THE UP AMIGOS PROJECT: TESTING THE PREDICTIVE VALIDITY OF THE 2007 PEDIATRIC EXPERT COMMITTEE RECOMMENDATIONS IN LATINOS

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Kinesiology in the Graduate College of the University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

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ABSTRACT

Background. Mexicans are disproportionately affected by cardiovascular disease and there is mounting evidence that Mexicans may be genetically prone to the development of cardiovascular disease (CVD) risk factors.

Objective. There were three aims of study. The first aim was to identify the prevalence of three CVD risk factors in Mexican young adults: (1) non-alcoholic fatty liver disease (NAFLD), (2) dyslipidemia, and (2) impaired fasting glucose. The second aim was to test the sensitivity and specificity of the Pediatric Expert Committee Recommendations (PECR) in identifying Mexicans with these three cardiovascular disease risk factors. Finally, the third aim was to explore ways to improve the clinical screening algorithm.

Methods. In this cross-sectional study, data for UP AMIGOS were collected from 9,974 participants (age 18- to 21-years-old) living in Central Mexico. Participants underwent a health screen that included: a questionnaire, anthropometric measurements (i.e. height, weight, waist circumference, blood pressure), a physician-conducted history and physical, and venipuncture for blood biomarkers.

Analysis. In order to determine prevalence of CVD risk factors, descriptive statistics were run making comparisons in prevalence by sex and weight category: normal weight, overweight, or obese. The value of the PECR was measured with sensitivity, specificity, and positive predictive value, with additional tests for significant associations. Alternative algorithms were explored using classification and regression tree analysis.
**Results.** NALFD (17.1 to 45.5%) and dyslipidemia (44.8%) were fairly prevalent. In contrast, impaired fasting glucose (IFG) was rare (4.0%). Each CVD risk factor increased with increasing levels of adiposity. The PECR provided a reasonable clinical screen for NALFD, but was fairly insensitive in detecting those with dyslipidemia or IFG. Multiple exploratory analyses revealed more sensitive screening solutions for each individual CVD disease risk factor, but at the cost of having a less parsimonious clinical screen.

**Significance.** Mexican adolescents and young adults already have a high prevalence of CVD risk factors. These risk factors may go unnoticed and eventually convert to irreversible disease, unless a valid, predictive screening protocol is established. Based on this analysis, screening recommendations are three-fold: (1) Universal screening for dyslipidemia is recommended for Mexican young adults, (2) IFG screening is not recommended in adolescents or young adults, (3) the PECR may be a reasonable clinical screen for NALFD, but more data is needed.
# Table of Contents

List of Abbreviations Used ........................................................................................................vii

## CHAPTER 1: JUSTIFICATION .................................................................................................1

- Background of Childhood Obesity .........................................................................................2
- Diseases Related to Insulin Resistance .....................................................................................6
  - T2DM ..................................................................................................................................7
  - Dyslipidemia ........................................................................................................................8
  - Hypertension .......................................................................................................................8
  - The Metabolic Syndrome .....................................................................................................9

- Pediatric Screening Recommendations for Mexico .................................................................9

- Study Goals ..........................................................................................................................12

## CHAPTER 2: REVIEW OF LITERATURE ..............................................................................15

- Global Increase in Childhood Obesity ...................................................................................16
- Mexico Will Be Hit the Hardest ..............................................................................................20
  - The Burden of Disease in Mexico .......................................................................................21
  - Screening for Obesity-Related Disease in Mexico .................................................................23
  - The Genetic Vulnerability of Mexicans ..............................................................................24

- The Pathophysiology of Childhood Obesity .........................................................................27
  - Childhood Overweight and Atherosclerosis: Epidemiological Evidence .........................28
  - Childhood Overweight and Atherosclerosis: The Pathophysiology ..................................30
  - Diseases Caused by Insulin Resistance ..............................................................................38
  - The Metabolic Syndrome .....................................................................................................45
  - The Early Stages of Atherogenesis are Reversible .................................................................46
  - Mexicans are Prone to Developing Insulin Resistance ..........................................................47

- The Feasibility of Pediatric Screening for Obesity .................................................................48
  - The 2007 Pediatric Expert Committee Recommendations .................................................49
  - The Importance of Pediatric Screening .................................................................................54
  - Validating Pediatric Screening Recommendations ............................................................57

- Study Objective ....................................................................................................................58

## CHAPTER 3: THE PEDIATRIC EXPERT COMMITTEE RECOMMENDATIONS AS A CLINICAL SCREENING TOOL FOR ELEVATED LIVER ENZYMES .........................................................61

- The Natural History of NAFLD .............................................................................................62
- Epidemiology of NAFLD .......................................................................................................64
- Clinical Diagnosis of NAFLD ..................................................................................................67
- Study Aims ..........................................................................................................................72
- Methods ................................................................................................................................72
  - Location ..............................................................................................................................73
  - Participants and Matching ..................................................................................................73
  - Protocol ................................................................................................................................74
  - Variables in the Algorithm .................................................................................................75
  - Analysis ...............................................................................................................................79

- Results ....................................................................................................................................81
  - Dependent Variables ..........................................................................................................83
  - The PECR Clinical Screen ....................................................................................................84
  - Exploratory Analysis to Improve the Algorithm .................................................................86
CHAPTER 4: DEVELOPING A CLINICAL SCREENING TOOL FOR THE IDENTIFICATION OF DYSLIPIDEMA ...................................................... 97
CVD Risk Factors ............................................................................. 97
Dyslipidemia .................................................................................... 98
Screening for Dyslipidemia ............................................................... 100
Definitions of Dyslipidemia .............................................................. 103
Study Aims ...................................................................................... 105
Methods .......................................................................................... 106
Participants ...................................................................................... 106
Protocol ............................................................................................ 106
Variables in the Algorithm ............................................................... 108
Dyslipidemia .................................................................................... 110
Clinical Screening Algorithms ......................................................... 110
Analysis ........................................................................................... 111
Results ............................................................................................ 112
Descriptive Statistics ...................................................................... 113
Clinical Screening Algorithms ......................................................... 116
Classification and Regression Tree Analysis .................................... 118
Discussion ....................................................................................... 120
The Prevalence of CVD Risk Factors ............................................... 120
GOAL 1: Dyslipidemia Increases with Increasing Adiposity ............... 122
GOAL 2: Simple Algorithms based on Anthropometrics Outperform Others ................................................................. 123
GOAL 3: The inclusion of Waist Circumference with BMI data .......... 125

CHAPTER 5: THE PEDIATRIC EXPERT COMMITTEE RECOMMENDATIONS AS A CLINICAL SCREENING TOOL FOR IMPAIRED FASTING GLUCOSE .......... 128
Pathophysiology of T2DM ................................................................ 128
Diabetes in Mexico ............................................................................ 132
Methods .......................................................................................... 135
Participants ...................................................................................... 136
Protocol ............................................................................................ 136
Variables in the Algorithm ............................................................... 138
Analysis ........................................................................................... 141
Results ............................................................................................ 143
Descriptive Statistics ...................................................................... 145
Pediatric Expert Committee Recommendations .............................. 146
Classification and Regression Tree Analysis .................................... 149
Discussion ....................................................................................... 154
The Prevalence of CVD Risk Factors ............................................... 154
Goal 1: Low Impaired Fasting Glucose Prevalence Rates .................... 155
Goal 2: The PECR is Not a Sensitive Screen ..................................... 155
Goal 3: The Challenge of Finding a Parsimonious Screen .................. 156
Conclusions ..................................................................................... 160

CHAPTER 6: CONCLUSIONS ................................................................ 162
Brief Review of The Three Analyses .................................................. 163
Risk Factor Descriptive Analysis ................................................................. 165
GOAL 1: Establishing the Prevalence of Cardiometabolic Abnormalities .......... 166
GOAL 2: Testing the PECR Recommendations .............................................. 170
GOAL 3: Exploration of Alternative Algorithms ........................................... 173
Study Strengths & Limitations ....................................................................... 175
Conclusions .................................................................................................. 178
Final Recommendations for Screening .......................................................... 179
Future Directions ......................................................................................... 180
REFERENCES .............................................................................................. 182
APPENDIX A: 2009 QUESTIONNAIRE ......................................................... 238
### List of Abbreviations Used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>%ile</td>
<td>Percentile</td>
</tr>
<tr>
<td>Add Health</td>
<td>The National Longitudinal Study of Adolescent Health</td>
</tr>
<tr>
<td>AAP</td>
<td>American Association of Pediatrics</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transaminase</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index $\text{kg/m}^2$</td>
</tr>
<tr>
<td>BMIZ</td>
<td>BMI Z-score</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CA</td>
<td>Coronary artery</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CART</td>
<td>Classification and Regression tree</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CE</td>
<td>Consistent evidence</td>
</tr>
<tr>
<td>CRP</td>
<td>C Reactive Protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>EBP</td>
<td>Elevated blood pressure ($\text{BP} \geq 130/85$)</td>
</tr>
<tr>
<td>ELE</td>
<td>Elevated liver enzymes</td>
</tr>
<tr>
<td>Evid</td>
<td>Evidence</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FMHx</td>
<td>Family medical history</td>
</tr>
<tr>
<td>FMHx CVD</td>
<td>Family medical history of cardiovascular disease</td>
</tr>
<tr>
<td>FMHx T2DM</td>
<td>Family medical history of type 2 diabetes mellitus</td>
</tr>
<tr>
<td>FMHx OB</td>
<td>Family medical history of obesity.</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>Glucose transporter 4</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein cholesterol</td>
</tr>
<tr>
<td>HTN</td>
<td>Hypertension (BP ≥ 140/90)</td>
</tr>
<tr>
<td>HSI</td>
<td>Hepatic steatosis index</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IDH</td>
<td>Ischemic Heart Disease</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischemic heart disease</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>INF</td>
<td>Interferon</td>
</tr>
<tr>
<td>IPAQ</td>
<td>International physical activity questionnaire</td>
</tr>
<tr>
<td>KLF-6</td>
<td>Krueppel-like factor 6</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>ME</td>
<td>Mixed evidence</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NASH</td>
<td>Non-alcoholic steatohepatitis</td>
</tr>
<tr>
<td>NCEP</td>
<td>National cholesterol education program</td>
</tr>
<tr>
<td>NHANES</td>
<td>The National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NHBPEP</td>
<td>The National High Blood Pressure Education Program</td>
</tr>
<tr>
<td>NW</td>
<td>Normal weight</td>
</tr>
<tr>
<td>OB</td>
<td>Obese</td>
</tr>
<tr>
<td>OW</td>
<td>Overweight</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>PECR</td>
<td>Pediatric Expert Committee Recommendations</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>PNPLA3</td>
<td>Patatin-like phospholipase domain-containing protein 3</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator characteristics</td>
</tr>
<tr>
<td>SAP</td>
<td>Spanish Association of Pediatrics</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Sens</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Spec</td>
<td>Specificity</td>
</tr>
<tr>
<td>T2DM</td>
<td>T2DM Mellitus</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor alpha</td>
</tr>
<tr>
<td>UASLP</td>
<td>Autonomous University of San Luis Potosi</td>
</tr>
</tbody>
</table>
UP AMIGOS  University of San Luis Potosi and Illinois: A Multidisciplinary Investigation on Genetics, Obesity, and Social-environment

USPTF  United States Preventive Service Task Force

vasc dz  vascular disease

VAT  Visceral adipose tissue

VLDL  Very low density lipoprotein

WC  Waist circumference

WHO  World Health Organization
CHAPTER 1: JUSTIFICATION

“This is the first generation where children may die before their parents”

-Paul Zimmet (International Diabetes Federation, 2007)

The International Obesity Task Force currently estimates that one in ten children in the world are either overweight or obese (Lobstein, Baur, Uauy, IASO International Obesity Task Force, 2004). Most of these children are from developing countries (World Health Organization [WHO], 2010) that have limited healthcare provisions (Peters et al., 2008) and still struggle with lingering problems of infectious disease and perinatal mortality (Stevens et al., 2008). Therefore, these countries suffer a double burden and may not be able to cope with either the short-term or long-term consequences of childhood overweight and obesity. While some consequences are reversible with early intervention (Battista, Murray, & Daniels, 2009), few developing counties have published recommendations for pediatric obesity screening.

One of the developing countries without recommendations is Mexico. This is particularly concerning, because Mexico is now one of the most obese countries in the world, and obesity-related diseases have swiftly become the leading causes of death within Mexico (Ogden et al., 2006; Olaiz et al., 2003 as cited by¹ García-García et al., 2006; Rivera et al., 2002). New research is emerging that shows the Mexican people may be genetically prone to fat-induced diseases (Aguilar-Salinas et al.,

Footnote

¹ “as cited by” indicates a citation where the original article was in Spanish, but was described in English by another author.
2009). Mexicans develop these conditions at higher rates, at lower weights, and at much younger ages than Caucasians (Sanchez-Castillo et al., 2005).

Clearly, a protocol for pediatric screening is needed within Mexico. One possible solution is to adapt a pre-existing screening recommendation from a developed country. The Pediatric Expert Committee Screening Recommendations published by the American Academy of Pediatrics in 2007 has special provisions for people of Hispanic ethnicity (Barlow & The Expert Committee, 2007). Before implementation would be possible, these recommendations would need to be validated and adapted for the pediatric population of Mexico. Therefore, the objective of this study is to validate the 2007 Pediatric Expert Committee Recommendations in a Mexican population. The expectation is that early pediatric screening will lessen the burden of disease in this developing country.

**Background of Childhood Obesity**

Childhood obesity is increasing globally and carries both short- and long-term consequences. The short-term consequences of childhood overweight and obesity are a myriad of diseases occurring in childhood that substantially reduce quality of life (Ludwig, 2007) (See Table 1.1). The prevalence of some diseases is high. For example, it is estimated that 20 – 30% of overweight schoolchildren are hypertensive and 20% of overweight 5- to 10-year-olds have elevated cholesterol (Barlow, Dietz, Klish, & Trowbridge, 2002; Daniels, 2006). There have also been antecedents of fat-induced liver failure in 30% – 50% of obese children (Barlow, Dietz, Klish, & Trowbridge, 2002; Daniels, 2006). Other conditions, while less common, occur at an increased rate in the obese. Many of these conditions such
### Table 1.1

*Complications of Childhood Obesity*

<table>
<thead>
<tr>
<th>Body System</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurologic</td>
<td>Pseudotumor cerebri</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Dyslipidemia</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>Chronic inflammation</td>
</tr>
<tr>
<td></td>
<td>Endothelial dysfunction</td>
</tr>
<tr>
<td></td>
<td>Coagulopathy (i.e. clotting disorder)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Sleep apnea</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
</tr>
<tr>
<td></td>
<td>Exercise intolerance</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Gastroesophageal reflux (i.e. heart burn)</td>
</tr>
<tr>
<td></td>
<td>Steatohepatitis (i.e. fatty, inflamed liver)</td>
</tr>
<tr>
<td></td>
<td>Gallstones</td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
</tr>
<tr>
<td>Renal</td>
<td>Glomerulosclerosis</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Slipped capital femoral epiphysis</td>
</tr>
<tr>
<td></td>
<td>Blount’s disease*</td>
</tr>
<tr>
<td></td>
<td>Forearm fracture</td>
</tr>
<tr>
<td></td>
<td>Back pain</td>
</tr>
<tr>
<td></td>
<td>Flat feet</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td></td>
<td>T2DM</td>
</tr>
<tr>
<td></td>
<td>Precocious puberty</td>
</tr>
<tr>
<td></td>
<td>Polycystic ovarian syndrome (girls)</td>
</tr>
<tr>
<td></td>
<td>Hypogonadism (boys)</td>
</tr>
<tr>
<td>Psychosocial</td>
<td>Poor self-esteem</td>
</tr>
<tr>
<td></td>
<td>Anxiety</td>
</tr>
<tr>
<td></td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td>Eating disorders</td>
</tr>
<tr>
<td></td>
<td>Social isolation</td>
</tr>
<tr>
<td></td>
<td>Lower educational attainment</td>
</tr>
</tbody>
</table>

*Blount’s disease is a growth disorder of the tibia that causes the lower leg to angle inward (tibia vara).*

Note: Adapted from Ludwig (2007).
as type 2 diabetes mellitus (T2DM), place a great burden on the child’s body and can decrease life expectancy by as much as 20- to 28-“life-years” (Ludwig & Ebbeling, 2001). Other co-morbidities exact their toll by creating an increased risk of sudden death (sleep apnea and obesity-associated hypoventilation syndrome), potentially permanent bone deformations (slipped capital femoral epiphysis and Blount’s disease), infertility (polycystic ovarian syndrome), or inoperable brain tumors (pseudotumor cerebri) (Ludwig, 2007; Barlow & The Expert Committee, 2007).

Perhaps more worrisome than the short-term implications are the long-term consequences of childhood overweight and obesity. Many obesity-associated diseases lead to premature death in early adulthood. Indeed, the overweight or obese child has two- to three-times the risk of early mortality compared to normal weight counterparts (Bjorge, Engeland, Tverdal, & Smith, 2008; van Dam, Willett, Manson, & Hu, 2006). The risk of early death disappears if an overweight child becomes a normal weight adult (Engeland, Bjorge, Tverdal, & Sogaard, 2004); but, unfortunately, most overweight children remain overweight or become more overweight in adulthood (Guo, Wu, Chumlea, & Roche, 2002; Srinivasan, Bao, Wattigney, & Berenson, 1996; Steinberger, Moran, Hong, Jacobs, & Sinaiko, 2001; Wang, Chyen, Lee, & Lowry, 2008; Whitaker, Wright, Pepe, Seidel, & Dietz, 1997).

Once a child becomes an overweight or obese adult, it is likely that he or she will remain so. Adult weight loss interventions result in only modest long-term decreases in weight (-1.5 kg [-3 lbs]), and rates of recidivism after weight loss are high (Shaw, Gennat, O’Rourke, & Del Mar, 2006). Therefore, early prevention,
identification, and treatment of overweight and obesity earlier in life may be the most promising solution (Whitlock, O'Connor, Williams, Beil, & Lutz, 2010). In the hopes of diagnosing and treating obesity and obesity-related diseases early, several developed countries have written pediatric screening recommendations (Delgado-Noguera, Tort, Bonfill, Gich, & Alonso-Coello, 2009). Unfortunately, few pediatric screening recommendations exist in developing countries (Delgado-Noguera et al., 2009).

Screening recommendations are urgently needed in the developing country of Mexico. Mexico has the highest rates of overweight in the world (Ogden et al., 2006; Olaiz et al., 2003 as cited by Garcia-Garcia et al., 2006) and the inhabitants in this country are prone to fat-related diseases (Gardner et al., 1984; Aguilar-Salinas et al., 2009). Currently, about one third of children and two thirds of adults in Mexico are either overweight or obese (del Rio-Navarro et al., 2004; Olaiz et al., 2003 as cited by Garcia-Garcia et al., 2006) and rates are still increasing (Rivera et al., 2001 as cited by Martorell, 2005). Paralleling increasing obesity trends are deaths from obesity-related diseases such as ischemic heart disease and diabetes (Rivera et al., 2002).

Cross-country comparisons have revealed that Mexicans develop cardiovascular disease and T2DM at body mass indices (BMI = kg/m²) in the upper normal range (BMI ≥ 23). In addition, these diseases emerge at higher prevalence rates in Mexican young adults (aged 20 to 49), as compared to US counterparts, who do not develop the diseases until decades later (Sanchez-Castillo et al., 2005). So
Mexicans acquire obesity-related diseases much younger, and at lower levels of adiposity than non-Hispanic blacks and non-Hispanic whites.

One possible explanation why Mexican’s may be more prone to obesity-related diseases is that Mexicans are genetically more responsive to the “toxic” effects of carrying access fat (Gardner et al., 1984; Aguilar-Salinas et al., 2009). Some of the toxic effects of obesity have been attributed to insulin resistance. Unfortunately, Mexicans develop insulin resistance more frequently than non-Hispanic whites and non-Hispanic blacks of similar BMI values (Lee, Okumura, Davis, Herman, & Gurney, 2006).

Once an individual gains weight and develops insulin resistance, he or she becomes prone to many cardiometabolic derangements such as type 2 diabetes (T2DM), dyslipidemia, and hypertension. All of these conditions are risk factors for cardiovascular disease (CVD), and are described in more detail in the section below. Because Mexicans are more prone to developing insulin resistance, they are also more vulnerable to the diseases outlined in the following section, and, ultimately to cardiovascular morbidity and mortality.

**Diseases Related to Insulin Resistance**

Insulin resistance is often initiated by increases in adipose tissue, particularly around the waist (D’Adamo, Santoro, & Caprio, 2009). Insulin is a hormone that is released by the pancreas after meals. It tells your body to store glucose and fat. When fat cells become too large, they become resistant to the storage signal of insulin and begin to haphazardly release their fat stores into the bloodstream. This drenches the body in fatty acids and sets into motion a cascade of
events rendering many organs, including the liver and skeletal muscle, resistant to insulin. Meanwhile, the pancreas tries to increase the output of insulin in order to compensate for peripheral resistance. For a pictorial description of this process, see Figure 1.1. Insulin resistance causes many maladaptive cardiometabolic derangements that ultimately damage blood vessels and lead to ischemic heart disease and stroke. Specifically, these include T2DM, dyslipidemia, and hypertension.

Figure 1.1

The Pathogenesis of Insulin Resistance Syndrome

Note: Adapted from Kirk & Klein (2009).

T2DM

T2DM occurs when the pancreas is no longer able to produce enough insulin to overcome the body’s insulin resistance. When this happens, blood glucose levels begin to rise. Blood glucose increases first after meals and then continuously in the fasting state. Once fasting blood glucose reaches 100 mg/dL and above, the patient is diagnosed with pre-diabetes, also known as impaired fasting glucose (IFG). When
fasting blood glucose is 126 mg/dL or above, the patient has diabetes. The persistence of high levels of blood glucose over several years can damage small nerves and blood vessels leading to kidney damage, limb amputations, and ischemic heart disease.

**Dyslipidemia**

Dyslipidemia literally means “abnormal lipid in the blood.” It is defined by having high levels of “bad” cholesterol and triglycerides in the bloodstream and not enough “good” cholesterol known as High Density Lipoprotein (HDL). HDL-C acts by clearing excess cholesterol from the blood. Lipids or fats can come from the diet, but can also be made and destroyed by the liver. In the insulin resistant state, the liver creates more triglycerides and destroys HDL. The result is that “bad” lipids increase, HDL-C decreases, and the individual develops dyslipidemia. Over many years, dyslipidemia contributes to the buildup of atherosclerotic plaques in large blood vessels, decreasing blood flow and increasing the risk of ischemic heart disease and stroke.

**Hypertension**

Hypertension, also known as high blood pressure, occurs when there is an abnormally high amount of pressure within the arteries. This can be due to having too much blood volume or having vessels that are too rigid or chronically flexed. Hypertension has been linked to obesity and insulin resistance, but the mechanism through which insulin resistance leads to hypertension is still a matter of debate.
(Kirk & Klein, 2009). If left untreated, hypertension damages blood vessels and leads to stroke and ischemic heart disease.

**The Metabolic Syndrome**

Taken together, high blood glucose, dyslipidemia, and high blood pressure are all known as Metabolic Syndrome (MetS). Researchers now often refer to MetS as insulin resistance syndrome, because insulin resistance is the underlying cause. Each component of MetS contributes independently to the risk of cardiovascular disease mortality from ischemic heart disease or stroke, even in normal weight individuals (Camhi et al., 2010). Because Mexicans are prone to developing insulin resistance, they are more prone to MetS and, ultimately, to ischemic heart disease and stroke. Some sources estimate that the prevalence of MetS in Mexicans adolescence is 25% higher than non-Hispanic white and 280% than non-Hispanic black (Johnson, et al., 2009).

MetS is largely asymptomatic in the early stages. Therefore, screening tests are needed to identify those with high blood glucose, dyslipidemia, and hypertension. In Mexico, despite health care being a constitutional right, screening rates for diabetes or hypertension in the adult population are relatively low: about 12% and 29% of Mexico (ENSANUT, 2007). To our knowledge, no routine screening is being conducted for these conditions in the pediatric population of Mexico.

**Pediatric Screening Recommendations for Mexico**

Mexico would undoubtedly benefit from a pediatric screening protocol. In 2007, the American Academy of Pediatrics published recommendations for a
screening protocol for the prevention, assessment, and treatment, of child and adolescent overweight and obesity. The 2007 screening recommendations were a revision from the 1998 recommendations and, like the previously published recommendations, were written to guide health care providers in the diagnosis and treatment of obesity-related disease. Included in the screening recommendations are provisions for children and adolescents of Hispanic ethnicity (Barlow & The Expert Committee, 2007).

Outlining the recommendations, The Pediatric Expert Committee suggests that at every clinic visit, for all pediatric patients, a qualitative assessment of dietary patterns is recommended. At least once a year, for all pediatric patients, it is recommended there be an assessment of physical activity levels and sedentary behaviors such as TV viewing. Also at least once per year, physicians should measure the BMI of all patients and plot the BMI age- and sex-specific percentile on a Centers for Disease Control (CDC) BMI percentile chart. When a child is found to have a high BMI, the physician is recommended to obtain a family medical history of obesity, T2DM, cardiovascular disease (especially hypertension) in first-degree relatives (i.e. parents, grandparents), as well as conduct a review of systems and physical exam for weight-related problems. Appropriate laboratory tests are recommended according to BMI percentile and stratification of risk. The laboratory evaluation algorithm is depicted in Table 1.2 (Barlow & The Expert Committee, 2007).

Screening recommendations have the potential to vastly improve identification of overweight and obese children, as well as uncover asymptomatic
### Table 1.2

*Algorithm for laboratory evaluation*

<table>
<thead>
<tr>
<th>BMI Percentile</th>
<th>Risk Factors</th>
<th>Recommended Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight (≤85th %ile)</td>
<td>0</td>
<td>BP</td>
</tr>
<tr>
<td></td>
<td>1*</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>Overweight (&gt;85th – 94th %ile)</td>
<td>&lt;2**</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td></td>
<td>≥2**</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td>Obese (≥95th %ile)</td>
<td>≥0</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(microalbumin/creatinine ratio)</td>
</tr>
</tbody>
</table>

Note: Adapted from Barlow & The Expert Committee (2007).

BP – Blood pressure

* Family medical history of dyslipidemia

** Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease, hypertension), ethnic minority [African, Hispanic], elevated BP, dyslipidemia, tobacco use, and signs of insulin resistance.

The identification of overweight and obesity in children is surprisingly difficult. This has been studied in developed countries with a long-standing obese pediatric population and previously established screening recommendations, such as the United States. Currently, in the United States, 97% of physicians use visual assessment to determine the weight status of their pediatric patients (Klein et al., 2010). However, the accuracy of visual assessment is poor. Huang and colleagues report a median of 58% of pediatricians correctly identifying the weight status of children (Huang et al., 2009). Inaccurate measurement techniques yield low identification rates: Data from one large Midwest academic center revealed that between 1999 and 2007, only 10% of overweight (as defined by BMI 85th -94th percentile) children aged 2 to 18 years were correctly identified as being overweight (Benson, Baer, & Kaelber, 2009). In contrast, the use of BMI percentile
charts as recommended by the 2007 Pediatric Expert Committee has been shown to dramatically improve identification of overweight children (Perrin, Flower, & Ammerman, 2004). Identification of at-risk children may increase the rate of screening and treatment. A chart review by O’Brien, Holubkov, and Reis (2004) showed that less than 5% of obese children received the appropriate referrals and laboratory assessment, but that referrals and assessment improved when obese children were correctly identified. Therefore, countries such as Mexico, with a high prevalence of overweight, obesity, and related diseases, would benefit from having national screening recommendations.

**Study Goals**

The major goal of the current study was to test the 2007 Pediatric Expert Committee Recommendations (PECR) in Mexico for three CVD risk factors (1) NALFD, (2) dyslipidemia, (3) impaired fasting glucose (IFG). To our knowledge, the PECR has not been tested on a Hispanic population or any population of children to date. In order to test the epidemiological value of screening recommendations, cross-sectional data from the UP AMIGOS project were be analyzed.

The UP AMIGOS study is a multidisciplinary collaboration between the Autonomous University of San Luis Potosi (UASLP) and the University of Illinois. The collaboration includes researchers from psychology, nutrition, genetics, chemistry, community health, and medicine at both institutions. The collaborative agreement allows The University of Illinois to submit questions for a questionnaire that is administered to applicants to UASLP during a routine medical visit. In addition, there is an open exchange of all collected, de-identified data. Three waves
of data collection have occurred to date and were all approved by Institutional Review Boards at both institutions.

Participants were adolescents and young adults (aged 18- to 21-years-old) in the Central Mexican State of San Luis Potosi. All participants completed a health screen that included: a questionnaire, anthropometric measurements (height, weight, waist circumference), blood pressure, a physician-conducted history and physical, and venipuncture for blood biomarkers.

The analysis had three subgoals. The first aim was to identify the prevalence of three CVD risk factors in Mexican young adults: (1) NAFLD, (2) dyslipidemia, and (2) IFG. The second aim was to test the sensitivity and specificity of the PECR in identifying Mexicans with the three aforementioned cardiovascular disease risk factors. Finally, the third aim was to explore ways in which to improve the clinical screening algorithm.

In order to accomplish these goals, three separate lines of analyses were conducted on (1) NALFD, (2) dyslipidemia, and (3) IFG. Each analysis began with descriptive statistics comparing the relative prevalence of CVD risk factors in males and females, and normal weight, overweight, and obese participants. Next, the PECR was tested for sensitivity, specificity, and positive predictive value (PPV). Univariate and multivariate tests of significance were used to test for associations between a the PECR screen and CVD risk factors. Finally, alternative algorithms were explored using classification and regression tree analysis where power allowed. In the case of NALFD, power was not sufficient and manual exploration of alternative algorithms was performed.
The main research question was: *Are the PECR a valuable screening test for (1) NALFD, (2) dyslipidemia, and/or (3) IFG in a population of college applicants in Central Mexico?* Whether or not the PECR was a valuable screening test ultimately depended on whether or not it met the following criteria:

- The screen was able to accurately identify a condition
- The condition of interest is serious
- The condition of interest is treatable
- The condition of interest has a long latency period
- The condition is sufficiently common to justify the cost of screening

*The screening test* of interest in the PECR clinical screen. *The condition* was defined as elevated liver enzymes, dyslipidemia, or IFG. *Accurate identification* was considered as having a sensitivity and specificity of > 0.80 and significant associations on multivariate analysis.
CHAPTER 2: REVIEW OF LITERATURE

"The childhood obesity epidemic is a tsunami.

We’re beginning to see the wave hitting the shore."

– David Ludwig MD/PhD

Children’s Hospital Boston

Childhood obesity is a global health concern that increases the risk of death in early adulthood. Obesity-related diseases are the world’s leading killers today (Lopez & Mathers, 2006; Mathers, Boerma, & Ma Fat, 2009). As childhood obesity rates continue to rise, so too will diseases that are potentially preventable. The result will be a generation of children who may die before their parents (International Diabetes Federation [IDF], 2007).

The first section of this literature review will discuss world obesity and mortality data, focusing on Latin America and, specifically, on Mexico. The second section will explain how Mexico may be positioned to be hit hardest by the incoming “tsunami” for reasons that are threefold: geographic, economic, and genetic. The third section will focus on the pathophysiological link between childhood obesity, disease, and early death. This will highlight the value of screening for obesity and obesity-related diseases in the pediatric population. The fourth section will describe the current Pediatric Expert Committee Recommendations (PECR) published in 2007 by the American Academy of Pediatrics and discuss barriers to implementation in Mexico. The fifth and final section will introduce the goal of the current study, which will be to validate the 2007 PECR in a population of adolescents and young adults from Central Mexico.
Global Increase in Childhood Obesity

The world is undergoing a dramatic health transition and children are at the forefront. Undernutrition is rapidly shifting to overnutrition. Although children of developing countries may live to adulthood, they may have an increased risk of early mortality as a result of childhood obesity. The rapid shift is most pronounced in Latin America, where rates of obesity-related diseases are already high and continue to climb.

Now, at the first decade of the millennium, undernutrition is waning and fewer children are dying before they reach 5-years of age (WHO, 2010). Despite this major stride forward in world health, the pendulum is swiftly swinging to another extreme. Globally, childhood underweight may be decreasing, but overweight and obesity are on the rise. In the last 5 years, the WHO has doubled its estimation of children less than 5-years-old who are overweight or obese from 20 million children in 2005 to over 42 million in 2010 (WHO, 2006; WHO, 2010). The International Obesity Task Force estimates that 10% of children aged five to 17 are either overweight or obese (Lobstein, Baur, Uauy; IASO International Obesity TaskForce, 2004) (see Table 2.1) and most are living in developing countries (WHO, 2010).

Overweight and obesity in childhood is associated with an increased risk of disease and death in adulthood (Daniels, 2006; Gunnell, Frankel, Nanchahal, Peters, & Smith, 1998; Must, Jacques, Dallal, Bajema, & Dietz, 1992; van Dam, Willett, Manson, & Hu, 2006). The increased risk of mortality from overweight and obesity in childhood lays dormant and becomes apparent after the age of 30, when mortality levels begin to rise (Engeland, Bjorge, Sogaard, & Tverdal, 2003).
Table 2.1

Definitions of overweight and obesity

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Pediatric definitions*</th>
<th>Adult definition (BMI)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>BMI &lt; 5th percentile</td>
<td>BMI ≤ 18.5</td>
</tr>
<tr>
<td>Healthy weight</td>
<td>BMI 5th – 84th percentile</td>
<td>BMI 18.5 – 24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>BMI 85th – 94th percentile</td>
<td>BMI 25 – 29.9</td>
</tr>
<tr>
<td>Obese</td>
<td>BMI ≥ 95th percentile</td>
<td>BMI ≥ 30</td>
</tr>
</tbody>
</table>

Note: Adapted from Barlow and the Expert Committee (2007)

* Based on standardized BMI percentile charts for age and sex

** Obesity Task Force classification

BMI = Body mass index (kg/m²)

Figure 2.1 depicts the risk of adult mortality for children based on BMI percentile and cause of death. The rates of increased death associated with childhood overweight and obesity are similar to those of being a lifetime smoker (Neovius, Sundstrom, & Rasmussen, 2009).

Figure 2.1

*Increased mortality in middle age for overweight and obese (BMI ≥ 85th percentile) 14- to 19-year-olds.*

Note: Subjects with BMI 25th to 75th percentile (CDC charts) were the referent group.

