RISK FACTORS OF TYPE 2 DIABETES IN MEXICAN AND U.S. PIMA INDIANS: ROLE OF ENVIRONMENT

By

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A Dissertation Submitted to the Faculty of the

DIVISION OF EPIDEMIOLOGY AND BIOSTATISTICS (GRADUATE)
UA ZUCKERMAN COLLEGE OF PUBLIC HEALTH

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN EPIDEMIOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2010

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we read the dissertation prepared by Julian Esparza-Romero

entitled Risk Factors of Type 2 Diabetes in Mexican and U.S. Pima Indians: Role of Environment

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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ACKNOWLEDGEMENTS

I would like to thanks to My Committee members, Professor Eyal Shahar, MD, MPH, Professor Antonio Estrada, PhD, and Professor M. Elena Martinez, PhD, MPH, for their continuous support and constructive insights. I also wish to thank the members of the Maycoba Pima Community and the Gila River Indian Community for their participation in these studies. Our gratitude goes to Bertha Isabel Pacheco, Ana Cristina Gallegos and Hortensia Montesinos for data collection in Mexico and the staff of the Phoenix Epidemiology and Clinical Research Branch (NIDDK) for data collection in Sacaton, Arizona. Special thanks are extended to Nurse Eremita Perez Ruiz for facilitating work with our Mexican communities.

I would like to gratefully Dr. Mauro E. Valencia, Dr. Peter H. Bennett, Dr. Leslie O. Schulz, and Dr. Eric Ravussin for their collaboration in the critical review of two out of three papers included in this dissertation as well as for allows me to use their Pima Indians information as part of my dissertation. I am also indebted to Mirna Ruiz for helping me with the edition of the literature review. Thanks to Samantha Sabo for helping me with the edition of the second paper (Appendix 3). Many thank to Sonia Medina for helping me with the edition of my pre-doctoral proposal. Thanks to Chris Tish for helping me with scholarship received through the Mel and Enid College of Public Health. Many thank to Dr. Maria Teresa Velez for helping me during my PhD program. Many thanks also to all my Professors.

I am also grateful to CONACYT for the scholarship granted; To CIAD and specially the Nutrition Division to allow me to return to my work and continue doing my

dissertation work from Mexico. Many thank to Dr. Inocencio Higuera Ciapara for his support to get the CONACYT scholarship. Thank to Dr. Alfonso Gardea Bejar, Dr. Ramon Pacheco Aguilar and Dr. Heliodoro Aleman Mateo for their support to complete my dissertation.

Finally, many thank to Angelica Espinosa Plascencia, my wife for your love and unconditional support no matter how many miles separated us during most of the time in Tucson; to my children Priscila and Julian; to my parents, grandparents, brothers and sisters for staying by my side always.

The study in Mexico was supported partially by grant number NIH-DK-49957. Publications contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIH.

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ABSTRACT

Introduction. Pima Indians living in the United States (U.S.) have the highest prevalence of type 2 diabetes mellitus in the world. Their Mexican counterparts, living a traditional lifestyle in the mountain of Sonora, Mexico, have at least five times less diabetes than the U.S. Pima Indians. The effects of a traditional lifestyle in reducing type 2 diabetes risk factors and the association of factors to type 2 diabetes were evaluated in a sample of 1211 genetically related Pima Indians living different lifestyles (224 from Mexico and 887 from U.S.). Subsets of these populations were used to address specific questions. First, differences in insulin resistance between subjects with normal glucose tolerance (194 Mexican *versus* 449 U.S. Pima) were evaluated. Second, the effect of physical activity and obesity explaining differences in metabolic syndrome prevalence were evaluated in 224 and 447 Mexican and U.S. Pima Indians. Third, factors associated with type 2 diabetes were evaluated in each Pima Indian population (224 from Mexico and 887 from U.S.).

Methods. Demographic, physical, biochemical, and lifestyle factors were measured in 1996 in a cross-sectional study of Pima Indians 20 years of age or older living in Maycoba, Sonora Mexico and contrasted to results from a sample of U.S. Pima Indians participating in an ongoing epidemiological study that used similar methods and selection criteria. Insulin resistance was estimated by both fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR). Metabolic syndrome was defined using the Third Report of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP III) criteria. Body mass index (BMI) was calculated by dividing weigh in

kilograms by the square of height in meters (Kg/m^2). Physical activity was measured using a questionnaire developed for the U.S. Pima Indians and adapted to the Mexican Pima Indian population. Type 2 diabetes was defined according to the 1999 WHO criteria after an oral glucose tolerance test. Multiple linear regression analysis was used to answer the first question (related to differences in insulin resistance) and multiple logistic regressions analysis to answer the second (related with differences in metabolic syndrome) and third questions (related to factors associated with type 2 diabetes).

Results. Insulin resistance was much lower in the Mexican Pima Indians than in genetically related U.S. counterparts, even after controlling for differences in obesity, age and sex. In addition, the unadjusted prevalence of metabolic syndrome was 24.1% and 56.6 % in the Mexican and U.S. Pima Indians, respectively. However, most of the difference in metabolic syndrome prevalence was explained by differences in obesity and physical activity. Furthermore, in Mexican Pima Indians, type 2 diabetes was independently associated with age, fasting insulin, and waist circumference. In the U.S. Pima Indians, type 2 diabetes was associated with with age, sex, fasting insulin, total cholesterol, blood pressure and physical activity.

Conclusion. The findings underscore the importance of lifestyle in the prevention of type 2 diabetes risk factors, such as insulin resistance and metabolic syndrome, even in individuals with high propensity to develop diabetes.

INTRODUCTION

I. Explanation of the problem

Diabetes is a major public health concern worldwide, with the rates of diabetes reaching pandemic proportions. The disease causes a huge impact to individuals and their families and to the national health systems because of its associated premature morbidity and mortality. The burden of diabetes has dramatically increased and is expected to worsen because of its increased incidence [1, 2].

In terms of number of cases, projections estimate 366 million people diagnosed with diabetes by the year 2030; most of whom living in India, China and the United States (U.S.) [2]. These cases are associated with increased health care costs; in 2002 in the U.S., a total cost of $132 billion ($92 billion as direct costs and $40 billion as indirect costs) related to type 2 diabetes was estimated as being due to diabetes and its complications [3]. In 2007, the total cost due to diabetes and its complications increased to $174 billion (116 billion as direct cost and 58 billion as indirect cost) [4]. Certain population groups in the U.S., such as Native Americans and Mexican populations, are of great concern since they seem particularly susceptible to type 2 diabetes, especially when exposed to a modern lifestyle [5].

The overall goal of the main proposal ("NIDDM and Obesity in Pima Indians: Environment *vs.* Genetics") was to identify the effect of living in contrasting lifestyles to explain the variation in prevalence of type 2 diabetes in two genetically related groups of Pima Indians (Mexican *versus* U.S. Pima Indians). The existence of two populations that are genetically related but live contrasting lifestyles provides a natural design to study the

influence of lifestyle related risk factors in the development of type 2 diabetes. The U.S. Pima Indians live a Western lifestyle in Arizona, whereas the Mexican Pima Indians reside in the Sierra Madre Mountains of Northern Mexico live under more traditional conditions.

The specific strategy for this dissertation proposal was to test several hypotheses regarding physiologic, metabolic, and physical and lifestyle risk factors already known to predict type 2 diabetes in U.S. Pima Indians and how these risk factors are influenced by differences in lifestyles. The data base used for these analyses includes 224 Mexican and 887 U.S. Pima Indians from the project "NIDDM and Obesity in Pima Indians: Environment vs. Genetics". This project was completed during the period from 1995 to 1997 under the grant funded by the National Institute of Health DK-49957. The data base used for this project includes information from Pima Indians living in the U.S. and Mexico obtained under similar recruitment and data collection procedures and common laboratory measurements performed in the same centralized laboratory at the Phoenix Epidemiology and Clinical Research Branch. Data from the U.S. Pima Indians are part of an ongoing cohort study, which coincides with the same period of time as that in the Mexican Pima Indians collection times (from 1995 to 1997).

This dissertation research is based on prior findings that 1) U.S. Pima Indians have the highest reported prevalence of type 2 diabetes worldwide [6] 2) that diabetes is independently predictive by insulin resistance, metabolic syndrome and its components, not only in U.S. Pima Indians but in other populations [7-12], and 3) that U.S. Pima Indians have a marked increase prevalence of type 2 diabetes and obesity in comparison

with their closely genetically related Mexican Pima Indians [13, 14]. We hypothesize that the difference in type 2 diabetes prevalence between these two populations is attributed to their contrasting lifestyles, expressed by: a) a higher insulin resistance in the U.S. Pima Indians, b) a higher prevalence of the metabolic syndrome in the U.S. Pima Indians and c) a higher additional type 2 diabetes risks factors in the U.S. Pima Indians.

This study assessed the following specific aims:

1) Whether Mexican Pima Indians have lower insulin resistance evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) than U.S. Pima Indians.
2) The prevalence of metabolic syndrome in Mexican and U.S. Pima Indians as well as whether physical activity and obesity explain differences of metabolic syndrome between the two groups.
3) Ascertain the variables independently associated with type 2 diabetes in Mexican and U.S. Pima Indians.

II. Dissertation format

This dissertation is presented as two chapters and five appendices. The first chapter presents an explanation of the problem and study aims, dissertation format and literature review. The second contains study methods, statistical analysis plans and a summary of results of the three hypotheses tested. The appendices are manuscripts in the process of submittal for publication (one of them already published) as well as additional tables not included in the papers. All manuscripts have been written by the degree candidate with input from the dissertation committee and co-authors. All papers are based on one epidemiological study in which the candidate substantively contributed in these areas: questionnaire and clinical record development, subject recruitment, database development and maintenance, and study management. All analyses and interpretations represent the candidate's independent and original contribution, completed with the advice of the dissertation committee and co-authors. The candidate has been involved in the Binational Pima Indians Study since first preliminary information was gathered in the first team trip to the Maycoba Mountains in 1991. Since 1992, the candidate has worked with this project as part of his master's studies (completed at 1994). Some of these analyses were then used to complete a large proposal ("NIDDM and Obesity in Pima Indians: Environment vs. Genetics") which was awarded for the period from 1995 to 1997 (Dr. Leslie O. Schulz as principal investigator in the U.S. side and Dr. Mauro E. Valencia in the Mexican side). The candidate was part of the staff during the three years period of the study and his role was to coordinate the field work as well as data entry and cleaning along with the PIs. Since then, six different

papers have been published from which the candidate has been co-author or primary/first author.

Appendix A is the first manuscript, “Differences in Insulin Resistance in Mexican and U.S. Pima Indians with Normal Glucose Tolerance”. This manuscript contains a complete description of the study population, methods, results and discussion regarding insulin resistance status in Mexican and U.S. Pima Indians with normal glucose tolerance. Appendix A1: contains a shorter version of the manuscript in appendix A, as it was submitted to the *Journal of Clinical Endocrinology & Metabolism* on February 05, 2010 and published as a brief report 2010 Nov; 95 (11):E258-E362 (Published ahead of print July 28, 2010 as doi:10.1210/jc.2010-0297). *Copyright 2010, The Endocrine Society* (“Differences in Insulin Resistance in Mexican and U.S. Pima Indians with Normal Glucose Tolerance”). Appendix B is a second manuscript “Metabolic syndrome in the Mexican and U.S. Pima Indians: The importance of physical activity”. Appendix C is the third manuscript titled “Risk factors associated with type 2 diabetes in Mexican and U.S. Pima Indians”.

III. Literature review

A. Epidemiology of type 2 diabetes

1. Definition

Diabetes mellitus is described as a group of metabolic disorders caused by many different mechanisms and characterized by chronic hyperglycaemia or high levels of glucose with disturbances of carbohydrate, fat, and protein metabolism on target tissues, caused by deficient insulin secretion and/or resistance to its action (hepatic and peripheral glucose uptake) [15].

Two major types of diabetes are commonly described. Type 1 diabetes is characterized by having virtually complete lack of endogenous pancreatic insulin production due mainly to autoimmune-mediated destruction of pancreatic cell islet. Consequently, people with this disease require insulin to survive [16-18]. Type 2 diabetes is characterized by relative hyperinsulinemia or insulin resistance. Although in general, people with type 2 diabetes do not require insulin to survive, some of them will require it as adjunctive alternative to reach a better blood glucose control [16-18]. Hyperglycaemia in type 2 diabetes is considered as an abnormal elevation in blood glucose level, which is caused by impaired insulin-stimulated glucose uptake and uncontrolled hepatic glucose production [15].

2. Type 2 diabetes and health related problems

Type 2 diabetes is a chronic disease associated with vast premature morbidity and mortality [16-18]. It is one of the principal causes of death in several countries and

contributes significantly to the development of specific long-term damage, dysfunction and failure of multiple organs including retinopathy with potential blindness, nephropathy with a risk of progression to renal failure, neuropathy with risk for foot ulcers, amputation, charcot joints (Neuropathic osteoarthropathy) and autonomic dysfunction (sexual impairment). Because of this, diabetes contributes to disability, poor quality of life and loss of potential years of life [16]. Its related mortality is mainly caused by the high contribution of type 2 diabetes to cardiovascular, cerebrovascular, and peripheral artery disease [16, 19, 20] which together account for two-third of death in people with type 2 diabetes [17].

Type 2 diabetes results from a combination of genetic predisposition, unhealthy diet, and physical inactivity [21]. The mechanisms for the genetic explanation have not been completely described, but different studies give support to this hypothesis. Among the supported findings are the strong family associations (higher type 2 diabetes in relatives and similar in twins), the high variability in prevalence in different populations and the fact that certain subgroups (Pima Indians, Naurans, etc) have a disproportionate prevalence [16].

Regarding lifestyle influence, the increase in type 2 diabetes appears to be related in great degree to aging of the population and urbanization; this latter factor is associated with more sedentary lifestyles and higher caloric intake and subsequently with increasing obesity [16-18, 22]. Additional explanations for the increase in type 2 diabetes are associated with improvements in survival rates and changes in diagnostic criteria and screening practices [22].

3. Magnitude of type 2 diabetes

Diabetes is a major public health issue worldwide. It causes a huge impact to individuals and their families and to the national health system because of its associated premature morbidity and mortality. Its burden has been dramatically increased and is expected to worsen due to its increased incidence. In terms of number of cases, diabetes has reached pandemic proportions [1, 2, 17, 18, 23].

a. Worldwide and U. S. type 2 diabetes prevalence, mortality, and cost

According to a widely accepted estimation, in adults aged 20 years and older, estimates project a doubling in the number of total diabetes cases worldwide (300 million) between the years 1995 to 2025 [23]. Similar updated estimations project 366 million people with diabetes by the year 2030, most of them (90-95%) as type 2 diabetes. It is considered that India, China and the U.S. will be the three countries with the highest number of cases [2]. As was mentioned before, one of the problems related to diabetes is the increase in premature mortality associated with this condition. In this regard, estimations of diabetes worldwide indicated that for the year 2000, it would be the fifth leading cause of death, with 2.9 million, corresponding to 5.2% of total deaths. Excess in mortality due to diabetes will be greater in countries such as U.S., Canada, and the Middle East, accounting for 8% of all deaths in these countries [24].

In the U.S., diabetes is also considered a major health problem, as it has become a leading cause of morbidity and mortality [1, 22, 25, 26]. A substantial segment of the U.S. population has suffered this disease over the last decade [25] and this trend will

continue if no action is taken now in order to decrease the expected upsurge in the number of cases of the disease in the next 25 years [26]. Regarding the magnitude of the disease, the National Health and Nutrition Examination Survey (NHANES) conducted by the National Center for Health Statistics in 1999-2002, reported a crude prevalence of type 2 diabetes in adults aged ≥ 20 years of 9.3%, representing 19.3 million diabetic subjects for the year 2002, with 6.5% characterized as physician diagnosed and 2.8% as undiagnosed type 2 diabetes [25]. The most recent information indicates an increase in the number of individuals with diabetes in 2007 with a prevalence of 10.7% in the adult population (20 years and older), representing 23.5 million individuals with diabetes [27].

In the previous report [25], type 2 diabetes cases increased in older age groups and it was similar by sex. In comparison with information reported in a previous national survey (1988-1994), it appears that diabetes increases over time (7.8% *vs.* 9.3% and 8.2% *vs.* 9.3%, respectively for the crude and standardized prevalence) in both males and females, although no significant differences (p=0.06) were found for the standardized values [25]. However, when comparisons were done by sex between the two surveys, both the crude and standardized changes in percentages were significant in males (7.9% *vs.* 10.2%, and 8.7% *vs.* 10.6%, respectively for crude and standardized), but not in females (7.8% *vs.* 8.5% and 7.8% *vs.* 8.2%, respectively for crude and standardized) [25]. Similarly, the prevalence in diagnosed type 2 diabetes increased significantly from 5.1% to 6.5%, but not for undiagnosed type 2 diabetes, which was similar for the two national surveys [25]. Undiagnosed cases represented one-third of the total type 2 diabetes population.

A variety of reports predicted a considerable increase of diabetes prevalence in the adult U.S. population. For instance, one study from the NHANES (1999-2002) projected 14.5 and 17.4 million people with diabetes for the years 2010 and 2020, respectively [3]. Similarly, two different WHO reports using diabetes data from the NHANES II (1976-1980) and the NHANES III (1988-1994) projected 21.9 (prevalence of 8.9%) and 30.3 (prevalence of 11.2%) million people with diabetes in the U.S. for the years 2025 and 2030 [2, 23].

Furthermore, in a very recent report [26] that used new modeling techniques (multivariable risk score) and included information taken from a variety of U.S. National Surveys from individuals at high risk of developing diabetes, it was projected that 37.7 million people (14.5%) would have diabetes by the year 2031. This same report [26] also showed projections for total diabetes, both diagnosed and undiagnosed, by 2011 of 25.4 million or 11.5% and by 2021 of 32.6 million or 13.5%.

In 2002, the American Diabetes Association estimated a total cost of diabetes of $132 billion, including the cost of medical expenditures (direct cost) and loss in productivity (indirect cost). The direct cost represented $92 billion and included the cost associated with diabetes care, management of chronic complications and excess in prevalence of some related medical conditions. The indirect cost represented $40 billion and it was associated with a higher rate of lost work time, disability, and premature mortality from diabetes. When total cost, expressed as per capita, was compared among people with diabetes and people without, the cost in the former group was 2.4 times higher [3, 28, 29]. In 2007, the total cost due to diabetes and its complications increased

to $174 billion (116 billion as direct cost and 58 billion as indirect cost). The increase ($21 billion in 2007 dollars after adjustment for inflation) between the 2002 estimate and new 2007 estimate reflects three factors: 1) growth in diabetes prevalence, 2) medical costs rising faster than general inflation, and 3) improvements made in the methods and data sources to estimate the cost of diabetes [4].

The total cost of type 2 diabetes was based on three general components, a) the size of the population with diabetes, b) the health care use and the total heath care expenditure attributable to diabetes, and c) the value of lost productivity attributable to diabetes [3]. Using these three components and an estimated 12.1 million people with type 2 diabetes for the year 2002, projections of type 2 diabetes for the year 2010 and 2020 were estimated at 14.5 and 17.4 million people in the U.S., representing a correspondingly total cost of 156 and 192 billion U.S. dollars for each of those two years, respectively [3].

The growing prevalence of type 2 diabetes and its related increase in health costs explain the greater costs from previous estimations (98 *vs*. 132 billion in 1997 and 2002, respectively) [3]. It is important to mention that the estimation for the 2002 and the projection for the 2010 and 2020 may be underestimated because the size of the population used (projection of the Census Bureau) for the year 2000 was smaller than the actual population based on the U.S. 2000 census and also because the prevalence of type 2 diabetes was estimated using information from the National Health Interview which was based on self reported diabetes. However, it is also estimated that around one-third of diabetics are unaware of their condition [3].

b. Mexican type 2 diabetes prevalence, mortality, and cost

In Mexico, type 2 diabetes is also considered a major public health problem because of an increase in prevalence in the last four decades, the associated high co-morbidities, the increased mortality rate, and its associated socio-economic burden, not only at the individual but at the health care service level [30-33]. Furthermore, in comparison with other countries, Mexico was included among the 10 top countries with the highest rates of type 2 diabetes for both 1995 and 2025 and an estimated move up from the ninth place in 1995 to seventh place by 2025 [23].

Two main national surveys [National Survey of Chronic Disease (NSCD 1993) and National Health Survey (NHS 2000)] have evaluated type 2 diabetes prevalence along with some related risk factors in Mexico. At the national level, the age- and sex-adjusted prevalence has increased from 6.7% in 1993 to 8.2% in 2000 (a 22% increase in a 7 year period), although the difference may be greater. In the NHS 2000, a large group of people had marginal glucose; if complementary testing using OGTT were used, the prevalence of type 2 diabetes would be 12.6% [31]. Also, the NSCD only includes urban populations while the NHS includes both urban and rural populations.

Under the same diagnostic criteria, however, a national prevalence of 10.7 % of diabetes (5.3 million of diabetics) has been published elsewhere [34], for people ages 20 to 69 years and weighted for the year 2000 national population distribution regarding age and sex and using data from the NHS. In an additional report based on an urban population >20 years of age, the type 2 diabetes prevalence was 10.7% for the year 2002 (14.0% and 9.6% for cities located in northern states *versus* those in center states) using

capillary glucose and the same criteria as in NHS [35]. Of note, in both national surveys (NSCD 1993 and NHS 2000), results indicated that type 2 diabetes and related risk factors were higher in people living in the northern Mexican states, in people with lower socioeconomic status and in females. Type 2 diabetes also was associated with advanced age and increased body mass composition [34, 36, 37].

Aguilar-Salinas et al. (2003) used data from NHS 2000 to analyze all men and women diagnosed with diabetes that formed part of this population-based survey and were 20 years of age or older. Their findings indicated that all diabetics surveyed were classified as having type 2 diabetes. Of the number of people diagnosed at time of the study (20% of the total surveyed), 38% were at younger ages, and most of them were either overweight or obese. Of the total diabetic population (3,597) 13% were younger than 40 years, 75% with BMI >25 and 87% of women and 43% of men had abdominal obesity. In addition, half of all diabetic persons had hypertension and a third of them were smokers [31].

In the most recent national surveys (Mexican National Health and Nutrition Survey (ENSANUT 2006) using a sample of 6,021 subjects in fasting conditions, the overall prevalence of type 2 diabetes was 14.42% (7.31 millions of cases), with 7.34% characterized as physician diagnosed and 7.07% as undiagnosed type 2 diabetes (38). The prevalence of type 2 diabetes was higher in urban than in rural population (15.48% *vs.* 10.39%); higher in male than in female (15.82% *vs.* 13.20%); it also increased with age and with SES. By regions, the prevalence of type 2 diabetes was lower in the South-southeast region and higher in the Center-west region. The Northern and center regions

had an intermediate prevalence (38). Type 2 diabetes was defined as either, subjects who declared to have a previously established diagnosis of diabetes by a physician independently of the survey glucose concentration or subjects whose glucose concentration in the fasting blood sample (measured in serum by the glucose hexokinase method) was ≥ 126 mg/dL (38).

Causes related to the increased prevalence in type 2 diabetes in Mexico have been usually associated with demographic, nutritional transition and decreased physical activity. Demographic transition in Mexico is explained by the increase in the proportion of older individuals in the population and a shift from high to low fertility. Nutritional transition is a shift from high prevalence of undernutrition to predominance of diet-related chronic disease explained as the changes in dietary patterns (from a traditional diet to a more diabetogenic dietary pattern), resulting from modernization and urbanization (a large proportion of the population have moved from rural to urban areas). This transition appears to have resulted in increased consumption of kilocalories, total and saturated fats, and refined sugars while decreasing their consumption of complex carbohydrates; a trend that has been accompanied by a parallel rise in their hours of sedentary lifestyle [39-42].

An additional non- modifiable risk factor that seems to play an important role in the epidemic of type 2 diabetes in Mexico is a genetic propensity of Mexican people to develop type 2 diabetes as reported elsewhere [43]. Although reductions in lifestyle risk factors seems to overcome genetic factors as shown in different studies [14, 44], it is important to mention that Mexican environmental conditions, along with their genetic

propensity, are favorable to the development of type 2 diabetes and may explain its epidemic [33, 45).

Diabetes is a chronic disease that requires lifelong, continuous medical care. In Mexico, however, many people with diabetes have limited access to health care. In the year 2000, for instance an estimated 44% of adults who had been previously diagnosed with diabetes in Mexico reported having no health insurance [46] and although the access to health care have improved since 2004 with a health care reform, the improvement in controlling diabetes and its complications are modest [47]. Under this reality, diabetes is still considered to have considerable medical, social, and economic consequences in Mexico and other developing countries. In Latin America and the Caribbean for instance, the indirect costs due to diabetes exceed direct health care costs. The annual number of deaths in 2000 caused by diabetes was estimated at 339,035, which represented a loss of 757,096 discounted years of productive life among persons younger than 65 years (U.S. $3 million). Permanent disability due to diabetes in the working population was related with loss of 12,699,087 discounted years of productive life ($50.6 billion) and temporary disability with loss of 136,701 discounted years of productivity ($763 million) [48].

Regarding direct cost, it was estimated that costs associated with insulin and oral medications represented U.S. $4,720 billion, hospitalizations $332 million, consultations $888 million and care for complications $2.4 billion. Thus, in Latin America and the Caribbean, the total annual cost associated with diabetes was estimated at $65,216 billion, of which $ 10,721 billion were direct costs and $ 54,496 billion were indirect costs [48]. In this same report [48], the estimate for Mexico was $15,118 billion (1,974

billions and 13,144 billion for direct and indirect costs, respectively), representing the second largest cost for these countries. The diabetic population in Mexico was estimated at 3.7 million (4.1% of the population) for the year 2000.

On the other hand, in Mexico, mortality due to type 2 diabetes has increased in the last decades. Barquera et al. (2003) reported an increased mortality rate due to type 2 diabetes from 1980 to 2000 [45]. Their findings also indicated a rapid increase from 1980 to 1990, with a slighter increase from 1990 to 2000. In a more recent report the standardized mortality rate changed from 77.1 to 88.3 deaths per 100, 000 inhabitants during the period corresponding to 2001 and 2005 [49]. This trend has led to the consideration that type 2 diabetes is now one of the main causes of death [40, 42]; in 2003 for instance, diabetes was the leading cause of death in women and the second in men [50].

Factors explaining the high morbidity-related problems and the increase in mortality rates are the increase in prevalence and the greater number of diabetic people younger than 40 years old. Additional factors are the large numbers of diabetic people with uncontrolled glucose, the large numbers of undiagnosed people and the fact that many diabetics also have a high prevalence of other risk factors for chronic complications [31, 33, 42]. Aguilar-Salinas et al. (2003) using data from NHS 2000 reported that a very low percent of people with diabetes followed a dietary and physical activity program as an alternative way to reduce glucose [31].

As was the case for diabetes prevalence, cities from the northern states also have a higher mortality rate than cities from the southern states of Mexico. Comparing diabetes

associated mortality rates (standardized to the 2000-2025 age and sex worldwide distribution and expressed per 100, 000 inhabitants) between Sonora and Chiapas as representatives of these states, the mortality rate was 56.6 in 2001 and 64.0 in 2005 for Chiapas and 78.6 in 2001 and 81.3 in 2005 for Sonora [49]. Additionally, for both 2001 and 2005 the mortality rate in Sonora and Chiapas, as well as at the national level, was higher for women than for men [49]. Previous reports have also shown higher standardized mortality ratios in the northern Mexican states [45].

Type 2 diabetes is the primary cause of premature disability, blindness, and kidney failure [3]. The complications of diabetes will be further increased by different factors characteristic of the epidemic of type 2 diabetes in Mexico. These include the early appearance of the disease, its under-diagnosis, under-treatment and the high prevalence of important coronary risk. Type 2 diabetes will affect most middle aged people (45-64 years) in developing countries in comparison with developed countries and will therefore, be exposed for a longer time to the disease increasing their risk of complications, disabilities, and premature mortality. This will also represent an increase in expenses and costs for the health care system. Another factor that may further complicate the issue is that rural areas may be more affected [23].

4. Mexican health care system

Mexico has various types of health services, both public and private. The public health services is attended by two major agencies: the *Instituto Mexicano del Seguro Social* (Mexican Institute for Social Security, IMSS) and the *Instituto de Seguridad y*

Servicios Sociales de los Trabajadores del Estado (Institute for Social Security for Government Employees, ISSSTE) and complemented by other smaller agencies directed to the armed forces, oil workers and local governments employees. In 2004, the proportion of beneficiaries of these social security agencies was 45.4% (47.7 million people: 80% and 16.7% from IMSS and ISSSTE, respectively) of the total population (51). In addition, people from the informal sector of the economy (underserved and uninsured population) are attended by government sponsored facilities through the Secretaria de Salud (ministry of health). Finally, a small segment of the population (~2.0%) received health care from the private sector (51).

In 2004, the Mexican government established a new health option named *Seguro Popular de Salud* (Popular Health Insurance, SPS) aimed to serve the underserved and uninsured population (most of those people covered by the *Secretaria de Salud*). The service is voluntary and free for families categorized as being in the lowest socioeconomic status, but some payment is required as the socioeconomic status of families increase, although cheaper than that of private services. Receipt of this health service is contingent upon filing a family application to the SPS system (52).

Applications to the SPS are reviewed and if accepted, registered in the system, where special ID Cards are delivered to family members in whom the health service are controlled by means of a smart chip integrated to each ID card. For all patients, information on basic socio-demographic variables, health related problems, as well as the prescribed drugs are stored into the ID card, using a special program created for this particular national service. Thus, using their ID card, registered individuals receive the

needed health service and prescribed drugs through the public health system such as the IMSS and certified drug stores with no extra payment. It was estimated that through the first quarter of 2007, approximately 5.2 million (44%) of the estimated 11.9 million eligible households nationwide had enrolled in the SPS program [53] and this had increased to 20 million by the end of 2007 (51).

B. Insulin Resistance

The role of insulin in people with normal glucose tolerance is to maintain the glucose homeostasis mainly through controlling the glucose uptake into peripheral tissues and the hepatic glucose production, as well as by suppressing the release of stored lipids from adipose tissue [15]. In people with normal glucose tolerance, insulin affects cells through binding to its receptors on the surface of insulin-responsive cells. The stimulated insulin receptor phosphorylates itself and several substrates, including members of receptor substrates family (IRS), thus initiating downstream signalling events [54], which activate glucose transporter 4 (GLUT4) allowing the transport of glucose into cells where it is phosphorylated by hexokinases. Glucose-6-phosphate is then either utilized in the glycolytic pathway or incorporated into glycogen by glycogen synthase [55].

Insulin resistance is explained as a decreased sensitivity to metabolic actions of insulin such as insulin-mediated glucose disposal, inhibition of hepatic glucose production and suppression of the release of stored lipids as previously mentioned, resulting in a secondary stimulus to the β-cells and hyperinsulinemia and eventually in type 2 diabetes and other associated diseases [56]. Therefore, it is important to identify

person at this pre-diabetes status, mainly in individuals considered at high risk in order to be able to delay or even prevent the development of these diseases [56, 57].

