Assessing the Linkages Between a Processing-Based Dietary Index and Nutrient Intakes, Obesity, and the Gut Microbiome Among Bolivian Women in a Region Undergoing the Nutrition Transition

by

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Dedication

This dissertation is dedicated to Ilona –
for your immeasurable support, encouragement, and guidance.

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Table of Contents

List of Tables

List of Appendices

Abstract

Low- and middle-income countries (LMICs) are undergoing a rapid dietary transition from traditional foods to highly processed Western diets. Conventional analyses of dietary patterns do not account for the level of processing of foods in the diet, yet methods of food processing and ingredients introduced into foods to make them more palatable, nutrient-dense, and/or shelf-stable have been hypothesized to increase individuals' risk for obesity, in part through disruptions to the gut microbiome.

This dissertation implemented the use of two food processing-based indices, the Processed Food Dietary Index (PFDI) and NOVA, to characterize the level of processing of diets among women recruited from the baseline participants of a three-year longitudinal cohort study in Montero, Bolivia, a country experiencing a rapid dietary transition, to further examine the associations between the extent of processing of diets, obesity, and gut microbiome composition. With the linkages between the nutrition transition in LMICs and obesity best established in women, we collected three 24-hr dietary recalls, anthropometric measurements, and two fecal samples from 160 women of reproductive age (18-49 years) based on the extent of processing in the diet using data from the aforementioned baseline assessment data of the longitudinal study.

The first aim utilized both the PFDI and NOVA as a single measure of diet quality to examine and compare relationships between nutrient intakes and the dietary share of processing level groups; it also examined the distribution of nutrient means across quintiles of the dietary share of ultra-processed foods and drinks (UPFDs) and unprocessed/minimally processed foods. We found statistically significant linear relationships between various nutrient means and PFDI and NOVA scores, although not always in the hypothesized directions. There were also a number of nutrients typically associated with processing that did not exhibit statistically significant linear relationships with processing levels (i.e., saturated fat, *trans* fat, sodium).

The second aim examined the association between the processing level of the diet and measures of obesity (i.e., body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR)) using the PFDI and NOVA as a single measure of overall dietary quality. Although daily caloric intake increased across quintiles of PFDI and NOVA scores, there were no differences found in BMI, WC, or WHR between quintile pairs. Neither processing level of the diet nor the consumption of UPFDs were associated with obesity.

The third aim evaluated the association between the processing level of the diet, obesity, and the gut microbiome. We found that the processing level of the diet, as measured by the PFDI, may influence the *Firmicutes/Bacteroidetes (F/B)* ratio and that the proportion of UPFD intake in the diet is associated with the diversity of microbiota present in the gut. We also observed that *F/B* ratios were not different between obese and lean individuals; however, obese individuals had a less diverse microbiome than those who were lean.

These studies provide information regarding the use of the PFDI and NOVA classification systems in future studies assessing the impact of food processing on human health, as well as novel insight into the relationships between the level of processing in the diet, obesity, and the composition of the gut microbiome.

Chapter 1

Introduction

Dietary pattern analysis

In examining the relationship between diet and the risk of chronic disease, the field of nutritional epidemiology has shifted from assessing diet from a single nutrient approach to one that captures the diet in its entirety through dietary pattern analysis. Dietary patterns can be empirically derived from dietary data *a posteriori*, utilizing exploratory data analysis methods such as factor analysis, cluster analysis, and reduced rank regression. Alternatively, dietary patterns can be theoretically derived *a priori* based on measures of diet quality. This dissertation will focus on *a priori* methods of diet analysis.

A priori dietary pattern analysis involves utilizing pre-defined indices in which quantitative estimates of nutrients, foods, and/or food groups derived from diet records/diet history, food frequency questionnaires, or 24-hour dietary recalls are scored according to a metric of diet quality, such as dietary diversity, government nutrient and food intake recommendations, or favored dietary patterns (e.g. Mediterranean dietary pattern). It is important to note, however, that it is the investigator that defines "diet quality" and these definitions change over time (1). For example, attributes of a low-fat diet, which were emphasized in the 1980s and 1990s, would possibly be defined and scored differently on indices utilized during that time period than they would be on indices developed today given advances in our understanding of the relationship between fat intake and cardiometabolic outcomes (2).

The most well-known and widely applied dietary index is perhaps the Healthy Eating Index (HEI) developed by the U.S. Department of Agriculture in 1995 (3). The HEI is a summary measure of diet quality based on both food groups and nutrients. Briefly, the HEI assesses: 1) conformity to the serving recommendations of the USDA Food Guide Pyramid (4) for grains, vegetables, fruits, milk, and meat; 2) the Dietary Guidelines for Americans (5) nutrient

recommendations for total fat, saturated fat, cholesterol, and sodium; and, 3) dietary variety (3). Additional examples of indices previously utilized to measure dietary quality include: the Healthy Diet Indicator (HDI) (6), Healthy Food Index (HFI) (7), Recommended Food Score (RFS) (8), Diet Quality Index (DQI) (9), Diet Quality Score (DQS) (10), and Mediterranean Diet Score (MDS) (11). A number of these indices, including the HEI, have been revised since their initial inception and have alternative versions that have been employed in more recent years as the paradigm of "diet quality" has shifted. For example, adapted versions of the HEI include the Alternative Healthy Eating Index (AHEI) (12), the Healthy Eating Index from Food Frequency Score (HEI-f) (13), the HEI-2005 (14), the HEI-2010 (15), and the AHEI-2010 (16).

From the time the analysis of dietary patterns first began in the 1940s through the mid-1990s, *a priori* derived dietary patterns were primarily used to assess nutritional adequacy (1), but they are now frequently utilized to examine associations between the human diet and health outcomes (17), including obesity (18,19). Two recent analyses examined the association between *a posteriori* and *a priori* dietary patterns and the risk of obesity in adults (18,19). A meta-analysis of 39 *a posteriori* studies found a significant inverse association between "healthy/prudent dietary patterns" and obesity; however, no significant association was found between "unhealthy/Western dietary patterns" and obesity (18). A systematic review of 34 *a priori* studies found mixed results regarding the association of dietary patterns and obesity (19). However, among studies that utilized the HEI or its alternate versions (n=13), 10 demonstrated a significant inverse relationship with obesity; this correlation was stronger in males than females (19).