Adapted from Bjorge, Engeland, Tverdal, & Smith (2008).
The risk for adult mortality has been shown to begin at thresholds below the standard definitions of childhood overweight and obesity, in the upper ranges of normal weight. The Harvard Longitudinal Study found increased all-cause mortality in teenage boys with BMIs greater than the 75th percentile for age (unstandardized curve) (Must et al., 1992). In a large male and female cohort from Norway, a BMI between the 75th and 84th percentile in childhood, was associated with an increased risk of mortality in middle age compared to those in the 25th to 75th percentile (CDC curve) (Bjorge, Engeland, Tverdal, & Smith, 2008).

The most likely explanation for increased mortality within the upper normal BMI ranges is not that small amounts of childhood fat are “toxic,” but rather that children who are able to gain weight early in life have the tendency to keep gaining. Epidemiological evidence has found that overweight children, who become normal-weight adults, do not have an increased risk of early death (Engeland, Bjorge, Tverdal, & Sogaard, 2004).

The probability of reaching a normal weight in adulthood can be estimated with growth charts. Ventura, Loken, & Birch (2009) found that children with BMI values between the 50th and 60th BMI percentile, tend to track (i.e. remain) on that growth trajectory, staying within the normal weight range in adulthood. In contrast, children with BMI values above the 75th percentile tend to accumulate more fat as they age. The ultimate result is that these children become overweight or obese adults (Guo, Wu, Chumlea, & Roche, 2002; Srinivasan, Bao, Wattigney, & Berenson, 1996; Steinberger, Moran, Hong, Jacobs, & Sinaiko, 2001; Wang, Chyen, Lee, & Lowry, 2008; Whitaker, Wright, Pepe, Seidel, & Dietz, 1997).
The older and more overweight a child is, the more likely it is for adiposity to persist. For instance, an overweight or obese child between the ages of 6- to 9-years-old has a 55% chance of remaining obese as an adult (Whitaker et al., 1997). An older, obese adolescent (16- to 18-years-old) has a 69% to 89% chance of remaining obese (Guo et al., 2002). Once a child becomes an overweight or obese adult, it is likely that he or she will remain so. Adult weight loss interventions result in only modest long-term decreases in weight (-1.5 kg [-3 lbs]) and rates of recidivism after weight loss are high (Shaw, Gennat, O'Rourke, & Del Mar, 2006). Therefore, early prevention, identification, and treatment of overweight and obesity in childhood may offer the most promising solution (Whitlock, O'Connor, Williams, Beil, & Lutz, 2010), because, if left uninterrupted, the overweight or obese child will enter a trajectory of adult obesity and early death.

Currently, the world’s leading causes of death are the obesity-related diseases IHD and stroke (Lopez & Mathers, 2006; Mathers et al., 2009). The first swell of disease hit the countries where obesity emerged first. These countries were the developed countries and the former socialist economies (Kelly, Yang, Chen, Reynolds, & He, 2008). But now, trends show signs of a shift. Obesity rates within these countries are plateauing (Lissner, Sohlström, Sundblom, & Sjöberg, 2010; Ogden, Carroll, Curtin, Lamb, & Flegal, 2010; Olds, Tomkinson, Ferrar, & Maher, 2010) with a concomitant stabilization of death rates from IHD and stroke (Yusuf, Reddy, Ounpuu, & Anand, 2001).

As obesity and related-diseases plateau in developed countries, it is anticipated that the waistlines of developing countries will continue expanding
(Kelly et al., 2008). Eventually, overall obesity rates in developing countries will equate to or surpass those of developed countries. Based on current trends in rising obesity rates, it is projected that by 2030, Latin America and the Caribbean will be the most obese regions of the world (Kelly et al., 2008). Death rates from IHD will likely parallel the rise of overweight and obesity. Over the next 30 years, ischemic heart disease mortality in Latin America is expected to increase nearly 150%, compared to a 40% increase in developed countries (Yusuf et al., 2001).

The prospects of the developing countries of Latin America are grim. Already, death rates from obesity-related diseases are high. Diabetes, an obesity-related disease, causes more deaths in Latin America than any other part of the world (Danaei, Lawes, Vander Hoorn, Murray, & Ezzati, 2006) and cardiovascular disease kills 1 in every 3 Latin Americans (Yusuf et al., 2001). Unfortunately, in low-income countries, these emergent diseases often coexist with the lingering problems of infectious disease, malnutrition, and perinatal death. Thus, poorer countries face a “double burden” of both historic and emergent diseases (WHO, 2010). Perhaps hardest hit of the Latin American countries will be our neighbor to the south: Mexico (Yusuf, et al., 2001).

**Mexico Will Be Hit the Hardest**

Of all the Latin American countries, Mexico is positioned to be hit hardest by the incoming ‘tsunami’ of childhood obesity. Mexico is not only quickly becoming a world leader in rates of overweight and obesity, but diseases of overnutrition are now summating with diseases of poverty, straining an already overstretched health system. Additionally, the Mexican people may be genetically vulnerable to obesity-
related diseases (Aguilar-Salinas et al., 2009) and experience them at higher prevalence rates, at younger ages, and lower levels of adiposity than other ethnicities (Sanchez-Castillo et al., 2005). For these reasons, Mexico may soon feel a powerful impact from the wave of childhood obesity.

**The Burden of Disease in Mexico**

At the turn of the millennium, rates of overweight in Mexico surpassed those of the United States (Ogden et al., 2006; Olaiz et al., 2003 as cited by Garcia-Garcia et al., 2006). Currently, about one third of children and two thirds of adults in Mexico are either overweight or obese (del Rio-Navarro et al., 2004; Olaiz et al., 2003 as cited by Garcia-Garcia et al., 2006) and rates are still increasing (Rivera et al., 2001 as cited by Martorell, 2005).

Overnutrition and diseases related to overnutrition are not evenly distributed throughout Mexico. Northern Mexico has the highest rates of overweight and obesity (Sanchez-Castillo et al., 2005; del Rio-Navarro et al., 2004) and this has been attributed to the influence of the United States (Deckelbaum & Williams, 2001), along with increasing “westernization” and urbanization (Yusuf et al., 2001). Unfortunately, areas in Mexico experiencing rises in overweight and obesity have also experienced a steep increase in deaths from obesity-related diseases such as ischemic heart disease, T2DM, and hypertension (Rivera et al., 2002). The highest mortality rates from diabetes in Mexico exist in the North Pacific Region, considered to be most “westernized” region of Mexico (Stevens et al., 2008).
In the south of Mexico, malnutrition rates remain similar to those seen in some parts of Sub-Saharan Africa (Rivera et al., 2001 as cited by Martorell, 2005). In addition to malnutrition, Southern regions are still struggling with conditions of poverty such as communicable, maternal, and perinatal diseases. These diseases remain over 5 times those in the north of Mexico and over 50 times those seen in high-income countries (Stevens et al., 2008). Despite persisting poverty, the nutritional transition in Mexico is occurring so rapidly that malnutrition now coexists with overweight and obesity. This can be seen by measuring stunting. *Stunting* occurs when severe undernutrition during childhood causes the bones in the legs to stop growing and for the child to never reach his or her potential adult height.

Stunting is still seen in 10-20% of Mexican children, and occurs primarily in Central and South Mexico (del Rio-Navarro et al., 2004). Now, adolescents, who were undernourished as children and experienced stunting, are subsequently *over*nourished in adolescence and develop obesity. In fact, the prevalence rates of obesity in stunted adolescents are similar to rates seen in the rest of Mexico (del Rio-Navarro et al., 2004).

With the onset of obesity comes the potential for obesity-related diseases. T2DM and IHD are also filtering to the south of Mexico. Southern inhabitants suffer more disability-adjusted life years from these diseases compared to more developed areas of Mexico (Stevens et al., 2008). This is perhaps due to the unavailability of healthcare in these areas. These obesity-related diseases summate with the burden of diseases of poverty, to cause higher overall mortality in these regions.
Screening for Obesity-Related Disease in Mexico

It is clear that Mexico has been struck with the “double burden” of disease, but it is unclear whether appropriate healthcare provisions exist in Mexico to cope with both historic and emergent diseases. The following, emergent, obesity-related diseases are now the three leading causes of death in Mexico: ischemic heart disease, T2DM, and stroke (Stevens et al., 2008). All three are preventable diseases and have many modifiable risk factors such as hypertension and dyslipidemia. If screening programs were in place, and if these modifiable risk factors were identified and treated, the risk of early death could decrease (American Heart Association, 2010).

Screening programs exist in Mexico, but rates of screening are low: About 12% and 29% of the population have been screened for T2DM or hypertension (ENSANUT, 2007). In the six major cities of Mexico, less than half of those with hypertension were aware of their disease (Meaney, et al., 2006). In rural villages and tribes, where there is not regular access to healthcare, hypertension-awareness may be even lower.

Access to healthcare is a problem in Mexico. Although healthcare is a constitutional right and several public insurance plans are available, in 2006, 51.4% of Mexican residents did not have insurance or receive medical attention (ENSANUT, 2007). When compared to the 15.4% of Americans who did not have insurance in 2009 (DeNavas-Walt, Proctor, & Smith, 2009), 51.4% is an alarming percentage of individuals without medical care. As a result of suboptimal distribution of healthcare and prevention services, many Mexicans with
asymptomatic risk factors for CVD are unaware of their risk and, therefore, remain untreated. This is of particular concern as Mexicans are considered to be a population that is genetically vulnerable to the development of obesity-related diseases.

The Genetic Vulnerability of Mexicans

Mexicans are prone to diseases related to obesity (Aguilar-Salinas et al., 2009) and increasingly more Mexicans are becoming exposed to obesogenic environments. This occurs either through urbanization of Mexican cities or through emigration to westernized countries such as the United States. The vulnerability of the Mexican people is perhaps easiest seen in cross-ethnic comparative studies conducted within the United States. In these studies, Mexicans have higher rates of disease, perhaps because of higher rates of obesity, because of genetic vulnerability, or due to some combination of both.

When Mexicans live in obesogenic environments such as the United States, disease rates of Mexicans surpass those of other ethnic groups living in comparable conditions. This is true of (a) T2DM, (b) dyslipidemia, and (c) hypertension. First, rates of T2DM are high in Mexicans (Sanchez-Castillo et al., 2005). In the United States, cross-ethnic comparisons have estimated that T2DM will develop in 1 in 3 of the overall population, and in 1 in 2 Hispanics (Narayan, Boyle, Thompson, Sorensen, & Williamson, 2003). Others estimate that compared to non-Hispanic whites, Mexicans are over two-times as likely to have T2DM (Martorell, 2005; The Writing Group for The SEARCH for DIABETES in Youth Study Group, 2007). Second,
dyslipidemia is also common in Mexicans. Half of the entire Mexican population is estimated to have low HDL-C (defined as HDL-C <35 mg/dL for both genders) (Aguilar-Salinas et al., 2009). HDL-C is the “good” cholesterol and is protective against cardiovascular disease. Cross-ethnic comparisons within the United States show that Mexicans have the lowest levels of HDL-C compared to other United States ethnic groups (Carroll et al., 2005).

Finally, hypertension is also prevalent, and affects half of Mexicans over 60 (Olaiz et al., 2003 as cited by Garcia-Garcia et al., 2006). In comparisons of ethnic groups, Mexicans do not have the highest rates of hypertension. Non-Hispanic blacks have the highest rates (Johnson et al., 2009). Compared to the overall US population, however, Mexicans have significantly higher prevalence rates of hypertension at ages below 50-years-old (Sanchez-Castillo et al., 2005). Additionally, hypertension is seen in more in the 2nd generation than in the 1st generation of Mexican Americans (Morales, Leng, & Escarce, 2009) suggesting that obesogenic environments tend to uncover the underlying genetic susceptibility of Mexicans.

One possible explanation for why Mexican Americans are hardest hit by obesity-related diseases is, simply, that Mexican Americans have higher rates of obesity. Related to this, obesity rates in Mexican Americans are higher than non-Hispanic Whites (Flegal, Ogden, & Carroll, 2004; Ogden et al., 2006). However, obesity rates cannot fully explain the Mexican predisposition to diabetes, dyslipidemia, and hypertension. Independent of weight, Mexican American adolescents more frequently show signs of diabetic disease processes compared to
non-Hispanic whites and non-Hispanic blacks (Lee, Okumura, Davis, Herman, & Gurney, 2006). Additionally, cross-country comparisons have revealed that Mexicans develop hypertension and diabetes at lower BMI values and decades younger than their counterparts living in the United States (Sanchez-Castillo et al., 2005).

The second, and more probable explanation for why Mexican Americans are impacted so strongly by obesity-related diseases is that there is a genetic predisposition to develop these conditions at lower levels of adiposity (i.e. BMI ≥ 23 kg/m²)(Sanchez-Castillo et al., 2005). It is currently believed that the degree of Native American ancestry is a risk factor for T2DM (Gardner et al., 1984) as well as other metabolic abnormalities such as having low HDL-C (Aguilar-Salinas et al., 2009). Most Mexicans have a heritage of Native American ancestry, as admixture was common among early Spanish settlers (Wikipedia, 2010).

Therefore, genetically, the Mexican people may be prone to the development of obesity-related diseases. Dr. Elliot Joslin once said, “Genetics loads the gun, lifestyle pulls the trigger.” As the westernized lifestyle continues to spread, the trigger will be pulled and obesity-related diseases will summate with diseases of poverty (Stevens et al., 2008); and the Mexican medical system will face substantial screening and treatment challenges. It is clear that the public health strategies for curbing the incoming tsunami are needed within Mexico. But before proposing a screening protocol, the pathophysiology of how childhood obesity leads to diseases and increases in adult mortality must be understood.
The Pathophysiology of Childhood Obesity

Ischemic heart disease and stroke are the two leading causes of death in the world today (Danaei et al., 2006). Both conditions fall under the category of CVD because they result from damaged vasculature causing poor blood circulation to the heart and brain, respectively. Typically, the cause of the damage is atherosclerosis (Kumar, Abbas, & Fausto, 2005). Atherosclerosis forms on the inside of arteries. Arteries deliver oxygen and essential nutrients to the tissues of the body just as pipes bring water to homes. Using this analogy, atherosclerosis would be similar to large blisters of rust forming on the inside of a main pipe. The rust deposits may grow so big that they slow water flow into the house; or, alternatively, a piece of the rusty buildup may break free and lodge itself in a smaller pipe downstream. This would cause a sudden and complete blockage of water flow. In a similar way, atherosclerotic plaques can both build up slowly and also suddenly break free, halting blood flow to a vital organ. If this were to happen in the coronary arteries of the heart, it would cause what is known as ischemic heart disease (IHD) (e.g. heart attack). When this happens to the carotid arteries supplying the brain, it is known as a stroke.

The risk for atherosclerosis increases with age, but precursor lesions begin to form years before the development of overt disease and are associated with several risk factors. The major three risk factors for atherosclerosis are diseases that have been mentioned previously are diabetes, dyslipidemia, and hypertension (i.e. high blood pressure). Other risk factors include: male gender, smoking, and, of course, obesity (American Heart Association, 2010).
When many risk factors coexist within the same person, it is often referred to as metabolic syndrome (MetS). MetS is most frequently defined as: large waist circumference, prediabetes or diabetes, dyslipidemia, and high blood pressure (IDF, 2007). Metabolic syndrome was originally uncovered as a statistical anomaly. The constellation of risk factors seemed to always coexist in epidemiological or clinical studies. Regardless of etiology, each component of metabolic syndrome contributes independently to the risk of atherosclerosis and cardiovascular disease. Both epidemiological and molecular evidence now link childhood obesity with MetS, atherosclerosis, and cardiovascular disease. The following subsections will explore the epidemiology and biological processes that link childhood obesity with early death.

**Childhood Overweight and Atherosclerosis: Epidemiological Evidence**

Cardiovascular disease is caused by atherosclerosis, and atherosclerosis has many preventable risk factors such as diabetes, dyslipidemia, and hypertension; together known as MetS. Childhood obesity has been linked to risk factors for atherosclerosis, atherosclerosis itself, and death from cardiovascular disease.

Overweight and obesity in childhood have been associated with cardiometabolic risk factors, both contemporaneously in the obese child, and decades later in adulthood. Childhood adiposity is associated with increased incidence of T2DM, dyslipidemia, and hypertension in childhood (Ludwig, 2007; Barlow, Dietz, Klish, & Trowbridge, 2002). But, even if the overweight child remains healthy, these diseases may emerge later. Longitudinally, adiposity in childhood or
adolescence was associated with adult dyslipidemia, hypertension (Srinivasan et al., 1996), T2DM (Koplan, Liverman, & Kraak, 2005), as well as the full constellation of MetS (Sun et al., 2008; Ventura et al., 2009). Children within the upper normal ranges of normal weight are also at risk for future MetS. Sun and colleagues (2006) found that a BMI around the 60th to 75th percentile (CDC curves) in boys as early as 8 and girls as early as 13 was significantly associated with developing metabolic syndrome after the age of 30 (Sun et al., 2008). Childhood BMI values around the 75th percentile may represent a threshold for disease. These diseases lead to death, and childhood BMI values greater than the 75th percentile have also been associated with increased mortality in adulthood (Must et al., 1992; Bjorge et al., 2008).

Perhaps through an increasing incidence of diabetes, dyslipidemia, and hypertension, or perhaps independent of those risk factors, childhood obesity is associated with atherosclerosis. Atherosclerotic lesions begin as small, precursor lesions before growing into advanced atherosclerotic lesions. These precursor lesions exist more frequently and are more advanced in adolescents with higher BMI values (McGill et al., 2002). Over many years, precursor lesions develop into advanced atherosclerotic lesions. Adiposity in childhood and adolescence has also been associated with future, advanced atherosclerotic lesions in both the coronary arteries (Mahoney et al., 1996) and carotid arteries (Oren et al., 2003).

Atherosclerosis leads to cardiovascular disease. Childhood obesity has been associated with death from cardiovascular disease (Baker, Olsen, & Sørensen, 2007; Must et al., 1992; Gunnell et al., 1998; Bjorge et al., 2008). Bjorge and colleagues
(2008) found that 14- to 19-year-olds who were overweight or obese grew up to have approximately 3 times the risk of IHD and sudden death in middle age.

Therefore, obesity has been linked epidemiologically to atherosclerotic risk factors, atherosclerosis itself, and death from diseases caused by atherosclerosis. In this way, the overweight child gets a head start in the pathogenesis of atherosclerosis and cardiovascular disease. Previously, researchers did not know what biological mechanisms linked obesity to these diseases, but thanks to the work of many laboratories in the first decade of the 21st century, the pathophysiological processes are finally becoming clear.

**Childhood Overweight and Atherosclerosis: The Pathophysiology**

Being overweight or obese contributes to atherosclerosis in two ways: (a) Directly through various adipose tissue factors acting independently of other risk factors (McGill et al., 2002); and (b) indirectly by increasing the risk of the triumvirate dyslipidemia, hypertension, and diabetes (AHA, 2010; Danaei et al., 2006). The biological link between obesity, metabolic syndrome, and atherosclerosis may be a derangement in the signaling cascade of one single hormone: insulin. Initiating the entire process is a simple energy imbalance.

Obesity is a condition caused when calorie intake exceeds energy expenditure over time. Undoubtedly, there is genetic component to the propensity of individuals to store excess calories, but for a large majority of individuals, obesity results from this primary nutritional imbalance (Kumar, Abbas, & Fausto, 2005). After a meal, food is digested and glucose is absorbed from the intestinal lumen and
put into the bloodstream. The nutrient-filled blood then travels through the body. The pancreas senses the rise in blood glucose and releases a hormone called insulin. Insulin acts on striated muscle, fat cells, and liver cells by triggering them to pull glucose from the bloodstream and into the cell bodies for storage as either glycogen (muscle cells, liver cells, fat cells), or fatty acids (mainly adipose tissue). Other cells, such as brain cells, also use glucose, but do not need a signal from insulin to pull glucose from the bloodstream. The insulin-dependent organs, therefore, play the primary role in the development of insulin resistance.

During periods of fasting, when there is no insulin, fat cells and the liver are capable of releasing stored energy to nourish the rest of the body. In order to do this, fat cells release fatty acids via lipolysis (i.e. lipid breakdown); and liver cells release glucose in a process known as gluconeogenesis. After a meal, insulin is released once again, triggering the cessation of both lipolysis and gluconeogenesis. In this way, insulin serves as an energy sensor, telling the fat and liver to store rather than release fatty acids and glucose into the bloodstream. Insulin “holds back the floodgates,” so to speak, keeping the blood free of unneeded nutrients.

The beginning: Old, overworked fat cells

When more calories are consumed than can be used, the calories are stored as fatty acids in fat cells. After puberty, the number of fat cells in the human body remains relatively constant, despite constant and natural cell turnover and replacement (Arner et al., 2010). When more storage is needed, either the existing fat cells can increase in size to become bigger in a process known as hypertrophy; or new, small fat cells can be made, which is called hyperplasia (Arner et al., 2010).
Regardless of BMI, individuals have a combination of small and large cells in their adipose tissue.

Hyperplasia may be a healthier process than hypertrophy. In other words, creating newer, smaller fat cells in which to store access fat may be preferable to overstressing the preexisting, older, and larger fat cells. The preferred process of hyperplasia begins when precursor cells known as pre-adipocytes mature into differentiated fat cells (i.e. adipocytes) in a process known as adipogenesis (Heilbronn & Campbell, 2008). Unfortunately, adipogenesis does not always occur when extra fat storage is needed. The reasons for this are still only conjecture and include: reduced preadipocytes activation, low numbers of preadipocytes, or inhibitory signals from local immune cells known as macrophages. Regardless of mechanism, when adipogenesis does not occur, fat expands by hypertrophy instead and preexisting fat cells must become bigger (Heilbronn & Campbell, 2008). This leads to an older population of large fat cells (Arner et al., 2010).

Unfortunately, a large fat cell is a stressed fat cell. Not only do larger cells have lower oxygen diffusion, but they also secrete more chemoattractants and proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Heilbronn & Campbell, 2008; O'Connell et al., 2010). These cytokines attract macrophages to the area. While it is unknown if resident macrophages are a cause or the result of the large cells, there is a positive association between fat cell size and the number resident macrophages (Heilbronn & Campbell, 2008). In children, subcutaneous fat cell diameter was more strongly associated than overall fat mass with TNF-α and IL-6 (Maffeis et al., 2007).
Most human studies rely on data from subcutaneous fat because it is readily accessible under the skin. However, the fat surrounding the internal organs known as visceral adipose tissue (VAT) secretes more proinflammatory cytokines than subcutaneous fat. It also has a higher concentration of resident macrophages (Heilbronn & Campbell, 2008). The larger the waistline is, the higher the concentration of macrophages residing in the VAT tissue. Fat cells and macrophages both secrete cytokines in order to “talk” to one another, but macrophages are believed to be the main source of cytokines in the bloodstream of obese individuals (Heilbronn & Campbell, 2008). Indeed VAT as measured by waist circumference is suspected to be a better predictor than BMI in regards to early cardiometabolic derangements (Yamamoto-Kimura et al., 2006; D'Adamo, Santoro, & Caprio, 2009; Savva et al., 2000), perhaps because of these histological features. Once released into the bloodstream, TNF-α and IL-6 trigger the immune system, mimicking a mild infection. Therefore, excessive fat deposition, characterized by large fat cells, particularly those residing in the abdomen, produces a condition of long-term, low-grade inflammation (Heilbronn & Campbell, 2008).

**The development of insulin resistance in fat cells**

Fat cells have insulin receptors on their membrane. Insulin binds to these receptors and triggers many signaling cascades within the cell, promoting fat storage and inhibiting lipolysis. Both stored fatty acids and cytokines such as TNF-α, have the ability to inactivate the insulin receptor via the addition of a phosphate group to the receptor (Boppart, 2010). This is known as phosphorylation. Without functioning insulin receptors, fat cells become “deaf” to the insulin signal, even
when insulin is present. The result is the lipolysis is no longer inhibited by insulin. This occurs mainly in large, inflamed cells.

The larger the cell, the more fatty acids are stored, the more cytokines are released (D'Adamo et al., 2009; Heilbronn & Campbell, 2008; Maffeis et al., 2007), and the more resistant the cell is to insulin (Maffeis et al., 2007; O’Connell et al., 2010). In children, there is an association between subcutaneous fat cell diameter and insulin resistance independent of whole body fat mass (Maffeis et al., 2007). Longitudinal studies conducted with Pima Indians have demonstrated that fat cell size may predict future onset of T2DM in Pima Indians even after adjusting for overall percent body fat (Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). This suggests that there is a special subset of patients who develop “toxic adiposity”, characterized by old, large, fat cells that secrete cytokines and are prone to insulin resistance (O’Connell et al., 2010).

Fat cell insulin resistance is the coup de grace in obesity-related pathology, because it sets in motion a voracious cycle of cardiometabolic derangements. Once the fat cell becomes resistant to insulin, the floodgates are opened. Lipolysis is no longer inhibited, so the bloodstream becomes awash with released fatty acids (FA) (Kirk & Klein, 2009). FA overwhelm organs such as the liver and muscle. While the pancreas attempts to compensate by increasing insulin output, it cannot always overcome the receptor defect (Kirk & Klein, 2009).

**The effect of free fatty acids on the liver**

The FA released from fat cells are delivered to the liver by the blood. About 20% of FA delivered to liver is from lipolysis of visceral adipose tissue, the rest from
subcutaneous adipose tissue. The liver takes up the FA, but cannot process them fast enough and FA accumulate within the liver cells. Through a process that is not well understood, the lipid-laden liver cells develop insulin resistance. The liver cells can no longer “hear” insulin; and, since insulin’s job in the liver is halt gluconeogenesis, gluconeogenesis resumes. This increases basal blood glucose levels.

There are other effects as well. For instance, without insulin’s signal, the FA-filled liver also increases the production of triglycerides (TG) (Kirk & Klein, 2009). The increasing concentration of TG in the bloodstream then triggers the liver to destroy a blood borne particle known as high density lipoprotein (HDL) (Kirk & Klein, 2009). HDL-C is known as the “good” cholesterol. It acts by cleaning up extra cholesterol esters in the blood, and is inversely associated with risk of ischemic heart disease. So as a result of insulin resistance in the liver, the blood has more “bad” TG, less “good” HDL, and higher glucose levels. Meanwhile, the fat cells continue to release FA.

**The effect of free fatty acids on skeletal muscle**

Fortunately, skeletal muscle is capable of “mopping up” excess glucose from the bloodstream. This is fortunate because long-term exposure to high glucose levels can be damaging to the body, destroying small nerves and blood vessels. Both insulin and exercise trigger glucose uptake by muscle cells via increasing the number of GLUT-4 glucose channels on the cell membrane. This restores blood glucose to normal levels.

Skeletal muscle can also store small amounts of free fatty acids for use in oxidative muscle fibers. Unfortunately, in the same way that increased FA storage
leads to insulin resistance in liver cells, high levels of FA can overwhelm the storage capacity of skeletal muscle cells and they, too, become insulin resistant. When muscle cells become insulin resistant, they no longer take in glucose in response to insulin, although exercise-induced glucose uptake is still intact. Therefore, in sedentary individuals, the deleterious effects of fatty acids storage are more pronounced, and glucose levels rise.

The process of FA induction of insulin resistance in skeletal muscle is more thoroughly understood than in the liver and molecular cascades that have been proposed. Current research suggests that the cascade begins when increased FA storage leads to increases in FA metabolites (diacylglycerol, ceramides and long-chain acyl-coenzyme A) creating reactive oxygen and nitrogen species. Through an unknown mechanism, Protein Kinase C (PKC) is phosphorylated. Phosphorylation activates PKC. The role of PKC is to phosphorylate various other downstream proteins subsequently activating or inactivating them. Among the proteins phosphorylated by PKC are Insulin Receptor substrate-1 and the NFκB inhibitor (IκB) (Kirk & Klein, 2009; Silveira et al., 2008). Insulin Receptor substrate-1 phosphorylation leads to the inactivation of insulin-signaled GLUT-4 translocation to the membrane. The result is that glucose is not taken up by the muscle cells in response to insulin. Phosphorylation of IκB releases the inhibition of NFκB. NFκB travels to the nucleus to increases increase muscle cell production of the inflammatory cytokines TNF-α and IL-6 (Silveira et al., 2008). In addition to these short-term effects, long-term FA exposure decreases the oxidative capacity of muscle cells (Silveira et al., 2008), possibly via mitochondria dysfunction (Kirk &
Klein, 2009). So the muscle’s ability to break down its increasing FA stores is reduced, leading to the stagnation of the large FA pool, and a worsening of insulin resistance. Thus FA accumulation leads to insulin resistance, inflammation, FA accumulation, and worsening insulin resistance. The cycle has become self-perpetuating, furthering the need for the pancreas to increase insulin production.

The final step: The pancreas increases insulin output

In response to peripheral insulin resistance in the fat, liver, and muscle, the pancreas produces more insulin. The goal is to keep blood glucose levels within normal values, but the task is not easy. In the insulin-resistant state, the muscle cells are indifferent to glucose, and the liver cells are needlessly continuing gluconeogenesis, churning out new glucose molecules. Blood glucose levels are high. Despite this, the pancreas initially succeeds. Higher and higher insulin levels produced by the pancreas are able to overcome peripheral resistance and blood glucose levels are maintained within normal limits.

Often, children with insulin resistance have normal glucose levels (Cali & Caprio, 2008). This suggests that the pancreas’ compensatory efforts are successful in the early stages; increasing insulin enough to maintain normal blood glucose levels, and overcoming multiple organ insulin resistance. When insulin itself is measured, children who are overweight (BMI 85 – 94th percentile) are much more likely to have high insulin levels than non-overweight children (Freedman, Dietz, Srinivasan, & Berenson, 1999). In obese adolescents, increasingly higher BMI quartiles have increasingly higher levels of post-meal insulin levels (Cali & Caprio,
2008). However, even when glucose levels are maintained, insulin resistance has devastating effects on the metabolic balance of the body, resulting in disease.

**Diseases Caused by Insulin Resistance**

Derangements in insulin signaling cause widespread disruptions in metabolism and increase the chances of developing diabetes, dyslipidemia, and hypertension (D’Adamo et al., 2009). All three are independent risk factors for atherosclerosis.

**Diabetes**

When insulin resistance develops and the pancreas is unable to compensate with enough insulin, blood glucose levels begin to rise. This occurs first after meals, and then in the fasting state. This is called impaired fasting glucose pre-diabetes. Pre-diabetes then progresses to T2DM (Daniels, 2006), a dangerous disease that can lead to blindness, kidney failure, limb amputation, and cardiovascular disease.

On the positive side, overt diabetes in children and adolescents is still rare: only 1-15/100,000 have the condition (Adair, 2008; Dolan et al., 2005), but almost all childhood cases are in those who are obese (Daniels, 2006; Barlow, Dietz, Klish, & Trowbridge, 2002). On the negative side, once a child develops insulin resistance, it may progress to T2DM much more quickly than in adults (D’Adamo et al., 2009). In those who are insulin resistant, but not diabetic, there is still an increased risk dyslipidemia, hypertension, and fatty liver disease.

**Dyslipidemia**
Dyslipidemia is an important risk factor for atherosclerosis, and half of all deaths from ischemic heart disease are attributable to high cholesterol (Danaei et al., 2006). Dyslipidemia literally means “abnormal lipid in the blood.” There are many sources of fat or lipid in the bloodstream. The body can absorb lipids from foods, release them from adipose tissue, and also make them de novo (from scratch) in the liver. These lipids may be in the form of cholesterol, triglycerides, or free fatty acids. Triglycerides are actually the storage and carrier form of fatty acids and are composed of three fatty acids attached by a glycerol backbone. Most lipids, cholesterol and triglycerides from food and free fatty acids from adipose tissue, are sent to the liver for processing. The liver then sends the processed lipids into the bloodstream for use by other tissues. Lipids, however, are not soluble in blood and must travel within specialized proteins known as lipoproteins. Lipoproteins are like balloons. The rubber of the balloon is analogous to the protein matrix and the inside is not filled with air, but with lipid. In this way, lipids are carried in the blood, contained within lipoproteins. Free fatty acids are unique in that they are small and can attach to globular blood proteins such as albumin to be carried in the blood directly to other tissues for use as fuel (Barter, 2005).

Initially, the liver processes and packages lipids into very low-density lipoproteins (VLDL). VLDL leave the liver filled primarily with triglycerides and small amounts of cholesterol. As the VLDL travels through the bloodstream, triglycerides (TG) are released to tissues such as skeletal muscle, which use the component fatty acids for fuel. Once most of the triglycerides are released, the lipoproteins become low-density lipoproteins (LDL) filled primarily with the
remaining cholesterol. Total blood cholesterol is usually estimated by the amount of circulating LDL. Cholesterol is then delivered to cells, which use it to construct cell membranes. Any remaining, unused cholesterol is scavenged by another species of lipoprotein, known as high-density lipoproteins (HDL). If the amount of unused cholesterol exceeds the scavenging ability of HDL, then it is deposited in vessel walls, contributing to the buildup of atherosclerotic plaques (Barter, 2005). Atherosclerosis is associated with high levels of total cholesterol, TG, LDL, and low levels of the scavenging HDL. Having high levels of “bad” cholesterol and low levels of “good” cholesterol is known as dyslipidemia.

When insulin resistance triggers fat cells to stop storing lipid, but to instead release large amounts of free fatty acids, the liver attempts to process these fatty acids and shifts metabolism towards TG production and HDL-C particle destruction as previously mentioned (Kirk & Klein, 2009). Therefore, high TG and low HDL-C are the dyslipidemia of insulin resistance. While free fatty acids are rarely measured, they also exist in higher levels in the bloodstream in the insulin resistant state.

Children who are overweight (BMI 85th – 94th percentile) have a 7-fold increased risk of having elevated TG, and a 3-fold increased risk of having lower HDL-C than non-overweight children (Freedman et al., 1999). LDL-C is also affected by adiposity and abnormally high levels of total cholesterol are present in about 20% of overweight children (Barlow, Dietz, Klish, & Trowbridge, 2002), double the rate of all children (5-10%) (Daniels, 2006).

**Hypertension**
Hypertension is an elevation of blood pressure, which occurs when the heart pumps blood into arteries that do not distend properly or when the blood volume is too high. Globally, nearly half of all deaths from ischemic heart disease and stroke can be attributed to hypertension (Danaei et al., 2006). Obesity has long been linked to hypertension (Daniels, 2006). The mechanism by which obesity causes hypertension is still a matter of conjecture. Several hypotheses have been made linking fat-derived hormones known as adipokines to hypertension. Other hypotheses implement insulin resistance directly.

