EXAMINING THE RELATIONSHIP BETWEEN PROTEIN INTAKE AND SOURCE AND ACANTHOSIS NIGRICANS AMONG YOUNG CHILDREN IN THE CHILDREN'S HEALTHY LIVING PROGRAM IN THE UNITED STATES AFFILIATED PACIFIC

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DEDICATION

This thesis is dedicated to my family, who has encouraged and supported me from the very start, to expand my knowledge and pursue my academic and career goals. First and foremost, I thank God for blessing me with the gift to learn and share my passion with others. To my dad, Claude Calabrese, you have always been my mentor and guide in all aspects of my life. Thank you for always being there, regardless of the circumstance and being my number 1 supporter. To my mom, Rose Ann Calabrese, you are the most selfless, kind hearted, and hard-working person I know. Thank you for instilling those values in me and continuing to be a strong female role mode in my life. To my brother and sister, Kelsie and Jason, though we could not be any more different, you two are my best friends and the ones I can always count on to be in my corner. Last, but not least, to my boyfriend, Ian Kukahiko, you have and continue to shower me with love and support in all of my life goals. I would not be the person I am today without you, I cannot wait to see what the next chapter of our lives has in store for us.

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ABSTRACT

BACKGROUND: Acanthosis Nigricans (acanthosis) is a dermatological condition that is characterized by a symmetrical, velvety, light brown to black pigmentation of the skin. Acanthosis has been reported to be linked to obesity and is a physical marker on the skin for insulin resistance. Previous research has focused on the relationship of high carbohydrate and fat diets with insulin resistance. However, there are no studies to date analyzing the relationship between protein intake and food sources to acanthosis in children.

OBJECTIVE: The purpose of this study was to examine if the presence of acanthosis is related to dietary protein consumption and protein source among children in the United States Affiliated Pacific.

METHODS: A secondary analysis was conducted on data collected from 3468, 2 to 8-year-old children in 11 jurisdictions from the Children's Healthy Living Program. One way ANOVA was conducted to determine the relationship between acanthosis and total energy intake (kcal), mean protein (g), fat (g) and dairy (servings) intake of the two days weighted for weekday/weekend, mean meat (oz) intake and lean meat equivalents from egg (oz), poultry (oz), franks and luncheon meats (oz), fish and seafood (oz), beef, pork and lamb (oz), nuts and seeds (oz), soy (oz), and dry beans and peas (oz) weighted for weekday/weekend days and adjusted for within person variance, and age (yrs). Binary logistic regression was used to analyze the protein sources and whether children consuming protein intake within the age appropriate US Dietary Guidelines and Dietary Reference Intakes were less likely to screen positive for acanthosis. All models were adjusted for age, sex, overweight/obesity, energy intake (kcal) and mean total fat intake (oz).

RESULTS: There were 191 (5.5%) children that screened positively for acanthosis. The prevalence of acanthosis was higher in overweight and obese children than in healthy weight children (63.4% vs 35.6%). The total intake for protein was not significantly associated with acanthosis. However, for every 1 oz increase in intake of meat per day, the risk of acanthosis significantly increased by 16% ($p = 0.009$). Lean meat from meat, poultry, and fish (oz) was significantly associated with acanthosis ($p = 0.000$). Poultry and meat intake were no longer significantly associated with acanthosis in logistic regression models after controlling for confounding variables. However, for every 1 oz increase in fish and seafood intake the risk of acanthosis significantly increased by 22% ($p=0.001$). Mean total dairy intake had a protective effect against the risk of acanthosis, where for every ½ serving increase in dairy, the risk decreased by 40% ($p<0.001$). There was no significant difference in acanthosis risk between children who consumed protein within the age appropriate Dietary Guidelines and Dietary Reference Intakes as compared to those who did not.

CONCLUSION: Total protein intake did not affect the likelihood of screening positive for acanthosis in young children from the United States Affiliated Pacific. However, meat intake, specifically from fish and seafood did increase the risk for having acanthosis while dairy consumption was shown to have a protective effect. Further study analyzing the different nutrient components of protein sources such as fish, seafood, and dairy sources are warranted.

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CHAPTER 1: LITERATURE REVIEW

Introduction

There are currently over 1.3 million Native Hawaiians and other Pacific Islanders (NHOPI) residing in the United States, a 40% increase since 2000.[1] Up until 2000, the United States categorized American Asians and Pacific Islanders together as "Asian and Pacific Islanders". The NHOPI population was shown to have higher estimated mortality rates from cardiovascular disease, stroke and cancer than Asian Americans and therefore should no longer be categorized together. [2] Due to significant health disparities between the two ethnic groups, American Asians lower rates have masked the severity of obesity and chronic diseases in the NHOPI population.[2] With this recent understanding, there is a growing need to analyze the NHOPI population's health.

Chronic diseases such as diabetes, cardiovascular disease, obesity, and cancer are responsible for 7 out of 10 deaths per year and account for 86% of the health care costs in the United States.[3] The 2010 National Health Interview Survey (NHIS) found that about 42% of the NHOPI participants self-reported they were obese.[4] The NHOPI population also has the highest age-adjusted percentage of type 2 diabetes mellitus (T2DM) amongst all racial groups with 20.6 % of the population diagnosed.[5] Native Hawaiian men and women in a multiethnic prospective cohort study had the second highest mortality rates due to chronic diseases such as obesity, cardiovascular disease, diabetes and cancer, following closely behind African American men.[6] With such prevalent rates of chronic disease and mortality in the NHOPI populations, prevention and early onset indicators are important in reducing morbidity rates.

Acanthosis Nigricans Overview

Acanthosis Nigricans (acanthosis) is a dermatological condition that is characterized by a symmetrical, velvety, light brown to black pigmentation of the skin observed in obese individuals.[7] The exact prevalence of acanthosis in adults and children is unknown. Studies done in different regions of the world have all reported different prevalence rates. A study in New Mexico reported that the prevalence of acanthosis was 49.2% in obese adolescents compared with 7.7% in not obese adolescents [8] whereas the prevalence of acanthosis in Texas middle school children was about 7.1%. [9] Acanthosis was first associated with cancer in the obese adult population and considered to be rare. Recently, acanthosis has become a comorbidity associated with obesity, insulin resistance, and hyperinsulinemia in children and adolescents.[10] According to the Center for Disease Control and Prevention (CDC) the prevalence of obesity amongst youth aged 2-19y was 18.5% in 20015-2016, which is about 13.7 million adolescents and children per year.[11] Childhood obesity is a major concern because it leads to non-communicable diseases (NCD) that can carry on into adulthood - such as cancer, diabetes, and heart disease. With the rapid increase in childhood and adolescent obesity, acanthosis has become prevalent in the younger population as a key indicator and predictor of pre-diabetes.[10]

Pre-diabetes Overview

Pre-diabetes is a condition that comes before an individual develops T2DM when blood glucose levels are higher than normal but are not high enough to be considered T2DM. Individuals with pre-diabetes have fasting plasma glucose levels that are between 100 to 125 mg/dL or a Hemoglobin A1C level of 5.7- 6.4%.[12] According the American Diabetes Association (ADA), it is estimated that 33.9% of US adults (84.1 million people) aged 18 or older had pre-diabetes in 2015.[13] Although no cure for T2DM exists, health professionals are able to detect pre-diabetes before diabetes develops. Unlike T2DM, pre-diabetes is reversible with early intervention and treatment. Early treatment and prevention methods can return blood glucose levels to the normal range of 70 to 100 mg/dL. Research shows that risk of T2DM can be significantly lowered by a healthy diet, losing body weight, and exercising.[12]

Insulin Resistance Overview

Insulin Resistance (IR) is a condition that can increase an individual's risk for developing T2DM. Insulin is a hormone made by the pancreas that assists in the transportation of blood glucose into body cells, where it is used as energy. Insulin resistance occurs when muscle, fat and liver cells do not respond well to insulin and cannot uptake blood glucose.[14] In response to IR, beta cells in the pancreas will make more insulin to help glucose enter the body's cells. Individuals who have pre-diabetes usually have some IR occurring in the body as IR can keep blood glucose levels elevated.[14] The homeostatic model of assessment (HOMA) is the most common way of detecting insulin resistance. HOMA involves measuring glucose and insulin levels and using both to estimate the function of the beta cells in the pancreas to produce insulin and insulin sensitivity.[14]