Insulin resistance was first suggested in 1925 by Bainbridge based on its works in rats and mice [56]. Himsworth, in the year 1936 further suggested an association of insulin resistance with obesity and mild diabetes in human, which was supported by Berson and Yallow several years later, after their introduction of radioimmunoassay in 1960 and using postprandial hyperinsulinemia as a marker of insulin resistance [56]. Although, the putative role of insulin resistance in type 2 diabetes remained controversial since no direct method to assess insulin resistance was available at that time [56], to date it is widely recognized that insulin resistance is a critical step in the pathogenesis of type 2 diabetes [7, 8, 58, 59].

1. Mechanisms of insulin resistance

Insulin resistance has been associated with obesity, commonly explained as the result of chronic exposure of tissues to elevated dietary nutrients, resulting in accumulation of lipid by-products such as diacylglycerol and fatty acyl CoAs and subsequent increase in protein kinases activity. This explanation is called the lipid induced insulin resistance [15, 60]. The increased level of circulating free fatty acids in obesity and its resulting by-products is recognized as the major contributor to the development of insulin resistance in insulin sensitive tissues [61]

Lipid accumulation in adipocytes is associated with adipocyte dysfunction and subsequent delivery of fatty acids to the liver and skeletal muscle leading to increases in

intracellular lipid content [55, 60, 62] and subsequent peripheral insulin resistance [54, 55]. Many insulin-resistant states have been associated with increased plasma free fatty acids and this association is explained by the fact that free fatty acids affect parallel insulin-signalling pathways leading to inhibition of glucose transport activity [63]. Exposure of insulin sensitive cells to elevated levels of free fatty acids stimulate inhibitory phosphorylation of serine residues of IRS-1, which reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signalling and insulin action [54].

Shulman et al. (2000), have described the mechanism by which free fatty acids cause insulin resistance in skeletal muscle, and probably in liver and adipocytes [63]. Specifically, increases in intracellular fatty acid metabolites such as diacyl-glycerol, fatty acyl CoA, and ceramides are due to the increase free fatty acids delivery and/or to a decrease in intracellular metabolism of fatty acids (Figure 1). These toxic metabolic by-products activate a serine/threonine kinases cascade, initiating with protein kinase C (PKC-θ) and possibly involving the inhibitor-kappa kinase (IKK-beta) and/or c-Jun N-terminal kinase (JNK-1), which cause phosphorylation of serine/threonine sites on insulin receptor substrates (IRS-1 and IRS-2), which in turn reduces the ability of the insulin receptor substrate to activate phosphatidylinositol 3-kinase (PI 3-kinase), resulting in decreased GLUT 4 activity [63].

The base of these mechanisms is explained by the existence of alterations in the partitioning in fat (reduced ability to metabolize fatty acids) in insulin sensitive tissues leading to the accumulation of triglycerides and fatty acid metabolites mentioned above

[63]. In a more recent review by Savage et al (2007) [55], two mechanisms were proposed to explain insulin resistance caused by free fatty acids, one for muscle cells and another for liver cells (Figure 2). The new mechanisms proposed in muscle cells is similar to that proposed by Shulman (2000), although Shulman's mechanism was a general proposal for muscle, liver and adipocytes cells.

The mechanism proposed for the liver cells has small differences in comparison with that for muscle cells (Figure 2). In the liver cells, long-chain acyl CoA and diacylglycerol are produced by increased lypogenesis in addition to the increased delivery of free fatty acid and/or decreased mitochondrial fatty acid oxidation. These metabolites activate PKC-ε, which binds and inactivates the insulin receptor kinase, resulting in reduced insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation and subsequent reduced insulin activation of PI 3-kinase and protein kinase β (PK β [AKT2]). Reduced AKT2 activation further results in lower glycogen synthase kinase-3 phosphorylation and lower forkhead box protein phosphorylation, which in turn results in lower insulin-stimulated liver glycogen synthesis and decreased suppression of hepatic gluconeogenesis, respectively [55].

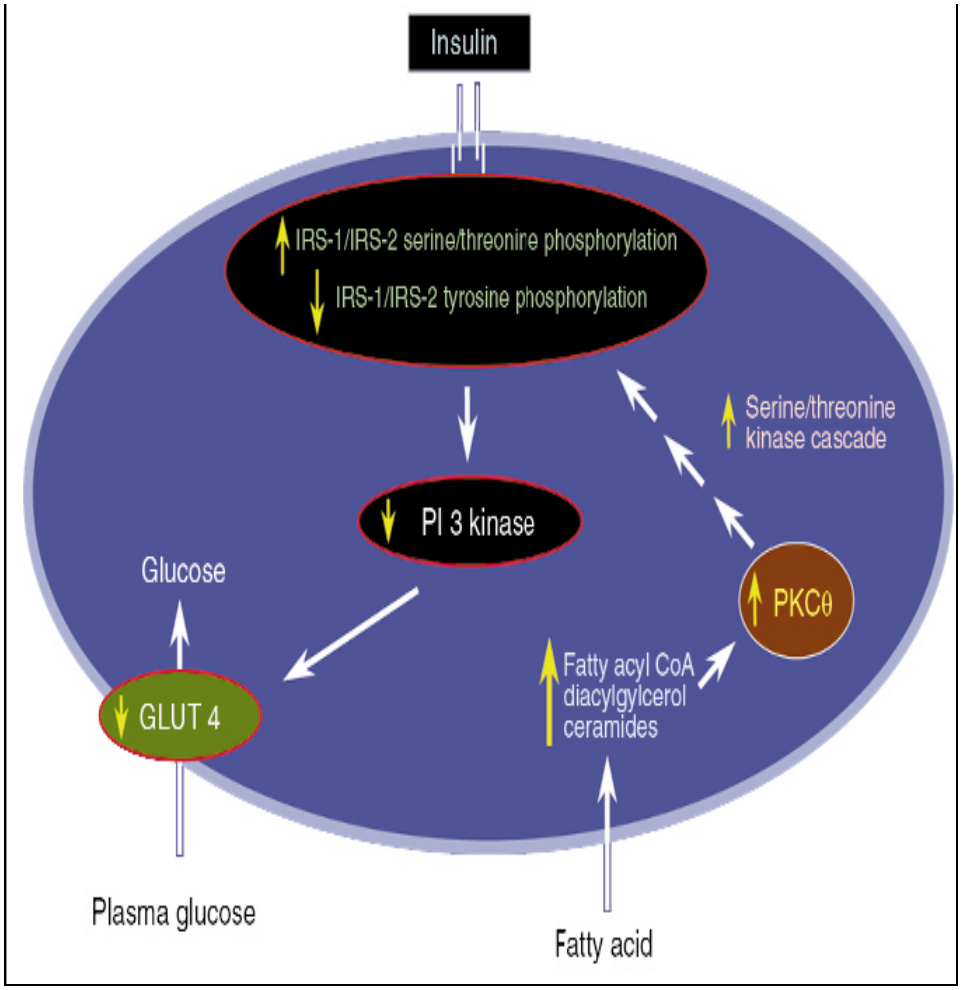

Figure 1. Mechanism of fatty acid-induced insulin resistance in skeletal muscle and liver. PKCØ, protein kinase CØ; PI 3 kinase, phosphatidylinositol 3-kinase; IRS, insulin receptor substrate; GLUT, glucose transporter.
From reference [63].

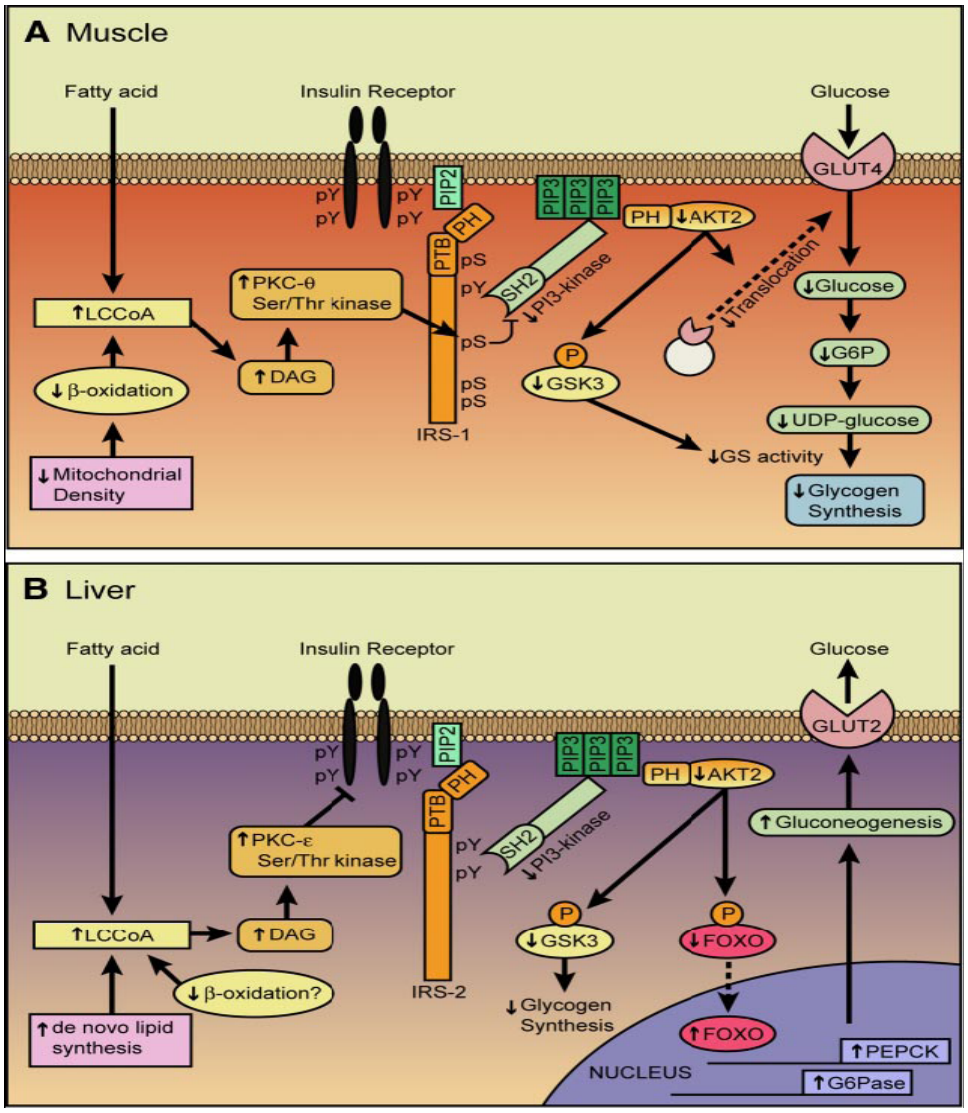

Figure 2. Mechanism of fatty acid-induced insulin resistance in skeletal muscle (A) and liver (B). DAG, diacylglycerol; FOXO, forkhead box protein O; GLUT, glucose transporter; G6P, glucose 6-phosphate; GSK3, glycogen synthase kinase-3; IRS, insulin receptor substrate; IKK-ß, Ikß kinase-ß; JNK-1, Jun kinase-1; LCCoA, long-chain acylcoenzyme A; nPKCs, novel protein kinase Cs; PEPCK, phosphoenolpyruvate carboxikinase; PI-3-kinase, phosphoinositol 3-kinase; PTB, phosphotyrosine binding domain; PH, pleckstrin homology domain; SH2, src homology domain.
From reference [55]

More recently, new insights related to the etiology of insulin resistance have indicated an additional mechanism involving hormonal factors and inflammatory molecules [15, 55, 60, 64] in which adipocytes with its endocrine/paracrine function play a central role. This new mechanism of insulin resistance is explained because in addition to the well known function of adipocytes as storage for lipids, adipocytes are also a source of several factors collectively called adipokines. These adipokines among others include inflammatory molecules such as TNF-α and IL-6, as well as hormonal molecules such as leptin and adiponectin [15, 60].

Adipose tissue is a specialized connective tissue that comprises adipocytes as well as many additional cell types, referred as stromal vascular cells, including pre-adipocytes, vascular smooth muscle cells, endothelial cells, leukocytes, percytes, monocytes, and macrofaphages [65, 66]. The adipocyte is a cell in which the lipid droplet encompasses greater than 95% of the entire cell body. The lipid droplet serves as a storage vessel for triglycerides that can be released through lipolysis and added to by the process of triglycerides synthesis. As explained before, adipocyte with its remained 5% of mass cell has additional roles such as endocrines and autocrine/paracrines functions, releasing factors referred as adipokines. This additional function allows adipocytes to regulate processes in peripheral tissues and neighbouring adipocytes and other local cell types within the adipose tissue [65].

Chronic energy imbalance produces adipocyte hypertrophy, endoplasmic reticulum stress, and mitochondrial dysfunction which increase intracellular and systemic release of adypokines and free fatty acids and consequently induce adverse effects in the

liver, pancreatic beta-cells, and skeletal muscle, as well as the heart, and vascular beds [62]

The association of inflammation molecules with insulin resistance has been explained by a) decreasing the action of insulin receptor substrate (IRS-1 or 2) and peroxisome proliferators-activated receptor gamma (PPARγ) that are important in the insulin signaling pathway, and b) through increasing circulation fat free acids by stimulation of lipolysis and decreasing adiponectin in the blood and finally decreasing the action of IRS-1 or 2 or PPARγ (skeletal muscle and liver insulin resistance) [54, 60, 67]. In both cases the effect is explained through activation of inflammation mediators as was explained earlier [55, 62, 67]. Serine kinases [IKK (inhibitor-kappa B kinase), JNK (c-Jun N-terminal kinase), protein kinase C (PKC), and S6K (ribosomal protein S6 kinase)] are chief examples of these inflammation mediators [54, 67]. PPARγ appears to be a key player in the ability of adipose tissue to buffer fatty acid influx [55].

In obesity states, the normal function of adipose tissue is disturbed due to interrelated factors such as enlarged adipocytes, impaired adipocyte tissue blood flow, adipose tissue hypoxia, adipose tissue macrophage infiltration and local inflammation in adipose tissue leading to disturbances in excessive fat storage and lipid overflow in non-adipose tissues (ectopic fat deposition), and adipokines secretion [66]. Ectopic fat deposition is characteristic of obesity and is mainly explained by chronic imbalances, in which energy intake exceeds adipose tissue storage capacity leading to increased delivery/synthesis or reduced oxidation of free fatty acids within the tissue involved [55].

In a recent report, inflammation response was associated with increased free fatty acids, endoplasmic reticulum stress, reactive oxidative species, and adipocyte death, which in turn appear to be explained by adipose tissue hypoxia through inhibition of adipogenesis and triglycerides synthesis [67]. Hypoxia has been demonstrated by a reduction in the level of oxygen in adipose tissue of obese animals and humans [67].

It has been demonstrated that enlarged adipocytes cause functional abnormalities of endoplasmic reticulum (ER) leading to ER stress and subsequent activation of inflammatory signalling pathways [54]. ER stress also cause oxidative stress in the mitochondria due to its increased activity because of greater circulating (and intracellular) lipid concentrations. The ER is responsible for synthesizing proteins, forming lipids droplets, and sensing and regulating cholesterol. Oxidative stress produces reactive oxygen species and ultimately apoptosis. ER stress also may result in systemic release of free fatty acids and inflammation molecules and ultimately insulin resistance which cause subsequent ER stress [62].

Inflammatory response is initiated in the adipocytes themselves, as they are the first cells affected by the development of obesity, or potentially in neighboring cells that may be affected by adipose growth [54]. Increased adipokines in enlarged adipocytes have local and systemic effects. Most of the local inflammatory response in obesity states is the result of increased accumulation of macrophages in the adipose tissue (macrophage infiltration), with exception of leptin and adiponectin that are primarily synthesized and secreted by adipocytes [60, 66]. Macrophages are non-adipocyte cells derived from bone marrow precursors and obesity states cause macrophage infiltration [55, 66].

Among the local effects it has been reported inhibition of adypocyte differentiation and induction of apoptosis in pre-adipocytes and mature adipocytes, resulting in enlargement of the remaining fat cells, leading to a reduction of the capacity of adipocyte tissue to handle lipids and further increasing of adipokines. Other local effects are the increased lipolysis in adipocytes resulting in the release of fatty acids from adipose tissue into circulation and subsequent ectopic effect in skeletal muscle and liver. With regard to the systemic effects of inflammation, results appear to indicate that there is a cross-talk between insulin receptor signalling and inflammatory pathways leading to insulin resistance [66].

TNF-α is a pro-inflammatory cell surface transmembrane protein mainly produced by infiltrated macrophages within adipose tissue; although some amount is produced by the adipocytes itself [60, 64]. This protein was recognized as the first cytokine that could induce insulin resistance [60]. It is stimulated by endoplasmatic reticulum stress due to obesity [60, 62]. Obesity increases the numbers and activation state of macrophages [68]. TNF-α appears to play a pivotal role with respect to the production of several others adipokines [66]. TNF-α causes IR by inhibition of lipoprotein lipase activity and increased lipolysis (increases circulating fatty acid level) [60, 62], by increased leptin and decreased adiponetin secretions, decreased glucose transporters 4 expression, and antagonisms of PPARγ [65] and by the phophorylation of the IRS-1 on serine residues leading impaired insulin signalling [62, 64]. It also inhibits differentiation of human adipocytes precursor cells and induces apoptosis in both human pre-adipocytes and mature adipocytes [66].

IL-6 is also a proinflammatory protein which is positively associated with insulin resistance and is released by several types of cells including those from the liver and adipose tissue. In obesity status, one-third of the circulating IL-6 is released in the adipose tissue [60, 62], mainly by the stromal vascular cells including pre-adipocytes, endothelial cells and monocytes macrophagues, where it can exert paracrine effects directly on adipocytes [64, 65]. It has direct effects on insulin signalling in adipocytes and hepatocytes [60, 62]. IL-6 also causes increased leptin and lipolysis and decreases lipoprotein lipase activity in adipocytes with increasing free fatty acids and subsequent insulin resistance, in addition to decreases in adiponectin [65]. In the liver it also induces the CRP protein release (64).

Adiponectin is a protein that is produced by adipocytes and is highly expressed in adipose tissue of non obese persons; however this protein decreases in obesity state. There is an increasing correlation between adiponectinemia and insulin sensitivity and inverse association with obesity and more particularly with central obesity. The insulin sensitivity action of adiponetin is explained by the activation of AMP-activated protein kinase (AMPK), which is know to regular cellular malony CoA concentrations by inhibiting acetil CoA carboxilase and subsequent lipogenesis decrease and increase beta oxidation [64]. Increased adiponectin is associated with decreased glucose (by inhibits liver gluconeogenesis), fat free acids, and triglycerides, as well as increased glucose uptake and fatty acid oxidation in skeletal muscle [64, 65] and modulates the release of TNF-α of macrophagues [64]. In addition, decreased adiponectin is at least partly explained by the action of the over expressed TNF-α and IL-6 [66].

Leptin is a product of the obese (ob) gene from the adipocyte cells [69]. Its function is at the level of central nervous system, associated with the control of energy metabolism by decreasing energy expenditure and increasing food intake [64]. Leptin is also capable of controlling TNF-α and macrophage activation. Both TNF-α and IL-6 stimulate leptin production by adipocytes [69]. Furthermore, leptin and adiponectin are associated with decreasing triglycerides synthesis, increasing β-oxidation and insulin sensitivity in both skeletal muscle and liver in normal persons. In states of insulin resistance, leptin is increased possibly due to leptin resistance in obesity and adiponectin is decreased [15]. Regarding the order of events related to insulin resistance, Savage et al [55] proposed that insulin resistance in muscle is the earliest event, which is in turn associated with peripheral and portal vein hyperinsulinemia leading to hepatic steatosis due to increased lipogenesis and reduced fatty acids oxidation.

2. Methods of measurements of insulin resistance

The euglycemic hyperinsulinemic clamp is recognized as the gold standard method for the measurement of insulin resistance. The method consists in measuring peripheral glucose uptake under situations of elevated insulin concentrations. The rate of exogenous glucose infusion needed to keep plasma glucose concentrations from changing is indicative of the degree of insulin sensitivity (the inverse of insulin resistance). If a person is totally insulin resistant the glucose infusion rate is near zero; while if a person is very insulin sensitive, the glucose infusion rate will be substantial [56, 70]. Measurements of insulin resistance using the euglycemic hyperinsulinemic clamp

however, are technically difficult (labor-intensive), impractical (frequent monitoring of blood glucose and a well trained individual to control the glucose infusion rate as a function of time are needed), and not feasible for epidemiological studies involving many individuals due to high costs in time and money [56, 70-72].

Several authors have proposed diverse indices validated against the gold standard method in order to measure insulin resistance and sensitivity in large population studies [15, 73]. These indices are based on knowledge of the interrelation between insulin and glucose and the fact that basal glucose and insulin interactions are mainly determined by a simple feed back loop [73]. Some authors have grouped these indices as those based on fasting glucose and insulin in comparison to those based in the oral glucose tolerance test which require measures of glucose and insulin as response to a load of 75g of glucose [56, 57].

Fasting insulin, the homeostasis model assessment of insulin resistance (HOMA-IR) [73] and the quantitative insulin sensitivity check index (QUICKI) [74] are among the methods based on fasting measurements. These methods give information related to hepatic insulin sensitivity and basal hepatic glucose production. When these indices are used, however, care must be taken when comparing groups with differing β-cell function, since estimation of insulin resistance will be underestimated [56].

Insulin sensitivity indices which use a combination of information regarding fasting and two hours glucose and insulin from the oral glucose tolerance test (OGTT) include the Matzuda Index [75], Cederholm Index [76] and the Gutt Index [77]. The first method (Matzuda Index) gives information related to hepatic and peripheral tissue insulin

sensitivity or whole-body insulin sensitivity and include in its calculation endogenous insulin as a response of glucose load and fasting glucose ad insulin; however, β-cell response from insulin sensitivity can not be excluded per se. The second indices represent mainly peripheral insulin sensitivity and muscular glucose uptake due to the dominant role of peripheral tissue in glucose disposal after an oral glucose load [75].

Fasting insulin and HOMA-IR are the most commonly used methods or indices to estimate insulin resistance in large epidemiological studies [78] and have also been widely reported as good predictors of type 2 diabetes [9, 70, 71,79, 80]. Similar to both methods, higher values mean higher insulin resistance or lower insulin sensitivity while lower values mean lower insulin resistance o higher insulin sensitivity [56]. Fasting insulin and HOMA-IR have been validated against the gold standard method in adult men and women, obese and non obese with normal glucose [71, 81, 82]. Furthermore, there is a high correlation of fasting insulin and the HOMA-IR index (r=0.98-0.99) in non-diabetic populations [70].

The HOMA-IR is calculated as the product of the fasting concentration of plasma insulin (μU/ml) and plasma glucose (mmol/L) divided by 22.5, assuming that a normal-weight normal person aged <35 years has a 100% β-cell function and an insulin resistance of 1 [73]. It was proposed in 1985 by Matthews and colleagues [73] as a computer-based model taking into account that basal glucose and insulin concentrations are largely determined by a simple feedback loop.

The accuracy of the methods was evaluated by comparing the gold standard method of hyperinsulinemic euglycemic clamp in normal and diabetic patients (12 and

11, respectively) aged 46-68 years, and within 100-176% ideal weight [73]. Well accepted correlation values were reported in diabetics and normal subjects together ($r=0.88$, $p<0.0001$) and in normal and diabetics separately ($r=0.83$, $p<0.01$ and $r=0.92$, respectively) [73]. This index is widely used due to the simplicity of the estimates since it just requires information regarding fasting glucose and insulin concentrations [73], several other studies have supported its use [83-85] and has been suggested as the best surrogate index in a very recent report [78].

C. Metabolic syndrome

The metabolic syndrome describes an entity, characterized by a cluster of central obesity, hypertension, dyslipidemia (elevated triglycerides and decreased HDL-cholesterol), and hyperglycemia [86, 87], that increases the risk to type 2 diabetes in different populations [88-90], including the U.S. Pima Indians [10], even after adjusting by insulin resistance [91]. The etiologic factor of this syndrome is not yet well understood, despite the existence of several definitions [90, 92]. Insulin resistance was first proposed as the central underlying factor and thus the cluster of metabolic variables has been used as a surrogate variable for insulin resistance [93, 94]; an idea supported by the fact that most of the metabolic syndrome components are strongly inter-correlated [70]. However, the etiologic explanation for the cluster appears more complicated and several different factors are probably involved, many related to changes in lifestyles [61, 87, 89]; the most favored hypothesis suggests that central distribution of fat and its ectopic deposition in liver may be the core component of the syndrome [70, 95], although

further work needs to be done to determine which factor(s) are central to the development of the metabolic syndrome [87].

In the U.S. Pima Indians for instance, the hypothesis of a single underlying factor explaining the cluster of metabolic syndrome was not supported and four major components (insulinemia, body size, blood pressure, and lipid metabolism) were reported, each with its particular independent underlying etiologic factor [10]. Based on their findings, Hanson et al. (2002) argued that much of the information related to metabolic syndrome may be lost if its explanation is reduced to a single central physiologic process. Their finding was additionally supported by genetic linkage analyses which suggested separate major genetic determinants related to the four factors mentioned above [10].

Regarding predictors of type 2 diabetes incidence in the U.S. Pima Indians, insulinemia was the strongest risk factor while blood pressure was the weakest [10]. Both the World Health Organization (WHO) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definitions of metabolic syndrome also predicted type 2 diabetes in this group; a high prevalence of metabolic syndrome was reported in U.S. Pima Indians in the Hanson et al. (2002) study, similar to the prevalence reported by utilizing the WHO and NCEP ATP III definitions (31%) [10].

Most of the individual variables such as insulin resistance, obesity, central obesity, hypertension, hypertriglyceridemia, and hypoalphalipoproteinamia have all been described as risk factors for type 2 diabetes in most populations, including the U.S. Pima Indians [21, 90, 96]. However, it is generally accepted that the metabolic syndrome is a

predictor of type 2 diabetes in U.S. Pima Indians independent of its individual components, although more information in different populations needs to be generated [90]. In the Mexican Pima Indians, information regarding the metabolic syndrome and whether it is influenced by a traditional lifestyle is lacking although differences in the expression of the components of this syndrome has been reported in different populations. For instance, hypertension has been consistently associated with insulin resistance in whites, but not in American Indians or African-Americans [97]. Furthermore, obesity is particularly prevalent in the U.S., whereas hypertension is particularly prevalent in European populations [88, 98].

1. Definition of metabolic syndrome

Metabolic syndrome refers to a cluster of several metabolic abnormalities considered independent risk factors for cardiovascular disease and type 2 diabetes in patients, which then may be at increased risk for these diseases when two or more of these factors co-occur in the same individual [61, 87, 92]. There are multiple working definitions of metabolic syndrome that have been described by various health organizations; although there is controversy as to what components constitute the syndrome, the most widely recognized definitions include impaired glucose metabolism or insulin resistance, central obesity (except the AACE/ACE), dyslipidemia, and elevated blood pressure [61, 87, 92].

Definitions of the metabolic syndrome include those proposed by the World Health Organization (WHO) [93], the European Group for the Study of Insulin

Resistance (EGIR) [99], the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) [86, 100, 101], the American Association of Clinical Endocrinologist / American College of Endocrinology (AACE/ACE) Task Force on Insulin Resistance Syndrome in 2003 [94, 102] and most recently, the International Diabetes Federation (IDF) [93] (Table 1). Both the WHO and EGIR definitions include either glucose intolerance or insulin resistance as an essential component while the NCEP ATP III and the IDF definitions do not. The number of definitions and lack of standardized criteria have been barriers to epidemiological studies and have contributed to the growing confusion around how best to define the metabolic syndrome [61].

a. WHO definition

In 1998, the WHO proposed a consultation group to develop the first operational and internationally recognized definition of the metabolic syndrome with the primary goals of achieving agreement on definitions as well as to provide a tool for clinicians and researchers [61, 87, 92]. The WHO criteria required the presence of insulin resistance and/or its surrogates, impaired glucose tolerance or diabetes, which is proposed to play a central role on the syndrome, and at least two or more additional factors from the following: obesity (BMI>30 kg/m^2 and/or waist-to-hip ratio:>0.90 in men and > 0.85 in women); elevated plasma triglycerides:≥150 mg/dL (1.7 mmol/L), and/or low HDL cholesterol: <35 mg/dL (0.9 mmol/L) in men and <39 mg/dL (0.9 mmol/L) in women; elevated arterial pressure:≥160/≥90 mm Hg, and microalbuminuria: urinary albumin excretion rate≥20 μg/min or albumin:creatinine ratio≥20 mg/g [93].

Table 1. Current definitions of the metabolic syndrome.

WHO 1999	EGIR 1999	ATPIII 2001	IDF 2005
Diabetes or impaired glucose tolerance or insulin resistance* Plus two or more of the following: 1. Obesity: BMI>30 kg/m^2 or waist-hip-ratio>0.90 (M) >0.85 (F) 2. Dyslipidaemia: triglycerides≥150 mg/dL (1.7 mmol/L) or HDLC<35 mg/dL (0.9 mmol/L) (M) or <39 mg/dL (1.0 mmol/L) (F) 3. Hypertension: blood pressure≥140/90 mmHg or medication 4. Microalbuminuria: albumin excretion≥20 μg/min or albumin:creatinine ratio≥30 mg/g	Insulin resistance† or hyperinsulinaemia (only non-diabetic subjects) Plus two or more of the following: 1. Central obesity: waist circumference≥94 cm (M), ≥80 cm (F) 2. Dyslipidaemia: triglycerides>177 mg/dL (2.0 mmol/L) or HDLC<40 mg/dL (1.0 mmol/L) 3. Hypertension: blood pressure≥140/90 mmHg or medication 4. Fasting plasma glucose≥110 mg/dL (6.1 mmol/L)	Three or more of the following: 1. Central obesity: waist circumference>102 cm (M), >88 cm (F) 2. Hypertriglyceridaemia: triglycerides≥150 mg/dL (1.7 mmol/L) 3. Low HDL-C: <40 mg/dL (1.03 mmol/L) (M), <50 mg/dL (1.29 mmol/L) (F) 4. Hypertension: blood pressure≥130/85 mmHg or medication 5. Fasting plasma glucose≥110 mg/dL (6.1 mmol/L)	Central obesity‡: Waist circumference (ethnicity specific) Plus any two of the following: 1. Raised triglycerides≥150 mg/dL (1.7 mmol/L) or specific treatment for this abnormality. 2. Reduced HDL-C<40 mg/dL (1.03 mmol/L) (M) <50 mg/dL (1.29 mmol/L) (F) or specific treatment for this abnormality 3. Hypertension: blood pressure> 130/85 mmHg or medication. 4. Fasting plasma glucose≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes§

*Defined under hyperinsulinaemic euglycaemic conditions, glucose uptake below lowest quartile for background population. †Defined as the top quartile of fasting insulin in the non-diabetic population. ‡If BMI is>30 kg/m^2 then central obesity can be assumed, and waist circumference does not need to be measured. §In clinical practice, IGT is also acceptable, but all reports of the prevalence of the metabolic syndrome should use only the fasting plasma glucose and presence of previously diagnosed diabetes to assess this criterion. If fasting plasma glucose is above 5.6 mmol/L, OGTT is strongly recommended but is not necessary to define the presence of the syndrome. WHO=World Health Organization; EGIR=the European Group for the Study of Insulin Resistance; the American college of Endocrinology Task Force on the Insulin Resistance Syndrome; ATPIII=National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III); IDF=International Diabetes Association; F=female; M=male.