Fat cells release many adipokines, some of which may cause increases in blood pressure. TNF-α and IL-6 are adipokines that have been mentioned previously. Additional adipokines are leptin, adiponectin, and resistin. When the body's fat stores increase, levels of leptin, TNF-alpha, and IL-6 also increase; but adiponectin decreases. The first theory linking obesity to hypertension is that leptin is released at higher levels and can activate the sympathetic nervous system via the hypothalamus (Kirk & Klein, 2009), increasing blood pressure.

A second theory is that low levels of inflammation from TNF-α and IL-6 are the mechanism by which adiposity causes hypertension. Adipose TNF-α and IL-6 travel to the liver. In response, the liver increases the production of C Reactive Protein (CRP) (Heilbronn & Campbell, 2008). At the level of the vessels, CRP decreases endothelial nitric oxide synthase resulting in decreases in nitric oxide. Nitric oxide is a vasodilator. Therefore, inflammation may inhibit the vessel's ability to dilate (Virdis et al., 2009).
The third hypothesis is that insulin resistance and FA elevate blood pressure. FA have intrinsic vasoconstrictive properties and insulin typically causes vasodilation, increasing blood flow. Insulin also acts at the kidneys to increase renal sodium reabsorption and water retention. In the context of fat cell insulin resistance, FA are released causing vasoconstriction. Additionally, insulin resistance does not only develop in fat, muscle, and liver, it can also develop in other organs such as blood vessels. Insulin-resistant blood vessels do not dilate. The kidney, however, remains sensitive to insulin, so as insulin levels rise, sodium and water are retained (Kirk & Klein, 2009). In this way, insulin resistance leads to both vasoconstriction and water retention.

Rates of hypertension are estimated to be 2-4% in all children (Daniels, 2006), and 20-30% in obese children (Barlow, Dietz, Klish, & Trowbridge, 2002). But a child does not have to be obese to be at risk for hypertension. At all levels of adiposity, an increasing BMI z-score is continuously associated with increases in fasting insulin and blood pressure even within the normal weight range (Bell et al., 2007). In longitudinal studies, fasting insulin in 6- to 9-year-old children has been shown to precede and predict blood pressure 6 years later (Taittonen et al., 1996).

**Non-alcoholic fatty liver disease**

The increased prevalence of obesity has uncovered additional insulin-resistance-related diseases that are associated with atherosclerosis, though may not provide a causal link. One such disease is nonalcoholic fatty liver disease (NAFLD). While little is known about NAFLD, it has recently been correlated to atherosclerotic
plaques in the carotid artery (Sookoian & Pirola, 2008) independent of BMI and other components of metabolic syndrome (Choi et al., 2008; Wang et al., 2009).

When hepatic fat cells become entrenched in fatty acids, there is an accumulation of fat stored in the liver (Kirk & Klein, 2009). This is not a benign process. It is toxic to the liver cells. When levels of fatty acids comprise 5.5% the total tissue mass, NAFLD is diagnosed. NAFLD represents the entire continuum from simple fatty liver, known as steatosis; to fatty liver with inflammation, known as steatohepatitis or NASH; all the way to cirrhosis and liver failure. NAFLD progresses to NASH when stored FA cause oxidative stress within the cell, triggering the activation of TNF-α and an inflammatory cascade. The inflammatory process then creates reactive oxygen species that react with stored FA to create destructive peroxides. Peroxides lead to cell injury. Inflammation from cell injury, and from the response of resident stellate cells, causes irreversible fibrosis and cirrhosis (D’Adamo et al., 2009). Cirrhosis will lead to death, unless a liver transplant is performed.

NAFLD is seen in 2.6% of normal weight children, but in as many as 77% of obese children (D’Adamo et al., 2009). Increasing BMI z-score in children is continuously associated with increasing levels of fasting insulin and liver aminotransferases (AST, ALT), markers of liver cell damage (Bell et al., 2007). Independent to levels of obesity, fatty liver severity has been associated with insulin resistance and could be a childhood risk factor for the future development of T2DM (D’Adamo et al., 2009) and atherosclerosis (Choi et al., 2008; Wang et al., 2009).

**Obesity as an independent risk factor for atherosclerosis**
Most overweight children remain overweight as adults, and obesity can directly increase the risk of atherosclerosis independent of other risk factors. This is believed to occur as the result of adipokines released from the fat cells such as the previously mentioned TNF-α and adiponectin (Virdis et al., 2009). First, TNF-α released from adipose tissue triggers the formation of atherosclerotic plaques by initiating a cascade of growth factors and proinflammatory cytokines, which activates macrophages in the periphery and other atherogenic processes such as smooth muscle cell migration and increased adhesion molecules in the endothelial surface of vessels (Virdis et al., 2009). Second, adiponectin, released from fat cells, decreases atherosclerosis, diabetes, and inflammation (D’Adamo et al., 2009). The release of adiponectin is inversely related to fat mass. Unfortunately, as fat stores go up adiponectin decreases (Virdis et al., 2009). When adiponectin decreases, it is no longer able to protect against atherosclerosis, nor decrease diabetes or inflammation. So by increasing TNF-α and decreasing adiponectin, fat cells contribute directly to atherosclerosis.

Perhaps due to the effects of TNF-α and adiponectin, adolescent BMI has been associated with atherosclerotic lesions in human subjects both cross-sectionally and longitudinally. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group found that the BMI of adolescents was cross-sectionally associated with atherosclerotic precursor lesions, independent of other cardiovascular risk factors (McGill et al., 2002). A retrospective longitudinal study by Oren and colleagues (2003) demonstrated that adolescent BMI was
independently associated with adult (27-30 y/o) carotid intima-media thickness (CIMT), a marker of atherosclerosis measured with ultrasound technology.

**The Metabolic Syndrome**

When all of the conditions described above (central adiposity, impaired fasting glucose, dyslipidemia, high blood pressure, and perhaps NAFLD) are combined in a single person, it is known as metabolic syndrome. It is largely recognized that the underlying cause of MetS is insulin resistance. Insulin resistance and its associated syndrome have been found in the pediatric population, and its development may distinguish those who are most at risk of future cardiovascular disease and those who are not (Camhi et al., 2010).

Currently, it is estimated that the prevalence of MetS in adolescents is 0.1% in normal weight adolescents, 7% in overweight, and 29% in obese adolescents (Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003). Continuous increases in adiposity are associated with continuous increases in insulin resistance (Bell et al., 2007) and insulin resistance that is the harbinger of MetS syndrome. Indeed, childhood fasting insulin independently predicts components of MetS in childhood, adolescence (Bao, Srinivasan, & Berenson, 1996), and adulthood (Camhi et al., 2010). Once a child develops MetS, they are likely to continue having MetS as an adult (Bao et al., 1996; Bao, Srinivasan, Wattigney, & Berenson, 1994; Lauer, Clarke, Mahoney, & Witt, 1993; Morrison, Friedman, Wang, & Glueck, 2008; Webber, Srinivasan, Wattigney, & Berenson, 1991), and they have a 12-fold risk of developing T2DM (Morrison et al., 2008). Conversely, if a child never develops MetS,
he or she is less likely to get MetS in middle age (Chen, Srinivasan, Li, Xu, & Berenson, 2005; D'Adamo et al., 2009).

Individually, the elements of insulin resistance syndrome: IFG, low HDL, high TG, and elevated blood pressure all contribute independently to the risk of atherosclerosis. Therefore, obesity increases atherosclerosis both indirectly by increasing MetS and directly through various fat-derived apipokines. Atherosclerosis is the final convergent pathway, linking obesity to early death from cardiovascular disease.

**The Early Stages of Atherogenesis are Reversible**

The path between childhood insulin resistance and eventual cardiovascular disease is not unalterable. The pathogenic processes of insulin resistance and atherogenesis are reversible if caught early. According to Battista, Murray, and Daniels (2009), the cardiovascular system of children and adolescents is resilient enough to allow for reversal of early atherosclerotic changes if changes to weight and health behaviors are made (Battista et al., 2009). In large retrospective cohorts, the relationship between child and adolescent adiposity and mortality was attenuated after adjusting for adult BMI (Engeland et al., 2004). Additionally, Oren and colleagues (2003) found that subjects who remained in upper BMI distribution from adolescence to young adulthood had higher carotid intima-media thickness (CIMT) than those who had lost weight over time. Those experiencing weight loss had CIMT values comparable to those with constantly low BMI (Oren et al., 2003). This suggests that weight loss and maintenance may be the key to reversing the
atherosclerotic process, especially in those who are most susceptible to insulin resistance, such as Mexicans.

**Mexicans are Prone to Developing Insulin Resistance**

Previous sections discussed how Mexicans throughout North America have high levels of T2DM, hypertension, and dyslipidemia at younger ages and lower levels of adiposity. The pathophysiology revealed how insulin resistance is the underlying cause of all of these conditions, all of which contribute to atherosclerosis and cardiovascular disease. Insulin resistance has a strong genetic susceptibility (D’Adamo et al., 2009), and while most insulin resistance occurs in overweight individuals, not all overweight individuals become insulin resistant (O’Connell et al., 2010). There is now evidence that Mexicans are more prone to becoming insulin resistant, therefore they are more prone to developing the constellation of diseases associated with insulin-resistance, namely MetS.

Mexicans of all ages are genetically vulnerable to developing insulin resistance compared to other ethnic groups (Ford, Giles, & Dietz, 2002; Lee et al., 2006; Park et al., 2003). Children with lower insulin sensitivity may develop MetS (Shaibi & Goran, 2008), Perhaps not surprisingly, MetS is highest among Hispanic American adolescents compared to white and black counterparts (Cook, Auinger, Li, & Ford, 2008; de Ferranti, Gauvreau, Ludwig, & Neufeld, 2004; Johnson et al., 2009; Messiah et al., 2009). Some sources estimate that the prevalence of MetS in adolescent Mexican Americans is 25% higher than non-Hispanic White and 280% than non-Hispanic Black (Johnson et al., 2009). Adult Mexicans more also more
prone to MetS compared to other ethnicities (Park et al., 2003; Ford et al., 2002). In addition, the highest risk for NAFLD is seen in people of Hispanic ethnicity (Schwimmer et al., 2003).

In summary, Mexico will bear much of the incoming onslaught from the tsunami of childhood obesity for three reasons: The first, is that Mexico is in close proximity to the obesiogenic environment of the United States. Not only is the United States exporting many cultural changes into Mexico, but many Mexicans are emigrating into the United States, increasing the obesiogenic exposure of this population. The second, is that while many obesity-related diseases can be prevented with good healthcare provisions, the Mexican health care system is already strained with the “double burden” of diseases of overnutrition and diseases of poverty such as infectious disease and perinatal mortality. The third reason that Mexico will bear much of the burden of childhood obesity, is that Mexicans are genetically prone to insulin resistance and the subsequent development of obesity-related diseases such as MetS, and resultant atherosclerosis, IHD, and stroke. One can anticipate that over the next several years, this economically and genetically vulnerable population will experience a sharp incline in obesity-related diseases (Rivera et al., 2001 as cited by Martorell, 2005). Screening protocols are urgently needed, because, without them, the steady rise in life expectancy seen in the modern era may reverse (Olshansky et al., 2005).

**The Feasibility of Pediatric Screening for Obesity**

Mexico would benefit from establishing a pediatric protocol for the screening of obesity and obesity-related diseases. The precursors of cardiovascular disease
begin in childhood and can be detected early by screening. Countries such as the England, Scotland, Canada, and Australia have each published various strategies for screening children in the clinic for overweight and obesity, and cardiovascular disease risk. The number of screening protocols originating from Latin American countries is limited (Delgado-Noguera, Tort, Bonfill, Gich, & Alonso-Coello, 2009). In 2007, the American Academy of Pediatrics published screening recommendations that included special provisions for children of Mexican Hispanic ethnicity (Barlow and the Expert Committee, 2007). Implementing such pediatric screening recommendations is promising, but may be challenging, as most recommendations remain to be externally validated. However, without such recommendations, physician identification of the overweight child is surprisingly low. Recommendations such as those published in 2007 are clearly needed, but also require validation in understudied populations such as those within Mexico.

**The 2007 Pediatric Expert Committee Recommendations**

In 2007, the American Academy of Pediatrics published recommendations for a screening protocol on the prevention, assessment, and treatment of child and adolescent overweight and obesity. The 2007 screening recommendations were a revision of the recommendations published in 1998. The original recommendations were a consensus statement from experts belonging to the American Academy of Pediatrics, American Heart Association, the National Institutes of Health, the CDC, and other governmental and professional organizations (Barlow & Dietz, 1998).
At the time of its inception, there was little evidence on which to base recommendations. The results of having limited evidence were threefold. First, there were no consensus cutoffs for defining pediatric overweight and obesity. This was a substantial limitation. Standardized BMI percentile curves would not be published by the CDC until 3 years later (Kuczmarski et al., 2000). Second, it was recommended that risk behaviors such as diet and physical activity should be assessed, but their inclusion was merely speculative. For example, physicians were instructed to ask families to describe dietary intake, but no references were cited providing evidence on what behaviors or foods were obesiogenic. Third, the algorithm for laboratory assessment was vague and did not provide diagnostic criteria for IFG, dyslipidemia, or hypertension. For example, it was recommended to screen for T2DM, however, laboratory cutoffs were not provided (e.g. “Diabetes is defined as having two fasting glucose values ≥ 126 mg/dL”). As a result of these limitations, external reviewers discredited the recommendations stating that they were not evidence-based (Delgado-Noguera et al., 2009).

Ten years later, a second writing group headed by Dr. Sarah E. Barlow met to rewrite the recommendations. The 2007 writing group also consisted of a panel of expert committee members from many professional organizations, and included physicians and scientists with expertise in childhood obesity (Barlow and the Expert Committee, 2007). The result of this second meeting was a 124-page, 4-part document containing over 1,000 cited references (Barlow and the Expert Committee, 2007; Davis et al., 2007; Krebs et al., 2007; Spear et al., 2007). Not only were overweight and obesity defined according to standardized percentile charts
(Kuczmarski et al., 2000), but also an extensive review of the literature was conducted. This review of literature included cross-sectional evidence, longitudinal studies, and randomized controlled trials in the area of diet and physical activity. From 1997 to 2007, the number of cited studies for dietary behaviors increased from zero to over 100, and citations on physical activity behaviors increased over 4-fold (Barlow & Dietz, 1998; Barlow and the Expert Committee, 2007). Recommendations for laboratory assessment also became more well-defined and were largely based upon previously published guidelines from the American Heart Association, the American Academy of Pediatrics, and the American Diabetes Association (Krebs et al., 2007). Needless to say, the 2007 Pediatric Expert Committee Recommendations (PECR) represented an improvement over the original 1997 recommendations in the area of anthropometric definitions, diet and physical activity parameters, and laboratory assessment.

As mentioned above, the 2007 PECR were evidence-based and included many specific screening recommendations for physicians and healthcare providers. The goal was to give guidance to providers as to what questions to ask, what labs to order, and when to order them (See Table 2.2). Outlining the recommendations, The Pediatric Expert Committee states that at every clinic visit, for all pediatric patients, it is recommended to perform a qualitative assessment of dietary patterns. This includes, but is not limited to, asking about the frequency of eating outside of the home, excessive consumption of sugar-sweetened beverages, and breakfast consumption. At least once a year, at every well-child visit, for all pediatric patients, it is recommended there be an assessment of physical activity levels, determining
Table 2.2

*Recommended Diet and Physical Activity Assessment (Krebs et al., 2007).*

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<thead>
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<th>Assessment</th>
<th>Description of Recommendations</th>
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<tr>
<td>Health Behavior</td>
<td>A brief clinical assessment to gauge a patient’s motivation to change</td>
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<tr>
<td>Dietary Assessment</td>
<td>Assessment of self-efficacy &amp; readiness to change</td>
<td>-</td>
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<tr>
<td>(all patients as each clinic visit)</td>
<td>Qualitative assessment of dietary patterns</td>
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<td>Address certain patterns for which evidence supports a positives association with energy intake:</td>
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<tr>
<td></td>
<td>1. Frequency of eating outside the home at restaurants or fast food</td>
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<td>2. Excessive consumption of sweetened beverages</td>
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<td></td>
<td>3. Consumption of excessive portion sizes for age</td>
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<td>4. Excessive consumption of 100% fruit juice</td>
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<tr>
<td></td>
<td>5. Breakfast consumption (frequency and quality)</td>
<td>ME</td>
</tr>
<tr>
<td></td>
<td>6. Excessive consumption of foods that are high in energy density</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7. Low* consumption of fruits and vegetables</td>
<td>ME</td>
</tr>
<tr>
<td></td>
<td>8. Meal frequency and snacking patterns (include quality)</td>
<td>-</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>Self-efficacy and readiness to change</td>
<td>-</td>
</tr>
<tr>
<td>Assessment</td>
<td>Environment and social support</td>
<td>-</td>
</tr>
<tr>
<td>(all patients, once a year at each well-child visit)</td>
<td>Assessment of social and environmental barriers and facilitators for PA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Level of Physical Activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screen for whether or not child is meeting recommendations of 60 minutes of at least moderate physical activity/day</td>
<td>ME</td>
</tr>
<tr>
<td></td>
<td>Level of Sedentary Behavior</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Assessment of sedentary behavior such as watching TV in comparison with &lt;2hrs/day.</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>2. DVDs, playing videogames, and using the computers in comparison with &lt;2hrs/day.</td>
<td>-</td>
</tr>
</tbody>
</table>

Evid – Strength of the evidence supporting this behavior as obesiogenic (Barlow et al., 2007).
CE – Consistent evidence
ME – Mixed evidence
* Low is defined as < 5 servings of fruits and vegetables a day (Barlow and The Expert Comm. 2007)
whether the patient gets at least 60 minutes of moderate to vigorous physical activity per day; and also an assessment of sedentary behaviors such as TV viewing to assure that it does not exceed 2 hrs/day. Also, at least once per year, physicians should measure the BMI of all patients and plot the BMI age- and sex-specific percentile on the standardized CDC BMI percentile chart (Kuczmarski et al., 2000).

When a child is found to have a high BMI, it is recommended that the physician obtain a family medical history (FMHx) of obesity, T2DM, CVD (especially hypertension) in parents and grandparents, as well as conduct a review of systems and physical exam for weight-related problems. Appropriate laboratory tests are recommended according to BMI percentile and stratification of risk (see Table 2.3) (Barlow and the Expert Committee, 2007).

Table 2.3

<table>
<thead>
<tr>
<th>BMI Percentile</th>
<th>Risk Factors</th>
<th>Recommended Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight (≤85th %ile)</td>
<td>0</td>
<td>BP</td>
</tr>
<tr>
<td></td>
<td>1*</td>
<td>BP, Fasting lipids</td>
</tr>
<tr>
<td>Overweight (&gt;85th – 94th %ile)</td>
<td>&lt; 2**</td>
<td>BP, Fasting lipids</td>
</tr>
<tr>
<td></td>
<td>≥ 2**</td>
<td>BP, Fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td>Obese (≥95th %ile)</td>
<td>≥ 0</td>
<td>BP, Fasting lipids, fasting glucose, AST, ALT (microalbumin/creatinine ratio)</td>
</tr>
</tbody>
</table>

Note: Adapted from Barlow & The Expert Committee (2007).
BP – Blood Pressure
* Family Medical History of high cholesterol
** Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease, hypertension), ethnic minority [African, Hispanic], elevated BP, elevated lipids, tobacco use, and signs of insulin resistance.
**The Importance of Pediatric Screening**

Currently, the leading causes of death in Mexico are from preventable diseases that can be screened for in childhood. A screening protocol such as the 2007 PECR could potentially benefit Mexico, which currently does not have national recommendations. In order for screening to be successful in this developing country, Mexico-specific screening recommendations must be written and validated. Recommendations are crucial, because, without them many children with potentially underlying diseases remain unrecognized.

There is evidence that suggests screening protocols improve physician identification and management of the overweight child. Evidence for this comes from developed countries with a long-standing population of overweight and obese children, such as the United States. In the United States, 1 in 10 pediatric well-child exams are with a child who is obese (O’Brien, Holubkov, & Reis, 2004) and a large majority of physicians recognize the growing health burden of childhood obesity (Story et al., 2002). Despite this, providers document obesity in only half of all cases (O’Brien et al., 2004; Barlow, Trowbridge, Klish, & Deitz, 2002; Eneli, Keast, Rappley, & Camargo, 2008; Benson, Baer, & Kaelber, 2009).

Obesity may be poorly documented, but documentation rates for less severe forms adiposity, such as those in the overweight range, are much lower. Data from one large Midwest academic center revealed that between 1999 and 2007, only 10% of overweight (BMI 85th to 94th percentile) children aged 2- to 18-years-old were correctly identified as overweight. Identification rates were higher in obese children (BMI ≥95th percentile): 54% of obese, and 76% of severely obese children.
were correctly identified (Benson et al., 2009). Similar results on low identification of overweight children have been described elsewhere (Barlow, Bobra, Elliott, Brownson, & Haire-Joshu, 2007).

Why are so few overweight and obese children being identified? According to questionnaire data, 97% of physicians report visuially assessing their patients for overweight status at most or every well-child visit (Klein et al., 2010). Unfortunately, there is a major problem with visual assessment of children: Physicians are exceedingly poor at it. The accuracy of visual assessment is barely better than flipping a coin. Huang and colleagues (2009) reported that 58% of pediatricians correctly identify the weight status of children in pictures. During visits with patients, visual assessments were only slightly better, with approximately 60% of children’s weight status being correctly reported (Chaimovitz, Issenman, Moffat, & Persad, 2008). Almost all identification errors are due to underestimation, and normal weight children are sometimes identified as underweight (Chaimovitz et al., 2008). Experience does not improve estimation. In fact, experienced pediatricians were less accurate than their younger colleagues at identifying the weight status of children (Huang et al., 2009).

Visual assessment is exceedingly poor, yet the use of BMI percentile charts as described in the screening recommendations can drastically improve identification of overweight children (Perrin, Flower, & Ammerman, 2004), particularly those who are overweight, but yet not obese (Barlow et al., 2007). Despite improved identification with BMI percentile charts, only 6.1% of medical charts contained up-to-date plots of BMI percentiles (Barlow et al., 2007). Poor physician identification
of overweight and obesity may be a matter of logistics rather than a lack of knowledge. Simply providing physicians tools to calculate and plot BMI percentile charts improves documentation (Barlow, Dietz, Klish, & Trowbridge, 2002; Dunlop, Leroy, Trowbridge, & Kibbe, 2007; Spurrier, Magarey, & Wong, 2006). In theory, disseminating tools would improve the identification of children who are overweight or obese (Barlow et al., 2007; Perrin, Flower, & Ammerman, 2004), and perhaps improve the management of these patients.

Identification is the key to improving management of the overweight or obese child. This is especially true for in-office counseling for diet and physical activity (Dunlop et al., 2007; Jonides, Buschbacher, & Barlow, 2002). For instance, in obese children correctly identified as obese, 71% received dietary counseling and 33% were advised to increase physical activity (PA). In obese children who were not identified, counseling occurred in only 6% and 0% of patients respectively (O’Brien et al., 2004). However, even in those identified, management of obese patients prior to the publication of 2007 PECR was subpar.

While on-the-spot counseling was high in correctly-identified patients, appropriate follow-up was low. Only half of all physicians self-reported routinely initiating treatment in obese patients (Jonides et al., 2002). After examining medical records, O’Brien and colleagues (2004) found that even for obese children who were properly identified, appropriate follow-up with evaluation and management occurred less than 5% of the time (O’Brien et al., 2004). Laboratory exams were most frequently ordered only for the most severely obese and oldest adolescents. Younger children and those with less severe obesity were often overlooked. The
study did not examine the charts of overweight children, though one can deduce that management of these children would be even lower. Others have documented that only the oldest and most obese patients are managed by physicians (Barlow et al., 2007; Jelalian, Boergers, Alday, & Frank, 2003; Benson et al., 2009; O’Brien et al., 2004).

Without good recommendations, most physicians practice “eyeballing,” and only the older, most severely obese children are identified and managed. Unfortunately, the risk for cardiovascular disease has been associated with BMI values much lower than severe obesity, even at values within the upper-normal weight range (Messiah, Arheart, Lipshultz, & Miller, 2008). The risk was also present in children as young as 8-years-old, which is clearly a decade sooner than late adolescence (Sun et al., 2008). Therefore, when “eyeballing” is practiced, many children who are at risk are missed. Recommendations are clearly needed to improve identification and management, but these recommendations need to be evidence-based.

**Validating Pediatric Screening Recommendations**

The importance of valid screening recommendations has become evident in developed countries where pediatric obesity management is low. When screening recommendations are not evidence-based, even clinicians who are aware of recommendations are not more likely to follow them (Kolagotla & Adams, 2004). Delgado-Noguera and colleagues (2009) reviewed clinical guidelines published around the world between 1998 to 2007, and found that as few as half were
evidence-based, and only a quarter of existing recommendations could be recommended by independent evaluators.

Now that the evidence-based 2007 Pediatric Expert Committee Recommendations have been published, it is important to test these recommendations on real patients. If the recommendations are valid, the algorithms will discern patients who are at risk for obesity-related diseases from those who are not. Valid recommendations are especially needed in high-risk countries such as Mexico. Therefore, if the 2007 PECR could be validated and adapted to a Mexican population, they could be disseminated and reinforced, creating a weir against the storm of childhood obesity.

**Study Objective**

The efficacy of existing screening recommendations for Hispanics must be validated, before recommendations can be made for Mexico. The 2007 PECR by Barlow and the Expert Committee (2007) are the only available, evidence-based set of guidelines written specifically for those of Mexican ethnicity. While the writing of the guidelines was evidence-based, to our knowledge (last search May 2010), the epidemiological parameters of the PECR have not been tested on a Hispanic population or any population of children, adolescents, and young adults to date. The current study proposes to do so. The subjects will be adolescents in the Central Mexican State of San Luis Potosí. Data were collected as recommended by the Pediatric Expert Committee and subjected to a classification and regression tree analysis in order to determine the predictive validity of the recommendations.
The main research question was: Are the PECR a valuable screening test for (1) NALFD, (2) dyslipidemia, and/or (3) IFG in a population of matriculating college students in Central Mexico? Whether or not the PECR was a valuable screening test ultimately depended on whether or not it met the following criteria:

- The screen was able to accurately identify a condition
- The condition of interest is serious
- The condition of interest is treatable
- The condition of interest has a long latency period
- The condition is sufficiently common to justify the cost of screening

The screening test of interest in the PECR clinical screen. The condition was defined as elevated liver enzymes, dyslipidemia, and IFG. Accurate identification was considered as having a sensitivity and specificity of > 0.80 and significant associations on multivariate analysis.

The hypothesis was that the PECR would be moderately sensitive and specific, but would require adjustment for the Mexican population, given the predisposition for Mexicans to develop obesity-related diseases at lower BMI levels compared to other ethnicities (Sanchez-Castillo et al., 2005).

In order to answer this research question, there was a three-stepped analysis for each of the three CVD risk factors (NAFLD, dyslipidemia, and IFG). The first step was to identify the prevalence of the three CVD risk factors in Mexican young adults. The second aim was to test the sensitivity and specificity of the PECR in identifying Mexicans with the CVD risk factors. Finally, the third aim was to explore ways in which to improve the clinical screening algorithm (Figure 2.2).
By testing and adjusting a screening protocol for Mexican adolescents, individuals who are at risk for MetS and future cardiovascular risk will, hopefully not be missed, but will be identified early. In this way, the incoming tsunami in Mexico may be averted.

Figure 2.2

The plan for data analysis.

Elevated Liver Enzymes

- n = 63, matched
- GOAL 1
- GOAL 2 incl. ethnicity vs. not
- GOAL 3 via manual exploration

Dyslipidemia

- n = 829
- GOAL 1
- GOAL 2
- GOAL 3 via CART Analysis

Impaired Fasting Glucose

- n = 5455
- GOAL 1
- GOAL 2 incl. ethnicity vs. not
- GOAL 3 via CART Analysis

CART – classification and regression tree
incl. – including
vs. – versus
CHAPTER 3: THE PEDIATRIC EXPERT COMMITTEE RECOMMENDATIONS AS A CLINICAL SCREENING TOOL FOR ELEVATED LIVER ENZYMES

Non-alcoholic fatty liver disease (NAFLD) is currently believed to be the most common liver disease in children, adolescents, (Schwimmer et al., 2006; Clark, 2006; Mager & Roberts, 2006; Riley, Bass, Rosenthal, & Merriman, 2005) and adults (Grattagliano et al., 2007). It is associated with higher mortality rates compared to the general population (Adams et al., 2005; Rafiq et al., 2009; Ekstedt et al., 2006; Soderberg et al., 2010; Ahmed, Abu, & Byrne, 2010; Musso, Gambino, Cassader, & Pagano, 2010) and its incidence in children, adolescents, and young adults has risen in parallel with increasing rates of obesity (Koebnick, et al., 2009).

NAFLD, therefore, constitutes an emerging global health problem (Barshop, Sirlin, Schwimmer, & Lavine, 2008). Biopsy, postmortum, and imaging studies have estimated prevalence rates of NAFLD to be 9.6% in adolescents (Schwimmer et al., 2006) and 15 to 40% in adults in western countries (Hultcrantz et al., 1986; Ground, 1982; Loguercio et al., 2004; Szczepaniak, et al., 2005; Krawczyk, Bonfrate, & Portincasa, 2010; Williams et al., 2011). Obesity and insulin resistance are the strongest risk factors for NAFLD (Ahmed, Abu, & Byrne, 2010) and both are associated with the presence of the disease and its severity (Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine 2003; Patton et al., 2010). This relationship is so strong that NAFLD has been dubbed the hepatic manifestation of the syndrome of insulin resistance known as MetS (NCEP, 2002; Shulman & Mangelsdorf, 2005; Rector, Thyfault, Wei, & Ibdah, 2008).
The Natural History of NAFLD

NAFLD defines a continuum of disease that begins with a relatively benign and reversible accumulation of triglycerides in liver cells and ends in irreversible liver failure death. When excess triglycerides are stored in the liver cells, this subtype of NAFLD is known as simple steatosis. Simple steatosis can overburden the liver and lead to cell damage and inflammation. Once inflammation exists, the NAFLD subtype is known as non-alcoholic steatohepatitis (NASH). Simple steatosis and NASH are both potentially reversible. However, if inflammation leads to fibrosis (i.e. scarring), then NASH progresses to the subtype of cirrhosis. Cirrhosis is irreversible and can lead to liver failure and death (Farrell & Larter, 2006), unless a liver transplant can be arranged. In the pediatric population, the full spectrum of disease exists and cases of cirrhosis have been reported (Sathya, Martin, & Alvarez, 2002; Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine 2003; Molleston et al., 2002; Rashid & Roberts, 2000; Molleston, White, Teckman, & Fitzgerald, 2002). When transplantation is necessary, NAFLD tends to recur in the transplanted liver (Feldstein, et al., 2009).

It is estimated that approximately 4% of patients with NAFLD will progress to cirrhosis within 20 years (Matteoni et al., 1999; Dam-Larsen et al., 2004). In patients holding the diagnosis of inflammatory NASH (this is 20-30% of all NAFLD cases), progression to cirrhosis occurs more frequently (Matteoni et al., 1999; Grattagliano et al. 2007). Approximately 15 to 39% of patients with NASH develop cirrhosis within 10 years (Bacon, Farahvash, Janney, & Neuschwander-Tetri, 1994; Matteoni, et al., 1999), making NASH the leading cause of crypogenic cirrhosis.
(Caldwell, et al., 1999). The prognosis for those with cirrhosis is grim: Over a third of patients with cirrhosis die from liver-related causes within 10 years (McCullough, 2006). In addition to the development of cirrhosis, those with NASH are at a higher risk for hepatocellular cancer (Adams et al., 2005). With higher rates of cirrhosis, cancer, and liver failure, approximately 12% of those with NASH succumb to death within 10 years of the initial diagnosis (Schuppan, Gorrell, Klein, Mark, & Afdhal, 2010). In contrast, those in whom simple steatosis never progresses to inflammation, have better prognosis (Musso, Gambino, Cassader, & Pagano, 2010). However, in the pediatric population, the progression of simple steatosis to NASH may be more common than in the adult population (Schwimmer, Behling, Newbury, Deutsch, Nievergelt, Schork, & Lavine, 2005).

While liver disease a significant cause of death in those with NAFLD (Ong, Pitts, & Younossi, 2008; Rafiq et al., 2009; Ekstedt et al., 2006), it is the heart and not the liver that constitutes the greatest mortality risk. For patients with NAFLD, the leading cause of death is coronary artery disease (Treeprasertsuk, Lopez-Jimenez, & Lindor, 2010; Targher, Day, & Bonora, 2010). Coronary artery disease (CAD) accounts for 1 in 4 deaths in those with NAFLD (Adams et al., 2005, Rafiq et al., 2009). In contrast, liver-related deaths account for just over 1 in 10 deaths (Schuppan, Gorrell, Klein, Mark, & Afdhal, 2010). Further, NAFLD has been strongly associated with other risk factors for coronary artery disease, such as the MetS (Marchesini et al., 2001; Pagano et al., 2002; Patton et al., 2010). However, even after controlling for components of the metabolic syndrome and other well-established CAD risk factors, the presence of a fatty liver remains independently
associated with increased cardiovascular risk (Targher, Day, & Bonora, 2010; Targher et al., 2007; Chen, Nien, Yang, & Yeh, 2010; Arslan et al., 2007). The pathophysiology of this relationship remains unknown, but research has elucidated several links between NAFLD and cardiovascular derangements such as autonomic instability (Newton, 2010); cardiac left ventricular diastolic and systolic dysfunction (Fotbolcu et al., 2010); impaired flow-mediated arterial vasodilatation (Brea et al., 2005; Fracanzani et al., 2008; Kim, Kim, & Huh, 2009; Targher et al., 2004; Targher et al., 2006; Volzke et al., 2005; Vlachopoulos et al., 2010); and an increase in subclinical and clinical atherosclerotic lesions (Fracanzani et al., 2008; Volzke et al., 2005; Sookoian & Pirola, 2008). The severity of NAFLD correlates to the severity of CAD independent of other CAD risk factors (Mirbagheri, et al., 2007). Despite disease severity, meta-analysis has shown that both simple steatosis and NASH to carry an increased risk for CVD death above those without NALFD (Musso, Gambino, Cassader, & Pagano, 2010).

