory components such as TNF-alpha.

Additionally, Zhou et al. (2017) determined the effects of MPO-modified oxHDL (nitrated and chlorinated oxHDL) on smooth muscle cell (SMC) migration and atherosclerotic plaque stability in vivo using a carotid artery collar implantation mice model. Native HDL promoted SMC proliferation and migration, whereas nitrated oxHDL and chlorinated oxHDL inhibited SMC migration and reduced capacity of stimulating
SMC proliferation as well as migration, respectively, with no significant influence of oxHDL on SMC apoptosis.

Some metabolites from oxidative modifications of HDL, such as tyrosyl radical-oxHDL (tyrHDL) by MPO, have increased the availability of cellular lipids for apo-mediated HDL particle formation. These metabolites would be expected to increase total reverse cholesterol transport and decrease the development of atherosclerosis. In a series of studies, Francis et al. showed that tyrHDL markedly enhanced depletion of the regulatory pool of cell cholesterol available for esterification by acyl-CoA:cholesterol acyltransferase (ACAT), compared to native HDL, in cultured human fibroblasts, mouse peritoneal macrophages, and human arterial smooth muscle cells. In another study, the same research group found a marked increase in efflux to apoAI after incubation of fibroblasts with tyrHDL.

In line with same previous research, Wang (1998) investigated the structural modifications in tyrHDL, in which apoAI-apoAII monomer and apoAI-(apoAII) heterodimers showed a markedly increased ability to prevent the accumulation of LDL-derived cholesterol by sterol-depleted fibroblasts, compared to other apolipoprotein species of tyrHDL. These results indicated a novel product of HDL oxidation, apoAI-apoAII heterodimers within tyrHDL, with a markedly enhanced capacity to deplete cells of the regulatory pool of free cholesterol and total cholesterol mass. Moreover, Macdonald et al. (2003) injected 150 µg of tyrHDL intraperitoneally twice weekly in apoE-deficient mice. A maximum 2.3-fold increase in endogenous HDL cholesterol levels was observed, and tyrHDL treatment showed 37% less aortic lesion development than control HDL-treated mice (p<0.001). This study demonstrated that treatment with
tyrHDL raised endogenous HDL-c levels and decreased atherosclerosis development in apoE-deficient mice.

In regard to the degree of oxidation, Gao et al. (2008) analyzed the effects of copper and hypochlorite (that preferentially oxidize lipids and proteins, respectively) on thermal stability of plasma spherical HDL. The study showed that mild oxidation destabilized HDL and accelerated protein dissociation and lipoprotein fusion, lowering kinetic barriers for HDL remodeling. In contrast, advanced oxidation inhibited protein dissociation and HDL fusion. Gao et al. concluded that mild oxidation of HDL promoted remodeling of human HDL in vitro, which may benefit HDL functions, while advanced oxidation impaired it. These findings helped to clarify the apparent controversy regarding structural modifications of oxHDL that promote HDL remodeling.

In addition, Valiyaveettil et al. (2008) reported that oxHDL, but not native HDL, was a potent inhibitor of platelet activation and aggregation induced by physiologic agonists. This effect was especially important since activated platelets and platelet extracellular vesicle (PL-EV) release are essential factors in atherosclerosis, promoting thrombosis and plaque formation. The anti-thrombotic effect was concentration- and time-dependent and positively correlated with the degree of lipoprotein oxidation. Similarly, an in vitro study carried out by Tafelmeier et al. (2017) focused on the effect of mildly oxHDL and native HDL on platelet aggregation and PL-EV release. Mildly oxHDL significantly decreased PL-EV release by -36% and partially reversed agonist-induced platelet aggregation, in comparison to native HDL. In addition, mildly oxHDL improved platelet membrane lipid homeostasis through increased uptake of lysophospholipids and their remodeling to corresponding phospholipid species.
Furthermore, Talens et al. (2013) investigated the possible function of oxHDL in coagulation and fibrinolysis by thromboelastography. Addition of oxHDL resulted in reduced clot firmness in a time-dependent effect, while oxLDL and native HDL did not show any effect. The effect was correlated with a shift in the apolipoprotein AI protein band around 25 kDa. These studies support the notion that mildly oxHDL has inhibitory abilities on platelet activation and aggregation, as well as beneficial effects on coagulation and fibrinolysis, which represent anti-atherosclerotic properties.

The experimental evidence here discussed suggests that oxHDL elicits different anti- and pro-atherogenic properties, which depend on factors like the cell lines used (human or animal) during in vitro studies, the time of exposure and dose of oxHDL on assays, but mainly on the degree of oxidation of HDL. Most of the research from mildly oxHDL previously mentioned established beneficial effects on remodeling HDL reverse cholesterol transport, cholesterol efflux, platelet aggregation and release of pro-coagulant platelet extracellular vesicles, as well as coagulation and fibrinolysis. The increases in oxHDL levels after the lifestyle intervention, although non-significant, might indicate the possibility that oxHDL has antiatherogenic properties. It would be interesting to elucidate the degree of oxidation in HDL from participants after the current intervention, as well as some other chemical characteristics of oxHDL particles that could lead to the mechanism by which oxHDL influence the risk of CV.

There are few cross-sectional human studies regarding HDL function and oxidation. Concerning oxidation, Peterson et al. (2016) compared levels of oxHDL between lean and obese adult females with no CVD (total n=16). Obese patients showed
6-fold higher levels of oxHDL compared to normal weight, as well as higher oxHDL/total HDL ratio as BMI was higher. In youth, similar results were reported by Marin et al. (2015), who analyzed levels of both oxLDL and oxHDL in 37 normal weight, 38 obese, and 42 obese T2D adolescents, aged 11–18 y. Greater concentrations of oxHDL were reported in obese T2D children (65%), and oxHDL was not correlated with insulin resistance (HOMA-IR), while it was positively associated with oxLDL and lean body mass. Based on these studies that showed greater levels of oxHDL in obese compared to normal weight, it was hypothesized that oxHDL levels would decrease after the present intervention. However, contrary to the hypothesis, oxHDL levels increased yet not significant.

Two cross-sectional investigations from The Young Finns Study have been documented. Kresanov et al. (2013) investigated the associations of oxHDL lipids (based on conjugated dienes) with known risk factors for atherosclerosis in 1395 Finnish adults ages 24-39 y (54.9% women). A significant inverse association was found between oxHDL and age, and a direct association with oxLDL lipids after adjustment for Apo-A1. Additionally, in men oxHDL lipids were inversely associated with insulin. In women, oxHDL lipids were inversely associated with waist circumference and daily smoking, and directly with CRP and alcohol use. The study carried out by Kresanov et al. allowed to conclude that an elevated risk profile characterized primarily by advanced age was associated with lower oxHDL lipid levels in this sample. In parallel with these studies, lower levels of oxHDL in the present sample were detected at baseline, compared to increases observed in oxHDL levels after intervention and improvements in cardiometabolic outcomes. Results from the present study were consistent with those
reported by Kresanov et al.\textsuperscript{110} although not totally comparable because of the nature of each study (cross-sectional vs. interventional).

Another study from the aforementioned group and published by Kaikkonen et al. (2016),\textsuperscript{98} determined circulating levels of oxidized lipids in LDL and HDL (through conjugated dienes and a method based on antibodies against oxLDL) and their associations with fatty liver, in 1286 middle-aged participants with normal liver and 288 with fatty liver. Lower significant levels of oxHDL were reported as the degree of severity of fatty liver increased, Moreover, participants with elevated oxLDL lipids had an increased risk for fatty liver (p=0.011), and a high oxidation score (oxLDLlipids + oxLDLprot) was directly associated with fatty liver (p=0.012). These data indicated that oxLDL lipids were directly linked with risk of fatty liver in middle-aged adults, while oxHDL levels were inversely linked to the severity of the disease. Findings from The Young Finns Study agreed with results from the present study regarding levels of oxHDL related to CVD risk. It might be that compensatory mechanisms attempting to reduce CV risk provoke increases in oxHDL after the intervention here presented, such as higher rates of remodeling HDL, reverse cholesterol transport, and cholesterol efflux, in an attempt to prevent and decrease the accumulation of oxidized lipids and other oxidants. All these aspects could explain why oxHDL levels were lower at baseline and increased after the intervention.

The number of interventional studies regarding HDL function and/or oxidation is limited. Among them, Roberts et al. (2006)\textsuperscript{171} examined the effects of a lifestyle intervention in obese men (n =22) allocated to a 3-week residential program providing a high-fiber, low-fat diet ad libitum, and daily aerobic exercise. Lipid hydroperoxides and
the HDL inflammatory/anti-inflammatory indexes were measured pre- and post-intervention. Significant decreases in lipid hydroperoxides were shown, as well as in the HDL inflammatory index, with significant increases in HDL-anti-inflammatory index. Moreover, in a randomized controlled trial with sedentary women aged 43–63 y, the effect of 6-month aerobic exercise on lipid peroxide transport function of HDL was analyzed by Tiainen et al. (2016), compared to a control group. Similar to increases found in the present intervention, both the levels of oxHDL and the ratio of oxHDL/HDL-c significantly increased 5% in the exercise group, and decreased 2% and 1.5% respectively, in the control group, while CETP concentrations remained unchanged, implicating that the transfer of cholesteryl esters and triglycerides between lipoproteins remained stable. Findings from the current study were similar to the elevation in oxHDL after the intervention by Tiainen, in which the present intervention resulted in 8.3% increases in oxHDL levels (p=0.056) and 13.3% of oxHDL/HDL-c ratio (p=0.009) were found after the intervention.