In 1999, the WHO definition was modified by reducing the threshold values for blood pressure from ≥160/≥90 to ≥140/≥90 mmHg and by increasing the albumin:creatinine ratio from ≥20 to ≥30 mg/g [16]. The core of the WHO definition is the biological and physiological significance of insulin resistance while the main goal of the definition is to detect people who have insulin resistance in combination with two or more other abnormalities [16, 87]. Impaired glucose tolerance requires an oral glucose tolerance test and precise measurements of insulin resistance require an insulin clamp study, which is of limited practical value. Since the WHO definition criteria require the presence of insulin resistance and/or impaired glucose tolerance, this definition is impractical for epidemiological studies and in clinical settings [61, 87].

b. European Group for the Study of Insulin Resistance definition

In 1999, recognizing the complexity of using the euglycemic clamp to measure insulin sensitivity, especially in diabetic patients, the European Group for the Study of Insulin Resistance (EGIR) proposed a modified version of the WHO definition, excluding diabetic individuals and using fasting insulin as a proxy measure for insulin resistance instead of the euglycemic clamp to estimate insulin resistance, as well as impaired fasting glucose as a substitute for impaired glucose tolerance measured with the oral glucose tolerance test. In addition, the EGIR suggested that as the syndrome includes non-metabolic features, a more appropriate term would be insulin resistance syndrome instead of metabolic syndrome [87, 92, 99].

Using the EGIR definition, an individual is diagnosed with the insulin resistance syndrome when the presence of insulin resistance or hyperinsulinemia (defined as the top quartile of fasting insulin in the non-diabetic population) plus two or more of the following risk factors: central obesity: waist circumference ≥94 cm in male or ≥80 cm in female; dyslipidemia: triglycerides >177 mg/dL (2.0 mmol/L) or HDL-cholesterol <40 (1.0 mmol/L); hypertension: systolic/diastolic blood pressure ≥140/≥90 mm Hg or medication; and fasting plasma ≥110 mg/dL (6.1 mmol/L); however, this definition does not apply to diabetics [99].

The core of the EGIR definition is that insulin resistance or hyperinsulinemia is identified as having ≥75% (top quartile) of the value of the fasting insulin concentration in the non-diabetic population. The EGIR also modified the WHO definition by using the waist circumference, rather than the waist-to-hip ratio, to define central obesity and by raising the triglycerides cutoff value for dyslipidemia. A simple cutoff value of HDL-cholesterol was used for both men and women. In addition, receiving medical treatment was included in the definition for hypertension and dyslipidemia. The EGIR definition of the metabolic syndrome was restricted to non-diabetic people, however [99].

c. National Cholesterol Education Program Adult Treatment Panel III definition.

In 2001, the NCEP ATP III provided a working definition of the metabolic syndrome, which was planned to be simple, allowing its widespread use directed to prevention of CVD and type 2 diabetes. This definition is based on five commonly measured clinical criteria that clinicians could implement in their clinical practices to

identify people at high risk of CVD and type 2 diabetes. According to the NCEP ATP III definition, an individual is diagnosed with the metabolic syndrome if 3 or more of the following 5 risk determinants are present: abnormal waist circumference: >102 cm (40 in) in men or >88 cm (>35 in) in women; high triglycerides levels: ≥150 mg/dL (1.7 mmol/l); low HDL-cholesterol levels:<40 mg/dL in men (1.03 mmol/l) or <50 mg/dL in women (1.29 mmol/l); high blood pressure: ≥130/≥85 mm Hg, or high fasting plasma glucose concentration: ≥110 mg/dL (6.1mmol/l). Under this definition, all five of its components are of equal importance and does not require measurements of insulin sensitivity [86].

The fasting glucose level was later modified from 110 (6.1 mmol/l) to 100 mg/dL (5.6 mmol/l) according to the recommendations of the American Diabetes Association [100]. More recently, the American Hearth Association/National Heart, Lung, and Blood Institute further modified the NCEP ATP III definition by incorporating drug treatment for elevated triglycerides or reduced HDL-cholesterol, antihypertensive drug treatment in a patient with history of hypertension, and drug treatment for elevated glucose as alternative criteria to those cutoff values for the clinical diagnosis of metabolic syndrome [101].

Waist circumference was used to measure abdominal obesity, and the cutoff values from men and women were higher than those suggested by the EGIR. The cutoff value of triglycerides was the same as that suggested by the WHO, and the cutoff value of HDL-cholesterol was specific for men and women and was higher than those suggested by the WHO consultation group. In addition, the threshold blood pressure

values used by the NCEP ATP III were lower than those used by both the WHO and the EGIR [86].

The NCEP ATP III definition is recognized as a preferred tool by clinicians due to its simplicity since it includes fasting glucose in addition to other criteria commonly measured in a clinical practice [100]. In addition, it is considered less glucocentric than the definition of the WHO and EGIR. Finally, it is most commonly used in medical research because its five components can be easily measured in large epidemiologic studies. However, because the definition does not include a component for insulin resistance, it may not identify all people with insulin resistance. In addition, the cutoff value of waist circumference for defining central obesity may not apply to all ethnic groups, particularly Asians [87, 100]. Furthermore, it has also discussed that the five criteria and the cutoff values appear to be arbitrary and not evidence-based [61, 92]. Finally, the NCEP ATP III definition has a clinical strategy whereas the WHO definition has a more pathophysiological approach [88].

d. American Association of Clinical Endocrinologist/American College of Endrocrinology (AACE/ACE) definition

In 2003, the American Association of Clinical Endocrinologist/American College of Endrocrinology (AACE/ACE) Task Force on Insulin Resistance Syndrome published a position statement recommending that the appropriate term should be the insulin resistance syndrome. Under this definition insulin resistance is the core feature of the syndrome, aimed at identifying individuals with insulin resistance. The AACE/ACE

definition was created as a modification of the NCEP ATP III definition and it meant to be more an explanation of the mechanisms of the syndrome, rather than another competing definition [92, 94, 102].

The AACE/ACE definition relies strongly on clinical judgment rather than satisfying specific criteria and list four clinical components. Thus, under this definition, an individual is diagnosed with the insulin resistance syndrome if at least one of the following factors is present: diagnosis of CVD, hypertension, polycystic ovarian syndrome, nonalcoholic fatty liver disease or acantosis nigricans; family history of type 2 diabetes, hypertension, or CVD; history of gestational diabetes or glucose intolerance; non-Caucasian origin; sedentary lifestyle; BMI>25 and/or waist circumference >102 cm (40 in) in men or >88 cm (>35) in women; age >40 years; plus any 2 or more of the following 4 risk determinants: high triglycerides levels: ≥150 mg/dL (1.7 mmol/L); low HDL-cholesterol levels:<40 mg/dL in men (1.03 mmol/L) or <50 mg/dL in women (1.29 mmol/L); high blood pressure: ≥130/≥85 mm Hg, and impaired fasting plasma glucose concentration: 110-125 mg/dL or impaired glucose tolerance: 140-200 mg/dL (7.8-11.1 mg/dL) [94, 102].

In comparison with the NCEP ATP III definition, the AACE/ACE does not include obesity or abdominal obesity as a component, since abdominal obesity is considered a contributing factor of insulin resistance rather than a consequence of the insulin resistance [87]; however, the cutoff value for triglycerides, HDL-cholesterol and blood pressure are based upon the NCEP ATP III first definition [Einborn et al. 2003] as reported elsewhere [86]. Furthermore, the insulin resistance syndrome is used to describe

the cluster of abnormalities that are more likely to occur in insulin resistant/hyperinsulinemic individuals [94], rather than on CVD [102].

e. International Diabetes Federation definition

In 2005, a group of experts directed by the International Diabetes Federation released a new global definition of the metabolic syndrome, emphasizing the importance of central obesity, and allowing for differences in waist circumference cutoff points among populations of different ethnicity. The IDF group based its decision to propose a new global definition on the observation that most of the definitions of the metabolic syndrome explained above are currently in use despite their limitations in addition to the urgent need for comparison data on the metabolic syndrome measured with a common tool across countries and populations as well as the need for having a tool suitable for use in primary, specialist care, and in resource-poor settings worldwide [61, 87, 88].

The IDF is considered the most recent definition and is similar to the NCEP ATP III definition but has different cutoff values for abdominal obesity which is a prerequisite risk factor for the diagnosis of the syndrome, with the aim of identifying people at high risk for CVD and type 2 diabetes [87, 103]. The consensus group was formed by members of the IDF from all seven world regions of which it is comprised and representatives from several organizations including those who had contributed to the previous definitions.

As in the NCEP ATP III and EGIR definitions, waist circumference was used to define central obesity; however, because of racial differences in the relationship between

level of adiposity and risk of co-morbidities, ethnic-specific cutoff values, differentiated by sex, were proposed for waist measurements. For the Europids, Sub-Saharan African, Eastern Mediterranean and Middle East (Arab) population, the cutoff value was the same as that used in the EGIR (central obesity: waist circumference ≥94 cm in male or ≥80 cm in female). For South Asians, Chinese, Japanese and Ethnic South and Central Americans, specific cutoff values for central obesity with a waist circumference of ≥90 cm in males and ≥80 cm in females are proposed [103]. The cutoff values of the remaining components were identical to those reported by the original NCEP ATP III definition except for glucose, where the cutoff value of 100 mg/dL (5.6 mmol/L) was used instead, according to the NCEP ATP III modified version in 2004 [100]. Under these characteristics, this new metabolic syndrome definition may be potentially more flexible for use in many settings than previous definitions, particularly in diverse populations.

In the IDF definition, central obesity was given a pivotal position due to its association with CVD and the other metabolic syndrome components as well as its recognized importance as an important determinant in the causative pathway of the metabolic syndrome. Thus, under this definition, metabolic syndrome is diagnosed if at least central obesity (specific for ethnic group and sex) is present, in addition to any two or more of the following criteria: high triglycerides: ≥150 mg/dL (1.7 mmol/L) or medication; low HDL-cholesterol:<40 mg/dL (1.03 mmol/L) in males or <50 mg/dL (1.29 mmol/L) in females or medication; hypertension with blood pressure ≥130/≥85 mm

Hg or medication; high fasting plasma glucose: ≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes [103].

The difficulty of conducting an oral glucose tolerance test in large numbers of subjects contributes to the decision of the IDF working committee to omit OGTT as a component in this definition at the possible expense of missing individuals who would otherwise be diagnosed as having the metabolic syndrome had impaired glucose tolerance be included as a qualifying factor [87, 103], although it is considered a reasonable decision given that other included components such as waist circumference and triglycerides were so highly correlated with insulin resistance and few of those with insulin resistance would be missing [87]. The WHO and EGIR definitions have as a core feature impaired glucose regulation and insulin resistance, respectively. In contrast, the IDF definition has abdominal obesity as a core component, with different waist circumference cutoff values depending on race/ethnicity, while the structure of the NCEP ATP III definition is a simple scoring system with no single dominant component [87].

D. Risk factors for type 2 diabetes

Type 2 diabetes is a multifactorial disease known to have both genetic and environmental influences [104, 105]. Genetic influence is supported by studies completed in identical twins in comparison to dizygotic twins which indicate a higher concordance in type 2 diabetes in identical twins. Additional support regarding the role of genetics on type 2 diabetes come from observations of differences in type 2 diabetes prevalence in

populations with mixed racial background in comparison with that from their parent populations [104, 106].

Environmental influence on type 2 diabetes, however, appears to have a determinant role as suggested in a variety of epidemiologic studies [17, 105, 106]. The recent global epidemic of type 2 diabetes worldwide appears most likely explained by the rapid changes to a more “Westernized” lifestyle over the last several decades while genetic changes in such a relatively short time as an explanation for the epidemic of type 2 diabetes are less probable [17, 104]. Strongly associated with increased risk of type 2 diabetes, lifestyle-related risk factors such as total and central obesity and sedentary lifestyle are well established [17, 107]. Some components of dietary intake, however, are suggested as additional lifestyle-related factors that appear to be associated to type 2 diabetes. Dietary fiber, whole-grains and fat, as well as some markers of carbohydrate quality intake such as the glycemic index/glycemic load are among the most widely studied components of dietary intake [108, 109]. Additional factors related to type 2 diabetes include age, race/ethnicity, family aggregation and low birth weight [18].

Western lifestyle, characterized by low levels of physical activity and a diet high in saturated fat, low in carbohydrates and fiber has been associated with increased type 2 diabetes as demonstrated by migration studies and studies of secular trends [110]. Furthermore, many surveys have confirmed that increasing urbanization and industrialization is associated with an increased prevalence of type 2 diabetes because of a tendency towards low levels of physical activity and higher consumption of fat-rich

diets in urbanized societies [17, 18]. These factors are extremely common in western countries and are increasing in most developing countries [18].

1. Obesity

Obesity, a result of an imbalance between energy intake and energy expenditure, is one of the major risk factors for the development of type 2 diabetes [107, 111-113]. Although understanding the role of genetics in obesity is increasing, the rapid rise in prevalence of overweight and obesity throughout the world demonstrates that environmental changes are the major determinants of this epidemic, with genetic factors likely modifying individual susceptibility to these environmental factors [62].

Several findings support the role of obesity as an important risk factor to type 2 diabetes [17, 110]. The increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity [114]. It is widely reported that the increase in prevalence and incidence of type 2 diabetes is parallel to the increase in overweight and obesity [115-118]. A review by Wild and Byrne (2006) clearly indicated a much higher risk of type 2 diabetes in both obese women and men in comparison to people of normal weight. This review also reported that obesity is one of the main factors related to the increased risk of early onset of type 2 diabetes as well as the increased prevalence of type 2 diabetes in younger people [119].

Findings of intervention studies have demonstrated that it is possible to prevent or delay type 2 diabetes by weight reduction through lifestyle management give considerable support to the theory that obesity is an important risk factor for type 2

diabetes [107]. In addition, obesity is an epidemic in many developing populations, particularly for Pacific Islanders [17]. Both general and abdominal obesity are associated with type 2 diabetes [17, 95, 120-122]. In Mauritius, obesity, waist and waist-to-hip ratio were associated with increased risk of type 2 diabetes; among these factors, waist-to-hip ratio was the strongest predictor [95], although in a very recent analysis of the same population total obesity was as strongly correlated as central obesity [120]. Previous studies reported that waist-to-hip ratio and BMI independently predicted type 2 diabetes in this population [121]. In the U. S. Pima Indians, BMI was found to be a strong predictor of type 2 diabetes [122].

Approximately 70 to 80 percent of people with type 2 diabetes are either overweight or obese. People who are overweight have 3.8 times greater prevalence of type 2 diabetes in comparison with those of normal weight [107, 112, 123]. However, in a review by Simpson et al. (2003), a wide range of risk ratios (between 2 and 60) were reported comparing overweight *vs.* normal weight people; the highest ratios generally corresponding to the very obese in comparison to normal weight people [124].

The mechanism explaining the deleterious effect of obesity on type 2 diabetes appears to be related with increasing insulin resistance and hyperinsulinemia, mediated by the increased levels of free fatty acids or singling proteins (adipokines) in the obese person secreted by adipose tissue [60, 110]. Adipose tissue was considered in the past as simple lipid storage depot; however, to date it is well recognized as an important place where daily influx of dietary fat is buffered and where a variety of proteins or adipokines are secreted due to its autocrine, paracrine and/or endocrine functions (66). In a non-

obese person, the buffering action of adipocytes (the capacity to store the daily influx of dietary fat in adipose tissue) is reached by suppressing the release of non-esterified fatty acids into circulation and by increasing the clearance of triacylglycerol (66). However, in obese people the normal function of adipose tissue is disturbed due to increased lipid accumulation in adipocytes causing adipocyte dysfunction (decreasing the buffering action of adypocytes) and subsequent delivery of fatty acids and adipokines secretions (55, 60, 62, 66].

2. Physical inactivity

Type 2 diabetes is positively associated with physical inactivity [125]. Changes in work patterns from heavy labor to a more sedentary lifestyle, the increase in computerization and mechanization, and improved transport are just a few of the changes that have had an impact on the increase in physical inactivity [18]. Simpson et al. (2003) reviewed a variety of cross-sectional and prospective studies and concluded that the independent negative association between physical activity and type 2 diabetes reported is strongly evident. An additional finding in this review was the dose response relationship between exercise and type 2 diabetes and the fact that it is possible to reduce the risk of type 2 diabetes by 30-50% by doing moderate to vigorous exercise [124]. The importance of physical activity was additionally reviewed by van Dam RM (2003), including mechanistic, observational and interventions studies [110] supporting evidence of the protective effect of physical activity against type 2 diabetes. Additionally, strong

evidence of the capability of physical activity to prevent type 2 diabetes was demonstrated in different intervention studies in different populations [107].

The mechanisms explaining the association of physical activity and the reduced risk in type 2 diabetes are both related to the direct action of improving insulin sensitivity and indirectly through the reduction in weight [17, 110, 123, 124]. The mechanisms of improving insulin sensitivity appear to be related with increased rate of insulin-stimulated glucose transport/phosphorilation, increased activity of GLUT4 glucose transporters, capillary proliferation in muscles, a higher proportion of more insulin sensitive type of muscle fibers, increased muscle mass [110] and increased number and function of mitochondria [55].

3. Diet composition

Although diet is not considered the most important risk factor to type 2 diabetes, intake of certain dietary components have recently been associated with increased risk of type 2 diabetes [105, 107, 126-128]. Among the dietary components associated with increased risk of type 2 diabetes include low intakes of dietary cereal fiber, and whole-grain cereals as well as high intake of high-fat diets, rich in saturated fatty acids [129 130].

A variety of studies have consistently associated whole grain cereals and cereal fiber with lower risk of type 2 diabetes [110, 128]. Whole grain cereal was inversely associated with risk of type 2 diabetes in the Health Professionals Follow-Up Study [127], the Iowa Women's Health Study [131] and the Finnish Prospective Study [132].

Cereal fiber was positively associated with lower type 2 diabetes risk in the Atherosclerosis Risk in Communities (ARIC) Study [133], and the Black Women's Health Study [134], supporting other findings of the Nurse's Health Study [135], the Health Professionals Follow-Up Study [136] and the Iowa Women's Health Study [131] as well as in the in the Finnish Prospective Study [132].

Furthermore, the effect of carbohydrates on type 2 diabetes is measured through glycemic index (GI) and/or glycemic load (GL), where GI is the relative measure of postpandrial glucose to a given carbohydrate intake and GL is the mathematical product of the GI of a food and its carbohydrate content. Studies have indicated that people with high GI and/or GL diets have a greater risk of type 2 diabetes as was shown in the women from the Nurse's Health Study and in men from the Health Professionals Follow-up Study [107] and in four other studies [137]. Although the association of GI and/or GL and type 2 diabetes risk was not supported in two other studies such as the Iowa Women's Health Study [110] and the ARIC Study [133], a recent meta-analysis reported that low GI and/or low GL are associated with reduced risk comparable to that reported for whole grain and high fiber intake [137]. The Nurse's Health Study II, which included younger and middle-aged women, found a positive association between GI and diabetes risk [138]. The same positive results were demonstrated in a large prospective study in middle-aged Chinese women [139] and in the Black Women's Health Prospective Study [134].

Two suggested mechanisms have been proposed to explain why high GI foods may alter the risk of type 2 diabetes. First, the same amount of carbohydrates from high-

GI foods produces higher blood glucose concentration and higher insulin demand than low-GI foods. The increased insulin demand over time results in pancreatic exhaustion and it eventually leads to glucose intolerance and type 2 diabetes. Second, high-GI foods may directly increase insulin resistance due to an increased production of fatty acids [138, 134]

A number of studies in a variety of populations have shown a positive association between fat intake, measured as total fat and saturated fat, with type 2 diabetes. Among these studies are the San Luis Valley Diabetes Study and the Finnish and the Dutch cohorts of the Seven Countries Study. In addition, results from the Nurse's Health Study and in the Iowa Women's Health Study demonstrated a protective effect of polyunsaturated fat intake against type 2 diabetes [107]. In the case of total fat intake however, the association is not conclusive [107, 124], although evidence appears to indicate a detrimental effect of saturated fat on type 2 diabetes [110]. In fact, evidence indicates that replacing saturated fat with polyunsaturated fat will lower the risk of type 2 diabetes [105]. Dietary fat components as fatty acids appear to influence insulin sensitivity by modifying glucose metabolism by altering fatty acid composition and function of cell membranes, enzyme activity, insulin signalling, and gene expression, in addition to the effect on obesity status [105, 110].

Finally, although most studies have examined the effects of individual dietary components, as demonstrated in the above-mentioned studies, said effects are difficult to investigate in epidemiological studies because of several factors, including the complexity of the "Westernized diet" [126], the vast number of tools to obtain dietary

information [124], and the high correlation among similar dietary components [110]. Therefore, examinations of dietary patterns have been used as an alternate option. For instance, the Western dietary pattern has been identified with a substantially higher risk for type 2 diabetes [110, 140], while rural dietary patterns have been identified as protective against type 2 diabetes [140].

4. Other risks factors

Type 2 diabetes prevalence has been correlated to increased age; however, its incidence varies considerably by age. In high risk populations of similar composition, young adults between 20 and 35 years of age have an increased prevalence, whereas in other populations the prevalence is higher at older ages (55-74 years of age). In most populations the prevalence is low in people older than 74 years of age [106]. In the study published by Montonen et al. (2003), type 2 diabetes risk was positively correlated to age, where people ages 60 to 69 (RR=6.81) had almost twice the relative risk of people 50 to 59 years of age (RR= 3.73) in comparison to people < 50 years old [132].

Type 2 diabetes also appears to be associated with race/ethnicity. Different minority groups have experienced a noticeable increase in type 2 diabetes prevalence, as evidenced by many studies worldwide [17, 18]. Among these are the Pima Indians from the U.S., the Naurans from the Pacific islands of Nauru, and the Mauritians from Asia. Some other minority groups in the U.S. (Mexican-Americans and African-Americans) as well as Australian aborigines have also experienced an increase of this disease [17, 18].

In 1999-2002, the prevalence of type 2 diabetes was almost double in the African American and Mexican American populations than that in non-Hispanic white [25]. In Mexican-Americans, type 2 diabetes has almost twice the standardized prevalence as that of the white population (7.8 *vs.*13.5%); however, it has not increased since the last decade for the Mexican American population (14.0% *vs.* 13.5% standardized) [25]. American Indians have one of the highest rates of type 2 diabetes in the world. On average, American Indians and Alaska Natives are 2.2 times more likely to have type 2 diabetes than non-Hispanic whites. Some groups, such as Native Hawaiians, Asians, and other Pacific Islanders residing in Hawaii are more than twice as likely to have type 2 diabetes as the non-Hispanic white population [141].

Regarding familiar aggregation of type 2 diabetes, a review by Steyn et al (2004), showed that people who have a parent or sibling with type 2 diabetes have a 2 to 6-fold increased risk of type 2 diabetes. Although family history of type 2 diabetes may be a marker of genetic susceptibility, it also may also occur by non-genetic factors associated to lifestyle, which is similar among family members [106].

Low birth weight has also been proposed as a new and important risk factor for type 2 diabetes [142]. Hales et al. (1991) also suggested that low birth weight, a reflection of nutritional deficiency in uterus, is related to the later development of glucose intolerance, both impaired glucose tolerance and type 2 diabetes, independent of current BMI and social class. Their interpretation of these findings was that impaired function of the endocrine pancreas and other tissues involved in insulin sensitivity results from the long term effects of the nutritional deprivation affecting fetal and infant growth [143].

They suggested that this scenario applies in high-prevalence communities such as the Nauruans [142].

In the U.S. Pima Indians aged 20 to 39, the association of birth weight with type 2 diabetes was U-shaped, with higher rates of type 2 diabetes in those with high as well as low birth weights. The association with high birth weight is mainly explained by maternal diabetes during pregnancy because of abnormalities in uterus regarding metabolic fuel, while the association with low birth weight was explained as the result of selective survival of children by the genetic predisposition to type 2 diabetes and/or other metabolic syndromes [144]. The epidemiological association between low birth weight and type 2 diabetes appears well accepted [17]; however, in the U.S. Pima Indians low birth weight accounts for only 6% of the total diabetes population [144] and about 10% of adults had low birth weight [17].

F. Pima Indians in U.S. and Mexico

The U.S. Pima Indians participating in this study represent a group of people who reside in the Gila River Indian Community in Arizona (Figure 3). The ancestors of the present group of U.S. Pima Indians are believed to have lived for >2000 years in the valleys of the Gila and Salt Rivers in what is now Arizona. The Pimas probably descended from the Hohokam, who in ~200 BC moved into the Gila River valley from Mexico [6, 13].

These prehistoric people were derived from Paleoindians, who were the American Indians whose ancestors moved from Asia to North America in the first of three

migrations across the Bering land Bridge. The Hohokam developed advanced agricultural techniques, supported by the construction of an elaborated irrigation system from the Gila River. They successfully cultivated their own crops and complemented it by hunting and gathering until the end of the 19th century. However, their lifestyle changed drastically after European colonization and the establishment of indigenous reservations [6, 13].

The European immigrant diverted the Pimas' water supply, which disrupted their traditional agriculture and led to fundamental changes in their way of life. Irrigation was gradually reintroduced as a result of water allotments made upon completion of the Coolidge Dam on the Gila River in 1929. Although agriculture is now the mayor tribal industry, the type of farming has changed, with most crops being fed to livestock or sold in the market rather than being directly consumed. Currently, their lifestyle is very similar to that of the overall U.S. population, characterized by a diet rich in fat that includes more animal fat, more simple carbohydrates and less fiber, with lower levels of physical activity [6, 13]. The high prevalence of type diabetes and obesity appears to be a recent phenomenon that became apparent only in the 1950s but has continued to increase, and parallels to changes in the population's lifestyle [13].

The Mexican Pima Indians are a small group of people who live in a remote location in northwestern Mexico; specifically in the southeastern region of the state of Sonora. Most of their population is concentrated in the village of Maycoba (Figure 3), situated in the Sierra Madre Mountains, 340 km southeast of Hermosillo, Sonora [13].

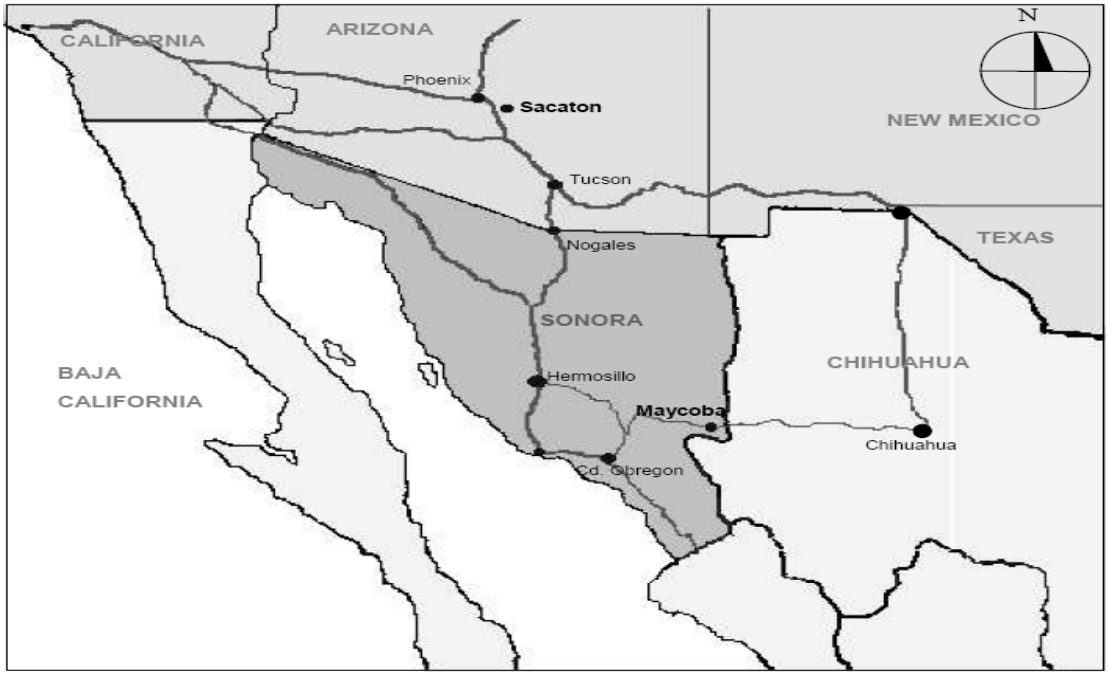

Figure 3: Map of location of the studied Pima population in U.S. and Mexico.

Archeological, anthropological and language studies suggest that Mexican Pima Indians and U.S. Pima Indians share a common ancestry and that they were separated 700-1000 years ago [13]. Genetic studies confirmed that the two groups share similar genetic background as show in Figure 4 [14, 145, 146].

The Mexican Pima Indians are still dedicated to a traditional subsistence cultivation of corn, beans, and potatoes in hill-side small parcels of land as their most important agricultural products. Complementary crops are tomatoes, green beans, chiles, onions, and peach which are planted in small fenced gardens near their dwellings. Their economy is complemented by additional subsistence activities including a very small livestock breeding industry, wood milling, and mining to a lesser degree. The Mexican Pima have lifestyle characterized y greater energy expenditure in physical labor and a traditional diet very low in fat, especially animal fat [13, 14, 147]

Figure 4 A least-squares tree for 37 populations based on 80 independent loci (41 haplotyped loci, 36 biallelic loci and 3 STRPs) with ~620 statistically independent alleles. This is the best fit found among several exact least-squares evaluations of the similar trees found by a heuristic search algorithm, improving on the neighbor-joining tree. Under the assumption of random genetic drift and no migration or selection (clearly not applicable to African Americans), the branch lengths are proportional to $t/2N_e$ (ref. 16). Because the time from the root (in Africa) to all terminals (modern populations) is the same, the increasing distance from populations in Africa to those in East Asia and the Americas represents increasing drift caused by decreasing effective population sizes. Within a geographic region, the genetic clustering of populations often parallels the linguistic clustering because stochastic factors affect both as populations diverge[19]. SF, San Francisco; TW, Taiwan; AZ, Arizona; MX, Mexico; R, Rondonian. The symbols represent bootstrap values (based on 1,000 replicates): circles, >95%; diamonds, 90–95%; triangles, 85–90%.

From reference: Tishkoff and Kidd 2004 (146).

1. Type 2 diabetes and related risk factors in Pima Indians

The U.S. Pima Indians of part of the Gila River Indian community in Arizona have been enrolled in an extensive type 2 diabetes related epidemiologic study conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Phoenix Arizona since 1965. This ongoing study was approved by the review boards of the NIDDK and by the Tribal Council. Pima Indians and the closely related Tohono-O'odham (Papago) > 5 years of age are asked to have a research examination every 2 years. Informed consent is given at each examination. Findings appear to indicate that the high prevalence and incidence of type 2 diabetes in this population parallels their changes from traditional to more modern lifestyles [6, 13].

