

INFLAMMATORY EFFECTS OF FOOD AVAILABLE THROUGH THE FOOD
DISTRIBUTION PROGRAM ON INDIAN RESERVATIONS (FDPIR;
COMMODITIES)

by

Melinda Sue Smith

A thesis submitted in partial fulfillment of
the requirements of the degree

of

Master of Science

in

Health and Human Development

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2018

©COPYRIGHT

by

Melinda Sue Smith

2018

All Rights Reserved

TABLE OF CONTENTS

1.	INTRODUCTION	1
	Development of the Problem	1
	Purpose.....	3
	Hypotheses	3
	Research Questions	4
	Operational Definitions	5
	Abbreviations	6
	Delimitations	6
	Limitations	7
2.	LITERATURE REVIEW	9
	Introduction.....	9
	Food Distribution Program	10
	on Indian Reservations	
	Dietary Recommendations.....	11
	for Carbohydrates	
	Carbohydrates and Appetite.....	13
	FDPIR Commodity Foods	13
	Physiological Effects of	17
	Carbohydrate Consumption	
	Summary	21
3.	INFLAMMATORY EFFECTS OF FOOD AVAILABLE	23
	THROUGH THE FOOD DISTRIBUTION PROGRAM	
	ON INDIAN RESERVATIONS (FDPIR; COMMODITIES)	
	Contribution of Authors	23
	and Co-Authors	
	Manuscript Information Page	24
	Introduction.....	25
	Methods.....	28
	Participants.....	28
	Research Design.....	28
	Experimental Protocol	29
	Baseline Assessment.....	32
	Saliva Sample Protocol	32
	Assessment of Hunger and Satiety	33

TABLE OF CONTENTS CONTINUED

Diet Composition	34
Salimetrics IL-1 β and IL-6 ELISA Analysis	36
Statistical Analysis.....	37
Results.....	37
Participants.....	37
Inflammatory Biomarkers	38
Analysis of Appetite	39
Total Daily Energy Consumption	41
Post-Condition Questionnaire	42
Discussion	44
4. DISCUSSION AND	50
CONCLUSIONS	
REFERENCES CITED.....	53
APPENDICES	60
APPENDIX A: Institutional Review Board	61
Consent Form	
APPENDIX B: Screening Questionnaire	67
APPENDIX C: Visual Analogue Scale.....	70
Appetite Questionnaire	
APPENDIX D: Post-Condition Questionnaire.....	72
APPENDIX E: Self-Collection of Saliva Samples	74

LIST OF TABLES

Table	Page
2.1 Food Distribution Program on Indian Reservations Carbohydrate (CHO) Foods	14
3.1 Experimental Protocol and Time Course	31
3.2 Participant Characteristics	38
3.3 Total Daily Energy Consumption	42

LIST OF FIGURES

Figure	Page
2.1 Conceptual framework describing the influence of FDPIR on metabolic disturbances and health outcomes.	21
3.1 Diagram of Research Design	29
3.2 Diagram of Research Protocol	31
3.3 Interleukin 1 beta	39
3.4 DGA vs TYPICAL Mean Appetite Scores	40
3.5 Total Mean Appetite Scores.....	41
3.6 Post-Condition Questionnaire Data	43

ABSTRACT

American Indian and Alaskan Native (AI/AN) populations experience some of the greatest health disparities and the lowest life expectancy in the United States compared to all other races and ethnicities. The Food Distribution Program on Indian Reservations (FDPIR) is a primary food supplement program that serves AI/AN communities in the United States. Recent studies have reported that FDPIR monthly food packages do not meet Dietary Guidelines for Americans (DGA). This study measured the effects of two FDPIR diets on inflammation, appetite, and energy intake to better understand potential health outcomes of each FDPIR diet. A within-subjects, randomized, crossover design was used to compare two dietary conditions: 1) FDPIR diet that met Dietary Guidelines for Americans (DGA); and 2) a typical FDPIR diet. Participants were AI/AN and non-AI/AN men and women (n=13), 18-55 years of age, with a waist circumference of > 94 cm for men and > 80 cm for women. Salivary interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6) were collected at six separate time points over 24 hours to determine inflammatory response for each condition. Self-reported visual analogue scale (VAS) appetite questionnaires were used to gauge the effect of each condition on specific appetite sensations (hunger, fullness, satiety, desire to eat, and prospective consumption). Daily energy intake was calculated by weighing food in grams before and after each test day. There were no significant differences in inflammatory response and appetite sensations between the two dietary conditions found by RMANOVA ($p < 0.05$). Participants ate 14% more ($p < 0.01$) kilocalories on a typical FDPIR diet compared to FDPIR diet that met DGA. Higher energy intakes during a typical FDPIR diet compared to a FDPIR diet that meets DGA may increase risk for obesity and nutrition-related diseases, including type 2 diabetes, cardiovascular disease and other chronic inflammatory conditions. This project was approved by Montana State University Institutional Review Board (IRB) and is supported by National Institute of General Medical Sciences of the National Institutes of Health award number P20GM103474.

CHAPTER ONE

INTRODUCTION

Development of the Problem

American Indian and Alaskan Native (AI/AN) populations experience some of the greatest health disparities and the lowest life expectancy in the United States compared to all other races and ethnicities.^{1, 2,3, 4} AI/AN die at higher rates in many categories, including type 2 diabetes, chronic liver disease, cirrhosis, chronic lower respiratory diseases, and injury (assault, suicide, and accidents) than other ethnicities in the nation.^{4, 5} Compared to non-Hispanic whites, AI/AN have a 50% higher chance of becoming obese and are twice as likely to become type 2 diabetic over the age of 20 years old.^{6,7} Obesity is a strong predisposing factor for type 2 diabetes. Insulin resistance, activation of the body's innate immune system, and the subsequent expression of inflammatory cytokines are identified as important links between obesity and type 2 diabetes.⁸ A number of factors play a part in obesity. Energy intake, appetite, and food security have been associated with obesity and chronic diseases.^{3, 9, 10}

The household food insecurity rate in AI/AN households is more than two times higher than the non-Hispanic white rate, and four times higher in AI/AN households that receive food from the Food Distribution Program on Indian Reservations (FDIPR) compared to the national average.^{1, 10, 11} Household food insecurity has been associated with stress, depression and dependence on inexpensive, high-energy dense foods. Stress

and energy dense foods both can cause increased visceral adiposity, which has a direct role in the development of type 2 diabetes and other chronic inflammatory conditions.¹²⁻¹⁴

The FDPIR, also known as Commodities, is the primary food supplement program that serves AI/AN communities in the United States. FDPIR is one of the United States Department of Agriculture (USDA) food supplement programs.¹¹ An assessment of the dietary quality of FDPIR revealed that typical monthly food packages do not meet the Dietary Guidelines for Americans (DGA).¹⁵ The DGA is a tool for policymakers and health professionals to help Americans make healthy food choices to help prevent chronic disease.¹⁶ On average, the FDPIR monthly food packages were found to have excess amounts of refined grains and lack fresh fruits and vegetables.¹⁵ Consumption of low-quality carbohydrates has been found to induce inflammation, oxidative stress and lipogenesis. Interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6) are two common pro-inflammatory biomarkers used to measure changes in inflammation. Increased inflammation can cause a cascade of physiological events that results in reduced insulin sensitivity and the subsequent development of chronic inflammatory diseases, including type 2 diabetes and cardiovascular disease.^{17, 18}

Current research on FDPIR has focused on food security within FDPIR recipient households, DGA adherence, and cultural appropriateness of commodity foods.^{10, 15, 19, 20} There is a concern that low-quality FDPIR foods contribute to the rate of prevalent chronic inflammatory conditions in AI/AN communities.¹² Further research is needed to determine the health impacts of FDPIR commodity foods on program recipients and the AI/AN communities that FDPIR serves. Awareness about the health impacts of FDPIR

commodity foods will enable policymakers to make decisions to support the health status of the AI/AN communities that receive FDPIR foods. There has been little to no research on how foods from the FDPIR affect inflammation, energy intake, and appetite in AI/AN communities. Determining inflammatory effects of a typical FDPIR commodity food diet will provide better understanding of the health consequences that the diet may have on cellular and metabolic function. Investigating differences in appetite and energy intake between a typical FDPIR diet and a FDPIR diet that meets DGA may corroborate the identified relationship between nutrient density of food and obesity, and support research that has highlighted the nutrient shortcomings of FDPIR and the need to improve the nutrient quality of FDPIR commodity foods. This study is a first step toward understanding how FDPIR commodity foods may impact inflammation, energy intake, and appetite in a sample of AI/AN and non-AI/AN individuals.

Purpose

Learning how FDPIR foods influence inflammation, appetite, and energy intake could aid in better understanding how FPDIR impacts the health and wellbeing of AI/AN communities. The purpose of this investigation was to determine if the inflammatory effects of a typical FDPIR diet differed from a FDPIR diet that met DGA standards. Secondary purposes were to determine appetite and energy intake differences between a typical FDPIR diet and a FDPIR diet that met DGA standards.

Hypotheses

PRIMARY: It was hypothesized that the inflammatory response would be significantly

different between the typical FDPIR diet and the FDPIR diet that met DGA standards in all participants.

Ho: $\mu\text{IL-1}\beta$ and $\mu\text{IL-6}$ TYPICAL FDPIR = $\mu\text{IL-1}\beta$ and $\mu\text{IL-6}$ DGA FDPIR

Ha: $\mu\text{IL-1}\beta$ and $\mu\text{IL-6}$ TYPICAL FDPIR $\neq$ $\mu\text{IL-1}\beta$ and $\mu\text{IL-6}$ DGA FDPIR

SECONDARY1: It was hypothesized that a diet that met DGA standards would better satisfy participants' appetites compared to the typical FDPIR condition.

Ho: appetite TYPICAL = appetite DGA

Ha: appetite TYPICAL < appetite DGA

SECONDARY2: It was hypothesized that the total energy intake would be higher during the typical FDPIR condition compared to the condition that met DGA standards.

Ho: energy intake TYPICAL = energy intake DGA

Ha: energy intake TYPICAL > energy intake DGA

Research Questions

Q1 – Is there a difference in inflammatory response between a typical FDPIR diet and a FDPIR diet that meets DGA standards?

Q2 – Does a FDPIR diet that meets DGA standards better satisfy a person's appetite compared to a typical FDPIR diet?

Q3 – Is there a difference in total energy intake between a typical FDPIR diet and a FDPIR diet that meets DGA standards?

Operational Definitions

Inflammation - An innate immune response to tissue damage or aggression.

Food Distribution Program on Indian Reservations – The primary United States Department of Agriculture food supplement program that serves American Indian and Alaskan Natives in the United States. Also known as Commodities.

American Indian and Alaskan Natives – Indigenous people of North America.

United States Department of Agriculture – United States Federal Executive Department responsible for developing, and executing federal policies related to food agriculture, natural resources, rural development, and nutrition.

Food and Nutrition Services – A federal agency responsible for administering the U.S. domestic nutrition assistance programs.

Indian Tribal Organizations – Organizations managed by federally recognized AI/AN communities.

Dietary Guidelines for Americans – A tool used to help American make healthy, daily nutritional choices.

Interleukin 1 beta – A key pro-inflammatory cytokine and mediator in cytokine network.

Interleukin 6 – A secondary pro- and anti- inflammatory cytokine.

Body Mass Index – Ratio of weight to height

Waist Circumference – Measurement of the waist in centimeters.

Abbreviations

FDPIR: Food Distribution Program on Indian Reservations

AI/AN: American Indian / Alaskan Native

USDA: United States Department of Agriculture

FNS: Food and Nutrition Services

DGA: Dietary Guidelines for Americans

CDC: Centers for Disease Control and Prevention

GI: Glycemic Index

HEI-2010/2015: Healthy Eating Index 2010/2015

IL-1 β : Interleukin 1 beta

IL-6: Interleukin 6

BMI: Body mass index

WC: Waist Circumference

Delimitations

Participants between the ages of 18-55 years old were recruited from the city of Bozeman and Montana State University campus. Participants were comprised of females and males that met these inclusion criteria: 1) having a waist circumference ≥ 94 cm for men and ≥ 80 cm for women; 2) absence of self reported acute and chronic inflammatory conditions including diabetes, kidney problems and heart disease; and 3) having an activity level between sedentary or moderate. Exclusion criteria included: 1) having a

waist circumference < 80 cm in females and < 94 cm in males; 2) taking prescribed or over the counter anti-inflammatory medication or lipid lowering medication; 3) having an acute or chronic inflammatory disease; 4) under or over the age range of 18-55 years; and 5) females who are pregnant. Participants were asked to refrain from strenuous activities, alcohol consumption, and smoking 24 hours before and on the test day.