In athletes, Valimaki et al. (2016) examined the effects of endogenous oxidative stress induced by acute exhaustive physical exercise on the concentration of oxHDL lipids in 24 male national top-level endurance runners who performed a maximal run on a treadmill until exhaustion. Immediately after the treadmill run, the concentration of oxHDL lipids and the ratio of oxHDL lipids/oxLDL lipids increased by 24 % (p<0.01) and 55 % (p<0.001), respectively, while oxLDL lipids levels decreased by 19 % (p<0.001). Simultaneously, MDA and serum total peroxyl radical trapping antioxidant potential (TRAP) increased by 54% (p < 0.001) and 29% (p<0.01), respectively. After the 90 min recovery, the concentration of oxHDL lipids decreased towards the pre-exercise
level, but oxLDL lipids remained decreased below pre-exercise values (p<0.001).

Although with different participant characteristics, current outcomes in the present study partially agreed with increases in oxHDL after exercise by Valimaki et al., suggesting that during physical exercise, HDL has an active role in the removal of lipid peroxides, showing elevated concentrations compared to baseline.

Respect to dietary modification, a randomized controlled trial by Hernáez et al. (2017) assessed the effects of traditional Mediterranean diet (TMD) on HDL function in a random subsample of 296 adult volunteers from the PREDIMED Study, after one year of intervention. Two variations of the diet were compared: one enriched with virgin olive oil (TMD-VOO; n=100) and the other enriched with nuts (TMD-Nuts; n=100), compared to a low-fat control diet (n=96). Both diets significantly increased cholesterol efflux capacity relative to baseline (p<0.05). The TMD-VOO intervention decreased CETP activity (p=0.028) and increased HDL ability to esterify cholesterol, paraoxonase-1 arylesterase activity, and HDL vasodilatory capacity (all p<0.05). Adherence to the Mediterranean diet, especially when enriched with virgin olive oil, was associated with increases in the key HDL functions here analyzed. Intervention by Hernáez et al. improved HDL function through an antioxidant-rich dietary pattern such as the traditional Mediterranean diet, which promotes frequent intake of fresh F&V.

In pediatric interventions, outcomes were mainly concerning HDL function and particle size. Albeit difficult to directly compare these interventions with the present study, these studies can be used to highlight the effect of interventions on HDL function. In a randomized trial Wesnigk et al. (2016) analyzed the effect of a 10-month lifestyle
intervention on HDL-mediated eNOS activation and HDL-reverse cholesterol transport with either intervention group (restricted diet and exercise) or usual care group (UC) in obese adolescents of 15±1 y old. The HDL-mediated phosphorylation at position Ser\textsuperscript{1177} of eNOS and the efflux capacity of HDL significantly increased after the intervention (p<0.05), both important factors involved in reverse cholesterol transport, while the UC group showed no difference in phosphorylation of eNOS (p= 0.08) and a significant decline in efflux HDL capacity (p=0.05). Additionally, the lifestyle intervention resulted in reductions in BMI and body fat (p< 0.001), with a 10% increase in maximal oxygen uptake (p=0.002), while the UC group did not show changes. This study denotes the importance of promoting a restricted diet and exercise in obese adolescents to improve HDL function.

Moreover, a study carried out by Davidson et al. (2017)\textsuperscript{122} tested the hypothesis that atherogenic HDL profile (HDL subspecies and HDL function) would improve with VSG bariatric surgery in adolescent males with severe obesity (mean age 17.4±1.6 y). Outcomes were analyzed at baseline and after 1 year, compared to a lean group of adolescents. Decreases in HDL lipid peroxidation potential by 30%, and significant increases in large apoE-rich HDL subspecies, as well as 12% increased in cholesterol efflux capacity and 25% increased in HDL anti-oxidative capacity were found.\textsuperscript{122} Considering both pediatric interventions by Wesnigk and Davidson et al., as well as the present study, benefits related to HDL function and oxHDL levels were found with improvements in anthropometric and cardiometabolic outcomes, despite participants kept obese after the intervention. Future research must focus on elucidating the effects of lifestyle modification on the function, structure, and composition of oxHDL. Outcomes
such as changes in the degree of oxidation and function, as well as structural
modifications and some other important lipoprotein characteristics must be taken into
account during future T2D prevention interventions. Differences in atherogenic
properties have been observed depending on the chemical characteristics of oxHDL.
HDL that is mildly oxidized have exerted beneficial effects regarding HDL reverse
cholesterol transport, cholesterol efflux, decreasing platelet aggregation and release of
pro-coagulant platelet extracellular vesicles, as well as coagulation and
fibrinolysis. Detecting these modifications on oxidized lipoproteins will allow
understanding physiologically and chemically how lifestyle modification can reduce
atherosclerotic risk at early stages.

5.4 Changes in Fresh Fruit and Vegetable Intake and its Correlation with Changes
in Oxidized Lipoproteins

In the present study, dietary intake of fresh F&V was significantly increased, with
values of 116.4 (97.0) g/d at baseline and 165.8 (91.0) g/d after the intervention, p=0.025.
It was interesting to observe that although each component of this dietary composite
outcome increased after the intervention, individual food changes were non-significant
(p>0.05, Table 5). Weak and non-significant within-subjects correlations were detected
between changes in oxLDL and fresh F&V intake (R=-0.15, p=0.52), and between
changes in oxHDL and fresh F&V intake (R=0.22, p=0.25), both adjusted for VO$_2$max
changes (Table 7).
It is worth discussing here that studies regarding dietary assessment have found that children and adolescents are prone to report errors during dietary intake records and questionnaires at both group and individual level, with the existence of subject-specific responding in dietary assessments. Most of the errors were related to under-reporting, since dietary reports are influenced by body weight status, especially in obese children like participants in this study. Furthermore, this error does not occur systematically across different age groups or different dietary survey techniques. Nevertheless, it is important to note that even with the possibility that under-report could occur, significant increases in fresh F&V intake as a whole food group were detected.

The dietary modification has been one of the most extensive recommendations for CVD prevention. Diverse nutrients in F&V have been associated with lower risk for CVD; however, it is important to consider fresh F&V as a whole food group due to its physical and biochemical complexity and the interaction between their nutrients. In recent years diet-based approaches have been more effective in reducing CV risk than single nutrient-approaches. Intake of F&V in adults has been associated with a reduced rate of developing CVD, such as CHD and stroke. There is scientific evidence in both adults and children that show that dietary patterns characterized by a high intake of F&V are associated with a lower risk of hypertension, obesity, and T2D, all of which are established risk factors for CVD. Besides, Dauchet et al. (2006) in a meta-analysis that included nine studies with 91,379 men, 129,701 women, and 5,007 CHD events, showed that the risk of CHD was decreased by 4% (p=0.0027) for each additional portion per day of F&V.
Concerning cross-sectional studies in regards to F&V intake and oxidative stress biomarkers, a higher F&V intake was independently associated with lower oxLDL, 8-OHdG, and 8-iso-PGF2α (p< 0.05) in 296 healthy middle-aged men with BMI of 25.8±3.5 kg/m². In another study, 266 healthy subjects (age=22±3 y; BMI=22.0±2.7 kg/m²) were enrolled to investigate the potential relationships of dietary TAC with oxidative stress markers. Dietary TAC was inversely associated with oxLDL concentration (p<0.05), suggesting a putative role of antioxidant rich-diet in the linkage between redox state and atherogenesis at an early stage. Moreover, in a epidemiological study carried out by Pitsavos et al. (2005), the effect of a diet score based on the Mediterranean diet on total antioxidant capacity (TAC) was examined in 3042 participants aged 18–89 y from Greece who had no clinical evidence of CVD. Outcomes revealed that TAC was positively correlated with diet score, and participants in the highest tertile of the diet score had, on average, 11% higher TAC levels and 19% lower oxLDL concentrations than participants in the lowest tertile (p<0.01). TAC was positively correlated with consumption of F&V (R=0.34 and R= 0.31 respectively, p<0.001 for both). In case of adolescents, Holt et al. (2009) determined whether greater intakes of F&V were inversely associated with urinary 8-iso prostaglandin F2α in 285 adolescent boys and girls aged 13 to 17 y. Although weak, urinary F2-isoprostane was significantly inversely correlated with intakes of total F&V (R=−0.13, p<0.05).

Based on this kind of cross-sectional studies, interventions designed to increase F&V intake have been promoted in all age groups. In a systematic review of the effectiveness of interventions, 44 studies (mainly from developed countries) were analyzed and stratified by study setting. In primary prevention interventions in healthy
adults, F&V intake was increased by approximately 0.1–1.4 serving/d, while larger effects were found in individuals with preexisting health disorders. Consistent beneficial effects were observed during different interventions, suggesting that small increases in F&V intake were possible in population subgroups by a variety of approaches.

Few interventions have centered on dietary patterns that increase F&V intake in regards to oxidative stress biomarkers. For instance, 79 adult participants with metabolic syndrome and hyperglycemia were randomly assigned to two low-calorie diets (−30% energy): the control diet based on the AHA criteria; and the RESMENA diet (Metabolic Syndrome Reduction in Navarra diet), characterized by an increase of meal frequency (seven-times/day), low glycemic load, high antioxidant capacity, and high n-3 fatty acids content. Changes in oxLDL were inversely associated to dietary TAC ($R=-0.310$, $p = 0.032$) and fruit consumption ($R=-0.304$, $p=0.028$). However, correlations in the RESMENA study were simple and partial correlations, without taking into account the correlation within subjects, which is the correct analysis for the repeated measures design in this interventional study due to the dependence of data among time measurements for each participant.

In children, there are enough interventions centered on increasing F&V intake. Although with the same main aim of augmenting F&V dietary intake, pediatric interventions have differed in several aspects such as the settings, with some based on the school environment, others on community-based and family-based environments, and some others using both schools- and community-based approaches. Another aspect is the method of delivery, either using motivational intervention, telephone-based, peer-led, rewards-led, social messages-
led, gardening, etc. Some interventions have focused on specific ethnic groups such as African-American, Chinese-American, Hispanics, children with migration background, etc. Furthermore, outcomes have differed with some interventions showing no F&V intake increases over time, while others showing successful increases.