**Epidemiology of NAFLD**

NAFLD is common, affecting up to 40% adults in westernized countries (Krawczyk, Bonfirate, & Portincasa, 2010). Obesity is the most frequently cited risk factor, but after controlling for measures of adiposity, NAFLD differentially affects those who are male (Barshop, Sirlin, Schwimmer, & Lavine, 2008; Browning et al., 2004; Lavine & Schwimmer, 2004), those who are older (Schwimmer et al., 2006), and those of Hispanic ethnicity (Browning, Kumar, Saboorian, & Thiele, 2004; Browning et al., 2004; Caldwell, Harris, & Hespenheide, 2002; Ioannou, Boyko, & Lee
The association of Hispanic ethnicity and NAFLD exists in children, adolescents, and adults (Ruhl & Everhart, 2003; Weston et al., 2005; Schwimmer, Behling, Newbury, Deutsch, Nievergelt, Schork, & Lavine, 2005; Schwimmer, McGreal, Deutsch, Finegold, & Lavine, 2005). In adult samples from the United States, biopsy and magnetic resonance spectroscopy (MRS) studies estimate that NAFLD affects approximately 1 in every 2 Hispanic adults compared to 1 in 3 adults overall (Browning et al., 2004; Williams et al., 2011). In an autopsy study of children and adolescents (2- to 19-years-old) NAFLD existed in 11.8% of Hispanics compared to 1.5% of non-Hispanic blacks and 8.6% of non-Hispanic whites (Schwimmer et al., 2006). Compared to non-Hispanic whites and non-Hispanic blacks, Hispanic children and adolescents have been found to be the most prone to severe forms of steatosis and advanced liver fibrosis (Schwimmer, Behling, Newbury, Deutsch, Nievergelt, Schork, & Lavine, 2005; Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003), and Hispanic adults have the highest rates of cirrhosis (Caldwell, Harris, & Hespensheide, 2002; Browning, Kumar, Saboorian, & Thiele, 2004).

Mortality from hepatocellular cancer has also been associated with Hispanic ethnicity (Younossi, & Stepanova, 2010). It was originally supposed that the higher prevalence of NAFLD in Hispanics was largely due to a higher prevalence of obesity (Patton et al., 2006; Schwimmer, Burwinkle & Varni 2003; Schwimmer, McGreal,
Deutsch, Finegold, & Lavine, 2005). However, the propensity of Hispanics to get NAFLD tends to persist even after controlling for measures of adiposity (Quiros-Tejeira et al., 2005; Schwimmer et al., 2006) and individual features of MetS (Kallwitz et al., 2009). It has been estimated that being Hispanic puts a child at 5 times greater the risk of having steatosis versus being non Hispanic black (Schwimmer et al., 2006).

The reasons for ethnic disparities remain unknown. A combination of genetics and lifestyle undoubtedly play a role. As Dr. Elliot Joslin once said, “Genetics loads the gun, lifestyle pulls the trigger.” Ethnic differences between Hispanics and non-Hispanic whites may not exist in the normal weight ranges and insulin sensitive states (Browning et al., 2004). Rather, differences in susceptibility may emerge only after overnutrition puts stress on the body. Candidate genes associated with NAFLD have recently emerged such as the PNPLA3 variant. This variant exists in Hispanics and other ethnicities (Wagenknecht et al., 2011). Polymorphisms have also been found in TNF-alpha, IL-6, and KLF6 sequences (De Bruyne, Fitzpatrick, & Dhawan, 2010). Genetics may not tell the whole story and cultural differences in food and lifestyle cannot be excluded. Lifestyle habits, such as eating fast food, exercising less, being more sedentary, having a diet higher in fructose, saturated fat, cholesterol, and lower in polyunsaturated fat, were more frequently found in those with NAFLD than those without (Williams et al., 2011; Ouyang et al., 2008; Musso et al., 2003; Mager et al., 2010; Yki-Järvinen, 2010).

Most studies indicating the high prevalence of NAFLD in Hispanics have been conducted in the United States. Several studies have been conducted in Latin
American looking at NAFLD in Hispanic adults in countries such as Chile (Riquelme et al., 2009) and Mexico (Gutierrez-Grobe, Ponciano-Rodríguez, Ramos, Uribe, & Méndez-Sánchez, 2010; Rodriguez-Hernandez et al., 2010; Rodríguez-Hernández et al., 2008). Fewer studies in Latin America have examined NAFLD in children and adolescents (Fernandes, Ferraro, de Azevedo, & Fagundes Neto, 2010; Flores-Calderon, Gomez-Diaz, Rodriguez-Gomez, & Moran-Villota, 2005; Lira, Oliveira, Escrivão, Colugnati, & Taddei, 2010). One study from Brazil compared obese and overweight students to normal weight controls and found NALFD to be 10 times more prevalent in obese and overweight students (Lira et al., 2010). To our knowledge, no studies have been conducted in Mexican adolescents and young adults comparing the risk of NAFLD in normal weight and overweight or obese individuals. In Mexico, rates of overweight and obesity are similar to those of the United States (Ogden et al., 2006; Olaiz et al., 2003 as cited by Garcia-García et al., 2006) justifying the need to properly diagnose NALFD in the pediatric population.

**Clinical Diagnosis of NAFLD**

It is critical to put epidemiological evidence into the context of clinical practice. In the physician’s office, NALFD is notoriously difficult to diagnosis both in the adult and pediatric population. Sometimes the disease is asymptomatic (Manton et al., 2000; Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003) and progresses silently. In children and adolescents, symptoms are often present but vague. The most common symptoms are fatigue or abdominal pain. Hepatomegaly is often present but difficult to assess on physical exam (Feldstein et al., 2009).
NAFLD is suspected, the recommended laboratory evaluation includes liver transaminases (AST, ALT) as well as alkaline phoshatase, bilirubin, albumin, and prothrombin time (Dowman, Tomlinson, & Newsome, 2011). The liver enzymes ALT and, to a lesser extent, AST are both frequently-used biomarkers for NAFLD. Levels ALT are closely associated with liver fat content (Westerbacka et al., 2004; Radetti, Kleon, Stuefer, & Pittschieler, 2005). Liver enzymes are relatively inexpensive first-line tests, but results must be interpreted with caution because they tend to underestimate the prevalence of NALFD. Elevated liver enzymes clearly indicate the presence of liver damage, and liver enzymes increase with increasing amounts of fat content (Figure 3.1)(Kotronen et al., 2008; Radetti, Kleon, Stuefer, & Pittschieler, 2005), inflammation, and fibrosis (Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003). However, normal enzymes do not necessarily indicate a normal liver. A large proportion of those with NALFD, particularly those with early or mild NALFD, have transaminases within the normal range (Browning, et al., 2004; Dunn & Schwimmer, 2008; Riquelme et al., 2009; Franzese, et al., 1997; Manco, Alisi, & Nobili, 2008; Rashid & Roberts, 2000; Radetti, Kleon, Stuefer, & Pittschieler, 2005; Schwimmer, Behling, Newbury, Deutsch, Nievergelt, Schork, & Lavine, 2005). This is especially true in those with insulin resistance, who have 40-200% more fat in their livers than do insulin-sensitive patients with the same BMI, AST, and ALT levels (Kotronen et al., 2008). Despite these limitations, the use of liver enzymes in Hispanic populations is promising because both AST and ALT correlate highly with hepatic triglyceride content in Hispanics. This is in contrast to
The relationship between liver fat content and liver enzymes (Kotronen et al., 2008).

A finding of elevated liver enzymes should prompt imaging such as ultrasound or MRS (Dowman, Tomlinson, & Newsome, 2011). As an imaging modality, ultrasound is convenient and portable, but only detects fat infiltration >30% (Saadeh et al., 2002). A fat infiltration of >5.5% is indicative of steatosis. This can be determined more accurately with MRS (Radetti, Kleon, Stuefer, & Pittschieler, 2006; Fishbein et al., 2005). Neither MRS nor U/S, however, can determine the extent of inflammation and fibrosis, necessitating liver biopsy for definitive staging (De Bruyne, Fitzpatrick, & Dhawan, 2010). Once diagnosis occurs, treatment options exist. Particularly effective is weight loss (Franzese et al., 1997; Kocak, Yuce, Gurakan, & Ozen, 2000; Palmer & Schaffner, 1990; Manton et al., 2000; Nobili et al.,
In Mexican samples, weight reduction has been shown to improve biochemical and imaging markers of NAFLD in Mexicans (Méndez-Sánchez, González, Chávez-Tapia, Ramos, & Uribe, 2004). Weight reduction can be accomplished with physical activity and diet, with physical activity reducing liver triglycerides independent of any weight effects (Centis, Marzocchi, Di Domizio, Ciaravella, & Marchesini, 2010).

In adults, no international guidelines exist to guide physicians when to initiate the workup for NAFLD by ordering liver biomarkers (Kallman et al., 2009). The American Gastroenterological Association recommends that clinicians have a high level of clinical suspicion for NAFLD in adults, when obesity, diabetes, and/or high TG are present (American Gastroenterological Association, 2002). Despite these recommendations, screening rates and diagnosis of adult NAFLD by healthcare providers remains low (Sharp, Santos, & Cruz, 2009). Pediatric NAFLD is also underdiagnosed (Mencin & Lavine, 2010; Riley, Bass, Rosenthal, & Merriman, 2005). In 2007, guidelines were published outlining a clinical screening algorithm as an indication for laboratory assessment of liver transaminases (Krebs et al., 2007) (See Figure 3.2). This algorithm divides patients into normal weight, overweight, and obese categories, further subdividing them based on risk factors. Laboratory assessment is recommended in all obese patients and overweight patients with two or more risk factors. Mexico is a population known to be at high risk for this treatable, but dangerous disease.
Figure 3.2

*The Pediatric Expert Committee Recommended algorithm for laboratory evaluation*

Note: Adapted from Krebs et al. (2007).

FMHx – Family Medical History

%ile - percentile

* Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease, hypertension), ethnic minority [African, Hispanic], elevated BP, elevated lipids, tobacco use, and signs of insulin resistance.
**Study Aims**

The current study examined elevated liver enzymes in a group of Mexican adolescents and young adults aged 18 to 21-years-old. Obese participants were matched to overweight and normal weight controls by age and sex, then examined for the presence of elevated liver enzymes (ALT and AST). The goals of the analysis were three-fold: The first goal of the analysis was to describe the relative prevalence of elevated liver enzymes in obese, overweight, and normal weight participants. The second goal was to test the sensitivity and specificity of The PECR in detecting those with elevated liver enzymes. The third goal was to explore ways in which to improve the sensitivity and specificity by adjusting the algorithm using a decision tree analysis. An improvement would be defined as a more sensitive and specific test and/or a simpler screening protocol. A sensitivity of $\geq 0.80$ and a specificity $\geq 0.80$ was considered an acceptable screening tool.

**Methods**

Data for this cross-sectional study were collected as part of the UP AMIGOS project (University of San Luis Potosí and Illinois: A Multidisciplinary Investigation on Genetics, Obesity, and Social-environment). The UP AMIGOS project is an international collaboration between researchers at the University of Illinois in the United States and the Autonomous University of San Luis Potosí (UASLP) in Mexico.
Location

A large majority of participants were from the city of San Luis Potosi and surrounding area. San Luis Potosí is the name of the capital city of a centrally located state in Mexico that shares the name San Luis Potosí. The city has a population of 730,950 (INEGI Census, 2005). The university is located in the capitol city and is one of the largest universities in the state. It is also one of the oldest having been founded in 1624 (Wikipedia, 2010).

Participants and Matching

The participants were a quota-matched sample of applicants to the UASLP for the 2009 academic year. The data collection included participants aged 18- to 21-year-olds (n=9,974). Some participants had also applied the previous year (2008) when the UP AMIGOS pilot study was being conducted.

Using stored blood samples, liver enzymes were analyzed on a select number of cases in the Fall of 2010. Select cases were chosen from the total sample of 9,974 if they met the criteria of having complete biological data in both 2008 and 2009. This would allow for a future longitudinal analysis. In those with complete data, there were a small number of obese (BMI ≥ 30 kg/m²) participants (n < 30). Of those obese participants, 14 could be matched by age, sex, and height to one overweight (BMI 25 to 29.9 kg/m²) and one normal weight (BMI <25 kg/m²) participant with complete data. There were additional overweight individuals (n=12) with complete data, who could be matched to normal weight controls. In total, this included 14 obese participants, 26 overweight participants, and 26
participants with normal weight. Three participants had to be excluded due to poor quality of data. The final sample comprised 63 participants. Only cross-sectional 2009 data were used in the current analysis.

**Protocol**

Between February and March 2009 participants completed a questionnaire and underwent a health screen. The health screening was completed by trained health care professionals and included: anthropometric measurements (e.g., blood pressure, height, weight, waist circumference), blood pressure, a physician-conducted history and physical exam, and venipuncture for blood biomarker analysis. The protocol was reviewed and approved by the UASLP Institutional Review Board (IRB) and the University of Illinois Champaign-Urbana IRB. Informed consent was obtained from every subject prior to data collection.

**Anthropometric Measurements.** During the health screen, blood pressure (BP) was measured according to a common protocol adapted from procedures recommended by the American Heart Association. BP was taken on the dominant arm (right in most of the cases) in sitting position. Height was measured via stadiometer and recorded to the nearest 0.5 centimeter. Weight was assessed with a scale graduated to the nearest 0.1 kg. Body mass index (BMI) was calculated from the height and weight (BMI= kg/m²) and BMI percentiles were calculated using the Centers for Disease Control and Prevention 2000 BMI growth charts (Kuczmarski et al., 2000) as recommended by The PECR. Waist circumference (WC) was measured with the subject standing. A flexible steel tape was used to measure the WC to the
nearest 0.1 cm at the level of the iliac crest at the end of normal expiration. As a comparison, PECR cite that WC be taken at just above the ileum (Krebs et al., 2007).

**Blood Biomarkers.** Participants arrived at the health screen following an overnight fast. After the screen, trained health professionals drew fasting blood samples by venipuncture. On the day of collection, samples were refrigerated at 4°C. At the end of the day, serum was frozen at 20°C. Samples were thawed prior to assay. Plasma fasting lipid levels (total cholesterol, HDL, triglycerides) and liver enzymes (AST and ALT) were measured using the RD/Hitachi 902 analyzer (Roche Diagnostics, Indianapolis, IN). Serum fasting glucose was determined using the glucose oxidase peroxidase method (reagents from Biosystems, autoanalyzer Alcyon 300 from Abbott). It is recognized by the researchers that freezing serum to -20°C leads to a 48% mean loss in ALT activity (Gunter, Lewis, & Koncikowski, 1996). Despite decreases in ALT activity in all samples, the statistical relationships and associations should remain intact based on The National Health and Nutrition Examination Survey (NHANES) data (1988-1994 and 1999-2004) (Ioannou, Boyko, & Lee, 2006). Elevated liver enzymes were defined according to The PECR as AST(TGO) > 40 U/L or ALT(TGP) >56 U/L (Krebs et al., 2007).

**Variables in the Algorithm**

The PECR is an algorithm meant to guide a physicians’ decision on whether or not to order laboratory assessment of liver transaminases for suspected NAFLD. The algorithm includes sorting patients into BMI categories and the further subdivision of patients based on the presence of risk factors such as positive family
medical history for weight-related diseases (obesity, T2DM, cardiovascular disease), elevated blood pressure, dyslipidemia, tobacco use, and ethnic minority (African, Hispanic). Excessive alcohol use causes simple steatosis and excessive alcohol use must be ruled out as a cause for elevated liver transaminases before the diagnosis of NAFLD can be assumed.

**BMI Categories.** BMI Percentile categories were defined according to Krebs et al. (2007). Normal was defined as <85\(^{th}\) percentile, overweight was defined as \(\geq85^{th}\) percentile but < 95\(^{th}\) percentile, and obese was defined as \(\geq95^{th}\) percentile. Z-scores were not available for the 21-year-old participants. In those cases, adult BMI categories were used. Adult BMI categories were defined as normal weight (<25 kg/m\(^2\)), overweight (25 to 29.9 kg/m\(^2\)), and obese (\(\geq30.0\) kg/m\(^2\)).

**Family Medical History of Obesity.** Having a family medical history (FMHx) of obesity in first degree relatives was reported by participants using two questions from the questionnaire assessing the weight status of the mother and father: “Please indicate how you would classify your mother’s/father’s weight currently.” Responses were: markedly underweight, underweight/thin, average weight, overweight, obese, and don’t know. For this analysis, weight was coded dichotomously (1 if at least one parent was obese and 0 otherwise). “Don’t know” was coded as missing.

**Family Medical History of T2DM and CVD.** During the health screen, healthcare providers asked participants whether anyone on their maternal or paternal side had diabetes. They were then asked whether anyone had cardiovascular disease. Answering that a family history existed on the maternal
side, paternal side, or both was coded as 1. A noted deviation from The PECR is that dyslipidemia was not specifically assessed. Therefore, in the analysis, cardiovascular disease was used as a surrogate for a FMHx of dyslipidemia.

**Elevated blood pressure.** Elevated blood pressure (EBP) was defined as \( \geq 130/85 \) mmHg. This is the International Diabetes Federation (IDF) definition (IDF, 2009). This definition differs from the PECR, where it was suggested that BP be defined as \( \geq 95^{th} \) percentile for height, age, and sex. However, standard percentiles are only published for ages \( \leq 17 \)-years-old, and our study sample includes those who are older. Therefore, an alternative definition was used. The more severe form of EBP known as hypertension (HTN) was defined as \( \geq 140/90 \) mmHg.

**Dyslipidemia.** Dyslipidemia was defined according to a hybrid definition described in greater detail in the next chapter: Dyslipidemia defined as total cholesterol \( \geq 200 \) mg/dL, LDL-C \( \geq 130 \) mg/dL, TG \( \geq 110 \) mg/dL, and HDL-C \( \leq 40 \) mg/dL. PECR as HDL-C \( \leq 40 \) mg/dL (females and males) and/or TG \( \geq 110 \) mg/dL (Krebs et al., 2007).

**Smoking.** Smoking is considered a risk factor for cardiovascular disease and The PECR empirically recommended that it be assessed as a risk factor in the clinical screening algorithm. In the questionnaire, smoking was assessed with the question adapted from the ENCUESTA survey: “Have you smoked at least 100 cigarettes (5 packs) during your life? (Yes, No, I have never smoked),” Yes was coded as 1.

**Alcohol.** Excessive alcohol in the pediatric population has been defined as less than 10 g of alcohol per day (Feldstein et al., 2009). This equates to approximately 1 standard drink per day. Alcohol consumption was assessed with
the question adapted from ENCUESTA: “How frequently do (did) you have 5 or more alcoholic drinks at one time?” The response scale was: (a) I never drink more than 5 glasses, (b) Daily or almost every day, (c) 3 or 4 times per week, (d) 1 or 2 times per week, (e) 1 or 3 times monthly, (f) 7 to 11 times per year, (g) 3 to 6 times per year, (h) 1 to 2 times per year, (i) Prefer to not respond. Responses indicating excessive alcohol consumption were coded as 1 (b, c, d); non responders were coded as missing data (i); and the remaining responses were coded as 0 (a, e, f, g, h).

**Risk Categories**

The PECR is provided in Table 3.1. It splits participants into five risk categories: (1) Normal weight with no FMHx of CVD, (2) Normal weight with a FMHx of CVD, (3) Overweight with <2 risk factors, (4) Overweight with ≥2 risk factors, (5) Obese. In order to create these risk categories, the number of risk factors were summed (FMHx of obesity, FMHx of T2DM, FMHx of CVD, EBP, dyslipidemia, smoking). In the first analysis, Hispanic ethnicity was not counted as a risk factor. In the second analysis, Hispanic ethnicity was included as a risk factor. The PECR recommend that all obese participants and participants who were overweight with ≥2 risk factors be tested for elevated liver enzymes. Therefore, members of these two groups were coded as having a “positive” clinical screen and an indication for laboratory assessment.
Analysis

The three goals of the study were accomplished using descriptive statistics, epidemiological estimations of sensitivity and specificity, as well as exploratory analysis using receiver operator curve (ROC) analysis.
Table 3.1

*Complete Algorithm for laboratory evaluation from Barlow & The Expert Committee (2007).*

<table>
<thead>
<tr>
<th>Risk Categories</th>
<th>Risk Factors</th>
<th>Recommended Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal weight (≤85th %ile)</td>
<td>0</td>
<td>BP</td>
</tr>
<tr>
<td>2) Normal weight (≤85th %ile)</td>
<td>1*</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>3) Overweight (&gt;85th – 94th %ile)</td>
<td>&lt;2**</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>4) Overweight (&gt;85th – 94th %ile)</td>
<td>≥2**</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td>5) Obese (≥95th %ile)</td>
<td>≥0</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT (microalbumin/creatinine ratio)</td>
</tr>
</tbody>
</table>

BP – Blood pressure

* Family Medical History of dyslipidemia

** Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, CVD), ethnic minority (African, Hispanic), EBP, dyslipidemia, tobacco use, and signs of insulin resistance.

---

**Descriptive Statistics.** Statistical analysis was performed with SPSS (version 18.0). Descriptive statistics were run for all variables. Sex and age differences were determined with t-tests and ANOVA respectively for continuous variables and χ² tests for categorical variables. Differences between BMI (percentile) categories were determined with ANOVA and χ² tests. Alpha was set at 0.05.

Those with excessive alcohol use were compared to those without and continuous ALT and AST values were compared between the two groups using t-tests. The prevalence of the dependent variable of elevated liver enzymes was
conducted according to PECR criteria. Sex, age, and BMI differences were assessed.

**Testing the Clinical Screening Algorithm.** The screening algorithm was analyzed for sensitivity, specificity, and the positive predictive value. The relationship between liver enzymes and PECR was then tested using univariate analysis (χ² tests) and logistic regression initially controlling for alcohol intake and then removing alcohol as a control. The data were not analyzed on a matched analysis approach, as sufficient statistical power existed without doing so.

**Exploratory Analysis.** In an attempt to improve upon the existing algorithm, an exploratory analysis was conducted using ROC analysis for continuous variables as well as the sensitivities and specificities of various risk factor combinations in a decision tree analysis.

**Results**

Descriptive statistics for all independent variables were presented in Table 3.2. Approximately half of the participants were female (49.2%), most were 19-years-old (44.4%) with the second most common age being 18-years-old (33.3%). Matching was successful in that there were no significant differences for age and sex across the BMI Percentile Categories.

Most of the participants had FMHx of T2DM (60.3%). A smaller portion had a FMHx of CVD (33.3%) and a very low percentage reported having a parent who was obese (1.6%). Elevated blood pressure (≥130/80) was found in 15.9% of participants with significantly (P<0.05) higher prevalence of EBP in males (28.1%) compared to females (3.2%). Rates of dyslipidemia were high (65.1%).
Table 3.2
Descriptive statistics by sex

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean and SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age</td>
<td>18.90 ± 0.89</td>
<td>18.66 ± 0.75</td>
<td>19.16 ± 0.97</td>
<td>0.024</td>
</tr>
<tr>
<td>height</td>
<td>1.67 ± 0.10</td>
<td>1.74 ± 0.06</td>
<td>1.59 ± 0.07</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3.39 ± 0.52</td>
<td>3.65 ± 0.48</td>
<td>3.15 ± 0.43</td>
<td>0.004</td>
</tr>
<tr>
<td>WC</td>
<td>89.14 ± 12.23</td>
<td>91.00 ± 12.78</td>
<td>87.23 ± 11.52</td>
<td>0.224</td>
</tr>
<tr>
<td>BMI</td>
<td>26.19 ± 4.31</td>
<td>26.46 ± 4.28</td>
<td>25.91 ± 4.40</td>
<td>0.615</td>
</tr>
<tr>
<td>SBP</td>
<td>112.94 ± 12.63</td>
<td>118.91 ± 11.20</td>
<td>106.77 ± 11.07</td>
<td>0.000</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>174.43 ± 29.12</td>
<td>179.97 ± 30.65</td>
<td>168.71 ± 26.74</td>
<td>0.126</td>
</tr>
<tr>
<td>LDL-C</td>
<td>102.23 ± 25.06</td>
<td>107.45 ± 26.16</td>
<td>96.83 ± 23.06</td>
<td>0.093</td>
</tr>
<tr>
<td>triglycerides</td>
<td>130.84 ± 55.80</td>
<td>140.94 ± 63.95</td>
<td>120.42 ± 45.85</td>
<td>0.146</td>
</tr>
<tr>
<td>HDL-C</td>
<td>46.03 ± 9.87</td>
<td>44.33 ± 11.34</td>
<td>47.79 ± 7.88</td>
<td>0.166</td>
</tr>
<tr>
<td>AST U/L TGO</td>
<td>34.46 ± 23.50</td>
<td>35.59 ± 25.75</td>
<td>33.29 ± 21.28</td>
<td>0.701</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>27.05 ± 23.93</td>
<td>31.44 ± 28.98</td>
<td>22.52 ± 16.55</td>
<td>0.140</td>
</tr>
<tr>
<td><strong>Prevalence (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI percentile</td>
<td></td>
<td></td>
<td></td>
<td>0.067</td>
</tr>
<tr>
<td>NW</td>
<td>55.6 ± 59.4</td>
<td>51.6</td>
<td></td>
<td>0.575</td>
</tr>
<tr>
<td>OW</td>
<td>27.0 ± 15.6</td>
<td>38.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OB</td>
<td>17.5 ± 25.0</td>
<td>9.7</td>
<td></td>
<td>0.730</td>
</tr>
<tr>
<td>FMHx OB</td>
<td>1.6 ± 3.1</td>
<td>0.0</td>
<td>-</td>
<td>0.446</td>
</tr>
<tr>
<td>FMHx T2DM</td>
<td>60.3 ± 65.6</td>
<td>54.8</td>
<td>0.446</td>
<td>0.466</td>
</tr>
<tr>
<td>FMHx CVD</td>
<td>33.3 ± 31.3</td>
<td>35.5</td>
<td>0.793</td>
<td>0.567</td>
</tr>
<tr>
<td>EBP</td>
<td>15.9 ± 28.1</td>
<td>3.2</td>
<td>0.013</td>
<td>0.232</td>
</tr>
<tr>
<td>HTN</td>
<td>6.3 ± 12.5</td>
<td>0.0</td>
<td>0.113</td>
<td>0.855</td>
</tr>
<tr>
<td>Smoker</td>
<td>9.5 ± 9.4</td>
<td>9.7</td>
<td>1.000</td>
<td>0.002</td>
</tr>
<tr>
<td>Dyslipidemia*</td>
<td>65.1 ± 71.9</td>
<td>58.1</td>
<td>0.297</td>
<td>0.438</td>
</tr>
<tr>
<td>High Alcohol</td>
<td>33.3 ± 55.6</td>
<td>20.0</td>
<td>0.099</td>
<td>0.552</td>
</tr>
</tbody>
</table>

Pediatric Expert Committee Recommendations
no ethnicity         | 31.7 ± 28.1   | 35.5          | 0.595          | 0.299   |
incl ethnicity       | 41.3 ± 37.5   | 45.5          | 0.613          | 0.083   |

Note: p-values from ANOVA for continuous variables and Pearson χ² for categorical variables.

* Dyslipidemia defined as total cholesterol ≥200 mg/dL, LDL-C ≥ 130 mg/dL, TG ≥ 110 mg/dL, and HDL-C ≤ 40 mg/dL.; BMI – body mass index; EBP – elevated blood pressure (BP≥130/85)
FMHx OB – Family medical history of obesity in a parent
FMHx T2DM – Family medical history of diabetes
FMHx CVD – Family medical history of cardiovascular disease
HTN – hypertension (BP≥140/90); NW – Normal weight (BMI <85 th percentile)
OW – Overweight (BMI 85 th to 94.9 th percentile); OB – Obesity (BMI ≥ 95 th percentile)
SBP – systolic blood pressure; SD – Standard deviation; WC – Waist circumference
Approximately a tenth of the sample reported being smokers (9.5%) or heavy drinkers (12.7%).

There was no association between high alcohol intake and AST or ALT in univariate analysis separating those with and without excessive alcohol intake.

**Dependent Variables**

The mean ALT value was 27.05 ± 23.93 U/L, the mean AST was 34.46 ± 23.50 U/L. There were no significant age or sex differences for either enzyme in the sample, but ALT was significantly (P<0.05) higher between the BMI groups (NW 23.03 ±14.94; OW 24.65 ± 15.12; OB 43.55 ±45.38). Mean ALT and AST increased with increasing risk factor counts, but none of these continuous relationships were significant. The relationship between risk factor counts and the binomial variable of elevated liver enzymes was significant (χ², P<0.05) (See Figure 3.3).

**Krebs AST and ALT.** Overall, 25.4% of the sample met the PECR criteria for elevated liver enzymes (ALT>56 U/L and/or AST>40 U/L). This accounted for 16 participants total: 1 participant had isolated elevations in ALT>56 U/L, 10 had isolated AST elevation, and 5 had elevations in both AST and ALT. There were no significant differences in the prevalence of elevated liver enzymes by age, sex, or BMI percentile category (NW, OW, OB). While not statistically significant, there was a trend towards increasing rates of elevated liver enzymes for increasing adiposity. Those who were normal weight, overweight, and obese had rates of elevated liver enzymes of 17.1%, 29.4%, and 45.5% respectively.
The PECR Clinical Screen

Approximately one third (31.7%) of the participants had a positive clinical screen if Hispanic ethnicity was not included as a risk factor. When Hispanic ethnicity was included as a risk factor, more overweight participants had ≥2 risk factors and the percent with a positive clinical screen rose to 41.3%.

The PECR, had a 0.625 sensitivity, 0.787 specificity, and 0.500 positive predictive value for detecting elevated liver enzymes. This indicates that the screen accurately detected 62.5% of those with elevated liver enzymes. Out of those with
normal liver enzymes, 21.3% had a false positive screen. The PECR screen was significant on univariate $\chi^2$ test ($P<0.01$) and multivariate logistic regression. After logistic regression, the model was not significant when alcohol was included as a cofactor, therefore, it was dropped from the final model. In the final model, having a positive PECR screen was associated with a 6-fold increase in odds of having elevated liver enzyme. When Hispanic ethnicity was counted as a risk factor, the $\chi^2$ test was not significant, nor was the logistic regression (Table 3.3). Therefore, the decision tree in Figure 3.4 describes only the PECR without ethnicity.

Table 3.3
*Univariate and multivariate statistics for the PECR clinical screening algorithm in testing for elevated liver enzymes.*

<table>
<thead>
<tr>
<th>Clinical Screen</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>$\chi^2$ test</th>
<th>95% CI</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elevated liver enzymes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.625</td>
<td>0.787</td>
<td>0.500</td>
<td>0.004</td>
<td>6.17</td>
<td>1.80</td>
<td>21.1</td>
</tr>
<tr>
<td>PECR screen (ethnicity)</td>
<td>0.625</td>
<td>0.660</td>
<td>0.385</td>
<td>0.076</td>
<td>3.23</td>
<td>0.99</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Note: Logistic regression with elevated liver enzymes as a function of the PECR clinical screen. No controls were entered.

PECR – Pediatric Expert Committee Recommendations
CI – Confidence interval
PPV – positive predictive value
SD – Standard deviation
Sens – sensitivity
Spec – specificity
Figure 3.4
Decision Tree for Elevated Liver Enzymes

Note: Adapted from Krebs et al. (2007).
FMHx - Family Medical History
%ile - percentile
* Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, CVD, hypertension), EBP, elevated lipids, and tobacco use. Ethnic minority (Hispanic) was not included.

Exploratory Analysis to Improve the Algorithm

As mentioned above, the PECR clinical screening algorithm was moderately sensitive and specific in this population. In order to improve upon the current screen, the decision tree in Figure 3.4 was examined to see the distribution of those with abnormal liver enzymes. Out of those participants who were normal weight, 6
out of 35 (17.1%) had elevated liver enzymes as defined by Krebs *et al.* (2007). A closer look at these cases revealed that 5 out of the 6 had two or more risk factors.

**ROC curves.** ROC analysis was performed on all integral level risk factor variables, as a function of elevated liver enzymes. Variables and area under the curves (AUC) were as follows: BMI (AUC=0.645), BMI Z-score (AUC=0.637), waist circumference (AUC=0.681), risk factor count (AUC=0.709), systolic blood pressure (AUC=0.708), diastolic blood pressure (AUC=0.612), total cholesterol (AUC=0.431), triglycerides (AUC=0.534), HDL-C (AUC=0.337).

On the ROC curve, the BMI value that maximized sensitivity (.750) and minimized 1-specificity (.488) was ≥25.34. The BMI percentile coordinate that maximized sensitivity (.750) and minimized 1-specificity (.512) corresponded to ≥ the 75.8th BMI percentile. Similarly, A WC of 81.5 cm maximized sensitivity (.750) and minimized 1-specificity (.651). A systolic pressure of ≥117.5 maximized sensitivity (.688) and 1-specificity (.298). When a simple summation of risk factors was set to a ROC curve analysis, having 2 or more risk factors (not including Hispanic ethnicity) had a good sensitivity (.938) and 1-specificity (.512).

**Step 3 Categorical Risk Factors.** After ROC curves were performed with continuous variables, the sensitivity and specificity of individual risk factors was explored (See Table 3.4).

**FMHx.** A FMHx of DM was reasonably sensitive (.750) for elevated liver enzymes while a FMHx CVD was specific (.723). When the variables were combined, having both a FMHx of CVD and DM was not significantly associated with elevated liver enzymes, but having *either* a FMHx of CVD or DM was significantly
associated with elevated liver enzymes (p<0.05) on $\chi^2$ analysis with a high sensitivity (.938) and low specificity (.298).

**Blood Pressure.** A SBP of $\geq 130$ was very specific for elevated liver enzymes (0.915), but not sensitive (0.375). A $\chi^2$ test demonstrated a significant relationship between a SBP of $\geq 130$ and elevated liver enzymes (P<0.05).

**Dyslipidemia.** When individual risk factors were explored through multiple $\chi^2$ tests, lipids (total cholesterol, TG, HDL) by the Krebs et al. (2007) definitions failed to correspond significantly with having elevated liver enzymes.