Type 2 Diabetes Mellitus Overview

T2DM occurs overtime as IR continues resulting in high blood glucose levels. With repeated exposure to high glucose levels in the bloodstream, beta cells are unable to produce enough insulin and the muscle, fat and tissue cells become starved for energy which, overtime can lead to damage of the eyes, kidneys, nerves and heart.[12] Traditionally, T2DM was diagnosed and seen in individual's ages 40 years or older with fasting glucose levels of 126 mg/dL or greater.[12] However, with the recent increase in obesity rates, T2DM is becoming more prevalent in children with approximately 193,000 children and adolescents 20 years and younger diagnosed with this disease in 2017.[12]

Risk factors for T2DM are obesity, physical inactivity, race, and low socioeconomic status.[15] Underprivileged areas with lower socioeconomic status have shown to have an increased prevalence of T2DM.[16] According to the NHIS, the NHOPI population has higher poverty rates, lower incomes, lower educational attainment and larger families.[2] These risk factors put the NHOPI population at a greater risk for developing T2DM. Further studies addressing the NHOPI population are needed to prevent the increase in T2DM.

Current studies suggest obesity is significantly associated with the development of acanthosis and is the only predictive factor for acanthosis.[17] The severity of acanthosis is also a predictor of the severity of obesity in an individual, where the greater severity of acanthosis, the greater obesity.[17] Although acanthosis is not found in every obese individual, obese children with acanthosis have shown to have higher IR compared to those without.[17] Obesity is associated with an increased risk of developing IR due to the adipose tissue releasing factors such as non-esterified fatty acids, glycerol, hormones and pro-inflammatory cytokines that are involved in the development of IR.[18] Acanthosis has been found to reliably diagnose a subgroup of obese children with pre-diabetes. Figure 1 shows the relationship between obesity, acanthosis, IR, pre-diabetes, and T2DM.[10]

Methods for Screening Acanthosis

Acanthosis is a physical indicator on the skin that serves as a marker for insulin resistance.[10] A study done by Kluczynik et al (2012) analyzed the association between the presence of acanthosis and the development of T2DM in overweight children and adolescents. The cross-sectional study performed a variety of tests associated with diabetes such as IR to determine its relationship to acanthosis. The results showed that acanthosis was identified in 58% and IR in 42% of overweight children and adolescents.[19] Based on these results, the authors concluded the presence of acanthosis was associated with being between 10-19 years of age, being a non-white girl and with the presence of IR.[19] Koh et al (2016) examined the relationship between acanthosis and IR further to determine the severity of acanthosis as an index for predicting IR. The results of this study revealed that IR worsens with an increase of acanthosis severity.[10] IR plays a pivotal role in the pathophysiology of developing T2DM.[15] These findings stress the need for health professionals to be trained for acanthosis screening and identification.

With a significant relationship with IR and T2DM, proper screening for acanthosis can be important in early detection and possible reversal of IR. The detection and screening process of acanthosis is simple and non-invasive. Due to the visible skin pigment or discoloration usually located in and around a crease area, a health professional can easily detect acanthosis on an individual. Burke et al., (1999) developed an easy- to -use screening and severity scale for acanthosis. Acanthosis can be screened for on the neck, axilla, knuckles, elbows, and knees. The severity grading scale ranges from 0-4 with zero showing an absence of acanthosis in the specific area and increasing numerically to 4, showing a severe case of acanthosis with >6 inches of visible acanthosis. A score greater than or equal to 3 is considered a severe acanthosis diagnosis.[10] The grading scale also analyzes the neck texture when screening for acanthosis to further indicate the severity of this condition. Scales ranging from 0-3 with zero having a smooth touch, and 4 showing coarseness with portions of the skin clearly raised above other areas.[20] In this qualitative screening tool, the higher numbers indicate the severity of acanthosis, which is correlated with a worsened IR.[10] Images 1 - 4 below are examples of the different screening severities of acanthosis.

Dietary Guidelines and Recommendations

In order to ensure that Americans are consuming a healthy, balanced and adequate diet, there are specific dietary guidelines and recommendations for healthy individuals. The Dietary Guidelines (DG) provides food-based recommendations for Americans who are more than 2 years old.[21] These guidelines are updated every five years by the United States Department of Agriculture (USDA) with the purpose of helping health professionals and policymakers to advise Americans about healthy choices for their diet.[21] MyPlate is a resource of the DG that offers information, messages and tools to help make healthy eating choices.[22] Protein recommendations for MyPlate are 2 oz per day for children 2-3 years old and 4 oz per day for children 4-8 years old. [22] The Dietary Reference Intakes (DRI) is a general term that is used to assess nutrient intake in healthy people which varies by age and sex. Under the DRI, the Recommended Dietary Allowance (RDA) is the average daily level of intake sufficient to meet the nutrient requirements of nearly all healthy people.[23] The RDA for protein intake is 1.1g/kg per day for children ages 1-3 years old and 0.95 g/kg per day for children ages 4-8 years old. [23] Also under the DRI, the Acceptable Macronutrient Distribution Range (AMDR) expresses intake recommendations as a percentage of total caloric intake.[23] The range of protein intake for children ages 1-3 years old is 5-20% of total caloric intake per day and 10-30% of total calories per day for children ages 4-8 years old. [23]

Diet and Insulin Resistance

Diet is a modifiable factor that has been linked to IR. Current research has focused on high carbohydrate or fat diets and their relationship to IR.[24, 25] Consumption of simple carbohydrates like sugar-sweetened beverages have been shown to be associated with IR, whereas complex carbohydrates have resulted in opposite results.[26] High fat diets with excessive total fat intake, greater than 37% of daily energy, also increase IR in individuals.[27] High intakes of *trans* unsaturated fatty acids have shown to induce IR in individuals who are genetically predisposed when compared to other types of fat such as monounsaturated fatty acids, polyunsaturated fatty acids and saturated fatty acids.[28]

Protein and Insulin Resistance

Research has shown that short-term high protein diets are effective for weight loss in obese children.[29] Although this type of diet has been shown to significantly reduce weight, little is known on the long-term impacts of a high protein diet, especially in children, on IR. In order to assess the prevention methods of diabetes in adolescents and children, it is important to analyze the role that dietary protein plays in childhood obesity and IR.

Early identification of metabolic markers in children and adolescents are key to preventing chronic diseases such as diabetes. However, traditional risk factors for T2DM such as obesity, age and family history are weak predictors of changes in glucose tolerance and IR. Trico et al. conducted a cross sectional and longitudinal study to detect early metabolic features of IR and the effect on predicting glucose intolerance. The results revealed that elevated fasting alpha-hydroxybutyrate and branched-chain amino acids (BCAA) levels were associated with IR in youth ages 8 to 18 years old.[30] A similar study done by McCormack et al. (2012) evaluated the association between obese adolescents ages 8 to 18 years old with fasting concentrations of circulating BCAAs to determine if they were a predictor of future IR. This cross sectional study found that elevations in plasma BCAAs were significantly associated with obesity in youth.[31]

These results warrant for further investigation to determine the role of dietary proteins on plasma concentrations of BCAAs.

A randomized controlled study conducted with obese rats analyzed the effects of supplementation of BCAAs with a high fat diet. The first intervention group of rats fed a high fat diet gained the highest percent body weight after 13 weeks while the second intervention group, which was given the same high fat diet along with BCAA supplements, gained a slightly lower percent body weight.[32] The control group was fed the standard chow and gained the least amount. The difference in body weight between the two intervention groups was due to different rates of food intake where the BCAA group consumed about on average 100 kcals less per week than the high fat and standard chow group. Although the BCAA supplementation group consumed fewer calories, the two intervention groups were equally IR. This experiment demonstrates that BCAAs contribute to the development of IR that is independent of bodyweight and kcal intake in rats.[32]

Zheng et al. (2016) used a randomized controlled intervention study on adult men and women to examine the effect of weight-loss diets on long-term changes in plasma amino acids and the relationship with IR. This 2-year trial analyzed both BCAA’s and aromatic amino acids (AAA) to determine which specific plasma amino acids played a role in IR. The results showed that the specific AAA tyrosine was significantly related to IR and, when plasma levels of tyrosine were low, IR decreased.[33] The study found that the diets with a lower amount of protein consumption, consisting of 15% of daily energy intake, had significantly stronger effects in reducing concentrations of diabetes-associated AAA tyrosine in comparison to the control.[33] These findings inform the relationship between protein and IR in adults but further studies examining children and adolescents are needed.