Recently, the examination of dietary patterns has focused on the role of food processing – the alteration of food from the point of harvest (or slaughter) to increase its shelf-stability, nutrient profile, palatability, and/or digestibility. The interest in food processing emerged as evidence concerning the relationship between the elements of food processing (e.g., sodium, *trans* fats, added sugars, refined grains, processed meats, food additives) and health outcomes has increased in recent years, as well as recognition regarding the speed at which global food systems and supplies are being transformed (20). A number of food classification indices based on processing have been developed and applied in various contexts around the world (21), though the extent of

examining industrial food processing in dietary patterns has been carried out almost exclusively using the NOVA classification index (22) **(Appendix A)**. Investigators have utilized the NOVA to classify and score foods to examine *a priori* dietary patterns based on the extent of processing in the diet and various chronic disease outcomes. These studies have presented findings largely focused on the dietary share of ultra-processed foods and drinks (UPFDs) and their association with health-related outcomes.

To the best of our knowledge, there are no published studies that have calculated an average NOVA score as a single measure of overall diet quality based on processing. However, a number of studies have asserted a positive association between the purchase or consumption of UPFDs, the most extreme degree of processing, and obesity (23–29). A study conducted in the UK, however, did not find any such relationship between UPFDs and obesity (30).

As stated earlier, one of the limitations of *a priori* dietary pattern analysis is that the investigator predefines how diet quality is measured (1) and arbitrarily determines a scoring methodology and cut-off values (31). While dietary patterns defined *a priori* are generally based on current nutrition knowledge, the NOVA classification system is particularly novel in that there were no previously agreed upon "levels" or "degrees" of food processing in the scientific community upon which to discriminate between processing categories (32). Another limitation of *a priori* dietary pattern analysis is validation (1,31). Indices can be validated by assessing nutrient adequacy; biochemical, anthropometric, and/or clinical parameters of nutritional status; or health outcomes in relation to overall diet scores (1,31). In a review of 20 *a priori* diet quality indices that were utilized in 39 studies examining associations between overall diet scores with nutrient adequacy or health outcomes, the investigators found that although the indices were commonly associated with higher intakes of micronutrients, and lower risks of mortality and cardiovascular disease, the magnitudes of these effects were modest in most published studies, casting doubt on their validity (31).Therefore, indices based on food processing, such as NOVA, may accurately measure the extent to which individuals eat a range of processed foods, but this does not mean the index is a good predictor of morbidity or mortality associated with diet. Many issues that traditional diet quality indices face in regards to their construction (i.e., defining quality, cut-off points, scoring methodology) extend to NOVA as well. Investigators utilizing NOVA, therefore,

must remain cautious in regards to how NOVA is used and how associations are interpreted. Despite widespread use, there has been no critique of the NOVA food processing categories, the rationale behind the categories, or whether it accurately measures the processing level of the diet.

The nutrition transition, obesity, and food processing

The nutrition transition, characterized by rapid shifts in dietary and physical activity patterns (33) due to increasing national income and urbanization (34), has been implicated in the rising prevalence of obesity observed in low- and middle-income countries (LMICs) (35). The principal defining characteristic of these dietary shifts has been the rapid transformation of traditional dietary patterns to Western dietary patterns marked by an abundance of highly processed foods and beverages (33).

This nutrition transition has been especially prominent in Latin America (36). In Bolivia, a lower middle-income country in South America and the setting of this dissertation, this nutrition transition began in the mid-1990's, later than other countries in the region (37). Bolivia has experienced tremendous economic growth and urbanization in the last 25 years. The economy has grown from a GDP of 6.7 billion in 1995 to 33.0 billion USD in 2015 (38). The urban population proportion has similarly grown rapidly, nearly doubling between 1992 and 2009 from 59 to 68% with rural-to-urban migration contributing to over a third of that increase (39). Bolivia is expected to further urbanize with 79% of its population living in urban areas by 2050 (40).

Correspondingly, the prevalence of obesity more than doubled from 7.8 to 17.4% between 1994 and 2008 (41,42). By 2013, this prevalence increased to 24.5% (43), more than tripling in a span of 19 years. The most recent Demographic and Health Survey (DHS) data revealed that overweight and obesity prevalence is highest among women living in the Bolivian lowlands (i.e., Department of Santa Cruz), the wealthiest part of the country, and is more prevalent in urban (51.4%) than rural (46.4%) areas (44) (although also notably high for what might be expected in rural areas). The overall overweight and obesity prevalence in Bolivia is 62.0% (43).

The pathophysiology of obesity is complex and multifactorial as the state of positive energy balance resulting in excess body weight is a result of the interaction between genetic, hereditary

(e.g., parental diet, lifestyle and other exposures via epigenetic mechanisms), environmental (e.g., the "built" environment, viruses, the gut microbiome, social networks), and socioeconomic factors (e.g., income, education), as well as individual behaviors (e.g., diet, physical activity, sleep) (45). **Figure 1.1** illustrates the mechanisms in which one factor, the processing level of the diet, influences obesity.