The development of insulin resistance is a critical step in the pathogenesis of type 2 diabetes. Several prospective studies have shown that insulin resistance and fasting serum insulin are independent predictors of type 2 diabetes in diverse populations [7, 58, 59] including U.S. Pima Indians [8, 9, 148], a population reported to have the highest prevalence of type 2 diabetes and obesity in the world [6]. A prospective study aimed at investigating the pathogenesis of type 2 diabetes in this population has characterized this disease by obesity, insulin resistance, insulin secretory dysfunction, and increased rate of endogenous glucose production [8, 149].

Individuals from diverse populations with metabolic syndrome, characterized by a cluster of central obesity, hypertension, dyslipidemia, and hyperglycemia [86], have an elevated risk of type 2 diabetes [11, 89, 90], including the U.S. Pima Indians [10], even after adjusting for insulin resistance [150]. A high prevalence of metabolic syndrome

(31%) was reported in U.S. Pima Indians [10]. The etiologic factor of this syndrome is not yet well established [90] and appears complicated, with several different factors probably involved, many related to changes in lifestyles [89].

In addition, most of the individual variables such as insulin resistance, obesity, central obesity, hypertension, hypertriglyceridemia, and hypoalphalipoproteinamia have all been described as independent risk factors for type 2 diabetes in most populations, including the U.S. Pima Indians [10, 21, 90, 96]. However, more information in different populations needs to be generated [90]. In the Mexican Pima Indians, information regarding insulin resistance, the metabolic syndrome, and additional type 2 diabetes risk factors and how they are influenced by a traditional lifestyle is lacking.

During the period from 1995 to 1997, a cross-sectional study was completed to study differences in prevalence of type 2 diabetes and related risk factors between Mexican Pima Indians and U.S. Pima Indians 20 years of age or older. The age–and sex-adjusted prevalence for type 2 diabetes was 6.9% and 38.0%, for Mexican Pima Indians and U.S. Pima Indians respectively [14]. Regarding lifestyle differences, Mexican Pima Indians have much higher physical activity [151] and a diet lower in fat and higher in fiber and complex carbohydrates than U.S. Pima Indians (14, 147]. Differences in type 2 diabetes in these two groups have been attributed largely to differences in obesity, which in turn has been attributed to differences in lifestyle [14].

Additional support to further explain differences in type 2 diabetes between Mexican and U.S. Pima will be gained by studying the influence of lifestyle factors on risk factors already shown to be predictive factors for type 2 diabetes in the U.S. Pima

Indians. A complementary objective of the cross-sectional study completed during the period from 1995 to1997 was to examine some physiologic, metabolic, demographic, physical and lifestyle habit determinants to type 2 diabetes already studied in the U.S. Pima Indians such as insulin resistance, metabolic syndrome and its components, as well as additional possible risk factors such as age, sex, weight, obesity (BMI), percent body fat, waist circumference, fasting and two hours glucose and insulin, triglycerides, blood pressure, total and HDL-cholesterol, as possible individuals contributors to differences observed between Mexican Pima Indians and U.S. Pima Indians with respect to prevalence of type 2 diabetes.

PRESENT STUDY

I. Research design and methods

The methods, results, discussion, and conclusions of this study are presented in the three papers appended to this dissertation. The following is a summary of the methods and most important findings in these papers.

A. Overview

A cross-sectional study of Mexican (n=224) and U.S. (n=887) Pima Indians was completed during the period from 1995 to 1997 under the grant funded by the National Institute of Health Grant DK-49957 ("NIDDM and Obesity in Pima Indians: Environment versus Genetics"). This original study proposed to compare the prevalence of type 2 diabetes and obesity in Pima Indians living in Arizona (data already available) in an affluent environment to Pima Indians living under markedly contrasting conditions in a remote, mountainous region of Norwest Mexico. To accomplish the previously proposed goal, the study collected type 2 diabetes and obesity related information in the Mexican Pima Indians and compared to similar information already available on the U.S. Pima Indians (n= 887).

Results of this study showed that Mexican Pima Indians have a much higher physical activity [14, 151], a diet lower in fat and higher in fiber and complex carbohydrates in comparison to the U.S. Pima Indians [14]. Mexican Pima Indians also have an age- and sex-standardized prevalence of type 2 diabetes at least 5 times lower than that U.S. Pima Indians [14]. Differences in type 2 diabetes in these two genetically

related populations have been attributed largely to differences in body weight and body fat probably due to differences in diet and physical activity [14].

In the present dissertation proposal, a database containing 224 Mexican and 887 U.S. Pima Indians from the study: “NIDDM and Obesity in Pima Indians: Environment versus Genetics”, were used to test several hypotheses regarding physiologic, metabolic, demographics, physical and lifestyle risk factors already known to predict type 2 diabetes in the U.S. Pima Indians and how they are influenced by differences in lifestyles. The following sections describe the study design, subject recruitment, and methods for this study.

B. Populations

The Mexican Pima Indians is a small group of people who live in a remote location in northwestern Mexico; specifically in the southeastern region of the state of Sonora. Most of their population is concentrated in the village of Maycoba, situated in the Sierra Madre Mountains, 340 km southeast of Hermosillo, Sonora at an altitude of 1400 to 1800 m. The access to this region is from Hermosillo or Ciudad Obregon. In spring of 1992, a winding, paved road was completed and inaugurated, allowing the journey from Hermosillo to Maycoba that takes 5-6 hours [13].

Results of a census completed during the period from 1992 to 1993 as part of the preliminary information obtained in the village of Maycoba and the surrounding communities are presented in Figure 5. Most of the people were concentrated in the village of Maycoba (38 families and 222 people) and El Kipor (12 families and 74

people). Surrounding communities were also Mexican Pima Indians live are El Llano (5 families and 10 people), Maycobita (1 family and 5 people), Agua Fria (4 families and 14 people), Santa Rosa (1 family and 8 people), La Minita (1 family and 1 person), Encinal 1 (8 families and 48 people), Encinal 2 (5 families and 36 people), Los Alisos (3 families and 26 people), Junta de Jimenez (1 family and 2 people), El Duraznito (2 families and 5 people), Cieneguita (3 families and 19 people), Tierra Panda (4 families and 20 people), Cueva Prieta (2 families and 6 people), and La Dura (5 families and 22 people).

U.S. Pima Indians participating in this study represent a group of people who reside in the Gila River Indian Community in Arizona. The ancestors of the present group of U.S. Pima Indians developed an advanced agriculture, supported by the construction of an elaborated irrigation system from the Gila River. They successfully cultivated their own crops and complemented it by hunting and gathering. However, their lifestyle changed drastically after European colonization and the establishment of reservation. Currently, their lifestyle is much similar to that of the overall U.S. population [6].

Study subjects

1. Mexican Pima subjects

The project “NIDDM and Obesity in Pima Indians: Environment versus Genetics” which include 224 Mexican and 887 U.S. Pima Indians and completed during the period from 1995 to 1997 have been reported previously as a population-based cross-sectional study aimed at measuring the prevalence of type 2 diabetes and obesity in the Pima Indians from Mexico and U.S. [14, 147, 151].

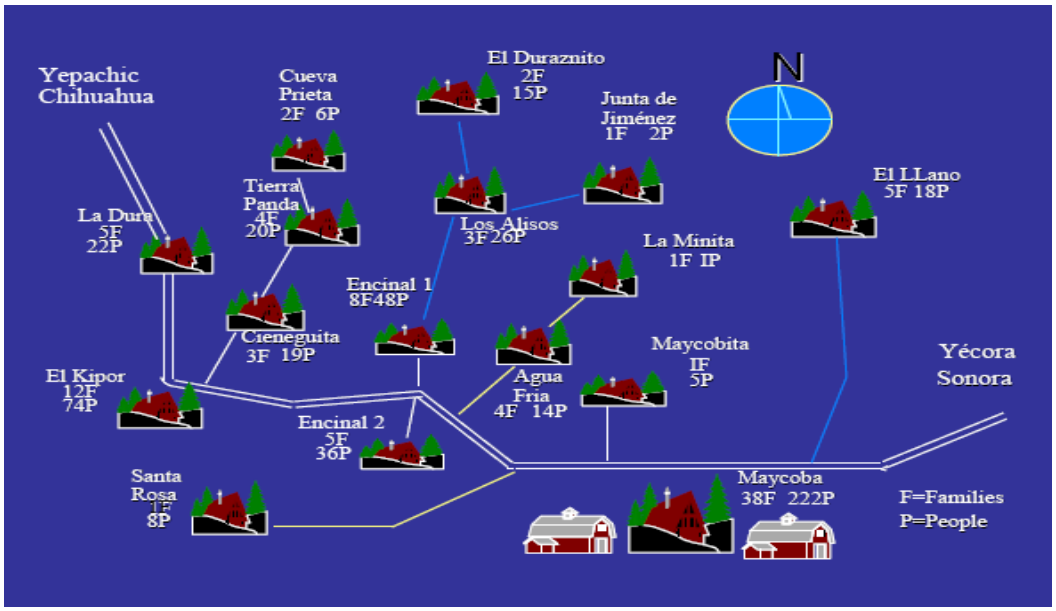

Figure 5. Distribution of the Mexican Pima Indians communities (1992 to 1993).

All potential subjects living in Maycoba, Sonora in Mexico and surrounding areas, 20 years of age and older, were visited in their homes and invited to participate in a health examination at a research clinic in the village of el Kipor, 10 Km east of Maycoba (Table 2). The health examination included a set of physical measurements, such as anthropometrics [weight, height, waist circumference, and body composition (percent body fat)], systolic and diastolic blood pressure, and blood draws to measure glucose, insulin, and lipid profile (total and high density lipoprotein (HDL) cholesterol and triglycerides). A final set of questionnaires was also included related to assess socio-demographic, medical history, personal antecedents of diabetes mellitus and hypertension, current medication, and physical activity [14, 147, 151].

All examinations were conducted by experienced and trained Spanish speaking interviewers and technicians, following the same protocol in both Pima populations. In addition, all biological laboratory analyses were conducted in a central laboratory at the Phoenix Epidemiology and Clinical Research Branch of the National Institute of Health in Phoenix following standard procedures [6, 14].

2. U.S. Pima subjects

Since 1965, a longitudinal study of type 2 diabetes and its complications has been conducted in the Gila River Indian Community by the National Institute of Diabetes and Digestive and Kidney Diseases. Approximately every two years, each resident of this community who is at least 5 years old is invited to participate in examinations conducted at the study clinic located in the community [6, 14].

At each examination, a 75-g oral glucose tolerance test is performed, in which venous serum insulin and plasma glucose concentrations are determined after an overnight fast and 2 hours postload. In addition, at each examination, anthropometrics [weight, height, waist circumference, and body composition (percent body fat)], systolic and diastolic blood pressure, lipid profile (total and high density lipoprotein (HDL) cholesterol and triglycerides), socio-demographic, medical history and physical activity are also performed [6, 14].

Based on this ongoing epidemiological study, 887 U.S. Pima Indians were included for comparison with the Mexican Pima Indians regarding prevalence of type 2 diabetes and obesity. Thus, in the project "NIDDM and Obesity in Pima Indians: Environment versus Genetics", 887 U.S. Pima participants who were 20 years of age or older and with enough information to be diagnosed with and without type 2 diabetes were selected as having been examined during a similar year period as in the Mexican Pima Indians [14].

Recruitment

In the phase of subject selection, recruiters explained the focus of the study to families and for those subjects who expressed their willingness to participate in the study, they made arrangements to transport them to the clinic at the scheduled visit. At the clinic, technicians explained the study again and obtained signed informed consent. Procedures, discomforts, risk, benefits and subjects' rights were described in the subjects' consent form. Importantly, voluntary participation was underlined as well as the facts that

if denied participation or withdrawal from the study at any point would not have any implication related with their or family health services at the local clinic or any other places. Study protocol, including consent forms were previously reviewed and approved by the Ethics Committee at Centro de Investigacion y Desarrollo, A.C. and the Institutional Review Board of the University of Wisconsin, Milwaukee.

Data management & quality control

All data were kept confidential. Test results, measurements and interview information were stored in subjects' records and maintained in a secured place with restricted access. A computerized database were created using codes with unique identifying subject ID numbers.

Data were collected from different sources such as questionnaires, data forms, and laboratories. To assure that all questionnaires and forms were completed and without errors, a check list form was included in the protocol, which needed to be filled up and reviewed by the field responsible at the end of the day of work and before subjects were transported to their homes. Laboratory results were reported in Excel files to the principal investigator and maintained in Excel files. Data entry was processed as follow: 1) first, data entry of questionnaires and forms was completed in paralleled of the field work by the principal investigators of the project in Excel files, 2) second, at the end of the field work, the staff of the project checked the data entered (Excel files) by comparing the entry information with those in each questionnaires and forms, 3) third, basic statistic were conducted to assure that each value fell into a plausible range.

Table 2. Schedule for a working day in the Maycoba Clinic

Time	**Activity**
Early morning	-Transport to clinic by field worker
7:30	-Informed consent process
7:45 am	-Brief medical history
8:00 am	-Fasting blood sample drawn, then drink sweet solution
8:00-10:00 am	-Height, weight, body composition by impedance, waist circumference, blood pressure and physical activity
10:00 am	-Second blood sample
10:10 am	-Snacks provided
10:30	-Return home
10:40	-Sample processing and storing

C. Study variables

1. Physical and biochemical measures

Weight was measured on a battery-operated electronic scale and height by a portable stadiometer. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (Kg/m^2); Central obesity was assessed by waist circumference in centimeters, measured in supine position at the level of the umbilicus. Percent body fat was estimated from bioelectrical impedance (BIA-103; RJL Systems, Detroit, MI) using an equation developed for the U.S. Pima Indians [152]. Blood pressure was measured to the nearest 2 mm Hg with a mercury sphygmomanometer (Desk Model Mercury Sphygmomanometer, model 100; Liberator Medical Supply, Stuart, FL) in the right arm while the subjects rested in a sitting position. Diastolic blood pressure was measured at the fourth Korotkoff sound. Physical activity was measured using a questionnaire developed for the U. S. Pima Indians and adapted to the Mexican Pima Indian population (14, 151). This questionnaire was previously shown to be both reliable and valid in the U.S. Pima Indian population [153, 154]; it measures leisure and occupational physical activity over the past year, which are expressed as hour per week (hour/week) and metabolic equivalent hours per week (MET-hour/week) averaged over the past year as reported elsewhere [153, 154].

Biochemical measures were in serum for fasting and 2-h post load glucose for concentrations of glucose, insulin, triglycerides, total and high density lipoprotein (HDL) cholesterol. Biochemical analyses were conducted in a central laboratory at the Phoenix Epidemiology and Clinical Research Branch. Plasma glucose concentrations were

measured with an autoanalyzer using a glucose hexokinase (Ciba Corning Express, Norwood, MA). Plasma insulin concentrations were determined using an automated radioimmunoassay analyzer (Concept 4; INCBiomedicals, Horsham, PA). Triglycerides, total and HDL-cholesterol were measured by enzymatic methods [155-157]

Some continuous variable were also expressed as categorical. Thus, participants were considered to have hypertension if they were taking antihypertensive medication or had a systolic blood pressure greater than or equal to 140 mmHg or a diastolic blood pressure of greater than or equal to 90 mmHg at the time of examination [158]. Overweight was defined as a BMI greater than or equal to 25 kg/m^2 and less than 30 kg/m^2. Obesity was defined as a BMI greater than or equal to 30 kg/m^2 [159]. Central obesity was defined as a waist circumference greater than or equal to 88 cm in women and greater than or equal to 102 cm in men. Low HDL-cholesterol was defined if values were less than or equal to 35 mg/dl in men and less than or equal to 45 mg/dl in women. High total cholesterol was defined if values were greater than or equal to 240 mg/dl. High triglyceride concentration was defined if values were greater than or equal to 150 mg/dl [86].

2. Definition of dependent variables

a. Insulin resistance

For the present analyses, insulin resistance was evaluated separately using fasting insulin as well as the homeostasis model assessment-insulin resistance (HOMA-IR) variables. The formula to estimate HOMA-IR, a relatively simple mathematical index,

requires both fasting glucose and insulin. “HOMA-IR = fasting insulin* fasting glucose/22.5, where fasting insulin is expressed in microunits per milliliters and fasting glucose in millimoles per liter” [73]. In both cases, higher scores indicate increasing insulin resistance [81].

b. Metabolic syndrome

Metabolic syndrome status was defined under the Third Report of the National Cholesterol Education Program’s Adult Treatment Panel (NCEP) criteria, which has proposed a definition that only requires readily available clinical variables [86]. In the revised NCEP, diagnosis of metabolic syndrome requires a combination of at least three of the following categorical and borderline risk factors: central obesity (waist circumference >102 cm in men and >88 cm in women), high triglycerides [≥1.7 mmol/l (≥150 mg/dL)], low HDL cholesterol [<1.03 mmol/l (<40 mg/dL) in men and <1.29 mmol/l (<50 mg/dL) in women], high blood pressure (≥130/≥85 mm Hg or pharmacological treatment of hypertension), and fasting glucose [≥6.1mmol/l (≥110 mg/dL) or current treatment with antidiabetic medications or insulin [100].

c. Type 2 diabetes

Subjects were considered to have diabetes if they have either of the following: 1) self-report of a physician diagnosis with currently on therapy (either oral or insulin); 2) fasting plasma glucose level was greater than or equal to 140 mg/dl (7.8mmol/l); 3) two-hour plasma glucose level was greater than or equal to 200 mg/dl (11.1mmol/l) [16].

D. Statistical analysis

Frequency of responses for variables related to demographic, physical and biochemical measurements were compared between groups (Mexican and U.S. Pima Indians) using either chi-square tests (for categorical data) or independent t-tests (for continuous variables). Variables with skewed distribution were either log transformed before analysis or tested by a non-parametric Mann-Witney test.

For all hypotheses, a series of multivariate models (linear and logistic) were constructed including variables significantly associated ($p<0.05$) with the dependent variables of interest (insulin resistance, metabolic syndrome and type 2 diabetes) using stepwise analysis in its forward option or known to be biologically important to the dependent variable. Interaction terms between the group variable (Mexican and U. S. Pima Indians) and selected covariates were tested for each model. In addition, models were evaluated for lineal or logistic models assumptions. All analyses were performed using STATA software (version 8.0; Stata Corp, College Station, TX). Statistical significance of the testing was assessed by p-values of $p<0.05$ and $p<0.1$ for significance of selected covariates and their interaction terms, respectively.

1. Hypothesis #1 (Manuscript #1). Insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

The hypothesis was evaluated in 194 Mexican and 449 U.S. Pima Indians with normal glucose tolerance diagnosed by the oral glucose tolerance test using a 75 g

glucose load after 10-12 hour fasting according to World Health Organization recommendations [16]. The response variable was insulin resistance measured by fasting insulin (microunits per milliliters) and by homeostasis model assessment-insulin resistance (HOMA-IR), as previously defined [73]. In addition, a subset of Mexican and U.S. Pima Indians was selected, pair-matched on the basis of age, sex and percent body fat (n=79 pairs) to confirm the hypothesis.

Differences in physical and biochemical characteristics between Mexican and U.S. Pima Indians were tested by independent t-test. Variables with skewed distribution were log transformed before analysis. Multiple linear regression analysis was used to assess differences between groups in insulin resistance by fasting insulin and HOMA-IR in separate models. Multivariate models were constructed by stepwise model selection methods in its forward option and using a p-value of 0.05. In addition, preliminary models were evaluated for possible interaction by testing all the possible combinations of first degree product terms interactions with the group population one by one. The final models were tested to evaluate linear regression assumptions and corrections were implemented when necessary. Linearity of continuous variables was evaluated. Based on graphical results, it was decided to use BMI and waist circumference as categorical variables. BMI was categorized in three categories according to World Health Organization Criteria [159] identifying individuals as normal (BMI< 25), overweight (BMI≥25 and <30), or obese (BMI≥30). Waist circumference was categorized into tertiles. In addition, differences in insulin resistance were also analysed in a pair-matched subset (n=79) by paired t-test.

2. Hypothesis #2 (Manuscript #2). The metabolic syndrome will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

In order to test this hypothesis, analyses were conducted in 224 Mexican and 447 U.S. Pima Indians who had data on the components necessary for the metabolic syndrome diagnosis. Thus, the response variable was a dichotomized variable defined as the presence or not of the metabolic syndrome [86, 100].

Descriptive statistics such as means and standard deviations (median and 95% CI for skewed variables) for continuous variables and proportions for categorical variables were compared using independent t-test (Mann-Witney test for median comparison) and chi-square test, respectively. To analyze the individual association of metabolic syndrome with age, sex, weight, height, BMI, percent body fat and physical activity (MET-hours per week), non adjusted logistic regression analyses were applied for each covariate, stratified by population group. Odds ratios (OR) and 95% confidence intervals were calculated.

In multivariate logistic regression, the likelihood of having metabolic syndrome in the U.S. Pima Indians compared to Mexican Pima Indians was further assessed after adjustment for covariates. Multivariate logistic models were constructed by stepwise model selection methods in its forward option and using a p-value of 0.05, as the criterion to allow covariates in the model. Non-significant variables considered of physiologic importance such sex were also included in the final model. The preliminary model was evaluated for possible interaction by testing all the possible combinations of first degree product terms interactions with the group population one by one and using lr-test and p-

value of 0.1. The model was evaluated for goodness-of-fit using Hosmer-Lemeshow statistic and the receiver operating characteristic (ROC) curve. Furthermore, assumptions of the logistic regression were also evaluated and corrections were implemented in necessary cases. Linearity in the log odds of continuous variables was evaluated based on graphical results.

In addition, differences in physical characteristics were analysed between the groups who had data on the components necessary for the metabolic syndrome diagnosis (n=447) and the group who did not have enough data on the components necessary for the metabolic syndrome diagnosis (n=440) by paired t-test.

3. Hypothesis #3 (Manuscript #3). Variables independently associated with type 2 diabetes status will be different between Mexican and U.S. Pima Indians.

In order to test this hypothesis, analyses were conducted in 224 Mexican and 887 U.S. Pima Indians. The response variable was a dichotomized variable defined as the presence or absence of type 2 diabetes [16]. Mean and standard deviations or geometric means [95% confidence interval] were generated for physical and biochemical characteristics.

Association between type 2 diabetes and risk factors of interest were assessed separately for U.S. and Mexican Pima Indians using logistic regression analysis and the odds ratio (OR). In addition, a first degree product term interactions between group (Mexican and U.S. Pima Indians) and the corresponding variable was added to each models in order to test the null hypothesis of no differences between the groups regarding

the association of the above-mentioned variables with type 2 diabetes. Differences in association between Mexican and U.S. Pima Indians were considered when p-values for the corresponding interaction term were smaller than or equal to 0.1

Multiple logistic regression was used for Mexican and U.S. Pima Indians to ascertain the group of variables independently associated to type 2 diabetes prevalence. Multivariate models were constructed by stepwise model selection methods in its forward option and using a p-value of 0.05. Non significant variables considered of physiologic importance such as sex was also included in the final model. In addition, preliminary models were evaluated for possible interaction by testing all the possible combinations of first degree product terms interactions with the group population one by one and using p-value of 0.1. Models were finally evaluated for goodness-of-fit using Hosmer-Lemeshow statistic and the receiver operating characteristics (ROC) curve. Furthermore, assumptions of the logistic regression were also evaluated and corrections were implemented in necessary cases. Linearity in the log odds of continuous variables was evaluated. Based on graphical results in each adjusted population model, it was decided to dichotomize age to correct for non linearity relationship.

II. Results

This dissertation proposal used data from 224 Mexican and 887 U.S. Pima Indians from a cross-sectional study funded by the National Institute of Health Grant DK-49957 ("NIDDM and Obesity in Pima Indians: Environment versus Genetics"). Three different hypotheses were tested, each one corresponding to a separated manuscript. The three manuscripts are titled as follows: 1) "Differences in Insulin Resistance in Mexican and U.S. Pima Indians with Normal Glucose"; 2) "Metabolic syndrome in the Mexican and U.S. Pima Indians: The importance of physical activity"; and, 3) "Risk factors associated with type 2 diabetes in Mexican and U.S. Pima Indians".

Hypothesis #1 was tested in persons with normal glucose tolerance (fasting plasma glucose <126mg/dl and 2h plasma glucose <140mg/dl). Thus, the subjects who met this criterion from the original database above-mentioned were 194 Mexican Pima Indians and 449 U.S. Pima Indians. Hypothesis #2 was tested in persons who had data on the metabolic syndrome components. Subjects who who had enough data on the components necessary for the metabolic syndrome diagnosis under the Third Report of the National Cholesterol Education Program's Adult Treatment Panel criteria were 224 Mexican Pima Indians and 447 U.S. Pima Indians. Finally, hypothesis #3 was tested in persons who had enough information to be diagnosed with type 2 diabetes (self-reported physician diagnosed with current therapy, either oral or insulin or if fasting plasma glucose level was greater than or equal to 140 mg/dl, or if two-hour plasma glucose level was greater than or equal to 200 mg/dl). Subjects who had enough information to be diagnosed with or without diabetes were 224 Mexican Pima and 887 U.S. Pima Indians.

1. Hypothesis #1 (Manuscript #1). Insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

Detailed results and discussion of the analysis of insulin resistance and associated variables in Mexican and U.S. Pima Indians are presented in Appendix A (a long version of the manuscript, "Differences in Insulin Resistance in Mexican and U.S. Pima Indians with Normal Glucose Tolerance") and appendix A1 (a shorted version of the first manuscript, published as a brief report at the *Journal of Clinical Endocrinology & Metabolism* 2010 Nov; 95 (11):E258-E362 (Published ahead of print July 28, 2010 as doi:10.1210/jc.2010-0297). *Copyright 2010, The Endocrine Society.*

The study population consisted of 194 Mexican Pima Indians (94 men and 100 women) and 449 U.S. Pima Indians (203 men and 246 women) with normal glucose tolerance (fasting plasma glucose <126mg/dl and 2h plasma glucose <140mg/dl). As shown in Table 1 (in appendix A), physical and biochemical characteristics were compared between Mexican and U.S. Pima Indians. Mexican Pima Indians were older and had higher physical activity ($p<0.0001$) but had lower BMI, percent body fat and waist circumference values than the U.S. Pima Indians ($p<0.0001$). Fasting and 2-h glucose as well as 2-h insulin and HDL-cholesterol were also significantly lower in the Mexican Pima Indians compared to U.S. Pima Indians ($p<0.0001$). There were no statistically significant differences in total cholesterol, triglycerides, systolic and diastolic blood pressure ($p>0.05$) between the groups (Mexican *versus* U.S. Pima Indians). Similar results were found when comparisons between groups were stratified by sex, except for

HDL-cholesterol and systolic blood pressure values (Table 1, Appendix E). In comparison to the U.S. Pima Indians, HDL-cholesterol was statistically significantly lower in the Mexican Pima Indian women, but similar values were observed for men. In the case of systolic blood pressure, similar values were observed for women, but lower values were found in the Mexican Pima Indians compared to U.S. Pima Indians.

Correlations between physical and biochemical measures are shown in Table 2 (in Appendix A). Fasting insulin was correlated to weight, BMI, waist circumference and total cholesterol in the Mexican Pima Indians. In the U.S. Pima Indians, all variables except total cholesterol were associated with fasting insulin concentrations and HOMA-IR. When obesity was taken into account, however, the magnitude of the association of total and HDL-cholesterol, triglycerides, systolic and diastolic blood pressure were "attenuated", and just HDL-cholesterol remained statistically significant in the U.S. Pima (partial r=-0.18, p=0.04, for both outcomes) and total cholesterol in the Mexican Pima (partial r=0.15, p=0.04, for both outcomes).

Results of the association between insulin resistance and group (U.S. Pima compared to Mexican Pima Indians) are presented in Table 3. The unadjusted analysis indicates that Mexican Pima Indians have lower fasting insulin and HOMA-IR concentrations as demonstrated by the positive coefficient for group population, taking the Mexican group as the reference. Differences in insulin resistance between groups remained significant either after adjustment for age, sex or in a full model including BMI and waist circumference (either expressed as continuous or categorical) in addition to age and sex in both outcome variables; however, in the fully adjusted model, although still

significant, differences in insulin resistance were attenuated in both groups as demonstrated by the lower value of the beta-coefficients (Table 3 and Tables 2, 3 and 4 for more detailed models in Appendix E). Furthermore, using the mean level of age (32.8 years), in the female group with BMI classified as overweight and using the second tertile of waist circumference, predicted mean concentrations of fasting insulin and HOMA-IR in Mexican Pima Indians were approximately half those of the U. S. population in the fully-adjusted model (fasting insulin: 12.75 *vs.* 5.85 μU/mL and HOMA-IR: 2.82 *vs.* 1.28) as shown in Table 3.

Similar results of the association between insulin resistance and group (U.S. Pima *vs.* Mexican Pima Indians) are presented in Figure 1 (in Appendix A1: short version of the paper) as exponentiated means and 95% confidence interval (95% CI) for male and female together. The unadjusted analysis indicates that Mexican Pima Indians have lower mean fasting insulin (4.17; 95% CI, 3.64-4.77 *vs.* 16.35; 95% CI, 14.87-17.97 μU/mL) and HOMA-IR (0.91; 95% CI, 0.79-1.05 *vs.* 3.73; 95% CI, 3.38-4.11) than U.S. Pima. Differences between groups remained significant either after adjustment for age, sex (fasting insulin: β=1.339; 95% CI, 1.172-1.506) and HOMA-IR: (β=1.387; 95% CI, 1.216-1.559) or in a full model including BMI and waist circumference in addition to age and sex (fasting insulin: β=0.779; 95% CI, 0.579-0. 979) and HOMA-IR: (β=0.785; 95% CI, 0.581-0. 989), taking Mexican group as the reference.

Table 3. Association between measures of insulin resistance (IR) and Pima Indian population (n=560)

Model	β coefficient	95% CI	Predicted IR *
Fasting Insulin (μU/mL)			
Unadjusted			
Mexican Pima Indians	reference	-	4.00
U.S. Pima Indians	1.368	1.203-1.532	16.25
Adjusted†			
Mexican Pima Indians	reference	-	4.17
U.S. Pima Indians	1.339	1.172-1.506	15.93
Adjusted‡			
Mexican Pima Indians	reference	-	5.85
U.S. Pima Indians	0.779	0.579-0.979	12.75
HOMA-IR			
Unadjusted			
Mexican Pima Indians	reference	-	0.86
U.S. Pima Indians	1.409	1.239-1.579	3.64
Adjusted†			
Mexican Pima Indians	reference	-	0.89
U.S. Pima Indians	1.387	1.216-1.559	3.58
Adjusted‡			
Mexican Pima Indians	reference	-	1.28
U.S. Pima Indians	0.785	0.581-0.989	2.82

*****Covariates set to: mean age = 32.8, gender=female, BMI=overweight, waist circumference=second tertile; †Adjusted for age and sex; ‡Adjusted for age and sex, BMI and waist circumference.