A high protein intake during the ages of 5 to 6 has been associated with a higher risk of obesity.[34] Voortman et al. (2016) furthered this line of research to determine if there was an association of protein intake in early childhood with metabolic insulin levels. This prospective cohort study concluded that protein intake in early childhood was linked to a high body fat percentage and insulin levels in 6-year-old girls but not in boys. Although the evidence in protein intake and IR remains unclear in children, there is evidence that dietary protein stimulates the secretion of insulin in adults.[35] A similar study done with adults was able to conclude that high total dietary protein consumption in both male and females was associated with an elevated risk in developing T2DM.[36] With differing results, more studies on children and adolescents are needed.

Currently, there are limited studies examining different protein sources and their relationship to IR in children and adolescents. One of the few studies done on children and adolescents by Hoppe et al. (2004) examined the protein intake from milk or meat in 8-year-old boys. This randomized control study analyzed the effect of a diet high in either skimmed milk or low fat meat for one week on insulin concentrations. This short-term study concluded that the milk-group had an increase in serum insulin concentrations, which caused IR to increase.[37] However, the meat-group did not show any increase in serum insulin and IR. Although this study found a significant relationship between IR and different animal protein products, the study period was too short to assess the long-term consequences of protein intake and sources on IR. A similar study done by Turner et al. (2015) analyzed the difference of dairy and red meat consumption over a four-week period. The results obtained showed IR in the group eating a high lean red meat diet while the group consuming high amounts of dairy had a decrease in IR.[38]

Further research is needed to identify the long-term effect of high protein intake and different protein sources in children and adolescents.

Different protein sources have been studied in adults to determine the effect on IR. A study by Nielen et al. (2014) investigated the long-term effects of different types of protein sources on IR. In this prospective study, high consumption of animal protein was associated with a higher risk of T2DM, but the plant-based protein was not. Obese women who consumed high animal protein had the highest risk. Another similar study also done on adults analyzed the intake of animal and plant-based protein and drew the same conclusions.[39] Although these studies show the difference between animal and plant protein sources, analysis of different animal protein sources could have a different effect on IR.

A study conducted by Mandani et al. (2012) tested the effects of sardine protein and casein protein on IR in obese rats. The rats were fed a high fructose diet to induce a metabolic syndrome of hyperinsulinemia, insulin resistance, and hypertension. The results of this randomized intervention study showed that rats given the sardine protein had significantly lower plasma glucose concentrations and IR than rats on the casein protein diet.[40] Although conducted on rats, this study informs the relationship between different protein sources and IR.

A study analyzed the relationship between meat intake and the occurrence of diabetes in adults. Vang et al. (2008) used a prospective cohort study to examine meat consumption, including the type, with T2DM risk. The study showed that men who were weekly consumers of all meats had a 74% increased risk for developing T2DM than men with a vegetarian diet. In addition, individuals who consumed any processed meats were 38% more likely to develop T2DM.[41] This study suggests the possibility that meat intake, particularly processed meats, are a dietary risk factor for T2DM in adults. A comparable cohort study done on women also examining the effects of different meat consumption concluded that diets high in processed meats increased the risk for developing T2DM.[42]

Another study analyzed the effects of codfish protein compared to other animal proteins, and their effects on insulin sensitivity, on adults who already presented signs of IR.[43] The participants either consumed a high codfish protein diet or BPVEM diet containing lean beef, pork, veal, eggs, milk or milk products. 58-68% of the daily dietary protein came from either the cod fish or BPVEM proteins whereas the remaining protein consumption was from vegetables. The dietary cod protein group had improved IR while the BPVEM group did not.[43] Although both groups were consuming the same proportions of protein and other macronutrients, the BPVEM protein group contained a variety of different protein sources, which makes it difficult to conclude if a specific animal protein source contributed more to the results than other proteins. The study, however, does suggest the benefits of fish protein on improving insulin sensitivity.

While there is evidence that protein intake affects IR and the risk of T2DM, there is a lack of research on children and adolescents. The recent childhood obesity epidemic and the relationship between acanthosis and IR require further research regarding dietary protein consumption and its relationship with acanthosis. Because acanthosis is an early indicator of IR, understanding the dietary patterns that affect acanthosis and IR are crucial. This is especially true for populations such as the NHOPI that suffer a greater disparity for IR and T2DM in comparison to other populations.

Methods for Assessing Diet

Diet is a major lifestyle-risk factor for NCD diseases like T2DM. Methods used for assessing dietary intake can be helpful for prevention and treatment of many NCDs. Self-report dietary assessment methods are the most common and include the 24-hour dietary recall (24HR), dietary record (DR), dietary history, and food frequency questionnaire (FFQ).

24-Hour Dietary Recall (24HR) Overview

The 24HR is an open-ended method that collects detailed information about foods and the estimated amounts consumed over the previous 24-hours. Additional information about food preparation methods and ingredients used are also included, as needed (Shim, 2014). An advantage of this method is that there is a minimal burden placed on the respondent but the accuracy depends on the respondent's memory.

Dietary Record (DR) Overview

The DR is similar to the 24HR where the method also documents the consumption of food on specific days. The DR requires participants to record (self or surrogate) at the time food is consumed. This minimizes the reliance on the participant's memory but requires the respondents to be literate and trained before recording.

Food Frequency Questionnaire (FFQ) Overview

The FFQ is a questionnaire containing foods and/or amounts representing primary foods of interest to a practitioner or researcher. A FFQ requires recall of frequency and in some cases, portion sizes over a specific time period (e.g. the previous 12 months) and can be self-administered. The FFQ enables the assessment of long-term dietary intakes to be simple, cost-effective, and time-efficient.[44] However, the FFQ requires that the foods on the FFQ be validated for the race/ethnic population under study.

Dietary History

Dietary history is a detailed retrospective method of diet assessment that is used to describe usual food or nutrient intake over a specific period of time and consists of three parts. A skilled professional conducts an interview to estimate the habitual consumptions of foods and portion sizes which can be done by a 24 HR. Following this interview, a food frequency questionnaire is completed to verify the frequency of food consumption. Lastly, a 3 day dietary record is completed [45]. Although this method can be very accurate; there is a burden on the participant and requires highly skilled professionals to collect the information.

Dietary Assessment in Children

Dietary assessment is crucial in examining the nutritional status of a child. However, it can be difficult due to the lack of familiarity among children concerning foods and ingredients. Typically, in younger populations, parents or guardians will complete dietary assessments such as an FFQ, 24HR, or DR. Recent studies show that dietary records are useful and valid in assessing energy and nutrient intake in toddlers.[46] This, however, may also lead to inaccuracy because the parent or guardian is not with the child at all times. Cheng, 2013 found that parents and caregivers were also more likely to overestimate food or beverages in their child's dietary record than underestimate and it is important to take into consideration that family and child characteristics may impact the reporting error.

The Pacific Region

The islands located in the Pacific Region referred to as Oceania, consists of approximately 10,000 tropical islands and extends between Asia and America. The region of Oceania is divided into 3 sub-regions know as Polynesia, Melanesia, and Micronesia.[47] The indigenous people of Oceania are referred to as the Native Hawaiian and other Pacific Islanders today. Due to the vast geography of Oceania, NHOPI's from different islands faced contact with European or Westerners at different time points. For example, Pohnpei, an island state of the Federated States of Micronesia, first contact with the Spaniards occurred in 1526.[48] Table 1. Identifies the year in which first contact was made by Westerners in the islands located in Oceania.

Records from early Westerners along with indigenous oral histories indicated that the NHOPI populations were healthy and hardy individuals.[49] However, within the last 2 decades the NHOPI populations health has dramatically shifted to one of the highest incidence of preventable chronic diseases in the world such as diabetes, hypertension, cardiovascular disease, and cancer.[50] These health outcomes of the NHOPI population are attributed to a multitude of complex factors. The colonization and westernization in the Pacific Region adversely affected many of the social, cultural, and economical traditions that once supported the health of the indigenous people.