"Food processing" includes one or more physical and/or chemical operations in which a plant or animal-based food is modified from its original state to change or preserve it (46); it also encompasses the combination of plant and/or animal derivatives (i.e., ingredients), possibly with artificial ingredients, to create new food and beverage products. Even the most minimal forms of food processing/preparation (e.g., applying thermal processes, removing an edible peel, grinding nuts or seeds) alter the nutrient content (i.e., vitamins, minerals, phytochemicals, dietary fiber) of raw food commodities, whereas moderate and higher levels of processing (e.g., addition or combination of culinary processed ingredients, refinement of grains, the use of multiple methods of processing (e.g., cheesemaking)) alter the nutrient content as well as add energy density and constituents that should be limited in the diet (i.e., added sugars, saturated fat, sodium) (47). UPFDs, the most extreme form of processing, are essentially industrial concoctions of numerous ingredients that are not typically consumed on their own or even derived from raw food commodities (e.g., fruit drinks, pre-packaged cookies, reconstituted meat products, candies, etc.), often adding substances to the diet (e.g., food extracts/derivatives, dyes, stabilizers, artificial flavors, artificial sweeteners, emulsifiers, humectants) that would otherwise not be consumed (48).

The primary determinant of the global obesity epidemic has been the transformation of dietary patterns (33,49); specifically, the displacement of lower energy density foods (i.e., calories per unit weight (kJ/g)) with those of higher energy density **(Figure 1.1 (Pathway A))**. Foods with lower energy density (i.e., vegetables, fruits, legumes, whole grains) tend to be higher in water content (i.e., denser in volume and weight) and naturally nutrient-dense as compared to foods with high energy density that are more refined and contain larger quantities of fats and sugars. Correspondingly, foods with lower energy density tend to be foods that have been minimally processed (or not processed at all), whereas foods with higher energy density tend to be foods

that have undergone multiple or industrial forms of food processing. Studies have demonstrated that people generally consume a constant weight/bulk/volume of food rather than a constant quantity of energy (50–55); therefore, the displacement of low energy dense foods with high energy dense foods has been hypothesized to be a key contributor of excessive caloric intake and the development of obesity. A systematic review by Rouhani et al. of 37 studies that directly or indirectly examined this hypothesis found 18 studies with a positive association, 15 studies with no association, and 2 studies with a negative association between consumption of a high energy dense diet and obesity; 2 studies were not included (56). It is important to note, however, the heterogeneity of these studies in regards to study design (cross-sectional, cohort), dietary assessment tools (food-frequency questionnaires, dietary records, dietary recalls) utilized, age and sex of study participants, and the calculation of energy density (e.g., solid foods only vs. beverages included) (56). Rouhani et al. also performed a meta-analysis on 23 of these studies, stratified by study design, and found that in cohort studies, high energy dense diets were significantly associated with greater weight gain, adjusted mean BMI, and adiposity risk (56). No significant association was found between high energy dense diets and obesity measures among cross-sectional studies (56).

The gut microbiome

The microbial community of the large intestine, consisting of trillions of bacteria, but also archaea, viruses, parasites, and fungi, is relatively stable within each individual (57). The phyla *Firmicutes* and *Bacteroidetes* generally constitute over 90% of adult gut bacteria cells; however, there are over 1,000 different bacterial species present (58,59). Among the many environmental factors (e.g., sanitation, hygiene, climate, geography, urbanicity) that influence the relatively stable and diverse adult gut microbiome, habitual diet is recognized as a key regulator of its composition (i.e., density and diversity of taxa) (60–68). Therefore, as illustrated by **Figure 1.1 (Pathway B)**, as the processing level of the diet naturally influences the proportion of macronutrients, dietary fiber, and other dietary constituents in the diet, it consequently also modulates the composition of the gut microbiome. Studies examining the impact of long-term dietary patterns on the gut microbiome have detected taxonomic differences in gut microbiota related to the composition of the diet. These differences, perhaps, are most discernable when examining the diet and microbiota between Western and non-Western populations.

In a comparative study of rural Burkina Faso children who consumed a diet low in fat and animal protein and high in starch, fiber, and plant polysaccharides, with Italian children who consumed a diet high in fat, animal protein, sugar, starch, and low in fiber, significant differences of gut flora were found in relationship to the types of food consumed and proportion of macronutrients in the diet (69). The gut microbiomes of the Burkinabe children were also found to be more diverse and complex (69). Briefly, at the phyla level, the *Firmicutes* to *Bacteroidetes* ratio was significantly different, with *Firmicutes* twice as abundant in the Italian children, suggesting dramatically different bacterial colonization of the human gut (69). The microbiomes of the Burkinabe children were dominated by *Prevotella*, the Italian children by *Bacteroides* (69). Similarly, in a separate study, significant differences in the phylogenic composition of microbiota were discovered between US residents as compared to Malawians and Amerindians with complementary dietary differences (70). Analogous differences in taxonomic composition have been observed in additional studies examining the impact of Western and non-Western diet on the gut microbiome (71,72). Researchers have also suggested that long-term dietary patterns tend to derive one of three specific genera that dominate the composition of the gut microbiome– *Bacteroides*, *Prevotella*, or *Ruminococcus* (73), regardless of nationality, gender, age, or BMI (74). These genus clusters, or 'enterotypes' have been associated with specific diet characteristics similar to the Burkina Faso/Italy study (69). The *Bacteroides* enterotype is associated with a diet rich in saturated fat and protein, similar to a Western dietary pattern; whereas the *Prevotella* enterotype is rich in carbohydrates and dietary fiber and low in animal protein (73).