Limitations

Due to time and financial resources, there were multiple anticipated limitations to this study. Limitations included having limited access to AI/AN communities, lack of access to commodity foods, and participant compliance. Participants were recruited from the city of Bozeman and Montana State University campus. The population of the city of Bozeman, including the Montana State University student body, is not diverse in regards to race and ethnicity. According to the US Census Bureau, AI/AN fall into the “other” category and make up only 1.8% of Bozeman’s population.¹³ At the university level, AI/AN students make up 2% of the student body.¹⁴ Due to the small AI/AN population in the area, participants were AI/AN and non-AI/AN individuals. This is a limitation because FDPIR recipients are primarily AI/AN living on or near a reservation. Another limitation was lack of access to commodity foods for the study. Although all food items for the study were selected based on the FDPIR Exhibit O food list from the Food and Nutrition Services (FNS) Handbook 501, the food items were purchased from a local grocery store, not from a FDPIR distribution site.²⁶ Relying on participant compliance was an additional challenge in receiving accurate inflammatory measurements and

calculating total energy consumption. Capturing accurate inflammatory responses required all participants to follow saliva self-collection directions and limit their food consumption during each test day to the provided foods specific to the condition. This study is a first step in understanding inflammatory effects of a typical FDPIR diet, as well as the effects that a typical FDPIR diet has on appetite and energy consumption.

CHAPTER TWO

LITERATURE REVIEW

Introduction

American Indian and Alaskan Native (AI/AN) populations have high rates of chronic inflammatory conditions such as type 2 diabetes and obesity.^{4, 5} Household food insecurity is two times higher in AI/AN populations compared to non-Hispanic whites, making it another major concern for AI/AN communities. Food insecurity is associated with stress and reliance upon inexpensive, energy dense foods.^{3, 10} Energy dense foods are associated with increased visceral adiposity and result in obesity-associated insulin resistance and activation of the body's innate immune system, which play a direct role in the development of type 2 diabetes and other chronic conditions.¹²⁻¹⁴ The Food Distribution Program on Indian Reservations (FDPIR), also known as Commodities, was established to alleviate food insecurity in AI/AN communities.^{11, 19} Unfortunately, the average FDPIR monthly food package has multiple nutritional shortcomings based on HEI scores, which measure how well a diet adheres to DGA.¹⁵ According to a previous assessment, the average monthly FDPIR food package exceeds the recommended limits for refined grains and falls short on nutrient dense carbohydrate options like fresh fruits and vegetables.¹⁵ Diets that are high in low-quality carbohydrates can stimulate the production of pro-inflammatory molecules and induce oxidative stress and fatty acid

synthesis; all of which reduce insulin sensitivity and contribute in the development of type 2 diabetes, atherosclerosis, and other chronic inflammatory conditions.^{17, 21}

Food Distribution Program on Indian Reservations (FDPIR)

The Food and Nutrition Services (FNS) is an agency under the USDA whose mission is to increase food security and reduce hunger by providing low-income people access to healthy food and nutrition education.²² At the federal level, the FNS works to ensure the quality, cultural appropriateness, and accessibility of FDPIR products. There are a number of Indian tribal organizations (ITO) and state government agencies that implement FNS changes, determine applicant eligibility, provide nutrition education, and manage orders, storage, and distribution of FDPIR products. Available foods and nutrition education vary across FDPIR distribution sites. Not all distribution sites have the capacity to order all of the food items that are available. This may leave the sites with a limited variety of healthy food choices for the program recipients. Although there are educational materials provided on the USDA website, the extent of education provided to FDPIR recipients for each community is heavily determined by the FDPIR site managers and local nutrition education programs.^{11, 22} FDPIR nutritional improvements within the last decade included decreasing sodium content (36-140 mg per serving) in canned vegetables to better meet the recommended sodium intake of <2300mg/day; and the addition of bison meat, wild rice, and frozen salmon to increase cultural appropriateness of the program.^{23 24}

Despite recent improvements, FDPIR food packages are not meeting dietary recommendations according to the DGA. Byker Shanks et al. assessed FDPIR monthly packages using Healthy Eating Index 2010 (HEI-2010). HEI-2010 was developed to measure dietary quality in accordance to DGA. HEI scoring range from zero to 100.²⁵ A score of 100 means that the diet/meal meets all standard recommendations set by the DGA. The study simulated five monthly food packages based on specific FDPIR foods and allowances. The average monthly food package scored 66.38 ± 11.60 , which is significantly lower than the maximum HEI score. No FDPIR food packages in the assessment met the standard recommendations for total fruit, total vegetables, greens and beans, dairy, total protein foods, refined grains or sodium.¹⁵

Dietary Recommendations for Carbohydrates

Carbohydrate, fat and protein are the three organic nutrients that our bodies use for energy. Carbohydrates contribute about half of the body's energy and consist of sugar, starches, and fiber.²⁶ Dairy and all plant foods (whole grain, vegetables, legumes, and fruits) are considered high-quality carbohydrate sources because they are nutrient dense, which means the carbohydrate source provides fiber, vitamins, and minerals. Foods with added sugar (refined grains, candies, sweetened juices, and soda) are considered low-quality carbohydrates sources because they have low nutrient density, high energy density, and a high glycemic index (GI).^{17, 26}

GI is a measure of the food's blood glucose raising ability, and is measured in relation to pure glucose (100).^{27, 28} For example, a food item that has a GI of 60 means

that the food raises blood glucose levels 60% of ingested glucose. Low-GI foods have a GI 55 or less, medium-GI foods have a GI of 56 to 69, and high-GI foods have a GI 70 or more. A food with a high GI results in a quick spike in blood glucose; while food with a lower GI has a slower and smaller rise in blood glucose. GI is important when assessing dietary quality of carbohydrate foods. Diets that consist of primarily high-GI foods elicit metabolic stresses, such as inflammation, and increase risk for developing chronic inflammatory diseases. Diets that have large portions of low-GI foods are associated with reduced risk for chronic diseases.²⁹ The American Diabetes Association recommends choosing food that have low to medium GI, and pairing high GI foods with foods that have a low GI. Nutrient density influences the GI of carbohydrate foods. For instance, increased micronutrient and fiber content in the food can lower the GI score of the food.^{17, 30}

Energy dense foods, such as low-quality carbohydrates, are foods that are high in kilocalories and have been directly linked with weight gain and obesity. Energy density has an inverse relationship to nutrient density.³¹ In an attempt to reduce prevalence of type 2 diabetes, cardiovascular disease and obesity in the United States, the Dietary Guidelines Advisory Committee recommends decreasing consumption of energy-dense foods and increasing consumption of nutrient-dense foods. The Dietary Reference Intake (DRI) sets nutritional standards for the general, healthy population to meet energy and nutrient needs. The DRI recommends 45% -65% of energy come from carbohydrates, with a maximum of 25% of carbohydrates from added sugar.^{26, 31,16}

Carbohydrates and Appetite

High nutrient dense carbohydrate foods may prolong the secretion of appetite regulating hormones, increase viscosity of intestinal content and reduce the rate of gastric emptying. Multiple studies have used visual analogue scale (VAS) appetite surveys to compare sensation ratings between whole grain and refined grain foods.³²⁻³⁴ One study reported that whole grain bread increased satiety and fullness and decreased hunger compared to refined grain bread.³⁴ Another study found the same results associated with whole grain versus refined grain pasta.³³ Indeed, highly nutrient dense carbohydrates including whole grains better satisfy appetite sensations compared to low-quality carbohydrate options such as refined grains.

FDPIR Commodity Foods

Exhibit O of the FNS Handbook 501, which can be found on the USDA website, provides a list of foods distributed through the FDPIR and the monthly allowance per person. The Exhibit O food list separates each food by macronutrient (carbohydrates, protein, and fat) and then further subcategorizes them (vegetables, fruits, grains, dairy etc.).³⁵ Table 2.1 displays the GI ranges for each carbohydrate food category listed in Exhibit O, the monthly allowance, the maximum HEI-2010 scores (best possible scores), and the average HEI-2010 scores (actual scores) for each carbohydrate category.^{15, 35}

The GI ranges for Table 2.1 were calculated using online GI calculators.^{36, 37} The HEI-2010 data is from *Assessing the Foods from the Food Distribution Program on*

*Indian Reservations (FDPIR) using the Healthy Eating Index 2010 study.*¹⁵ The purpose of Table 2.1 is to show the nutritional variability in carbohydrate rich foods in FDPIR monthly food packages.

Table 2.1 Food Distribution Program on Indian Reservations (FDPIR)
Carbohydrate (CHO) Foods

CHO Categories	Number of Items per month allowed (Any combination for each category; cannot exceed allowed units)	Glycemic Index (Range) High = H Medium = M Low = L	Maximum HEI- 2010 score (Byker Shanks et al.)	HEI-2010 Mean Score (Byker Shanks et al.)
Vegetables: Canned vegetables (15.5 oz. can) Fresh Vegetables	Up to 11 units per person	10 (L) – 95 (H)	Total vegetables: <u>5</u>	FDPIR Total Vegetables: <u>2.58</u>
Fruit: Canned Fruit (15.5 oz. can) Fresh Fruit Dried Fruit (15-16 oz.)	Up to 10 units per person	10 (L) – 65 (M)	Total Fruit: <u>5</u>	FDPIR Total Fruit: <u>3.52</u>
Juices (64 oz. bottle)	2 units per person	38 (L) – 76 (H)		
Cereal Dry (all sizes)	1 unit per person	45 (L) -70 (H)		
Quick oats (42 oz. package) Or Farina (14 oz. package)	1 unit per person	66 (M) – 74 (H)		

Table 2.1 Continued

Macaroni & Cheese (7.25 oz. treated as 1 lb.) Macaroni (1 lb.) Whole Grain Rotini (1 lb.) Spaghetti (1 or 2 lb. package) Egg Noodles (1 lb. package) Rice (2 lb. package)	Cannot exceed 5 lb. per person, with a limit of 1 lb. of macaroni and cheese	40 (L) – 70 (H)	Whole Grains: <u>10</u> Refined Grains: <u>10</u>	FDPIR Whole Grains: <u>7.88</u> FDPIR Refined Grains: <u>3.04</u>
Cornmeal / Flours (5 lb. or 2 lb. bag)	2 units per person	40 (L) -70 (H)		
Bakery Mix (5lb. bag)	1 unit per 4 months 1 unit per 2 months	30 (L) – 50 (L)		
Saltine Crackers (16 oz.) /Whole Grain Tortillas (1 lb. package)	1 unit per person	72 (H) – 80 (H)		
Beans: Canned Beans (15.5 oz.) Dry Beans (2 lb. package)	4 units per person	18 (L) – 59 (M)	Greens & Beans: <u>5</u> (Grouped w/leafy greens and peas)	FDPIR Greens & Beans: <u>0.92</u> (Grouped w/leafy greens and peas)
Smooth Peanut Butter (18oz.) Roasted Peanuts (12 or 16 oz.)	1 unit per person	15 (L) – 40 (L)		

Table 2.1 Continued

Block Process American Cheese Or Sliced Reduced-fat Cheese Blend (5 lb. loaf)	1 per 2 months	0 – 30 (L)	Total Dairy: <u>10</u>	FDPIR Total Dairy: <u>5.12</u>
Skim evaporated milk (12 oz.)	1 per 2 months			
Instant Nonfat Dry Milk (25.6 oz. box)	1 per 2 months			
OR 1% Ultra High Temperature (UHT) Milk (32 fl oz. box)	4 units per person			

Table 2.1. The food categories and monthly allowance comes from Exhibit O of the FNS Handbook 501.³⁵ GI online calculators montignac.com and medindia.net calculated GI min and max scores;^{36,37} HEI-2010 data comes from the *Assessing the Foods from the Food Distribution Program on Indian Reservations (FDPIR) using the Healthy Eating Index 2010* study.¹⁴

The GI ranges in Table 2.1 highlight the wide range of nutrient quality of the commodity foods. For example, a FDPIR recipient could select canned fruit cocktail instead of fresh apples (GI 60 vs. 39), and macaroni and cheese instead of whole grain rotini (GI 64 vs. 32). All food categories in Table 2.1 fell short from meeting DGA recommendations. For instance, refined grains exceed DGA recommendations for total

grains (HEI-2010 score of 3.04 out of 10).¹⁵ All carbohydrate foods did not meet DGA recommendations.

Physiological Effects of Low-Quality Carbohydrates

Low-quality carbohydrates can directly and indirectly cause metabolic imbalances. An example of an indirect pathway would be the excess consumption of low-quality carbohydrate foods leading to obesity, which consequently results in obese-associated insulin resistance and activation of the body's innate immune system.^{8, 38} Excess carbohydrate consumption can increase oxidative stress, stimulate the production of inflammatory cytokines, and increase fatty acid synthesis.^{17, 38} A surge in one of these physiological responses often leads to the increase of another. For example, increased production of fatty acids can increase both oxidative stress and inflammation.³⁹

Increased Oxidative Stress

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defense. ROS, or free radicals, are chemical molecules that have unpaired electrons. Due to the presence of an unpaired electron, ROS are typically unstable and can result in tissue damage. Tissue damage occurs when a ROS steals an electron from various parts of a cell via oxidation. This disrupts the cell's ability to function normally and leads to cell death.^{40, 41} Elevated blood glucose increases production of superoxide radicals, a type of ROS, by the mitochondria.^{17, 21} One research study reported that an acute ingestion of 75 grams of glucose resulted in production of ROS and the subsequent activation of NF-kB, which leads to inflammation.²¹

Increased Inflammation

Inflammation can be caused by physical damage such as a broken bone, or by the body's own innate immune system.^{32, 33} Inflammation resulting from the body's innate immune system can be measured in different ways. Interleukins mediate communication between cells and stimulate the body's inflammatory response. Interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) cytokines are crucial in regulation of the innate immune response and are commonly used as biomarkers of inflammation.³² Irrefutably, the role that each cytokine plays in inflammation is unique and important.