In the aforementioned pilot study performed by our research group in 15 obese participants using the ELSC program that was applied during this study, outcomes showed that responders and non-responders did not differ with regard to F&V intake. Comparing with the present study, significant increases in fresh F&V intake were found, with significant decreases in oxLDL, as well as no significant increases in oxHDL. It is unknown if differences in the intake of fresh F&V observed in both studies were related to the awareness of the disease. Sample from this intervention was at high-risk, due to the severity of prediabetes and obesity together, compared to obese participants from Ryder et al. The prediabetes condition in the present study might lead to an increased awareness of both the disease severity and the necessity of making lifestyle changes. Although not measured during the present intervention, awareness of the disease has been identified in health conditions such as heart diseases as one of the main factors involved in seeking prompt treatment. In a study detecting sociocultural and familial factors to prevent childhood obesity in 6-to-10 y-old children, Bruss et al. (2003) identified limited awareness of disease and its relationship to diet as stress factors for appropriate child-feeding practices, factors that seem to create personal, intergenerational, and intra-family conflict, with consequent ineffective child-feeding practices.
On the other hand, adherence may be another factor that could influence F&V intake in the pilot intervention compared to the present study. However, both the pilot study reported by Ryder et al.\textsuperscript{120} and the current intervention lacked a gold-standard measure of behavior to infer adherence. There is a lack of effective methods to measure adherence to diet in the childhood obesity context.\textsuperscript{379} Besides, insights from six European studies helped to identify barriers to the implementation of dietary and PA recommendations in women and children. Psychological factors in women, such as a greater sense of control and having higher levels of food involvement allowed women to be empowered to eat more healthily and to feed their children better. Moreover, older women and those with higher educational attainment had better diets for themselves and for their children. In addition, living in more disadvantaged circumstances was associated with poorer diets, and mothers in such circumstances appear to have lower levels of food involvement.\textsuperscript{380} All these factors could differentially influence both the present study and the pilot study. Therefore it would be important in future studies to take into account these psychological factors in the family involved in the intervention, especially in the mother.

Correlations between changes in dietary intake of F&V and changes in oxidative biomarkers during lifestyle interventions in children are very scarce. Only the study carried out by Rendo-Urteaga et al. (2014)\textsuperscript{113} evaluated oxidative status (TAC) in overweight/obese children after an aforementioned 10-week weight loss intervention in which individualized fixed full-day meal diet was provided. Interestingly, basal serum TAC values significantly predicted the decreases in urinary F2 isoprostane after the program in the HR group, and the relationships between oxidative markers and
antioxidants dietary intake were more favorable in the HR than in the low responders (LR). Non-significant associations between oxidative status markers and changes in adiposity and biochemical parameters were found. Outcomes indicated that in terms of oxidative status, basal levels of TAC were more important predicting change in oxidative markers. These results and findings from the present study suggested that frequent intake of specific foods with a high TAC content (such as fruits and vegetables, among others) should be encouraged through life, but especially at young ages, to assure optimal consumption of antioxidants.

Similar to the statistical correlation analysis used by the previously mentioned RESMENA study in adults, Rendo-Urteaga et al. also used partial correlations to calculate the association between changes, which according to the assumption of the dependence of subjects within the time measurements, it is not the correct statistical correlation analysis for this kind of within-subjects design.

Despite not focused on an oxidative marker, Woolcott et al. (2013) centered to determine if changes in PA were associated with changes in F&V intake in a prospective cohort with 700 adults from Hawaii. Covariance Model Analysis revealed no mean within-individual correlation between changes in PA and changes in F&V intake over time (R=0.03, p>0.05), even though between-individual Pearson’s correlations showed that individuals with a higher mean PA duration tended to eat more F&V (R=0.30, p<0.0001). It is important to note that unlike some studies previously discussed and similar to the statistical analysis used by Woolcott et al., within-subjects correlations between changes in each oxidized lipoprotein and changes in fresh F&V intake, adjusted for changes in VO2max, were calculated during the present study. Since participants were
analyzed at each time (pre- and post- intervention), using within-subjects correlation analysis did not violate the assumption of the dependence of measurements. This analysis is especially relevant considering that most studies that reported correlations between changes used Pearson or Spearman correlation, which was not the correct statistical analysis.

In the present intervention, it was hypothesized that changes in both oxLDL and oxHDL would be related to intake of fresh F&V, independent of the improvements in VO$_2$max. However, weak and non-significant correlations were detected between changes in fresh F&V intake and oxLDL, and with oxHDL, both adjusted for VO$_2$max changes. Although correlations were weak and not significant, this analysis allowed concluding that the ELSC lifestyle intervention applied on obese prediabetic Latino youth promoted significant reductions in oxLDL, and no significant increases in oxHDL, with significant increases in both fresh F&V intake and VO$_2$max. Additionally, the intervention was able to improve anthropometric and cardiometabolic outcomes. These findings suggest that changes in oxidized lipoproteins were promoted by mechanisms different that only those provided by the intake of fresh F&V, and that effects were not independent of improvements on CRF.

5.5 Strengths and Limitations

The present study has a number of strengths worth mentioning. No attrition rate was documented since all participants were included as completers. The range of attendance was between 58-100% of sessions, which was similar to the attendance reported in other community-based family interventions that have shown success.  382
Sample was homogeneous in terms of ethnicity, age, prediabetes and degree of obesity. The homogeneity of the sample was due to the rigorous inclusion criteria used during the present study.

In terms of the study design, the quasi-experimental study has advantages and disadvantages. Focusing on the strengths of the study, this design used repeated measures (also called within-subjects design), which establishes that each participant takes part of each measurement time, allowing for an adequate control of individual differences and consequently producing small random error effects. A medium effect size for one sample t-test of $d=0.5675$ was calculated, using a total sample size of 35 participants, a significance level of 0.05, and power =0.95. Concerning the statistical analysis, paired t-test for comparisons between pre-and post-intervention measurements was used, and within-subjects correlations were calculated between changes in oxidized lipoproteins and changes in fresh F&V intake adjusted for changes in VO2max. Using this design allowed to take into account the dependence of subjects, since participants were the same in both pre- and post-intervention measurements. This strength is especially meaningful since most reported correlations between changes in lifestyle interventions have used Pearson or Spearman correlation, assuming independence of measurements and therefore violating the assumption of within-subjects or repeated-measures correlation.

Another strength is the use of official laboratory measurement techniques according to the respective guidelines, since this study included a comprehensive evaluation of cardiometabolic outcomes, as well as anthropometric and behavioral variables. Moreover, with pre- and post-intervention measurements of dietary intake and
CRF, the potential influence of dietary intake of fresh F&V was taken into account on changes in oxidative stress biomarkers, adjusted for VO$_2$max.

Concerning limitations, the most important disadvantage of quasi-experimental designs is lack of a control group. It is well know that randomized control trials are the gold standard for interventional studies. However, the inclusion of controls is not ethically valid in studies with pediatric population at high risk for cardiometabolic conditions, owing to ethical concerns of treatment delay.$^{121}$ Thus, although not optimal, quasi-experimental designs with paired samples are frequently used in studies with the population at high risk. One possibility to improve the design in the future to be more rigorous in testing our intervention in children at-risk could be the inclusion of usual care as an alternative of comparison group. In this way a randomized trial could be implemented to test the present intervention, compared to the usual care program. Other designs such as the crossover design in prediabetic youth are not useful since the potential long-term effect of the intervention may represent a carry over effect.

Another limitation of quasi-experimental pre- and post-intervention studies is that repeated-measures designs have a risk of order effects, with participants maybe starting to suffer fatigue and/or carryover effects. Constant psychological motivation was offered throughout the current intervention to counteract order effects, as well as compensations and rewards for attendance. In addition, although within-subjects designs allow for small sample sizes, a large sample would have resulted in a higher effect size. The current sample size of the present study was part of the bigger sample from the parent study, thus a small sample size was expected considering the prevalence of prediabetes in this young population.
Another limitation is the elevated daily variability of the lipid outcome measures. Biological rather than analytical variability is a major contributor to the inaccuracy of CV risk assessments based on measurement of lipids and lipoproteins. Although in this study multiple-days samples could not been collected due to the time and cost implications, other strategies were used as an attempt to reduce within-person variability, such as fasting for at least 10 hours and the recommendation of a low-fat diet the previous day of blood sampling. Also, in order to reduce between-persons variability, participants were recruited with strict inclusion criteria to have a homogeneous sample regarding age, ethnicity, and obesity degree.

With respect to dietary intake, a food screener for ages 2-17 y was used, the Brief Dietary Assessment tool for children 2-to-17 y. Since this is a FFQ for self-administration by adolescents, the present study relied on self-report data to estimate children's intake by food group. This tool has few disadvantages, mainly related to the limited checklist of foods and beverages, however this screener was especially designed to assess children's intake by food group, with focus on the intake of F&V. Among cons, this questionnaire relies upon the respondent’s memory for being a retrospective method, and arbitrary groupings of foods may not correspond to the perception of the respondent. Moreover, exclusion of foods popular to specific ethnic minority groups such as Mexican-Americans, which represents most of the participants, would skew the data. Nevertheless, this questionnaire was an adequate option since it is adapted to children and adolescents and the food checklist is based on NHANES dietary data and mainly focused on F&V consumption, which was one of the main outcomes of this intervention. Moreover, this is a semi-quantitative FFQ with collected portion size information as standardized portions.
or as a choice of portion sizes. A representative of “habitual” intake, this is the preferable method of measuring food intake with very high day-to-day variability.