The gut microbiome is also influenced by individual dietary components and food processing methods and processing components. Raw and unprocessed plant foods (i.e. fruits, vegetables) contain autochthonous, diverse bacterial communities that colonize their surfaces and tissues (68). Plant polyphenols, lignin, carotenoids, and tannins are also recognized for their ability to stimulate the growth of commensal bacteria (68,75–77). However, modern food production practices (e.g., heat processing, addition of preservatives) meant to decrease pathogenic and spoilage bacteria and thus prolong shelf-life, may decrease beneficial bacterial that aids in digestion, crowds out pathogens, and synthesizes vitamins as well (68). Thus, frequent

consumption of highly processed and preserved foods reduces the intake of these commensal, food-associated microbes (68). Research examining whole grains and dietary fiber (i.e., resistant starch, inulin, fructo- and galacto-oligosaccharies, polydextrose, arabinoxylans) suggest a beneficial bifidogenic effect (78–80), as well as an increased ability to fuel the growth and activity of butyrate-producing bacteria following an increase in consumption (67). Food preparation methods also influence food-associated microbes. For example, frying, more so than boiling, increases the abundance of pathogenic bacteria in the gut microbiome (81). Recent literature also suggests that certain food preservation methods, such as fermentation, beneficially modulate the gut microbiome (66,82). Food additives, such as artificial sweeteners (e.g., saccharin, sucralose, aspartame) and dietary emulsifiers (e.g., carboxymethylcellulose (CMC), polysorbate-80 (P80)), are ubiquitous within highly processed foods. Animal studies have found that artificial sweeteners promote the growth of pathogenic bacteria that are able to utilize these additives (83–86), whereas emulsifiers weaken the mucosal barrier of the intestinal epithelium and facilitate pathogenic bacteria translocation across the intestinal epithelium (87–89). Long-term consumption of other dietary additives, including food coloring compounds, azo polymer coatings (90), and inorganic sulfur (e.g., sulfite and sulfate) (63) are also hypothesized to selectively inhibit the growth of commensal bacteria and/or promote the growth of pathogenic bacteria, respectively. Therefore, not only is the composition of the gut microbiome impacted by the nutritional quality of the diet (i.e., proportions of macronutrients), but it is also affected by various degrees of modern food production practices that may also alter its composition and function and correspondingly contribute to the pathogenesis of obesity.

While specific groups of colonic bacteria can rapidly change in abundance in response to changes in dietary intake of carbohydrates, protein, and fat without changing the ‘enterotype’ of the microbiome (73), thousands of years of change and adaptation of the human diet brought on by the introduction of fire, cooking techniques, development of agriculture, domestication of animals, and food preservation methods (e.g., drying, fermentation) has led to evolutionary changes of human GI physiology, its corresponding microbial communities, and function over time (63,91). Therefore, it is plausible that the introduction of new dietary exposures (i.e., frequent consumption of highly- and ultra-processed foods) that become long-term dietary habits

for certain populations (e.g., rural-to-urban migrants in LMICs) may contribute to changing the 'enterotype' in respective hosts.

It is increasingly recognized that the microbial community of the large intestine (i.e., the gut microbiome) plays a critical role in modulating metabolism and energy balance. Numerous controlled and comparative studies in mice (92–97) and humans (58,59,98) have found different taxonomic proportions (i.e., phyla *Firmicutes/Bacteroidetes*) and decreased diversity (i.e., number of distinct species), complexity (i.e., gene richness) and function of gut microbiota among subjects who are obese vs. lean.

A number of mechanisms have been proposed as to how an imbalance in the composition of the gut microbiome (e.g., 20% increase in *Firmicutes* with a corresponding 20% decrease in *Bacteroidetes* (99)) influences the obesogenic potential of the host **(Figure 1.1 (Pathway C))**, including: fermentation of indigestible polysaccharides; bile acid metabolism; disruption of the gut mucosal barrier; production of the angiopoietin-like protein 4 (ANGPTL4); and, changes in appetite and food intake (100). Two of these mechanisms – the fermentation of indigestible polysaccharides and the disruption of the gut mucosal barrier – are the most directly linked with the processing of foods.

Gut bacteria are able to metabolize indigestible polysaccharides (e.g., cellulose, carrageenan, hemicellulose, polydextrose, beta-glucan, pectin, galactomannans (gums), xylans, resistant starch, inulin) to short-chain fatty acids (SCFAs) (i.e., butyrate, acetate, and propionate). These indigestible plant components, both naturally occurring and artificially produced, are available in a range of foods, from more minimally processed whole grains, fruits, and vegetables to food additives found in more highly processed food products. In normal healthy adults, SCFAs provide 80 – 200 kcal/day (101); dysbiosis of the gut microbiome can result in an additional 150 kcal/day of energy harvest (99) and potentially increase fat deposition (102). SCFAs also modulate the secretion of gut hormones that directly influence satiety (103).

It has been proposed that low-grade inflammation that is associated with obesity is initiated with the development of gut barrier dysfunction (104). Studies have demonstrated that dietary

emulsifiers weaken the gut mucosal barrier, facilitating the translocation of pathogenic microbes across the intestinal epithelium, which drives intestinal inflammation and changes to the gut microbial composition (87,105); this was seen to induce obesity/metabolic syndrome in mice (87).

Finally, as illustrated by **Figure 1.1 (Pathway D)**, the composition of the gut microbiome may mediate the association between the processing level of the diet and obesity through the displacement of low energy dense foods with high energy dense foods (via energy harvest from the diet and energy storage in the host) by modulating the expression of host genes (106). Fecal transplant studies in mice have demonstrated that gut microbiota alter adiposity. Gut microbiota from obese mice induce weight gain and increased adiposity in lean, germ-free mice (92,94). Furthermore, gut microbiota from lean mice induce weight loss in obese mice (107,108). Additional rodent students have also found that mice microbiota responds to reduced caloric intake (109) and that it is able to rapidly change nutrient load (110). A limited number of studies in humans have also demonstrated an interrelation between energy balance, diet, as well as the composition (59,111) and gene pool (112) of the gut microbial community (112). Significant changes in the gut microbial community (increases in *Bacteroidetes* and reductions in *Firmicutes*) have been documented after gastric bypass surgery (113) and weight loss studies (111,114,115) in humans; however, some human studies have failed to find differences in the *Firmicutes/Bacteroidetes* ratio between obese and lean individuals (112–117) or found a predominance of *Bacteroidetes* in overweight and obese individuals (98). Therefore, it is unclear whether the gut microbiota contributes to obesity in humans.