NHOPI population are also more likely to have higher poverty rates, lower income, larger families, and lower educational status.[2] According to the 2015 Census Bureau report, 17.3% of NHOPI were living in poverty in comparison to 10.4% of non-Hispanic whites. NHOPI's also have lower education attainments with 28% of the population containing a bachelor's degree or higher compared to Whites with 47% containing a bachelor's degree or higher.[1] These socioeconomic factors have contributed to the decline in NHOPI's health.

The NHOPI populations that have continued to reside in the region have gone through a significant nutrition transition that is related to their decline in population health.[51] Pre-colonized NHOPI diets consisted of fresh fruits, vegetables, and fish but recent research has shown that their diets now largely consist of processed meats and fast foods.[52] Processed and fast foods are low-cost goods that appeal to the lower income NHOPI households. Along with economic issues, colonization of the Pacific Islands has affected the food supply in other ways. For example, the Marshall Islands was used by the United States for nuclear weapons testing on their atolls in the 1940's until 1950's. Due to the radioactive contamination from the nuclear bombing, the islanders are no longer able to practice their traditional diets but instead rely on imported foods.[53] This high demand for low cost meats in the Pacific Islands has led to an excess amount of imports of high fat mutton flaps, turkey tails, chicken backs, and Spam® from countries like the United States, New Zealand and Australia.[54]

The NHOPI that have adopted a diet high in meat and processed meat, and are now at a much greater risk for developing T2DM.[55] Large multi-cohort studies done found that dietary patterns high in fat and meat were linked to increased diabetes risk in the NHOPI and other minority populations such as Japanese Americans.[55,56] NHOPI populations coming from a lower socio-economic status are making imported processed foods a part of their diet.[54] Investigating the relationship between processed meats and health outcomes has been done in adults but due to the recent epidemic in childhood diseases, further studies on children and adolescents are needed.

Physical Activity

Although race and dietary factors play a role in the increased risk for T2DM, lifestyle factors like physical activity are also important. With prevalence levels of obesity, T2DM, and heart disease increasing among the NHOPI populations, weight loss through physical activity has been clinically helpful in preventing and lowering the risk for these diseases.[57] Grandinetti et al. (2007) examined multi-ethnic adults in Hawaiʻi to determine if physical activity played a role in IR. The study found that an increased heart rate and low levels of physical activity were independently associated with IR.[58] Amongst NHOPI, few studies are currently available that have examined physical activity and its association with IR. However, one pilot study on Samoan college-aged women found an associated between sedentary, low physical activity level with higher body fat percentage.[59]

The Children's Healthy Living Program

The Children's Healthy Living Program for Remote Underserved Minority Populations in the Pacific Region (CHL) is a partnership in the United States Affiliated Pacific (USAP) that's mission is to build and sustain a healthy food and physical environment to help maintain weight and prevent obesity among young children in the Pacific region.[60] The USAP region includes Hawaiʻi, Alaska, American Samoa (AS), Commonwealth of Northern Marianas (CMNI), Guam, the Federated States of Micronesia (FSM), the Republic of the Marshall Islands (RMI) and the Republic of Palau. Through a combination of efforts, CHL had the following objectives: 1) Conduct program/data inventories and situational analysis; 2) Train 22 public health nutrition workers in obesity prevention; 3) Develop a Pacific food, nutrition, and physical activity data management and evaluation system; 4) Develop and conduct a community-based environmental intervention to prevent, maintain, or reduce childhood obesity; 5) Evaluate the environmental intervention; 6) Incur at least one obesity prevention policy change per jurisdiction. To address the child obesity epidemic in the Pacific, CHL was a multi-dimensional strategy of environmental changes to promote healthy food intake and physical activity in children ages 2-8 years old in the USAP to prevent childhood obesity, as seen in Figure 2. CHL was sponsored by the United States Department of Agriculture (USDA), Agriculture and Food Research Initiative grant number 2011-68001-30335. CHL continues its mission as the CHL Center of Excellence (CHL Center) funded by the USDA National Institute of Food and Agriculture, Agriculture and Food Research Institute Grant no. 2018-69001-27551.

CHL Study Design and Schematics

Children 2 to 8 years old were recruited from 51 communities. The communities were chosen for CHL based on four criteria; census data for a population >1000, relative accessibility and representativeness, having >25% of the population of indigenous/native descent (15% in Alaska due to focus on accessible communities), and having >10% of the population under age 10.[61]

Communities in Alaska, American Samoa, CNMI, Guam and Hawaiʻi were selected to participate in the intervention trial. Only 2 communities in Alaska were selected due to large distances between sites while 4 communities were selected in the remaining 4 jurisdictions. Each community was matched to form intervention and control pairs based on percentage in poverty and population density based on U.S. census data, distance from urban cities, and percentage of overweight/obesity, if available. From each pair, one community was randomly assigned to an intervention group while the other to a delayed optimized intervention. The intervention study was assessed for outcomes at baseline and a follow up at 24 months.

An additional 2 communities were selected to serve as temporal indicators of anthropometry status. The Freely Associated States of Micronesia which includes FSM, the Republic of Palau, and RMI along with the temporal communities did not receive the intervention but were included in the baseline measurements, see Figure 3.

CHL Prevalence Study

The purpose of the CHL Prevalence study was to estimate the prevalence of obesity and acanthosis among young children in the USAP.[62] A total of 27 communities were selected from the CHL Intervention study jurisdictions and 24 communities were selected for the CHL Prevalence study. A total of 5775 children participated in the CHL Prevalence Study with 4488 children from the baseline of the CHL intervention study and 1287 children from the Prevalence study.

Anthropometric measurements of height and weight were taken and Body Mass Index (BMI) categories were defined by the Center of Disease Control reference BMI percentile data according to age and sex.[62] Within this population 14% were classified as obese, 14.4% as overweight, 68.9% healthy and 2.6% underweight. Obesity significantly differed by age group with 12.9% in the 2-5 years old and 16.3 % in the 6 to 8-year-olds. The prevalence of acanthosis was 4.7% with a majority scored at 1 (60.3%), 20.8% had a score of 2, 12.2% a score of 3 and 6.8% a score of 4, based on the Burke screening scale.[11] The study also found that obesity was highly associated with acanthosis with the strongest relationships demonstrated in Asians, then in Native Hawaiians, and then in other Pacific Islanders.

This CHL study, which is the only study of its kind on young children in the USAP with a high proportion of NHOPI, suggests that acanthosis may represent an important indicator of early metabolic change. With an increase in obesity rates with age, the data suggests that these children will have a higher risk for developing T2DM. The early detection of acanthosis in a population at higher risk is crucial in preventing and treating the childhood T2DM epidemic. Given the relationship between childhood obesity and acanthosis, established by Novotny, et al 2016, further research is needed to examine lifestyle factors that may be influencing this relationship. Evidence suggests that protein intake and sources may influence IR. As acanthosis is a risk factor for IR and T2DM, examining the association between dietary protein and acanthosis will be important in understanding the recent increase of health disparities in NHOPI children.

Problem Statement

Based on the results of this literature review, the aim of this thesis is to examine the relationship between dietary protein intake and the risk of acanthosis in children ages 2-8 years old. Due to the high risk for T2DM in NHOPI, it is critical to examine early detection methods and possible factors associated with the disease.[11] Acanthosis is an early warning sign of T2DM that can indicate a need for lifestyle changes such as dietary behavior modification. With previous studies suggesting that high fat meats and processed meats increase the risk of IR, this thesis hypothesizes the following:

1. Children who consume a diet characterized by low fat protein sources are less likely to have acanthosis.
2. Children who consume dietary protein within current dietary guidelines recommendations will be at a lower risk of having acanthosis than children exceeding dietary guidelines for protein.

3. Children who consume dietary protein within current dietary reference intakes recommendations will be at a lower risk of having acanthosis than children exceeding DRI.