In the pair-matched subset (n=79), large differences were confirmed (fasting insulin: 3.99; 95% CI, 3.14-5.08 *vs.* 9.25; 95% CI, 7.36-11.63 μU/mL) and HOMA-IR: 0.89; 95% CI, 0.70-1.14 *vs.* 2.02; 95% CI, 1.60-2.56). Moreover, even though U.S. Pima had similar age (31.18 *vs.* 30.99 years), body fat (31.15 *vs.* 31.24%) and sex distribution than Mexican Pimas, the groups differed considerably in physical activity expressed as metabolic equivalent hours per week (U.S. Pima: 46.16; 95% CI, 32.22-66.14 *vs.* Mexican Pima: 133.93; 95% CI, 113.70-152.40, P<0.0001).

In summary, the results indicate that Mexican Pima Indians with normal glucose tolerance have lower insulin resistance in comparison to their genetic related counterparts U.S. Pima Indians, even after controlling for differences in obesity, age and sex.

2. Hypothesis #2 (Manuscript #2). The metabolic syndrome will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

Detailed results and discussion of metabolic syndrome and associated factors in Mexican and U.S. Pima Indians are presented in manuscript form in Appendix B, "Metabolic syndrome in the Mexican and U.S. Pima Indians: the importance of physical activity". This section summarizes results of this analysis as well as additional tables not included in the manuscript, but presented in Appendix E.

Socio-demographic, physical, and lifestyle characteristics are presented in Table 1 (in Appendix B). In both populations, individuals with metabolic syndrome were older, and had higher weight, BMI and percent of body fat. Physical activity was lower in individuals with metabolic syndrome in both groups (Mexican and U.S. Pima Indians).

The prevalence of metabolic syndrome and its component factors in the Mexican and U.S. Pima Indians are presented in Table 2 (in Appendix B). The unadjusted prevalence of metabolic syndrome was 2.5-fold higher in the U.S. Pima Indians than in the Mexican Pima Indians ($p<0.0001$). Likewise, higher prevalences were found in the U.S. Pima Indians for elevated fasting glucose, central obesity and high blood pressure ($p\leq0.0001$). However, the prevalences of high triglycerides and low HDL-cholesterol were similar (Table 2 in Appendix B). After adjustment for age, sex, BMI and physical activity, the likelihood of having elevated fasting glucose and central obesity remained higher in the U.S. Pima Indians ($p<0.0001$); however, no differences were shown for prevalence of high blood pressure or high triglycerides. The likelihood of having low HDL-cholesterol was lower in the U.S. Pima Indians ($p=0.002$) (Table 5, in Appendix E).

Mexican Pima Indians classified as having high physical activity (MET-hour/week) (using the values above the median) had lower prevalence of metabolic syndrome and central obesity as well as lower prevalence of HDL-cholesterol than those with low physical activity. Furthermore, the U.S. Pima Indians in this study classified as having a high physical activity (using the median) had a lower prevalence of metabolic syndrome, elevated fasting glucose, central obesity as well as lower prevalence of elevated blood pressure in comparison to people with low physical activity (Table 6, in Appendix E).

Variables significantly associated with metabolic syndrome among Mexican Pima Indians include older age (per 10 years: OR=1.5; 95% CI, 1.2-1.8), female sex: (OR=0.37; 95% CI, 0.19-0.71), greater weight (per 5 kg: OR=1.4; 95% CI, 1.2-1.6),

height (per one m: OR=0.96; 95% CI, 0.92-0.99), BMI (per 5 kg/m^2: OR=4.5; 95% CI, 2.8-7.3) and percent body fat (per 5%: OR=1.9; 95% CI, 1.6-2.4) and lower physical activity (per 30 MET-hour/week: OR=0.87; 95% CI, 0.79-0.95). In U.S. Pima Indians, variables significantly associated with metabolic syndrome were older age (per 10 years: OR=2.3; 95% CI, 1.9-2.7)], female sex: (OR=0.49; 95% CI, 0.34-0.73), greater weight (per 5 kg: OR=1.15; 95% CI, 1.1-1.2), BMI (per 5 kg/m^2: OR=4.5; 95% CI, 2.8-7.3) and percent body fat (per 5%: OR=1.9; 95% CI, 1.6-2.4) and lower physical activity (per 30 MET-hour/week: OR=0.88; 95% CI, 0.83-0.93), but not height (per one m: OR=0.98; 95% CI, 0.96-1.00) (Table 7, in Appendix E).

The likelihood of having metabolic syndrome was analyzed by multiple logistic regression using the Mexican Pima Indians as the reference group. As presented in Table 4 and (Table 8 in Appendix E for more detailed data), after adjustment for age and sex, the odds of having metabolic syndrome was 4.8 (95% CI, 3.2-7.1) times higher in U.S. Pima Indians *versus* Mexican Pima Indians. In the second model, after further adjustment for BMI (classified as BMI <25 *versus* BMI ≥ 25) along with age and sex, attenuation in the magnitude of association of metabolic syndrome between groups was found (OR=2.4; 95% CI, 1.5-3.7). In the model that included physical activity (MET-hour/week), in addition to age, sex and BMI, the odds ratio was substantially attenuated and not statistically significant (OR=1.4; 95% CI, 0.8-2.3). Sex was not significantly associated with metabolic syndrome in the final model, but was retained, although similar results were found when sex was not included in the final model (Table 8 in Appendix E). Interestingly, in the group having BMI<25 the differences in the prevalence of metabolic

syndrome between the groups was completely explained by physical activity [OR=1.05; 95% CI, 0.3-4.3).

Physical characteristics were analysed between the groups who had data on the components necessary for the metabolic syndrome diagnosis (n=447) and the group who did not have enough data on the components necessary for the metabolic syndrome diagnosis (n=440). Triglycerides and HDL-Cholesterol were the components which contributed with the greatest degree of missing values. The results indicate similar proportion of female/male and similar values of height, percent of body fat and physical activity. However, the group who had data on the components necessary for the metabolic syndrome diagnosis was younger, and had larger values of weight and BMI in comparison to the group who did not have enough data on the components necessary for the metabolic syndrome diagnosis (Table 5).

In summary, the results indicate that differences in the prevalence of metabolic syndrome exist between the Mexican and U.S. Pima Indians and that these are mostly explained by the populations' contrasting differences in obesity and physical activity.

Table 4. Adjusted comparison of metabolic syndrome prevalence between Mexican and U.S. Pima Indians

Variable	Model 1: OR (95% CI)	Model 2: OR (95% CI)	Model 3: OR (95% CI)
Group			
Mexican Pima	reference	reference	reference
U.S. Pima	4.77 (3.19-7.13)	2.37 (1.51-3.71)	1.35 (0.80-2.29)

OR, Odds Ratio (95% confidence intervals); Model 1 (n=671): adjusted for age and sex; Model 2 (n=662): adjusted for age, sex, and BMI (dichotomized as BMI<25 *vs.* BMI>=25); Model 3 (n=579): Adjusted for age, sex, BMI (as previously defined), and physical activity (METs-hr/week).

Table 5. Physical characteristics between the groups who had data on the components necessary for the metabolic syndrome diagnosis and the group who did not have enough data on the components necessary for the metabolic syndrome diagnosis

Variable	Group diagnosed with or without MS	Group with missing values*	p-value
N	447	440	
Female (%)	58.6	59.6	0.7780
Age (year)	40.2±14.0	36.9±13.1	0.0003
Weight (kg)	92.2±24.4	95.6±23.3	0.0358
Height (m)	164.4±8.4	164.5±8.3	0.8869
BMI (kg/m^2)	33.9±8.0	35.3±7.9	0.0121
Body Fat (%)	41.0±9.5	41.9±8.4	0.1475
PA (MET-hour/week)	23.7 (18-31)	22.5 (18-31)	0.8813**

BMI, body mass index; PA, physical activity; MS, metabolic syndrome; Median (95% CI); All other data are presented as mean ± SD; a t test was done with independent samples (**Mann-Whitney); *Group not diagnosed with or without the MS because of missing values in triglycerides and/or HDL-cholesterol.

3. Hypothesis #3 (Manuscript #3). Predictors variables associated with type 2 diabetes status will be different between Mexican and U.S. Pima Indians.

Detailed results and discussion of variables associated with type 2 diabetes in Mexican and U.S. Pima Indians are presented in Appendix C. This section summarizes results of this analysis as well as additional tables not included in the manuscript, but presented in Appendix E.

Demographic, physical, and biochemical characteristics in Mexican and U.S. Pima Indians are shown in Table 1 (in Appendix C). Compared to Mexican Pima Indians, all variables had higher values/concentrations in U.S. Pima Indians with the exception of age and diastolic blood pressure, which had similar values in both groups and physical activity, which was lower in U.S. Pima Indians. Variables were additionally analyzed stratified by sex and are presented in Table 9 (in Appendix E). In comparison with Mexican Pima Indian women, U.S. Pima Indians women were significantly older and significantly heavier, as determined by weight, BMI, percent body fat, and waist circumference. U.S. Pima Indian women also had higher levels of fasting and two hours post load glucose and insulin, systolic blood pressure and HDL-cholesterol. However, similar levels were found regarding total cholesterol, triglycerides and diastolic blood pressure.

In the case of men, U.S. Pima Indians were also heavier, had higher levels of fasting and two hours post load glucose and insulin, and systolic blood pressure, but similar levels of total and HDL-cholesterol, triglycerides, diastolic blood pressure and age in comparison with Mexican Pima Indians men (Table 9, in Appendix E).

In the Mexican Pima Indians, variables positively associated with type 2 diabetes were age, weight, BMI (overweight and obesity), percent body fat, waist circumference, fasting and two hours insulin, total cholesterol, triglycerides and hypertension. HDL-cholesterol and sex were negatively associated to type 2 diabetes. The associations were all significant, except for sex and total cholesterol (Table 2 in Appendix C). Although physical activity (MET-hours per week) was not significantly associated with type 2 diabetes in the Mexican Pima population (Table 2 in Appendix C), in secondary analysis stratified by sex, a significant inverse association was observed in Mexican Pima males (OR=0.71; 95% CI, 0.52-0.96), but not females (OR=1.16; 95% CI, 0.87-1.55), expressed as per 20 MET-hours per week and type 2 diabetes in men but not in women. When physical activity was dichotomized using the median (≥120 Mets-hours-week), similar results were found (Table 10, 11 and 12, in Appendix E).

Among U.S. Pima Indians (Table 2, in Appendix C), positive associations with type 2 diabetes were shown for age, waist circumference, percent body fat, fasting insulin, total cholesterol, triglycerides, and hypertension, while negative associations were found for HDL-cholesterol and physical activity (MET-hours per week). All the associations were significant, except for weight, BMI and two hours insulin. Furthermore, unlike Mexican Pima, no interaction was found between physical activity and sex ($p>0.1$). Significant interactions between the two Pima groups were observed for associations between type 2 diabetes and age, weight, fasting and two hour insulin concentrations, BMI, waist circumference and percent of body fat.

As presented in Table 6, type 2 diabetes was independently and positively associated with older age (>=50 years; OR=3.70; 95% CI, 1.03-13.27), waist circumference (per 5 cm; OR=2.60; 95% CI, 1.45-4.65) and fasting insulin (per 1 uU/mL; OR: 1.09; 95% CI, 1.03-1.15) in the Mexican Pima Indians. In the U.S. Pima Indians, type 2 diabetes was independently and positively associated with older age (>=50 years; OR=4.60; 95% CI, 2.50-8.46), higher fasting insulin (per 1 uU/mL; OR=1.02; 95% CI, 1.01-1.03), higher cholesterol (≥240 mg/dl; OR=3.62; 95% CI, 1.59-8.25) and having hypertension (OR= 1.69; 95% CI, 1.03-2.76), and negatively associated with higher physical activity (≥ 22.85 Mets-hours-week; OR=0.45; 95% CI, 0.30-0.66).

In subgroup analysis stratified by sex, physical activity (Mets-hours per week) and triglycerides were the only significant factors associated to type 2 diabetes in Mexican Pima males. The same was true when physical activity (Mets-hours per week) and triglycerides were expressed as categorical (Table 13, in Appendix E). In the case of women the only variables significantly associated to type 2 diabetes were age and obesity (waist circumference, BMI, and percent body fat) (Table 14, in Appendix E).

In summary, results indicate that risk factor patterns associated to type 2 diabetes were different in Mexican and U.S. Pima Indians. Only age and fasting insulin were common risk factors in both populations.

Table 6. Multivariate Odds ratio (95% confidence intervals) for diabetes status in Mexican and U.S. Pima Indians

Variable	**Mexican Pima N=224**	**U.S. Pima N=630**
Age (years)		
<50 *vs.* ≥50	3.70 (1.03-13.27)	4.60 (2.50-8.46)
Male *vs.* Female	0.99 (0.26-3.89)	0.77 (0.51-1.15)
Waist circumference (cm)		
Per 5 cm	2.60 (1.45-4.65)	-
Fasting insulin (uU/mL)	1.09 (1.03-1.15)	1.02 (1.01-1.03)
Cholesterol (mg/dL)		
High *vs.* normal	-	3.62 (1.59-8.25)
Hypertension		
Yes *vs.* No	-	1.69 (1.03-2.76)
PA (Mets-hrs-week)		
High *vs.* low	-	0.45 (0.30-0.66)

Data are odds ratio (95% CI); Female as reference group; [†]High cholesterol ≥240 mg/dl; Hypertension; systolic blood pressure ≥140 mmHg or diastolic blood pressure≥90 mmHg or under antihypertensive medication; PA, physical activity in U.S. Pima, high ≥ 22.85 Mets-hours-week (median).

III. Conclusions

Brief overview

The main goal of the project "NIDDM and Obesity in Pima Indians: Environment *versus* Genetics" was to identify the effect of living in contrasting lifestyles in explaining differences in the prevalence of type 2 diabetes in two genetically related Pima Indian populations (Mexican *versus* U.S. Pima Indians). The existence of two populations genetically related, but living contrasting lifestyles, provides a natural design to study the influence of lifestyle related risk factors in the development of type 2 diabetes. The U.S. Pima Indians live a Western lifestyle in Arizona, whereas the Mexican Pima Indians live under more traditional conditions in the Sierra Madre Mountains of Northern Mexico.

Because U.S. Pima Indians have a marked increased prevalence of type 2 diabetes and obesity in comparison with their closely genetically-related Mexican Pima Indians [14], in the present dissertation research we hypothesized that: 1) Insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians; 2) The metabolic syndrome will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians; and, 3) Variables independently associated with type 2 diabetes status will be different between Mexican and U.S. Pima Indians.

The findings of this study support the hypothesis that contrasting differences in lifestyle factors between Mexican and U.S. Pima Indians explain the differences in type 2 diabetes risk factors, mainly insulin resistance and metabolic syndrome as well as the

differences in factors associated to type 2 diabetes between Mexican and U.S. Pima Indians.

Hypothesis #1 (Manuscript #1). Insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

The primary finding of the present analysis was that Mexican Pima Indians are less insulin resistant than their U.S. Pima Indian counterparts. Previous reports showed that Mexican Pima Indians have a much higher physical activity [14, 151], a diet lower in fat and higher in fiber and complex carbohydrates [14] in comparison to the U.S. Pima Indians. Part of the differences in insulin resistance between groups seems to be explained by the greater degree of obesity in the U.S. Pima Indians. The difference in insulin resistance between groups, although still significant, was reduced by about 44% after adjusting for obesity. With respect to fasting insulin, the adjusted predicted mean difference between groups population was 6.9 μU/mL (unadjusted mean difference: 12.19 μU/mL) whereas that for HOMA-IR was 1.54 (unadjusted mean difference: 2.82).

Additional support is derived from the contrasting differences in obesity and central obesity between both groups, judged by the higher levels of BMI, percent of body fat and waist circumference in the U.S. Pima Indians in comparison with the Mexican Pima Indians and the much stronger association of obesity (BMI, percent of body fat and waist circumference) with insulin resistance in the U.S. Pima Indians in comparison with the Mexican Pima Indians, even after adjusting for age and sex. Similarly, Dowse et al.

1993 [160] compared fasting insulin in different Asian populations and found that differences in insulin resistance between groups, although still significant, decreased considerably after controlling for total and central obesity as compared with that seen when controlling only for age. Furthermore, and consistent with this observation, different studies have reported that obesity is one on the most important factors leading to insulin resistance [66].

Increasing age and female sex were also found to be significantly associated with insulin resistance in the total population. However, these variables explained a small amount of the difference in insulin resistance between both Pima groups. Both age and sex, however, are non-modifiable risk factors and inconsistent results have been found regarding their association with insulin resistance. In accord with our findings, an inverse association between age and insulin concentration in both non diabetic U.S. Pima Indians and Mauritius [161]. However, no association between fasting insulin and age was found in a Native Canadian population [162]. Regarding the association between sex and insulin resistance, Hunt et al. 2007 [163] showed lower fasting insulin concentrations in non diabetic women without metabolic syndrome in comparison to that in non diabetic men, but similar values in non diabetic subjects with metabolic syndrome. Conversely, Dowse et al. 1993 [160] showed that fasting insulin level was higher in women than that in men.

Difference in insulin resistance between Mexican and U.S. Pima Indians may be additionally explained by differences in physical activity and dietary intake between both groups as reported elsewhere [14, 151]. The increased physical activity in Mexican Pima than

in U.S. Pima Indians, even in those of similar age, body fat and sex may also explain part of the differences in insulin resistance. Accordingly, physical activity was associated to insulin resistance in non diabetic U.S. Pima Indians and Indians from Mauritius [161]. Dowse et al. 1993 [160], suggested that differences in fasting insulin levels among the four Asian populations may be explained by environmental factors (diet and physical activity) closely related with ethnicity.

Hypothesis #2 (Manuscript #2). The metabolic syndrome will be significantly lower in Mexican Pima Indians *versus* U.S. Pima Indians

Differences in the prevalence of metabolic syndrome exist between the Mexican and U.S. Pima Indians, with lower prevalence in the Mexican Pima Indians. Almost all of the difference in the prevalence of metabolic syndrome between the Mexican and U.S. Pima Indians can be explained by the contrasting levels in obesity and physical activity between groups. Age and sex did not explain much of the difference in the likelihood of having the metabolic syndrome between groups. The likelihood of having a metabolic syndrome in the U.S. Pima Indians was a halved in after adjustment for obesity and none statistically significant after further adjustment for physical activity.

The high prevalence of metabolic syndrome in Pima Indians confirmed data by Resnick [164] showing that American Indian men and women from the Strong Heart Study, have a 2.18-fold and a 2.45-fold higher prevalence of the metabolic syndrome when compared to men and women from the NHANES III, which include white, black and Hispanic populations [164]. Ethnicity, however, cannot be implicated for the

difference in metabolic syndrome in the present study because of the genetic similarity of the two populations [14].

A couple of studies have reported positive associations between age and obesity and metabolic syndrome and a negative association between physical activity and metabolic syndrome [165, 166]. In addition, prospective cohort studies [167, 168] have identified a low physical activity as a predictor of metabolic syndrome. Palaniappan et al. 2004 [166] investigated predictors of the metabolic syndrome and concluded that obesity was a significant predictor of the incidence of metabolic syndrome. Erikson et al. 1997 [165] reviewed the role of physical activity and exercise in the prevention and management of metabolic syndrome. The authors concluded that low levels of physical activity are related to most of the components of metabolic syndrome. Furthermore, in a large cross-sectional study, inactivity was directly associated to metabolic syndrome as well as with its components [168].

It has been suggested that most of the differences in obesity between Mexican and U.S. Pima Indians are due to their contrasting differences in physical activity levels and diet [151]. Our results indicated that physical activity is associated with metabolic syndrome independently of obesity in both populations. However in those with BMI<25, differences in physical activity and not BMI accounted for the difference in metabolic syndrome prevalence in the Mexican Pima Indians. Furthermore, our finding appears to indicate that the association between physical activity and metabolic syndrome may be explained by the direct influence of physical activity on obesity as well as by the independent effects of physical activity on the individual components of metabolic

syndrome. Mexican Pima Indians classified as having high physical activity (MET-hour/week) (using the median) had lower prevalence of metabolic syndrome and central obesity as well as lower prevalence of HDL-cholesterol than Pima Indians with low physical activity. Furthermore, the U.S. Pima Indians in this study classified as having a high physical activity (using the median) had a lower prevalence of metabolic syndrome, elevated fasting glucose, central obesity as well as lower prevalence of elevated blood pressure in comparison to people with low physical activity (data not shown).

Hypothesis #3 (Manuscript #3). Predictors variables associated with type 2 diabetes status will be different between Mexican and U.S. Pima Indians.

Risk factors pattern associated to type 2 diabetes is different in Mexican and U.S. Pima Indians. Only age and fasting insulin were common risk factors in both groups (Mexican and U.S. Pima Indians). In the Mexican Pima Indians, type 2 diabetes was independently associated with older age, higher waist circumference and fasting insulin. In the U.S. Pima Indians, in addition to older age and higher fasting insulin, type 2 diabetes was also independently associated with higher total cholesterol, hypertension and lower physical activity. In a different analysis using a cohort design, Hanson et al. reported that type 2 diabetes incidence was independently associated with insulinemia, body size, lipids and blood pressure factors in the U.S. Pima Indians [10]. However, blood pressure was only weakly associated with type 2 diabetes in this analysis [10].

Physical activity was not included in Hanson's et al. report [10]. However, in a different report from the U.S. Pima Indians cohort study [154], lower physical activity

was associated with type 2 diabetes incidence. In accordance with our findings, in the Mexican Pima Indians, results from the Mexican-American and non-Hispanic whites showed that type 2 diabetes incidence was associated with older age, higher BMI, higher fasting insulin but not with sex [11].

Insulin resistance, BMI, abdominal obesity, hypertension, hypertriglyceridemia, and low HDL-cholesterol all been associated with type 2 diabetes [21, 90]. The fact that in our analysis body size was not associated with type 2 diabetes in the U.S. Pima Indians may be explained because we are using prevalent data and the knowledge that type 2 diabetes can lead to weight reduction [169]. Knowler et al. 1991, showed a weak association between BMI and type 2 diabetes prevalence and a strong association between BMI and type diabetes incidence [169]. The weaker association between BMI and type diabetes prevalence was explained because in prevalence studies weight loss might occur after the diagnosis of diabetes due to treatment, calorie loss as glycosuria, or the high resting metabolic rate in diabetes [169].

Significance of the study

This study is unique due to the ability to establish a natural design comparing two populations genetically related, but living contrasting lifestyles in order to study type 2 diabetes and its major risk factors. This study results contributes to the understanding of the role of lifestyle in explaining the differences in type 2 diabetes risk factors and finally the differences in type 2 diabetes in the Pima Indians, a population with highly propensity to obesity and type 2 diabetes. In addition, the results are significant since they can be

used by health authorities and health educators as an example on how to prevent type 2 diabetes and its major risk factors.

Strengths and limitations

The main strength of this study is the fact that we are comparing two genetically related populations living with naturally contrasting lifestyles. Additional strengths are the facts that all the analysis followed similar measurement protocols and that all biological analyses were carried out in a central laboratory at the Phoenix Epidemiology and Clinical Research Branch. Nevertheless, our study is limited by the cross-sectional nature of the data, which do not allow the demonstration any of temporal effect of the factors studied on risk of insulin resistance and metabolic syndrome and type 2 diabetes.

An additional limitation of the study, mainly related with manuscript 2, is the large number of subjects with missing information to be diagnosed with or without the metabolic syndrome in the U.S. Pima Indians and has the potential of bias the result. However, the similar values in percent of body fat and physical activity as well as in the proportion of female/male between the groups of U.S. Pima Indians who had data on the components necessary for the metabolic syndrome diagnosis (n=447) and the group who did not have enough data on the components necessary for the metabolic syndrome diagnosis (n=440) may support the finding of the manuscript 2 that U.S. Pima Indians have 56% of metabolic syndrome. Although the group who had data on the components necessary for the metabolic syndrome diagnosis was younger, and had larger values of weight and BMI in comparison to the group who did not have enough data on the

components necessary for the metabolic syndrome diagnosis, the magnitude of the difference found may not support the idea that prevalence of the metabolic syndrome may be different to that found in the present study. Supporting the finding is the similar values in high triglycerides and low HDL-cholesterol between Mexican and U.S. Pima Indians.

Future research

Since rural communities are changing to a more Occidentalized lifestyle in Mexico, as demonstrated by the national studies results, it will be important to establish a cohort study in the Mexican Pima Indians in order to evaluate if changes in lifestyle such as a decreasing in physical activity and increased in fat intake will be associated with increasing in the incidence and prevalence of obesity and type 2 diabetes in this group. The result of this future studies will provide the health authorities with information to establish interventions programs directed to decrease obesity and type 2 diabetes in population with highly propensity to obesity and type 2 diabetes.

In conclusion, the findings underscore the importance of lifestyle in the prevention of type 2 diabetes risk factors, such as insulin resistance and metabolic syndrome, even in individuals with high propensity to develop diabetes.

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APPENDIX A: Differences in Insulin Resistance in Mexican and U. S. Pima Indians with Normal Glucose Tolerance

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Abstracts: 267

Total words number: 2,799

Number of tables: 3

RUNNING TITLE: Lifestyle and Insulin Resistance

ABSTRACT

CONTEXT: Insulin resistance is a mayor risk factor for the development of type 2 diabetes in Pima Indians, a population with the higher prevalence of type 2 diabetes in the world. Their Mexican counterpart living a traditional lifestyle in the mountain of Sonora have at least 5 times less diabetes than the U.S. Pima Indians.

OBJECTIVE: We evaluated whether Mexican Pima Indians had lower insulin resistance than U.S. Pima Indians.

DESIGN AND PATIENTS: We compared fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) in 194 Mexican Pima Indians (100 Females, 94 Males) vs. 449 U.S. Pima Indians (246 Females 203 Males) with normal glucose tolerance. Adjusted differences of the log transformed outcomes (fasting insulin and HOMA-IR) between groups were evaluated using multiple linear regression models.

RESULTS: Unadjusted fasting insulin and HOMA-IR were much lower in the Mexican Pima Indians than in their U.S. counterparts. Differences were attenuated after adjusting for obesity, age and sex, but still remained significant. For fasting insulin, the adjusted coefficient for group population was β=0.78 (95%CI, 0.58-0.98) and the adjusted mean difference between groups was 6.9 μU/mL. For HOMA-IR, the adjusted coefficient for group population was β=0.79 (95%CI, 0.58-0.99) and the adjusted predicted mean difference between groups was 1.54. Results were confirmed in subset matched for age, sex and body fat.

CONCLUSION: Our results indicate that Mexican Pima Indians have lower insulin resistance in comparison to their genetically related U.S. counterparts, even after

controlling for differences in obesity, age and sex. This finding underscores the importance of lifestyle factors as protective factors against insulin resistance in individuals with high propensity to develop diabetes.

Insulin resistance is a mayor risk factor in the pathogenesis of type 2 diabetes mellitus. Prospective studies have shown that insulin resistance is an independent predictor of type 2 diabetes (1) including the U.S. Pima Indians (2,3), a population with the highest reported prevalence of type 2 diabetes and obesity in the world (4). A longitudinal study designed to investigate the pathogenesis of type 2 diabetes in this population has characterized independent predictors of diabetes including obesity, insulin resistance, insulin secretory dysfunction, and increased rate of endogenous glucose production (2,5).

Mexican Pimas, a population living a traditional lifestyle in the mountains of northwestern Mexico, have a much higher physical activity (6,7), a diet lower in fat and higher in fiber and complex carbohydrates (6) in comparison to the U.S. Pima. Mexican Pima Indians also have an age- and sex-standardized prevalence of type 2 diabetes at least 5 times lower than that U.S. Pima Indians (6). Differences in type 2 diabetes in these two genetically related populations (8) have been attributed largely to differences in body weight and body fat probably due to differences in diet and physical activity (6). Although insulin resistance has been widely studied in the U.S. Pima Indians (2,3), information is lacking in Mexican Pima Indians.

The aim of the present work was to investigate whether Mexican Pima Indians have lower insulin resistance evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) than U.S. Pima Indians.

PATIENTS AND METHODS

Subjects

The Mexican Pima study have been previously described (6,7). Briefly, residents of Maycoba, Sonora in Mexico and surrounding areas were invited to participate in a health examination at our research clinic in the village of El Kipor, 10 Km east of Maycoba.

Only subjects with normal glucose tolerance were included in this analysis. The Mexican Pima sample consisted of all subjects 20 years of age and older, who took part in a population-based cross-sectional study aimed at measuring the prevalence of type 2 diabetes and obesity. A total of 224 Mexican Pima Indians of both sexes participated in the study of whom 194 had normal glucose tolerance. The study was approved by the ethics committees of the University of Wisconsin, Milwaukee and the Centro de Investigacion en Alimentacion y Desarrollo, Asociacion Civil. All subjects gave written informed consent prior to participation.

U.S. Pima Indians participating in this study represent a group of people who reside in the Gila River Indian Community in Arizona. The ancestors of the present group of U.S. Pima Indians developed an advanced agriculture, supported by the construction of an elaborated irrigation system from the Gila River. They successfully cultivated their own crops and complemented it by hunting and gathering. However, their lifestyle changed drastically after European colonization and the establishment of reservation. Currently, their lifestyle is much similar to that of the overall U.S. population (4).

Since 1965, a longitudinal study of type 2 diabetes and its complications has been conducted in the Gila River Indian Community. Approximately every two years, each resident of this community who is at least 5 years old is invited to participate in examinations (4,6). Based on this ongoing epidemiological study, 887 U.S. Pima Indians

were included for comparison with the Mexican Pima Indians regarding prevalence of type 2 diabetes and obesity. Thus, U.S. Pima participants who were 20 y old or more were selected as having been examined during a similar year period as in the Mexican Pima study (6). For current analysis all subjects with normal glucose tolerance (n=449) from the U.S. Pima Indians sample (n=887) were included. In addition a subset of Mexican and U.S. Pima Indians was selected, pair-matched on the basis of age, sex and percent body fat (n=79 pairs).

Biochemical Measures

Oral glucose tolerance tests were performed using a 75 g glucose load after 10-12 hour fasting according to World Health Organization recommendations (9). Biochemical measures were in serum for fasting and 2-h post glucose for concentrations of glucose, insulin, triglycerides, total and high density lipoprotein (HDL) cholesterol. All measures followed the same protocol as those in the U.S. Pima cohort study; biochemical analyses were conducted at the Phoenix Epidemiology and Clinical Research Branch. Plasma glucose concentrations were measured with an autoanalyzer using a glucose hexokinase (Ciba Corning Express, Norwood, MA). Type 2 diabetes and IGT were defined according to the World Health Organization criteria (9) but only persons with normal glucose tolerance (Fasting plasma glucose <126mg/dl and 2h plasma glucose <140mg/dl) were included in the present analysis. Plasma insulin concentrations were determined using an automated radioimmunoassay analyzer (Concept 4; INCBiomedicals, Horsham, PA). The response variables was insulin resistance measured by fasting insulin (microunits per

milliliters) and by HOMA-IR = [(Fasting Insulin (microunits per milliliters) x Fasting Glucose (millimoles per liter)]/22.5 (10). Triglycerides, total and HDL-cholesterol were measured by enzymatic methods (11-13).