This dissertation will address limitations of the NOVA food processing-based classification system with the development of the Processed Food Dietary Index (PFDI). Using a novel approach, the PFDI and NOVA will be utilized as single measures of diet quality to investigate and compare the dietary share of processing and nutrient means. The direction and trend of nutrients associated with food processing will be examined in an effort to validate the indices. Intrinsically linked with the nutrition transition, food processing has provided freedom from the daily need to process raw commodities, contributing to societal growth and development. However, elements of food processing have also been linked to the development of obesity

through the displacement of low energy dense foods with high energy dense foods, as well as the composition of the gut microbiome. There have been mixed results in examining the relationship between dietary energy density and obesity as well as the composition of the gut microbiome and obesity in humans. Therefore, the PFDI and NOVA will also be utilized to examine the association between the processing level of the diet, obesity, and the composition of the gut microbiome in humans.

The objective of this dissertation is to examine the extent to which the processing level of diets of women of reproductive age (18-49 years) in Bolivia is associated with obesity and gut microbiome composition. The first aim assesses the nutrient adequacy of the Processed Food Dietary Index (PFDI) and NOVA processing classification index and characterizes the processing level of diets among women of reproductive age in Bolivia. The second aim explores the association between the processing level of the diet and obesity among these same women, using the PFDI and NOVA as a single measure of overall dietary quality. The third aim examines the association between the PFDI, obesity, and gut microbiome composition.

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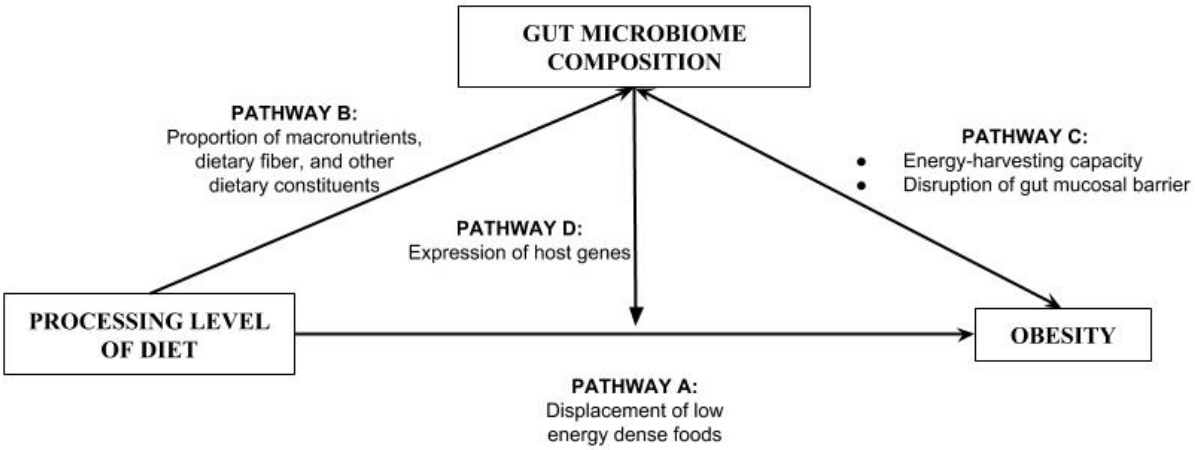
Figure 1.1 Mechanisms in which the processing level of the diet influences obesity.



Chapter 2

Two Food Processing Classification Systems Show Heterogeneous Associations with Macro- and Micronutrient Intakes Among a Population of Bolivian Women of Reproductive Age

Introduction

In the last 20 years there has been an increased interest in examining associations between dietary patterns and health outcomes rather than focusing solely on isolated nutrients (1,2). As the global disease burden has shifted from undernutrition and nutritional deficiencies to overnutrition and chronic disease, it has become clear that studying the synergistic and antagonistic interactions between nutrients and other biological components of foods may provide the strongest epidemiological evidence for diet-health relationships (1–4). Consequently, observational *a priori* and *a posteriori* dietary patterns have been increasingly used to investigate associations with chronic disease risk (1,2). *A priori* dietary patterns are typically derived from pre-defined indices utilizing dietary recommendations as their constructs whereas *a posteriori* dietary patterns are derived from dietary data utilizing data reduction techniques such as cluster and factor analysis with *post hoc* interpretation of the dietary patterns observed (1). Recently, the examination of dietary patterns has focused on the role of food processing – the alteration of food from the point of harvest or slaughter to increase its shelf-stability, nutrient profile, palatability, and/or digestibility. A number of food classification indices based on processing have been developed and applied in various contexts around the world (5), though the extent of examining food processing in dietary patterns has been carried out almost exclusively using the NOVA classification index (6) **(Appendix A)**.

NOVA classifies foods and beverages into four groups according to the nature, extent, and purpose of industrial processing used in their production: Group 1: Unprocessed or minimally processed foods; Group 2: Processed culinary ingredients; Group 3: Processed foods; Group 4: Ultra-processed food and drink products (UPFDs) (7). Scrutiny of the NOVA classification system from the nutrition community has been absent until recently (6,8). The majority of studies

that have utilized NOVA have focused on associations between the consumption of ultra-processed products, dietary nutrient profiles, and chronic diseases such as obesity (9). While these studies have provided important new insights that contribute to our understanding of dietary patterns, the NOVA classification system also has limitations (6,8), including the inability to account for variability of processing and to discriminate nutrient intakes across a spectrum of processing.

To address these potential limitations of the NOVA classification system we developed an alternative schema named the Processed Food Dietary Index (PFDI) **(Appendix B)**. Briefly, the PFDI is a food processing-based classification system built on the foundation of the NOVA system with some key differences in the number and nomenclature of food processing groups and the classification of specific foods and beverages within these groups. **Appendix B** provides a description of the five PFDI groups, as well as examples of the types of foods and beverages that fall within each group. In short, the PFDI classification groups are meant to incrementally represent a higher level of processing for distinct food groups from one classification group to the next. This differs from NOVA, which considers the processing group "processed culinary ingredients" more as a processing step needed to transform unprocessed or minimally processed foods to processed foods rather than a distinct processing group of foods that people directly consume. The PFDI processing groups are meaningful in that they more comprehensively consider the various methods of processing different food groups undergo, as well as the impact on their nutritive value.