Regarding the panel of biomarkers used here, they were not exhaustive and other potential markers of oxidative stress and lipoprotein function were not measured. However, there is a paucity of evidence in oxidized lipoproteins as early markers of oxidative stress and atherosclerotic risk, thus in this sense this study and statistical analysis was novel. Moreover, the inclusion of conventional cardiometabolic variables suggested that the intervention was successful in reverting the prediabetic status of most participants and consequently decreasing CVD risk.

5.6 Future Research

The present study was design to evaluate the effects of a lifestyle and T2D-prevention intervention for obese prediabetic Latino adolescents on levels of oxidized lipoproteins as early biomarkers of oxidative stress. Future research evaluating the present intervention should improve the study design including a randomized design in which a usual care group could be compared to the intervention group. In addition to this, a larger sample size could be included to have a higher effect size. Moreover, long-term effects could be evaluated including follow-up measurements in the future design.

Decreases in oxLDL were observed during the present study according to the proposed hypothesis. However, results from oxHDL were unexpected, showing non-significant increases after the intervention. Future research evaluating oxHDL should include other biomarkers of lipoprotein metabolism and function such as the degree of oxidation, functionality of HDL, cholesterol efflux capacity, enzymatic activity during
reverse cholesterol transport, apoproteins, and some others. Although few, evidence from studies regarding HDL have shown these potential variables as better indicators of the antiatherosclerotic properties of HDL. Regarding the effect of interventions on the oxidative stress process, meaningful variables related to the overproduction of ROS may be included, such as MPO and PON enzymes and oxidant metabolites. It would also be interesting to consider the effects of the intervention on some other indicators of more direct CVD risk, such as endothelial dysfunction and antithrombotic properties, among others.

Diet assessment using the corresponding FFQ may allow evaluating some other nutrients and compounds, such as fiber intake. Fiber is recognized as a source of diverse antioxidants and as a component that affects the glycemic response and blood lipids of individuals. Therefore, future approaches may include the effects of fiber dietary intake on the antioxidant status of participants, as well as its influence on cardiometabolic variables. Moreover, dietary patters instead of individual food groups or nutrients are recently recognized as the main contributors to the decrease in CVD risk, thus future studies should investigate the effects of dietary patterns on the risk for CVD.

Finally, future research should be done applying translational science to implement lifestyle modifications to more people at the community, to get meaningful health outcomes in this young group at high risk for T2D and CVD.
CHAPTER 6. CONCLUSION

Effective interventions for improving oxidative stress and atherosclerotic biomarkers are necessary as CVD represents the leading cause of morbidity and mortality worldwide. Lifestyle modification constitutes an excellent approach to prevent T2D and its concomitant CVD complications. In youth, lifestyle interventions are outstanding options since pharmacological and surgical treatments are limited. The purpose of the present study was to evaluate the effects of a lifestyle T2D-prevention intervention for obese Latino adolescents with prediabetes on levels of oxidized lipoproteins as early biomarkers of oxidative stress. We chose oxLDL and oxHDL as these early biomarkers would better reflect both oxidative stress and atherosclerotic risk. We hypothesized that levels of both lipoproteins would be significantly lower after the intervention, and that changes in fresh F&V intake will be inversely and independently correlated with changes in oxLDL and oxHDL, after controlling for changes in VO$_2$max. Previous investigations have analyzed the effects of lifestyle modification in obese but otherwise healthy kids. To our knowledge, this study is the first specifically designed to test the synergistic effect of diet and PA modification on both oxidized lipoproteins in prediabetic obese adolescents. Additionally, this research adds to previous work by including measurements of behavior such as changes in fresh F&V intake, as potential correlates of changes in each oxidized lipoprotein.

The present study showed significant decreases in oxLDL levels; however, the intervention failed to show reductions in oxHDL levels. Following the intervention, dietary fresh F&V intake was significantly increased, as well as VO$_2$max.
significant within-subjects correlations were found between changes in oxidized lipoproteins and fresh F&V intake after adjustment for VO$_2$\textsubscript{max} changes. In addition to these outcomes, the lifestyle intervention resulted in improvements in most anthropometric and cardiometabolic variables suggesting a reduced risk of CVD. Main cardiometabolic changes were observed in 2-hour glucose and 2-hour insulin during an OGTT, resulting in 23 out of 35 participants (66%) considered as normal glucose tolerant immediately after the intervention. All variables in the lipid panel significantly decreased except for LDL-c, which was not statistically changed.

Lack of detecting a significant correlation between changes in oxidized lipoproteins and changes in fresh F&V intake could be related to the fact that, although components of F&V have antioxidant properties that counteract oxidative stress, overall diet patterns rather than single foods have been recently recognized as the leading dietary factor reducing CVD risk. New research challenges must focus on elucidating behavior change and subsequent biochemical mechanisms by which healthy diet and PA patterns may reduce CV risk. In addition, biochemical and functional aspects of lipoproteins would be meaningful variables worth including in future studies of this early age group. With these mechanisms defined, their promotion in practical, real-life lifestyle interventions can be supported.
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APPENDIX A

IRB APPROVAL
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

PARENTAL CONSENT
PARENTAL/LEGAL GUARDIAN INFORMED CONSENT for RESEARCH

Study Title: Community-based diabetes prevention program for obese Latino youth.

Principal Investigator: Gabriel Q. Shaibi, PhD
Arizona State University
Day Time Phone: (502) 496-0909

INTRODUCTION
The purpose of this form is 1) Give you information to help you decide whether to let your child participate in this project and 2) Record your agreement for your child to be part of this study.

RESEARCHERS
Gabriel Shaibi, PhD, and colleagues from Arizona State University (ASU), are inviting your child to be part of a research study.

STUDY PURPOSE
The purpose of this study is to see if a 12-week lifestyle education and exercise program can improve the health of obese Latino teenagers. We are asking your child to take part in this study because you identify yourself as Latino or Hispanic and your child is obese. These characteristics place your child at risk for developing type 2 diabetes. This project will compare changes in health between two groups of teenagers over the next year. One group will be asked to participate in a 12-week education and exercise program at the YMCA in Phoenix and the other group will be put on a waiting list to complete a health program in a year from now. Group selection will be random and your child has a 50/50 chance of being in each group. Up to 160 teenagers will be in this study and everyone will undergo the same testing described below.

DESCRIPTION OF RESEARCH STUDY
If you agree to let your child participate, we will complete a health screening on your child to make sure he/she is healthy and able to participate in this study. This visit will last about 4.5 hours and will take place at ASU in Downtown Phoenix. During this visit, we will ask you to fill out a questionnaire about your household, your child’s health, and your family’s health. The rest of this visit is described in detail below. After this visit, your child will be selected at random to one of two groups. No matter what group your child is in, we will ask them to come to ASU for testing a total of 4 times over the next year. These visits will take place now, in 3-months, 6-months, and 12-months from now and will be exactly the same.

Health Examination and Diabetes Test:
You and your child will be scheduled to come to ASU in the morning. Your child will be asked not to eat or drink anything other than water after 10 PM the night before. We will do the following:

- Ask you and your child questions about their medical and family history.
- Ask your child questions about family/friends, culture, exercise and nutrition, their bodies, and how they feel about themselves and their bodies.
- Measure your child’s height, weight, blood pressure, pulse, temperature, and waist.
- Nurses will insert a plastic needle in a vein in your child’s arm to draw blood. This will stay in for about 2.5-3 hours. We will take blood 6 times for a total of about 5 tablespoons (75 mL) from your child over the course of 2.5 hours. During this time we will measure health indicators such as cholesterol, lipids (blood fat), glucose (sugar), insulin, and liver markers to make sure your child is healthy. We will perform a diabetes-screening test called an Oral Glucose Tolerance Test (OGTT) where your child will drink a sugar drink. We will measure glucose and insulin during this test to tell us how well your child’s body uses sugar and if your child has diabetes. During this test we will ask your child to sit in a chair or
bed and not get up except to use the bathroom. Your child can watch TV, read, or play video games if they want.

- We also are asking your permission to draw and store an extra tablespoon (10 mL) of blood so that, as new indicators related to diabetes are discovered, we can measure them in your child’s blood without having you come back for another blood draw and sign another permission form. We may share this information with other researchers who work with us, but we will never give out your child’s name. If you agree to have your child’s blood stored for future use, you give us permission to share this blood with other researchers without telling you.
- We will ask your child to walk on a treadmill for about 10 minutes to measure their fitness level.

The total time you and your child will spend will be about 4-5 hours. A light snack will be given at the end of the visit. This visit will tell us if your child is eligible to participate. You will be given the results of the diabetes screening tests. If these tests suggest that your child may be diabetic or has some other health problem, they will not be eligible for the study. We will recommend that you talk with a doctor right away.

Program Description
After the first visit, your child will be selected at random to participate in one of two groups (described below).

Group One: Will enter into a 12-week education and exercise program shortly after testing. This 12-week program includes weekly, one-hour lifestyle education classes held at the Lincoln Family YMCA in downtown Phoenix. We will talk about healthy eating, exercise, and ways to prevent diseases such as diabetes. We will also talk about the roles and responsibilities of parents and children, and self-esteem.

You must be able to attend these sessions with your child in order for your child to be able to participate in the program. Your child will also attend 3 one-hour exercise sessions per week at the YMCA with other kids in the program. These sessions are designed to be fun and to help your child enjoy exercise. You do not need to attend the exercise sessions. Upon enrolling in the program, your child will receive a YMCA membership, which is good for one year at any YMCA in Phoenix. After the 12-week program, there will be a monthly follow-up program for three more months. The follow-up program will include 3 education sessions that last about an hour where we will ask you to update us on how things are going and if it has been hard to keep up with the changes made during the program. All program sessions will be in the evenings. Your child will be asked to participate in at least 75% of the education and exercise sessions. Your child must have positive behavior in order to remain in the program.