Physical Measures

Weight was measured on a battery-operated electronic scale and height by a portable stadiometer. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (Kg/m^2); Central obesity was assessed by waist circumference in centimeters, measured in supine position at the level of the umbilicus. Percent body fat was estimated from bioelectrical impedance (BIA-103; RJL Systems, Detroit, MI) using an equation developed for the U.S. Pima Indians (14). Blood pressure was measured to the nearest 2 mm Hg with a mercury sphygmomanometer (Desk Model Mercury Sphygmomanometer, model 100; Liberator Medical Supply, Stuart, FL) in the right arm while the subjects rested in a sitting position. Diastolic blood pressure was measured at the fourth Korotkoff sound. Physical activity was assessed by a questionnaire (6,7).

Statistical Analysis

Differences in physical and biochemical characteristics between Mexican and U.S. Pima Indians were tested by independent t-test. Variables with skewed distribution were log transformed before analysis. We used multiple linear regressions analysis to assess differences between groups in insulin resistance in fasting insulin and HOMA-IR

in separate models. Multivariate models were constructed by stepwise model selection methods in its forward option and using a p-value of 0.05. In addition, preliminary models were evaluated for possible interaction by testing all the possible combinations of first degree product terms interactions with the group population one by one. The final models were tested to evaluate linear regression assumptions and corrections were implemented when necessary. Linearity of continuous variables was evaluated. Based on graphical results, it was decided to categorize BMI and waist circumference. BMI was categorized in three categories identifying individuals as normal (BMI< 25), overweight (BMI≥25 and <30), or obese (BMI≥30). Waist circumference was categorized into tertiles. In the pair-matched subset (n=79) differences in insulin resistance were analysed by paired t-test. All analyses were performed using STATA software (version 8.0; Stata Corp, College Station, TX) and p-values of 0.05 and 0.1 were used to test significance of selected covariates and their interaction terms.

RESULTS

The study population consisted of 194 Mexican Pima Indians (94 men and 100 women) and 449 U.S. Pima Indians (203 men and 246 women) with normal glucose tolerance. As shown in table 1, physical and biochemical characteristics were compared between Mexican and U.S. Pima Indians. Mexican Pima Indians were older and had higher physical activity ($p<0.0001$), but had lower BMI, percent body fat and waist circumference values than the U.S. Pima Indians ($p<0.0001$). Fasting and 2-h glucose as well as 2-h insulin and HDL-cholesterol were also significantly lower in the Mexican

Pima Indians *vs.* U.S. Pimas (P<0.0001). There were no significant differences in total cholesterol, triglycerides, systolic and diastolic blood pressure (p>0.05). Similar results were found when comparison between groups was done stratified by sex, except for HDL-cholesterol and systolic blood pressure values (Table 1, Appendix E). In comparison to the U.S. Pima Indians, HDL-cholesterol was significantly lower in the Mexican Pima Indian women, but similar values were found in men. In the case of systolic blood pressure, similar values were found in women, but lower values were found in the Mexican Pima Indians compared to U.S. Pima Indians.

Correlations between physical and biochemical measures are shown in Table 2. Fasting insulin were correlated to weight, BMI, waist circumference and total cholesterol in the Mexican Pima Indians. In the U.S. Pima Indians, all variables except total cholesterol were associated with fasting insulin concentrations and HOMA-IR.

When obesity was taken into account however, the magnitude of the association of total and HDL-cholesterol, triglycerides, systolic and diastolic blood pressure were "attenuated", and just HDL-cholesterol remained significant in the U.S. Pima (partial r=-0.18, p=0.04, for both outcomes) and total cholesterol in the Mexican Pima (partial r=0.15, p=0.04, for both outcomes).

Results of the association between insulin resistance and group (U.S. Pima *vs.* Mexican Pima Indians) are presented in Table 3. The unadjusted analysis indicates that Mexican Pima Indians have lower fasting insulin and HOMA-IR concentrations as demonstrated by the positive coefficient for group population, taking the Mexican group as the reference. Differences between groups remained significant either after adjustment

for age, sex or in a full model including BMI and waist circumference in addition to age and sex in both outcome variables; however, in the fully adjusted model, although still significant, differences were attenuated in both groups as demonstrated by the lower value of the beta-coefficient. Furthermore, using the mean level of age (32.8 years), in the female group with BMI classified as overweight and using the second tertile of waist circumference, predicted mean concentrations of fasting insulin and HOMA-IR in Mexican Pima Indians were approximately half those of the U.S. population in the fully-adjusted model (fasting insulin: 12.75 *vs*. 5.85 μU/mL and HOMA-IR: 2.82 *vs.* 1.28).

In the pair-matched subset (n=79), large differences were confirmed [fasting insulin: 3.99, 95% CI 3.14-5.08 *vs.* 9.25, 95% CI 7.36-11.63 μU/mL and HOMA-IR: (0.89, 95% CI 0.70-1.14 *vs.* 2.02, 95% CI 1.60-2.56]. Moreover, even though U.S. Pima had similar age (31.18 *vs.* 30.99 years), body fat (31.15 *vs.* 31.24 %) and sex than Mexican Pimas, the groups differed considerably in physical activity expressed as metabolic equivalent hours per week (U.S. Pima: 46.16, 95%CI 32.22-66.14 *vs.* Mexican Pima: 133.93, 95% CI 113.70-152.40, P<0.0001).

DISCUSION

Because U.S. Pima Indians have a marked increased prevalence of type 2 diabetes and obesity in comparison with their closely genetically-related Mexican Pima group (6), we hypothesized that insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) would be lower in the Mexican Pima Indians in comparison to that in the U.S. Pima Indians. Results of the present analysis showed

that Mexican Pima Indians are less insulin resistant, than their counterparts U.S. Pima Indians, even after adjusting for differences in age, sex and obesity. Furthermore, this finding was supported by the more than two-fold difference in insulin resistance found using the pair-matched subset. Previous reports showed that Mexican Pima Indians have a much higher physical activity (6,7), a diet lower in fat and higher in fiber and complex carbohydrates (6) in comparison to the U.S. Pima Indians.

Part of the differences in insulin resistance between groups seems to be explained by the higher obesity status in the U.S. Pima Indians. The difference in insulin resistance between groups, although still significant, was reduced by about 44% after adjusting for obesity. With respect to fasting insulin, the adjusted predicted mean difference between groups population was 6.9 μU/mL whereas that for HOMA-IR was 1.54. Additional support is derived from the contrasting differences in obesity and central obesity between both groups, judged by the higher levels of BMI, percent of body fat and waist circumference in the U.S. Pima Indians in comparison with the Mexican Pima Indians and the much stronger association of obesity (BMI, percent of body fat and waist circumference) with insulin resistance in the U.S. Pima Indians in comparison with the Mexican Pima Indians, even after adjusting for age and sex. Similarly, Dowse et al. (15) compared fasting insulin in different Asian populations and found that differences in insulin resistance between groups, although still significant, decreased considerably after controlling for total and central obesity as compared with that seen when controlling only for age. Furthermore, and consistent with this observation, different studies have reported that obesity is one on the most important factors leading to insulin resistance (16).

Increasing age and female sex were also found to be significantly associated with insulin resistance in the total population. However, these variables explained a small amount of the difference in insulin resistance between both Pima groups. The adjusted coefficient and the adjusted predicted mean difference between groups were attenuated by just 3.5 % after adjusting by age and sex, a value much lower in comparison to the 44% difference after adjustment by obesity. Both age and sex, however, are non-modifiable risk factors and inconsistent results have been found regarding their association with insulin resistance. In accord with our findings, an inverse association between age and insulin concentration in both non diabetic U.S. Pima Indians and Mauritius (17). However, no association between fasting insulin and age was found in a Native Canadian population (18). Regarding the association between sex and insulin resistance, Hunt et al. (19) showed lower fasting insulin concentrations in non diabetic women without metabolic syndrome in comparison to that in non diabetic men, but similar values in non diabetic subjects with metabolic syndrome. Conversely, Dowse et al. (15) showed that fasting insulin level was higher in women than that in men.

Difference in insulin resistance between Mexican and U.S. Pima Indians may be additionally explained by differences in physical activity and dietary intake between both groups as reported elsewhere (6,7). The increased physical activity in Mexican Pima than in U.S. Pima Indians, even in those of similar age, body fat and sex may also explain part of the differences in insulin resistance. Accordingly, physical activity was associated to insulin resistance in non diabetic U.S. Pima Indians and Indians from Mauritius (17). Dowse et al. (15), suggested that differences in fasting insulin levels among the four Asian populations may

be explained by environmental factors (diet and physical activity) closely related with ethnicity.

Several reports have indicated racial/ethnic variation in triglycerides, total and HDL-cholesterol, diastolic and systolic blood. Among these variables however, only HDL-cholesterol was different between both Pima groups in the present analysis. Of note is the fact that U.S. Pima Indians had higher values of HDL-cholesterol, which was not expected because of the high level of obesity and inactivity in this particular population (6) and the lower mean values reported in the U.S. Pima Indians, compared to Caucasians (20). Similar unexpected results were the lack of differences in levels of triglycerides, total cholesterol, systolic and diastolic blood pressure between both groups. Accordingly, Vozarova et al. (21) U.S. Pima Indians have low sympathetic nervous system activity which appears to contribute to their higher risk of obesity and the lower risk of hypertension. Lower total cholesterol levels have been reported in the U.S. Pima in comparison to Caucasian (20). In addition, values of over 40 mg/dL for HDL-cholesterol have been previously reported in the U.S. Pima Indians (20), which is similar to the mean value found in the present study of 44.7 mg/dL for the same population.

Triglycerides have been related with obesity in the U.S. Pima Indians; however, the association was not as strong as in other populations. High levels of carbohydrates intake have been associated with higher levels of triglycerides (22), and higher levels of triglycerides has been associated with lower level of HDL-cholesterol (20). This phenomenon may also explains the lower levels of HDL-cholesterol, as well as the lack of association with insulin resistance in the Mexican Pima Indians, since a large amount

of the energy intake in this population is from carbohydrates (6). The association of insulin resistance and hypertension is controversial. Based in the Insulin Resistance Atherosclerosis Study, Saad et al. (23) found a positive association of insulin resistance and hypertension in Mexican American, but not in Caucasian and African American after adjusted by age, sex, BMI, and waist. In the U.S. Pima Indians, a non significant association with insulin resistance was also reported after adjustment for age, sex, body weight, and percent of body fat (24).

The main strength of this study is the fact that we are comparing two genetically related populations living with contrasting lifestyles. Additional strengths are the facts that all the analysis followed similar measurement protocols in a central laboratory. Nevertheless, our study is limited by the cross-sectional nature of the data, which do not allow the demonstration any of temporal effect of the factors studied on risk of insulin resistance.

In conclusion, our results indicate that Mexican Pima Indians with normal glucose tolerance have lower insulin resistance in comparison to their genetic related counterparts U.S. Pima Indians, even after controlling for differences in obesity, age and sex. This finding underscores the importance of lifestyle factors as direct protecting factors against insulin resistance in non diabetic populations.

ACKNOWLEDGMENTS

We wish to thank the members of the Maycoba Pima Community and the Gila River Indian Community for their participation in these studies. Our gratitude goes to Bertha Isabel Pacheco, Ana Cristina Gallegos and Hortensia Montesinos for data collection in Mexico and the staff of the Phoenix Epidemiology and Clinical Research Branch, NIDDK for data collection in Sacaton, Arizona. Special thanks are extended to Nurse Eremita Perez Ruiz for facilitating work with our Mexican communities. The study in Mexico was supported partially by grant number NIH-DK-49957. Part of this work was presented in abstract form at the Congress of Epidemiology, Seattle, WA, 21-24 June 2006.

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Table 1. Physical and biochemical characteristics by population group with normal glucose tolerance

Variable	Mexican Pima	U.S. Pima	p-value
n	194	449	
Age (years)	36.8±14.7	32.5±10.0	<0.0001
BMI (Kg/m^2)	24.6±4.2	34.1±8.0	<0.0001
Body fat (%)	26.9±11.0	39.6±9.5	<0.0001
Waist circumference (cm)	83.1±11.0	107.5±19.4	<0.0001
Fasting glucose (mg/dL)	89.0±8.8	92.9±8.8	<0.0001
Two hrs glucose (mg/dL)	94.6±21.4	104.0±20.3	<0.0001
Two hrs insulin (μU/mL)*	21.8 [18.8,25.4]	64.1 [57.6,71.4]	<0.0001
HDL-cholesterol (mg/dL)	39.1±9.4	44.7±12.5†	<0.0001
Total Cholesterol (mg/dL)	170.6±37.1	173.0±32.6	0.4234
Triglycerides (mg/dL)*	104.4 [96.8,112.7]	101.0 [93.4,109.3]‡	0.5588
SBP (mmHg)	115.7±13.7	118.0±15.3	0.0729
DBP (mmHg)	71.3±10.6	70.5±11.1	0.3869
PA* (METs-hour/week)	131.7 [121.5,142.8]	32.7 [27.9,38.8]	<0.0001

BMI, body mass index; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; PA, physical activity; a t test was done with independent samples; *Geometric means [95% CI]; †n=168; ‡n=143; All other data are presented as mean ± SD. To convert glucose to mmol/L, multiply by 0.0555; Insulin to pmol/L, multiply by 6.945; HDL-cholesterol to mmol/L, multiply by 0.0259; Total Cholesterol to mmol/L, multiply by 0.0259; Triglycerides to mmol/L, multiply by 0.0113

Table 2. Correlation between physical and biochemical characteristics by population group

Variable	Fasting Insulin* (μU/ml)		HOMA-IR*	
	MXPI	USPI	MXPI	USPI
Age (years)	-0.04	-0.12†	-0.01	-0.11†
Weight (kg)	0.21†	0.43†	0.23†	0.45†
BMI (Kg/m^2)	0.17†	0.44†	0.20†	0.46†
Body fat (%)	0.09	0.33†	0.10	0.33†
Waist circumference (cm)	0.18†	0.45†	0.20†	0.47†
HDL-cholesterol (mg/dL)	-0.09	-0.31†	-0.09	-0.31†
Total Cholesterol (mg/dL)	0.19†	0.07	0.20†	0.07
Triglycerides (mg/dL)†	0.10	0.18†	0.12	0.19†
SBP (mmHg)	0.08	0.20†	0.11	0.22†
DBP (mmHg)	0.05	0.13†	0.08	0.16†

Abbreviations: MXPI, Mexican Pima Indians; USPI, U.S. Pima Indians; SBP, systolic blood pressure; DBP, diastolic blood pressure; *log transformed; Sample size: MXPI=193 and USPI=393; †$p \leq 0.05$ based on Pearson correlation

Table 3. Association between measures of insulin resistance (IR) and Pima Indian population (n=560)

Model	β coefficient	95% CI	Predicted IR *
Fasting Insulin (μU/mL)			
Unadjusted			
Mexican Pima Indians	reference	-	4.00
U.S. Pima Indians	1.368	1.203-1.532	16.25
Adjusted†			
Mexican Pima Indians	reference	-	4.17
U.S. Pima Indians	1.339	1.172-1.506	15.93
Adjusted‡			
Mexican Pima Indians	reference	-	5.85
U.S. Pima Indians	0.779	0.579-0.979	12.75
HOMA-IR			
Unadjusted			
Mexican Pima Indians	reference	-	0.86
U.S. Pima Indians	1.409	1.239-1.579	3.64
Adjusted†			
Mexican Pima Indians	reference	-	0.89
U.S. Pima Indians	1.387	1.216-1.559	3.58
Adjusted‡			
Mexican Pima Indians	reference	-	1.28
U.S. Pima Indians	0.785	0.581-0.989	2.82

*****Covariates set to: mean age = 32.8, gender=female, BMI=overweight, waist circumference =second tertile; †Adjusted for age and sex; ‡Adjusted for age and sex, BMI and waist circumference

APPENDIX A1: Differences in Insulin Resistance in Mexican and U.S. Pima Indians with Normal Glucose Tolerance

Short title: Insulin Resistance in Pima Indians

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Disclosure statement: The authors have nothing to disclose.

Number of words: Text: 1869; Abstract: 256

ABSTRACT

CONTEXT: Insulin resistance is a major risk factor for the development of type 2 diabetes in Pima Indians, a population with the highest prevalence of type 2 diabetes mellitus in the world. Their Mexican counterpart, living a traditional lifestyle in the mountain of Sonora, have at least 5 times less diabetes than the U.S. Pima Indians.

OBJECTIVE: We evaluated whether Mexican Pima Indians had lower insulin resistance than U.S. Pima Indians.

DESIGN AND PATIENTS: We compared fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) in 194 Mexican Pima Indians (100 Females, 94 Males) and 449 U.S. Pima Indians (246 Females, 203 Males) with normal glucose tolerance from a cross-sectional study. Adjusted differences of log transformed outcomes (fasting insulin and HOMA-IR) between groups were evaluated using multiple linear regression models and paired t-test in a matched subset.

RESULTS: Unadjusted fasting insulin and HOMA-IR were much lower in the Mexican Pima Indians than in their U.S. counterparts. After adjusting by obesity, age and sex, mean [95% confidence interval] for fasting insulin were 6.22 [5.34-7.24] *vs.* 13.56 μU/mL [12.27-14.97] and for HOMA-IR 1.40 [1.20-1.64] *vs.* 3.07 [2.77-3.40], respectively, for Mexican Pima and U.S. Pima Indians. Results were confirmed in subset matched for age, sex and body fat.

CONCLUSION: Our results indicate that Mexican Pima Indians have lower insulin resistance in comparison to their genetically related U.S. counterparts, even after controlling for differences in obesity, age and sex. This finding underscores the

importance of lifestyle factors as protecting factors against insulin resistance in individuals with high propensity to develop diabetes.

Insulin resistance is a major risk factor in the pathogenesis of type 2 diabetes mellitus. Prospective studies have shown that insulin resistance is an independent predictor of type 2 diabetes (1) including the U.S. Pima Indians (2,3), a population with the highest reported prevalence of type 2 diabetes and obesity in the world (4). A longitudinal study designed to investigate the pathogenesis of type 2 diabetes in this population has characterized independent predictors of diabetes including obesity, insulin resistance, insulin secretory dysfunction, and increased rate of endogenous glucose production (2,5).

Mexican Pima Indians, a population living a traditional lifestyle in the mountains of northwestern Mexico, have a much higher physical activity (6,7), a diet lower in fat and higher in fiber and complex carbohydrates (6) in comparison to the U.S. Pima. Mexican Pima Indians also have an age- and sex-standardized prevalence of type 2 diabetes at least 5 times lower than that U.S. Pima Indians (6). Differences in type 2 diabetes in these two genetically related populations (8) have been attributed largely to differences in body weight and body fat probably due to differences in diet and physical activity (6). Although insulin resistance has been widely studied in the U.S. Pima Indians (2,3), information is lacking in Mexican Pima Indians.

The aim of the present work was to investigate whether Mexican Pima Indians have a lower insulin resistance, evaluated by fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR), than U.S. Pima Indians.

PATIENTS AND METHODS

Subjects

The Mexican Pima study have been previously described (6,7). Briefly, residents of Maycoba, Sonora, Mexico, and surrounding areas were invited to participate in a health examination at our research clinic in the village of El Kipor, 10 Km east of Maycoba.

Only subjects with normal glucose tolerance were included in this analysis. The Mexican Pima sample consisted of all subjects 20 years of age and older, who took part in a population-based cross-sectional study aimed at measuring the prevalence of type 2 diabetes and obesity in the year 1996. A total of 224 Mexican Pima Indians of both sexes participated in the study of whom 194 had normal glucose tolerance. The study was approved by the ethics committees of the University of Wisconsin, Milwaukee and the Centro de Investigacion en Alimentacion y Desarrollo, Asociacion Civil. All subjects gave written informed consent prior to participation.

Since 1965, a longitudinal study of type 2 diabetes and its complications has been conducted in the Gila River Indian Community. Approximately every two years, each resident of this community who is at least 5 years old is invited to participate in examinations (4,6). Based on this ongoing epidemiological study, a sample of 887 U.S. Pima Indians were selected for comparison with the Mexican Pima Indians regarding prevalence of type 2 diabetes and obesity (6). Thus, U.S. Pima participants who were 20 y old or more were selected as having been examined during a similar time period as in the Mexican Pima study (6). For current analysis all subjects with normal glucose tolerance (n=449) from the U.S. Pima Indians sample (n=887) were included. In addition a subset of Mexican and U.S. Pima Indians was selected, pair-matched on the basis of age, sex and percent body fat (n=79 pairs).

Biochemical Measures

Oral glucose tolerance tests were performed using a 75-g glucose load after 10-12 hour fasting according to World Health Organization recommendations (9). Biochemical measures were in serum for fasting and 2-h after glucose load for concentrations of glucose, insulin, triglycerides, total and high density lipoprotein (HDL) cholesterol. All measures followed the same protocol as those in the U.S. Pima cohort study; biochemical analyses were conducted at the Phoenix Epidemiology and Clinical Research Branch. Plasma glucose concentrations were measured with an autoanalyzer using a glucose hexokinase (Ciba Corning Express, Norwood, MA). Type 2 diabetes and IGT were defined according to the World Health Organization criteria (9) but only persons with normal glucose tolerance (Fasting plasma glucose <126mg/dl and 2h plasma glucose <140mg/dl) were included in the present analysis. Plasma insulin concentrations were determined using an automated radioimmunoassay analyzer (Concept 4; INC Biomedicals, Horsham, PA). The response variables were insulin resistance measured by fasting insulin (microunits per milliliters) and by HOMA-IR = [(Fasting Insulin (microunits per milliliters) x Fasting Glucose (millimoles per liter)]/22.5 (10). Triglycerides, total and HDL-cholesterol were measured by enzymatic methods (11-13).

Physical Measures

Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Central obesity was assessed by waist circumference in centimeters. Percent body fat was estimated from bioelectrical impedance (BIA-103; RJL

Systems, Detroit, MI) using an equation developed for the U.S. Pima Indians (14). Blood pressure was measured to the nearest 2 mm Hg with a mercury sphygmomanometer (Desk Model Mercury Sphygmomanometer, model 100; Liberator Medical Supply, Stuart, FL) in the right arm while the subjects rested in a sitting position. Diastolic blood pressure was measured at the fourth Korotkoff sound. Physical activity was assessed by a questionnaire (6,7).

Statistical Analysis

Differences in physical and biochemical characteristics between Mexican and U.S. Pima Indians were tested by independent t-test. Variables with skewed distribution were log transformed before analysis. We used multiple linear regressions to assess differences between groups in fasting insulin and HOMA-IR in separate models. Multivariate models were constructed by the stepwise method in its forward option using a *P*-value of 0.05. In addition, preliminary models were evaluated for possible interaction. The final models were tested for linear regression assumptions. In the pair-matched subset differences in insulin resistance were analysed by paired t-test. All analyses were performed using STATA software (version 8.0; Stata Corp, College Station, TX) and *P*-values of 0.05 and 0.1 were used to test significance of selected covariates and their interaction terms.

RESULTS

The study population consisted of 194 Mexican Pima Indians (94 men and 100 women) and 449 U.S. Pima Indians (203 men and 246 women) with normal glucose tolerance. As

shown in table 1, physical and biochemical characteristics were compared between Mexican and U.S. Pima Indians. Mexican Pima Indians were older and had higher physical activity (P <0.0001), but had lower BMI, percent body fat and waist circumference values than the U.S. Pima Indians (P <0.0001). Fasting and 2-h glucose as well as 2-h insulin and HDL cholesterol were also significantly lower in the Mexican Pima Indians *vs.* U.S. Pimas (P <0.0001). There were no differences in total cholesterol, triglycerides, systolic and diastolic blood pressure (P >0.05).

Results of the association between insulin resistance and group (U.S. Pima *vs.* Mexican Pima Indians) are presented in Fig. 1 as exponentiated means and 95% confidence interval (95% CI). The unadjusted analysis indicates that Mexican Pima Indians have lower mean fasting insulin [4.17, 95% CI 3.64-4.77 *vs.* 16.35, 95% CI 14.87-17.97 μU/mL] and HOMA-IR (0.91, 95% CI 0.79-1.05 *vs.* 3.73, 95% CI 3.38-4.11) than U.S. Pima. Differences between groups remained significant either after adjustment for age, sex [fasting insulin: β=1.339, 95% CI 1.172-1.506] and HOMA-IR: [β=1.387, 95% CI 1.216-1.559] or in a full model including BMI and waist circumference in addition to age and sex [fasting insulin: β=0.779, 95% CI 0.579-0. 979] and HOMA-IR: [β=0.785, 95% CI 0.581-0. 989], taking Mexican group as the reference. In the pair-matched subset (n=79), large differences were confirmed [fasting insulin: 3.99, 95% CI 3.14-5.08 *vs.* 9.25, 95% CI 7.36-11.63 μU/mL and HOMA-IR: (0.89, 95% CI 0.70-1.14 *vs.* 2.02, 95% CI 1.60-2.56]. Moreover, even though U.S. Pima had similar age (31.18 *vs.* 30.99 years), body fat (31.15 *vs.* 31.24 %) and sex than Mexican Pimas, the groups differed considerably in physical activity expressed as metabolic equivalent hours per week (U.S.

Pima: 46.16, 95% CI 32.22-66.14 *vs.* Mexican Pima: 133.93, 95% CI 113.70-152.40, P<0.0001).

DISCUSION

Because U.S. Pima Indians have a marked increased prevalence of type 2 diabetes compared with their closely genetically-related Mexican Pima group (6), we hypothesized that insulin resistance would be lower in the Mexican Pima Indians in comparison with that in the U.S. Pima Indians. Results of the present analysis showed that Mexican Pima Indians are less insulin resistant than their counterpart U.S. Pima Indians, even after adjusting for age, sex and obesity. Furthermore, this finding was supported by the more than two-fold difference in insulin resistance found using the pair-matched subset.

Part of the differences in insulin resistance between groups seems to be explained by the greater degree of obesity in the U.S. Pima Indians. The difference in insulin resistance between groups, although still significant, was reduced by about 40% after adjusting for obesity. With respect to fasting insulin, the adjusted predicted mean difference between groups population was 7.34 μU/mL (unadjusted mean difference: 12.19 μU/mL) whereas that for HOMA-IR was 1.67 (unadjusted mean difference: 2.82). Similarly, Dowse et al. (15) compared fasting insulin in different Asian populations and found that differences in insulin resistance between groups, although still significant, decreased considerably after controlling for total and central obesity as compared with that seen when controlling only for age. Furthermore, and consistent with this

observation, different studies have reported that obesity is one on the most important factors leading to insulin resistance (16).

Increasing age and female sex were also significantly associated with lower insulin resistance in the entire population. However, these two variables explained only a small amount of the difference in insulin resistance between both Pima groups. The adjusted coefficient value and the adjusted predicted mean difference between groups were attenuated only by 1.5 % after adjusting for age and sex, a value much lower than the 40% difference after adjustment for obesity. Both age and sex, however, are non-modifiable risk factors and inconsistent results have been found regarding their association with insulin resistance. In accord with our findings, an inverse association was shown between age and insulin concentration in both non diabetic U.S. Pima Indians and Indians from Mauritius (17). However, no association between fasting insulin and age was found in a Native Canadian population (18).

Regarding the association between sex and insulin resistance, Hunt et al. (19) found lower fasting insulin concentrations in non diabetic women in comparison to that in non diabetic men, but similar values in non diabetic subjects with the metabolic syndrome. Conversely, Dowse et al. (15) showed that fasting insulin level was higher in women than that in men.

Physical activity was associated to insulin resistance in non diabetic U.S. Pima Indians and Indians from Mauritius (17). Accordingly, the increased physical activity in Mexican Pima than in U.S. Pima Indians, even in those of similar age, body fat and sex may also explain part of the differences in insulin resistance.

The main strength of this study is the fact that we are comparing two genetically related populations living with naturally contrasting lifestyles. Additional strengths are the facts that all the analysis followed similar measurement protocols in a central laboratory. Nevertheless, our study is limited by the cross-sectional nature of the data, which do not allow the demonstration any of temporal effect of the factors studied on risk of insulin resistance.

In conclusion, our results indicate that Mexican Pima Indians with normal glucose tolerance have lower insulin resistance in comparison to their genetic related counterparts U.S. Pima Indians, even after controlling for differences in obesity, age and sex. This finding underscores the importance of lifestyle factors as direct protecting factors against insulin resistance in non diabetic populations.

ACKNOWLEDGMENTS

We wish to thank the members of the Maycoba Pima Community and the Gila River Indian Community for their participation in these studies. Our gratitude goes to Bertha Isabel Pacheco, Ana Cristina Gallegos and Hortensia Montesinos for data collection in Mexico and the staff of the Phoenix Epidemiology and Clinical Research Branch, NIDDK for data collection in Sacaton, Arizona. Special thanks are extended to Nurse Eremita Perez Ruiz for facilitating work with our Mexican communities.