**Step 4 Risk Factor Combinations.** Risk factors and the BMI, BMI percentile, and WC cutoffs as recommended by the ROC analysis were combined in multiple ways and tested for significance. The combination with the highest sensitivity and specificity in Mexican adolescents was the following: elevated blood pressure, with a positive FMHx of either CVD or DM, and a BMI over 25. If a participant had any 2 out of 3, then it was considered a positive clinical screen and it is recommended to test for liver enzymes. This clinical screen was 0.813 sensitive and 0.740 specific for elevated liver enzymes in this sample.
Table 3.4

*Exploration of variables as predictors of elevated liver enzymes*

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>Inflection point*</th>
<th>Sens</th>
<th>1-sp</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous Risk Factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>.645</td>
<td>≥25.34</td>
<td>.750</td>
<td>.488</td>
<td>.512</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>.637</td>
<td>75.8th percentile</td>
<td>.750</td>
<td>.512</td>
<td>.488</td>
</tr>
<tr>
<td>WC</td>
<td>.681</td>
<td>81.5 cm</td>
<td>.750</td>
<td>.651</td>
<td>.349</td>
</tr>
<tr>
<td>Risk Factor count</td>
<td>.709</td>
<td>≥1.5</td>
<td>.938</td>
<td>.512</td>
<td>.488</td>
</tr>
<tr>
<td>SBP</td>
<td>.708</td>
<td>≥117.5</td>
<td>.688</td>
<td>.298</td>
<td>.702</td>
</tr>
<tr>
<td><strong>Categorical Risk Factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMHx T2DM</td>
<td>.750</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMHx CVD</td>
<td>.500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMHx CVD and/or DM</td>
<td>.938</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP ≥130</td>
<td>.375</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Risk Factor Combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Risk Fact count (not incl chol) + &gt;81WC</td>
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<td>.875</td>
<td>.558</td>
<td>.442</td>
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<td>Risk Fact count (not incl chol) + BMI&gt;25 kg/m²</td>
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<td>.875</td>
<td>.442</td>
<td>.558</td>
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<td>EBP+FMHx CVD or FMHx DM+ &gt;81 WC</td>
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<td>≥1.5</td>
<td>.750</td>
<td>.419</td>
<td>.581</td>
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<tr>
<td>EBP+FMHx CVD or FMHx DM + BMI&gt;75th %ile</td>
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<td>≥1.5</td>
<td>.813</td>
<td>.279</td>
<td>.721</td>
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<tr>
<td>EBP+FMHx CVD or FMHx DM + 25 BMI</td>
<td>.793</td>
<td>≥1.5</td>
<td>.813</td>
<td>.256</td>
<td>.740</td>
</tr>
</tbody>
</table>

1-sp – one minus specificity
AUC – Area under the Curve
BMI – Body mass index
CVD – cardiovascular disease
DM – diabetes mellitus
EBP – elevated blood pressure (≥130/85)
FMHx – Family medical history
not incl chol – not including cholesterol (TG, HDL, total cholesterol)
WC – waist circumference

* The point at which sensitivity is maximized and 1-sp is minimized
Discussion

Our study aims were three-fold. The first was to describe the prevalence of elevated liver enzymes in obese, overweight, and normal weight young adults from Mexico. The second was to demonstrate the sensitivity and specificity of the 2007 PECR in this population. The third aim was to explore whether a more sensitive and specific clinical screen was desirable. Those who were normal weight, overweight, and obese had ALT>30 U/L of 26.6%, 17.6%, 45.5% respectively. Overall, The PECR was 0.625 sensitive and 0.787 specific and correlated significantly with elevated liver enzymes in the logistic regression. The newly proposed protocol was a simple summation of several risk factors (EBP [≥130/85], a FMHx of either CVD or T2DM, or a BMI≥25). This new protocol was 0.813 sensitive and 0.740 specific for elevated liver enzymes in Mexican adolescents and young adults. Thus, a simple assessment of risk factors and measurement of adiposity is a feasible method with which to screen young adults at risk of NAFLD.

The mean ALT value was 27.05 ± 23.93 U/L and the mean AST was 34.46 ± 23.50 U/L. ALT was significantly (P<0.05) higher between the BMI percentile categories (NW 23.03 ± 14.94; OW 24.65 ± 15.12; OB 43.55 ± 45.38). These means were higher than the mean ALT seen in non-obese and obese (BMI≥95th percentile) Hispanic children and adolescents ages 4 to 19 y/o in the United States: ALT 16.6 ± 11.8 U/L and 31.3 ± 29.7 U/L respectively (Quirós-Tejeira, Rivera, Ziba, Mehta, et al., 2007). This may not be surprising given the higher age of our sample. Notably, there were no sex or age differences in mean AST, ALT or binomial determinations of elevated liver enzymes. This is in contrast to previous studies in which NAFLD was
more common in males (Barshop, Sirlin, Schwimmer, & Lavine, 2008; Browning et al., 2004; Lavine & Schwimmer, 2004) and among those who were older (Schwimmer et al., 2006). Large, multiethnic studies utilizing MRS as a diagnostic modality, have also found a 2:1 male to female ratio. However, this sex-discrepancy was seen only in non-Hispanic whites. In Hispanics and non-Hispanic blacks, males and females had similar rates of NAFLD (Browning et al., 2004).

**Goal 1: The Prevalence of Elevated Liver Enzymes**

A previous study in Mexico City examined liver enzymes in elementary students (n=125, ages 6 to 12 years) and found the prevalence of ALT>40 U/L to be 42% in overweight and obese (BMI >85th percentile) (Flores-Calderon, Gomez-Diaz, Rodriguez-Gomez, & Moran-Villota, 2005). Using the same definition, the prevalence of ALT>40 U/L in the current sample of adolescents and young adults was 8.6%, 17.6%, 36.4% for normal weight, overweight, and obese participants respectively. Rates in those who were obese were comparable to those in Mexico City. In the United States, data from NHANES(1999-2004), indicated that 3.1% of white adolescents (12-19 year olds), 2.3% of black adolescents, and 6.1% of Mexican Americans had ALT>40 U/L (Fraser, Longnecker, & Lawlor, 2007). Direct comparisons are not possible as these are prevalence rates of a representative U.S. sample and the current sample was quota matched. Still, overall rates of ALT>40 U/L in the Mexican Americans were comparable to those seen in normal weight participants in this study.
ALT has also been defined as >30 U/L. Using this definition, Strauss, Barlow, and Dietz (2000) stratified 1988-1994 NHANES data by adiposity. Combining all ethnicities, 1.5% of normal weight has ALT>30 U/L, 5.0% of overweight (85th to <95th BMI percentile) and 9.5% of obese (≥95th BMI percentile). In the current study, those who were normal weight, overweight, and obese had ALT>30 U/L rates of 26.6%, 17.6%, 45.5% respectively. The rates seen in this study are higher than those reported by Strauss and colleagues (2000).

A case control study in Brazil that screened students for NAFLD using ultrasound, found the prevalence of NAFLD to be 3.4% in normal weight participants and 27.7% in overweight and obese students. The rates of NALFD, as diagnosed by ultrasound, in overweight and obese Brazilian samples are similar to rates in the current sample; but rates of NAFLD in the current sample may be twice as high as rates reported in Brazil (Lira, Oliveira, Escrivão, Colugnati, & Taddei, 2010). Unfortunately, direct comparison is difficult due to differing diagnostic modalities.

Rates of elevated liver enzymes in the current sample could not be compared to rates in representative samples in Mexico, because rates in Mexico are currently unknown. The most recent estimate is that 20% of the adult population has NALFD. This figure supposes that NAFLD would be found in two thirds of the obese, and that 30% of Mexican population were obese (Almeda-Valdés, Cuevas-Ramos, & Aguilar-Salinas, 2009). Our data suggest that elevated liver enzymes can be found in normal weight individuals. This been reported elsewhere (Bacon, Farahvash, Janney, & Neuschwander-Tetri, 1994; Sharp, Santos, & Cruz, 2009). Therefore, if liver
transaminases underestimate the prevalence of NAFLD (Browning, et al., 2004; Dunn & Schwimmer, 2008) and our results show that elevated liver enzymes can exist in normal weight, young adult Mexicans, than the prevalence of NAFLD in Mexico may be higher than the estimated 20%.

Goal 2: The Pediatric Expert Committee Recommendations

The PECR separated participants into Five Risk Categories defined by BMI percentile and risk factors: (1) Normal weight with no FMHx of CVD, (2) normal weight with a FMHx of CVD, (3) overweight with <2 risk factors, (4) overweight with ≥2 risk factors, (5) obese. Those in the top two categories (4 and 5) were considered to have a positive clinical screen. In the original protocol written for a U.S. population, Hispanic ethnicity was included as a risk factor. When Hispanic ethnicity was counted among the risk factors in this sample, the previously significant relationship between the PECR and liver enzymes was no longer significant. Using The PECR without Hispanic ethnicity as a risk factor, the clinical screening algorithm had a 0.625 sensitivity, 0.787 specificity, and 0.500 positive predictive value for detecting elevated liver enzymes. On logistic regression analysis, having a positive clinical screen was associated with 6.17 (95% CI 1.80, 21.10) times the odds of having elevated liver enzymes compared to those who had a negative screen. Despite reaching statistical significance, this was considered to be an unacceptable screen based on the a priori criteria of a minimum sensitivity of >0.80 and sensitivity of >0.80.
Goal 3: Exploratory Analysis

A decision tree revealed that of the 54% of those with elevated liver enzymes (ALT>56 U/L and/or AST>40 U/L) were normal weight. A majority of those who were normal weight with elevated liver enzymes had two or more risk factors. Exploratory ROC analysis showed risk factor count had a higher AUC for detecting elevated liver enzymes than measures of adiposity such as BMI, BMI-percentile, and WC. The exploration also revealed that a BMI of around 25 was an optimal cutoff point to optimize sensitivity and specificity, as was a BMI percentile of >75th percentile. This BMI percentile threshold may not be arbitrary, as having a BMI >75th percentile before adulthood has been the threshold of increasing morbidity and mortality in adulthood (Must et al., 1992; Bjorge, Engeland, Tverdal, & Smith, 2008).

Additional exploratory analysis showed that no measure of cholesterol (HDL, TG, total cholesterol) correlated with the Krebs et al. (2007) definition of abnormal liver enzymes. One hypothesis is that this was due to the high rates of dyslipidemia (71.4%). These rates did not significantly differ by BMI category and may be influenced greatly by the Mexican phenotype of dyslipidemia (Aguilar-Salinas et al., 2009). Currently, the PECR counts dyslipidemia as a risk factor for NALFD. In this population, dyslipidemia is a nondiscriminatory test, because it affects those with and without elevated liver enzymes. Therefore, cholesterol was dropped from further exploratory analysis of risk factor combinations.

The exploration of risk factor combinations revealed a clinical screen that was 0.813 sensitive and 0.740 specific for elevated liver enzymes. This clinical screen had sufficient sensitivity as a screen, but lacked sufficient specificity to meet
the 0.80 minimum cutoff. This may be an acceptable compromise. The proposed clinical screen was to assess for the following risk factors: EBP (≥130/85 mmHg), a FMHx of either CVD or T2DM, a BMI ≥25. If 2 or more are present, then clinical screen is positive and it is recommended to perform a laboratory assessment of liver enzymes. This screen may be more clinically feasible than the Krebs algorithm in that it does not require a previous cholesterol measurement; nor for the physician to plot BMI percentile, a practice that few physicians routinely do (Barlow, Bobra, Elliott, Brownson, & Haire-Joshu, 2007). Larger studies are needed to confirm these results.

While no studies have proposed clinical criteria, the American Gastroenterological Association recommends that laboratory assessment be conducted on adults who are obese, have T2DM, high triglycerides, weight loss suddenly, and syndromes of insulin resistance (American Gastroenterological Association 2002). Our data suggest that in Mexicans, those who are normal weight and overweight may still be at risk for NAFLD. In addition, our data do not support the use of triglycerides as a screen in the Mexican population and, in young adults prevalence of impaired fasting glucose may be too small to be considered a prerequisite for screening. For instance, in our sample, 6.35% had fasting glucose ≥100 mg/dL and no participants with elevated fasting glucose also had elevated liver enzymes.

Lee and colleagues (2010) proposed a clinical screen for Korean adults known as the hepatic steatosis index (HSI) using a large cohort of known NAFLD cases (n=5362) and matched controls. The HSI was a formula (8 x [ALT/AST] + BMI
+2[if female], +2[if T2DM]). Using the thresholds of <30.0 or 36.0, the HSI was 0.931 sensitive and 0.924 specific for steatosis (Lee et al., 2010). The findings of Lee and colleagues (2010) demonstrate that clinical information such as BMI and the presence of T2DM can be combined with laboratory values to predict NAFLD with greater accuracy.

The currently study had several limitations. Firstly, the sample size was small and results may not be generalizable to the population as a whole. Secondly, the study did not measure Hepatitis viral serologies, which may also affect liver enzymes. Despite these limitations, because the sample was age- and sex-matched and included normal weight controls, there was sufficient power to define significant relationships. To our knowledge, this is the first study testing the utility of a pediatric clinical screening algorithm for detecting abnormal liver enzymes in Mexico. It, therefore, represents an important step forward in understanding screening protocols in a Mexican population.
"The time for primary prevention beginning in childhood has come."

The American Heart Association (Kavey et al., 2003)

Worldwide, over one-fifth of deaths are attributed to coronary artery disease and stroke, together known as cardiovascular disease (CVD) (Lopez & Mathers, 2006; Mathers, Boerma, & Ma Fat, 2009). CVD is caused by atherosclerotic plaques that build up within the arteries. These plaques are thought to begin in childhood for those children who are exposed to CVD risk factors (Newman, Freedman, Voors, et al., 1986; Berenson, et al., 1998; McGill et al., 2001; McGill, McMahan, Malcolm, Oalmann, & Strong, 1997; Enos, Holmes, & Beyer, 1953; McGill & McMahan, 1998). In these children, vascular abnormalities can begin silently and remain subclinical for decades before the emergence of CVD clinical consequences such as myocardial infarction (MI) and sudden death. However, this silent disease is preventable, and also reversible in the early stages if risk factors are identified and controlled (Battista, Murray, & Daniels, 2009).

**CVD Risk Factors**

Risk factors for CVD include dyslipidemia, high blood pressure, diabetes, smoking, and obesity (Ross, 1986). Dyslipidemia, or the presence of abnormal lipid concentrations in the blood, is perhaps one of the strongest risk factors for CVD (Yusuf, Hawken, Ounpuu, et al., 2004), causing half of all coronary artery disease cases worldwide (Acosta-Cázares & Escobedo-de la Peña, 2010; Danaei, et al., 2006).
Dyslipidemia is an umbrella term that includes any derangement in total cholesterol, LDL-C, triglycerides, or HDL-C. The presence of dyslipidemia independently increases the odds of having a future MI by 3 times (Yusuf, Hawken, Ounpuu, et al., 2004). Despite improved interventions, 30% of initial MIs are fatal (Belay, Belamarich, & Racine, 2004).

**Dyslipidemia**

In children, adolescents, and young adults, dyslipidemia is the most frequently identified coronary artery disease risk factor, affecting an estimated 25% of the pediatric population (William & Bolella, 1995). Rates of dyslipidemia in adolescents are highest in overweight (22.3%) and obese (42.9%) youth, but can also be seen in normal weight individuals (14.2%) (Centers for Disease Control and Prevention (CDC), 2010). In children and adolescents, dyslipidemia is independently associated with concurrent measures of atherosclerotic disease such as carotid intimal media thickness (CIMT) measured in vivo by ultrasound (Ayer, et al., 2009; Davis, Dawson, Riley, & Lauer, 2001; Fang, Zhang, Luo, Yu, & Lv, 2010; Morrison, et al., 2010) and early atherosclerotic lesions found on autopsy (Berenson et al., 1998; McGill, McMahon, Zieske, et al., 2000).

Pediatric CVD risk factors are also associated longitudinally with atherosclerosis in young adulthood (Davis, Dawson, Riley, & Lauer, 2001; Gooding, de Ferranti, 2010; Juonala et al., 2008; Juonala et al., 2010; Li, et al., 2003; Mahoney et al., 1996; McMahon, et al., 2007; Oren et al., 2003; Raitakari et al., 2003). This could be due to tracking, which is the tendency of pediatric dyslipidemia to persist
into adulthood. While tracking of dyslipidemia from childhood to adulthood is well-reported (Camhi, et al., 2010; Fitzsimmons, Shively, & Verderber, 1992; Haney et al., 2007; Lauer, Lee, & Clarke, 1988; Lauer & Clarke, 1990; Mattsson, Rönnemaa, Juonala, Viikari, & Raitakari, 2008; McGill et al., 2000; Srinivasan, Frontini, Xu, & Berenson, 2006; Webber et al., 1991), the positive relationship between risk factors in childhood and adolescence and CIMT in adulthood persists even when controlling for adult CVD risk factors (Raitakari et al., 2003; Oren et al., 2003). In a pooled analysis of three population-based, prospective cohort studies, adolescents (12- to 18-years-old) were followed for approximately 20 years. It was found that adolescents with dyslipidemia (low HDL-C and high LDL-C as defined by both National Cholesterol Education Program [NCEP] and NHANES) had a significantly higher risk of having abnormal CIMT as adults even if in those who were normal weight (Magnussen et al., 2009). Regardless of adiposity, the presence of dyslipidemia can lead to asymptomatic, subclinical atherosclerotic lesions in early adulthood. These subclinical lesions, in turn, can build and eventually culminate into symptomatic CVD (e.g. sudden death, MI, angina). In follow-up studies lasting 30 years or more, pediatric and young adult dyslipidemia has been shown to be independently associated with CVD events, with the earliest CVD event occurring in participants before the age of 40 (Klag et al., 1993; Morrison, Glueck, Horn, Yeramaneni, & Wang, 2009).
Screening for Dyslipidemia

It is possible to decrease the risk of future CVD in those with occult dyslipidemia. Adult protocols for universal screening and treatment algorithms, including dietary regimens and lipid-lowering agents (i.e. statins), have proven to be an effective strategy in those older than 20-years-old (Unal, Critchley, & Capewell, 2005). Such strategies have contributed to the dropping CVD mortality in developed countries (Ford, Ajani, & Croft, 2007; Unal, Critchley, & Capewell, 2005).

Over the past 30 years, death from coronary artery disease has decreased by 57 to 63% in the United States and Canada (Rodríguez, Malvezzi, & Chatenoud, 2006). In the past 30 years, CVD in Mexico has not decreased, but, rather, has increased precipitously (Lozano, Escamilla, Escobedo et al., 1993; Rodríguez, Malvezzi, & Chatenoud, 2006). The increase in CVD in Mexico has paralleled local increases in obesity rates (Rivera, Barquera, Campirano, Campos, Safdie, & Tovar, 2002) with the highest rates seen in the north of the country (Sanchez-Castillo, Velasquez-Monroy, Lara-Esqueda, et al, 2005). CVD is now the leading cause of death in Mexico (Stevens, Dias, Thomas, Rivera, et al., 2008) and over a quarter of these deaths are in people under the age of 65-years-old (Secretaría de Salud, 2010).

CVD risk factors are prevalent in Mexico, and dyslipidemia exists in 40 to 60% of the Mexican adult population (Aguilar-Salinas et al., 2009). Of those with dyslipidemia, 72.1% are unaware of it (Acosta-Cázares & Escobedo-de la Peña, 2010). Evidence exists that there is an ethnic propensity of the Mexican people towards having dyslipidemia, particularly the combination of high TG and low HDL-C (Carroll, Lacher, Sorlie et al., 2005; de Ferranti, et al., 2004; Johnson, Kroon,
Greenway et al 2009; Harel, Riggs, Vaz, Flanagan, & Harel, 2010; López-Capapé, et al., 2006). However, in multiethnic samples, many discrepancies in rates of dyslipidemia diminish when controlling for education, income, healthy eating, and physical activity (Walker, Gurka, Oliver, Johns, & Deboer, 2010). In the United States, diet and PA have been shown to be major determinants of HDL-C (AHA, 2010). This suggests that lifestyle intervention, may be useful once dyslipidemia is identified.

Screening for dyslipidemia can begin early in life. In 2002, the National Education and Cholesterol Program (NCEP) recommended that universal screening on all individuals be started at age 20 (NCEP, 2002). Since dyslipidemia tracks throughout the lifespan, screening does not have to wait until the age of 20, which is why several organizations have published consensus statements recommending screening in select individuals between the ages of 2 and 21. The organizations that have published consensus statements include the National Education and Cholesterol Program (NCEP, 1992), The Pediatric Expert Committee Recommendations (PECR) (Barlow et al., 2007; Krebs et al., 2007), The Spanish Association of Pediatrics (SAP) (Dalmau Serra et al., 2007), The American Academy of Pediatrics (AAP) (Daniels & Greer 2008), and The American Heart Association (AHA) (Kavey et al., 2003). While the details of most recommendations differ (Table 4.1), all share the common protocol of identifying pediatric patients over the age of 2 with a family medical history (FMHx) indicative of dyslipidemia (i.e. premature CVD), and/or patient risk factors such as overweight, obesity, elevated blood pressure, or smoking. In such patients, physicians are recommended to obtain a
Table 4.1

**Clinical Screening Recommendations**

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Screen if</th>
<th>Laboratory Test</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCEP-adult</td>
<td>Screen all patients ≥20-years-old BMI ≥ 85th %</td>
<td>Fasting lipid profile</td>
<td>NCEP, 2002</td>
</tr>
<tr>
<td>NCEP-pediatric</td>
<td>1° with TC≥240 (or) 1° or 2° relative w/ CA bypass surgery CA angioplasty CAD, MI, angina, sudden death peripheral vasc dz cerebral vasc dz before age 55 years.</td>
<td>Fasting lipid profile (or) TC if only 1° with TC≥240</td>
<td>NCEP, 1992</td>
</tr>
<tr>
<td>PECR-Barlow</td>
<td>BMI ≥ 85th percentile</td>
<td>Fasting lipid profile</td>
<td>Barlow, et al., 2007</td>
</tr>
<tr>
<td>PECR-Krebs</td>
<td>BMI ≥ 85th percentile</td>
<td>Fasting lipid profile</td>
<td>Krebs, et al., 2007</td>
</tr>
<tr>
<td>SAP</td>
<td>BMI ≥ 95th percentile</td>
<td>Total cholesterol and triglyceride fraction fasting lipid profile</td>
<td>Dalmau Serra et al., 2007</td>
</tr>
<tr>
<td>AAP</td>
<td>BMI ≥ 85th percentile</td>
<td>Total cholesterol and triglyceride fraction fasting lipid profile</td>
<td>Daniels &amp; Greer 2008</td>
</tr>
<tr>
<td>AHA</td>
<td>BMI ≥ 85th percentile</td>
<td>Fasting lipid profile</td>
<td>Kavey et al., 2003</td>
</tr>
<tr>
<td></td>
<td>EBP &gt;90th</td>
<td></td>
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</tbody>
</table>

1° – first degree relative (parents, siblings)
2° – second-degree relative (grandparents)
AAP – American Association of Pediatrics
AHA – American Heart Association
BMI – body mass index (kg/m²)
CA – coronary artery
CAD – coronary artery disease
CVD – cardiovascular disease
MI – myocardial infarction
vasc dz – vascular disease
TC – total cholesterol
EBP – elevated blood pressure
HTN – hypertension
NCEP – National Cholesterol Education Program
PECR – Pediatric Expert Committee Recommendations
SAP – Spanish Association of Pediatrics
*CVD in males <55-yrs-old or CVD in females <65-yrs-old
fasting lipid and lipoprotein profile to test for dyslipidemia. To our knowledge, no one has tested any of these pediatric dyslipidemia screening protocols in Mexico.

Definitions of Dyslipidemia

Once fasting lipids and lipoproteins are detected, the clinician must make treatment decisions based upon whether the patient fits the criteria for dyslipidemia. The NCEP and AHA have defined cutoffs for normal, borderline, and abnormal lipids and lipoproteins based on data from the Lipid Research Clinic Pediatric Prevalence Study (Tamir et al., 1981). However, cutoff definitions vary. Recent longitudinal data by Magnussen and colleagues (2008, 2009) has shed light on what definitions of adolescent dyslipidemia are associated with continuing adult lipid abnormalities (Magnussen et al., 2008), CIMT (Magnussen et al., 2009), or CVD (Klag et al., 1993).

In longitudinal studies, Magnussen and colleagues (2008) found that a total cholesterol of ≥ 200 mg/dL in adolescence was likely to track and become hypercholesterolemia (≥240 mg/dL) in adulthood (Magnussen et al., 2008). Those with high total cholesterol (>207 mg/dL) as young adults also had approximately 5 times the risk of CVD on 40-year follow-up compared to those with total cholesterol levels in the normal range (<172 mg/dL) (Klag et al., 1993). With regard to LDL-C, having the widely-cited LDL-C cutoff of ≥130 mg/dL, and even the lower cutoff of ≥120 mg/dL in adolescence was significantly associated with adult (29- to 39-year-old) CIMT ≥ 90th percentile (Magnussen et al., 2009). Both total cholesterol and LDL-C track well within individuals and families (Tonstad, Leren, Sivertsen, & Ose,
1995; Haney et al., 2007; Camhi, et al., 2010), while the triglycerides and HDL-C may
be more closely related to measures of adiposity (Wong et al., 1992; D l'Allemand-
Jander, 2010; Haney et al., 2007; Lee, et al., 2009). Even so, both are strong,
independent predictors of future CVD (Morrison, Glueck, Horn, Yeramaneni, &
Wang, 2009). HDL-C levels are highly correlated with atherosclerosis. In
adolescence, having an HDL-C that was <40 mg/dL predicted future CIMT ≥ 90th
percentile in adulthood (Magnussen et al., 2009). When risk was stratified for
adiposity, normal weight individuals with low HDL-C (<35 mg/dL) remained at risk,
while cutoffs as high as <60 mg/dL conferred risk in overweight and obese
adolescents (Magnussen et al., 2009). Good longitudinal studies have been
conducted for HDL, but debate still surrounds cutoff values for TG. Cook, Weitzman,
Auinger, Nguyen, & Dietz, 2003 cited TG ≥ 110 mg/dL as corresponding to the 90th
percentile for age, which is a value that confers risk of hypertriglyceremia in
adulthood. Indeed, higher values (≥130 mg/dL) are neither sensitive nor specific for
predicting adult hypertriglyceridermia (Magnussen et al., 2008).

In light of the evidence regarding which lipid values confer longitudinal risk
of CVD and/or continuing dyslipidemia, the following cutoffs will be used in the
current study: TC ≥ 200 mg/dL, LDL-C ≥130 mg/dL, TG ≥ 110 mg/dL, and HDL-C ≤
40 mg/dL. A derangement in any one of these values warranted the diagnosis of
dyslipidemia in the study population.
Study Aims

Using the above definition of dyslipidemia, the current study examined dyslipidemia in a group of Mexican adolescents and young adults aged 18- to 21-years-old applying to college. In Mexico, entrance into college requires a complete health screen, making this transition an excellent opportunity for identifying those with dyslipidemia. The first goal was to describe the relative prevalence of dyslipidemia in normal weight, overweight, and obese participants. The second goal of the study was to test the following consensus statements for clinical screening algorithms: (1) The National Cholesterol Education Program adult recommendations (NCEP, 2002), (2) The National Cholesterol Education Program pediatric recommendations (NCEP, 2002), (3) The Pediatric Expert Committee Recommendations (PECR) as written by Barlow et al. (Barlow et al., 2007), (4) The second Pediatric Expert Committee Recommendations (PECR) as written by Krebs et al. (Krebs et al., 2007), (5) the American Academy of Pediatrics (Daniels & Greer, 2008), (6) the Spanish Association of Pediatrics (SAP) (Dalmau Serra et al., 2007), and (7) the American Heart Association (AHA) pediatric recommendations (Kavey et al., 2003). In 1992, the NCEP also published a set of pediatric recommendations (NCEP, 1992). Each screening recommendation was tested for epidemiological parameters of sensitivity, specificity, and positive predictive value (PPV) in the detection of dyslipidemia. The third goal was to explore solutions towards improving the sensitivity and specificity of the clinical screen through a classification and recession tree (CART) analysis of all variables. A sensitivity of ≥0.80 and a specificity ≥0.80 was considered an acceptable clinical screen.
Methods

Data for this cross-sectional study were collected as part of the UP AMIGOS project (University of San Luis Potosí and Illinois: A Multidisciplinary Investigation on Genetics, Obesity, and Social-environment). The UP AMIGOS project is an international collaboration between researchers at the University of Illinois in the United States and the Autonomous University of San Luis Potosí (UASLP) in Mexico.

Participants

Participants, aged 18 to 21, were applicants to the Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí [UASLP]) for the 2009 academic year. The full sample included 9,974 participants. Fasting cholesterol values were available for a subset of the sample (n=1,786) due to availability of resources. The final sample included only those 18- to 21-year-olds with complete data on all variables (n=829). A large majority of participants were from the city of San Luis Potosí and surrounding area.

Protocol

Between February and March 2009 participants underwent a health screen and electively completed a questionnaire. Informed consent was obtained from every subject prior to data collection. The health screen was completed by trained health care professionals and included anthropometric measurements (e.g., blood pressure, height, weight, waist circumference), blood pressure, a physician-conducted history and physical exam, and venipuncture for blood biomarker
analysis. The protocol was reviewed and approved by the UASLP Institutional Review Board (IRB) and the University of Illinois Champaign-Urbana IRB.

**Anthropometric Measurements.** During the health screen, blood pressure (BP) was measured according to a common protocol adapted from procedures recommended by the American Heart Association. BP was taken once on the dominant arm (right in most of the cases) in a sitting position. Height was measured via stadiometer and recorded to the nearest 0.5 centimeter. Weight was assessed with a scale graduated to the nearest 0.1 kg. Waist circumference (WC) was measured with the subject standing. A flexible steel tape was used to measure the WC to the nearest 0.1 cm at the level of the iliac crest at the end of normal expiration. As a comparison, Krebs and colleagues (2007) recommend WC be taken at just above the ileum. Waist to height ratio (WHtR) was calculated with the equation: WC in cm divided by height in cm. Body mass index (BMI) was calculated from height and weight (BMI= kg/m²) and BMI percentiles were calculated using the Centers for Disease Control and Prevention 2000 BMI growth charts (Kuczmarski, et al., 2000) as recommended by The PECR.

**Blood Biomarkers.** Participants arrived at the health screen following an overnight fast. After the screen, trained health professionals drew fasting blood samples by venipuncture. On the day of collection, samples were refrigerated at 4°C. At the end of the day, serum was frozen at 20°C. Samples were thawed prior to assay. Plasma fasting lipid levels (total cholesterol, HDL, triglycerides) were measured using the RD/Hitachi 902 analyzer (Roche Diagnostics, Indianapolis, IN).
Serum fasting glucose was determined using the glucose oxidase peroxidase method (reagents from Biosystems, Autoanalyzer Alcyon 300 from Abbott).

**Variables in the Algorithm**

Clinical screening algorithms are meant to guide a physicians’ decision on whether or not to obtain lipids and lipoproteins. Many algorithms include collecting data in regards to FMHx and identifying patients with additional risk factors such as BMI percentile, elevated blood pressure, and tobacco use.

**BMI categories.** BMI percentile categories were defined according to Barlow and colleagues (2007). Normal was defined as <85th percentile, overweight as ≥85th percentile but < 95th percentile, and obese as ≥95th percentile using percentile curves published in 2000 by the Centers for Disease Control. Z-scores needed to calculate percentile were not available for the 21-year-old participants. In 21-year-olds, BMI was used. BMI categories were defined as normal weight (<25 kg/m²), overweight (25 to 29.9 kg/m²), and obese (≥30.0 kg/m²).

**FMHx obesity.** Having a family history of obesity in first degree relatives was reported by participants on two questions from the questionnaire. The first question assessed the weight status of the mother and, the second, the father: “Please indicate how you would classify your mother’s (or your father’s) weight currently.” Responses were: markedly underweight, underweight/thin, average weight, overweight, obese, and don’t know. For this analysis, weight was coded dichotomously (1 if at least one parent was obese and 0 otherwise). “Don’t know” was coded as missing.
**FMHx T2DM and CVD.** During the health screen, healthcare providers asked participants whether anyone on their maternal or paternal side had diabetes. They were then asked whether anyone had cardiovascular disease. Answering in the affirmative for a family history of diabetes on the maternal side, paternal side, or both was coded as 1. The same was done for CVD. This is a noted deviation from the PECR screening algorithm, which requires that dyslipidemia be specifically assessed. Addressing dyslipidemia, however, was not thought to be feasible because a majority of Mexicans with dyslipidemia are not aware of their condition (Acosta-Cázares & Escobedo-de la Peña, 2010). Therefore, in the analysis, cardiovascular disease served as a surrogate for a FMHx of dyslipidemia.

**Elevated blood pressure.** Elevated blood pressure (EBP) was defined as SBP ≥ 130 and/or DBP ≥ 85. This is the IDF (International Diabetes Federation) definition (IDF, 2009). While the PECR, AAP, and AHA defined elevated blood pressure and hypertension to be 90th percentile and ≥95th percentile, respectively, standardized curves for age- and sex-specific percentiles have only been published for ages ≤17-years-old (NHBPEP, 1996). Therefore, we used the adult definition of EBP (BP ≥130/≥85), as cited by the IDF (IDF, 2007). Hypertension, a more extreme version of EBP, was defined as SBP ≥140 and/or DBP ≥90 (AHA, 2010).

**Smoking.** Smoking is considered a risk factor for cardiovascular disease and the ECR empirically recommended that it be assessed as a risk factor in the clinical screening algorithm. In the questionnaire, smoking was assessed with the question adapted from the ENCUESTA survey, “Have you smoked at least 100 cigarettes (5 packs) during your life? (Yes, No, I have never smoked),” Yes was coded as 1.
**Dyslipidemia**

The laboratory biomarker analysis of every participant included fasting total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald Formula (LDL-C = TC – HDL-C – [TG/5]) (Friedewald, Levy, Fredrickson, 1972). This has been cited by the National Cholesterol Education Program (1992) as an acceptable measure of LDL-C when blood samples are taken after a 12-hour fast (NCEP, 1992). Dyslipidemia was defined as any one of the following: Total cholesterol ≥200 mg/dL, LDL-C ≥130 mg/dL, triglycerides ≥110 mg/dL, or HDL-C ≤40 mg/dL (females and males) (Krebs et al., 2007). This is a hybrid definition combining NCEP (1992) and AHA values TC and LDL, with definitions by Cook and colleagues (2003) for HDL-C and TG as recommended by the PECR (Krebs et al., 2007). Values for TC, LDL, and HDL-C for this hybrid definition were chosen based on evidence for tracking (Magnussen et al., 2008), and correlations with adult CIMT (Magnussen, 2009) and CVD (Klag et al., 1993).