Research Objectives

The primary objective of this study is to determine if the presence of acanthosis nigricans (acanthosis) is related to dietary protein consumption among children in the United States Affiliated Pacific. The secondary objectives are to 1) Examine the relationship between different protein sources and the presence of acanthosis. 2) Determine the relationship between protein consumption within the age specific US Dietary Guidelines and Dietary Recommended Intakes and the presence of acanthosis in young children from the USAP.

CHAPTER 2: EXAMINING THE RELATIONSHIP BETWEEN PROTEIN INTAKE AND SOURCE AND ACANTHOSIS NIGRICANS AMONG YOUNG CHILDREN IN THE CHILDREN'S HEALTHY LIVING PROGRAN IN THE UNITED STATES AFFILIATED PACIFIC

This is a draft of the article that will be submitted to the Journal of the Academy of Nutrition and Dietetics (JAND) and is included here as chapter two.

Calabrese, A., Fialkowski, M., Boushey, C., Davis, J., Leon Guerrero, R., Novotny, R. (2019). "Examining the relationship between protein intake and source and acanthosis nigricans among young children in the children's healthy living program in the united states affiliated pacific." Journal of the Academy of Nutrition and Dietetics.

INTRODUCTION

This thesis includes the following chapters: a literature review, a manuscript that will be submitted for publication, and a conclusion.

Acanthosis Nigricans (acanthosis) is a dermatological condition that is characterized by a dark hyperpigmentation of the skin. The exact prevalence of acanthosis is unknown with studies showing different prevalence rates in different regions of the world.[8,9] Acanthosis is a comorbidity associated with obesity, insulin resistance and hyperinsulinemia in children and adolescents (Koh, 2016).[10] Insulin resistance overtime may lead to the development of Type 2 diabetes mellitus (T2DM). Traditionally, T2DM was diagnosed and seen in individuals ages 40 years or over. However, recent increases in childhood and adolescent obesity has increased the prevalence of T2DM in children and adolescents. [12]

Native Hawaiians and other Pacific Islanders (NHOPI) are the indigenous and primary ethnic groups of the US Affiliated Pacific region (USAP). Within the past 2 decades, the NHOPI population's health has dramatically shifted to one of the highest incidences of preventable chronic diseases in the world. [50] These chronic diseases include diabetes, hypertension, cardiovascular disease and cancer. Although these health disparities are extensive in the NHOPI populations, there is limited research available on the prevalence of acanthosis and obesity in childhood and adolescents.

Previous research has focused on the relationship between high carbohydrate and fat diets and acanthosis in children. [24,25] High protein consumption in children has been found to be associated with insulin resistance.[34] However, there are no studies analyzing the association between protein intake and acanthosis in children.

The Children's Healthy Living Program (CHL) is a partnership in the USAP that implemented a community randomized intervention trial and a prevalence survey to address childhood obesity in the region.[61] The CHL Prevalence Study (including the baseline of the trial) estimated the prevalence of obesity and acanthosis among young children ages 2-8 years old in the USAP. [62] The prevalence of acanthosis was 4.7% while the prevalence of obesity was 14.0%. Obesity was highly associated with acanthosis with the strongest relationships demonstrated in Asians, then in Native Hawaiians and then in Pacific Islanders. Diet data was collected from both CHL intervention and prevalence studies; however, there are no publications analyzing the relationship of acanthosis to protein intake.

The purpose of this study was to determine if children's protein intake affects the likelihood of having acanthosis in the USAP. Data were collected by the CHL Program in following USAP jurisdictions: Hawai‘i, Alaska, Commonwealth of the Northern Mariana Islands, Guam, American Samoa, Palau, Republic of the Marshall Islands (RMI), 4 Federated States of Micronesia (Pohnpei, Yap, Kosrae, Chuuk).[61]

METHODS

Study Design and Settings

This study is a secondary analysis of cross-sectional data from the CHL Prevalence Study of children ages 2 to 8 years old from 51 communities in the 11 United States Affiliate Pacific (USAP) jurisdictions (Alaska, American Samoa, Commonwealth of the Northern Marianas, Guam, Hawaiʻi, Palau, Pohnpei, Yap, Chuuk, Kosrae, and the Republic of the Marshall Islands). The communities were chosen for CHL based on four criteria; census data for a population >1000, relative accessibility and representativeness, having >25% of the population of indigenous/native descent (15% in Alaska due to focus on accessible communities), and having >10% of the population under age 10. [34]

A total of 5775 children participated in the CHL Prevalence Study. To be included in this analysis participants needed to have completed acanthosis nigricans screening and dietary data collection. The goal for the dietary data collection was 66% of the total sample. [34] Therefore, 3486 participants were included in this analysis.

Data Sources/ Measurements:

Anthropometry

Height and weight were measured using standardized protocols. [61,63] Body mass index (BMI) was calculated (kg/m^2) and overweight/obese was defined using sex- and age-specific BMI cut-points from the Centers for Disease Control and Prevention (CDC) Growth Charts. [64]

Acanthosis Nigricans

Acanthosis screening was conducted based on the Burke's quantitative scale from 0 to 4.[20] Participants' necks were examined for the presence of acanthosis and converted into a bivariate variable for analysis as positive (1-4) and negative (0).

Dietary Intake

Food logs (i.e. dietary records) were collected in order to assess total energy, nutrients, and food group intakes of the child. The dietary records were combined with an activity log, referred to as the Food and Activity Log (FAL). The FAL was completed by a surrogate (e.g., parent/caregiver) over 2 randomly assigned non-consecutive days, which included one weekday and one weekend. Surrogates were instructed on record keeping techniques with food model aids, service ware, and utensils. Tool kits of calibrated utensils and a Ziploc bag to place food wrappers, labels, and packages were provided for each child.[61]

Questionnaires

Questionnaires were completed by the surrogates to assess the child's demographics such as age, race/ethnicity, sex, household composition, education level and income of the parent/caregiver, household food security, religion, general health status, and early life feeding behaviors. [61]

Statistical Methods:

IBM SPSS Version 24 was used for the analysis. Pearson Chi-Square tests were used to determine the relationship between the positive and negative acanthosis screening groups with overweight/obesity and dietary intake (energy intake [kcal] and protein [g]). One-way ANOVA was conducted to determine the relationship between acanthosis and total energy intake (kcal), mean protein (g), fat (g) and dairy (servings) intake of the two days weighted for weekday/weekend, mean meat (oz) intake and lean meat equivalents from egg (oz), poultry (oz), franks and luncheon meats (oz), fish and seafood (oz), beef, pork and lamb (oz), nuts and seeds (oz), soy (oz), and dry beans and peas (oz) weighted for weekday/weekend days and adjusted for within person variance, and age (yrs). The likelihood of acanthosis being associated with protein or meat intakes was examined using binary logistic regression adjusting for age, sex, overweight/obesity, energy intake (kcal) and mean fat intake (g). Binary logistic regression was also used in determining whether children who consumed protein within the US Dietary Guidelines and Dietary Reference Intakes were less likely to screen positive for acanthosis. Odds ratios and 95% CI were the reported statistics from the analysis.

Hunan Studies:

Institutional Review Board (IRB) at the University of Guam and the Committee on Human Studies at the University of Hawaiʻi at Mānoa obtained human studies approval. The other participating jurisdictions relinquished IRB approval to the University of Hawaiʻi at Mānoa. Parents gave consent for child participation and children gave assent before measurements were taken. Participants were compensated for their time and participation.

RESULTS

Table 2, shows that a little over 5% of children screened positively for acanthosis. Approximately half of both groups were males. Children who screened positively for acanthosis were more likely to be in the overweight and obese categories (P=0.000, Table 2) with a prevalence over 60%. Those children who screened negative for acanthosis were more likely to be in the underweight and healthy weight categories. Children who screened positive were more likely to be older in age.

The mean daily intake of protein was similar for cases and non-cases (see Table 3). However, statistically significant differences were observed between cases and non-cases for dairy, meat, egg, and the combined meat, poultry, and fish intake. Mean energy, protein, and fat intake were not significantly different between cases and non-cases. Lean meat equivalents from nuts and seeds, soy and dry beans and peas were also not significantly different.