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Table 1. Physical and biochemical characteristics by population group with normal glucose tolerance

Variable	Mexican Pima	U.S. Pima	p-value
n	194	449	
Age (years)	36.8±14.7	32.5±10.0	<0.0001
BMI (Kg/m^2)	24.6±4.2	34.1±8.0	<0.0001
Body fat (%)	26.9±11.0	39.6±9.5	<0.0001
Waist circumference (cm)	83.1±11.0	107.5±19.4	<0.0001
Fasting glucose (mg/dL)	89.0±8.8	92.9±8.8	<0.0001
Two hrs glucose (mg/dL)	94.6±21.4	104.0±20.3	<0.0001
Two hrs insulin (μU/mL)*	21.8 [18.8,25.4]	64.1 [57.6,71.4]	<0.0001
HDL-cholesterol (mg/dL)	39.1±9.4	44.7±12.5†	<0.0001
Total Cholesterol (mg/dL)	170.6±37.1	173.0±32.6	0.4234
Triglycerides (mg/dL)*	104.4 [96.8,112.7]	101.0 [93.4,109.3]‡	0.5588
SBP (mmHg)	115.7±13.7	118.0±15.3	0.0729
DBP (mmHg)	71.3±10.6	70.5±11.1	0.3869
PA* (METs-hour/week)	131.7 [121.5,142.8]	32.7 [27.9,38.8]	<0.0001

BMI, body mass index; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; PA, physical activity; a t test was done with independent samples; *Geometric means [95% CI]; †n=168; ‡n=143; All other data are presented as mean ± SD. To convert glucose to mmol/L, multiply by 0.0555; Insulin to pmol/L, multiply by 6.945; HDL-cholesterol to mmol/L, multiply by 0.0259; Total Cholesterol to mmol/L, multiply by 0.0259; Triglycerides to mmol/L, multiply by 0.0113

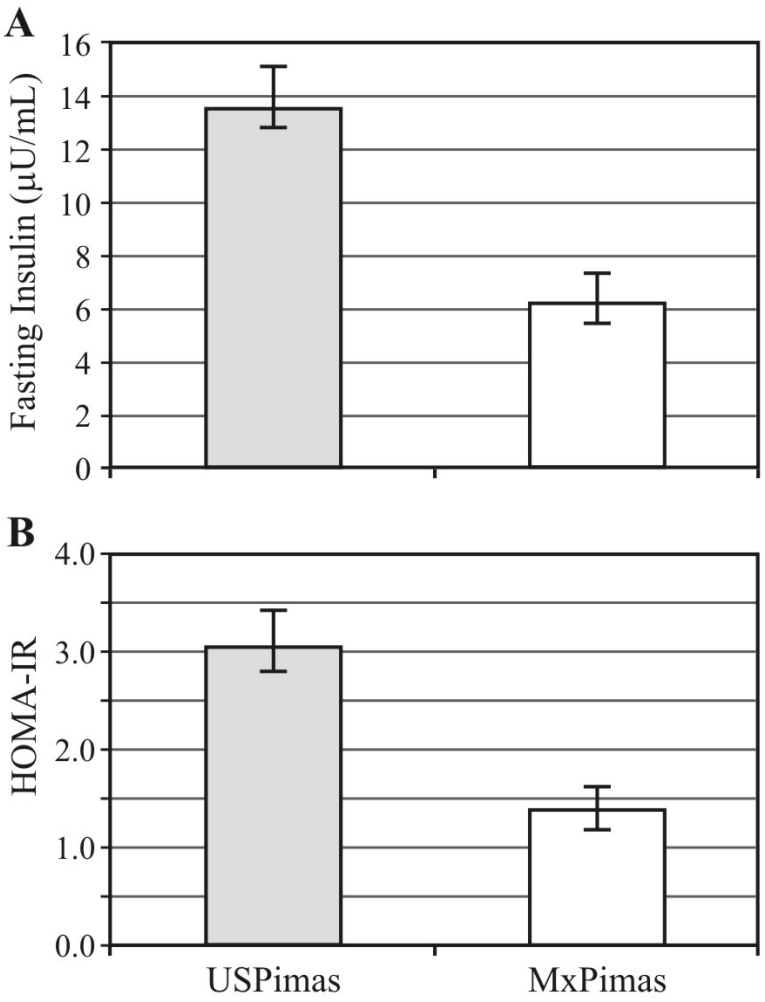

Figure 1. Insulin resistance in U.S. Pima and Mexican Pima Indians with normal glucose tolerance measured by fasting insulin and HOMA-IR

Fasting insulin (A) and HOMA-IR (B) values are presented as exponentiated means with 95% Confidence Intervals, adjusted for age, sex, BMI and waist circumference; USPimas, U.S. Pima Indians; MxPimas, Mexican Pima Indians

APPENDIX B: Metabolic syndrome in the Mexican and U.S. Pima Indians: the importance of physical activity

RUNNING TITLE: Metabolic Syndrome in Mexican and U.S. Pima Indians

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The study in Mexico was supported partially by grant number NIH-DK-49957.

Total words number: text= 2491; Abstract: 242

ABSTRACT

OBJECTIVE: The prevalence of the metabolic syndrome (MS) is very frequent in U.S. Pima Indians. However, in their genetically related Mexican Pima Indians counterparts, information on MS and how it is influenced by a traditional lifestyle is lacking. The aim of this study was to assess the prevalence of the MS in the genetically related Mexican Pima Indians and whether physical activity (PA) or obesity may explain the difference in prevalence in the two groups of Pima.

RESEARCH DESIGN AND METHODS: The present analysis included 224 Mexican Pima Indian subjects and 447 U.S. Pima Indians from a cross-sectional study. MS diagnosis was based on the Third Report of the NCEP. PA was assessed by a questionnaire and BMI by measured weight and height.

RESULTS: The unadjusted prevalence of MS was 24.1% and 56.6 % in the Mexican and U.S. Pima Indians, respectively. After adjustment for age and sex, the likelihood of having MS in the U.S. Pima Indians was 4.8-fold (95% CI 3.2-7.1) higher than in the Mexican participants. This association was attenuated but remained significant after further adjustment for BMI (OR=2.4, 95% CI 1.5-3.7). However, after further adjustment for PA, the difference disappeared (OR=1.4, 95% CI 0.8-2.3).

CONCLUSIONS: Our findings indicate that the difference in the prevalence of MS between the Mexican and U.S. Pima Indians is mainly explained by differences in obesity and PA and underscore the importance of lifestyle in the prevention of MS even in high risk populations.

Individuals with the metabolic syndrome (MS) are characterized by a cluster of central obesity, hypertension, dyslipidemia, and hyperglycemia (1), and have an elevated risk for type 2 diabetes (2,3). This knowledge has led some researchers to propose MS as an alternative predictor of type 2 diabetes (3,4). The etiological factors of this syndrome are not well understood but several different factors are probably involved, many related to lifestyle (5, 6), although genetic factors also seem involved (6). The U.S. Pima Indians have the highest reported prevalence and incidence of type 2 diabetes worldwide (7). They also have a high prevalence of obesity (8) and MS (2). In the Mexican Pima Indians, a population genetically related to the U.S. Pima Indians who live a traditional lifestyle in the mountains of northwestern Mexico (9), information on MS prevalence is lacking.

A cross-sectional study comparing prevalence of type 2 diabetes between the Mexican and U.S. Pima Indians, showed an age and sex-adjusted type 2 diabetes prevalence at least 5 times lower than that in U.S. Pima (9). Additional reports from this study showed that Mexican Pima Indians have a much higher level of PA (10), a diet low in fat and high in dietary fiber and complex carbohydrates (9). Differences in type 2 diabetes in these two populations have been attributed largely to differences in body weight and body fat probably due to differences in lifestyle (9).

The aim of the present study was to assess the prevalence of MS in Mexican and U.S. Pima Indians and to assess the importance of physical activity and obesity in explaining differences.

Research Design and Methods

Participants

The Mexican Pima Indian study population and data collection procedures have been described previously (9, 10). Briefly, each adult from Maycoba and surrounding areas in the state of Sonora, Mexico was invited to participate in a health examination at our research clinic in the village of El Kipor, 10 Km east of Maycoba. The Mexican Pima sample consisted of 224 men and women 20 years of age and older who agreed to participate in the study (9). The study was approved by the ethics committee**s** of the University of Wisconsin, Milwaukee and the *Centro de Investigación en Alimentación y Desarrollo*, AC. All of the subjects gave written informed consent prior to participation (9).

Since 1965, a longitudinal study of type 2 diabetes and its complications has been conducted in the Gila River Indian Community, where the U.S. Pima reside. Approximately every two years, each resident of this community who is at least 5 years old is invited to participate in the study (7,9). For comparison with the Mexican Pima Indians a sample of 447 U.S. Pima who were 20 years old or more and were examined between June, 1995 and June, 1996 and had data on the MS components were selected to coincide with the same time period as the data from Mexican study were collected (9).

Physical and Biochemical Measures

Examinations in the Mexican Pima were conducted in the morning under fasting conditions. The protocol included a brief medical history, demographic, physical and

lifestyle measures such as weight, height, waist circumference, percent body fat, systolic and diastolic blood pressure and physical activity. Biochemical measures were fasting and 2-h serum glucose and insulin, triglycerides, total and high density lipoprotein (HDL)-cholesterol. All measurements were made using the same protocol as those in the U.S. Pima Indian cohort study and the biochemical analyzes were conducted at the Phoenix Epidemiology and Clinical Research Branch.

Oral glucose tolerance tests were performed using a 75 g glucose load after 10-12 hour fasting according to World Health Organization (WHO) recommendations (11). Plasma glucose concentrations were measured in fasting and 2-h post-load using a glucose hexokinase method (Ciba Corning Express, Norwood, MA) and type 2 diabetes was defined according to the 1999 World Health Organization criteria (11).

Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (Kg/m^2); In Mexico, weight was measured on a battery-operated electronic scale and height by a portable stadiometer. Obesity was classified using BMI according to the World Health Organization criteria (12). Percentage of body fat was estimated from bioelectrical impedance (BIA-103; RJL Systems, Detroit, MI) using an equation developed for the U.S. Pima Indians (13). Waist circumference was measured in supine position at the level of the umbilicus. Blood pressure was measured to the nearest 2 mmHg with a mercury sphygmomanometer (Desk Model Mercury Sphygmomanometer, model 100; Liberator Medical Supply, Stuart, FL) in the right arm while the subjects rested in a sitting position. Triglycerides, total and HDL-cholesterol were measured by enzymatic methods (14-16). Physical activity was measured using a

questionnaire developed for the U.S. Pima Indians and adapted to the Mexican Pima Indian population (9,10). This questionnaire was previously shown to be both reliable and valid in the U.S. Pima Indian population (17). It measures leisure and occupational PA over the past year, which are expressed as hours per week (h/week) and as metabolic equivalent hours per week (MET-hour/week) averaged over the past year as reported elsewhere (17).

Metabolic syndrome

MS was defined using the Third Report of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP III) criteria, which propose a definition that only requires readily available clinical variables (1,18). The criteria include the presence of at least three of the following categorical risk factors: central obesity (waist circumference >102 cm in men and >88 cm in women), high triglycerides [≥1.7 mmol/l (≥150 mg/dL)], low HDL cholesterol [<1.03 mmol/l (<40 mg/dL) in men and <1.29 mmol/l (<50 mg/dL) in women], high blood pressure (≥130/≥85 mm Hg or pharmacological treatment of hypertension), and fasting glucose [≥6.1mmol/l (≥110 mg/dL) or current treatment with antidiabetic medications or insulin (1,18).

Statistical analysis

Descriptive statistics such as means and SD (median and 95% CI for skewed variables) for continuous variables and proportions for categorical variables were

compared using independent t-tests (Mann-Witney for median comparisons) and chi-square tests, respectively.

To analyze the individual associations of MS with age, sex, weight, height, BMI, percent body fat and PA (MET-hour/week), non-adjusted logistic regressions analyses were applied for each covariate, stratified by population group. For each model, the response variable was defined as the presence or absence of MS. The likelihood of having MS in the U.S. Pima Indians compared to Mexican participants was further assessed using multivariate logistic regression models with adjustment for covariates. Multivariate logistic models were constructed by stepwise model selection methods using the forward option and a p-value of 0.05 as the criterion to allow covariates to enter the model. Non significant variables considered to be of physiologic importance were retained in the final model. The preliminary models were evaluated for possible interactions by testing all the possible combinations of first degree product terms interactions with the group population one by one and using lr-test and p-value of 0.1. The model was evaluated for goodness-of-fit using Hosmer-Lemeshow statistic and the ROC curve. All analyses were performed using STATA software 8.0 and *P*-values of 0.05 and 0.1 were used to test significance of selected covariates and their interaction terms.

Results

Socio-demographic, physical, and lifestyle characteristics are presented in Table 1. In both populations, individuals with MS were older, and had higher weight, BMI and percent of body fat. Physical activity was lower in individuals with MS in both groups.

The prevalence of MS and its components (95% CI) in the Mexican and U.S. Pima Indians are presented in Table 2. The unadjusted prevalence of the MS was 2.5-fold higher in the U.S. Pima Indians than in the Mexican Pima Indians ($p<0.0001$). Likewise, higher prevalences were found in the U.S. Pima Indians for elevated fasting glucose, central obesity and high blood pressure ($p\leq0.0001$). However, the prevalences of high triglycerides and low HDL-cholesterol were similar. After adjustment for age, sex, BMI and PA, the likelihood of having elevated fasting glucose and central obesity remained higher in the U.S. Pima Indians ($p<0.0001$), but not for high blood pressure and for high triglycerides. Unexpectedly, the likelihood of having low HDL-cholesterol was lower in the U.S. Pima Indians ($p=0.002$).

The association of MS with each individual covariate was analyzed using the Odds Ratio (95 Confidence Interval) for each population. In Mexican Pima Indians variables significantly associated with MS were older age [per 10 years: 1.5 (95% CI, 1.2-1.8)], female sex: 0.37 (95% CI, 0.19-0.71), greater weight [per 5 kg: 1.4 (95% CI, 1.2-1.6)], height (per one m: 0.96 (95% CI, 0.92-0.99), BMI (per 5 kg/m^2: 4.5 (95% CI, 2.8-7.3)] and percent body fat [per 5%: 1.9 (95% CI, 1.6-2.4) and lower PA [per 30 MET-hour/week: 0.87 (95% CI, 0.79-0.95). In U.S. Pima Indians variables significantly associated with MS were older age [per 10 years: 2.3 (95% CI, 1.9-2.7)], female sex: 0.49 (95% CI, 0.34-0.73), greater weight [per 5 kg: 1.15 (95% CI, 1.1-1.2)], BMI (per 5

kg/m^2: 4.5 (95% CI, 2.8-7.3)] and percent body fat [per 5%: 1.9 (95% CI, 1.6-2.4) and lower PA [per 30 MET-hour/week: 0.88 (95% CI, 0.83-0.93), but not height (per one m: 0.98 (95% CI, 0.96-1.00)].

The likelihood of having MS was analyzed by multiple logistic regression using the Mexican Pima Indians as the reference group. As presented in Table 3, after adjustment for age and sex, the odds of having MS was 4.8 (95% CI, 3.2-7.1) times higher in U.S. Pima Indians *vs.* Mexican Pima Indians. In the second model, after further adjustment for BMI (classified as BMI <25 vs. BMI ≥ 25) along with age and sex, attenuation in the magnitude of association of MS between groups was found [odds ratio, 95% CI, 2.4 (1.5-3.7)]. In contrast, in a third model that included PA (MET-hour/week), in addition to age, sex and BMI, the odds ratio was substantially attenuated and not significant [odds ratio, 95%CI, 1.4 (0.8-2.3)] was found. Sex was not significantly associated with MS in the final model, but was retained, although similar results were found when sex was not included in the final model. Interestingly, in the group having BMI<25 the differences in the prevalence of MS between the groups was completely explained by PA [odds ratio, 95% CI, 1.05 (0.3-4.3)].

Conclusions

The aim of the present study was to calculate the prevalence of MS in Mexican and U.S. Pima Indians and to investigate the importance of PA and obesity in explaining possible differences. Our findings indicate that almost all of the difference in the prevalence of MS between the Mexican and U.S. Pima Indians can be explained by the

contrasting levels in obesity and PA between groups. Age and sex did not explain much of the difference in the likelihood of having the MS between groups. The likelihood of having a MS in the U.S. Pima Indians was a halved in after adjustment for obesity and none statistically significant after further adjustment for PA.

The high prevalence of MS in Pima Indians confirmed data by Resnick (6) showing that American Indian men and women from the Strong Heart Study, have a 2.18-fold and a 2.45-fold higher prevalence of the MS when compared to men and women from the NHANES III, which include white, black and Hispanic populations (6). Ethnicity, however, cannot be implicated for the difference in MS in the present study because of the genetic similarity of the two populations (9).

A couple of studies have reported positive associations between age and obesity and MS and a negative association between PA and MS (19,20). In addition, prospective cohort studies (21,22) have identified a low PA as a predictor of MS. Palaniappan et al (19) investigated predictors of the MS and concluded that obesity was a significant predictor of the incidence of MS. Erikson et al (20) reviewed the role of PA and exercise in the prevention and management of MS. The authors concluded that low levels of PA are related to most of the components of MS. Furthermore, in a large cross-sectional study, inactivity was directly associated to MS as well as with its components (21).

It has been suggested that most of the differences in obesity between Mexican and U.S. Pima Indians are due to their contrasting differences in PA levels and diet (10). Our results indicated that PA is associated with MS independently of obesity in both populations. However in those with BMI<25 differences in PA and not BMI accounted

for the difference in MS prevalence in the Mexican Pima. Furthermore, our finding appears to indicate that the association between PA and MS may be explained by the direct influence of PA on obesity as well as by the independent effects of PA on the individual components of MS. Mexican Pimas classified as having high PA (MET-hour/week) (using the median) had lower prevalence of metabolic syndrome and central obesity as well as lower prevalence of HDL-cholesterol than Pimas with low PA. Furthermore, the U.S. Pima Indians in this study classified as having a high PA (using the median) had a lower prevalence of MS, elevated fasting glucose, central obesity as well as lower prevalence of elevated blood pressure in comparison to people with low PA (data not shown).

It appears that the MS components may be different between the Mexican and U.S. Pima Indians. U.S. Pimas have higher fasting glucose, central obesity and blood pressure than Mexican Pimas, but similar triglycerides and HDL-cholesterol. Interestingly, after adjusting for age, sex, BMI, and PA, in the U.S. Pima Indians the prevalence of increased fasting glucose and central obesity remained higher, but was lower for low HDL-cholesterol.

Obesity and hyperinsulinemia/insulin resistance seem to have independent roles in the development of hypertension (23,24) and as a consequence, we expected a greater difference between the Mexican and U.S. Pima Indians regarding hypertension as the U.S. Pima exhibit greater insulin resistance than the Mexican Pima. Previous reports in U.S. Pima Indians indicated a relatively low prevalence of hypertension (25), even though there was a high prevalence of obesity (8). A suggested explanation for this

phenomenon was that the U.S. Pima Indians have lack an increase in sympathetic nerve activity with increasing obesity (25).

A limitation of this study is its cross-sectional nature which does not allow any assessment of the cause–effect relationships for reported associations. However, our findings are in accordance with the results of both prospective and cross-sectional studies as previously discussed (19-22). The strength of this study lies in the comparison of two genetically related populations living with naturally contrasting lifestyles. An additional strength is the fact that all of the analysis followed similar measurement protocols and that all biological analyses were carried out in the same laboratory.

In conclusion, our findings indicate that differences in the prevalence of MS exist between the Mexican and U.S. Pima Indians and that these are mostly explained by the populations' contrasting differences in obesity and PA. Furthermore, in the Mexican Pima with BMI<25 the prevalence of MS may be accounted for exclusively by differences in PA in those with and without MS. The finding underscores the importance of lifestyle, particularly PA in the prevention of MS even in high risk population.

Acknowledgments

We wish to thank the members of the Maycoba Pima and non-Pima Community and the Gila River Indian Community for their participation in these studies. Our gratitude goes to Bertha Isabel Pacheco, Ana Cristina Gallegos and Hortensia Montesinos for data collection in Mexico and the staff of the Phoenix Epidemiology and Clinical Research Branch. NIDDK for data collection in Sacaton, Arizona. Special thanks are extended to Nurse Eremita Perez Ruiz for facilitating work with our Mexican communities.

No conflict of interest

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Table 1. Socio-demographic and physical characteristics by metabolic syndrome status in Mexican and U.S. Pima Indians

	Mexican Pima Metabolic Syndrome Status			P-Value	U.S. Pima Metabolic Syndrome Status			P-value
	No	Yes	All	<	No	Yes	All	<
n	170	54	224		194	253	447	
Female (%)	46.5	70.4	52.2	0.05	49	66.0	58.6	0.001
Age (y)	36.2±14.8	45.2±13.8	36.6±15.1	0.001	33.2±10.3	44.4±14.1	40.2±14.0	0.001
Weight (kg)	62.0±11.5	71.7±12.2	64.3±12.4	0.001	83.9±21.3	98.5±24.7	92.2±24.4	0.001
Height (m)	160.9±8.6	158.0±7.2	160.2±8.4	0.05	165.1±7.6	163.9±9.0	164.4±8.4	NS
BMI (kg/m^2)	24.0±3.6	28.8±4.7	25.1±4.4	0.001	30.7±7.1	36.4±7.7	33.9±8.0	0.001
Body Fat (%)	25.1±10.2	37.5±9.3	28.0±11.3	0.001	36.0±10.5	44.9±6.4	41.0±9.5	0.001
PA (MET-hour/week)	134 (119-160)	90 (79-107)	120 (107-136)	0.001	52 (31-69)	12 (10-19)	22.5 (18-31)	0.001

Data are *n*, means ± SD, or median (95% CI); PA, physical activity; Comparison are between metabolic syndrome status using two-sample independent t-test (Rank-sum test) for continuous variables and chi-square for categorical variables; NS, non significant

Table 2. Prevalence of the metabolic syndrome and its components in Mexican and U.S. Pima Indians

Components	Mexican Pima Indians Percent (95% CI)	U.S. Pima Indians Percent (95% CI)
n	224	447
metabolic syndrome	24.1 (18.7-30.3)	56.6 (52.9-61.3)*
Elevated fasting glucose	8.9 (5.5-13.5)	48.6 (43.8-53.4)*
Central obesity	23.7 (18.3-30.0)	73.6 (69.3-77.7)*
High blood pressure	25.9 (20.3-32.2)	42.1 (37.5-46.9)*
High triglycerides†	30.6 (24.6-37.2)	32.5 (27.1-38.3)
Low HDL-cholesterol‡	73.1 (66.7-78.9)	74.8 (69.5-79.7)

*$P \leq 0.0001$; For U.S. Pima Indian group: †n=280; ‡n=298

Table 3. Adjusted comparison of metabolic syndrome prevalence between Mexican and U.S. Pima Indians

Variable	Model 1: OR (95% CI)	Model 2: OR (95% CI)	Model 3: OR (95% CI)
Group			
Mexican Pima	reference group	reference group	reference group
U.S. Pima	4.77 (3.19-7.13)	2.37 (1.51-3.71)	1.35 (0.80-2.29)

OR, Odds Ratio (95% confidence intervals); Model 1 (n=671): adjusted for age and sex; Model 2 (n=662): adjusted for age, sex, and BMI (dichotomized as BMI<25 *vs.* BMI>=25); Model 3 (n=579): Adjusted for age, sex, BMI (as previously defined), and physical activity (METs-hr/week)

APPENDIX C: Risk Factors Associated with Type 2 Diabetes in Mexican and U.S. Pima Indians

RUNNING TITLE: Factors associated with type 2 diabetes in Pima Indians

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Total words number: text= 2746; Abstract: 265

SUMMARY

OBJECTIVE- Prevalence of type 2 diabetes in Mexican Pima Indians is at least 5 times less than that of Unites States (U.S.) Pima Indians; however, information regarding factors associated to type 2 diabetes in the Mexican Pima Indians and whether these associations are different compared to those in the U.S. Pima Indians is lacking. The aim of the present analysis is to ascertain variables independently associated with type 2 diabetes in each population (Mexican and U.S. Pima Indians).

RESEARCH DESIGN AND METHODS- The study population consisted of 224 Mexican Pima Indians and 887 U.S. Pima Indian men and women 20 years of age and older. Type 2 diabetes was defined according to the 1999 WHO criteria after an oral glucose tolerance test.

RESULTS- The association of age, BMI, waist circumference, percent of body fat, fasting and two hour insulin with type 2 diabetes was different between Mexican and U.S. Pima Indians. The association of sex, total and HDL-cholesterol, triglycerides, hypertension and physical activity with type 2 diabetes was different between Mexican and U.S. Pima Indians. In multivariate analysis, age, fasting insulin, and waist circumference were independently associated with type 2 diabetes in the Mexican Pima Indians; the corresponding independent factors in U.S. Pima Indians were age, sex, fasting insulin, total cholesterol, blood pressure, and physical activity.

CONCLUSION- Risk factor patterns associated to type 2 diabetes were different in Mexican and U.S. Pima Indians. Only age and fasting insulin were common risk factors in both populations. In U.S. Pima Indians, type 2 diabetes was explained by larger number of factors than in the Mexican Pima Indians.

INTRODUCTION

Although type 2 diabetes is a major public health issue worldwide (1), certain groups are of major concern, such as Native Americans and Mexican-American populations since they are highly susceptible to type 2 diabetes, especially when they are exposed to a Western lifestyle (2). Studies in the Pima Indians comparing two genetically related populations (3) have shown that Mexican Pima Indians living in a contrasting traditional lifestyle in the Sierra Madre of Mexico have an age- and sex-adjusted prevalence of diabetes of at least 5 times less than their Unites States (U.S.) Pima Indians counterpart (3).

Type 2 diabetes risk factors are not equally distributed among American Indians (4) or between Mexicans living in U.S. and those living in Mexico (5). Based on this knowledge, it is important to assess risk factor profiles for type 2 diabetes by specific population group in order to establish future prevention strategies. Factors such as insulin resistance, total and central obesity, hypertension, hypertriglyceridemia, and hypoalphalipoproteinamia have all been described as risk factors for type 2 diabetes in most population, including the U.S. Pima Indians (6,7,8). However, in the Mexican Pima, related information is lacking. The aim of the present analysis was to ascertain variables independently associated with type 2 diabetes in both populations (Mexican and U.S. Pima Indians).

METHODS

Subjects

The Mexican Pima Indian study population and data collection procedures have been described previously (3,9). Briefly, all adult individuals from Maycoba, Sonora

Mexico and surrounding areas were invited to participate in a health examination at our research clinic in the village of El Kipor, 10 Km east of Maycoba. The Mexican Pima Indian sample consisted of subjects who were 20 years of age and older, having taken part of a population-based cross-sectional study (3). A total of 224 Mexican Pima Indian of both sexes participated in the study. The study was approved by the ethics committee**s** of the University of Milwaukee and the *Centro de Investigación en Alimentación y Desarrollo*, Asociacion Civil. All subjects gave written informed consent prior to participation (3).

Since 1965, a longitudinal study of type 2 diabetes and its complications has been ongoing in the Gila River Indian Community in Arizona, where a group of Pima Indians live. Approximately every two years, all residents of this community who are 5 years of age or older are invited to participate in the study (3,10). A sample of 887 U.S. Pima Indians was selected for comparison with the Mexican Pima Indians regarding prevalence of type 2 diabetes and obesity. Thus, U.S. Pima participants who were 20 years of age or older were selected on the basis of having been examined during similar year period as in the Mexican Pima study (3).

Biochemical Measures

All biochemical measures followed the same protocol as those in the U.S. Pima cohort study; biochemical analyzes were conducted at a central laboratory at the Phoenix Epidemiology and Clinical Research Branch. Oral glucose tolerance tests were performed using a 75 g glucose load after 10-12 hour fasting according to World Health Organization (WHO) recommendations (11). Biochemical measures were conducted in serum to assess fasting and 2-h post glucose for concentrations of

glucose, insulin, triglycerides, total and HDL-cholesterol. Plasma glucose concentrations were measured with an autoanalyzer using the glucose hexokinase (Ciba Corning Expresss, Norwood, MA). Plasma insulin concentrations were determined using an automated radioimmunoassay analyzer (Concept 4; INCBiomedicals, Horsham, PA). Triglycerides, total and HDL-cholesterol concentrations were measured by enzymatic methods (12-14).

Subjects were considered to have diabetes if they have either of the following: 1) self-report of a physician diagnosis with currently on therapy (either oral or insulin); 2) fasting plasma glucose level was greater than or equal to 140 mg/dl (7.8mmol/l); 3) two-hour plasma glucose level was greater than or equal to 200 mg/dl (11.1mmol/l) (11).

Physical Measures

All physical measures followed the same protocol. Weight was measured on a battery-operated electronic scale and height by a portable stadiometer. Body mass index (BMI) was calculated by dividing weigh in kilograms by the square of height in meters (Kg/m^2). Abdominal obesity was assessed by waist circumference in centimetres, measured in supine position at the level of the umbilicus. Percent body fat was estimated by bioelectrical impedance using an RJL impedance meter (model BIA-103; RJL Systems, Detroit, MI) using an equation developed for the U.S. Pima Indians (15). Blood pressure was measured to the nearest 2 mmHg with a mercury sphygmomanometer (Desk Model Mercury Sphygmomanometer, model 100; Liberator Medical Supply, Stuart, FL) in the right arm while the subjects rested in sitting position. Diastolic blood pressure was measured at the fourth Korotkoff sound.

Physical activity was measured using a questionnaire developed for the U.S. Pima Indians and adapted to the Mexican Pima Indians (3,9).

Participants were considered to have hypertension if they were taking antihypertensive medication or had a systolic blood pressure greater than or equal to 140 mmHg or a diastolic blood pressure of greater than or equal to 90 mmHg at the time of examination (16). Overweight was defined as a BMI greater than or equal to 25 kg/m^2 and less than 30 kg/m^2. Obesity was defined as a BMI greater than or equal to 30 kg/m^2. Abdominal obesity was defined as a waist circumference greater than or equal to 88 cm in women and greater than or equal to 102 cm in men. Low HDL-cholesterol was defined if values were less than or equal to 35 mg/dl in men and less than or equal to 45 mg/dl in women. High total cholesterol and high triglyceride were considered if values were greater than or equal to 240 mg/dl total cholesterol and if values were greater than or equal to 150 mg/dl for triglycerides. (17). Physical activity was expressed as hours per week and as metabolic equivalent (MET-hours per week) averaged over the past year (18). In addition to treating physical activity as continuous variable, it was also dichotomized at the median and categorized to tertiles.

Statistical Analyses

Physical and biochemical characteristics are presented as means and standard deviations or geometric means and their 95 confidence interval when variables where not normally distributed. Following recommendations (16,17), some continuous variables were also expressed as categorical.

Association between type 2 diabetes and risk factors of interest were assessed separately for U.S. and Mexican Pima Indians using logistic regression analysis and

the odds ratio (OR). In addition, a first degree product term interactions between group (Mexican and U.S. Pima Indians) and the corresponding variable was added to each models in order to test the null hypothesis of no differences between the groups regarding the association of the above-mentioned variables with type 2 diabetes. Differences in association between Mexican and U.S. Pima Indians were considered when p-values for the corresponding interaction term were smaller than or equal to 0.1

Multiple logistic regression was used for Mexican and U.S. Pima Indians to ascertain the group of variables independently associated to type 2 diabetes prevalence. Multivariate models were constructed by stepwise model selection methods in its forward option and using a p-value of 0.05. In addition, preliminary models were evaluated for possible interaction by testing all the possible combinations of first degree product terms interactions with the group population one by one and using p-value of 0.1. Models were finally evaluated for goodness-of-fit using Hosmer-Lemeshow statistic and the ROC curve. Furthermore, assumptions of the logistic regression were also evaluated and corrections were implemented in necessary cases. Linearity in the log odds of continuous variables was evaluated. Based on graphical results in each adjusted population model, it was decided to dichotomize age to correct for non linearity relationship. All analyses were performed using STATA software (version 8.0; Stata Corp, College Station, TX).

RESULTS

Demographic, physical, and biochemical characteristics in Mexican and U. S. Pima Indians are shown in Table 1. Compared to Mexican Pima Indians, all variables had higher values/concentrations in U.S. Pima Indians with the exception of age and diastolic blood pressure, which had similar values in both groups and physical activity, which was lower in U.S. Pima Indians.