Group Two: Will be asked to complete all 4 testing visits over the next year before being invited to participate in a program. This program will inform you and your child about healthy nutrition and the importance of exercise in preventing diseases such as diabetes. With this program, your child will also receive a 1-year YMCA membership good at any YMCA in Phoenix. Because you and your child will wait 1-year before receiving the program we will contact you on a monthly basis to stay in touch, make sure contact information is up-to-date, and update you on the next steps.
RISKS
There may be parts of this study your child may find uncomfortable or unpleasant. Your child will have to have a needle stick for blood drawing. Also, he/she will have to remain seated during the diabetes test (about 2-3 hours) and will not be allowed to walk around during this time. Your child may feel uncomfortable in answering some of the questions. Your child may be injured while exercising at the YMCA for this study.

General Discomforts
Your child will not eat breakfast and may feel hungry, dizzy, nauseous and weak or like passing out. These feelings are usually short and are not serious. In rare instances, your child may throw up or pass out. A light snack will be provided when study visits are over.

Risk of Blood Drawing and Needle Placement
Up to 75 ml of blood (5 tablespoons) will be drawn during each testing visit.

The most common problems from the placement of needles for drawing blood in the arm:
- Pain and bruising
- Feeling lightheaded or faint during the procedure
- Bleeding at the site where the catheters are placed
- Infection, bleeding, or clotting where the catheter enters the skin or inflammation of the vein

The risks are greatly reduced by having an experienced and trained person inserting the needle and by using sterile and clean techniques. Sometimes, a needle may need to be replaced because we cannot draw blood from it or it falls out. We will always ask your child’s permission before we do this.

Risk of Exercise
Your child may experience soreness, muscle strains, sprained ligaments, and be tired during and after the exercise. We will reduce these risks by developing a special program for their age and fitness level. A trained YMCA instructor or ASU student will lead the exercise program.

COMPENSATION FOR ILLNESS AND INJURY
If you give permission for your son/daughter to participate in this study, then your consent does not waive your legal rights. However, no funds have been set aside to compensate you or your child in the event of injury. However, if any injury occurs due to the experimental procedures, first aid will be provided. If there is a situation that the research nurse believes needs attention by a primary care practitioner, you will be referred to the Nurse Practitioner Clinic on the Downtown ASU campus. If any injury occurs after the Nurse Practitioner Clinic is closed you should seek attention at an urgent care facility. If a medical emergency were to occur during this study, we will call “911” to bring emergency medical personnel to ASU. You will be responsible for any costs incurred.

ALTERNATIVE TREATMENTS
There are no alternative procedures available for this study. If you do not want your child to participate in the study you can ask your doctor for a physical exam and enroll them in a weight loss program on your own.

BENEFITS
Your child will receive a diabetes screening from this study and will receive education or information about diabetes and health. Copies of his/her diabetes screening results will be given to you and any findings will be available to his/her doctor upon your request in writing. The tests we perform are for research purposes only and do not serve to diagnose a condition or disease. If the tests suggest your child has diabetes or other health condition, they will not be allowed to participate and we will recommend they go see a doctor. The information from this study will also be of benefit to researchers to better understand health in the Latino community.

Parent/legal guardian’s Initials

ELSC Randomized Control Trial 1-17-14

NEW INFORMATION
If we find new information during the study that would change your choice about letting your child participate, then we will give you this information.

CONFIDENTIALITY
All information obtained in this study is confidential unless the law makes us disclose it. The results of the study may be published or presented but your name or identity will be kept private. To keep information private, we will use codes for blood samples and laboratory results from all research volunteers and only the study team will have access to that information. The study records will be kept double-locked in a data storage room.

WITHDRAWAL PRIVILEGE
It is OK for you or your child to say no. Even if you say yes now you are free to say no later, and withdraw your child from the study at any time. Your decision will not affect your relationship with Arizona State University, St. Vincent DePaul Medical Clinic or the YMCA or otherwise cause a loss of benefits to which you might otherwise be entitled.

COST
The researchers want your decision about allowing your child to participate in the study to be your choice. There is no cost to you or your family to be part of this project.

COMPENSATION
Your child will receive compensation in the amount of $50.00 for each visit to ASU Downtown. If your child does not finish the tests, they will receive $10 per hour for every hour spent, but will not exceed $50.00. If your child completes the study, they will receive a 1-year membership to the YMCA. During the study, your child will be able to earn points for their attendance and participation that can be redeemed for prizes such as T-shirts, gift cards, and/or movie passes. The research team may ask your child to leave the study at any time without consent. Your child will be compensated for their time spent but may lose their YMCA membership.
VOLUNTARY CONSENT
Any questions you have concerning the research study or your child’s participation, before or after your consent, will be answered by Dr. Shaili, 500 N. 3rd St, Phoenix AZ 85002, 602-496-0909.

If you have questions about your child’s rights as a participant in this research, or feel he/she has been placed at risk you may contact the Chair of the Human Subjects Institutional Review Board through the Research Compliance Office at (480) 965-6788.

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

Your signature below indicates that you consent for your child to participate in the above study.

Your child’s name (please print): ________________________________

Parent/legal guardian’s Signature _____________________________
Printed Name _____________________________ Date _____________

Your initials here indicate that you give us permission to draw and store 10 mL (about 1 tablespoon) of your child’s blood for future use and allow us to share this blood with other investigators we work with without contacting you first.

I □ DO consent to have my child’s blood drawn and stored for future analyses.

_____________________________
Parent/legal guardian’s initials

Parent/legal guardian’s Initials _____________________________
ELSC Randomized Control Trial 1-17-14
We are asking your permission to collect additional blood samples for future genetic testing (11 ml or .75 tablespoons) related to diabetes research. This information will help us understand how genes affect diabetes risk. For example, information from genetic material such as DNA and RNA in blood may help researchers explain why children respond differently to the same intervention. This information may also help us identify children who are at greatest risk of developing type 2 diabetes. We hope that this part of the project will someday help doctors prevent and treat obesity-related disease such as type 2 diabetes. If you and your child agree to this collection, you are giving us permission to share these samples with other researchers without contacting you first. No identifiable information will be on the blood collection tubes and we will not share your personal information with anyone. If you decide in the future that you do not want these samples to be shared or used, you can tell us to destroy them. These tests are optional and you and your child do not have to agree to this collection to be in the project. These tests are for research only. We will not give the results to you or your doctor.

Do you agree to allow us to collect blood for genetic research related to diabetes risk?

Yes ☐

Parent/legal guardian’s Signature ____________________________  Printed Name ____________________________  Date ____________________________

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 offered the participant a copy of this signed consent document.”

Investigator’s Signature / Person Obtaining Consent ____________________________  Printed Name ____________________________  Date ____________________________

ELSC Randomized Control Trial 1-17-14
Consentimiento del Padre/Guardián Para Participar en un Estudio de Investigación

Título de la investigación: Programa comunitario para la prevención de la diabetes en jóvenes latinos obesos.

Investigador Principal: Gabriel Q. Shaibi, PhD
Arizona State University
Teléfono de día: 602-496-0909

INTRODUCCIÓN:
El propósito de esta forma de consentimiento es 1) para proveerle a usted, la información debida sobre la investigación para ayudarle a decidir si dejaría o no a su hijo/a participar en la investigación y 2) para documentar su consentimiento de la participación de su hijo/a en este estudio.

INVESTIGADORES:
Gabriel Shaibi, PhD, y sus colegas de Arizona State University (ASU) han invitado usted y a su hijo/a ser parte de este estudio.

PROPÓSITO DEL ESTUDIO:
El propósito de esta investigación es para ver si un programa de 12 semanas de educación de estilo de vida y ejercicio mejorará la salud de jóvenes latinos obesos. Le estamos pidiendo a su hijo/a que tome parte en este estudio porque usted se clasifica de ascendencia latina y su hijo/a es obeso. Estas características ponen a su hijo/a al riesgo de desarrollar la diabetes tipo 2. Este proyecto comparará los cambios en la salud de dos grupos de jóvenes durante el próximo año. A un grupo se le pedirá que participe en un programa de educación de estilo de vida y ejercicio y que dure 12 semanas en la YMCA en Phoenix y el otro grupo será puesto en una lista de espera para completar un programa de salud dentro de un año. Selección de grupo será al azar y su hijo/a tiene una oportunidad de 50/50 de estar en uno de estos dos grupos. Aproximadamente 160 jóvenes participarán en este estudio y se someterán a los exámenes descriptos al seguir.

DESCRIPCIÓN DEL ESTUDIO DE INVESTIGACIÓN:
Si usted da permiso a su hijo/a de participar en este estudio, haremos una evaluación de salud para asegurarnos que su hijo/a es saludable y capaz de participar en este estudio. Las visitas de evaluación de salud durarán entre 4.5-5 horas y se llevarán a cabo en ASU en el centro de Phoenix. Durante esta visita, le pediremos a usted que llene un cuestionario referente a su familia, la salud de su hijo/a, y la salud de su familia. El resto de esta visita es descrita en detalle debajo. Después de esta visita, su hijo/a será seleccionado al azar a uno de los dos grupos. No importa el grupo en que se encontrará, le pediremos que regrese a ASU para estas evaluaciones de salud un total de 4 veces durante el próximo año. Estas visitas se llevarán a cabo hoy, en 3 meses, en 6 meses, y en un año y serán exactamente iguales.