The objective of this study was to utilize both the NOVA and PFDI classification systems to examine and compare the relationships between the dietary share of processing level groups and nutrient intakes among a population of Bolivian women of child-bearing age. We hypothesized that higher processing levels would be associated with higher macronutrient and sodium intakes and lower micronutrient intakes (except sodium) and dietary fiber. We further hypothesized that these associations would be attenuated with the presence of fortified and enriched foods in the diet.

Methods

Study design and participant selection

Participants for this study were recruited from the baseline participants of a three-year longitudinal cohort study in Montero, Bolivia—a fast-growing metropolitan area in the eastern lowlands of Bolivia. The cohort study examined regional changes in food environments, diets, and nutritional status of women of reproductive age (18-49 years). The study design and sampling methods used for the cohort study have been described previously (10). Briefly, the baseline assessment of the cohort study was conducted from August to December 2015 and included 1,451 women from the city of Montero. Households within purposively selected urban, peri-urban and rural districts were randomly selected for participation.

Participants for the current study were eligible for recruitment based on the extent of processing in the diet using data from the 2015 baseline assessment of the longitudinal study. Our aim was to select groups that represented the extremes of processing in the diet. Utilizing food frequency data from the baseline study and the theoretical framework of the PFDI (described above), *ad hoc* scores (1: unprocessed/minimally processed; 2: moderately processed; or 3: highly/ultra-processed), rather than scores representing the categories of the PFDI, were assigned to 50 food groups and items based on the frequency of their consumption due to the limited description of the preparation of the foods in the food frequency data. These foods included: grains, starchy vegetables, legumes, fruits, noodles, breads, leafy vegetables, dairy products, meats, fats and oils, fried foods, and sugar-sweetened beverages.

Cumulative food frequency scores were then calculated by summing the processing scores of the food groups and items as indicated by the frequency in which they were consumed for each participant. Women with a cumulative food frequency score that fell in the lowest 10% of all scores (representing the most "minimally processed" diet) or the highest 5% of all scores in the sample (representing the most "highly processed" diet) were eligible for selection for the study and served as the sampling frame. In total, there were 127 women representing the "most minimally" processed diet and 269 women representing the most "highly processed" diet for a total of 396 women. From these, 160 women aged 18-49 years were randomly selected, 80 representing minimally processed diets and 80 representing highly processed diets (n=160 in total).

The sample size for this study was based on detecting a difference in mean body mass index (BMI) between women who consumed a highly versus minimally processed diet as determined by the PFDI. The focus on BMI as an outcome is related to an additional aim of the study that is reported elsewhere (Chapter 3). Previous studies examining differences in BMI using metrics of dietary patterns (11–13) informed us that with an expected mean difference in BMI of 1.1 and a standard deviation of 3.0, an estimated 80 women would be needed in total (assuming $1-\beta=0.8$; $\alpha=0.05$). To ensure sufficient statistical power accounting for potential noncompliance with study protocols, we aimed to enroll 160 women in the study.

Between August and October 2016, trained enumerators carried out in-person interviews with selected participants. At the time of recruitment, women who were known to be currently pregnant (n=7) or taking antibiotics (n=24) were excluded from participation. Women who could not be located at the time of recruitment or following a second contact attempt (n=108), as well as those who refused to participate (n=5) or were not available for all three visits (n=29), were replaced with another randomly selected participant. Two women began a course of antibiotics prior to the second interview and were also replaced. In total, three visits to the participant's home were carried out during a one-week period. All interviews were conducted in Spanish.

Measurement of variables

Recruited participants completed three in-person, non-consecutive, 24-hour dietary recall interviews on two weekdays and one weekend day spanning one week using the standard multiple pass method (14). Interviewers recorded information on the types and amounts of foods and beverages consumed, time and place of consumption, and where and how foods and beverages were prepared. For prepared foods and beverages, the enumerators used their knowledge of locally prepared dishes to further question participants regarding the ingredients used, amounts, and methods of preparation (e.g., boiled, grilled, fried). For self-contained foods and beverages (e.g., packaged, canned, bottled), enumerators collected information about the product (i.e., brand, flavor) and nutritional composition (i.e., facts from nutrition and ingredient labels). When available and with permission from the participant, labels of consumed commercial food and beverage items were photographed.

Reported food amounts were converted to grams or milliliters based on food portion tables compiled from previous work completed in Peru and Bolivia as well as commercial food product labels. Dietary energy and nutrient intakes were estimated based on Bolivian (15) and Peruvian (16) food composition tables and supplemented by the USDA National Nutrient Database for Standard Reference, Release 28 (SR28) (17). The following nutrients were included in the analysis: protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, trans fat, carbohydrates, dietary fiber, vitamin A (as retinol activity equivalents), vitamin C, thiamin, riboflavin, niacin, calcium, iron, magnesium, phosphorus, sodium, potassium, and zinc.

Reported food items were classified according to both PFDI (0 to 4) and NOVA (1 to 4) indices. When possible, mixed dishes prepared in the home were disaggregated into their constituent ingredients and scored individually. For reported mixed dishes in which recipes were not available, mean scores from existing recipes of mixed dishes were utilized. The scores were then weighed by calculating the PFDI and NOVA scores by the amount, in grams or milliliters, of each food item.

The three 24-hour recalls collected from each participant were used to estimate their average energy intake and nutrient consumption. We calculated average PFDI and NOVA scores for each participant using the weighted PFDI and NOVA score for each item consumed. PFDI and NOVA scores were then categorized into quintiles. The dietary share for each PFDI and NOVA food group, respectively, was calculated as a percentage of total energy intake. We also calculated the average dietary share of each nutrient. Macronutrient intake was calculated as a percentage of total energy; fiber and micronutrients as mean density (g, mg, or µg/1,000 kcal).