In the Mexican Pima Indians, variables positively associated with type 2 diabetes were age, weight, BMI (overweight and obesity), percent body fat, waist circumference, fasting and two hours insulin, total cholesterol, triglycerides and hypertension. HDL-cholesterol and sex were negatively associated to type 2 diabetes. The associations were all significant, except for sex and total cholesterol (Table 2). Although physical activity (MET-hours per week) was not significantly associated with type 2 diabetes in the Mexican Pima population (Table 2), in secondary analysis stratified by sex, a significant inverse association was observed in Mexican Pimas male [0.71, 0.52-0.96], but not females [1.16, 0.87-1.55], expressed as per 20 MET-hours per week and type 2 diabetes in men but not in women. When physical activity was dichotomized using the median (≥120 Mets-hours-week), similar results were found (data not shown). Among U.S. Pima Indians, positive associations with type 2 diabetes were shown for age, waist circumference, percent body fat, fasting insulin, total cholesterol, triglycerides, and hypertension, while negative associations were found for HDL-cholesterol and physical activity (MET-hours per week). All the associations were significant, except for weight, BMI and two hours insulin. Furthermore, unlike Mexican Pima, no effect modification was found by sex. Significant interactions between the two Pima groups were observed for associations

between type 2 diabetes and age, weight, fasting and two hour insulin concentrations, BMI, waist circumference and percent of body fat.

As presented in Table 3, type 2 diabetes was independently and positively associated with older age (>=50 years; OR=3.70; 95% CI, 1.03-13.27), waist circumference (per 5 cm; OR=2.60; 95% CI, 1.45-4.65) and fasting insulin (per 1 uU/mL; OR: 1.09; 95% CI, 1.03-1.15) in the Mexican Pima Indians. In the U.S. Pima Indians, type 2 diabetes was independently and positively associated with older age (>=50 years; OR=4.60; 95% CI, 2.50-8.46), higher fasting insulin (per 1 uU/mL; OR=1.02; 95% CI, 1.01-1.03), higher cholesterol (≥240 mg/dl; OR=3.62; 95% CI, 1.59-8.25) and having hypertension (OR= 1.69; 95% CI, 1.03-2.76), and negatively associated with higher physical activity (≥ 22.85 Mets-hours-week; OR=0.45; 95% CI, 0.30-0.66).

DISCUSSION

The present study examined differences between Mexican and U.S. Pima Indians for risk factor associations with type 2 diabetes. It also examined the group of variables independently associated with type 2 diabetes prevalence in Mexican and U.S. Pima Indians separately. Results show that most of the type 2 diabetes related factors assessed had lower values in the Mexican Pima Indians compared to U.S. Pimas, except for age and diastolic blood pressure. Lower prevalence of hypertension has been reported in the U.S. Pima Indians in comparison to the general U.S. population (19) in spite of having higher prevalence of obesity (20). Surprisingly, the mean of HDL-cholesterol was higher in the U.S. Pima Indians, despite their higher level of obesity (3), the negative correlation between obesity and HDL-cholesterol previously

reported in the U.S. Pima Indians (21) as well as the lower prevalence of low HDL-cholesterol level in comparison to the general U.S. population (22).

In a study that assessed factors explaining differences in type 2 diabetes prevalence between Mexican-American residents in San Antonio and Mexican residents from Mexico City, similar factors as those in the present analysis were assessed. The authors found that not all the type 2 diabetes risk factors studied were higher in Mexican-American participants compared to those living in Mexico. The Mexican-American group had lower fasting insulin, triglycerides and higher HDL-cholesterol (5). The authors suggested that these lower biochemical values might explain the contrasting differences in type 2 diabetes prevalence between these groups.

In the present study, although the U.S. Pima Indians are more obese, have higher consumption of dietary fat (3), and are more sedentary (9) than the Mexican Pima Indians, their level of plasma total cholesterol, triglyceride and diastolic blood pressure were similar. Lower levels in plasma total and LDL-cholesterol have been reported in the U.S. Pima Indians in comparison to Caucasians (23, 24). In a separate report (21), a positive correlation was reported between triglycerides and obesity in the U.S. Pima Indians, but not as strong as in other populations. The authors commented that there was little evidence for an association between hypertriglyceridemia and obesity (21). In addition, the high triglyceride values in the Mexican Indians may be due to the positive association of triglycerides with high dietary carbohydrates as reported elsewhere (25).

The relationship of the select risk factors with type 2 diabetes prevalence was evaluated separately for U.S. and Mexican Pima. Results indicated a different risk

factor pattern in the two groups. Most of the studied variables were significantly associated with type 2 diabetes in both groups, except for sex in the Mexican Pima Indians and weight, BMI, and two hour insulin in the U.S. Pima Indians. In a different analysis of U.S. Pima Indians using a prospective design, higher weight, BMI and waist circumference, were associated with type 2 diabetes risk (26), contrasting with our results. This finding underlines the importance of prospective analysis in studying cause-effect associations and the caution that needed to be taken when cross-sectional data are used.

Differences in the effect on type 2 diabetes was demonstrated for age, weight, fasting and two hours insulin, BMI (overweigh and obesity), waist circumference, high percent of body fat between Mexican and U.S. Pima Indians. On the other hand, not difference in the effect on type 2 diabetes was demonstrated for high total and HDL-cholesterol, triglycerides, hypertension and physical activity either expressed as continuous or categorical. It is important to note, however, the fact that in most of the cases the measures of association were more unstable in the Mexican Pima Indians, since the 95% confidence intervals were wider.

In the Mexican Pima Indians, type 2 diabetes was independently associated with older age, higher waist circumference and fasting insulin. In the U.S. Pima Indians, in addition to older age and higher fasting insulin, type 2 diabetes was also independently associated with higher total cholesterol, hypertension and lower physical activity. In a different analysis using a cohort design, Hanson et al. reported that type 2 diabetes incidence was independently associated with insulinemia, body size, lipids and blood pressure factors in the U.S. Pima Indians (27). However, blood pressure was only weakly associated with type 2 diabetes in this analysis (27).

Physical activity was not included in Hanson's et al. report (27). However, in a different report from the U.S. Pima Indians cohort study (18), lower physical activity was associated with type 2 diabetes incidence. In accordance with our findings in the Mexican Pima Indians, results from the Mexican-American and non-Hispanic whites showed that type 2 diabetes incidence was associated with older age, higher BMI, higher fasting insulin but not with sex (28).

Insulin resistance, BMI, abdominal obesity, hypertension, hypertriglyceridemia, and low HDL-cholesterol all been associated with type 2 diabetes (6,7). The fact that in our analysis body size was not associated with type 2 diabetes in the U.S. Pima Indians may be explained because we are using prevalent data and the knowledge that type 2 diabetes can lead to weight reduction (20). Knowler et al. showed a weak association between BMI and type 2 diabetes prevalence and a strong association between BMI and type diabetes incidence (20). The weaker association between BMI and type diabetes prevalence was explained because in prevalence studies weight loss might occur after the diagnosis of diabetes due to treatment, calorie loss as glycosuria, or the high resting metabolic rate in diabetes (20).

The strength of this study is due to several factors. First, we are comparing two genetically related populations living in naturally contrasting lifestyles (3). Second, all of the analysis followed similar measurement protocols and that all biological analyses were done at the same central laboratory at the Phoenix Epidemiology and Clinical Research Branch. Third, type 2 diabetes was determined by an oral glucose tolerance test and not by the self-report. Nevertheless, the present study is limited by the lack of dietary information in the U.S. sample population

which do not allow us to evaluate whether the contrasting differences in some dietary components such as fat, carbohydrates and fiber are associated with type 2 diabetes prevalence. Another major limitation is the cross-sectional nature of the data, which do not allow the demonstration any of temporal effect of the factors studied on risk of type 2 diabetes.

Risk factor patterns associated to type 2 diabetes were different in Mexican and U.S. Pima Indians. Only age and fasting insulin were common risk factors in both populations. In U.S. Pima Indians, type 2 diabetes was explained by larger number of factors than in the Mexican Pima Indians. Given the cross-sectional nature of the factors in this study, it is recommended to confirm these results in future longitudinal studies in the Mexican Pima Indians.

ACKNOWLEDGEMENT

We wish to thank the members of the Maycoba Pima and non-Pima Community and the Gila River Indian Community for their participation in these studies. Our gratitude goes to Bertha Isabel Pacheco, Ana Cristina Gallegos and Hortensia Montesinos for data collection in Mexico and the staff of the Phoenix Epidemiology and Clinical Research Branch. NIDDK for data collection in Sacaton, Arizona. Special thanks are extended to Nurse Eremita Perez Ruiz for facilitating work with our Mexican communities. The study in Mexico was supported partially by grant number NIH-DK-49957.

The authors have nothing to disclose.

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Table 1. Physical and biochemical characteristics in Mexican and U.S. Pima Indians

Variable	**Mexican Pima (n=224)**	**U.S. Pima (n=887)**
Age (years)	38.4±15.1	38.5±13.7
Weight (kg)	64.3±12.4	93.8±23.9
Height (m)	160.2±8.4	164.5±8.4
BMI (Kg/m^2)	25.1±4.4	34.6±8.0
Body fat (%)	28.1±11.3	41.4±9.1
Waist circumference (cm)	84.7±11.7	110.5±18.6
Fasting glucose (mg/dL)	96.8±31.5	135.1±71.5
Two hrs glucose (mg/dL)	117.2±79.3	188.6±125.1
Fasting insulin (uU/mL)	5.0 [4.0,6.7]	24.0 [22.8,26.0]
Two hrs insulin (uU/mL)	27.0 [24.0,31.0]	83.0 [77.2,90.0]
HDL-cholesterol (mg/dL)	38.4±9.4	41.3±11.7
Cholesterol (mg/dl)	174.3±38.9	177.0±38.5
Triglycerides (mg/dL)	111.5 [99.9,122.0]	118.0 [111.0,126.0]
SBP (mmHg)	117.3±15.7	123.3±19.0
DBP (mmHg)	72.0±10.6	73.3±11.8
PA (MET-hours per week)	120 [107.2,136.1]	22 [19.7,28.6]

Data are means ± SD, or geometric means [95% CI]; BMI, body mass index; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; PA, physical activity; Body fat measured by bioelectrical impedance.

Table 2. Odds ratios (95% confidence interval) for diabetes status and selected study variables in Mexican and U.S. Pima Indians

Variable	**Mexican Pima (n=224)**	**U.S. Pima (n=855)**	**Interaction (*p*-value)**[†]
Age (per 10 year)	1.58 [1.16-2.16]	2.43 [2.12-2.79]	0.013
<50 *vs.* ≥50	2.59 [0.92-7.32]	9.26 [6.22-13.80]	0.025
Male *vs.* Female	0.64 [0.22-1.81]	0.76 [0.57-1.00]	NS
BMI km/m^2			
< 25	1.00	1.00	<0.00001
25-29.99	6.41 [1.29-31.74]	1.08 [0.63-1.85]	
≥30	18.11 [5.4-92.76]	0.88 [0.55-1.42]	
Per 5 kg/m^2	2.75 [1.56-4.87]	0.97 [0.89-1.06]	0.0001
Body fat (Per 5%)	1.90 [1.34-2.70]	1.17 [1.08-1.27]	0.003
Waist circumference (cm)			
High *vs.* normal[††]	6.40 [2.20-18.58]	1.44 [1.03-2.02]	0.008
Per 5 cm	1.68 [1.32-2.12]	1.05 [1.02-1.09]	<0.00001
Fasting insulin (uU/mL)	1.11 [1.05-1.16]	1.02 [1.01-1.03]	0.0006
Two hrs insulin (uU/mL)	1.01 [1.00-1.02]	0.99 [0.99-1.00]	0.0032
Total Cholesterol (mg/dl)			
High *vs.* normal[††]	1.96 [0.40-9.49]	2.72 [1.50-4.94]	NS
Per 5 mg/dL	1.10 [1.03-1.17]	1.04 [1.02-1.06]	NS

(Table continued)

Table 2. (Continued)

Variable	**Mexican Pima (n=224)**	**U.S. Pima (n=855)**	**Interaction (*p*-value)[†]**
HDL-Cholesterol (mg/dL)			
High *vs.* low[††]	0.24 [0.07-0.86]	0.38[§] [0.24-0.61]	NS
Per 5 mg/dL	0.64 [0.45-0.91]	0.76 [0.68-0.86]	NS
Triglycerides (mg/dL)			
High *vs.* normal[††]	8.04 [2.49-25.96]	3.11[§§] [1.85-5.22]	NS
Per 5 mg/dL	1.05 [1.02-1.07]	1.05 [1.03-1.07]	NS
Hypertension			
Yes *vs.* No[††]	5.96 [2.03-17.47]	3.53 [2.52-4.96]	NS
PA (Per 20 MET-hours/week)	0.85 [0.71-1.02]	0.91 [0.87-0.96]	NS
High *vs.* low[††]	0.64 [0.22-1.87]	0.40 [0.29-0.55]	NS

BMI, body mass index; Body fat measured by bioelectrical impedance; [†]for interaction of each covariate with group; Female as reference group; [††] High waist circumference: men≥ 102 cm, women≥ 88 cm; High total cholesterol ≥240 mg/dl; HDL, low density lipoprotein: men≤ 35 mg/dl, women≤ 45 mg/dl; High triglycerides: ≥150 mg/dl; Hypertension; SBP, systolic blood pressure ≥140 mmHg or DBP, diastolic blood pressure ≥90 mmHg or under antihypertensive medication; PA, physical activity: in U.S. Pima, high≥ 22.85 Mets-hours-week (median), in Mexican Pima, high≥120 Mets-hours-week (median); NS (not significant)

Table 3. Multivariate Odds ratio (95% confidence intervals) for diabetes status in Mexican and U.S. Pima Indians

Variable	**Mexican Pima N=224**	**U.S. Pima N=630**
Age (years) <50 *vs.* ≥50	3.70 [1.03-13.27]	4.60 [2.50-8.46]
Male *vs.* Female	0.99 [0.26-3.89]	0.77 [0.51-1.15]
Waist circumference (cm) Per 5 cm	2.60 [1.45-4.65]	-
Fasting insulin (uU/mL)	1.09 [1.03-1.15]	1.02 [1.01-1.03]
Cholesterol (mg/dL) High *vs.* normal	-	3.62 [1.59-8.25]
Hypertension Yes *vs.* No	-	1.69 [1.03-2.76]
PA (Mets-hrs-week) High *vs.* low	-	0.45 [0.30-0.66]

Data are odds ratio [95% CI]; Female as reference group; [†]High cholesterol ≥240 mg/dl; Hypertension; systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or under antihypertensive medication; PA, physical activity in U.S. Pima, high ≥ 22.85 Mets-hours-week (median).

APPENDIX D: HUMAN SUBJECTS APPROVAL

THE UNIVERSITY OF ARIZONA.

Julian Esparza-Romero <esparzaj@email.arizona.edu>

Research Office Approval of PhD Internship Project for: Julian Esparza-Romero - *(Non-applicable)

1 message

Suzanna Trejo <Suzanna@email.arizona.edu> **Wed, Oct 27, 2010 at 10:03 AM**
To: Julian Esparza-Romero <esparzaj@email.arizona.edu>
Cc: Judy Goosherst <judyg@email.arizona.edu>

Dear Julian,

I have received your MEZCOPH form requesting Non-Applicable status approval for your PhD internship project titled, **"Risk factors of Type 2 Diabetes in Mexican and U.S. Pima Indians: Role of environment."**

I have reviewed all of your project materials, and since your project involves analysis of de-identified data, I do agree that it is "Non-Applicable" (to IRB Review) and should not require completion of the *IRB Application for Human Research Form*. You can pick up a copy of your approved project materials from Suzanna Trejo. (You will likely need this copy for your graduation paperwork.) Suzanna has a box just outside her desk that says "Student Pick-up" and a copy will be there for you to pick up at any time.

Good luck to you!

Dr. Sherrill

Duane Sherrill

THE UNIVERSITY OF ARIZONA.

Associate Dean for Research and Professor

Mel & Enid Zuckerman College of Public Health

APPENDIX E: ADDITIONAL TABLES

Table 1 (Manuscript 1). Physical and biochemical characteristics by sex and population group with normal glucose tolerance

Variable	**Women**			**Men**			**p-value, All**
	MxPI	**USPI**	***p-value***	**MxPI**	**USPI**	***p-value***	**MxPI *vs.* USPI**
N	100	246		94	203		194/449
Age (years)	35±12	32±9	0.05	39±17	33±11	0.0002	0.0001
BMI (Kg/m^2)	26±5	35±8	0.0001	23±3	33±8	0.0001	0.0001
Body fat (%)	35±7	46±6	0.0001	18±8	33±8	0.0001	0.0001
Waist circumference (cm)	84±13	110±20	0.0001	82±9	105±19	0.0001	0.0001
Fasting glucose (mmol/L)	4.9±0.5	5.1±0.5	0.0002	5.0±0.5	5.3±0.5	0.0004	0.0001
Two hrs glucose (mmol/L)	5.6±1.0	6.1±1.0	0.0001	4.9±1.3	5.4±1.2	0.001	0.0001
Two hrs insulin ()	40±37	111±81	0.0001	28±25	80±71	0.0001	0.0001
HDL (mg/dL)	38±10	47±12†	0.0001	40±9	41±13‡	0.607	0.0001
Cholesterol (mg/dl)	167±37	171±33	0.329	175±37	176±32	0.812	0.4234
Triglycerides (mg/dL)	121±83	105±51[#]	0.099	122±71	129±75[##]	0.568	0.3092
SBP (mmHg)*	112±12	112±14	0.562	120±14	125±14	0.01	0.0729
DBP (mmHg)**	70±9.3	68±11	0.151	75±11	74±10	0.229	0.0438

MxPI, Mexican Pima Indians; USPI, U.S. Pima Indians; *Systolic blood pressure; ** Diastolic blood pressure; T-test independent samples; †n=107; ‡n=61; [#]n=90; [#]n=53

Table 2 (Manuscript 1). Adjusted means differences in insulin resistance between Mexican and U.S. Pima Indians measured by log transformed fasting insulin and HOMA-IR (Model 1: age and sex adjusted)

Variable	Model*: ln fasting insulin		Model**: ln HOMA-IR	
	β	95% CI	β	95% CI
Group				
Mexican Pima Indians	(ref)		(ref)	
U.S. Pima Indians	1.34	1.17-1.51	3.84	3.10-4.58
Age (years)	-0.007	-0.013-(-0.001)	-0.027	-0.056-0.002
Sex				
Female	(ref)		(ref)	
Male	0.05	-0.10-0.21	0.46	-0.23-1.15
Intercept	1.66	1.38-1.93	2.29	1.05-3.52

*n=585 (R^2=0.32); **n=585 (R^2=0.17)

Table 3 (Manuscript 1). Adjusted means differences in insulin resistance between Mexican and U.S. Pima Indians measured by log transformed fasting insulin and HOMA-IR (Model 2: BMI and waist circumference as continue)

Variable	Model*: ln fasting insulin		Model**: ln HOMA-IR	
	β	95% CI	β	95% CI
Group				
Mexican Pima Indians	(ref)		(ref)	
U.S. Pima Indians	0.80	0.61-0.99	0.81	0.620-1.003
Age (years)	-0.008	-0.014-(-0.002)	-0.007	-0.013-(-0.001)
Sex				
Female	(ref)		(ref)	
Male	0.18	0.04-0.33	0.22	0.07-0.37
BMI	0.03	0.01-0.06	0.04	0.01-0.06
Waist circumference (cm)	0.01	-0.002-0.020	0.01	-0.002-0.021
Intercept	0.07	-0.38-0.53	-1.62	-2.09-(-1.16)

*n=560 (R^2=0.43); **n=560 (R^2=0.44)

Table 4 (Manuscript 1). Adjusted means differences in insulin resistance between Mexican and U.S. Pima Indians measured by log transformed fasting insulin and HOMA-IR (Model 3: BMI and waist circumference as categorical)

Variable	Model*: ln fasting insulin		Model**: ln HOMA-IR	
	β	95% CI	β	95% CI
Group				
Mexican Pima Indians	(ref)		(ref)	
U.S. Pima Indians	0.78	0.580-0.979	0.79	0.581-0.989
Age (years)	-0.01	-0.014-(-0.002)	-0.01	-0.013-(-0.001)
Sex				
Female	(ref)		(ref)	
Male	0.16	0.01-0.31	0.20	0.04-0.35
Total obesity	0.17	0.02-0.32	0.20	0.05-0.36
Central obesity	0.38	0.23-0.53	0.39	0.23-0.55
Intercept	1.10	0.81-1.40	-0.51	-0.81-(-0.21)

*n=560 (R^2=0.41); **n=560 (R^2=0.42); Central obesity (tertiles of waist circumference)

Table 5 (Manuscript 2). Adjusted comparison of metabolic syndrome components between Mexican and U.S. Pima Indians

Variable	**EFG** **OR (95% CI)**	**CO** **OR (95% CI)**	**HBP** **OR (95% CI)**	**HT** **OR (95% CI)**	**Low HDL-c** **OR (95% CI)**
Group					
Mexican Pima	(ref)	(ref)	(ref)	(ref)	(ref)
U.S. Pima	5.93 (2.98-11.78)	11.57 (6.95-19.25)	1.27 (0.77-2.12)	0.64 (0.38-1.11)	0.36 (0.19-0.69)
Age (years)	1.08 (1.06-1.10)	1.03 (1.02-1.05)	1.06 (1.04-1.08)	1.03 (1.01-1.05)	1.01 (0.99-1.03)
Sex	1.38 (0.87-2.19)	0.16 (0.10-0.26)	2.38 (1.57-3.64)	1.14 (0.69-1.87)	0.31 (0.19-0.52)
Overweight/obesity	6.05 (2.65-13.79)	-	2.70 (1.56-4.70)	4.41(2.40-8.10)	3.46 (1.90-6.29)
PA (Mets-hr-wk)	0.99(0.992-0.998)	0.99(0.993-0.999)	0.99(0.995-0.999)	1.00(0.998-1.004)	0.99(0.995-1.00)

EFG, elevated fasting glucose; CO, central obesity; HBP, high blood pressure; HT, high triglycerides; Low HDL-cholesterol; Female as a reference group; Non obese (normal) people as a reference group

Table 6 (Manuscript 2). Prevalence of the metabolic syndrome and its components in Mexican and U.S. Pima Indians stratified by physical activity

Components	Mexican Pima		U.S. Pima	
	Low PA	High PA	Low PA	High PA
n	110	111	184	180
Metabolic syndrome	34.6 **(**25.7-44.2)	13.5 (7.8-21.3)**	65.8 (58.4-72.6)	38.3 (31.2-45.9)**
Elevated fasting glucose	10.0 (5.1-17.2)	7.2 (3.2-13.7*).483*	56.7 (49.1-64.1)	29.8 (23.2-37.1)**
Central obesity	33.6 (24.9-43.3)	13.5 (7.8-21.3)**	83.0 (76.7-88.1)	61.5 (53.9-68.6)**
High blood pressure	24.6 (16.8-33.7)	27.0 (19.0-36.3).	32.3 (27.5-37.2)	31.6 (26.8-36.4)*
High triglycerides	33.6 (24.9-43.3)	27.3 (19.2-37.0)	28.5 (20.6-36.3) †	31.3 (21.8-40.7)
Low HDL-cholesterol	86.2 (78.3-92.1)	60.2 (50.3-70.0)**	78.4 (70.6-84.9) ‡	64.6 (55.7-73.6)

Data are percent (95% Confidence interval); PA, physical activity; Mexican and U.S. Pima Indians were classified either as having low or high PA in MET-hr/week units using the median in each group; Statistical comparison are between Low PA *vs.* High PA in each group; *p≤0.001 **p≤0.0001; †n=129 *vs.* 96; ‡n=139 *vs.*101

Table 7 (Manuscript 2). Relationship between metabolic syndrome status and independent variables in Mexican and U.S. Pima Indians

Variable	**Mexican Pima (n=224)** **OR (95% CI)**	**U.S. Pima (n=459)** **OR (95% CI)**
Age (years)	1.04 (1.02-1.06)	1.09 (1.06-1.11)
Sex[†]	0.37 (0.19-0.71)	0.49 (0.34-0.73)
Weight(kg)	1.07 (1.04-1.10)	1.03 (1.02-1.04)
Height (m)	0.96 (0.92-0.99)	0.98 (0.96-1.004)
BMI (Kg/m^2)	1.35 (1.23-1.49)	1.11 (1.08-1.15)
Body Fat (%)	1.14 (1.09-1.19)	1.13 (1.09-1.16)
PA (Met-h-week)	0.99 (0.998-0.997)	0.99 (0.991-0.996)

[†]Female as reference group; PA, physical activity; Using separated simple logistic regression for each variable and group population

Table 8 (Manuscript 2). Adjusted comparison of metabolic syndrome prevalence between Mexican and U.S. Pima Indians

Variable	**Model 1:** **OR (95% CI)**	**Model 2:** **OR (95% CI)**	**Model 3:** **OR (95% CI)**	**Model 3*:** **OR (95% CI)**
Group				
Mexican Pima	(ref)	(ref)	(ref)	(ref)
U.S. Pima	4.77 (3.19-7.13)	2.37 (1.51-3.71)	1.35 (0.80-2.29)	1.29 (0.78-2.16)
Age (years)	1.07 (1.05-1.08)	1.08 (1.06-1.10)	1.08 (1.05-1.09)	1.07 (1.05-1.09)
Sex	0.45 (0.32-0.64)	0.54 (0.37-0.79)	0.87 (0.57-1.35)	-
Overweight/obesity	-	11.43 (6.04-21.64)	14.37 (6.87-30.08)	14.78 (7.08-30.83)
PA (Mets-hr-wk)	-	-	0.99 (0.991-0.997)	0.99 (0.991-0.997)

Model 1 (n=671): adjusted for age and sex; Model 2 (n=662): adjusted for age, sex, and BMI (dichotomized as normal *vs.* overweight/obese); Model 3 (n=579): Adjusted for age, sex, BMI (as previously defined), and physical activity (Met-h/wk); Model 3* (n=579): sex removed; Female as a reference group; Non obese (normal) people as a reference group

Table 9 (Manuscript 3). Physical and biochemical characteristics by sex and combined both sexes in Mexican (n=117 and n=107, for women and men) and U.S. Pima (n=524 and n=363, for women and men) Pima Indians

Variable	Women			Men		
	MxPI	USPI	*p-value*	MxPI	USPI	*p-value*
Age (years)	36.3±13.0	39.1±14.0	0.0486	40.6±16.8	37.7±13.1	0.0589
BMI (Kg/m^2)	26.3±4.9	35.5±8.1	0.0001	23.8±3.4	33.3±7.6	0.0001
Body fat (%)	36.1±7.4	46.7±5.4	0.0001	19.3±7.8	34.3±7.9	0.0001
Waist circumference (cm)	86.2±13.5	113.1±18.4	0.0001	82.9±9.1	107.0±18.3	0.0001
Fasting glucose (mmol/L)	96.3±3.0	137.5±4.0	0.0001	97.3±2.9	131.7±3.9	0.0001
Two hrs glucose (mmol/L)	126.0±7.7	196.1±5.6	0.0001	107.5±7.2	178.5±6.8	0.0001
Fasting insulin (uU/mL)	7.6±8.1	33.4±32.8	0.0001	8.1±9.7	29.2±25.4	0.0001
Two hrs insulin (uU/mL)	46.7±48.4	129.5±116.1	0.0001	32.2±30.4	94.0±88.1	0.0001
HDL-cholesterol (mg/dL)	37.4±9.6	42.5±11.5&	0.0001	39.5±9.1	38.9±11.7	0.6899
Cholesterol (mg/dl)	171±39.2	175±38.8	0.3862	178±38.5	180±37.7	0.4847
Triglycerides (mg/dL)	135±90.2	140±97.0#	0.6378	132±78.2	153±109	0.1092
SBP (mmHg)*	113±14.4	119±16.6	0.0005	122±15.8	129±17.9	0.0003
DBP (mmHg)**	70.0±9.3	70.7±11.0	0.5466	76.5±11.0	77.0±11.8	0.6725

MxPI, Mexican Pima Indians; USPI, U.S. Pima Indians; *SBP, systolic blood pressure;** DBP, diastolic blood pressure; T-test independent samples

Table 10 (Manuscript 3). Relationship between diabetes status and physical activity in Mexican and U.S. Pima Indians men and women expressed as continues and categorical

Variable	**Mexican Pima (n=221)**		**U.S. Pima (n=728)**	
	OR (95% CI)	***p-value****	**OR (95% CI)**	***p-value***
PA (Mets-hrs-week)	0.992 (0.98-1.001)	0.0387	0.996 (0.993-0.998)	0.0001
PA**				
Sedentary	(ref)		(ref)	
Active	0.641 (0.220-1.867)	0.4105	0.402 (0.292-0.553)	0.00001
PA***				
Sedentary	(ref)		(ref)	
Moderate	1.911 (0.608-6.002)	0.267†	0.620 (0.429-0.8960)	0.011†
Active	0.192 (0.022-1.683)	0.136†	0.349 (0.236-0.520)	0.0001†

* By lrtest **Using the median in each population group to categorize; ***Using tertiles generated in each population to categorize; PA, physical activity; † By Wald test.

Table 11 (Manuscript 3). Relationship between diabetes status and physical activity in Mexican and U.S. Pima Indians men expressed as continues and categorical

Variable	**Mexican Pima (n=105)**		**U.S. Pima (n=316)**	
	OR (95% CI)	***p-value****	**OR (95% CI)**	***p-value***
PA (Mets-hrs-week)	0.982 (0.967-0.998)	0.006	0.996 (0.993-0.999)	0.002
PA**				
Sedentary	(ref)		(ref)	
Active	0.063 (0.007-0.59)	0.006	0.369 (0.223-0.611)	0.0001
PA***				
Sedentary	(ref)		(ref)	
Moderate	0.7 (0.088-5.578)	0.736†	0.657 (0.346-1.251)	0.201†
Active	0.116 (0.009-1.252)	0.075†	0.323 (0.177-0.591)	0.0001†

* By lrtest **Using the median in each population group to categorize; ***Using terciles generated in each population to categorize; PA, physical activity; † By Wald test

Table 12 (Manuscript 3). Relationship between diabetes status and physical activity in Mexican and U.S. Pima Indians women expressed as continues and categorical

Variable	**Mexican Pima (n=116)**		**U.S. Pima (n=412)**	
	OR (95% CI)	***p-value***	**OR (95% CI)**	***p-value***
PA (Mets-hrs-week)	1.007 [0.993-1.096]	0.337	0.994[0.990-0.999]	0.007
PA**				
Sedentary	(ref)		(ref)	
Active	3.24 (0.867-12.101)	0.086	0.402 (0.254-0.635)	0.0001
PA***				
Sedentary	(ref)		(ref)	
Moderate	2.85 (0.697-11.666)	0.145†	0.594 (0.378-0.933)	0.024†
Active ††	-	-	0.378 (0.209-0.683)	0.001†

* By lrtest **Using the median in each population group to categorize; ***Using tertiles generated in each population to categorize; PA, physical activity; † By Wald test; †† Program no presented results

Table 13 (Manuscript 3). Adjusted models explained diabetes prevalence in Mexican Pima Indians male

Variable	OR (95% CI)	P-value
Model 1, n=104		
PA (Mets-hr-wk)	0.984 (0.970-0.998)	0.038
Triglycerides (mg/dL)	1.011 (1.000-1.024)	0.004
Model 2, n=104		
PA (Mets-hrs-week) High	0.063 (0.006-0.646)	0.008
Triglycerides (mg/dL) High	12.620 (1.218-130.787)	0.016