Evaluación de Salud y Examen de Diabetes:
Se le dará a usted y a su hijo/a una cita de mañana para ir a ASU. Su hijo/a tendrá que ayunar esa mañana y también no podrá comer ni beber nada, con la excepción de agua, después de las 10 de la noche anterior. Haremos lo siguiente:

- Preguntarle a usted y a su hijo/a preguntas referentes a su historial médico y familiar.
- Le haremos preguntas a su hijo/a sobre sus amistades, su familia, su cultura, el ejercicio, la nutrición y sus pensamientos de su cuerpo y de sí mismo.
- Mediremos la estatura, peso, presión arterial, pulso, temperatura y cintura de su hijo/a.
- La enfermera insertará un catéter plástico en una vena del brazo de su hijo/a para sacar una muestra de sangre. Esto se mantendrá adentro aproximadamente 2.5-3 horas. Tomaremos 6 muestras de sangre por un total de aproximadamente 5 cucharadas (75 mLs) de su hijo/a por el curso de 2.5 horas. Durante este tiempo, mediremos indicantes de salud tal como el colesterol, lípidos (grasa en la sangre), glucosa,
(azúcar), insulina, y marcadores de hígado para asegurarnos que su hijo/a está saludable. Se le hará un examen de la diabetes llamado Oral Glucose Tolerance Test (OGTT) donde su hijo/a tomará una bebida azucarada. Mediremos la glucosa e insulina durante este examen para probar que tan eficaz el cuerpo de su hijo/a utiliza el azúcar y si su hijo/a tiene diabetes. Durante la prueba se le pedirá a su hijo/a que se siente en una silla o cama, y se le pedirá que permanezca sentado/a al menos que tenga que ir al baño. Su hijo/a puede ver televisión, leer, o jugar video juegos si gusta.

- También le estamos pidiendo permiso de sacar una cuadrada (10 mLs) extra de sangre para poder medir nuevos indicadores relacionados a la diabetes que puedan surgir sin que su hijo/a tenga que volver para dar otra muestra de sangre y usted firmar otro consentimiento. Podemos compartir esta información con otros investigadores quienes trabajan con nosotros, pero nunca daremos el nombre de su hijo/a. Si usted da permiso de poder sacar y guardar sangre extra para futuros análisis, nos está dando permiso de compartir esta sangre con otros investigadores sin notificarlo a usted.
- Le pediremos a su hijo/a que camine en una caminadora por aproximadamente 10 minutos para medir su nivel de condición física.

El tiempo total que usted y su hijo/a estarán en ASU será entre 4-5 horas. Se le dará un almuerzo ligero al terminar la cita. Esta visita nos informará si su hijo/a es elegible para participar. Se le darán los resultados del examen de diabetes. Si los exámenes indican que su hijo/a tiene diabetes o algún otro problema médico, no serán elegibles para participar. Le recomendaríamos que visite a su médico inmediatamente.

Descripción del Programa
Después de la primera visita, su hijo/a será seleccionado al azar para participar en uno de dos grupos (detalles debajo).

Grupo Uno: Entrará a un programa de educación de estilo de vida y ejercicio de 12 semanas un poco después de la evaluación de salud. Este programa de 12 semanas incluye clases semanales de educación de estilo de vida por una hora que se llevarán a cabo en el Lincoln Family YMCA en el centro de Phoenix. Queremos hablarle acerca de comer saludable, ejercicio, y maneras de prevenir enfermedades como la diabetes. También platicaremos de las responsabilidades de los padres e hijos y la autoestima.

Usted debe tener la habilidad de asistir estas clases con su hijo/a para que su hijo/a pueda participar en este programa. Su hijo/a también atenderá a una clase de ejercicio de una hora 3 veces a la semana en el YMCA con los otros jóvenes en el programa. Estas clases son diseñadas para ser divertidas y ayudarle a su hijo/a que disfrute el ejercicio. Usted no tendrá que participar en estas clases de ejercicio. Al inscribirse en este programa, su hijo/a recibirá una membresía al YMCA, que podrá usar por todo un año y puede ser usado en todos los YMCAs en Phoenix. Al terminar el programa de 12 semanas, habrá clases de seguimiento mensuales por 3 meses. El programa de seguimiento incluye 3 sesiones educativas que durarán una hora donde le preguntaremos que nos informe como ha estado y si los cambios hechos por medio del programa han sido difíciles de sostener. Todas las clases serán por la tarde. Le pediremos a su hijo/a que participe por un mínimo de 75% de las sesiones de educación y ejercicio. Su hijo/a tendrá que comportarse de una manera positiva para mantenerse en el programa.

Grupo Dos: Le pediremos que complete 4 visitas de evaluación de salud durante el próximo año antes de ser invitado a participar en un programa. Este programa le informará a usted y a su hijo/a acerca de la nutrición saludable y la importancia del ejercicio en prevenir enfermedades como la diabetes. En este programa su hijo/a también recibirá una membresía al YMCA por un año la cual servirá para ir a cualquier YMCA en la ciudad de Phoenix. Por la razón que usted y su hijo/a esperarán un año antes de poder recibir un programa, nos mantenemos en contacto con usted mensualmente para asegurarnos que tenemos su información al corriente y para informarse de los próximos pasos.

RIESGOS:

Iniciales del Padre/Guardián__________________________
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Podrán haber partes de la investigación que su hijo/la encuentre incomoda. Por ejemplo, se le pondrá una aguja para extraer sangre a su hijo/la. También, su hijo/la tendrá que mantenerse sentado durante la prueba de diabetes (casi 2-3 horas) y no se le permitirá caminar durante este tiempo. Su hijo/la podrá sentirse incomodo al contestar algunas de las preguntas que se le harán. Su hijo/la puede ser lastimado/a mientras este ejercitando en el YMCA durante el estudio.

Incomodidades Generales:
Su hijo/la no desayunará y podrá sentirse con hambre, mareos, náuseas y débil, o sensación de desmayo. Estas sensaciones usualmente son breves y no son serias. En raras ocasiones su hijo/la puede vomitar o desmayarse. Le proveeremos un bocadillo ligero cuando la visita termine.

Riesgos de la extracción de sangre y la colocación de la aguja
Un total de 75 mLs (5 cucharadas) de sangre serán extraídos durante cada visita de exámenes de laboratorio.

Los problemas mayormente conocidos de la colocación de la aguja en el brazo son:
- Dolor y molestias
- Mareos o sensación de desmayo durante el procedimiento
- Sangrado en el lugar donde los catéteres se colocan
- Infección, sangrado, o oclusión donde el catéter entra en la piel o inflamación de la vena

Se disminuyen tales riesgos cuando una persona con experiencia y entrenamiento pone la aguja y usan técnicas estériles. Si un catéter no puede extraer sangre o se cae de la vena se necesitará reemplazar. Si esto sucede, se le pedirá permiso a su hijo/la antes de hacerlo.

Riesgos del Ejercicio:
Existe la posibilidad de que su hijo/la sienta dolor, tensión muscular, esguince de ligamentos, y cansancio durante y después del ejercicio. Disminuiremos estos riesgos al desarrollar un programa especial y adecuado para la edad y el nivel de condición física de su hijo/la. El programa de ejercicio será dirigido por un entrenador profesional de la YMCA o un estudiante de ASU.

COMPENSACIÓN POR ENFERMEDAD Y LESIONES
Si usted da permiso para que su hijo participa en el estudio su consentimiento no renuncia a sus derechos legales. No se han asignado fondos para compensar a su hijo/la en caso de una lesión. Sin embargo, si alguna lesión ocurre debido a los procedimientos del experimento, los primeros auxilios estarán disponibles. Si hay una situación en que la enfermera de la investigación cree que necesita atención de un médico de atención primaria, usted será referido al Nurse Practitioner Clinic en el campus de ASU Downtown. Si alguna lesión ocurre después de las horas de servicios en el Nurse Practitioner Clinic, usted deberá buscar atención médica en un centro de atención de urgencia. Por si acaso ocurre una emergencia durante el estudio, hablaremos al “911” para traer personal médico de emergencia a ASU. Usted será responsable de cualquier costo eventual.

TRATAMIENTOS ALTERNATIVOS
No hay procedimientos alternativos para esta investigación. Si usted no quiere que su hijo/la participe en el estudio, usted puede pedirle al médico por un examen físico e inscribir su hijo en un programa de perder peso independientemente.

BENEFICIOS
Esta investigación le dará a su hijo/la un análisis de diabetes y educación de diabetes y salud. Las copias de los resultados de la evaluación de diabetes de su hijo/la se le dará a usted y cualquier resultado estará disponible para su doctor si usted lo solicita por escrito. Los análisis hechos son para fines de investigación solamente, y no son usados para una diagnosis médica. Si cualquier de los resultados de su hijo sugiere que su hijo tiene diabetes u otra condición de la salud, no se permitirá participar y le recomendaremos que haga una cita con un
médico. La información de este estudio será de beneficio para los investigadores al darles un mejor conocimiento sobre la salud de los Latinos.

INFORMACIÓN NUEVA
Si descubrimos nueva información durante el tiempo de la investigación que pueda cambiar su decisión de permitir a su hijo/a participar, entonces le daremos esta información.

CONFIDENCIALIDAD
Toda la información que se obtenga a través de esta investigación será confidencial, al menos que la ley nos haga divulgarla. Los resultados de esta investigación se podrán publicar o presentar pero se protegerá el nombre de su hijo/a y su identidad. Para mantener la información privada utilizaremos códigos para las muestras de sangre y los resultados de laboratorios para el uso de todos los voluntarios y solo el equipo de estudio tendrá acceso a esta información. Los archivos del estudio se mantendrán cerrados bajo doble llave en una sala de almacenamiento de datos.