Further investigation using binary logistic regression models was performed to determine the effects of intakes of different protein sources on the likelihood of the individuals screening positive for acanthosis after adjusting for sex, overweight/obesity, energy intake (kcal), and mean total fat intake. The results confirm that there was not a significant association between acanthosis and mean protein intake (Table 4). However, for every 1 oz increase in mean meat intake there was a 16% significant increase in risk for being positively screened for acanthosis. There was also a 21% increased risk for acanthosis for every 1 oz increase of mean meat, poultry, and fish intake. There were no differences in acanthosis risk among mean frank and luncheon meats, mean beef, pork and lamb, and mean poultry intake. There was a positive association between mean fish and other seafood intake and the risk of acanthosis with a 22% increase in risk for every 1 oz intake. Mean total dairy intake had a protective effect against the risk of acanthosis, where for every ½ serving increase in dairy, the risk decreased by 40%.

Binary logistic regression models were performed to determine if children who consumed protein within the US Dietary Guidelines (which for protein foods is 2 oz equivalents for ages 1-3 and 4 oz equivalents for ages 4-8 per day) and Dietary Reference Intakes (Acceptable

Macronutrient Distribution Range [AMDR] for protein is: 5-20% of total kcals for ages 1-3 and 10-30% of total kcals for ages 4-8; the Recommended Dietary Allowance [RDA] for protein is 1.1 g/kg of body weight for ages 1-3 and 0.9 g/kg body weight for ages 4-8) would be less likely to screen positive for acanthosis. The results show that there was no significant difference in acanthosis risk between children who consumed protein within the age appropriate US Dietary Guidelines or the Dietary Reference Intakes and those who did not (Table 5).

DISCUSSION

Protein intake as estimate in grams consumed per day was not shown to be significantly related to the likelihood of screening positive for acanthosis. These findings conflict with Voortman et al.who conducted a prospective cohort study and concluded that high protein intake in early childhood was associated with a high body fat percentage and insulin levels in 6-year-old girls but not in boys when adjusting for energy intake. However, a similar study done on adults concluded that high dietary protein consumption in both male and females was associated with an elevated risk in developing T2DM. [36]

Plant-based protein sources such as soy, beans, nuts and peas were not found to significantly influence the risk for screening positive for acanthosis. This is similar to previous research which found no association between intake of plant-based proteins and the risk of acanthosis or IR. [36,39] These results are surprising as previous research has found that plant based proteins have been found to be protective to other diseases related to IR and acanthosis such as obesity. [65]

Interestingly, an inverse relationship between dairy intake and acanthosis was found in this study. This is in congruence to findings from a randomized intervention study by Turner et al. where high dairy intake decreased risk for insulin resistance. Acanthosis is a physical indicator on the body that appears to be a marker for IR and the IR worsens with an increase of acanthosis severity.[10] Kluczynik et al. conducted a cross-sectional study to analyze the association between the presence of acanthosis and T2DM. The results showed that acanthosis was identified in 58% and IR in 42% of the population with T2DM. Based on these results, dairy intake can be a preventative dietary component to decreasing the risk for acanthosis and IR. Potential underlying mechanisms that may play a role in the protective factor of dairy include other dietary components such as calcium, vitamin D, and dairy fat.[66]

Contrary to previous research where meat intake was associated with IR in children[37,38,41] and increased the risk of T2DM and IR in adults[38,41], red meats, and processed meats like franks and luncheon meats showed no relationship with acanthosis in children. In contrast, research published examining the relationship between dietary fish protein and IR found an inverse relationship between the two.[40,43] In this study, for every 1 oz increase in fish and other seafood intake, the risk of screening positive for acanthosis increased by 22%. Further investigation is warranted to determine if there are specific types of fish and seafood that increase the risk of screening positive for acanthosis. The different nutrient compositions in which fish and seafood are comprised of may be associated with this relationship.

Protein consumption within the DG and DRI's were shown to have no significant relationship with screening positive for acanthosis compared to those exceeding the guidelines. To the authors knowledge, there are no studies analyzing this relationship.

The strengths of this study lie in its relatively large sample size of the USAP region. A limitation with this study is the quantitative scale used to measure acanthosis across the USAP.

Acanthosis was determined by trained professionals by examining the necks of the participants from different ethnic groups with varying skin tones. Novotny et al. (2015) proposed that higher proportions of acanthosis screened in level 1 category could be due to those populations having darker skin tones. Another limitation was that dietary intake for the children was reported by a surrogate. This cross-sectional study does not determine cause and effect but instead analyzes potential risk factors that may increase the risk of screening positive for acanthosis.

ACKNOWLEDGEMENTS

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CHAPTER 3: CONCLUSION

This study examined if the presence of acanthosis is related to protein consumption and different protein sources in young children from the United States Affiliated Pacific (USAP). This is a cross-sectional study was the first to evaluate the relationship between protein consumption and source in young children with acanthosis from the USAP. Additionally, this is was the first study to evaluate dietary protein intakes within the age appropriate US Dietary Guidelines (DG) and Dietary Reference Intakes (DRI) of young children ages 2-8 years old.

Total protein intake as estimated in grams consumed per day and plant-based protein sources (nuts, seeds, soy, beans and peas) as estimated in ounces consumed per day were not risk factors for children who screen positive for acanthosis. However, the mean meat consumption per day as estimated in ounces increased the risk for acanthosis. Further investigations into the mean meat intake variable showed that lean meat from meat, poultry and fish intake estimated in ounces per day increased the risk of acanthosis where other meat sources such as franks and luncheon meats, beef, pork and lamb did not. Individual lean meat variables poultry and eggs estimated in ounces per day were not risk factors for children with acanthosis. Interesting, lean meats from fish and seafood did increase the risk for screening positive for acanthosis in children. Dairy intake estimated in servings was shown to have a protective factor against acanthosis. This study also found that there was no significant association with acanthosis in children who consumed dietary protein within the age appropriate US DG and DRIs as compared to those who exceeded it.

These results underline the significance of considering different dietary protein sources and nutrient composition of foods when analyzing the relationship between acanthosis and diet in the USAP. The Native Hawaiian and other Pacific Islanders (NHOPI) populations located in the USAP have gone through a significant health transition in the past two decades with the highest age-adjusted percentage of Type 2 Diabetes Mellitus (T2DM) amongst all racial groups.[4] Diet is a modifiable factor that has been linked to IR and the development of T2DM. [12,24] Despite the health disparities that the NHOPI communities face, there is a lack of scientific research published analyzing the relationship between different diet components and the development of T2DM in NHOPI. Acanthosis is a visual indicator on the skin for IR that can potentially lead to the development of T2DM.[10] Therefore, it is important that future research pertaining to T2DM focuses on the risk factors of acanthosis to hopefully prevent T2DM. Identifying risk factors for acanthosis in NHOPIs can aid in implementing interventions to potentially reverse acanthosis.

This study has scratched the surface of the influence that dietary protein sources have on the young children with the presence of acanthosis. Future research analyzing the different nutrient composition of protein sources such as fish, seafood, and dairy are needed. Potential nutrient components needed to be analyzed further is the role of different fatty acids and tyrosine levels in IR. Previous research has found that specific fatty acids such as trans- palmitoleic acid have shown to be protective against type 2 diabetes. [66] Whereas the aromatic amino acid tyrosine has been significantly related to IR in that when plasma levels of tyrosine were low, IR decreased.[33] Protein sources with high levels of tyrosine include cheese, peanuts, pork, and chicken.[67] Next steps may include analyzing the tyrosine content in these protein sources to determine if consumption of high tyrosine sources decrease the risk of screening positive for acanthosis in young children from the USAP.