IL-1 β is a central mediator in the cytokine network. Although multiple cells can produce IL-1 β , it is commonly expressed in macrophages that infiltrate the blood and tissues. Several mechanisms control the production of IL-1 β . Microbial agents and ROS can stimulate the production of IL-1 β . Once a mature IL-1 β molecule is produced, it is free to activate surrounding type 1 IL-1 receptors (IL-1R1) that signal the expression of more pro-inflammatory molecules, including pro-inflammatory cytokine IL-6.⁴² In summary, microbial agents and ROS stimulate the production of IL-1 β , which subsequently stimulates the production of IL-6 and other pro-inflammatory molecules.

IL-6 can be a pro-inflammatory and anti-inflammatory cytokine. IL-6 is considered an anti-inflammatory cytokine when it is produced in absence of a pro-inflammatory stimulus. It is a pro-inflammatory cytokine when expressed in response to IL-1 β and tumor necrosis factor alpha (TNF α) activity. TNF α is another pro-inflammatory mediator in inflammatory production. Like IL-1 β , IL-6 can be expressed in a variety of cells, including adipocytes, muscle cells, endothelial cells, and leukocytes.⁴³

Hyperglycemia, or having excess glucose in the blood, stimulate the production of IL-6.^{44, 45}

Increased pro-inflammatory cytokine production correlates with elevated BMI, waist circumference, and higher percent body fat.^{17, 45} According to the International Diabetes Federation, criteria for central obesity are waist circumferences ≥ 80 cm (~ 31.5 inches) for women and ≥ 94 cm (37 inches) for men.^{46, 47} Research has shown that individuals with a bigger waist circumference have a greater inflammatory response compared to those with a smaller waist circumference.⁴⁶ A bigger waist circumference is an indicator of visceral adipose tissue, which is adipose located in the abdominal cavity. Compared to subcutaneous (under the skin) adipose tissue, visceral adipose tissue is a concern because of its close proximity to vital organs, high inflammation activity, contribution to insulin resistance, and ability to obstruct the function of hepatic cells.^{46, 48}

As adipose tissue increases, macrophage accumulation within the tissue occurs. The accumulated macrophages produce pro-inflammatory cytokines that obstruct adipocyte function, including glucose uptake and translocation of important insulin signaling molecules such as glucose transporter 4 (GLUT4) and insulin receptor substrate 1 (IRS1), both of which play a critical role in glucose uptake from the blood.⁴⁹ Consequently, prolonged inflammation in adipose tissue results in insulin resistance and release of fatty acids that can migrate to the liver and muscles and decrease insulin sensitivity in those tissues.^{46, 48, 50} In summary, low-quality carbohydrates can increase production of pro-inflammatory molecules from various cells, which can lead to the disruption of insulin signaling and glucose uptake from the blood.

Increased Fatty Acid Synthesis

Fatty acids are made from a two carbon, acetyl-coenzyme, which is a molecule in the fatty acid synthase pathway. Fatty acid synthesis is the creation of fatty acids via acetyl-CoA pathway. Acetyl-CoA can be derived from carbohydrates via the glycolytic pathway.⁵¹ Elevated fatty acids in the blood, or plasma free fatty acids, is more common in overweight and obese individuals. In obese individuals, fatty acid synthesis increases and fatty acid clearance from the blood is reduced.³⁹ A study done on overweight males showed increased fatty acids and triglycerides after a high-carbohydrate, low-fat meal.⁵² High-carbohydrate intake decreases fatty acid oxidation and increases lipogenesis. Plasma free fatty acids can circulate in the body and reduce insulin stimulated glucose uptake in skeletal muscles and the liver.⁵³⁻⁵⁵ The consequences of reduced glucose uptake in the liver are impaired gluconeogenesis, ketone body production and glucose dumping.¹⁷

In addition to insulin resistance, elevated free fatty acids can also increase expression of pro-inflammatory cytokines, such as IL-1 β and IL-6, through the activation of NF-kB pathway. Other research has demonstrated that fatty acids can activate protein kinase C (PKC), which subsequently activates NAD(P)H oxidase, a membrane bound enzyme that produces ROS. In short, fatty acids can be produced in response to a high-carbohydrate meal and is elevated in obese individuals, which consequently increases insulin resistance and the production of ROS and pro-inflammatory molecules.^{17, 21}

Summary

The FDPIR is the main food supplement program that serves AI/AN communities. There is large variability in quality and quantity of foods available to FDPIR recipients. Average FDPIR monthly food packages have large amounts of low-quality carbohydrate foods, such as refined grains, and lack nutrient dense carbohydrate options like fresh fruits and vegetables. Excess consumption of low-quality carbohydrate foods is indirectly and directly related to the development of chronic conditions. Indirectly, excess low-quality carbohydrates over time can cause increased visceral adiposity, which leads to metabolic and adipocyte stress. Directly, low-quality carbohydrates can fuel oxidative stress, inflammation and fatty acid synthesis; all of which decrease peripheral and hepatic insulin sensitivity, and increase risk for the development of chronic conditions, including type 2 diabetes, which is prevalent in AI/AN populations. The nutritional shortcomings in FDPIR are of concern due to the health disparities and pervasiveness of inflammatory diseases in AI/AN populations. Figure 2.1 is a conceptual framework that summarizes and describes how FDPIR and excess consumption of low-quality carbohydrates can lead to chronic conditions and health outcomes.

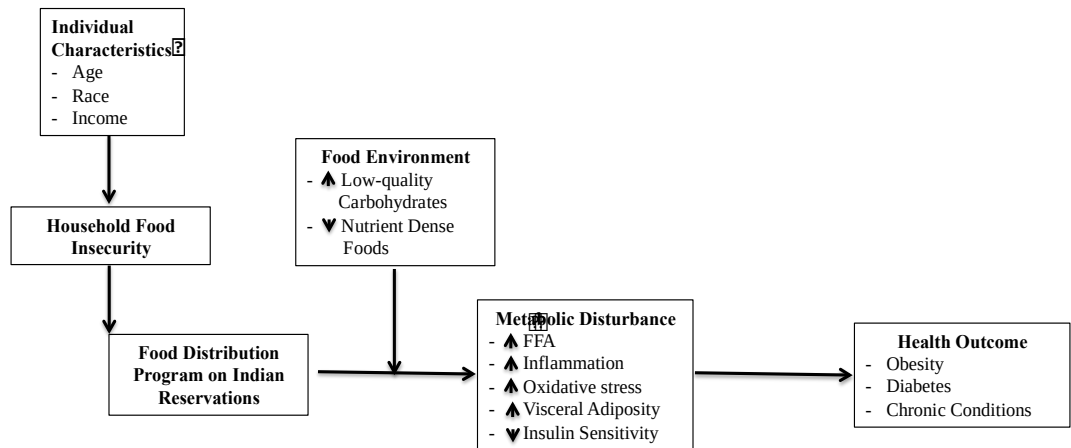


Figure 2.1. Conceptual framework describing the influence of FDPIR on metabolic disturbances and health outcomes.

CHAPTER THREE

INFLAMMATORY EFFECTS OF FOOD AVAILABLE THROUGH THE FOOD DISTRIBUTION PROGRAM ON INDIAN RESERVATIONS (FDPIR; COMMODITIES)

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Melinda Smith

Contributions: Conceived and implemented the study design. Collected and analyzed data. Ran the statistical analyses. Wrote the first draft of the manuscript.

Co-Author: Mary Miles, PhD

Contributions: Conceived and implemented the study design. Provided expertise and funding. Aided in statistical and data analyses. Provided feedback on the draft of the manuscript.

Co-Author: Elizabeth Rink, PhD

Contributions: Conceived and implemented the study design. Provided feedback on the draft of the manuscript.

Co-Author: Suzanne Held, PhD

Contributions: Conceived and implemented the study design. Provided feedback on the draft of the manuscript.

Manuscript Information Page

Melinda Smith, Mary P Miles, Elizabeth Rink, Suzanne Held

Status of Manuscript:

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-review journal
- ☐ Accepted by a peer-review journal
- ☐ Published in a peer-review journal

INFLAMMATORY EFFECTS OF FOOD AVAILABLE THROUGH THE FOOD
DISTRIBUTION PROGRAM ON INDIAN RESERVATIONS (FDPIR;
COMMODITIES)

Introduction

Chronic inflammatory diseases such as type 2 diabetes and cardiovascular disease are prevalent among American Indian and Alaskan Native (AI/AN) communities.^{2, 4} According to the Centers for Disease Control and Prevention (CDC) National Diabetes Statistics Report, AI/ANs had the highest rate (15.9%) of diabetes over the age of 18 years and older among all races and ethnicities.² Obesity is also a great concern for AI/AN communities as AI/AN populations have a 50% higher chance of becoming obese than non-Hispanic whites.^{6, 7} Food insecurity is closely associated with obesity and development of type 2 diabetes and other chronic diseases.^{13, 56} Household food insecurity is 200% higher in AI/AN households compared to non-Hispanic white households.¹ Supplemental food programs play a critical role in food accessibility for households with low food security.²⁰ The Food Distribution Program on Indian Reservations (FDPIR), also known as Commodities, is the USDA's primary supplemental food program that serves AI/AN communities. Although the FDPIR commodity foods are only meant to be supplemental, monthly food packages are the sole source of food for almost 40% of FDPIR recipients.¹⁹ This is a concern because the average FDPIR monthly food package does not meet Dietary Guideline for Americans (DGA). On average, the food packages have excessive amounts of low-quality

carbohydrate foods, such as refined grains, and lack nutrient-dense carbohydrate sources such as fresh fruits and vegetables.¹⁵

Quality of carbohydrate food is measured by nutrient and energy content. Nutrient dense carbohydrate sources are high in fiber, mineral, and vitamin content, all of which have the benefits of prolonging secretion of appetite regulating hormones, increasing viscosity of intestinal content and slowing gastric emptying.^{26, 33, 34} Low-quality carbohydrate foods have added sugar, are more energy dense, and have high glycemic indexes (GI).^{17, 26} GI is the measure of a food's blood glucose raising ability. Foods with a high GI cause a quick spike in blood glucose. In contrast, foods with a low or medium GI have a delayed rise in blood glucose. Low-quality carbohydrate foods like refined grains can directly and indirectly lead to metabolic disturbances that are important links in the development of type 2 diabetes and other chronic inflammatory diseases.^{17, 21, 30, 46, 57} For instance, low-quality carbohydrate foods can cause inflammation by increasing oxidative stress, which then stimulates the production of pro-inflammatory cytokines, such as interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6).^{17, 21} IL-1 β is a central mediator in the cytokine network; meaning that it is essential in the in stimulating further production of pro-inflammatory molecules. IL-6 is both an anti- and pro-inflammatory cytokine. Both cytokines are common pro-inflammatory biomarkers used to measure inflammation in humans.^{43, 44, 58, 59}

Indirectly, low-quality carbohydrate food can increase inflammation by elevating visceral adiposity.^{38, 46} Previous research studies have demonstrated that energy dense low-carbohydrate foods can induce overeating and cause weight gain.^{31, 38, 60, 61} Visceral

adipose tissue is elevated in obese individuals and is considered a direct risk for type 2 diabetes and other inflammatory conditions. Visceral adipose tissue can accumulate white blood cells, which produce pro-inflammatory cytokines.^{12, 47} Elevated pro-inflammatory cytokine production can obstruct adipocyte function by disrupting translocation of insulin signaling molecules that are crucial for glucose uptake. Inflammation in visceral adipose tissue can also disrupt free fatty acid (FFA) uptake from the blood, therefore leading to elevated FFA in the blood, which can cause hepatic insulin resistance and further production of pro-inflammatory molecules.³⁹ To summarize, low-quality carbohydrate food, like the refined grains offered through the FDPIR, can increase visceral adiposity and the production of pro-inflammatory molecules, which consequently reduces insulin sensitivity and increases the risk for developing chronic inflammatory conditions.

The purpose of this study was to determine the effects of a typical FDPIR commodity food diet on inflammation, appetite, and energy intake on a combination of AI/AN and non-AI/AN individuals. It was hypothesized that the inflammatory response would be greater for a typical FDPIR diet than for a FDPIR diet that met DGA standards. It was also hypothesized that the DGA condition would better satisfy participants' appetites compared to the typical FDPIR condition; and that the total energy intake would be higher after the typical FDPIR condition compared to the DGA FDPIR condition. Learning how FDPIR foods influence inflammation, appetite, and energy intake could aid in better understanding how FPDIR impacts the health and well being of AI/AN communities. Knowledge gained from this study will not only improve our understanding of the health impacts of FDPIR diets, but will also enable Indian Tribal Organizations

(ITO), state agencies, and FDPIR policymakers to make decisions that support the health status of the AI/AN communities that rely on the FDPIR.