PRIVILEGIO DE RETIRO
ESTÁ BIEN para usted o su hijo/a decir no. Aunque esté de acuerdo en este momento, usted y su hijo/a podrán decir no más tarde, y retirarse de la investigación en cualquier momento. Su decisión no afectará su relación con Arizona State University, St. Vincent de Paul Medical Clinic, o la YMCA ni de otra manera causará la pérdida de otros beneficios que podría recibir.

COSTO
Los investigadores desean que la decisión de permitir a su hijo/a participar en esta investigación sea absolutamente voluntaria. No habrá ningún costo para usted o su familia por participar.

COMPENSACIÓN
Su hijo/a recibirá una recompensa de 50 dólares por cada visita a ASU en el centro de Phoenix. Si su hijo/a no termina la evaluación de salud, entonces él/ella recibirá $10/hora por el tiempo que estuvo en la clínica, pero no puede sobrepasar $50. Si su hijo/a termina el estudio, recibirá membresía al YMCA por un año. Durante el estudio, su hijo/a podrá ganar puntos para su asistencia y participación que pueden ser redimbidos por premios tales como camisetas, tarjetas de regalo, y/o pases para el cine. El investigador le podrá pedir a su hijo/a que se retire de la investigación en cualquier momento sin consentimiento. Se le dará una recompensa a su hijo/a por su tiempo pero su hijo/a puede perder su membresía del YMCA.
CONSENTIMIENTO VOLUNTARIO
Cualquier preguntas que tenga correspondientes a la investigación o la participación de su hijo en el estudio, antes o después de su consentimiento, será contestada por el Dr. Shabii, 500 N. 3rd Calle, Phoenix, AZ 85004. Número de teléfono: 602-436-0909.

Si usted tiene preguntas sobre los derechos de su hijo como participante de esta investigación, o si usted siente que su hijo/a estuvo en peligro en algún momento, puede contactar a Chair de Human Subjects Institutional Review Board, por ASU Office of Research Integrity and Assurance, al número 480-965-6788.

Esta forma explica la naturaleza, las demandas, los beneficios y cualquier riesgo de la investigación. Al firmar esta forma usted acepta cualquier riesgo involucrado. Recuerde que la participación de su hijo/a es voluntaria. Usted puede decidir que no permite participar a su hijo/a o retirar su consentimiento y descontinuar la participación de su hijo/a en cualquier momento sin pena o pérdida de beneficios. Al firmar este consentimiento usted no renuncia a ningún reclamo legal ni derecho legal. Se le dará una copia de este consentimiento.

Su firma abajo indica que usted da consentimiento para que participe su hijo en en la investigación.

Nombre de Hijo/a: __________________________

_________ Firma del Padre/Guardián Legal ______ _______ Nombre _______ Fecha ______

Sus iniciales aquí indican si usted nos da permiso de sacar y guardar 10 ml (aproximadamente una cucharada) de la sangre de su hijo/a para uso futuro.

☐ Doy mi consentimiento para que extraigan y guarden sangre de mi hijo/a para uso futuro.

_________ Iniciales del Padre/Guardián ______
Estamos pidiendo su permiso para colectar muestras de sangre adicionales para usar en futuras pruebas genéticas (11 ml o .75 cucharadas) relacionadas a la investigación de la diabetes. Esta información nos ayudará a entender cómo los genes afectan el riesgo de diabetes. Por ejemplo, información de materiales genéticos como el ADN o ARN en la sangre puede ayudar a investigadores explicar porque algunos jóvenes responden diferente a la misma intervención. Esta información también puede ayudar a identificar a jóvenes que están en mayor riesgo de desarrollar diabetes tipo 2. Esperamos que esta parte del proyecto pueda algún día ayudar a médicos prevenir y tratar las enfermedades relacionadas con la obesidad como la diabetes tipo 2. Si usted y su hijo/a están de acuerdo con esta colección, nos están dando permiso a compartir estas muestras con otros investigadores sin que se les contacte primero. Ninguna información identificable estará en los tubos de colección de sangre y su información personal jamás será compartida. Si en el futuro usted desea que estas muestras de sangre no sean usadas o compartidas, usted nos puede pedir que estas muestras sean destruidas. Estas pruebas son opcionales y usted y su hijo/a no tienen que participar en esta colección para estar en el proyecto. Estas pruebas son sólo para investigación. Estos resultados no serán compartidos con usted o su doctor.

¿Está de acuerdo en permitirnos colectar estas muestras de sangre para investigaciones genéticas relacionadas a la diabetes?

Sí  [ ]

Firma del Padre/Guardián Legal  Nombre  Fecha

DECLARACIÓN DEL INVESTIGADOR
"Yo certifico que he explicado al individuo mencionado arriba la naturaleza y el propósito, los beneficios potenciales y los riesgos posibles asociados al participar en esta investigación, he contestado todas las preguntas que han hecho y soy testigo de su firma. Estos elementos de Consentimiento informado conforman la Garantía dada por Arizona State University a la Office for Human Research Protections para proteger los derechos de sujetos humanos. Yo le he dado participante una copia de este consentimiento firmado."

Firma de Investigador  Nombre  Fecha
Persona Obteniendo el Consentimiento

Iniciales del Padre/Guardián  
ELSC Ensayo Controlado Aleatorizado 2-12-14  
Child Assent for Research

Study Title: Community-based diabetes prevention program for obese Latino youth

Principal Investigator: Gabriel Q. Shaibi, PhD
Arizona State University
Day Time Phone: (602) 496-0909

What is the Project About?
We want to learn if a health education and exercise program can help Latino teens be healthier and feel better about themselves. This project will have 2 groups. One group will participate in the education and exercise program now and the other group will wait a year to receive education and a YMCA membership. You have a 50/50 chance of being selected to one of the two groups (like a coin flip). No matter what group you are put in, we will ask you to come to Arizona State University (ASU) for 4 visits over the next year. The first visit will be before group selection and the next three visits will be 3-months, 6-months, and 12 months after group selection. Each visit will be the same and will include blood tests, questions, and a health exam similar to when you go to the doctor. If you are selected to the health program, you will start a 12-week education and exercise program at the YMCA in Phoenix. If you are selected to the waiting group, you will wait a year for your program to start.

What Will the Four Visits at ASU be like for 4.5-5 hours?
Before you start the project, we need to make sure you are healthy so we will do the following:
1. Ask you and your parents questions about your health.
2. Measure your height, weight, blood pressure, temperature, heart rate, and waist.
3. Ask you questions about exercise and eating, culture, friends, family, school, and your body. Some of these questions may seem weird and personal because they are about your body. If you do not want to answer a question just skip it.
4. Measure the fats in your blood and do a diabetes test. Fats in the blood place you at risk for heart disease. Diabetes is when the sugar in your blood is too high. For the diabetes test we will take blood before and after you drink a really sweet drink. We will put cream on your arm so you do not feel the needle as much. We will put a small plastic tube in a vein in your arm that will stay in for about 2-3 hours. The tube is flexible so you can move your arm. We will take blood 6 times (about 5 tablespoons) from this tube. This test will tell us if you have diabetes and how well your body uses sugar. We will ask you to stay seated during this test but you can get up to go to the bathroom. You can read a book, play a video game or watch a movie so the visit does not feel long.
5. If you give us permission, we will collect and store blood for future use.
6. Ask you to walk on a treadmill for 10 minutes to see how fit you are.
7. Once the testing is done, we will give you a snack.

If I am selected to be in the health program, what will it be like at the YMCA?
1. You and your parent(s) will be in a group health education program with other families. The program will be once a week for about one hour. The classes will be held in the afternoon.
2. You will also exercise 3 times a week with a group of other kids your age at the YMCA.
3. Each session will last about one hour and will be taught by a YMCA trainer or ASU Student.
4. You and your trainer will keep track of how much you exercise.
5. The program will last 12 weeks (about 3-months).
6. You may be able to earn points for attendance and your participation in the program. These can be turned in for prizes like T-shirts, gift cards, or movie passes.
7. After the program is over, we will ask you to come back to ASU for follow-up tests like the ones you did before the program started.
8. We will ask you to come back to the YMCA once a month for 3-months for reminder classes. These classes will talk about healthy changes you made and anything that makes it hard to stay healthy.
9. You will be allowed to use the YMCA after the program is over for the rest of the year.

ELSC – Randomized Control Trial 1-17-14
What if I am selected to be in the waiting group?
If you are randomly selected to be in the waiting group then you will be asked to finish all 4 testing visits over the next year and then we will invite you to participate in a health program. At this time, you will also get a 1-year YMCA membership good at any YMCA here in Phoenix. But, before that happens, we want to keep in contact with you over the next year to get all the tests done. We will ask you to keep your regular lifestyle. We will contact you once a month to check in and so you can keep us updated on how things are going.

What are the Risks?
You might be sore from where we take blood and small bruises may appear. You might feel dizzy or like throwing up during the blood draw. When you come to ASU for testing, you will not be able to eat anything after about 8pm the night before. You won’t eat anything until after the tests are done the next morning (about 11 or 12 o’clock). You might feel hungry in the morning. Some of the questions we ask are personal so you may feel uncomfortable about answering them. During the exercise program, you may feel out of breath, light-headed, and your heart will be beating fast. After the exercise, your body may be sore and you might be tired.

What are the Benefits?
There may not be any direct benefits for participating. But, education and exercise programs can help kids feel better about themselves and become healthier.

Does it Cost Anything?
The project does not cost you or your family any money. We will give you $50.00 after each testing day at ASU. If you don’t complete a testing day, we will give you $10.00 per hour up to $50.00. You will get a 1-year membership to the YMCA if you complete the program. You will also be able to earn points for participation that can be redeemed for prizes like: gift cards, t-shirts, and/or movie tickets.

Do I have to do this?
You do not have to be in this project. The choice is yours. No one will be mad at you if you decide not to do this. Even if you start the project, you can stop later if you want.