APPENDIX

Figure 1. Flow chart indicating the pathway and diagnostic criteria to type 2 diabetes mellitus in children

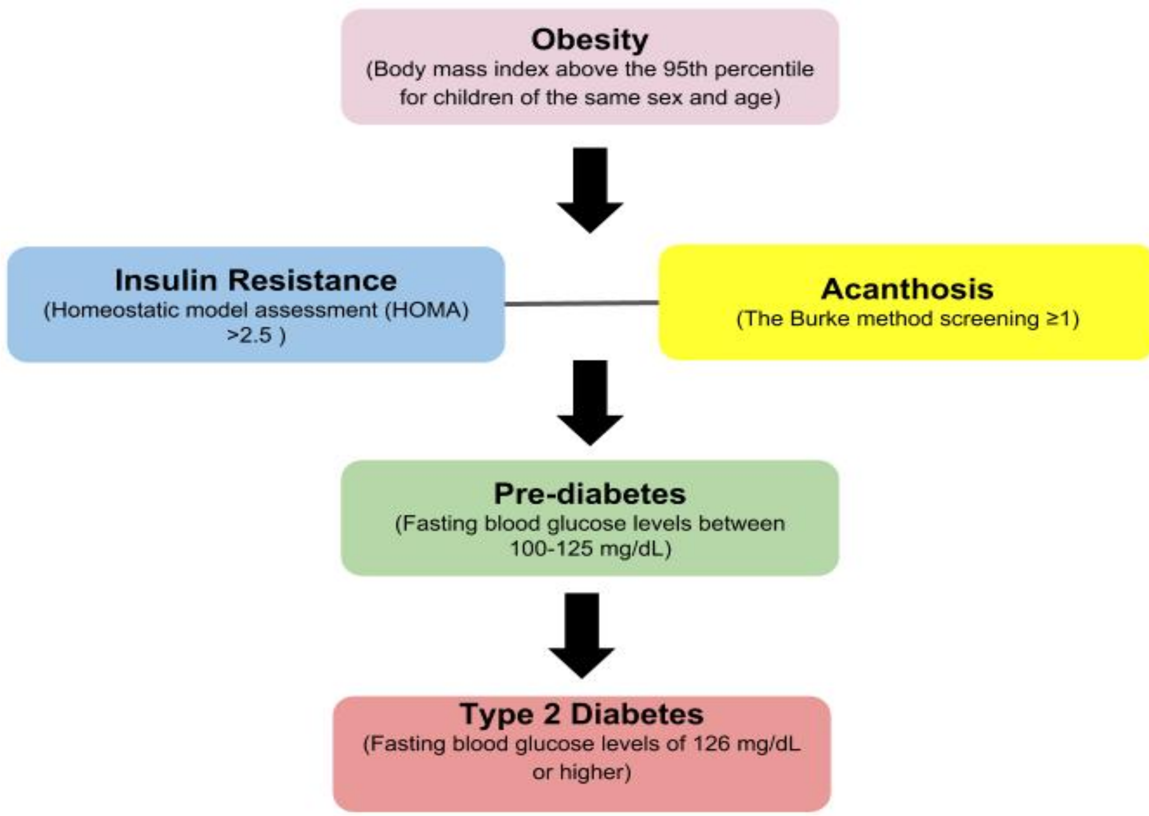

Figure 2. The Children's Healthy Living Program model to prevent childhood obesity in the USAP [61]

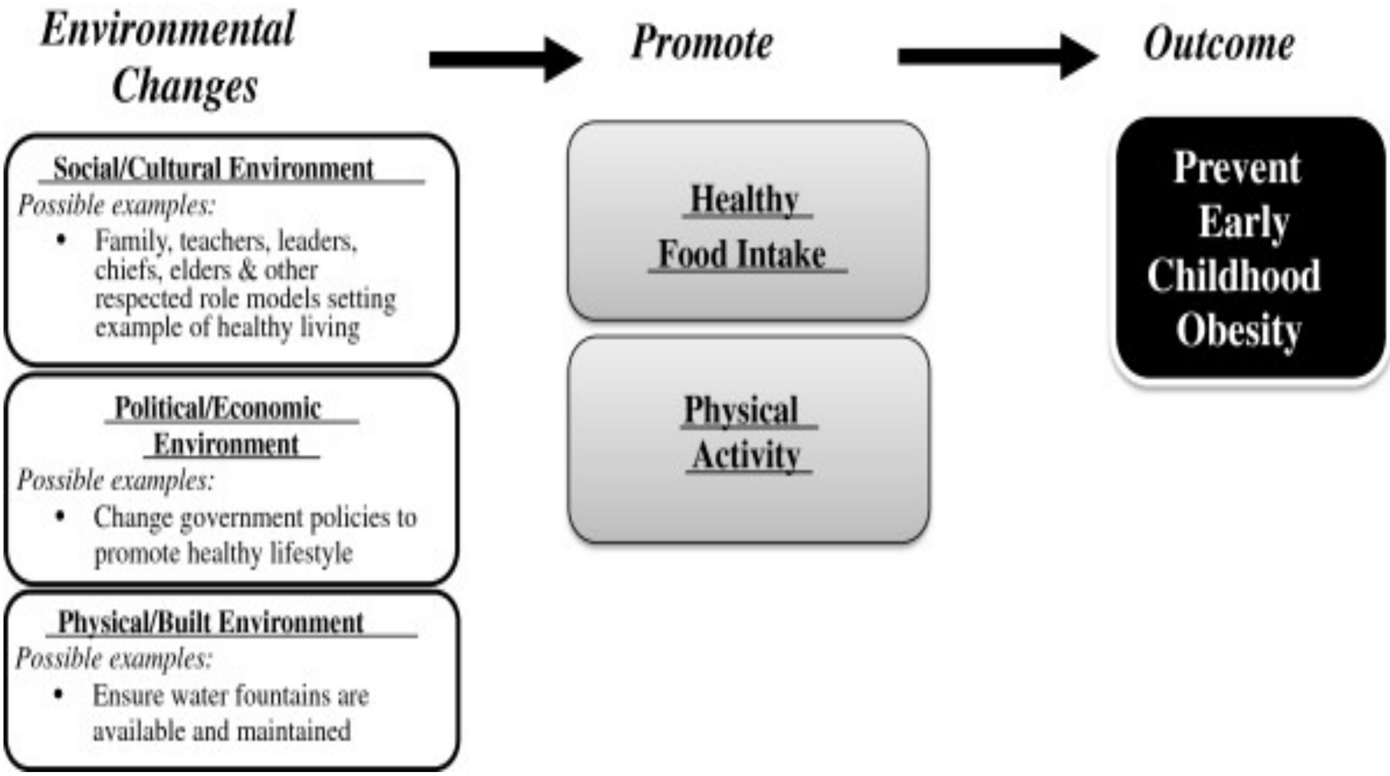

Figure 3. Children's Healthy Living Program Study Design Schematic [61]

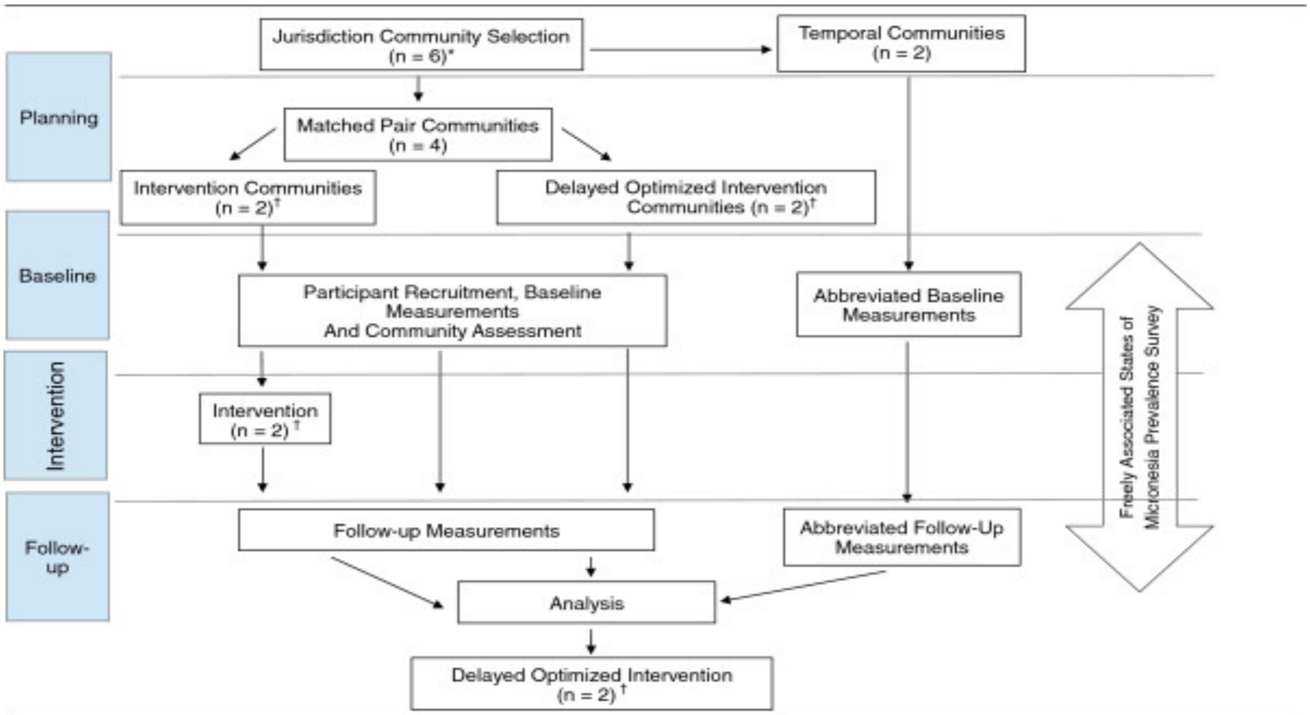

Table 1. Year of First Contact with Westerner's in the US Affiliated Pacific Islands[48]