Methods

Participants

Thirteen AI/AN and non-AI/AN participants, 18-55 years of age (male = 3, female = 10), enrolled in the study. Inclusion criteria included waist circumferences ≥ 94 cm for men and ≥ 80 cm for women, absence of self-reported acute and chronic inflammatory conditions including diabetes, kidney problems and heart disease, and having an activity level between sedentary or moderate. Exclusion criteria included waist circumferences < 80 cm in females and < 94 cm in males, taking prescribed or over the counter anti-inflammatory medication or lipid lowering medication, having an acute or chronic inflammatory disease, under or over the age range of 18-55 years, and females who are pregnant. Participants were informed of potential risks and discomforts of the study and signed an informed consent document approved by the Institutional Review Board (IRB) for protection of human subjects at Montana State University (MSU). Approval from the MSU IRB was received before recruitment for this study began (Appendix A). Participants were recruited within the MSU campus, the city of Bozeman, and surrounding areas using flyers, emails and word of mouth.

Research Design

A within, randomized, crossover design was used to compare two conditions: 1) FDPIR diet that met DGA (DGA); and 2) FDPIR diet that did not meet DGA (Typical).

Each participant served as their own control and was exposed to both conditions. The first condition was randomly assigned to participants upon completing their initial visit. The order of the two conditions was counterbalanced between participants and separated by at least seven days to allow variables to return to baseline between conditions. Figure 3.1 is a diagram of the project's randomized crossover design.

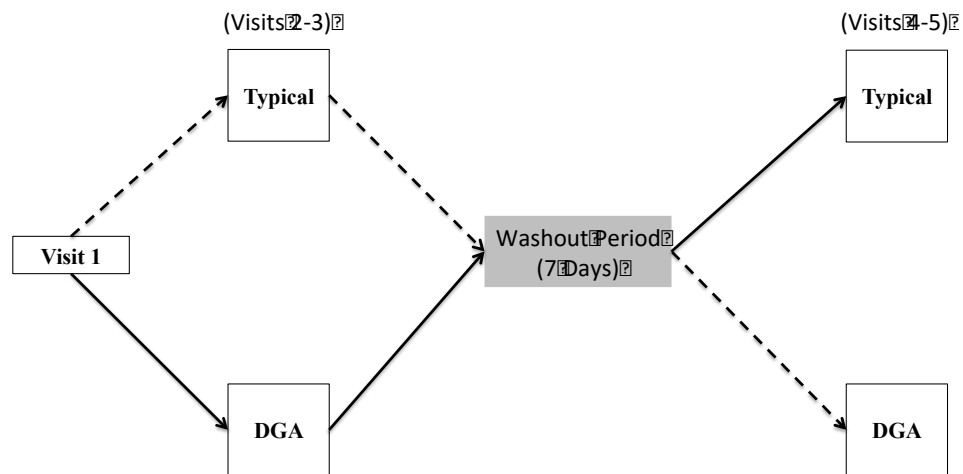


Figure 3.1. Visit 1 involved reviewing and signing the informed consent document and collection of anthropometric measurements (%fat, %lean, waist circumference, weight, and height). “Typical” represents the typical FDPIR dietary condition and “DGA” represents the dietary condition that meets DGA. Participants were randomly assigned to one of the conditions for visits 2-3, then there was a seven day washout period before crossing over to the second condition (visits 4-5).

Experimental Protocol

The study took place in the MSU Nutrition Research Laboratories and MSU Herrick Hall Food Laboratory on the MSU campus. Participants were asked to complete five visits. The initial visit involved signing the informed consent document, collecting anthropometric measurements, and reviewing the questionnaires. Visit two occurred two-

five days after visit one. Participants were asked to refrain from exercise and/or vigorous activity, consuming alcohol, and smoking 24 hours prior to and the day of each test day to minimize variability in inflammatory concentrations. Participants were also asked to log their diet the day before visit two and four. The goal of the dietary log was to help participants replicate their food choices the day before their next test day (visit four). Participants were instructed not to consume anything the morning of visits two through five, with the exception of water. Visit two took approximately one hour and entailed collecting participants' baseline saliva sample and appetite ratings, providing breakfast, and providing participants with saliva collection kits, appetite questionnaires, and food for the remainder of the day. Participants were instructed to eat ad libitum and to restrict food consumption to the food that was provided to them during the entirety of each test day. The following morning (visit three), participants returned to the MSU Nutrition Research Laboratory to deliver their saliva samples, completed appetite questionnaires, and uneaten food. During this visit, the participants collected their final saliva sample for the condition and completed an appetite questionnaire and the post-condition questionnaire. At least seven days after completing the first condition, participants crossed over to the second condition and repeated the procedures of visits two and three. For each condition, participants self-collected six saliva samples and completed six self-reported appetite questionnaires and one post-condition questionnaire (total of 12 samples, 12 appetite questionnaires, and two post-condition questionnaires). After completing their final visit, all participants were provided with a \$70.00 incentive. All five visits took approximately two to four weeks. Figure 3.2 displays a summary of the

experimental protocol, and Table 3.1 displays the experimental protocol and time course for collecting saliva samples and completing appetite questionnaires.

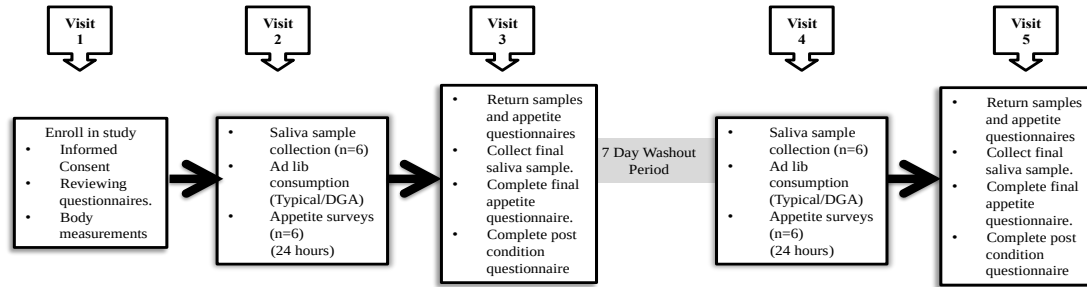


Figure 3.2. A diagram of the experimental protocol.

Table 3.1. Experimental Protocol and Time Course

Visit	Time	Activity or procedure
1	8:00 AM	Initial lab visit (informed consent, screening questionnaire, fat%, lean%, weight, height, waist circumference)
2	Waking to sleeping 7:00 AM	Controlled diet, limited physical activity 1 st Baseline Saliva Collection and Appetite Questionnaire
	11:00 AM	2 nd Saliva Collection and Appetite Questionnaire
	2:00 PM	3 rd Saliva Collection and Appetite Questionnaire
	5:00 PM	4 th Saliva Collection and Appetite Questionnaire
	8:00 PM	5 th Saliva Collection and Appetite Questionnaire
3	7:00 AM	6 th Final Saliva Collection and Appetite Questionnaire
4	Waking to sleeping 7:00 AM	Controlled diet, limited physical activity 1 st Baseline Saliva Collection and Appetite Questionnaire
	11:00 AM	2 nd Saliva Collection and Appetite Questionnaire
	2:00 PM	3 rd Saliva Collection and Appetite Questionnaire
	5:00 PM	4 th Saliva Collection and Appetite Questionnaire
	8:00 PM	5 th Saliva Collection and Appetite Questionnaire
5	7:00 AM	6 th Final Saliva Collection and Appetite Questionnaire

Baseline Assessment

The initial visit occurred in the MSU Nutrition Research Laboratory between 8:00 a.m. and 9 a.m. four days prior to the first condition. This visit took approximately one hour for each participant and included the following activities: 1) reading and signing the written informed consent document; 2) completing the screening questionnaire; 3) reviewing the appetite and post-condition questionnaires; 4) reviewing the instructions for self-collecting saliva samples; and 5) taking anthropometric measurements using a stadiometer (Perspective Enterprises, Portage, MI) to measure height, an ergonomic circumference measuring tape (Seca, Hamburg, Germany) to measure waist circumference, and a bioelectrical impedance analyzer (Seca, Hamburg, Germany) and an air displacement plethysmography (COSMED BodPod, Rome, Italy) to measure body composition, weight, resting metabolic rate (RMR), and total energy expenditure (TEE). Baseline saliva samples were collected at 7:00 a.m. on each test day prior to breakfast to measure pro-inflammatory biomarkers IL-1 β and IL-6. Baseline appetite ratings for hunger, fullness, satiety, desire to eat, and prospective consumption were collected immediately following the collection of each baseline saliva sample.

Saliva Sample Protocol

Salimetrics salivabio oral swab (SOS) collection instructions were used to create a simplified instruction document that was provided to each participant to educate them on how to self-collect saliva samples.⁶² The collection instructions required all participants to rinse their mouths, place the swab under their tongues, and minimize the movement of the swab for one-two minutes or until the swab was saturated.⁶² For each sample, two

swabs were collected to ensure adequate saliva for both IL-1 β and IL-6 analyses. The collection schedule for this study was based on the inflammatory response of two pilot participants who underwent each condition and collected 10 saliva samples for each condition. Following the analyses of the pilot participants' saliva samples, six collection times were identified and chosen based on the pilot participants' inflammatory responses for subsequent participants. Collection times were 7 am, 11 am, 2 pm, 5 pm, 8 pm, and 7 am the following morning. Immediately following the collection of a saliva sample, participants were asked to store them in a freezer (4°C). The following morning (visit three and five), participants brought their saliva samples into the MSU Nutrition Research Laboratory. There, the participants collected a final sample and then all samples were stored in an -80°C laboratory freezer until they were analyzed. A total of six collection tubes and 12 swabs were provided to the participants for each condition.

Assessment of Hunger and Satiety

The Self-Reported Appetite Questionnaire contained visual analogue scales (VAS) with words anchored to each end of a 100 mm scale. These words expressed the most positive and negative rating for each of these appetite sensations: hunger, satiety, fullness, desire to eat, and perspective consumption. For example, for hunger, 'Not at all' is anchored on one end of the scale, and 'Extremely (as hungry as I've ever been)' is anchored on the other end of the scale.^{49, 50} The goal of the Self-Reported Appetite Questionnaire was to determine if there was a difference in appetite scores between the two dietary conditions. The validity of this approach to collecting appetite data has been

established.⁶³ During visit one, the researcher reviewed the appetite questionnaire instructions and the required completion times with each participant. The appetite questionnaires were completed following self-collection of each saliva sample. There were four goals of the post-condition questionnaire: 1) assess participant compliance; 2) gauge the participants' overall satisfaction; 3) determine whether or not each participant felt that they received enough food; and 4) learn if the participants would eat the food on a normal day. Post-condition questionnaires contained five questions, three of them being Likert scale questions (1=Strongly Disagree; 2=Disagree; 3=Neither, 4=Agree, 5=Strongly Agree). The first three questions were Likert questions: 1) "I would eat the provided food on a normal day"; 2) "I had enough food to last me through the day"; and 3) "overall I am satisfied with the food that was provided". Question four and five were: "Did you eat or drink anything outside of the food and water that was provided to you? If yes, please list the food/beverage items that you consumed outside of the provided foods." and "Did you not eat a food item that was provided to you? If yes, please list the food/s that you did not eat." During the initial visit, the post-condition questionnaire was reviewed, and the expected time and place in which the participant was expected to complete the questionnaire was discussed.

Diet Composition

Food for both conditions was selected from the FDPIR food list from Exhibit O FNS Handbook 501.²⁶ The typical FDPIR diet was constructed to have comparable HEI scores to both the United States average adult HEI scores, and the HEI scores found by an assessment completed by Byker Shanks et al. (2015). The national average HEI score

for adults 18-64 years of age ($n=4,294$) between 2007-2008 was $54.3 (\pm 1.16 \text{ s.e.m})$.⁵¹ The average FDPIR monthly food package scored an average of 66.38 ± 11.60 . The typical FDPIR dietary condition consisted of 1% milk, cornflakes, canned fruit cocktail, saltine crackers, American cheese, macaroni & cheese, unsweetened applesauce, spam, spaghetti noodles, and spaghetti sauce with ground beef. The DGA FDPIR dietary condition was constructed to score a minimum HEI score of 80 out of 100. The DGA FDPIR dietary condition consisted of regular oatmeal, 1% milk, fresh grapefruit, chicken wraps (chicken breast, romaine lettuce, wild rice, black beans, American cheese, and whole wheat tortilla), fresh assorted fruit (apples and oranges), a fruit and nut trail mix, pork chops, steamed broccoli, and mashed sweet potatoes. The National Cancer Institute Automated Self-Administered 24 hour Dietary Recall System (ASA24) was used in developing each dietary condition. The ASA24 report provided essential nutrient information to calculate HEI scores. The typical FPDIR diet had a HEI score of 53.8 and the DGA FDPIR diet had a HEI score of 89.5.

All food was cooked, stored, and served in accordance to ServSafe Montana standard operating procedures. ServSafe is a food and beverage safety training and certificate program administered by the National Restaurant Association. Researchers handling the food for the study were ServSafe certified. Breakfast was served to participants in the MSU Herrick Hall Food Laboratory. After breakfast, participants were provided with food for the remainder of the day according to their estimated total energy expenditure (TEE) which was estimated by the air displacement plyphesmography machine. Kilocalories were calculated by weighing the food in grams and then

multiplying by the number of kilocalories per gram for each food. The kilocalories per gram for each food item were determined using the food item's nutrition labels and myfitnesspal.com. With the exception of water, participants were asked to restrict consumption to the provided food. The participants were allowed to eat ad libitum. Uneaten food was returned to the lab and weighed the following morning to determine total energy consumption.