**Clinical Screening Algorithms**

A total of seven clinical screening algorithms were tested: NCEP adult, NCEP pediatric, PECR-Barlow, PECR-Krebs, SAP, AAP, and AHA. The NCEP adult recommends universal screening on all patients starting at the age of 20 (NCEP, 2002). The NCEP pediatric recommendations (NCEP, 1992) are for pediatric patients whose parents had a total cholesterol ≥240 mg/dL or in patients whose parents or grandparents had coronary artery disease, peripheral vascular disease,
stroke, or sudden death (all indicative of CVD) before the age of 55. The PECR-Barlow suggests that all patients with a BMI ≥ 85th percentiles for age and sex be screened for dyslipidemia (Barlow et al., 2007). In a later chapter of these same recommendations (PECR-Krebs), the recommendations were amended slightly to include screening for all those of normal weight with a positive FMHx of dyslipidemia (Krebs, et al., 2007). Similar to the PECR-Barlow, The SAP also recommends screening solely based upon BMI percentile, but uses a cutoff of BMI ≥ 95th percentiles as a positive clinical screen. In contrast, the AAP recommends screening in all patients with a known FMHx of lipid abnormalities, a FMHx of premature CVD (CVD<55-years-old in men, <65-years-old in females), or if the patient has a BMI ≥ 85th percentile or other CVD risk factors such as diabetes, hypertension, or tobacco use. A combination of FMHx information and CVD risk factors was also used by the AHA, which recommended screening for dyslipidemia in all patients with a FMHx of any obesity-related disease (obesity, dyslipidemia, hypertension, diabetes, premature CVD, or smoking), or if the patient had a BMI ≥ 85th percentile or elevated blood pressure. In some cases, data were not available for specific FMHx variables such as lipids, hypertension, and smoking.

Analysis

In order to accomplish the three aims of the study, descriptive analysis, epidemiological parameters, and CART analysis were performed.

Descriptive Statistics. Statistical analysis was performed with SPSS (version 19.0). Descriptive statistics were calculated for all variables. Sex and age differences
were determined. ANOVA was used for continuous variables and \( \chi^2 \) tests for categorical variables. Alpha was set at 0.05.

**Cholesterol by age, sex, BMI.** The continuous means of elevated total cholesterol, LDL-C, TG, and HDL-C were calculated and tested for differences by age, sex, and BMI percentile category. Then, significant age, sex, and BMI differences were determined for the binomial variable of dyslipidemia using \( \chi^2 \) tests.

**Testing Clinical Screening Algorithms.** The seven clinical screening algorithms were analyzed for sensitivity, specificity, and PPV. Univariate relationships between each of the five clinical screening algorithms and dyslipidemia were then tested using \( \chi^2 \) tests. Following this, multivariate analysis was performed using logistic regression with dyslipidemia as a function of a positive or negative screen controlling for age and sex.

**Exploratory Analysis.** In an attempt to improve upon the existing clinical screening algorithms, an exploratory analysis was conducted using the SPSS 19.0 decision tree module for a CART analysis entering all variables as independent variables (age, sex, FMHx obesity, FMHx CVD, FMHx DM, EBP, HTN, tobacco use) as well as anthropometric variables such as BMI, BMI z-score, WC, and WHtR. After the CART analysis, nodes with the highest prevalence rates of dyslipidemia were chosen and coded as having a positive CART screen.

**Results**

Overall, 56.6% of the study samples were female (Table 4.2); and most were 18-years-old (69.2%), followed by 19-years-old (22.0%), while the remaining participants were 20- and 21-years-old (8.8%). A majority of participants were
normal weight (BMI <85\textsuperscript{th} percentile) (74.3%), and a smaller percentage was overweight (BMI 85\textsuperscript{th} to 94.9\textsuperscript{th} percentile) (15.9%), or obese (BMI ≥ 95\textsuperscript{th} percentile) (9.7%).

**Descriptive Statistics**

In regards to CVD risk factors, very few participants reported their parents as being obese (1.7%), but two thirds reported a FMHx of DM (60.4%), and a third reported having a FMHx of CVD (34.3%). There were no significant sex or age differences in reported of FMHx of obesity, DM, or CVD. There were significant sex differences in EBP, HTN, and smoking. A total of 9.2% of males had BP ≥130/85, while 2.8% of females had EBP (P<0.001). HTN was present in 5.8% of males and 1.1% of females (P<0.001). Males were also more likely to smoke (8.9%) as compared to females (4.5%) (P<0.05). Twenty-year-olds had the highest smoking rates (12.8%) and this difference was significant (P<0.05). Despite sex differences in risk factor prevalence, there was no significant difference in sex or age for those testing positive in most clinical screens. The exception was AHA, which had significantly more females than males with a positive clinical screen (P<0.05). The prevalence of positive screens ranged from 8.1% to 79.2% depending upon the screening algorithm.
Table 4.2
Descriptive statistics by sex

<table>
<thead>
<tr>
<th>Mean and SD</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±  SD</td>
<td>Mean ±  SD</td>
<td>Mean ±  SD</td>
<td>by sex</td>
</tr>
<tr>
<td>Age</td>
<td>18.43 ± 0.74</td>
<td>18.48 ± 0.79</td>
<td>18.38 ± 0.70</td>
<td>0.055</td>
</tr>
<tr>
<td>Height</td>
<td>1.65 ± 0.09</td>
<td>1.71 ± 0.07</td>
<td>1.59 ± 0.06</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3.23 ± 0.50</td>
<td>3.26 ± 0.51</td>
<td>3.20 ± 0.49</td>
<td>0.127</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>80.25 ± 11.86</td>
<td>82.94 ± 11.25</td>
<td>78.19 ± 11.91</td>
<td>0.000</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.49 ± 0.07</td>
<td>0.48 ± 0.06</td>
<td>0.49 ± 0.07</td>
<td>0.144</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.77 ± 0.09</td>
<td>23.92 ± 0.43</td>
<td>23.65 ± 0.46</td>
<td>0.386</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>111.24 ± 9.08</td>
<td>114.36 ± 8.95</td>
<td>108.84 ± 8.43</td>
<td>0.000</td>
</tr>
<tr>
<td>glucose (mg/dL)</td>
<td>88.63 ± 8.04</td>
<td>90.68 ± 7.83</td>
<td>87.06 ± 7.86</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Prevalence (%)

<table>
<thead>
<tr>
<th>BMI percentile</th>
<th>NW</th>
<th>OW</th>
<th>OB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>74.4</td>
<td>73.9</td>
<td>74.8</td>
<td>0.168</td>
</tr>
<tr>
<td>OW</td>
<td>15.9</td>
<td>14.4</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>OB</td>
<td>9.7</td>
<td>11.7</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>FMHx OB</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.000</td>
</tr>
<tr>
<td>FMHx T2DM</td>
<td>60.4</td>
<td>59.2</td>
<td>61.3</td>
<td>0.566</td>
</tr>
<tr>
<td>FMHx CVD</td>
<td>34.3</td>
<td>30.6</td>
<td>37.1</td>
<td>0.055</td>
</tr>
<tr>
<td>EBP (BP≥130/85)</td>
<td>5.5</td>
<td>9.2</td>
<td>2.8</td>
<td>0.000</td>
</tr>
<tr>
<td>HTN (BP≥140/90)</td>
<td>3.1</td>
<td>5.8</td>
<td>1.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Smoker</td>
<td>6.4</td>
<td>8.9</td>
<td>4.5</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Clinical Screens for dyslipidemia

| NCEP adult | 8.8 | 10.8 | 7.2 | 0.080 | 0 |
| NCEP peds  | 34.3 | 30.6 | 37.1 | 0.055 | 0.2 |
| PEGR Barlow | 25.6 | 26.1 | 25.2 | 0.810 | 0.3 |
| PEGR Krebs | 49.8 | 46.4 | 52.5 | 0.093 | 0.6 |
| SAP         | 9.7  | 11.7 | 8.1  | 0.097 | 1.0 |
| AAP         | 54.0 | 52.5 | 55.1 | 0.482 | 0.4 |
| AHA         | 79.2 | 75.6 | 82.0 | 0.025 | 0.6 |

Note: p-value based on ANOVA for continuous variables and Pearson χ² for categorical variables.

AAP – American Association of Pediatrics; AHA – American Heart Association; ANOVA – Analysis of variance; BMI – body mass index; BP – blood pressure; EBP – elevated blood pressure; FMHx OB – Family medical history of obesity in a parent; FMHx T2DM – Family medical history of diabetes; FMHx CVD – Family medical history of cardiovascular disease; HTN – hypertension; NCEP – National Cholesterol Education Program; NW – Normal weight (BMI <85th percentile); OW – Overweight (BMI 85th to 94.9th percentile); OB – Obesity (BMI ≥ 95th percentile); PEGR – Pediatric Expert Committee Recommendations; SAP – Spanish Association of Pediatrics; SBP – Systolic blood pressure; SD – Standard deviation; WC – Waist circumference; WHtR – Waist to height ratio
A total of 44.8% of participants had dyslipidemia defined as at least one lipid or lipoprotein abnormality. The most common lipid abnormality was hypertriglyceridemia (TG ≥ 110 mg/dL), which was found in over a third (38.7%) of participants. The prevalence of HDL-C ≤ 40 mg/dL was 24.2%; the prevalence of LDL-C ≥ 130 mg/dL was 12.4%; and the prevalence of total cholesterol ≥ 200 mg/dL was 17.9%. There were significant sex differences in TG (P<0.001) and HDL-C (P<0.001), with males having a higher prevalence of these abnormalities. No age differences were seen in the prevalence of dyslipidemia or its subcomponents (Table 4.3).

Table 4.3

*Dyslipidemia by BMI percentile category*

<table>
<thead>
<tr>
<th></th>
<th>NW mean ± SD</th>
<th>OW mean ± SD</th>
<th>OB mean ± SD</th>
<th>by BMI</th>
<th>by sex</th>
<th>by age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>169.2 ± 35.22</td>
<td>173.6 ± 30.68</td>
<td>172.9 ± 37.23</td>
<td>0.33</td>
<td>0.173</td>
<td>0.178</td>
</tr>
<tr>
<td>LDL-C</td>
<td>98.35 ± 30.71</td>
<td>102.4 ± 25.17</td>
<td>103.9 ± 34.10</td>
<td>0.16</td>
<td>0.610</td>
<td>0.356</td>
</tr>
<tr>
<td>TG</td>
<td>101.9 ± 43.88</td>
<td>126.6 ± 54.09</td>
<td>131.7 ± 60.51</td>
<td>0.00</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-C</td>
<td>50.51 ± 12.32</td>
<td>45.92 ± 10.57</td>
<td>42.69 ± 10.74</td>
<td>0.00</td>
<td>0.000</td>
<td>0.577</td>
</tr>
</tbody>
</table>

Prevalence (%)

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th>OW</th>
<th>OB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia</td>
<td>38.8</td>
<td>59.1</td>
<td>67.5</td>
<td>0.000</td>
</tr>
<tr>
<td>TC ≥ 200</td>
<td>16.7</td>
<td>19.7</td>
<td>23.8</td>
<td>0.254</td>
</tr>
<tr>
<td>LDL-C ≥ 130</td>
<td>12.7</td>
<td>9.8</td>
<td>15.0</td>
<td>0.516</td>
</tr>
<tr>
<td>TG ≥ 110</td>
<td>32.0</td>
<td>56.1</td>
<td>62.5</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-C ≤ 40</td>
<td>20.1</td>
<td>30.3</td>
<td>45.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: p-value based on ANOVA for continuous variables and Pearson χ² for categorical variables.

HDL-C – Low density lipoprotein cholesterol
LDL-C – Low density lipoprotein cholesterol
NW – Normal weight (BMI < 85th percentile)
OW – Overweight (BMI 85th to 94.9th percentile)
OB – Obesity (BMI ≥ 95th percentile)
TC – Total cholesterol; TG – Triglycerides
The NW, OW, and OB participants had significantly different prevalence rates of dyslipidemia: 38.8%, 59.1%, and 67.5% respectively (P<0.001). When dyslipidemia was separated and TC, LDL-C, TG, and HDL-C were analyzed separately, only TG and HDL-C were significantly (P<0.001) associated with adiposity (Figure 4.1).

Figure 4.1

*Prevalence of the components of dyslipidemia by BMI percentile category.*

HDL-C – Low density lipoprotein cholesterol
LDL-C – Low density lipoprotein cholesterol
NW – Normal weight (BMI <85th percentile)
OW – Overweight (BMI 85th to 94.9th percentile)
OB – Obesity (BMI ≥ 95th percentile)
TC – Total cholesterol
TG – Triglycerides

**Clinical Screening Algorithms**

An acceptable clinical screen was decided *a priori* to have a sensitivity ≥ 0.80 and a specificity ≥0.80. Using these criteria, none of the clinical screening algorithms were an acceptable screen. The algorithm with a sensitivity ≥0.80 was the AHA
(sens=0.817), with a substantial tradeoff in specificity (sp=0.228) (Table 4.4). The highest positive predictive values were seen with PECR (PPV=0.623) (Barlow et al., 2007) and SAP (PPV=0.675). On univariate analysis, PECR-Barlow, PECR-Krebs, SAP, and AAP were significantly associated with dyslipidemia (P<0.001).

Multivariate analysis was performed on only those that were significant on univariate analysis (Table 4.4). The PECR-Barlow and SAP recommendations were associated with over a 2-fold increase in the odds of having dyslipidemia (P<0.00). Notably, the NCEP adult recommendation of screening for dyslipidemia beginning at age 20, was not an acceptable screening strategy in this particular population.

Table 4.4

Univariate and multivariate statistics for the clinical screening algorithms (sensitivity, specificity, and positive predictive value).

<table>
<thead>
<tr>
<th>Clinical Screen</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>χ² test</th>
<th>β(exp)</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCEP adult</td>
<td>0.092</td>
<td>0.151</td>
<td>0.466</td>
<td>0.806</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP ped</td>
<td>0.332</td>
<td>0.648</td>
<td>0.433</td>
<td>0.557</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR Barlow</td>
<td>0.356</td>
<td>0.825</td>
<td>0.623</td>
<td>0.000</td>
<td>2.59</td>
<td>1.88</td>
<td>3.57</td>
</tr>
<tr>
<td>PECR Krebs</td>
<td>0.561</td>
<td>0.554</td>
<td>0.504</td>
<td>0.001</td>
<td>1.61</td>
<td>1.22</td>
<td>2.13</td>
</tr>
<tr>
<td>SAP</td>
<td>0.146</td>
<td>0.943</td>
<td>0.675</td>
<td>0.000</td>
<td>2.81</td>
<td>1.72</td>
<td>4.60</td>
</tr>
<tr>
<td>AAP</td>
<td>0.593</td>
<td>0.503</td>
<td>0.492</td>
<td>0.006</td>
<td>1.48</td>
<td>1.12</td>
<td>1.96</td>
</tr>
<tr>
<td>AHA</td>
<td>0.817</td>
<td>0.228</td>
<td>0.463</td>
<td>0.122</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CART Analysis

BMI > 23.33

BMI > 23.33 or WC > 66.5

Note: Logistic regression with dyslipidemia as a function of the clinical screen controlling for age and sex. Logistic regression only performed if univariate analysis was significant.

AAP – American Association of Pediatrics; AHA – American Heart Association;

CART – classification and regression tree; CI – Confidence interval;

NCEP – National Cholesterol Education Program;

PECR – Pediatric Expert Committee Recommendations; PPV – positive predictive value;

SAP – Spanish Association of Pediatrics; Sens – sensitivity; Spec – specificity

** those with BMI >23.33 or WC > 66.5
Classification and Regression Tree Analysis

The CART analysis created a tree with four nodes (Figure 4.2). The first node split participants as above or below a 23.33 BMI (kg/m$^2$). For those with a 23.33 kg/m$^2$ BMI or below, a second node separated participants with a WC > 66.5 cm from those with a WC ≤ 66.5 cm. In the first analysis, only those with BMI > 23.33 were included. Sensitivity, specificity, and PPV were calculated for those participants. In the second analysis, those with BMI ≤ 23.33 kg/m$^2$ and a WC > 66.5 cm were included as qualifying for a positive screen. Results were reported in Table 4.4.
**Classification and regression tree analysis**

Note: Variables entered into CART analysis were sex, age, family medical history (FMHx) of obesity, FMHx of diabetes, FMHx cardiovascular disease, and cardiovascular risk factors in the patient such as elevated blood pressure (≥130/≥85), hypertension (≥140/≥90), tobacco use, BMI age- and sex-specific percentile, BMI, waist circumference, waist-to-height ration.

0.00 – normal lipids
1.00 – dyslipidemia
BMI – body mass index (kg/m²)
WC – waist circumference (cm)
Discussion

The current study described dyslipidemia in normal weight, overweight, and obese Mexican youth aged 18- to 21-years-old. Nearly half of all participants had dyslipidemia, and prevalence rates increased with increasing adiposity. In order to test how to most accurately identify such patients with occult dyslipidemia, the performance of seven clinical screening algorithms was tested. Every clinical screen tested had substantial trade-offs between sensitivity and specificity. An exploration of alternative clinical screens was then completed using a CART analysis. The final clinical screen proposed by the CART analysis was clinically feasible, involving only two parameters (BMI and WC). Those with a positive clinical screen had 4 times the chance of having dyslipidemia as compared to those with a negative clinical screen, which represents an improvement above existing screens.

The Prevalence of CVD Risk Factors

A majority (74.4%) of the study sample was normal weight, and the remaining were overweight (15.9%) or obese (9.7%). These rates are comparable to nationally representative data from Mexico collected in 2000 (10- to 17-year-olds, n=16,809), which reported rates of overweight to be 10.8-19.1% and rates of obesity to be 6.8-14.7% depending on region (del Rio-Navarro, Velazquez-Monroy, Sanchez-Castillo et al 2004). In the current study sample, hypertension was prevalent in 5.8% of males and 1.1% of females. These rates were substantially lower than those reported in a representative sample of 20- to 29-year-olds in enrolled in The Institute of Mexican Social Security (IMSS) (Acosta-Cázares &
Escobedo-de la Peña, 2010). In the aforementioned sample, HTN (≥140/≥90) rates were 11.6% and 9.4% for males and females, respectively. The reason for this discrepancy is unknown, but previous studies have also demonstrated relatively low prevalence rates of hypertension in the state of San Luis Potosí compared to other states in Mexico (ENSANUT, 2006). The IMSS study also reported smoking rates of 38.5% for males and 12.7% for females aged 20 to 29, with significant differences between the sexes and significantly higher rates of smoking in the young. Similarly, rates of smoking were higher in our sample for males (8.9%) as compared to females (4.5%) with 20-year-olds having significantly higher rates (12.8%) than the other ages. But despite similar sex- and age-patterns, rates of smoking in our participants were lower than those reported by the IMSS. Therefore, in regards to personal risk factors, our study participants had comparable rates of overweight and obesity to the rest of the Mexican adolescent population, but lower rates of hypertension and smoking than Mexican samples of 20- to 29-year-olds.

**FMHx.** Having a family medical history of disease was quite prevalent in our sample. For instance, two thirds of young adults stated that they have a FMHx of diabetes, and one third stated that a FMHx of CVD existed. In contrast, a FMHx of obesity was cited by only 1.7% of participants. Reportedly, the adult prevalence of obesity in the Mexican population is 30% (Olaiz G, Rojas R, Barquera S, et al. 2003 as cited by Garcia-Garcia, Aviles-Gomez, Luquin-Arellano, et al., 2006). This suggests that youth-reported parental weight status may not be a valid measure of FMHx of obesity. The FMHx questions for CVD and DM used in the current study were broad, including all family members. This is closest to the FMHx protocol described by the
AHA, which includes all relatives and not only first-degree (parents) and second-degree (grandparents) relatives. While FMHx is not universally agreed upon, (Haney et al., 2007), this protocol is the most sensitive (>0.70) for detecting occult dyslipidemia in the pediatric population (Bell, Joseph, 1990; Diller, Huster, Leach, Laskarzewski, Sprecher, 1995 as cited by Haney et al., 2007). FMHx has had variable success in identifying children with dyslipidemia and it is estimated that 30-60% of children with dyslipidemia are missed when screening includes only with FMHx (Haney, et al., 2007) as was originally recommended by NCEP (1992). This is because FMHx is often not known (Soni, 2009).

**GOAL 1: Dyslipidemia Increases with Increasing Adiposity**

Dyslipidemia was seen in 44.8% of the sample. The most common lipid or lipoprotein abnormality was TG ≥110 mg/dL (38.8%), followed by HDL-C ≤ 40 mg/dL (24.2%). The NW, OW, and OB participants had significantly different prevalence rates of dyslipidemia, which were 38.8%, 59.1%, and 67.5% respectively. Looking at individual lipids and lipoproteins, TG and HDL-C remained significantly associated with BMI percentile category, whereas total cholesterol and LDL-C did not. Similar findings have been reported in previous studies (Lee, et al., 2009; D l’Allemand-Jander, 2010; Yamamoto-Kimura, et al., 2006; Wong et al., 1992). Some suggest that the relationship between HD, TG and adiposity may be mediated by insulin resistance (Dhuper, Sakowitz, Daniels, Buddhe, & Cohen, 2009).

In the United States, prevalence rates of having TG≥110 mg/dL was 18% for NW and 36% for OW or OB 12- to 18-year-olds included in the 1999 to 2002
NHANES data (Kranz Mahood, Wagstaff, 2007). The prevalence of TG ≥110 mg/dL in our sample was 32% for NW, and 56 and 61% for OW and OB respectively. These rates are almost double those seen in the United States sample. An ethnic discrepancy in TG levels was also reported by a large, multiethnic United States sample spanning 12- to 69-year-olds which reported statistically higher rates of hypertriglyceridemia (defined as TG ≥150) in Mexican American 20- to 34-year-olds that persists even after controlling for diet, physical activity, education, and income (Walker, Gurka, Oliver, Johns, & Deboer, 2010). In regards to HDL, NW, OW, and OB, our sample had prevalence rates of HDL-C ≤40 mg/dL of 20.1%, 30.3%, and 45.0% respectively. This is comparable to 12- to 18-year-old NHANES (1999-2002) data citing prevalence rates of HDL-C ≤40 mg/dL to be 18% in NW individuals and 36% of those who were OW or OB (Kranz Mahood, & Wagstaff, 2007).

**GOAL 2: Simple Algorithms based on Anthropometrics Outperform Others**

Out of the recommended clinical screening algorithms, none met the *a priori* criteria of having a sensitivity of ≥0.80 and a specificity of ≥0.60. The AHA recommended screen was the only screen with a sensitivity ≥ 0.80, but this came at a substantial cost to specificity, which was 0.228. This indicates most participants with dyslipidemia were identified by the screen, but 77.2% of healthy individuals were inappropriately tested for cholesterol values because they received a false positive clinical screen. The AHA recommendation, however, was not significantly associated with dyslipidemia on univariate analysis. The recommendations that were associated with dyslipidemia on univariate and multivariate analysis
(controlling for age and sex) were the PECR by Barlow et al. (2007), the PECR by Krebs et al. (2007), the SAP, and the AAP recommendations. The NCEP adult recommendations of universal screening in those 20-years-old and over was not significant on univariate analysis and not considered to be an acceptable protocol in this population as it failed to identify a majority of cases. Because of the nature of the Mexican healthcare system, failing to identify young adults during their college health screen may lead to a delay in diagnosis and an increased risk of developing symptomatic CVD (ENCUESTA Nacional de salud y nutricion, 2006).

The screening recommendations that were significant on univariate analysis were entered into multivariate analysis. Clinical screens with the highest OR of having dyslipidemia were PECR-Barlow (OR 2.59, 95% CI 1.88, 3.57) and SAP (OR 2.81, 95% CI 1.72, 4.60). Both of these clinical screening recommendations included only BMI percentile as a risk factor: The PECR-Barlow recommended screening those with BMI ≥ 85th percentile (OW/OB), and the SAP recommended screening those with BMI ≥ 95th percentile (OB). Other risk factors such as FMHx, blood pressure, and smoking were not included. Where these two recommendations fell short was in specificity. Both had specificities <0.40. Other clinical screening recommendations that included FMHx, or patient EBP, HTN, or smoking, did not perform as well (PECR Krebs, AAP, AHA) as the BMI-based recommendations. The recommendations that relied solely upon FMHx performed the most poorly (NCEP pediatric). The CART analysis affirmed these findings.
GOAL 3: The inclusion of Waist Circumference with BMI data

When all variables were entered into the CART analysis, a three-node tree was formed which first separated out all participants with BMI >23.33 kg/m². While this may seem like an arbitrary line drawn down the middle of the adult definition for the normal weight range (20 to 25 kg/m²), this cutoff point has been cited before. In 2005, Sanchez-Castillo and colleagues estimated that, in Mexicans, half (51%) of all type 2 DM and HTN risk was attributable to having a BMI ≥ 23 kg/m² (Sanchez-Castillo, et al., 2005). Other studies have also suggested lowering the testing threshold into the normal weight range.

Using child and adolescent NHANES data, researchers found that dyslipidemia, especially HDL-C and TG, correlated especially well to the 80th BMI percentile (Skinner, Mayer, Flower, Perrin, & Weinberger, 2009; Lee, Gebremariam, Card-Higginson, et al., 2009). The 75th percentile could also be used and had excellent sensitivity, but at the cost of an increasing number of false positives (Lee, Gebremariam, Card-Higginson, et al., 2009). Notably, the 75th BMI percentile is the point at which childhood weight becomes associated with higher adult morbidity and mortality (Must et al., 1992; Bjorge, Engeland, Tverdal, & Smith, 2008). In the pediatric population, BMI-z scores and lipids are continuously associated (Bell et al., 2007) at all levels of adiposity, including those in the normal weight ranges, but the presence of dyslipidemia is an independent predictor of future atherosclerosis (Raitakari et al., 2003). Normal weight patients had a higher risk of elevated CIMT in adulthood if they had dyslipidemia in adolescence (Magnussen et al., 2009).
Therefore, including individuals of normal weight in dyslipidemia screening protocols is not entirely unjustified.

In regards to the CART analysis, those with either a WC>66.5 or a BMI >23.33 were considered to have a positive clinical screen for dyslipidemia. The proposed clinical screen was 0.966 sensitive and 0.141 specific in detecting lipid and lipoprotein abnormalities. Having a positive screen in this test was associated with an OR of 4.60 (95% CI 2.44, 8.69). This was the highest OR of all of the clinical screening algorithms. There is often debate about whether WC or BMI are better predictors of dyslipidemia. Some cite that only WC is independently related to dyslipidemia (Yamamoto-Kimura, et al., 2006), while others have found that WHtR or BMI, but not WC were independently associated with outcomes of dyslipidemia such as CIMT (Maher, O’Dowd, Carey, Byrne, & Inerney 2008). The CART analysis suggested that both BMI and WC could be used in conjunction with each other to add diagnostic accuracy to the clinical screen. Again, all screens suffered from the trade-off between sensitivity and specificity. Because the continuous relationship between BMI and lipids, deciding upon screening recommendations may require deciding between catching as many cases as possible by increasing sensitivity versus sparing resources and only screening those who are highly likely to have abnormal lipids by increasing specificity.

One weakness of this study was that this is not a representative sample of Mexican adolescents and young adults. For instance, in the state of San Luis Potosí, nearly of third of residents have not finished grade school (Census, 2005). In contrast, ours is sample of high school graduates who have chosen to apply for
college. Results from this study are, therefore, not generalizable to the general population. A second weakness lies in the family history variables. Subtle differences between the screening algorithms may not have been appreciated because only three measures of FMHx were used (obesity, DM, CVD) when many more conditions were suggested by various recommendations (i.e. dyslipidemia, hypertension, FMHx of smoking, peripheral vascular disease, etc). Anthropometric indices were also a limitation as BP and WC were only measured once and inter-rater reliability was not assessed. In the end, BMI and WC overshadowed the three FMHx variables and so it is difficult to say what the impact would have been if more FMHx conditions had been included.

The current study was the first of its kind to test clinical screening algorithms in a population of Mexican students undergoing the transition from high school to college. Because requirements from the Mexican government, which recommends all college applicants undergo a health screen, this represents a unique window of opportunity to identify patients with asymptomatic dyslipidemia who would otherwise remain unidentified until the development of irreversible disease decades later. More research is needed to explore the impact of identifying such patients on improving lipid levels and health outcomes.
CHAPTER 5: THE PEDIATRIC EXPERT COMMITTEE RECOMMENDATIONS AS A CLINICAL SCREENING TOOL FOR IMPAIRED FASTING GLUCOSE

In the developed world, diabetes a leading cause of blindness, kidney disease, and limb amputations (CDC, 2005). These complications are known as the microvascular complications of diabetes. Diabetes also increases the risk of macrovascular complications such as cardiovascular disease (CVD). Currently, CVD is the world's leading killer (Lopez & Mathers, 2006; Mathers, Boerma, & Ma Fat, 2009) and up to a fifth of deaths from CVD have been attributed to diabetes (Danaei, Lawes, Vander Hoorn, Murray, & Ezzati, 2006). Increasing rates of T2DM have led to a 10% increase in death rates from cardiovascular disease (Ford, Ajani, & Croft, 2007). Between the microvascular and the macrovascular complications, those with diabetes are two-times more likely to suffer premature death as compared to those without diabetes (USPTF, 2008).

Pathophysiology of T2DM

T2DM is a metabolic disease continuum of disease characterized by a dysfunction of glucose and insulin. Insulin is a hormone secreted by the pancreas in response to any meal that increases levels of glucose in the blood. Insulin controls blood glucose by pushing glucose molecules out of the bloodstream and into tissues such as skeletal muscle, adipose tissue, and the liver. When these tissues become resistant to the effects of insulin, the pancreas will increase insulin levels to overcome that resistance. If pancreas begins to burnout and can no longer secrete enough insulin to control blood glucose, then blood glucose begins to rise, first after
meals, and then in the fasting state. When fasting plasma glucose (FPG) levels rise at or above 100 mg/dl, it is known as impaired fasting glucose (IFG) or prediabetes. Similarly, if FPG rises above 125 mg/dl the diagnosis of diabetes is made (American Diabetes Association [ADA], 2007).

The microvascular complications of diabetes are rarely present at the time of diagnosis. On the one hand, it takes decades of exposure to blood glucose levels within the diabetic range (FPG ≥ 126 mg/dL) to cause complications such as blindness and kidney failure. On the other hand, macrovascular complications begin to develop years before the diagnosis of diabetes. In patients with prediabetes (FPG 100 to 125 mg/dl), there is a substantially increased risk of CVD compared to those with normal fasting blood glucose levels (<100 mg/dL) (Coutinho, Gerstein, Wang, & Yusuf, 1999).

The risk for CVD conferred by insulin resistance exists regardless of age. A study of children living in China measured subclinical atherosclerosis via carotid intimal media thickness (CIMT). The investigators found that CIMT was independently associated with measures of insulin resistance, even after controlling for other cardiovascular disease risk factors such as dyslipidemia and adiposity (Fang, Zhang, Luo, Yu, & Lv, 2010). Therefore, for those with subclinical insulin resistance the process of CVD has already begun.

Age is a strong risk factor for insulin resistance, pancreatic burnout, and resultant T2DM (Paulweber et al., 2010). Thus, frank T2DM is not a common disease in the pediatric population and the projected national prevalence rate is estimated to be around 4.1 per 1,000 in the United States (Fagot-Campagna et al., 2000). Rates
of T2DM, however, are increasing in the pediatric population paralleling increases in childhood obesity (ADA, 2000; Fagot-Campagna et al., 2000; Lipton, Keenan, Onyemere, & Freels, 2002; Macaluso et al., 2002; Pinhas-Hamiel et al., 1996) and more sedentary lifestyles (Paulweber et al., 2010). It is currently estimated that, for children who were born in the year 2000, there is a 30% to 40% lifetime risk of diabetes, if obesity rates level off (Koplan et al., 2005).

While pediatric T2DM is not common, it may represent a more aggressive form of the disease. Pediatric T2DM is believed to progress rapidly to pancreatic dysfunction (Gungor & Arslanian, 2004), with the swift development of the CVD risk factors that are associated with insulin resistance such as elevated blood pressure and dyslipidemia (West et al., 2009; Bao, Srinivansan, & Berenson, 1996; Taittonen et al., 1996). Children will likely not outgrow the cardiometabolic derangements of insulin resistance. The insulin resistance syndrome, also known as Metabolic Syndrome (MetS) is comprised of IFG, elevated blood pressure, dyslipidemia, and abdominal obesity. This syndrome tracks from childhood to adulthood (Bao, Srinivansan, & Berenson, 1996; Mattsson, Rönnemaa, Juonala, Viikari, & Raitakari, 2008; Messiah et al., 2008; Kissebah et al., 1982; Morrison, Friedman, Wang, & Glueck, 2008). If the full syndrome associated with insulin resistance does not develop in childhood, it will likely appear in early adulthood. For example, on twenty-year follow-up, child and adolescent glucose levels were associated CVD risk factors in obese adults (Camhi et al., 2010) and higher fasting plasma glucose in childhood predicted the onset of prediabetes and diabetes decades later (Nguyen, Srinivasan, Xu, Chen, & Berenson, 2010).
Not everyone who is overweight or obese develops the MetS or T2DM. These diseases have a strong genetic component (D’Adamo, Santoro, & Caprio, 2009). For instance, reporting FMHx of T2DM in childhood has been independently associated with the development of T2DM and MetS as an adult (Morrison, Friedman, Wang, & Glueck, 2008). Because of the strong genetic component, some ethnicities are particularly prone to T2DM more so than others (Fujimoto, 1996). In particular, Hispanic or Latinos have high risk for T2DM (CDC, 2005).

Adult Hispanics are more prone to insulin resistance and have approximately 2 times the risk of T2DM compared to non Hispanic whites (Acosta-Cázares & Escobedo-de la Peña, 2010; CDC, 2004; Cowie et al., 2006; Ford, Giles, & Dietz, 2002; Park et al., 2003; Umpierrez, Gonzalez, Umpierrez, & Pimentel, 2007). The pattern is also seen in children (Williams, et al., 2005). Hispanics get T2DM at younger ages and lower levels of adiposity than do NHW (Sanchez-Castillo et al., 2005; Umpierrez, Gonzalez, Umpierrez, & Pimentel, 2007). When controlling for education, income, healthy eating index, and physical activity these associations diminish slightly, but the association between IFG and Mexican ethnicity still persists (Walker, Gurka, Oliver, Johns, & Deboer, 2010). The pediatric population has low rates of frank diabetes, but Hispanic children and adolescents have been found to be more prone to insulin resistance syndrome (Cook, Auinger, Li, & Ford, 2008; de Ferranti, et al., 2004; Johnson et al., 2009). Findings from North America, have been replicated in European studies of Hispanics and nonHispanic whites (López-Capapé et al., 2006). In addition to higher rates of insulin resistance, T2DM, and associated syndromes, Hispanics also have higher rates of complications and mortality from these diseases.
Globally, diabetes-death rates, after standardization by age, were the highest in men and women of Latin America compared to any part of the world (South Asia, Europe, Central Asia, East Asia, Pacific, Middle East, North Africa, Sub-Saharan Africa) (Danaei et al., 2006).