Pacific Island	Year
Hawai‘i	1778*
American Samoa	1722
The Federated States of Micronesia	1526
Guam	1521
Commonwealth of the Northern Mariana Islands	1521

*First contact may have occurred earlier

Table 2. Characteristics of the study participants ages 2 - 8 years categorized by screening status (positive and negative) of acanthosis nigricans (acanthosis) (n= 3468)

Descriptive		Acanthosis	
		Negative (n=3277)	**Positive (n= 191)**
		n (%)	
Sex	Male	1665 (50.8%)	97 (50.8%)
	Female	1612 (49.1%)	94 (49.2%)
BMI category[a]	Underweight	97 (2.9%)*	1 (0.5%)*
	Healthy weight	2379 (72.5%)***	68 (35.6%)***
	Overweight/obese	801 (24.4%)***	122 (63.8%)***
		Mean (sd)	
Age (years)		5.02 (1.64)***	5.57 (1.64)***

*P≤ 0.05
**P≤ 0.01
***P≤ 0.001
[a] BMI Categories was defined using sex- and age-specific BMI cut-points from the Centers for Disease Control and Prevention Growth Charts. BMI cut-points include: Underweight: less than the 5th percentile; Normal weight: 5th percentile to less than the 85th percentile; Overweight/Obese: 85th percentile or higher.

Table 3. Mean energy, fat, protein, dairy, and meat daily intake of study participants ages 2 - 8 years categorized by screening status (positive and negative) of acanthosis nigricans (acanthosis) (n= 3468)

	Acanthosis	
	Negative (n=3277)	**Positive (n= 191)**
	Mean (sd)	
Mean energy intake (kcal) [b]	1770.73 (673.33)	1777.17 (610.45)
Mean total fat intake (g) [b]	62.32 (31.28)	62.10 (30.03)
Mean protein intake (g) [b]	68.04 (27.98)	69.91(25.08)
Mean kcals from protein (%)	15.58 (0.06)	15.96 (0.27)
Mean protein servings (oz)	2.40 (0.98)	2.46 (0.88)
Total dairy intake (servings) [b]	1.20 (0.98)***	0.98 (0.68)***
Mean meat intake (oz) [c]	6.77 (7.82)**	6.96 (3.91)**
Lean meat equivalent from Egg (oz) [c]	0.39 (0.33)*	0.45 (0.41)*
Lean meat from meat, poultry, and fish (oz) [c]	4.69 (1.76)***	5.21 (1.64)***
Lean meat from Franks and Luncheon meat (oz) [c]	0.82 (0.80)	0.91 (0.91)
Lean meat from Beef, pork and lamb (oz) [c]	0.73 (0.67)	0.77 (0.75)
Lean meat from poultry (oz) [c]	0.99 (0.79)	1.04 (0.81)
Lean meat from Fish and other seafood (oz) [c]	0.87 (1.14)	0.99 (1.03)
Lean meat equivalent from nuts/seeds (oz) [c]	1.12 (2.51)	0.80 (1.45)
Lean meat equivalent from soy (oz) [c]	0.21(0.03)	0.019 (0.02)
Lean meat equivalent from dry bean and pea (servings)[c]	0.00 (0.02)	0.00 (0.03)

*P≤ 0.05
**P≤ 0.01
***P≤ 0.001
[b] Weighted for weekend and weekdays
[c] Weighted for weekend and weekdays and adjusted for within person variance

Table 4. Adjusted odds ratios for the association between acanthosis nigricans and fat, protein, dairy, and meat daily intake in study participants ages 2 - 8 years (n= 3468)

Variables[b]	**B**	**S.E.**	**OR**	**95% CI**	**P**
Mean protein intake (g) [b]	-0.008	0.00	0.92	(0.98- 1.00)	0.163
Total dairy intake (servings) [b]	-0.614	0.123	0.541	(0.42-0.68)	0.000***
Mean meat intake (oz) [c]	0.165	0.03	1.17	(1.09-1.27)	0.000***
Lean meat equivalent from Egg (oz) [c]	0.386	0.22	1.47	(0.94-2.29)	0.089
Lean Meat from meat, poultry, and fish (oz) [c]	0.217	0.056	1.24	(1.11- 1.38)	0.000***
Lean Meat from Franks and Luncheon meat (oz) [c]	0.180	0.104	1.19	(0.97- 1.46)	0.083
Lean Meat from Beef, pork and lamb [c]	-0.174	0.11	0.84	(0.67-1.05)	0.129
Lean meat from poultry (oz) [c]	-0.026	0.098	0.97	(0.80- 1.18)	0.793
Lean Meat from Fish and other seafood (oz)[c]	0.224	0.067	1.25	(1.09-1.42)	0.001***
Lean meat equivalent from nuts/seeds (oz)[c]	-0.052	0.052	0.950	(0.85-1.05)	0.321
Lean meat equivalent from soy (oz) [c]	-0.774	2.374	0.461	(0.00-48.33)	0.744
Lean meat equivalent from dry bean and pea (servings) [c]	-2.05	2.956	0.128	(0.00- 42.10)	0.487

*P≤ 0.05
**P≤ 0.01
***P≤ 0.001
[b] Weighted for weekend and weekdays
[c] Weighted for weekend and weekdays and adjusted for within person variance
[d]All variables were adjusted for age, sex, overweight/obesity, energy intake (kcal), and mean total fat intake

Table 5. Adjusted odds ratios for the association between acanthosis nigricans and dietary guidelines or dietary reference intakes in study participants ages 2 - 8 years (n= 3468)

Variables[d]	B	S.E.	OR	95% CI	P
RDA	-.567	0.46	0.56	0.22-1.4	0.228
AMDR (2-3y)	-1.02	0.58	0.37	0.11-1.13	0.082
AMDR (4-8y)	0.03	0.43	1.03	0.43-2.42	0.940
MyPlate (2-3y)	-0.556	0.43	0.57	0.24-1.34	0.201
MyPlate (4-8y)	0.001	0.29	1.00	0.55-1.79	0.998

*P≤ 0.05
**P≤ 0.01
***P≤ 0.001
[d] All variables were adjusted for age, sex, overweight/obesity, energy intake (kcal), and mean total fat intake

Image 1 Scale of Acanthosis Nigricans (Acanthosis) Class 1 Indicating that Acanthosis is Present on Close Inspection but the Extent is Not Measurable. (courtesy of M. Okihiro)

Image 2. Scale of Acanthosis Nigricans Class 2 Indicating that Acanthosis Does Not Extend to the Lateral Neck Margins and is <3 Inches from One Side to Another (Image courtesy of M. Okihiro)

Image 3. Scale of Acanthosis Nigricans Class 3 Indicating that Acanthosis Extends to the Lateral Margins of the Neck Between 3-6 Inches Wide but is Not Visible from the Front (Image Courtesy of M. Okihiro)

Image 4. Scale of Acanthosis Nigricans Class 4 Indicating that Acanthosis is Visible from the Front and Usually Larger than 6 Inches Wide (Image Courtesy of M. Okihiro)

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