Salimetrics IL-1 β and IL-6 ELISA Analysis

The Salimetrics IL-1 β and IL-6 ELISA kits have been designed and validated to quantitatively measure salivary IL-1 β and IL-6 cytokines. First, all reagents were prepared according to the Salimetrics reagent preparation instructions. Secondly, saliva samples were diluted with the instructed amount of the IL-1 β and IL-6 sample diluent, and then transferred to a microtitre plate in duplicates. The standards and controls were also added to the plates according to the procedure instructions and suggested plate layout. The plates then incubated for an hour on a plate rotator. Following the first incubation period, the plates were washed using a wash buffer. Then, antibody conjugate was added to the plates. The plates incubated for two hours at room temperature on a plate rotator. The wash procedure was repeated following the second incubation period. Streptavidin-HRP was immediately added to each well following the wash procedure. The plates were then place on a plate rotator for 20 minutes at room temperature. Upon completion of the 20-minute incubation period, the plates were washed again, and TMB Substrate Solution was added to each well. The plates were then placed on a plate rotator for an additional 20 minutes in the dark. Stop solution was added to each well

immediately after the final 20-minute incubation period. The plates were then placed on a plate rotator until all wells turned yellow. Within 10 minutes of adding the stop solution, the plates were analyzed using a μ -Quant plate reader with 450 nm and 620-630 reference filters (Bio-Tek Instruments Inc., Winooski, VT).

Statistical Analysis

Data was analyzed using the Statistical Package for the Social Sciences software (SPSS) and Microsoft Excel. Descriptive statistics were used to describe participant demographics and anthropometric measurements. A repeated measures analysis of variance (RMANOVA) was conducted to evaluate the null hypothesis that there is no inflammatory response difference between the two dietary conditions; and it was also used to examine the effect of each dietary condition on appetite sensations. Area under the curve (AUC) was calculated for IL-1 β concentrations for each participant and a paired t-test was conducted to determine if there were significant differences between each condition. A paired t-test was also used to compare daily energy intakes and mean appetite ratings for each condition. Bootstrapping was used to find the standard error of the median energy intake values. Criterion for statistical significance was $P < 0.05$.

Results

Participants

Thirteen AI/AN and non-AI/AN participants, 18-55 years of age (male = 3, female = 10), enrolled in the study. Participants ranged from 19 to 53 years of age, with the mean age of 32 ± 10.37 years of age. The mean BMI for the group was 28.69 ± 5.0

kg/m². None were smokers, pregnant, or had a history of diabetes or any other inflammatory illness. Participant anthropometric and demographic characteristics are shown in Table 3.2.

Table 3.2 Participant Characteristics

Variables	Males (n=3)	Female (n=10)
	Mean ($\pm$ SD)	Mean ($\pm$ SD)
Age (years)	37.6 $\pm$ 10.01	50.7 $\pm$ 26.7
Height (cm)	184.99 $\pm$ 12.73	167.51 $\pm$ 10.08
Body Mass (kg)	104.6 $\pm$ 13.76	78.49 $\pm$ 15.91
Body Fat %	34.18 $\pm$ 3.52	35.92 $\pm$ 8.29
Waist Circumference (cm)	110.92 $\pm$ 8.92	89.02 $\pm$ 8.92
BMI (kg/m ²)	27.79 $\pm$ 4.91	28.78 $\pm$ 5.84

Inflammatory Biomarkers

Mean IL-1 β measurements for each dietary condition are shown in Figure 3.3. The salivary IL-6 concentration did not reach detection level in the majority of participant saliva samples, thus IL-6 cytokine data was excluded. Salivary IL-1 β cytokines were detected in all participants' saliva samples. There were no significant differences in IL-1 β concentrations between the two conditions found by RMANOVA ($p = 0.591$). There were also no significant differences in AUC calculations between the two dietary conditions found by paired t-test ($p = 0.358$). Thus, there was no significant evidence to reject the primary null hypothesis that says that there is no difference in IL-1 β measurements between the dietary conditions.

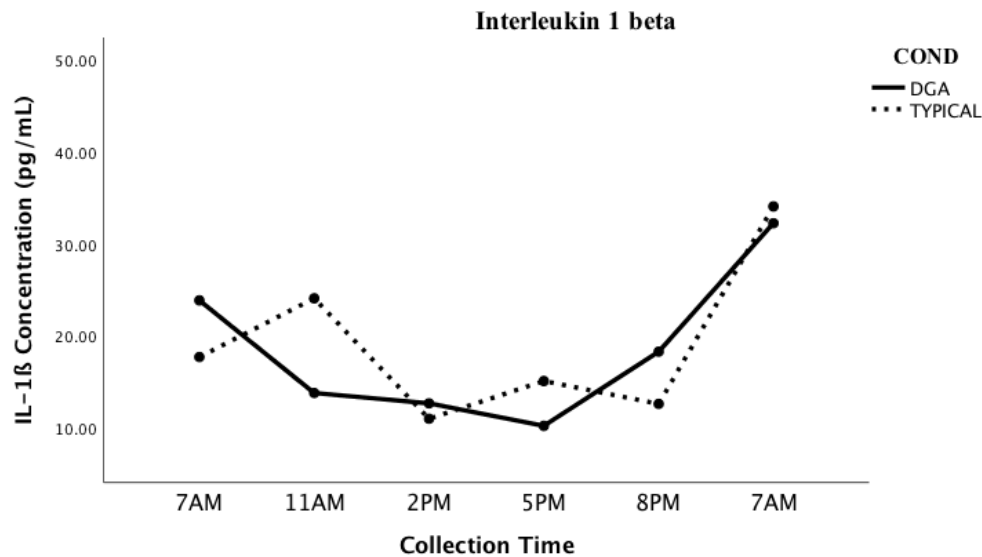


Figure 3.3. Values are the mean IL-1 β concentrations ($\pm$ 1 SE) for each of the six collection times. (DGA: solid line; TYPICAL: dashed line).

Analysis of Appetite

The response curves for each appetite sensation (hunger, satiety, fullness, desire to eat, and perspective consumption) are presented in Figure 3.4. The appetite profiles were similar after each dietary condition. Appetite scores were not significantly different between the two conditions in all sensations found by paired t-test ($P < 0.05$). Figure 3.5 presents the total mean appetite scores for each condition. The mean appetite sensation scores for each collection time were not significantly different between the two dietary conditions according to RMANOVA ($P < 0.05$). Therefore, there was no significant evidence to reject the null hypothesis that says that there is no difference in appetite between the dietary conditions.

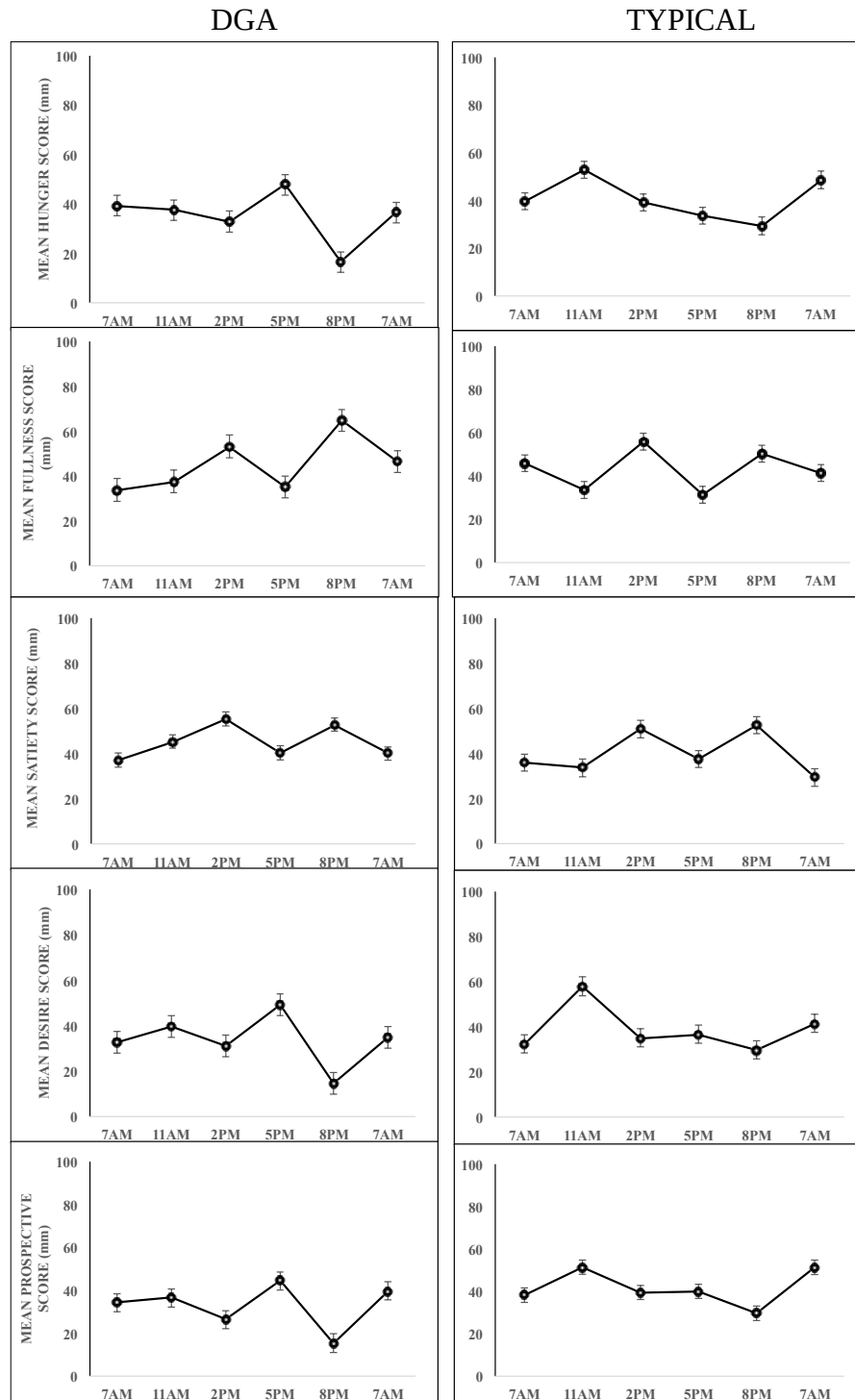


Figure 3.4. The values are the mean participant appetite scores for each sensation. The six survey completion times were completed over 24 hours (7AM, 11AM, 2PM, 5PM, 8PM, 7AM). The first value in each graph is the mean baseline score.

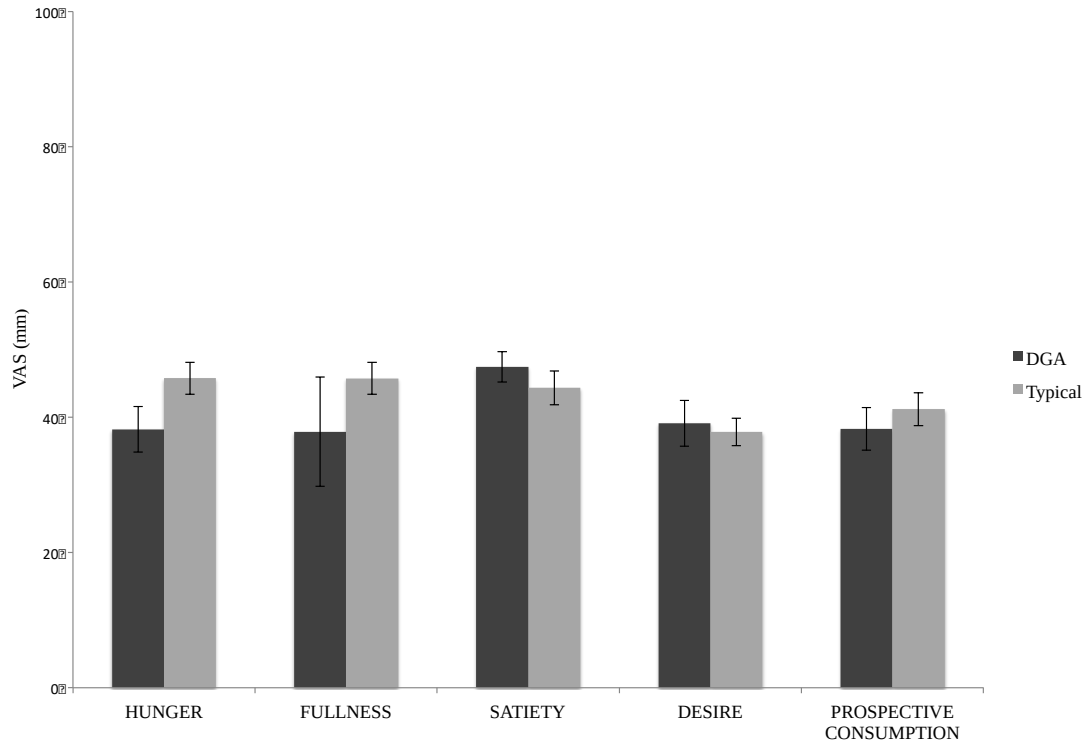


Figure 3.5. Total mean appetite scores for both conditions (DGA: dark bars; TYPICAL: light bars). The values are mean appetite sensation scores $\pm$ SE. $P < 0.05$ by paired t-test.