**Diabetes in Mexico**

In Mexico, diabetes is the second leading cause of death, with the highest rates of T2DM in the most westernized area of the country (Stevens et al., 2008). Rates of diabetes in westernized regions have paralleled increasing rates of obesity (Rivera et al., 2002; Meaney et al., 2006). Death from T2DM in Mexico is second only to CVD. Because T2DM also contributes to this disease, high blood glucose contributes to the highest number of deaths compared to any other risk factor (Stevens et al., 2008). A practical solution to this trend is to screen for prediabetes. Current screening rates in Mexican adults for diabetes range from 12.9 to 26.5% depending on the regional healthcare practices (ENSANUT, 2006).

Several adult screening recommendations exist. To prevent CVD complications associated with T2DM, The American Academy of Family Physicians and Canadian Task Force recommends screening adults with hypertension and dyslipidemia (AAFP, 2007; Feig, Palda, & Lipscombe, 2005). The United States Preventative Services Task Force found sufficient evidence to recommend screening only those with hypertension (USPTF, 2008). In contrast to these recommendations,
The American Diabetes Association (ADA) has taken a more liberal approach to screening. The ADA recommended screening for T2DM or prediabetes in all adults 45 or older. In adults less than 45-years-old, it was recommended to screen if BMI $\geq 25$ and another risk factor for T2DM was present. Risk factors included hypertension, dyslipidemia, a FMHx of T2DM, a personal history of gestational diabetes, or a history of vascular disease (ADA, 2007).

A European evidence-based guideline for the prevention of T2DM recommended even more liberal screening targeting patients with at least one clear risk factor for T2DM (Paulweber et al., 2010). For risk assessment the European guidelines recommended using The Finnish Diabetes Risk Score (FINDRISC), which is a scale based on age, body mass index (BMI), waist circumference, use of antihypertensive medication, history of high blood glucose, and family history of diabetes with the optional addition of physical activity and diet (Paulweber et al., 2010). Global recommendations from The International Diabetes Federation Clinical Guidelines Task Force supports such targeted screening, discourages universal screening, and states that each health service should decide whether to have a diabetes screening program based on the regional prevalence of undiagnosed T2DM as well as the resources available to screen and treat (IDF Clinical Guidelines Task Force, 2006).

Child and adolescent T2DM screening recommendations also exist. The ADA recommends screening the pediatric population as follows: Screen using a fasting plasma glucose if the patient has a BMI $>85^{th}$ percentile for age and sex, weight for height $>85$th percentile, or weight $>120\%$ of ideal (50th percentile) for height; and
also has two other risk factors. Risk factors include having a FMHx of T2DM in 1st and 2nd degree relatives, belonging to a high-risk race/ethnic group (American Indian, AA, Hisp, Asian/South Pacific Islanders), having clinical signs of insulin resistance (e.g. acanthosis nigricans, polycystic ovarian syndrome), or conditions associated with insulin resistance (e.g. hypertension, or dyslipidemia). Such patients should be tested every 2 years starting at the age of 10 or the onset of puberty if puberty occurs sooner than 10-years-old. In high risk patients not meeting criteria, clinical judgment can be used (ADA, 2000).

The American Diabetes Association recommendations have since been endorsed by The Pediatric Expert Committee (Barlow et al., 2007), that modified the recommendations to include only BMI percentile to measure adiposity and screening in for all obese children and adolescents regardless of the number of risk factors. FMHx of CVD, a FMHx of obesity, and smoking were also added as risk factors in order to unify the algorithm with an algorithm for dyslipidemia and fatty liver disease (Table 5.1). The Pediatric Expert Committee Recommendations (PECR) for screening for prediabetes has not yet been tested in Mexico. A pediatric screen for prediabetes would provide the earliest possible opportunity to uncover occult disease in a Hispanic population. This population suffers a large burden of T2DM and an early screen would be advantageous. And so, in accordance with the IDF recommendations of gathering regionally-based evidence, the current study sought to test the PECR in detecting IFG in a population from Mexico.
Table 5.1

*Complete Algorithm for laboratory evaluation from Barlow & The Expert Committee (2007).*

<table>
<thead>
<tr>
<th>Risk Categories</th>
<th>Risk Factors</th>
<th>Recommended Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal weight (≤85th %ile)</td>
<td>0</td>
<td>BP</td>
</tr>
<tr>
<td>2) Normal weight (≤85th %ile)</td>
<td>1*</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>3) Overweight (&gt;85th – 94th %ile)</td>
<td>&lt;2**</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>4) Overweight (&gt;85th – 94th %ile)</td>
<td>≥2**</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td>5) Obese (≥95th %ile)</td>
<td>≥0</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT (microalbumin/creatinine ratio)</td>
</tr>
</tbody>
</table>

BP – Blood pressure

* Family Medical History of high cholesterol

** Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease), ethnic minority (African, Hispanic), elevated BP, dyslipidemia, tobacco use, and signs of insulin resistance.

The goal of the study was three-fold. The first goal was to describe the relative prevalence of IFG in a population of adolescents and young adults from Central Mexico. The second was to test the PECR in detecting those with IFG assessing the clinical screening algorithm for sensitivity, specificity, and positive predictive value. The third goal was to perform exploratory classification and regression tree (CART) analysis in search of an improved clinical screening algorithm. Implementing such a screen would not only allow for the detection of this preventable disease, but may also improve patient motivation to lose weight regardless of the results (Rhodes et al., 2005).

**Methods**

Data for this cross-sectional study were collected as part of the UP AMIGOS project (University of San Luis Potosi and Illinois: A Multidisciplinary Investigation
on Genetics, Obesity, and Social-environment). The UP AMIGOS project is an international collaboration between researchers at the University of Illinois in the United States and the Autonomous University of San Luis Potosí (UASLP) in Mexico.

Participants

Participants were applicants to the Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí [UASLP]) for the 2009 academic year. The full sample included 9,974 participants. A large majority of participants were from the city of San Luis Potosi and surrounding area. The University of San Luis Potosi is located in the city of San Luis Potosi, which is the capitol of a centrally located Mexican state by the same name. The final sample included only those 18- to 21-year-olds with complete data on all variables (n=5,455). A fasting lipid profile was only analyzed on 828 participants and the PECR cites dyslipidemia as a risk factor for IFG. It was decided not to drop the variable, but to analyze those with those with complete data on every variable separately. Participants with complete data, but no fasting lipid profile were known as Group A (n=4,627). The group with complete fasting lipid profiles, were labeled Group B. For descriptive analysis, Groups A and B were analyzed together. The groups were analyzed separately when testing PECR and CART analysis.

Protocol

Between February and March 2009 participants underwent a health screen and electively completed a questionnaire. Informed consent was obtained from every subject prior to data collection. The health screen was completed by trained
health care professionals and included anthropometric measurements (e.g., blood pressure, height, weight, waist circumference), blood pressure, a physician-conducted history and physical exam, and venipuncture for blood biomarker analysis. The protocol was reviewed and approved by the UASLP Institutional Review Board (IRB) and the University of Illinois at Champaign-Urbana IRB.

**Anthropometric Measurements.** During the health screen, blood pressure (BP) was measured according to a common protocol adapted from procedures recommended by the American Heart Association. BP was taken on the dominant arm (right in most of the cases) in sitting position. Height was measured via stadiometer and recorded to the nearest 0.5 centimeter. Weight was assessed with a scale graduated to the nearest 0.1 kg. Waist circumference (WC) was measured with the subject standing. A flexible steel tape was used to measure the WC to the nearest 0.1 cm at the level of the iliac crest at the end of normal expiration. As a comparison, Krebs and colleagues (2007) recommend WC be taken at just above the ileum. Waist to height ratio (WHtR) was calculated with the equation: WC in cm divided by height in cm. Body mass index (BMI) was calculated from height and weight (BMI=kg/m²) and BMI percentiles were calculated using the Centers for Disease Control and Prevention 2000 BMI growth charts (Kuczmarski, et al., 2000) as recommended by Krebs and colleagues (2007).

**Blood Biomarkers.** Participants arrived at the health screen following an overnight fast. After the screen, trained health professionals drew fasting blood samples by venipuncture. On the day of collection, samples were refrigerated at 4°C. At the end of the day, serum was frozen at 20°C. Samples were thawed prior to
Plasma fasting lipid levels (total cholesterol, HDL, triglycerides) were measured using the RD/Hitachi 902 analyzer (Roche Diagnostics, Indianapolis, IN). Serum fasting glucose was determined using the glucose oxidase peroxidase method (reagents from Biosystems, Autoanalyzer Alcyon 300 from Abbott).

**Variables in the Algorithm**

The PECR clinical screening algorithm first sorts patients into the BMI percentile categories of normal weight (NW, BMI < 85\textsuperscript{th} percentile), overweight (OW, BMI 85\textsuperscript{th} to 94.9\textsuperscript{th} percentile), and obese (BMI ≥ 95\textsuperscript{th} percentile). The OW category is then further subdivided based on the presence of risk factors such as positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease), elevated blood pressure, tobacco use, dyslipidemia, and ethnic minority (i.e. African, Hispanic). A positive clinical screen is considered to be all participants who are OW with 2 or more risk factors (Category 4), or participants who are OB (Category 5).

**BMI categories.** BMI percentile categories were defined as previously described in accordance with PECR: NW was defined as < 85\textsuperscript{th} percentile, OW was defined as ≥ 85\textsuperscript{th} percentile but < 95\textsuperscript{th} percentile, and OB was defined as ≥ 95\textsuperscript{th} percentile using percentile curves published in 2000 by the Centers for Disease Control. BMI z-scores needed to calculate percentile were not available for the 21-year-old participants. In 21-year-olds, BMI was used. BMI categories were defined as normal weight (<25 kg/m\textsuperscript{2}), overweight (25 to 29.9 kg/m\textsupersquared), and obese (≥30.0 kg/m\textsupersquared).
**FMHx obesity.** Having a family history of obesity in first degree relatives was reported by participants using two questions from the questionnaire. The first questioned assessed the weight status of the mother, and the second used the same question stem to assess the weight status of the father. “Please indicate how you would classify your mother’s [or father’s] weight currently.” Responses to both questions were: markedly underweight, underweight/thin, average weight, overweight, obese, and don’t know. For this analysis, weight was coded dichotomously (1 if at least one parent was obese and 0 otherwise). “Don’t know” was coded as missing.

**FMHx T2DM and cardiovascular disease.** During the health screen, healthcare providers asked participants whether anyone on their maternal or paternal side had diabetes. They were then asked whether anyone had cardiovascular disease. Answering in the affirmative for a family history of diabetes on the maternal side, paternal side, or both was coded as 1. The same was done for CVD. This is a noted deviation from the ECR screening algorithm, which requires that dyslipidemia be specifically assessed. This was not thought to be feasible because a majority of Mexicans with dyslipidemia are not aware of their condition (Acosta-Cázares & Escobedo-de la Peña, 2010). Therefore, in the analysis, cardiovascular disease served as a surrogate for a FMHx of dyslipidemia.

**Elevated blood pressure.** While the ECR/AAP, and AHA defined elevated blood pressure and hypertension to be age-, sex- and height-specific 90th percentile and ≥95th percentile, respectively, the standardized curves have only been published for ages ≤17-years-old (NHBPEP, 1996). Therefore, we used adult definitions of
elevated blood pressure (SBP ≥ 130 and/or DBP ≥ 85) (IDF, 2007), and hypertension as defined by the AHA (SBP ≥ 140 and/or DBP ≥ 90) (AHA, 2010).

Smoking. Smoking is considered a risk factor for cardiovascular disease and the ECR empirically recommended that it be assessed as a risk factor in the clinical screening algorithm. In the questionnaire, smoking was assessed with the question adapted from the ENCUESTA survey: “Have you smoked at least 100 cigarettes (5 packs) during your life? (Yes, No, I have never smoked),” Yes was coded as 1.

Dyslipidemia. A fasting lipid profile was analyzed in Group B participants. The lipid profile included a fasting total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald Formula (LDL-C = TC – HDL-C – [TG/5]) (Friedewald, Levy, & Fredrickson, 1972). This has been cited by the National Cholesterol Education Program (1992) as an acceptable measure of LDL-C when blood samples are taken after a 12-hour fast (NCEP, 1992). Dyslipidemia was defined as any one of the following: Total cholesterol ≥ 200 mg/dL, LDL-C ≥ 130 mg/dL, triglycerides ≥110 mg/dL, HDL-C ≤40 mg/dL (females and males), or (Krebs et al., 2007). This is a hybrid definition combining NCEP (1992) and AHA values TC and LDL, with definitions by Cook and colleagues (2003) for HDL-C and TG as recommended by the PECR (Krebs et al., 2007). Values for TC, LDL, and HDL-C for this hybrid definition were chosen based on evidence for tracking (Magnussen et al., 2008), and correlations with adult CIMT (Magnussen, 2009) and CVD (Klag et al., 1993).
**Ethnicity.** In the PECR, OW participants are further subdivided based on having two or more risk factors. Hispanic ethnicity is generally used to describe those from Latin America and Mexico. Therefore, in this population, if Hispanic ethnicity was to be considered as a risk factor, then only 1 additional risk factor would be needed to categorize participants with a positive clinical screen. It is unknown whether the inclusion or exclusion of the ethnicity variable would improve the sensitivity or specificity of the PECR and so both possibilities were included in the analysis.

**Analysis**

There were three goals of the analysis. The first was to describe the relative prevalence of IFG in normal weight, overweight, and obese participants. The second was to test the sensitivity, specificity, and PPV of the PECR in detecting those with IFG. The third goal of the analysis was to explore ways in which to improve the sensitivity and specificity of a clinical screen by adjusting the algorithm using a CART analysis. A sensitivity of ≥0.80 and a specificity ≥0.80 was considered an acceptable clinical screen.

**Descriptive Statistics.** Statistical analysis was performed with SPSS (version 19.0). Descriptive statistics were run for all variables using pooled data from group a and b. Pooled data was not used when calculating the prevalence of dyslipidemia, which was only reported for group b. Sex and age differences were determined with ANOVA for continuous variables and χ² tests for categorical variables. Because of the large sample size, alpha was set at 0.001 for pooled data.
Glucose by sex, age, BMI. Significant age, sex, and BMI differences were determined for IFG (fasting glucose ≥ 100 mg/dL) using \( \chi^2 \) tests. Then ANOVA was used to compare the continuous variable of fasting glucose by age, sex, BMI percentile category. Continuous correlations between fasting glucose and BMI \( z \)-score were identified by Pearson’s \( r \) for those <21 years-old. The same was done using BMI (kg/m\(^2\)) for all ages.

Testing Clinical Screening Algorithms. In testing the PECR, separate analyses were run for Group A & Group B participants. First, the PECR was tested for sensitivity, specificity, and PPV with and without counting Mexican ethnicity as a risk factor. The univariate relationships between PECR and IFG was tested using a \( \chi^2 \) test. Following this, multivariate analysis was performed using logistic regression with IFG as a function of a positive or negative screen controlling for age and sex. A second logistic regression was run testing individual Risk Categories for significant associations with IFG.

Exploratory CART Analysis. In an attempt to improve upon the existing clinical screening algorithm, an exploratory analysis was conducted using the SPSS 19.0 decision tree module to perform CART analyses. Three CART analyses were run, one for Group A, one for Group B, and one for the pooled data (Group A+B). All independent variables were entered into the analysis (age, sex, FMHx obesity, FMHx CVD, FMHx DM, EBP, HTN, tobacco use, and dyslipidemia [Group B only], and number of risk factors), as well as the anthropometric variables BMI, BMI \( z \)-score, WC, and WHtR. The CART analysis output was then examined for nodes with the highest prevalence rates of IFG. These nodes were chosen and coded as having a
positive CART screen. Univariate and multivariate statistics were then run on pooled data in order to test for an association between a positive CART screen and IFG.

**Results**

Overall, 51.5% of the study sample was female (Table 5.2). Most participants were 18-years-olds (57.3%), followed by 19-years-olds (25.3%). The remaining participants were 20- and 21-years-olds (17.4%). Female participants tended to be younger ($\chi^2$, $P<0.001$). Groups A and B were significantly different on several independent variables. For instance, Group B was more likely to be female ($\chi^2$, $P<0.01$), were younger (18.4 $\pm$ 0.7 versus 18.7 $\pm$ 0.9 years-old, $P<0.001$), had higher mean SBP (111.2 $\pm$ 9.1 versus 109.7 $\pm$ 10.6 mmHg, $P<0.001$), were less likely to smoke ($P<0.001$), and were more likely to have a positive PECR screen ($P<0.001$).

Regarding the dependent variables, Group B had a higher mean fasting glucose (88.6 $\pm$ 8.0 versus 84.7 $\pm$ 7.7, $P<0.001$) and was more likely to have IFG ($P<0.001$). Pooled data from Groups A and B were reported for the remainder of the descriptive analysis. Because of differences in risk factors, Group A and Group B were analyzed separately when testing the PECR algorithm, and in the CART analysis.
Table 5.2
Descriptive statistics by sex

<table>
<thead>
<tr>
<th>Mean and SD</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>by sex</th>
<th>by age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.66 ± 0.91</td>
<td>18.74 ± 0.95</td>
<td>18.59 ± 0.86</td>
<td>&lt;0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.65 ± 0.09</td>
<td>1.71 ± 0.07</td>
<td>1.59 ± 0.06</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.20 ± 0.51</td>
<td>3.28 ± 0.54</td>
<td>3.15 ± 0.47</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>79.59 ± 14.46</td>
<td>82.31 ± 11.43</td>
<td>77.02 ± 10.88</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.48 ± 0.07</td>
<td>0.48 ± 0.06</td>
<td>0.48 ± 0.07</td>
<td>0.049</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.75 ± 4.52</td>
<td>24.10 ± 4.53</td>
<td>23.43 ± 4.48</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mg/dL)*</td>
<td>170.27 ± 34.75</td>
<td>168.38 ± 33.18</td>
<td>171.71 ± 35.89</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)*</td>
<td>99.53 ± 30.28</td>
<td>98.90 ± 28.62</td>
<td>100.01 ± 31.52</td>
<td>0.603</td>
<td>0.359</td>
</tr>
<tr>
<td>TG (mg/dL)*</td>
<td>108.57 ± 48.73</td>
<td>115.60 ± 53.49</td>
<td>103.17 ± 44.04</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-C (mg/dL)*</td>
<td>49.03 ± 12.19</td>
<td>46.36 ± 11.39</td>
<td>51.07 ± 12.40</td>
<td>&lt;0.001</td>
<td>0.565</td>
</tr>
<tr>
<td>glucose (mg/dL)</td>
<td>85.31 ± 7.92</td>
<td>86.78 ± 8.06</td>
<td>83.93 ± 7.53</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Prevalence (%)
BMI percentile

<table>
<thead>
<tr>
<th>BMI percentile</th>
<th>NW</th>
<th>OW</th>
<th>OB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>76.0</td>
<td>14.6</td>
<td>9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OW</td>
<td>14.6</td>
<td>74.3</td>
<td>11.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OB</td>
<td>9.4</td>
<td>15.0</td>
<td>7.5</td>
<td>0.796</td>
</tr>
</tbody>
</table>

FMHx OB

| FMHx OB | 2.8 | 2.3 | 3.2 | 0.049 | 0.796 |
| FMHx T2DM | 56.1| 53.6| 58.5| <0.001| 0.022 |
| FMHx CVD | 30.9| 28.1| 33.5| <0.001| 0.135 |
| EBP         | 5.3 | 8.7 | 2.0 | <0.001| 0.011 |
| HTN         | 3.6 | 6.0 | 1.4 | <0.001| <0.001 |
| Smoker      | 15.8| 21.5| 10.4| <0.001| <0.001 |
| Dyslipidemia* | 44.7| 47.8| 42.3| 0.121 | 0.035 |
| IFG         | 4.0 | 5.8 | 2.3 | <0.001| 0.368 |

Pediatric Expert Committee Recommendations

Group A
no ethnicity

| Group A no ethnicity | 15.1| 17.4| 12.8| <0.001| <0.001 |
| incl ethnicity      | 20.7| 22.6| 18.9| <0.001| <0.001 |

Group B*

| Group B* no ethnicity | 19.2| 19.7| 18.8| 0.790 | 0.604 |
| incl ethnicity*       | 23.8| 24.2| 23.5| 0.869 | 0.157 |

Note: p-value based on analysis of variance for continuous variables and Pearson χ² for categorical variables.

BMI – body mass index; BP – blood pressure; EBP – elevated blood pressure;
FMHx OB – Family medical history of obesity in a parent; FMHx T2DM – Family medical history of diabetes; FMHx CVD – Family medical history of cardiovascular disease;
HTN – Hypertension; NCEP – National Cholesterol Education Program; NW – Normal weight (BMI <85th percentile); OW – Overweight (BMI 85th to 94.9th percentile);
OB – Obesity (BMI ≥ 95th percentile); SBP – Systolic blood pressure; SD – Standard deviation;
WC – Waist circumference; WHtR – Waist to height ratio
* Group B only. When no asterick, Groups A and B pooled.
Descriptive Statistics

A majority of participants were NW (76.0%), a smaller percentage were OW (14.6%), or OB (9.4%). Females had higher prevalence rates of NW and OW, while males had higher rates of OB (P<0.001). Older participants tended to have higher rates of adiposity (P<0.001).

Most participants had at least one risk factor (86.2%), even in participants who did not have fasting lipids analyzed. A few participants reported their parents as being obese (2.8%); but over one half reported a FMHx of DM (56.1%), and a third reported having a FMHx of CVD (30.9%). There were no significant age differences in reported of FMHx of obesity, DM, or CVD, but females were more likely to report having a FMHx of DM or CVD (P<0.001). There were also significant sex and age differences in EBP, HTN, and smoking. A total of 8.7% males had EBP, while 2.0% of females had this condition (P<0.001). HTN was present in 6.0% of males and 1.4% of females (P<0.001). The prevalence of HTN increased with age (P<0.001). Males were also more likely to smoke (21.5%) as compared to females (10.4%) (P<0.001). Older participants had significantly higher rates of smoking, with rates being highest in 21-year-olds (26.9%) and lowest in 18-year-olds (12.2%) (P<0.001). In Group B, rates of dyslipidemia were (44.7%) with no significant age or sex differences. Overall, 15.1 to 23.8% of participants qualified as having a positive PECR screen. Prevalence rates depended upon the inclusion of ethnicity and the availability of dyslipidemia as a risk factor. In group a only, males and older participants were more likely to have a positive screen (P<0.001).
group b which included dyslipidemia as a risk factor, no significant differences existed for age and sex.

**IFG.** A total of 4.0% of participants had IFG defined as fasting glucose ≥ 100 mg/dL. There were significant sex differences in rates of IFG (P<0.001), with males having a higher prevalence of IFG. No age differences were seen. The NW, OW, and OB participants had significantly different prevalence rates of IFG (NW 3.2%, OW 5.0%, and OB 8.1%; P<0.001). Mean values of glucose for NW, OW, and OB participants were all between 80 and 90 mg/dL, but increased significantly with BMI percentile category (P<0.001): NW 84.89 ± 7.67; OW 86.35 ± 8.42; OB 87.36 ± 8.53 mg/dL. Continuous correlations measured by Pearson’s r were small (BMI z-score, glucose, r=0.067; BMI, glucose, r=0.10), but significant (P<0.001). IFG was significantly higher in participants with a positive PECR screen. In group A, the prevalence of IFG among those with a positive PECR versus those with a negative screen was 5.9% vs. 2.6% without counting ethnicity as a risk factor; and 5.3% vs. 2.5% when ethnicity was included. For participants belonging to group B, the prevalence of IFG among those with a positive PECR versus those with a negative screen was 14.5% vs. 7.3% when ethnicity was not included and 13.2% vs. 7.3% with the inclusion of ethnicity.

**Pediatric Expert Committee Recommendations**

An acceptable clinical screen was decided *a priori* to have a sensitivity of ≥0.80 and a specificity ≥0.80. Using these criteria, the PECR was not an acceptable screen for either Group A or Group B. In Group A, the algorithm had a sensitivity of
0.283, a specificity of 0.854, and PPV of 0.059 (Table 5.3) when ethnicity was not included as a risk factor; when ethnicity was included, the sensitivity increased to 0.355, and the specificity decreased to 0.798. PPV was 0.053. Logistic regression revealed a significant relationship between the PECR screen and the outcome of IFG (P<0.001) with a positive screen being associated with over a 2-fold increase in the risk of IFG. When risk categories were analyzed individually, Category 4 (P<0.05) and Category 5 (P<0.001) were associated with an increased risk of having IFG as compared to Category 1. This correlated to those who would be classified as having a positive PECR screen.

When participants in Group B were analyzed, the PECR algorithm had a sensitivity of 0.319, a specificity of 0.820, and PPV of 0.145 when ethnicity was not included; and a sensitivity of 0.361, a specificity of 0.774, and PPV of 0.132 when it was. On univariate analysis, PECR was significantly associated with IFG in Group B.
Table 5.3

*Univariate and multivariate statistics for the clinical screening algorithms (sensitivity, specificity, and positive predictive value).*

<table>
<thead>
<tr>
<th>Clinical Screen</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>p-value</th>
<th>$\chi^2$ test</th>
<th>$95%$ CI</th>
<th>p-value</th>
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<td>group a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.283</td>
<td>0.854</td>
<td>0.059</td>
<td>&lt;0.001</td>
<td>2.21 1.52 3.21</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Risk Category 1</td>
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<td></td>
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<tr>
<td>Risk Category 2</td>
<td>0.98</td>
<td>0.62</td>
<td>1.57</td>
<td>0.942</td>
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<tr>
<td>Risk Category 3</td>
<td>1.38</td>
<td>0.76</td>
<td>2.48</td>
<td>0.288</td>
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<tr>
<td>Risk Category 4</td>
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<td>3.76</td>
<td>0.023</td>
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<tr>
<td>Risk Category 5</td>
<td>2.43</td>
<td>1.53</td>
<td>3.84</td>
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<tr>
<td>PECR screen (ethnicity)</td>
<td>0.355</td>
<td>0.798</td>
<td>0.053</td>
<td>&lt;0.001</td>
<td>2.15 1.50 3.06</td>
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<tr>
<td>Risk Category 1</td>
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</tr>
<tr>
<td>Risk Category 2</td>
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<td>Risk Category 5</td>
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<td>1.57</td>
<td>3.93</td>
<td>&lt;0.001</td>
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<tr>
<td>group b</td>
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<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.319</td>
<td>0.820</td>
<td>0.145</td>
<td>0.007</td>
<td>2.16 1.26 3.70</td>
<td>0.005</td>
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</tr>
<tr>
<td>Risk Category 1</td>
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</tr>
<tr>
<td>Risk Category 2</td>
<td>.73</td>
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<td>2.87</td>
<td>.943</td>
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<td>.109</td>
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<tr>
<td>Risk Category 5</td>
<td>2.08</td>
<td>1.03</td>
<td>4.21</td>
<td>.042</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR screen (ethnicity)</td>
<td>0.361</td>
<td>0.774</td>
<td>0.132</td>
<td>0.014</td>
<td>1.96 1.16 3.21</td>
<td>0.011</td>
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</tr>
<tr>
<td>Risk Category 1</td>
<td></td>
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<tr>
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<td>.73</td>
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<td>.199</td>
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<td>Risk Category 5</td>
<td>2.08</td>
<td>1.03</td>
<td>4.21</td>
<td>.042</td>
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<td>CART Analysis</td>
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</tr>
<tr>
<td>(1) 0.083 0.981 0.159 &lt;0.001</td>
<td>3.61 2.09 6.23 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2) 0.477 0.666 0.056 &lt;0.001</td>
<td>2.33 1.76 3.10 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) 0.363 0.834 0.084 &lt;0.001</td>
<td>2.51 1.87 3.37 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Logistic regression with dyslipidemia as a function of the clinical screen controlling for age & sex.

PECR – Pediatric Expert Committee Recommendations

CART – classification and regression tree

CI – Confidence interval

PPV – positive predictive value

Sens – Sensitivity

Spec – Specificity

** variables: BMIZ, BMI, WC, WHtR, FMHx OB, FMHx DM, FMHx CVD, EBP, HTN, smoker, not sex, age, or risk factor count. Analysis of group b also included the presence of dyslipidemia.
On multivariate analysis, the PECR was significantly associated with approximately a 2-fold increased risk of having IFG (P<0.01) as compared to those with a negative screen. When risk categories were analyzed individually, only Category 5 (P<0.05) was associated with an increased risk of having IFG as compared to Category 1. This was true when ethnicity was included as risk factor and when it was not.

**Classification and Regression Tree Analysis**

Three CART analyses were run for (1) Group A, (2) Group B, and (3) then together. The models were then tested for sensitivity, specificity, and PPV. Univariate and multianalysis were run and reported in Table 5.3 for a comparison with the existing clinical screening algorithms. Because dyslipidemia was not a key node-splitting variable, pooled data were used in the analysis of each CART solution.

**CART analysis 1.** When Group A was included in the first CART analysis (Figure 5.1), there were four independent variables that had high normalized importance: BMI z-score, WHtR, BMI, and WC. A single node split the sample: BMI z-score >2.15 and 2.15 or less (Figure 5.1). This BMI z-score corresponded to a BMI percentile of 98.42%. The first CART screen was then applied to the entire sample and tested for sensitivity, specificity, and PPV.

**CART analysis 2.** In Group B, the five independent variables with the highest normalized importance were as follows: WC, female, HDL-C, WHtR, BMI z-score. A tree with four nodes was created. This was the second CART screen. In the second CART screen (Figure 5.2) the initial node split participants as male or female. Male
participants were then split by WC >88.5, which was an indication for screening. Female participants were split by BMI >25.33 and ≤ 25.33. In those in the lower weight category, it was recommended to screen those who had a BMI ≤ 19.30 kg/m². The sensitivity, specificity, and PPV were run on participants from the entire sample. Results reported in Table 5.3 for a comparison with existing clinical screening algorithms.

**CART analysis 3.** When Groups A and B were entered into the CART analysis together, the third CART screen was created (Figure 5.3). The variables with the highest normalized importance were WC, BMI, BMI z-score, sex, and WHtR. This tree had three nodes. The first node split participants according to a WC >90.5. In those with WC ≤ 90.5 and who were male, a BMI ≤ 17.92 was considered a screening indication. Results were reported for both univariate and multivariate analysis.
Results of the second classification and regression tree analysis.

<0.001 – Normal fasting glucose (< 100 mg/dL)
1.000 – Impaired fasting glucose (≥ 100 mg/dL)
Figure 5.2

Results of the second classification and regression tree analysis.

0.000 – Normal fasting glucose (< 100 mg/dL)
1.000 – Impaired fasting glucose (≥ 100 mg/dL)
Figure 5.3

Results of the third classification and regression tree analysis.

0.000 – Normal fasting glucose (< 100 mg/dL)
1.000 – Impaired fasting glucose (≥ 100 mg/dL)
Discussion

The current study is the first to screen for prediabetes using the PECR in a group of adolescents and young adults in Central Mexico. While a positive screen was associated with a 2-fold increase in the risk of prediabetes, as measured by IFG, the prevalence rates of prediabetes in this population was estimated to be low (4.0%). This was approximately half of rates measured in comparable populations in the United States. Exploratory CART analysis improved the sensitivity of the clinical screen by including waist circumference with BMI measurements in algorithms; as well as differentially screening male and female participants. Despite slight improvements and adequate specificity, the sensitivity of screens remained low, undoubtedly affected by low prevalence rates of the disease.

The Prevalence of CVD Risk Factors

Most of the participants in our sample were normal weight (76%). The remainder were either overweight (14.6%) or obese (9.4%). Obesity rates were similar to those previously found in representative Mexican samples of 20- to 29-year-olds (10.1% males and 15.3% females)(Acosta-Cázares & Escobedo-de la Peña, 2010). Even though most participants were normal weight, there was a high prevalence of having at least one T2DM risk factor (80%). In our participants, the most common risk factor was having a FMHx of T2DM. This was reported in 56.1% of participants. The next most common was risk factor was dyslipidemia, which affected 44.7% of participants in Group B. Comparing all risk factors, females were
more likely to report positive family histories, while males were more likely to have elevated blood pressure and hypertension. Previous studies with Mexican young adults have also found high rates of risk factors (Acosta-Cázares & Escobedo-de la Peña, 2010).

**Goal 1: Low Impaired Fasting Glucose Prevalence Rates**

In this population of adolescents and young adults from Central Mexico, overt IFG (fasting glucose ≥100 mg/dL) was not common and affected only 4.0% of participants. Rates were significantly higher in males (5.8%) as compared to females (2.3%). According to data from NHANCES 1999 to 2006, these rates were substantially lower than rates of IFG reported in a nationally representative sample of Mexican Americans (12- to 19-year-olds), which were 21.9% for males and 7.1% in females (Walker, Gurka, Oliver, Johns, & Deboer, 2010).

The prevalence of IFG increased significantly with increasing levels of adiposity. Prevalence rates were 3.2% in NW, 5.0% in OW, and 8.1% in OB participants. In the United States, rates of IFG are reported to be much higher: 12.3% in NW, 9.61% in OW, and 21.83% in OB 12- to 14-year-olds (Messiah, Arheart, Luke, Lipshultz, & Miller, 2008). Therefore, prevalence rates if IFG in the study sample were less than half of those seen in comparable samples from the United States.

**Goal 2: The PECR is Not a Sensitive Screen**

Regarding the PECR screen, a total of 15.1 to 23.8% of participants qualified as having a positive PECR screen. This would mean that approximately 1 in 5
participants would be recommended by their physician to have a fasting plasma glucose laboratory test. The prevalence of IFG among those with a positive PECR was 5.3% to 14.5% as compared to IFG prevalence rates of 2.5% to 7.3% in those with a negative screen. On multivariate analysis controlling for age and sex, having a positive clinical screen was associated with approximately a 2-fold increase in the risk of having IFG. This remained significant whether or not ethnicity or dyslipidemia were included as risk factors. The PECR screen was quite specific (>0.750), but suffered from low sensitivity (<0.400), indicating that many participants with IFG were not identified by the algorithm.