Information on the age, educational status, and physical activity level of the participating woman, as well as household food insecurity, wealth status, and urban residence (i.e., urban, peri-urban, and rural) was sourced from the baseline assessment of the longitudinal study. WHO's Global Physical Activity Questionnaire (GPAQ) was used to assess physical activity level by calculating the total time spent in physical activity during a typical week by the intensity of the physical activity for a total metabolic equivalent (MET) minutes per week (18). Household

food security level (food secure, or mildly, moderately, severely food insecure) was measured using the Latin American and Caribbean Household Food Security Measurement Scale (ELCSA) instrument (19). Household wealth was assessed using standardized asset scores generated from a principal component analysis to create an index that categorized households into five wealth quintiles (20). Upon enrollment, the date of birth of each participant was confirmed.

Statistical analyses

All analyses were performed using the statistical software package SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Average energy intake was calculated from the collected 24-hr dietary recall data in the present study. The mean percentage of energy intake was calculated for each PFDI and NOVA food processing group and across quintiles of PFDI and NOVA scores, respectively. Crude linear regression analysis was used to assess the statistical significance across said quintiles. The distribution of energy intake, as percent of total energy intake and kcal/day, was calculated for food groups within each PFDI food processing group. The average dietary share of each nutrient (listed previously) was calculated across quintiles of PFDI scores from women representing either the most minimally processed diet or the most highly processed diet from calculated cumulative food frequency scores. A two-sample t-test was conducted to compare nutrient means between the two groups that served as the sampling frame. The average dietary share of each nutrient was calculated across quintiles of PFDI and NOVA scores, as well as across the dietary share of ultra-processed and unprocessed/minimally processed foods. Crude and adjusted linear regression analyses were used to assess the associations between quintiles of the PFDI and NOVA scores and dietary intake of macronutrients (expressed as percent of total energy) and micronutrients (expressed as mean density per 1,000 kcal). Adjusted analyses controlled for age, educational attainment, and physical activity of the participating woman, as well as household wealth, food insecurity, urban residence, and total energy intake. Reported standardized regression coefficients were calculated by standardizing all variables to a mean of 0 and a SD of 1. Associations were considered consistent with random variation at $P>0.05$.

Results

The total analytical sample was 160 women. The average PFDI and NOVA scores were (mean ± SD) 1.56 ± 0.46 and 2.04 ± 0.40, respectively. PFDI scores ranged from 0.53 to 2.97; NOVA

scores ranged from 1.17 to 3.02. Both PFDI and NOVA scores were normally distributed. PFDI and NOVA scores were also highly correlated, r=0.94, p<.0001. The average daily energy intake of the women was 1,669 kcal/day.

The dietary share of macro- and micronutrients across quintiles of PFDI scores from the two groups (minimally processed group; highly processed group) serving as the sampling frame are outlined in **Tables 2.1** and **2.2**. We were particularly interested in examining differences in mean nutrient intakes between the two groups, summarized in **Table 2.3**. There were no statistically significant differences between mean caloric intake or macronutrient values between the two groups. Mean intake of dietary fiber (p=0.002), vitamins A (p=0.003) and C (p=0.04), as well as magnesium (p <.0001), phosphorus (p=0.001), and potassium (<.0001) were statistically significantly higher in the minimally processed group.

Using the PFDI, 1.9% of calories were attributed to unprocessed foods, 19.9% to minimally processed foods, 37.9% to moderately processed foods, 31.9% to highly processed foods, and 9.5% to UPFDs **(Table 2.4)**. Overall, the energy contribution from unprocessed, minimally processed, and moderately processed foods was lower among individuals moving from the first to fifth quintiles of PFDI scores. In contrast, the energy contribution from highly processed foods and UPFDs was higher among individuals with increasing quintiles of PFDI scores. Furthermore, this relationship between energy contribution and PFDI scores was observed to be monotonic among both unprocessed foods and UPFDs.

Using the NOVA classification system, 22.6% of calories were attributed to unprocessed or minimally processed foods, 27.9% to culinary processed ingredients, 40.1% to processed foods, and 9.5% to UPFDs **(Table 2.4)**. Overall, the energy contribution from unprocessed or minimally processed foods and culinary processed ingredients decreased among individuals with increasing quintiles of NOVA scores. In contrast, the energy contribution from processed foods and UPFDs increased from the first to fifth quintiles of NOVA scores. The relationship between energy contribution and NOVA scores was observed to be monotonic only for UPFDs.

The types of foods and beverages consumed by women in this sample in accordance with the PFDI food processing groups are described in **Table 2.5**. Among unprocessed foods, raw fruits provided twice the amount of energy (1.3%) of raw vegetables (0.6%). The majority of energy contributed by minimally processed foods was from simmered, boiled, and roasted meats (6.4%), rice (5.9%), and starchy vegetables (4.3%). Beverages in which sugar was added prior to consumption contributed the most energy among moderately processed foods (17.7%), followed by pan-fried, grilled, and barbecued meats (12.9%). Fresh breads (10.7%), homemade pastries (e.g., empanadas) (9.5%), French fries (4.2%), and fried chicken (3.6%) contributed the most energy among highly processed foods, while soft drinks (3.7%) and packaged, ready-to-eat flour-based confections (e.g., donuts, cakes, cookies) contributed the most energy among UPFDs (2.0%).