Total Daily Energy Consumption

Table 3.4 presents the mean and median daily energy intakes $\pm$ SE for the two conditions. Paired t-test showed significant differences in absolute daily energy (kcal/day) between the two conditions ($p = 0.006$). On average, the participants consumed 14.08% more kilocalories on the typical condition than on the DGA condition. The post-condition questionnaires revealed that three participants consumed food that was not provided to them during the DGA condition, and six participants during the

typical condition. The estimated kilocalories from those food items were added to the participant's total daily energy consumption.

Table 3.3 Total Daily Energy Intakes

Variables	Mean $\pm$ SE n = 13	Median $\pm$ SE n = 13
DGA_Daily Energy Intake (kilocalories/day)	1979.31 $\pm$ 129.75	1860 $\pm$ 125.11
Typical_Daily Energy Intake (kilocalories/day)	2303.73 $\pm$ 140.58**	2285 $\pm$ 157.37

P<0.01 compared to DGA condition.

Post-Condition Questionnaire

Participant responses to the post-condition questionnaire Likert scale (1 to 5) questions are presented in Figure 3.6. The participants' average response for whether or not they would eat the food from the typical condition on a normal day was 2.6, which is between disagreeing (2) and neither agreeing nor disagreeing (3). The average response for whether or not they would eat the food from the DGA condition on a normal day was 3.0. The participants' average response to describe their overall satisfaction with the food from the DGA condition was 3.6; and was 3.69 for the food from the typical condition. For the last Likert question, the participants agreed or strongly agreed (4 or 5) that they had enough food for the duration of both test days (DGA=4.5; typical=4.2). The responses for each of the three questions were not significantly different between the two conditions found by paired t-test (P<0.05).

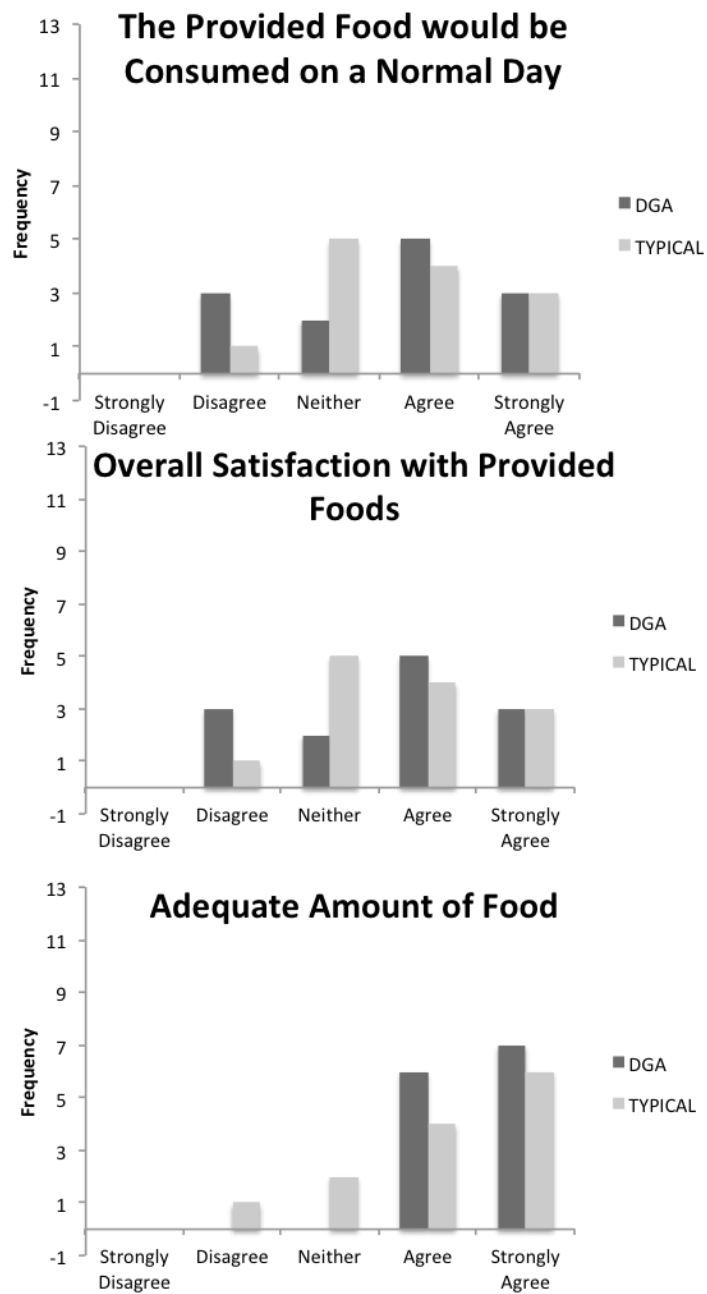


Figure 3.6. The graphs display the frequency of the participant responses to the post-condition questionnaire Likert scale questions (DGA: dark bars; Typical: light bars). There are no significant differences between the responses for each question found by paired t-test ($P < 0.05$).

Discussion

The objective of this study was to determine the influence of both a typical FDPIR diet and a FDPIR diet that met DGA standards on inflammation response, appetite, and daily energy intake. Understanding how food from the FDPIR influences these variables provides a broader view of how the FDPIR influences risk for nutrition-related diseases among AI/AN communities, including obesity and type 2 diabetes. Furthermore, understanding how aligning with the DGA is important with respect to nutrition related disease prevention and management among AI/AN communities. Awareness about these linkages will enable Indian Tribal Organizations (ITO), related state agencies, and FDPIR policymakers to better support the health status of many AI/AN communities that rely on FDPIR food.

The main finding of the study was mean daily energy intake was significantly higher after the typical condition compared to the DGA condition. On average, participants consumed over 14% more kilocalories upon completing the typical condition. Palatability, physical activity, energy availability, and nutrient density have been identified as factors that can affect energy intake. To account for palatability differences, the post-condition question measured how satisfied the participants were with the food. Satisfaction ratings were similar between the two dietary conditions. Similar satisfaction ratings for both diets reduced the likelihood that the energy intake differences were simply due to participants finding one dietary condition more palatable than the other. To control for physical activity, participants were instructed to avoid moderate to vigorous physical activities the day before and the day of each test day.

Participants' estimated total energy expenditure was used to determine the amount of energy provided to them during each condition. Balancing available kilocalories for each condition reduced the chance of energy intake differences due to dissimilarities in energy availability. Adequate energy was also confirmed by post-condition questionnaire responses. On average, participants agreed or highly agreed that they had enough food to last them throughout each test day. Hence, inadequate availability of kilocalories was eliminated as a potential explanation for daily energy intake differences.

We determined that the differences in energy intake between the dietary conditions were due to nutrient composition differences. A previous research study revealed that individuals are not sensitive to energy density and will eat the same volume of food when presented with diets that vary in energy and nutrient compositions. This same study also found that appetite (hunger and fullness) remained unchanged during ad libitum consumptions of diets that varied in energy density and macronutrient content. This is supported by the current study's second finding which was; appetite sensation ratings were not significantly different between the two dietary conditions. Similar to other studies, appetite sensation ratings remained the same among dietary conditions, despite differences in energy and nutrient densities of food.^{31, 60, 61} Previous studies have confirmed that balanced portion sizes result in no difference in appetite sensations, but does however, result in significant energy intake differences.^{38, 60, 64} We determined that appetite sensation ratings were similar between the two conditions because the participants consumed enough food during each condition to satisfy their appetites (hunger, fullness, satiety, desire to eat, and prospective consumption). Consequently, the

total daily energy intake was significantly higher post typical condition compared to post DGA condition. Further research investigating nutrient composition of each diet and energy intake is needed.

It was hypothesized that there would be a difference in inflammation response between a typical diet and a DGA diet. Based on prior research, it was expected that there would be a larger inflammation response after the typical diet, which provided a greater amount of low-quality carbohydrate foods than the dietary condition that met DGA standards.^{17, 21, 46} Low-quality carbohydrate foods can directly and indirectly cause inflammation.^{17, 38} Excess consumption of low-quality carbohydrate foods is associated with increased visceral adiposity, which is an important link to lower peripheral and hepatic insulin sensitivity, and elevated pro-inflammatory cytokine production.^{31, 39} Acutely, low-quality carbohydrates elevate blood glucose, which in turn increase production of reactive oxygen species (ROS), or oxidative stress, by the mitochondria of cells. Consequently, ROS stimulate the production of inflammatory cytokines, including IL-1 β .^{17, 21} This experiment did not confirm previous studies that have reported increased inflammation following consumption of low-quality carbohydrate food. Not detecting a significant difference in inflammation response between the two conditions may have been due to a small sample size. Increasing sample size may provide a better estimation of the mean measurements, thus allowing for more accurate comparisons of inflammation response between the two conditions.

There were limitations to the study design. First, the food items were purchased from a local grocery store, not from a FDPIR distribution site. Therefore, nutrient

composition was similar but not exactly the same as FDPIR foods. Future studies could include food directly from the FDPIR. Second, although participants agreed to adhere to several diet and physical activity restrictions 24 prior to and the day of each condition, the post-condition questionnaire revealed that multiple participants ate food outside of the food that was provided to them for each condition. The items were mostly small snack type foods. The estimated kilocalories from the added food were included in the total daily energy intake measurements. The effects of the added food on each variable were unknown and therefore are a limitation of the study. It was beyond the resources of the study to monitor the participants outside of the lab to ensure participant adherence. Third, participants were primarily non-AI/AN, which was a limitation of the study because FDPIR recipients are AI/AN and live on or near a reservation or in designated areas in Oklahoma. Thus, the majority of the participants lacked knowledge of, and exposure to FDPIR commodity foods prior to participating in the study. Lastly, available healthy food options vary among FDPIR distribution sites. The dietary conditions for this study were constructed using Exhibit O of the FNS Handbook 501, which contains all FDPIR food items. At the state and tribal level, FDPIR distribution site managers have the authority to decide which FDPIR food items to stock.⁶⁵ For example, one FDPIR site could offer all food items that are available through FDPIR, and another could opt out of ordering healthier foods, such as fresh produce. In that case, selecting foods that meet DGA standards may be limited. Therefore, the dietary conditions constructed for this study, particularly the condition that met DGA standards, may not be realistic for FDPIR recipients that are limited by the lack of healthy food options at their FDPIR distribution

site. Providing nutrition education to FDPIR distribution site managers and staff could aid in ensuring that all FDPIR fresh produce options are available at every site.

Despite the limitations to the study design, our finding of 14% higher caloric intake in a typical FDPIR diet versus a FDPIR diet that met DGA standards suggest that the FDPIR program has the potential to promote weight gain, which may increase risk for obesity and nutrition-related diseases among AI/AN communities, including type 2 diabetes. The results of the study indicate that it may be beneficial for the health status of many AI/AN communities for FDPIR distribution sites to align with DGA by providing an adequate amount of high-quality carbohydrate options, and by providing education on healthy diets to FDPIR recipients.

In conclusion, IL-1 β measurements and appetite sensation ratings were not significantly different between dietary conditions. Daily energy intake was significantly higher after the typical condition compared to the condition that met DGA standards. The combination of higher energy intake after the typical diet along with similar appetite sensations between both diets may be an indication that the participants adjusted their energy intake to satisfy feelings of hunger and satiety. Based on previous research, this may have been due to energy and nutrient density differences in food. The typical condition contained more low-quality carbohydrate foods and fell short on fresh fruit and vegetable options, which is similar to an average FDPIR monthly food package.¹⁵ The DGA condition had more fresh fruits and vegetables and limited added sugar. This is the first study that we know that has demonstrated the effects of both a typical FDPIR diet and a FDPIR diet that met DGA standards on inflammation, energy intake, and appetite

ratings. With respect to preventing and controlling type 2 diabetes and obesity in AI/AN communities, these are important findings because a typical FDPIR diet or a diet that has similar nutrient content may promote a positive energy balance, which can lead to weight gain and increased risk for developing or progressing nutrition-related diseases including type 2 diabetes. Conversely, a FDPIR diet that aligns with DGA reduces the risk for developing nutrition-related diseases by limiting FDPIR foods with added sugar and thus preventing excess energy intake. This study exposed the need to further research the long-term effects of food from the FDPIR on metabolic health. Expanding our knowledge on how FDPIR food affects inflammation, appetite, and energy intake is important for supporting chronic disease prevention and management in AI/AN communities, and enabling policymakers to make decisions regarding the FDPIR that supports the health status of AI/AN populations.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

The aim of this study was to determine if a typical FDPIR diet affects inflammation, appetite, and energy intake differently than a FDPIR diet that meets DGA standards. The primary hypothesis was that the inflammatory response would be significantly different between the typical FDPIR diet and the FDPIR diet that met DGA standards in all participants. Inflammatory responses were not significantly different between the two dietary conditions. Salivary interleukin 6 (IL-6) samples did not reach detection level and were omitted from this study. Salivary interleukin 1 beta (IL-1 β) responses showed no patterns or significant differences between the two dietary conditions. Thus, the primary hypothesis was not confirmed. Increasing sample size and reevaluating methods of collecting inflammatory measurements may be necessary to reveal mean differences in inflammatory response between a typical FDPIR diet and a FDPIR diet that meets DGA standards.