Can the Researchers ask me to Leave?
You may be asked to leave the study. Things like disruptive behavior, being mean to other participants, and not cooperating are examples of reasons that you may be asked to leave. Also, if you miss more than 25% of the classes, you may be asked to leave.

Agreement:
Signing here means that you have read this form or have had it read to you and you want to be in this project.

Signature of participant ________________________________

Participant’s printed name ___________________________ Date ______________________

Please mark the box below to indicate whether you allow us to collect and store extra blood for future use.

☐ I DO allow the researchers to collect and store an extra sample of my blood for future use.

Initials ____________________________

ELSC – Randomized Control Trial 1-17-14
We have another part of the project we would like you to be part of. We would like to measure genetic information related to diabetes (DNA and RNA) in your blood. This information will help us understand how genes affect diabetic risk. We will need to take some extra blood (11 ml or .75 tablespoons) before you drink the sugar drink. We will save this genetic information from your blood. We might share it with other researchers we work with. We will not put your name on this blood we will only put a number on it. You do not have to say yes to this additional blood draw to participate in the project. If you decide in the future that you do not want us to use these samples, you can tell us to destroy them.

Do you agree to allow us to collect blood for genetic research related to diabetes risk?

Yes □

____________________________________________________
Participant’s Signature

____________________________________________________
Printed Name

____________________________________________________
Date

____________________________________________________
Signature of Parent/Legal Guardian

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 offered the participant a copy of this signed consent document.”

____________________________________________________
Investigator’s Signature / Person

Obtaining Consent

____________________________________________________
Printed Name

____________________________________________________
Date

ELSC – Randomized Control Trial 1-17-14
Help Build Healthy Latino Families!

What will I learn?

- About my own health
- How to maintain a healthy lifestyle

Do I qualify?

- If you are Latino/Hispanic, 14-16 years old, and have a BMI of 95th percentile or above, please call us!

What are the benefits of my participation?

- Free YMCA Membership
- Free nutrition and exercise classes
- Free diabetes screening
- Free health information

For more information please call (623) 738-4278.
¡Sé parte del estudio ASU Every Little Step Counts!

¡Ayuda a edificar familias Latinas saludables!

¿Qué aprenderé?
- Sobre mi propia salud
- Sobre cómo mantener una vida saludable

¿Soy elegible?
- ¡Si eres Latino/Hispano, tienes entre 14 y 16 años, y tu porcentaje de IMC es 95% o más, llámanos!

¿Cuáles son los beneficios de mi participación?
- Membresía gratis para el YMCA
- Información gratis sobre la salud
- Clases gratis sobre la nutrición y el ejercicio
- Chequeo gratis para la diabetes

Para más información llame al (623) 738-4278.
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<th>Does the child's</th>
<th>1a. Mother have Type 2 diabetes?</th>
<th>2a. Father have Type 2 diabetes?</th>
<th>3a. Maternal grandmother have Type 2 diabetes?</th>
<th>4a. Maternal grandfather have Type 2 diabetes?</th>
<th>5a. Paternal grandmother have Type 2 diabetes?</th>
<th>6a. Paternal grandfather have Type 2 diabetes?</th>
<th>7a. Sibling (any) have Type 2 diabetes?</th>
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<td>2b.</td>
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<td>Age of diagnosis 1c.</td>
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<td>4c.</td>
<td>5c.</td>
<td>6c.</td>
<td>7c.</td>
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<tr>
<td>Treatment? Diet? Exercise Pills Insulin None/Unknown</td>
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</tr>
<tr>
<td>Gestational Diabetes 8. Did this child's mother ever have diabetes during pregnancy? Yes</td>
<td>No</td>
<td>Don't Know</td>
<td></td>
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<tr>
<td>8a. Was the child's mother diabetic during pregnancy with this child? Yes</td>
<td>No</td>
<td>Don't Know</td>
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<tr>
<td>8b. Was the diagnosis: Before this pregnancy? During 1st trimester? During 2nd trimester? During 3rd trimester?</td>
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<tr>
<td>8b. Did diabetes continue after this pregnancy? Yes</td>
<td>No</td>
<td>Don’t Know</td>
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<tr>
<td>9. Did the child's mother have gestational diabetes: a. Before this pregnancy? b. After this pregnancy? Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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</tbody>
</table>

Revised 5-6-13
APPENDIX F

FOOD FREQUENCY QUESTIONNAIRE
Think about everything you ate or drank last week. Remember what you had for breakfast, lunch, dinner, after school, while watching TV, at bedtime, and on the weekend. Please write your name in this box. **Use a pencil to complete this survey.**

<table>
<thead>
<tr>
<th>ID NUMBER</th>
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<tbody>
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</tbody>
</table>

**HOW MANY DAYS LAST WEEK DID YOU EAT OR DRINK IT?**

<table>
<thead>
<tr>
<th></th>
<th>Never last week</th>
<th>1 day last week</th>
<th>2 days last week</th>
<th>3-4 days last week</th>
<th>5-6 days last week</th>
<th>Every day last week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal, like corn flakes, Frosted Flakes</td>
<td></td>
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<tr>
<td>Cooked cereal, like oatmeal</td>
<td></td>
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<tr>
<td>Eggs, breakfast sandwiches or breakfast burritos</td>
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<tr>
<td>Breakfast bars, granola bars, Protein bars</td>
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<tr>
<td>Glasses of milk</td>
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<tr>
<td>Real fruit juice, like orange juice, apple juice, or Mexican fruit drinks like licuados (DO NOT include soda)</td>
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<tr>
<td>Drinks like Coke or 7-Up, Sunny Delight, Hawaiian Punch, or aguas frescas (DO NOT include diet soda)</td>
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<tr>
<td>Apples, bananas, or oranges</td>
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<tr>
<td>Applesauce, fruit cocktail</td>
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<tr>
<td>Any other fruit, like strawberries, grapes</td>
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<tr>
<td>French fries, hash browns, tater tots</td>
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<tr>
<td>Other potatoes, like mashed or boiled</td>
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<tr>
<td>Ketchup or salsa</td>
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<tr>
<td>Lettuce salad</td>
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<tr>
<td>Tomatoes, including on salad</td>
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<tr>
<td>Green beans or peas</td>
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<tr>
<td>Other vegetables, like corn, carrots, greens, broccoli</td>
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<tr>
<td>Vegetable soup, tomato soup, any soup or stew with vegetables in it</td>
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<tr>
<td>Chili beans, pinto beans, black beans, including in burritos</td>
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</tbody>
</table>

**HOW MUCH IN ONE DAY?**

<table>
<thead>
<tr>
<th></th>
<th>A little</th>
<th>Some</th>
<th>A lot</th>
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<tbody>
<tr>
<td>1 bowl</td>
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<td>2 bowls</td>
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<td>3 bowls</td>
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<td>A little</td>
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<td>Some</td>
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<tr>
<td>A lot</td>
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<td>1 egg</td>
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<td>2 eggs</td>
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<td>3 eggs</td>
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<td>1 glass</td>
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<td>2 glasses</td>
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<td>3 glasses</td>
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<td>1 pint</td>
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<td>2 pints</td>
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<td>Some</td>
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<tr>
<td>A lot</td>
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</table>

Turn this page over  

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<table>
<thead>
<tr>
<th>Food Item</th>
<th>None last week</th>
<th>1-2 days last week</th>
<th>3-4 days last week</th>
<th>5-6 days last week</th>
<th>Every day last week</th>
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</thead>
<tbody>
<tr>
<td>Refried beans</td>
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<tr>
<td>Hamburgers, cheeseburgers</td>
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<tr>
<td>Hot dogs, corn dogs, or sausage</td>
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<tr>
<td>Lunch meat like boloney, ham, Lunchables</td>
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<tr>
<td>Pizza or pizza pockets</td>
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<tr>
<td>Spaghetti or ravioli with tomato sauce</td>
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<td>Macaroni and cheese</td>
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<tr>
<td>Chicken, including nuggets, wings, tenders, also in sandwiches or stew</td>
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<tr>
<td>Fish, fish sticks or sandwiches, tuna, shrimp</td>
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<td>Burritos or tacos</td>
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<td>Beef like roast, steak or in sandwiches</td>
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<tr>
<td>Meat balls, meat loaf, beef stew, Hamburger Helper</td>
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<tr>
<td>Pork, like chops, roast, ribs</td>
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<tr>
<td>Popcorn</td>
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<tr>
<td>Snack chips like potato chips, Doritos, Fritos, tortilla chips</td>
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<tr>
<td>Ice cream</td>
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<tr>
<td>Candy, candy bars</td>
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<td>Cookies, donuts, cakes like Ho-Hos</td>
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<tr>
<td>Cheese, Remember cheese in sandwiches or nachos with cheese or quesadillas</td>
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<tr>
<td>Whole wheat bread or rolls (NOT white bread)</td>
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</tbody>
</table>

What kind of cereal did you eat? (MARK THE ONE YOU ATE THE MOST OF)
- Plain Cheerios, Grape Nuts, Shredded Wheat, Wheaties, Wheat Chex, Kix
- Honey Nut Cheerios, Cap’n Crunch, Lucky Charms, Life, Golden Grahams, Frosted Mini Wheats, Raisin Bran
- Other sweet cereals, like Frosted Flakes, Froot Loops
- Any other cereal, like Corn Flakes, Rice Krispies

What kind of milk did you drink? (MARK ONLY ONE)
- Whole milk
- Reduced fat 2% milk
- Low fat 1% milk
- Chocolate milk
- Nonfat milk
- Soy milk
- Lactaid milk
- Don’t know

Please tell us about yourself

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>How old are you?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17</td>
</tr>
</tbody>
</table>

DE-Nam-ReSeP EW-25709-1.0