**Goal 3: The Challenge of Finding a Parsimonious Screen**

In an attempt to offer an improved algorithm for detecting IFG, three CART analyses were run on Group A, Group B, and Groups A+B. All variables were included as independent variables, with the exception of the first CART analysis, which did not include dyslipidemia as an independent variable. Three separate solutions were produced, all of which hinged upon BMI, BMI z-score, WC, and sex. The first CART solution described screening participants with BMI percentile scores >98.42th percentile. This solution was incredibly specific (0.981), but not sensitive (0.083). Improved sensitivity was found with analyses two and three, which had nodes separating the sexes and utilized both BMI and WC. These two solutions had improved sensitivities above the PECR recommendations. Notably, the third CART solution had the highest sensitivity of all screens (PECR and CART) while maintaining a specificity >0.80. This solution first split participants according to a
WC >90.5. In those with WC ≤ 90.5 and who were male, a BMI ≤ 17.92 kg/m² was considered to be a positive screening. This solution has a sensitivity of 0.363 and a specificity of 0.834. So even after exploratory analysis, sensitivity still remained <0.50 for all solutions. One screening solution devised by researchers in Germany found that in prepubertal obese (BMI ≥ 90th percentile) children, measuring parental history of diabetes, pubertal stage, and extreme obesity detected almost 90% of children in the sample with prediabetes. Specificity, however, was reduced (~0.50) with this approach (Reinehr et al., 2009). To our knowledge, the current study is the first that has looked at sensitivity and specificity of screening techniques in NW, OW, and OB young adult participants.

Two of the three CART analyses utilized both WC with BMI to separate participants with high and low rates of IFG. There has been continued debate over the utility of these anthropometric measures in predicting IFG. In Mexican samples, WC has been particularly good at discriminating risk of T2DM more so than BMI (D’Adamo, Santoro, & Caprio, 2009; Guerrero-Romero, Rodríguez-Morán, Pérez-Fuentes, & Sánchez-Guillén, 2008; Lorenzo, Williams, & Gonzalez-Villalpando, 2005; Sánchez-Castillo et al., 2005; Sánchez-Viveros, Barquera, Medina-Solis, Velázquez-Alva, & Valdez, 2008). In children, frank T2DM is rare, but WC has also been associated with insulin resistance and the syndrome of insulin resistance (Cossio, Messiah, Garibay-Bieto et al 2009 [Hispanic children]; Fernández et al., 2004; Flodmark, Sveger, Nilsson-Ehle, 1994; Freedman, Serdula, Srinivasan, et al., 1999; Kahn, Imperatore, Cheng 2005). This holds true even within a given BMI category
(Janssen et al., 2005). Waist circumference in childhood also predicts cardiometabolic derangements of insulin resistance decades later (Sun, et al., 2008).

Continuous BMI z-score has also been associated with insulin resistance both crosssectionally (Bell et al., 2007) and longitudinally. In longitudinal studies, childhood BMI predicts insulin resistance and T2DM in adulthood (Morrison, Glueck, Horn, & Wang, 2010; Steinberger, Moran, & Hong, 2001; Sun et al., 2008). The specific thresholds at which BMI or BMI z-score becomes significant have not always been clear. For instance, the first CART analysis suggested a cutoff of >98.42 percentile be used for testing PFG. This was similar to what was seen by Skinner and colleagues (2009) who found that HbA1c levels (a measure of elevated blood glucose) increased around the 99th BMI percentile (Skinner, Mayer, Flower, Perrin, & Weinberger, 2009). While these high thresholds are certainly specific, our finding was that it was not sensitive. Previous studies examining 16- to 19-year old Hispanics cite that a BMI cutoff of 24.1-24.7 kg/m² is ideal for detecting an increase in CVD risk factors such as insulin resistance (Messiah, Arheart, Lipshultz, & Miller, 2008). Similarly, Sanchez-Castillo and colleagues (2005) reported that over half of all adult T2DM cases are attributable to Mexicans having a BMI≥23 kg/m². This is similar to our findings in the second CART analysis, where female participants were stratified by those with a BMI >25.33. The same CART split males by WC. This could open up the possibility that sex differences may exist in Mexican young adults between the efficacy of different anthroprometric measurements in detecting IFG.

It is interesting to note that the latter two CART solutions did not only recommend screening in participants with high BMI and WC values. The analysis
also recommended screening individuals with low BMIs, such as those with BMI $\leq 17.92$ kg/m$^2$. This appears to be a unique finding. Bell and colleagues (2007) did not measure PFG, but did measure insulin and found a u-shaped curve in the log of 120 min post-prandial insulin versus the BMI z-score of adolescents (Bell et al., 2007). Another study conducted by González-Barranco and colleagues (2003) in Mexico documents the metabolic consequences of the rapid nutritional transition in Mexico. In this study, glucose, insulin, TG, HDL, and BMI were measured in cases of adult males with documented malnutrition in their first year of life and compared them to levels of control subjects who did not experience malnutrition. Findings were that the area under the curve for glucose and insulin in relation to BMI was significantly different in cases versus controls. Of course, the finding of high rates of IFG in those of low weight may not be pathological, but, rather physiological. Pubetal growth, can temporarily decrease adiposity as linear growth ensues and the hormonal milieu can cause physiological insulin resistance. While this is one possible solution, it must be noted that this process usually occurs during early adolescents and is completed by late adolescents (Caprio et al., 1989).

The study had several limitations. The greatest limitation was that the sample was not representative of the young adult population of Mexico. Therefore, results may not be generalizable to the population as a whole. The second limitation was that lipid and lipoprotein data were only available on a subset of participants. The full sample, however, was quite large and allowed for three separate CART analyses. Suitable CART screening solutions could be made with pooled data regardless of fasting lipid profiles, because measures of dyslipidemia were not key
node-splitting variables in any of the solutions. The study could be vastly improved with the completion of fasting lipid profiles and also with the inclusion of fasting insulin levels. Having fasting insulin levels would allow for the calculation of HOMA-IR. Current screening recommendations are for assessing fasting glucose only (ADA, 2000; Barlow et al., 2007). However, in children, adolescents, and young adults, glucose levels are often normal in the setting of severe insulin resistance and rising insulin levels (Cali & Caprio, 2008). This is possibly due to the resilience of the adolescent physiology in overcoming high blood glucose levels through the production of increasing amounts of insulin. Once glucose is seen rising in the fasting state, it is late in the course of disease and prediabetes and resultant risk of CVD has already been initiated.

**Conclusions**

Hispanics are especially prone to insulin resistance and the development of T2DM. This population develops the disease at higher rates, at younger ages, at lower levels of adiposity, and with more severe consequences than do non-Hispanic white counterparts. Therefore, a screening program in Mexico is justified. Theoretically, the earlier the disease is identified, the sooner intervention could begin, and the less severe the CVD consequences of T2DM would be. This is the theory but may not be practical. The current study sought to screen for prediabetes in a group of adolescent and young adult Mexicans in Central Mexico using the PECR. A good screen must be able to accurately identify a serious condition that is not only treatable, but has a long, asymptomatic latency period. In addition, the
condition must be sufficiently common and serious to justify the cost of screening. Diabetes is undoubtedly a serious, and treatable, condition with a long asymptomatic period of insulin resistance and prediabetes. However, in this population of young adults, the prevalence of IFG may be too low to recommend screening. Further studies are needed of screening strategies in Mexico.
CHAPTER 6: CONCLUSIONS

Cardiovascular disease (CVD) is now the leading cause of death in Mexico (Stevens et al., 2008) and over a quarter of these deaths are in people under the age of 65-years-old (Secretaría de Salud, 2010). The Mexican ethnicity has been associated with high rates of cardiovascular risk factors such as nonalcoholic fatty liver disease (NAFLD), dyslipidemia, and prediabetes. Mexicans get these cardiometabolic derangements at younger ages and at lower levels of adiposity than do non-Hispanic whites or non-Hispanic blacks (Sanchez-Castillo et al., 2005; Umpierrez, et al., 2007).

In Mexico, screening for these diseases is poor and an estimated 72.1% of those with dyslipidemia are unaware of it (Acosta-Cázares & Escobedo-de la Peña, 2010). Without proper screening and preventative care, the risk for CVD increases. Therefore, studies on regional screening strategies are urgently needed. In 2007, the Pediatric Expert Committee published a screening protocol that would allow clinicians to use a single, unified algorithm for testing young patients for NAFLD, dyslipidemia, and prediabetes. This algorithm would provide early identification of covert disease, and could potentially be utilized by clinicians performing the required health screen for college admission.

The PECR clinical screening algorithm consists of five Risk Categories (Table 6.1). It is recommended that all patients in Risk Category 4 and 5 undergo laboratory assessment for fasting glucose and liver enzymes. This includes all OB individuals and those who are OW with two or more additional risk factors (e.g. FMHx of obesity-related diseases, elevated blood pressure, smoking). Fasting lipids
are recommended for all patients in Risk Category 2 through 5. This includes all OB and OW individuals and NW patients with a family history of cardiovascular disease (Krebs et al., 2007). This screening algorithm would be a useful clinical tool in Mexico, where the young adult population is burdened with a high prevalence of cardiovascular disease risk factors. Therefore, we sought to test the PECR screening algorithm in adolescents and young adults from Central Mexico.

Table 6.1

*Complete Algorithm for laboratory evaluation from Barlow & The Expert Committee (2007).*

<table>
<thead>
<tr>
<th>Risk Categories</th>
<th>Risk Factors</th>
<th>Recommended Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal weight (≤85th %ile)</td>
<td>0</td>
<td>BP</td>
</tr>
<tr>
<td>2) Normal weight (≤85th %ile)</td>
<td>1*</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>3) Overweight (&gt;85th – 94th %ile)</td>
<td>&lt;2**</td>
<td>BP, fasting lipids</td>
</tr>
<tr>
<td>4) Overweight (&gt;85th – 94th %ile)</td>
<td>≥2**</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT</td>
</tr>
<tr>
<td>5) Obese (≥95th %ile)</td>
<td>≥0</td>
<td>BP, fasting lipids, fasting glucose, AST, ALT (microalbumin/creatinine ratio)</td>
</tr>
</tbody>
</table>

BP – Blood pressure

* Family Medical History of high cholesterol

** Risk Factors: positive family medical history for weight-related diseases (obesity, T2DM, cardiovascular disease), ethnic minority (African, Hispanic), elevated BP, dyslipidemia, tobacco use, and signs of insulin resistance.

**Brief Review of The Three Analyses**

Three separate analyses were conducted for each laboratory test, which included liver enzymes, fasting lipids, and fasting glucose. These laboratory tests were for the detection of NALFD, dyslipidemia, and prediabetes, respectively. The goals of each analysis were the same, but the samples sizes differed. In the UP AMIGOS project, the total sample consisted of 9,974 people. Only those 18- to 21-
were chosen for analysis. Fasting glucose was performed on most participants in this age range (n = 5455). Fasting lipids were run on a random subset of individuals (n = 829). This subset was younger and was more likely to be female as compared to the sample as whole. Liver enzymes were analyzed on select individuals with complete data and who were matched by age, sex, BMI, and height (n = 63). The risk factor variables used in the PECR algorithm included BMI percentile, a FMHx of obesity-related diseases (obesity, CVD, T2DM), elevated blood pressure (BP ≥ 130/85 mmHg), Hispanic ethnicity, and tobacco use. The five Risk Categories were coded using BMI percentile and the sum of risk factors.

Each analysis had three goals. The first was to test for the relative prevalence of cardiometabolic derangements in NW, OW, and OB individuals. The second goal was to test the ability of the PECR clinical screen to identify participants with occult cardiometabolic derangements. The epidemiological parameters of sensitivity, specificity, and positive predictive value were used, as well as univariate and multivariate tests of significance. In some cases, variations of the PECR recommendations were tested, such as the inclusion or exclusion of Hispanic ethnicity as a risk factor. In other cases, the PECR algorithm was compared against other published screening recommendations. Finally, the third goal of each analysis was to explore ways in which to improve the PECR screening algorithm. Where power was sufficient (i.e. testing for dyslipidemia and prediabetes), a CART analysis was used for exploration. Where power was insufficient (i.e. elevated liver enzymes), explorations were performed manually.
Risk Factor Descriptive Analysis

Risk factors were present in a majority of participants (86.2%). Using data from Chapter 5, which had the largest and most representative sample, a majority of participants were NW (76.0%), a smaller percentage were OW (14.6%), or OB (9.4%). Rates of overweight and obesity in the study sample were similar to those previously found in representative Mexican samples of 10- to 17-year-olds, which reported rates of overweight to be 10.8-19.1% and rates of obesity to be 6.8-14.7% depending on region of Mexico (del Rio-Navarro, et al., 2004).

FMHx. A few participants reported their parents as being obese (2.8%); but over one half reported a FMHx of DM (56.1%), and a third reported having a FMHx of CVD (30.9%). It is unclear how accurate the FMHx reports of adolescents and young adults were, but it is likely that having a FMHx of obesity was underreported. The adult prevalence of obesity in the Mexican population was estimated to be 30%, while only 2.8% of participants reported that they had an obese parent (Olaiz, et al. 2003 as cited by Garcia-Garcia, et al., 2006). This suggests that youth-reported parental weight status may not be a valid measure of FMHx of obesity.

Blood Pressure. Males in our study had significantly higher rates of two risk factors: elevated blood pressure and smoking. In regards to blood pressure, a total of 8.7% males had EBP (BP ≥130/≥85 mmHg), while 2.0% of females had this condition. Diagnosable hypertension (BP ≥140/≥90 mmHg) was present in 6.0% of males and 1.4% of females (P<0.001). These rates were substantially lower than those reported in a representative sample of 20- to 29-year-olds enrolled in The Institute of Mexican Social Security (IMSS) (Acosta-Cázares & Escobedo-de la Peña,
In the aforementioned sample, hypertension (≥140/≥90) rates were 11.6% and 9.4% for males and females, respectively.

**Smoking.** The same IMSS study reported smoking rates that were over three times higher in males versus females (38.5% vs. 12.7%) in 20- to 29-year-olds. Similarly, in our sample, male tobacco-users were twice as prevalent as female users (21.5% vs. 10.4%). Rates were highest in 21-year-olds (26.9%) and lowest in 18-year-olds (12.2%). Smoking is a modifiable risk factor for CVD, yet rates of smoking in Mexico remain higher than rates U.S., and this includes rates among Mexican Americans (Acosta-Cázares & Escobedo-de la Peña, 2010). Therefore, in regards to personal risk factors, males were more likely to smoke and have high blood pressure. Comparing our study participants to representative Mexican samples of a similar age, rates of overweight and obesity and smoking were comparable, but our sample had lower rates of hypertension.

**GOAL 1: Establishing the Prevalence of Cardiometabolic Abnormalities**

The first goal of each analysis was to establish the prevalence of elevated liver enzymes, dyslipidemia, and impaired fasting glucose in the NW, OW, and OB BMI percentile categories (Table 6.2). The studies with sufficient power demonstrated significant associations between BMI percentile categories and cardiometabolic derangements. The most common cardiometabolic abnormality in all BMI percentile categories was dyslipidemia. This is consistent with previous research (Aguilar-Salinas et al., 2009), however, results should be interpreted with caution because the Cook et al., 2003 definition of abnormal TG (TG ≥ 100 mg/dL)
was used. This definition has a threshold in ranges that are considered normal by other definitions and its validity not been tested for longitudinal associations with adverse outcomes.

Table 6.2

*Relative Prevalence of elevated liver enzymes, dyslipidemia, and impaired fasting glucose in normal weight, overweight, and obese participants.*

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Total</th>
<th>NW</th>
<th>OW</th>
<th>OB</th>
<th>(\chi^2) test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated Liver Enzymes</td>
<td>63</td>
<td>25.4*</td>
<td>17.1</td>
<td>29.4</td>
<td>45.5</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>829</td>
<td>44.8</td>
<td>38.8</td>
<td>59.1</td>
<td>67.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Impaired Fasting Glucose</td>
<td>5455</td>
<td>4.0</td>
<td>3.2</td>
<td>5.0</td>
<td>8.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Note: elevated liver enzymes defined as ALT>56 U/L and/or AST>40 U/L; dyslipidemia was defined as TC ≥ 200 mg/dL, LDL-C ≥ 130 mg/dL, TG ≥ 110 mg/dL, and HDL-C ≤ 40 mg/dL; and impaired fasting glucose was defined as fasting glucose ≥ 100 mg/dL.

NW – Normal weight (BMI < 85th percentile)

OW – Overweight (BMI 85th to 94.9th percentile)

OB – Obesity (BMI ≥ 95th percentile)

*Number not representative of the sample population because it was a matched cohort.

**Elevated liver enzymes**, which were defined as ALT>56 U/L and/or AST>40 U/L, were present in 17.1%, 29.4%, and 45.5% of NW, OW, and OB individuals respectively. Most epidemiological studies that have been conducted previously rely solely upon ALT measurements as an indicator of liver disease and often use various cutoffs. One such study in the United States found that 6.1% of Mexican Americans (Fraser, Longnecker, & Lawlor, 2007) had ALT > 40 U/L, which was similar to rates seen in our NW participants (8.6%), but only a fraction of rates in the OW (17.6%) and OB (36.4%). Rates seen in our OB participants were comparable to the 42%
ALT>40 U/L seen in OW and OB elementary school children from Mexico City (Flores-Calderon, et al., 2005). Regardless of the definition, rates of elevated liver enzymes in OB participants were 2- to 4-times higher than rates seen in NW. Were this to be extrapolated to the population of Mexico, there is a possibility that rates of NAFLD may be higher in Mexico compared to the United States.

**Dyslipidemia** as defined with a hybrid definition was present in 44.8% of participants overall and 38.8%, 59.1%, and 67.5% of NW, OW, and OB participants. Hypertriglyceridemia was the most common lipid abnormality and rates of TG≥110 mg/dL were almost double those seen in 12- to 18-year-olds taking part in the 1999 to 2002 NHANES data (Kranz Mahood, Wagstaff, 2007). Ethnic discrepancies in hypertriglyceridemia between Mexicans and NHW have been shown to persist even after controlling for diet, physical activity, education, and income (Walker, Gurka, Oliver, Johns, & Deboer, 2010). In regards to low HDL, our sample had comparable rates to NHANES (1999-2002) data on 12- to 18-year-olds (Kranz Mahood, & Wagstaff, 2007). The higher rates of dyslipidemia in Mexican samples as compared to samples of different ethnicities is consistent with previous research (Carroll, Lacher, Sorlie et al., 2005; de Ferranti, et al., 2004; Johnson, Kroon, Greenway et al 2009; Harel, Riggs, Vaz, Flanagan, & Harel, 2010; López-Capapé, et al., 2006).

**Impaired fasting glucose.** In this sample of adolescents and young adults from Central Mexico, overt IFG (fasting glucose ≥100 mg/dL) was not common and affected 4.0% of participants. Rates were highest in OB (8.1%) and were lower in the OW (5.0%) and NW (4.0%) participants. All IFG rates were substantially lower than those reported in Mexican Americans (12- to 19-years-old), which were 21.9%
for males and 7.1% in females (Walker, Gurka, Oliver, Johns, & Deboer, 2010). When U.S. data were reported by BMI percentile category, rates were 21.8% in OB and 12.3% in NW 12- to 14-year-olds (Messiah, Arheart, Luke, Lipshultz, & Miller, 2008). Therefore, prevalence rates if IFG in the study sample were under half of those seen in comparable samples from the United States. This is in contrast to previous research, looking at cross-country comparisons. Such studies have found that Mexicans had higher prevalence rates of T2DM at younger ages and lower BMI and WC values than did Americans (Sanchez-Castillo, et al., 2005). When the U.S. population was split by ethnicity and subsequently compared to a representative sample in Mexico, the prevalence of T2DM in those aged 20 to 60 was highest in Mexicans compared Mexican Americans and NHW, despite higher rates of obesity in the U.S. population (Acosta-Cázares & Escobedo-de la Peña, 2010). Therefore, our sample has unexpectedly low rates of IFG than would be expected from previous research.

When the cardiometabolic profiles of study participants were compared to national Mexican and United States data, it was found that prevalence rates of NAFLD were slightly higher in our participants than those seen in Mexican Americans, while rates of hypertriglyceridemia were over twice those seen in the U.S. Unexpectedly, rates of IFG were much higher in samples from the United States than rates in our study sample.
GOAL 2: Testing the PECR Recommendations

The second goal of each analysis was to test the ability of the PECR recommendations to accurately detect participants with occult cardiometabolic derangements. Sensitivity, specificity, and positive predictive values were used to determine the utility of the PECR as a clinical screen. Univariate and multivariate analyses were performed to test for statistical differences in cardiometabolic outcomes between those with a positive and negative PECR screen and between those within each PECR Risk Category (Table 6.3).

Table 6.3
Univariate and multivariate statistics for the PECR clinical screening algorithm in testing for elevated liver enzymes, dyslipidemia, and impaired fasting glucose.

<table>
<thead>
<tr>
<th>Clinical Screen</th>
<th>sens</th>
<th>spec</th>
<th>PPV</th>
<th>χ² test</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elevated liver enzymes</strong></td>
<td></td>
<td></td>
<td></td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.625</td>
<td>0.787</td>
<td>0.500</td>
<td>0.004</td>
<td>6.17</td>
</tr>
<tr>
<td>PECR screen (ethnicity)</td>
<td>0.625</td>
<td>0.660</td>
<td>0.385</td>
<td>0.076</td>
<td>3.23</td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR-Barlow</td>
<td>0.356</td>
<td>0.825</td>
<td>0.623</td>
<td>0.000</td>
<td>2.59</td>
</tr>
<tr>
<td>PECR-Krebs</td>
<td>0.561</td>
<td>0.554</td>
<td>0.504</td>
<td>0.001</td>
<td>1.61</td>
</tr>
<tr>
<td><strong>Impaired Fasting Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group a no cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.283</td>
<td>0.854</td>
<td>0.059</td>
<td>0.000</td>
<td>2.21</td>
</tr>
<tr>
<td>PECR screen (ethnicity)</td>
<td>0.355</td>
<td>0.798</td>
<td>0.053</td>
<td>0.000</td>
<td>2.15</td>
</tr>
<tr>
<td>group b cholesterol incl.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECR screen (no ethnicity)</td>
<td>0.319</td>
<td>0.820</td>
<td>0.145</td>
<td>0.007</td>
<td>2.16</td>
</tr>
<tr>
<td>PECR screen (ethnicity)</td>
<td>0.361</td>
<td>0.774</td>
<td>0.132</td>
<td>0.014</td>
<td>1.96</td>
</tr>
</tbody>
</table>

Note: Logistic regression with dyslipidemia as a function of the clinical screen controlling for age and sex for dyslipidemia and impaired fasting glucose. No controls were entered for elevated liver enzymes.

PECR – Pediatric Expert Committee Recommendations
CART – classification and regression tree
Overall, the PECR had the best performance in detecting abnormal liver enzymes and the poorest in detecting IFG. Across all cardiometabolic diseases, the PECR had suboptimal sensitivity. Sensitivity was often poor suggesting that individuals with occult disease were not identified by the screen. The PECR was, however, quite specific. This would mean that individuals with a negative screen could rest easy in knowing that they have a low likelihood of disease. Low sensitivity could be easily explained for IFG given the low prevalence of disease, but the same cannot be said of dyslipidemia, which affected almost half of the population. Thus, further variations of the screen and alternative algorithms were explored.

**PECR Variations.** According to the PECR, those in Risk Category 4 (OW with two or more risk factors) and those in Risk Category 5 (OB) have a positive clinical screen for elevated liver enzymes and impaired fasting glucose. Risk factors were considered to be FMHx variables, elevated blood pressure, a positive screen for dyslipidemia, Hispanic ethnicity, and smoking. It was believed to be clinically cumbersome to have a laboratory test (i.e. dyslipidemia) as a prerequisite for another laboratory test, and so the PECR was tested both with and without including dyslipidemia. Overall, the result was a slight increase in sensitivity and decrease in specificity.
The PECR recommendation was also tested with and without including Hispanic ethnicity as a risk factor. The effect of including ethnicity as a risk factor in this population was a reduction in specificity and PPV with modest increases in sensitivity. Statistically significant associations between the disease and the screen often disappeared or diminished with the inclusion of ethnicity, suggesting that it should not be included in screens within Mexico. Within the United States, being of Hispanic ethnicity is a risk factor for CVD and T2DM (Krebs, et al., 2007). While the same risk may be conferred to the population of Mexico as a whole, the variable of ethnicity does not alter improve the predictive value of the PECR for this population.

The screen for dyslipidemia had two variations found within the published PECR themselves. One version, PECR-Barlow, tested all OW and OB participants for dyslipidemia. The second version, PECR-Krebs, also included participants of NW with a positive FMHx of CVD. The PECR-Krebs screen was more sensitive in that it included more individuals for screening and therefore “caught” more cases of dyslipidemia, but at the cost of testing many participants who did not have the condition. On multivariate analysis the PECR-Barlow screen had a stronger association with dyslipidemia than did the PECR-Krebs screen.

Other recommendations. In certain cases, the PECR was not the only published screening recommendation. Notably, evidence-based clinical screening recommendations for adolescents and young adults have been published for dyslipidemia for nearly a decade. Therefore, it was felt to be important to compare the PECR to other clinical screening recommendations. In comparison, PECR performed well and was comparable to recommendations by the American
Academy of Pediatrics. The recommendations most strongly associated with
dyslipidemia, were recommendations that relied solely upon BMI percentile and did
not include other risk factors. The recommendations that did include other risk
factors tended to be more sensitive and less specific. In all of the recommendations,
however, sensitivity was incredibly low, and over half of dyslipidemia cases were
missed by all screens. An exploratory analysis was then performed to test for ways
in which to improve the screen.

**GOAL 3: Exploration of Alternative Algorithms**

The third goal of each analysis was to explore alternative solutions to the
PECR using the same risk factors, but with the inclusion of WC, WHtR, and BMI.
Because of a lack of power, exploratory analysis was performed manually for
elevated liver enzymes; and a CART analysis was used for dyslipidemia and IFG.

**Elevated Liver Enzymes.** A manual exploration of risk factor combinations
revealed a clinical screen that was 0.813 sensitive and 0.740 specific for elevated
liver enzymes. The proposed protocol was to pick two: EBP (≥130/85 mmHg), a
FMHx of either CVD or DM, a BMI ≥25. If two or more were present, then the clinical
screen was considered positive. This screen may be more clinically feasible than the
Krebs algorithm in that it does not require a previous cholesterol measurement; nor
for the physician to plot BMI percentile, a practice that few physicians routinely do
(Barlow, Bobra, Elliott, Brownson, & Haire-Joshu, 2007). Larger studies are needed
to confirm these results.
**Dyslipidemia.** A CART analysis was performed for dyslipidemia and compared to existing screening recommendations. The CART solution was to screen all participants with a BMI >23.33. This solution was preferable to existing recommendations on the basis of sensitivity, specificity, and β. Other studies in Mexico have cited a BMI ≥ 23 kg/m² to be the point at which CVD risk factors begin to emerge (Sanchez-Castillo, et al., 2005). Despite the CART analysis offering the best solution of all previous recommendations, it still failed to identify 40.3% of those with dyslipidemia.

**Impaired Fasting Glucose.** In order to explore alternative screening solutions for IFG, three separate CART analyses were run in patients with available data on fasting lipids, patients without such data, and both. The results were heterogeneous but hinged largely upon the anthropometric variables of BMI, BMI percentile, and WC. The two CART screening solutions demonstrating superior epidemiological parameters utilized WC in addition to BMI and also recommended testing in those with low and high BMI values. For instance, one of the solutions suggested screening females with a BMI >25.33 or ≤19.3, but used a WC of >88.5 cm as indication for male screening. The other suggested screening all participants with a WC >90.5 cm and all male participants with a BMI ≤ 17.9. While sensitivity was improved with these two CART screening solutions, even the most sensitive solution failed to identify 52.3% of those with IFG. The inclusion of low BMI values as an indication for a screen was unexpected and could not be easily explained. These results could be due to lingering physiological insulin resistance of puberty or a response to undernutrition. The current results are in stark contrast to similar
studies performed in the U.S., which reported that screening for IFG should ideally be performed in participants with a BMI percentile in the 99th percentile or higher (Skinner, Mayer, Flower, Perrin, & Weinberger, 2009). Based on our data, this approach would have little to no sensitivity, but maximum specificity.

Exploratory analysis revealed that in this population of 18- to 21-year-olds, BMI was more predictive of the three cardiometabolic derangements than was BMI percentile. This is perhaps not surprising given that this age range is in the upper limits of BMI percentile curves, which, in the 2000 CDC version, do not align with adult cutoffs for overweight and obesity (Lobstein, Baur, & Uauy 2004). The inclusion of risk factors appeared to add little value to dyslipidemia screening protocols, while the risk factors of FMHx and EBP improved the sensitivity and specificity of a screen for elevated liver enzymes. In exploring IFG screens, WC served as an adjunct to BMI and its inclusion, improved specificity. However, even after epidemiological parameters were improved with exploratory solutions, 40.3% of those with dyslipidemia and 52.3% of those with IFG remained unidentified. For a summary of the three studies see Figure 6.1.

**Study Strengths & Limitations**

The study had several limitations. The greatest limitation was that the sample was not representative of the young adult population of Mexico. Therefore, results may not be generalizable to the population as a whole. The second limitation was that lipid and lipoprotein data and liver enzymes were only available on a subset of participants.
The study could be improved with the completion of fasting lipid profiles, liver enzymes, and, also with the inclusion of fasting insulin levels. Having fasting insulin levels would allow for the calculation of HOMA-IR. Current screening recommendations are for assessing fasting glucose only (ADA, 2000; Barlow et al.,
2007). However, in children, adolescents, and young adults, glucose levels are often normal in the setting of severe insulin resistance and rising insulin levels (Cali & Caprio, 2008). This is possibly due to the resilience of the adolescent physiology in overcoming high blood glucose levels through the production of increasing amounts of insulin. Once glucose is seen rising in the fasting state, it is late in the course of disease: Prediabetes and resultant risk of CVD has already been initiated.

A study strength of the study was its sample size and matching when sample size was small. This allowed for adequate power to detect significant differences in the sample, even when prevalence rates of diseases were low. The other strength was the richness of the data, which included questionnaire variables,
anthropometric variables, and blood biomarkers. Data for young adults from developing countries are scarce. Additionally, screening protocols originating from developed countries are rarely cross-validated in the developing world (Delgado-Noguera, Tort, Bonfill, Gich, & Alonso-Coello, 2009). Therefore, this study represents a unique glance into this understudied population.

**Conclusions**

After describing the prevalence of cardiometabolic abnormalities, testing the PECR, and exploring alternative solutions, we have found that dyslipidemia and NAFLD were very prevalent in 18- to 21-year-olds from Central Mexico. IFG was rare, affecting less than 5%. When screening for these conditions, the PECR screen was found to be a clinically feasible and statistically valid way to test for three different CVD risk factors using the same algorithm. While the PECR does have the sensitivity to detect all of those with occult disease, it has high specificity allowing clinicians to confidently proceed with patients who have a negative clinical screen.

Exploratory analysis revealed additional, alternative screening solutions. In summary, these solutions require clinicians to measure BMI, WC, BP, and assess for FMHx. If BMI ≥23 kg/m², test cholesterol; if BMI ≥ 25 kg/m² or WC >88.5 cm test fasting glucose. If two or more risk factors are present (BMI ≥ 25 kg/m² or EBP or FMHx of CVD/DM) then test liver enzymes. This solution is slightly more cumbersome than the PECR in that it requires different parameters for each disease. The tradeoff would be an improvement in sensitivity and case detection. Despite being statistically significant, this proposed screen would fail to identify approximately half of those with dyslipidemia and IFG. The prevalence of IFG in this
population is low (4.0%), and, therefore, this might be acceptable. However, the prevalence of dyslipidemia is high (44.8%) and such a screen would equate to approximately 18.0% of the total population having unidentified dyslipidemia despite undergoing a clinical screen. In light of this, one must consider implementing universal cholesterol screening in college applicants in Mexico. Universal screening has been debated for many years (McNeal et al., 2010), and has already been recommended in those 20 and older (NCEP, 2002).

**Final Recommendations for Screening**

In order to be a valuable screening test, the PECR must ultimately fulfill the expectations outlined in Table 6.4. Applying these expectations to our study population, the condition of dyslipidemia meets all criteria. As for elevated liver enzymes, knowledge about the progression of NAFLD, treatment, and the accuracy of screening tests is still in its infancy. Once the clinical impact of NALFD is more thoroughly understood, if screening were to be justified, the PECR would represent a valuable clinical screen. In regards to IFG, this condition is simply not common enough to justify screening young adults aged 18 to 21. Furthermore, there is insufficient evidence to suggest that screening asymptomatic individuals reduces clinical outcomes (USPTF, 2008). Theoretically, however, the lifestyle treatments for dyslipidemia would also improve liver pathology, and IFG. Therefore, it is recommended only that dyslipidemia screening be implemented in this population. Universal screening for dyslipidemia would be strongly recommended unless a more sensitive and specific clinical screen could be found.
Table 6.4

Criteria of a valuable screening test.

<table>
<thead>
<tr>
<th>Condition Description</th>
<th>NALFD</th>
<th>Dyslip</th>
<th>IFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>The screen must be able to accurately identify a condition of interest.</td>
<td>?</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>The condition of interest is serious</td>
<td>?</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>The condition of interest is treatable</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>The condition of interest has a long latency period</td>
<td>?</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>The condition is sufficiently common to justify the cost of screening</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Dyslip – dyslipidemia
IFG – impaired fasting glucose
NALFD – non alcoholic fatty liver disease
X – indicates criteria is met
? – indicated that there is insufficient evidence

Future Directions

Further research into screening protocols are clearly needed as patterns of disease in Mexico are not identical to those in the United States, where most recommendations originate. Future studies in college applicants should expand the screening protocol to include a diet and physical activity assessment (Barlow et al., 2007). A diet and physical activity assessment may be a good adjunct to the current PECR screening protocol. There should also be documented follow-up of those screened. This is especially important for NALFD, which is an understudied disease entity that affects Hispanics at a disproportionate rate. Research questions include:

Does AST and ALT correspond to percent hepatic triglycerides on MRS? What AST and ALT values are reasonable cutoffs for identifying hepatic triglycerides >5% in this
Is biopsy necessary for staging and treatment? Regarding blood glucose levels, there is currently insufficient evidence to suggest that early identification of prediabetes affects clinical outcomes (USPTF, 2008). Longitudinal follow-up of those with IFG, would, therefore, be a valuable addition to the pool of research. Finally, we have explored the PECR screening protocol. Treatment and follow-up recommendations were also published in the PECR. These recommendations were not addressed by this study. A future study could be a RCT assigning participants to the PECR treatment arm, and those with standard follow-up, measuring longitudinal clinical outcomes such as change in BMI and/or disease regression. Many possibilities exist, but the emphasis should be on improving clinical outcomes for Mexico, while recognizing the limited resources available in this country. After all, Mexico already suffers a high burden of CVD. As obesity rates in youth continue to rise, more research efforts are needed to avert the incoming tsunami.
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APPENDIX A: 2009 QUESTIONNAIRE

The 2009 UP AMIGOS Questionnaire in its translated English form is available as a supplementary document.