There were two secondary hypotheses. One secondary hypothesis was that the DGA FDPIR condition would satisfy all participants' appetites more than the typical FDPIR condition. Feelings of hunger, fullness, satiety, desire to eat, and prospective consumption were measured and all were not significantly different between the two conditions; therefore, this hypothesis was not confirmed. The other secondary hypothesis was that the typical condition would result in a greater daily energy intake than the DGA condition. The typical condition was found to have significantly higher daily energy

intakes than the DGA condition, and so this secondary hypothesis was accepted. These results support previous research that has demonstrated excess energy intake during a low-quality carbohydrate dietary condition.^{61, 64} The combination of no difference in appetite ratings between conditions along with higher daily energy intake measurements during the typical condition concurs with previous studies that have found that appetite sensations remain the same despite energy intake differences.⁶⁰

In conclusion, we measured inflammation response, appetite, and energy intake after two FDPIR commodity food conditions: 1) typical condition and 2) a DGA condition. The typical condition contained more low-quality carbohydrate foods and lacked fresh fruits and vegetables, which is similar to an average FDPIR monthly food package.¹⁵ The DGA condition met DGA standards and had more fresh fruit and vegetable options, along with limited added sugar compared to the typical FDPIR condition. The main finding of this study was that daily energy intake was 14% higher after the typical condition compared to the FDPIR condition that met DGA standards. IL-6 did not reach detection level in most participants' saliva samples and were not used to measure inflammatory response. IL-1 β measurements did not show any significant differences or evident patterns between the two conditions, and therefore, there was no significant evidence to reject the first null hypothesis that says that there was no significant difference in inflammation response between the dietary conditions. Appetite ratings were not significantly different between the two dietary conditions. The combination of higher energy intake after the typical condition along with no difference in appetite sensations between both conditions may be an indication that participants

adjusted energy intake to satisfy feelings of hunger and satiety. With respect to preventing and managing obesity and other nutrition-related diseases in American Indian and Alaskan Native (AI/AN) communities, this is an important finding because a typical FDPIR diet, or a diet that consists of the same nutrient composition, may have negative effects on health by promoting excess energy intake and weight gain, which can increase risk for developing chronic inflammatory conditions by increasing inflammation and decreasing insulin sensitivity. Conversely, our finding that daily energy intake measurements were significantly lower after a FDPIR diet that met DGA standards compared to a typical FDPIR diet is also an important finding with respect to preventing and controlling nutrition-related diseases in AI/AN communities. Aligning with DGA may have beneficial effects on health by preventing excess energy intake, thus preventing increased visceral adipose tissue and the consequent production and accumulation of pro-inflammatory molecules and decreased insulin sensitivity. The results of this study support research studies that have exposed the nutritional shortcomings of the FDPIR.^{15, 19, 23} To ensure clear understanding of how FDPIR foods impact the health status of AI/AN communities that rely on the FDPIR, further research on the long-term effects of FDPIR foods on inflammation, appetite, and energy intake should be conducted.

REFERENCES CITED

1. Prevention CfDca. Healthy People 2010 Final Review2016.
2. Prevention CfDca. National Diabetes Statistics Report2017.
3. Berryhill K, Hale J, Chase B, Clark L, He J, Daley CM. Food Security and Diet Among American Indians in the Midwest. *J Community Health*. 2018.
4. Services IH. Disparities2016.
5. Satterfield D. Health promotion and diabetes prevention in American Indian and Alaska Native communities—Traditional Foods Project, 2008–2014. *MMWR supplements*. 2016;65.
6. Health HaHSOoM. Chronic Liver Disease and American Indian and Alaskan Natives2016.
7. Kouvari M, Notara V, Kalogeropoulos N, Panagiotakos DB. Diabetes mellitus associated with processed and unprocessed red meat: an overview. *International Journal of Food Sciences and Nutrition*. 2016;67:735-743.
8. Grossmann V, Schmitt VH, Zeller T, et al. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. *Diabetes Care*. 2015;dc143008.
9. Blundell J, De Graaf C, Hulshof T, et al. Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews*. 2010;11:251-270.
10. Blue Bird Jernigan V, Salvatore AL, Styne DM, Winkleby M. Addressing food insecurity in a Native American reservation using community-based participatory research. *Health Education Research*. 2011;27:645-655.
11. Agriculture USDo. Food Distribution Program on Indian Reservations (FDPIR)2016.
12. Jones-Smith JC, Karter AJ, Warton EM, et al. Obesity and the food environment: income and ethnicity differences among people with diabetes: the Diabetes Study of Northern California (DISTANCE). *Diabetes Care*. 2013;36:2697-2705.
13. Laraia BA. Food insecurity and chronic disease. *Adv Nutr*. 2013;4:203-212.
14. Laraia B, Epel E, Siega-Riz AM. Food insecurity with past experience of restrained eating is a recipe for increased gestational weight gain. *Appetite*. 2013;65:178-184.

15. Byker Shanks C, Smith T, Ahmed S, Hunts H. Assessing foods offered in the Food Distribution Program on Indian Reservations (FDPIR) using the Healthy Eating Index 2010. *Public Health Nutr.* 2016;19:1315-1326.
16. Committee DGA. Report of the dietary guidelines advisory committee on the dietary guidelines for Americans 2010, to the Secretary of Agriculture and the Secretary of Health and Human Services 2010.
17. Miles MP. Carbohydrates for physical activity: a strategy to avoid undesirable health consequences. *American Journal of Lifestyle Medicine.* 2012;6:121-132.
18. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation.* 2005;112:2735-2752.
19. Pindus N. *Study of the Food Distribution Program on Indian Reservations (FDPIR) Final Report*: United States Department of Agriculture, Food and Nutrition Service, Office of Policy Support; 2016.
20. L. Dillinger SCJ, Martha J. Macri, Louis E. Grivetti, Teresa. Feast or famine? Supplemental food programs and their impacts on two American Indian communities in California. *International Journal of Food Sciences and Nutrition.* 1999;50:173-187.
21. Dandona P, Chaudhuri A, Ghanim H, Mohanty P. Proinflammatory effects of glucose and anti-inflammatory effect of insulin: relevance to cardiovascular disease. *The American journal of cardiology.* 2007;99:15-26.
22. Services FaN. About FNS 2015.
23. Finegold K, Pindus N, Levy D, Tannehill T. Tribal Food Assistance: A Comparison of the Food Distribution Program on Indian Reservations (FDPIR) and the Supplemental Nutrition Assistance Program (SNAP). *Washington, DC: The Urban Institute and Support Services International, Inc. November.* 2009.
24. Agriculture USDo. Update on low-sodium canned vegetables 2012.
25. Health NIo. Developing the Healthy Eating Index 2017.
26. Whitney EN, Rolfes SR. *Understanding nutrition*: Cengage Learning; 2018.

27. Jenkins D, Wolever T, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American journal of clinical nutrition*. 1981;34:362-366.
28. Dolson L. Glycemic Index Food Lists2018.
29. Bulló M, Casas R, Portillo M, et al. Dietary glycemic index/load and peripheral adipokines and inflammatory markers in elderly subjects at high cardiovascular risk. *Nutrition, Metabolism and Cardiovascular Diseases*. 2013;23:443-450.
30. Association AD. Glycemic Index and Diabetes. 2018.
31. Drewnowski A. Energy density, palatability, and satiety: implications for weight control. *Nutr Rev*. 1998;56:347-353.
32. Björck I, Östman E, Kristensen M, et al. Cereal grains for nutrition and health benefits: Overview of results from in vitro, animal and human studies in the HEALTHGRAIN project. 2012;25:87-100.
33. Cioffi I, Ibrugger S, Bache J, et al. Effects on satiation, satiety and food intake of wholegrain and refined grain pasta. 2016;107:152-158.
34. Kristensen M, Jensen MG, Riboldi G, et al. Wholegrain vs. refined wheat bread and pasta. Effect on postprandial glycemia, appetite, and subsequent ad libitum energy intake in young healthy adults. 2010;54:163-169.
35. Agriculture USDo. FNS Handbook 501 for FDPIR2017.
36. Team MC. Glycemic Index Calculator2016.
37. Montignac. Search for Specific Glycemic Index2016.
38. Stanhope KLJCricls. Sugar consumption, metabolic disease and obesity: The state of the controversy. 2016;53:52-67.
39. Boden G. Obesity and free fatty acids. *Endocrinology and metabolism clinics of North America*. 2008;37:635-646.
40. Betteridge DJ. What is oxidative stress? *Metabolism*. 2000;49:3-8.
41. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *The lancet*. 1994;344:721-724.

42. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Molecular cell*. 2002;10:417-426.
43. Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood*. 1994;83:113-118.
44. McCarty M. Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. *Medical hypotheses*. 1999;52:465-477.
45. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *The Journal of Clinical Endocrinology & Metabolism*. 1997;82:4196-4200.
46. Miles MP, Horrigan LC, Jay SE, Brown KM, Porter JW, Steward AN. Concentric and eccentric exercise, glycemic responses to a postexercise meal, and inflammation in women with high versus low waist circumference. *Appl Physiol Nutr Metab*. 2016;41:1262-1270.
47. Abete I, Goyenechea E, Zulet M, Martinez J. Obesity and metabolic syndrome: potential benefit from specific nutritional components. *Nutrition, metabolism and cardiovascular diseases*. 2011;21:B1-B15.
48. Garg A. Adipose tissue dysfunction in obesity and lipodystrophy. *Clinical cornerstone*. 2006;8:S7-S13.
49. Gao D, Madi M, Ding C, et al. Interleukin-1 β mediates macrophage-induced impairment of insulin signaling in human primary adipocytes. *American Journal of Physiology-Endocrinology and Metabolism*. 2014;307:E289-E304.
50. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007;56:1010-1013.
51. Wakil SJ. Mechanism of fatty acid synthesis. *Journal of Lipid Research*. 1961;2:1-24.
52. Marques-Lopes I, Ansorena D, Astiasaran I, Forga L, Martínez JA. Postprandial de novo lipogenesis and metabolic changes induced by a high-carbohydrate, low-

fat meal in lean and overweight men—. *The American Journal of Clinical Nutrition*. 2001;73:253-261.

53. Horton JD, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proceedings of the National Academy of Sciences*. 1998;95:5987-5992.
54. Yamamoto T, Shimano H, Inoue N, et al. Protein kinase A suppresses sterol regulatory element-binding protein-1C expression via phosphorylation of liver X receptor in the liver. *Journal of Biological Chemistry*. 2007;282:11687-11695.
55. Irimia JM, Meyer CM, Segvich DM, et al. Lack of liver glycogen causes hepatic insulin resistance and steatosis in mice. *Journal of Biological Chemistry*. 2017;jbc. M117. 786525.
56. Mason S, Santaularia N, Berge J, Larson N, Neumark-Sztainer DJPm. Is the childhood home food environment a confounder of the association between child maltreatment exposure and adult body mass index? 2018;110:86-92.
57. Donath MY. Inflammation as a sensor of metabolic stress in obesity and type 2 diabetes: Oxford University Press; 2011.
58. Salimetrics. Salivary Interleukin-6. 2018.
59. Salimetrics. Salivary Interleukin 1 beta. 2018.
60. Ello-Martin JA, Ledikwe JH, Rolls BJ. The influence of food portion size and energy density on energy intake: implications for weight management. *The American Journal of Clinical Nutrition*. 2005;82:236S-241S.
61. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. Energy density of foods affects energy intake in normal-weight women. *Am J Clin Nutr*. 1998;67:412-420.
62. Salimetrics. Collection Method: Salivabio Oral Swab (SOS)2018.
63. Jamison RN, Gracely RH, Raymond SA, et al. Comparative study of electronic vs. paper VAS ratings: a randomized, crossover trial using healthy volunteers. *Pain*. 2002;99:341-347.
64. Sclafani A. Carbohydrate taste, appetite, and obesity: An overview. *Neuroscience & Biobehavioral Reviews*. 1987;11:131-153.

- 65.** Services USDoAFaN. Food Distribution Program on Indian Reservations Regional Vendor Pilot Assessment. 2016.

APPENDICES

APPENDIX A

INSTITUTIONAL REVIEW BOARD CONSENT FORM

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH AT MONTANA
STATE UNIVERSITY

Study Title: Comparing the inflammatory effects of two Food Distribution Program on Indian Reservations (FDPPIR) commodity food diets

Investigators: Melinda Smith, MS Exercise and Nutrition Sciences Student, Mary Miles, Ph.D., Nicole Doherty, MS Exercise and Nutrition Sciences Student
Dept. of Health and Human Development
Herrick Hall, Montana State University
Bozeman, MT 59717-3540
Phone: (406) 994-5001
melinda.smith2@student.montana.edu; mmiles@montana.edu; ndoherty10@gmail.com

Summary:

You are being asked to volunteer as a participant in a research study that will measure the inflammatory response of two diets comprised of food from or similar to the commodity foods offered in the United States Department of Agriculture (USDA) Food Distribution Program on Indian Reservations (FDPPIR). Inflammation plays an important role in our body's immune response. Certain types of food can increase inflammation in the body. Prolonged inflammation in the body is linked to the development of chronic diseases, such as type II diabetes, which is prevalent in Native American communities that receive FDPPIR benefits. Two different dietary strategies with the same available overall number of calories but different sources of calories will be compared. Saliva samples will be used for measurement of substances that are indicators of inflammation. The samples will not be used to collect DNA.

Purpose:

The purpose of this study is to determine the inflammatory effects of two different diets. Specifically, we would like to know if there is a difference in inflammatory response between the two diets? Information from this study will help us to better understand the inflammatory effects of foods in the FDPPIR. In addition, this study has the potential to support the need to improve overall nutrient quality of commodity foods in the FDPPIR program.

Participants in the study:

You have been asked to participate in this study because you meet the following criteria: 18-55 years of age, with a body mass index 25 to 35 kg/m² and a waist circumference greater than 35 inches for women and 40 inches for men. You may not be a participant if you do not meet eligibility requirements of our screening questionnaire because of health history, symptoms, issues or risks, if you have an allergy to wheat, gluten, dairy, onions or peanuts, if you are taking blood pressure, cholesterol lowering or anti-inflammatory medications, if you have hypertension (high blood pressure), diabetes or heart disease, or if

APPROVED
MSU IRB
01/22/2018
Date approved

Informed Consent Document (continued)

you have other health concerns you feel may interfere with the study or that make it difficult for you to participate.

Procedures:

Participation is voluntary. If you agree to participate in this study, you will be asked to complete 5 visits to complete the procedures of the investigation. The first visit will be to the Nutrition Research Laboratory for an initial visit (Visit 1), then twice for each of the experimental conditions to be compared. In total, there will be 2 tests in which you will complete 5 self-collected saliva samples (a total of 10 samples over the course of the entire study). Collection kits (collection tubes and cotton swabs) and saliva collection directions will be provided. Completion of the study will take approximately 2 to 4 weeks, depending on scheduling of the visits.

Visit 1: You will need to refrain from eating or exercising for 2 hours prior to this visit, which will take approximately 1 to 1.5 hours and include the following activities:

- 1) **Informed consent.** Read and provide written informed consent (this form). We also will provide you with a copy of this form and discuss it with you prior to test day 1.
- 2) **Self-Collection of Saliva and Questionnaires.** Read and receive directions for collecting saliva properly. Read and receive questionnaires and directions for when to complete each questionnaire.
- 3) **Body size measurements.** The researcher will take baseline measurements of height, and circumferences of your waist and hips. You will remain clothed during these measurements, however, you will be asked to remove extra clothing items such as coats or sweatshirts.
- 4) **Body composition (%fat and %lean).** You will change into either a swimsuit or other tight fitting clothing such as compression shorts and a sports bra or tank top. You will stand on a scale, and then sit in an egg-shaped chamber for measurement of the volume of your body. To do this, we close the chamber door. There is a window for you to see the investigator at all times. The door will be closed for about one minute while the first measurement is taken and then opened again. The measurement will be repeated one or two times. This will also give us your estimated energy needs at rest. To do this test, you will be inside the chamber with the door closed and breathe in and out according to instructions for about one minute. This measurement may be repeated. After completing the measure in the egg-shaped chamber, you will stand on an additional scale that will provide a second measurement of percent fat and lean mass.

Condition A

Visit 2 will occur at least 4 days after visit 1, and you will be asked to not consume alcohol 24 h prior to or on the day of the exercise. We will ask you to write down what you eat and drink the day before you come to the lab so that you can replicate this for the next condition. You should drink plenty of water so that you are well hydrated throughout the study. This visit will take approximately 1 hour to collect your first saliva sample and for you to finish your meal.

Informed Consent Document (continued)

You will be given breakfast while you are in the laboratory. We also will provide you with food to take with you and consume during the remainder of the day. With the exception of water, you are asked not to consume any food or drinks other than what we give you for the remainder of the day. We ask that you consume as much food as you desire, but also to bring any food or beverage that you were not able to consume with you when you come to the lab the next morning. You are not obligated to eat all of the food. The diets will be slightly different between the two conditions. One condition will consist of foods such as a chicken wrap, fresh fruit, a fruit and nut mix, meat and vegetables, and water. The other condition will consist of macaroni and cheese, applesauce, meat, vegetables, spaghetti, and water. The food will be prepared and served in adherence to ServSafe Montana standards and policies. The amount of food will be based on the amount of food that fits your body size and composition (measured during visit 1).

With the randomized, counter-balanced, crossover design there is the possibility that condition A could happen before or after condition B.

Visit 3 will occur the following morning. Before consuming anything for that day, you will come to the Nutrition Research Laboratory at approximately 8:00 a.m. for the following procedures that will take about 20 minutes:

- 1) Delivery of uneaten foods and self-collected saliva samples.
- 2) Final saliva collection for that condition.

Condition B (visits 4 and 5): At least 1 week after the completion of condition A, you will repeat the procedures of visits 2 and 3.

If you volunteer to participate in this study, you will be asked to refrain from vigorous physical activity or any extracurricular physical activity you usually would not partake in for 24 hours before and during the protocol.

Risks:

No risks.

Benefits: You may gain some benefits by participating in this study, such as:

- 1) You will receive information about your estimated resting metabolic rate and current measurements such as waist circumference, weight, height, and body composition (fat% and lean%).

No other benefits are promised to you.

Compensation: You will receive a total of \$70.00 upon completion of your testing. You may withdraw from the study at any time. If you choose not to complete a condition, then the amount of money paid to

Informed Consent Document (continued)

you will be prorated depending on how much of the condition is completed. The breakdown of the cash incentive for each visit are as following: visit 1 = \$10.00, visit 2 = \$20.00, visit 3 = \$10.00 (Condition A – visits 2 and 3), visit 4 = \$20.00, and visit 5 = \$10.00 (Condition B – visit 4 and 5).

Freedom of Consent: You have the right to withdraw from participating in the study at any time with a no questions asked policy. You may withdraw in writing, over the phone (to Melinda Smith at (406-994-5001), or in person. If you withdraw, you will not lose any benefits you incurred up to the time of withdrawal. Your participation in this study is completely voluntary.

Funding: IDeA Network Biomedical Research Excellence (INBRE) funds this study.

Please ask any questions: You are encouraged by the researcher to ask any and all questions you may have, as well as addressing any concerns about the study. The researcher will answer your questions as fully and as accurately as possible. Your peace of mind and comfort in the study is of utmost importance to the researcher.

Confidentiality: All data and information received from you for this study will be kept completely confidential. You will be given a subject identification number that will be used to describe all data. This data will be kept locked in a file cabinet in the Nutrition Research Laboratory. This information could be published in scientific and/or medical journals, but your identity will remain confidential. If you withdraw from the study at any time, all of your information will be deleted from the study records, and you will not be contacted again regarding the study. There are absolutely no penalties for withdrawing.

In the unlikely event of injury to you in the Nutrition Research Laboratory due to participation in this study, medical treatments such as first aid and help getting to adequate health care providers (such as transportation to Bozeman Deaconess Hospital) will be provided, however, there is no compensation for any of this provided by Montana State University. You can access further information regarding this policy and treatment by contacting Mary Miles 406-994-6678, or email mmiles@montana.edu.

Any other question you may have regarding your rights as a participant may be answered by the chairman of Human Subjects Committee, Mark Quinn. You can reach him at 406-994-4707.

Informed Consent Document (continued)

STATEMENT OF AUTHORIZATION

Study Title: Comparing the inflammatory effects of two Food Distribution of Indian Reservation diets.

AUTHORIZATION: By signing this document, I acknowledge that I have read the above and I understand the discomforts, inconvenience, and risks associated with my participation in this study. I, _____ (name of subject), agree to participate in this study. I fully understand that I may later refuse participation at any time, and may withdraw from the study at that time. I have been given a copy of this consent form for my own records.

Signed: _____ Date: _____

Investigator: _____ Date: _____

APPROVED
MSU IRB
01/22/2018
Date approved

APPENDIX B

SCREENING QUESTIONNAIRE

Participant I.D. _____ Date: _____

Comparing Inflammatory Effects of Two Food Distribution on Indian Reservations (FDPIR) Diets

Screening Questionnaire

Instructions

Answer questions as they relate to you. Check the box that is most applicable to you and fill in the blank if applicable. Your information will remain confidential and will not be used for any study or publication that is not discussed or disclosed in the consent form.

About You

1. Your Age

_____ years old. D.O.B. ____/____/____

2. Your Gender

(Select only one.)

- ☐ Female
☐ Male
☐ Other

3. Are you pregnant?

- ☐ Yes
☐ No

4. Do you smoke?

- ☐ Yes
☐ No

5. Are you taking any antiinflammatory medications (including over the counter) and/or lipid-lowering medications? If yes to either, please list your medications

- ☐ Yes
☐ No

1. _____

2. _____

3. _____

Participant I.D. _____ Date: _____

6. Do you have any food allergies? If yes, please list food allergies.

☐ Yes

☐ No

1. _____

2. _____

3. _____

7. Do you have any inflammatory condition such as diabetes, heart disease, kidney problems (excluding kidney stones)? If yes, please list conditions.

☐ Yes

☐ No

1. _____

2. _____

3. _____

APPENDIX C

VISUAL ANALOGUE SCALE APPETITE QUESTIONNAIRE

Subject ID: _____

Date: _____

Time: _____

Self-Reported Appetite Questionnaire

Please complete questionnaire after each self-saliva collection

SCALE	QUESTION	For each question, please indicate how you feel by marking a line on the scales below.	
Hunger	How hungry are you?	Not at all	Extremely (As hungry as I have ever been)
			
Fullness	How full are you?	Not at all	Extremely (As full as I have ever been)
			
Satiety	How satiated are you?	Not at all	Extremely
			
Desire	How strong is your desire to eat?	Very weak/ Extremely low	Very strong / Extremely high
			
Prospective Consumption (Quantity)	How much do you think you could (or would want to) eat right now?	Nothing at all	Very large amount
			

APPENDIX D

POST-CONDITION QUESTIONNAIRE

Subject I.D. _____ Date: _____

Post – Condition Questionnaire**Instructions**

Answer questions as they relate to you. Check the box that is most applicable to you and fill in the blank if applicable.

		Strongly disagree	Disagree	Neither	Agree	Strongly agree
		1	2	3	4	5
1	I would eat the provided food on a normal day.					
2	I had enough food to last me through the day.					
3	Overall I am satisfied with the food that was provided.					
4	Did you eat or drink anything outside of the food and water that was provided to you? If yes, please list food/beverage items that you consumed outside of the provided foods.	☐ Yes ☐ No 1. _____ 2. _____ 3. _____				
5	Did you not eat a food item that was provided to you? If yes, please list the food/s that you did not eat.	☐ Yes ☐ No 1. _____ 2. _____ 3. _____				

APPENDIX E

SELF-COLLECTION OF SALIVA SAMPLES

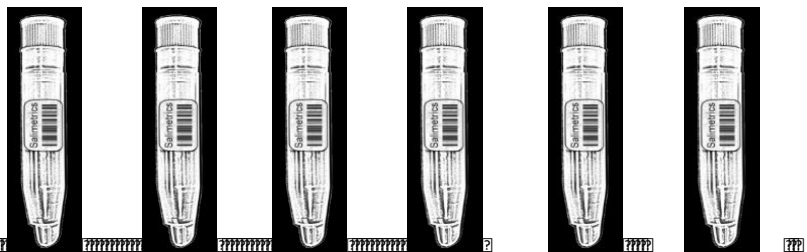
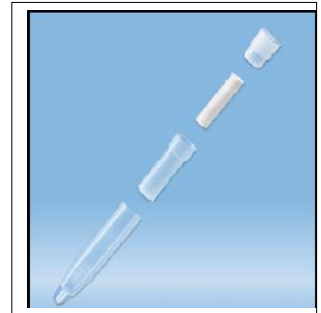
How to Self-Collect Saliva Samples

The SalivaBio swabs are made from non-toxic, inert polymer. To reduce risk of choking and for accurate measurements, please follow the directions and complete each collection at the indicated times.

INSTRUCTIONS:

Wait 10 minutes after eating or drinking before collecting your saliva. Tubes are labeled. Use the correct tube for each collection.

- 1.) Rinse mouth with water immediately before collection.
Do not move swab around in your mouth.
- 2.) Remove the swab from the packaging and place under your tongue.
- 3.) Keep swab in the mouth for 1-2 minutes to ensure adequate saturation.
- 4.) Place the saturated swab into the swab storage portion of the tube (upper portion of the tube) and push the cap on tight. **Repeat** (two swabs/sample).
- 5.) Immediately after collection, freeze samples at or below -4°F (-20°C). If freezing is not possible, refrigerate immediately at 39°F (4°C) until you can freeze it at -4°F (most domestic refrigerators and freezers meet these temperatures).



	#1	#2	#3	#4	#5	#6
Collection Times	7AM	11AM	2PM	5PM	8PM	7AM
	(In MSU Lab)					(In MSU Lab)

CAUTION:

- Use only as directed.
- Store out of reach of children.