The dietary shares of macro- and micro-nutrients across quintiles of PFDI and NOVA scores in this population are outlined in **Tables 2.6** and **2.7**. Among macronutrients, the adjusted mean intake of carbohydrates decreased significantly across PFDI quintiles (first quintile: 58.8%; fifth quintile: 56.3%). In contrast, the adjusted mean intake of carbohydrates increased across NOVA quintiles (regression coefficient: 0.02, $p<0.05$). The adjusted mean consumption of total fat increased significantly across both PFDI (first quintile: 28.1%; fifth quintile: 30.1%) and NOVA quintiles (first quintile: 27.5%; fifth quintile: 30.3%). A decreasing linear trend in micronutrient intakes was observed across both PFDI and NOVA quintiles. This trend was statistically significant in adjusted models for dietary fiber ($p<0.0001$), vitamin A ($p<0.0001$), vitamin C ($p<0.0001$), magnesium ($p<0.05$), and potassium ($p<0.0001$); the crude models for thiamin, niacin, iron; and the adjusted model for riboflavin ($p<0.05$). This trend monotonically decreased for dietary fiber and vitamin A across both PFDI and NOVA quintiles, as well as for vitamin C across the NOVA quintiles. Mean intake of sodium was highest in the first and third PFDI and NOVA quintiles and decreased from the first to fifth PFDI quintiles (first quintile: 1986.8; fifth quintile: 1599.7 mg/1000 kcals) and NOVA quintiles (first quintile: 2059.6; fifth quintile: 1543.4 mg/1000 kcals).

We examined the distribution of nutrient means across quintiles of the dietary share of UPFDs **(Table 2.8)** and unprocessed and minimally processed foods **(Tables 2.9** and **2.10)**. The dietary

share of UPFDs among the sample population ranged from a minimum of 0% to a maximum of 51.1%; the share of unprocessed and minimally processed foods ranged from 1.2% to 63.4%. Opposite trends in the direction of associations were observed for a number of nutrients across quintiles representing the extremes of consumption of processed foods. Among macronutrients, mean consumption of protein decreased while total fat and saturated fat increased as the overall share of UPFDs in the diet increased. Concurrently, intake of protein increased and total fat and saturated fat decreased as the overall share of unprocessed and minimally processed foods increased across both PFDI and NOVA indices. Among micronutrients, mean intake of dietary fiber, vitamin A, vitamin C, niacin, magnesium, phosphorus, sodium and potassium decreased across quintiles of UPFDs while intake of these micronutrients increased across PFDI and NOVA quintiles of unprocessed and minimally processed foods. These trends were observed to be statistically significant for total fat, dietary fiber, vitamin C, and potassium. Notably, the mean intake of sodium decreased between the first and fifth quintiles of UPFDs and increased between the first and fifth quintiles of unprocessed and minimally processed foods; these trends, however, were not statistically significant.

Discussion

In this study, we examined the association between the PFDI and NOVA classification systems and the dietary share of processing level groups and nutrient intakes among a population of Bolivian women of child-bearing age that were initially selected based on the extent of processing in their diet. We investigated how the dietary share of each processing group in the NOVA and PFDI classification systems was associated with individual nutrient intakes to understand the extent to which processing level groups were consistent with dietary attributes of processing. Overall, we found the strongest associations between crude and adjusted models of PFDI and NOVA scores with total fat, dietary fiber, vitamin A, vitamin C, magnesium, and potassium.

Upon examining the differences in nutrient means from the minimally processed and highly processed groups that served as the sampling frame, the finding of no statistically significant differences in macronutrient intakes between the two groups was expected since the PFDI scores for the entire sample population were normally distributed. While we did find statistically

significant differences in mean intake of dietary fiber, vitamin A, vitamin C, magnesium, phosphorus, and potassium between the two groups, these differences could have been driven by how the FFQ categorized foods and food groups rather than how processing was defined utilizing the FFQ questions.

Upon analyzing the distribution of energy, we found that only 9.5% of energy intake was attributed to UPFDs in this population. This proportion of UPFDs is low compared to other Latin American middle-income countries where studies have calculated the contribution of UPFDs in diets to range from 21.5 to 29.8% (21–23). This difference is likely attributable to the more limited availability of UPFDs in lower-middle income countries such as Bolivia, as evidenced by lower (but rapidly increasing) per capita sales of UPFDs as compared to higher income countries (24). Studies utilizing the NOVA classification system have asserted that increased purchasing or consumption of UPFDs is associated with obesity (22,25–28); however, limitations regarding study design and adjustment for confounding covariates in these studies, as well as a counter finding in a study from the United Kingdom (29), challenge these assertions. Despite a high prevalence of overweight among Bolivian women of child-bearing age (62% overweight or obese in 2013 (30)), our study results suggest that consumption of UPFDs in Bolivia may be quite low. This suggests that consumption of UPFDs is not sufficient to explain changing patterns of overweight and obesity in low- and middle-income countries and indicates the importance of evaluating how each food processing group, not only UPFDs, contributes to the overall dietary patterns and nutritional status.

In both the NOVA and PFDI classification systems, unprocessed and minimally processed food groups are considered “nutrient dense” (31) – contributing beneficial micronutrients with relatively little energy. These groups also contain the largest proportion of unrefined plant foods and therefore are likely to contribute the highest density of dietary fiber. As such, we hypothesized that the density of vitamins, minerals (excluding sodium), and dietary fiber would be lower with increasing PFDI and NOVA score quintiles. This trend was observed for vitamin A, vitamin C, magnesium, potassium, and dietary fiber, however, not for other relevant micronutrients, including thiamin, riboflavin, niacin, calcium, iron, and zinc. Enrichment and/or fortification of “processed foods” (32) may have attenuated the expected trend for these

nutrients. Bolivia began fortification of wheat flour with thiamin, riboflavin, niacin, folate, and iron in 1997 (33), though there is uncertainty regarding how widespread and to what degree other food products (i.e., maize flour, vegetable oils, milk products) may be fortified with various micronutrients in Bolivia (34). In addition, UPFD's are often "fortified" with nutrients as a selling point for their consumption.

Previous studies that have utilized the NOVA classification system commonly examined various mean nutrient intakes across quintiles of the dietary share of UPFDs. These studies found that adjusted intakes of dietary fiber (4 studies), potassium (2 studies), zinc (2 studies), riboflavin (2 studies), and niacin (2 studies) were statistically significantly lower across increasing quintiles of UPFDs (35–38). These results are consistent with the direction of trends observed in our study, however, these trends were not statistically significant in our study for magnesium, phosphorus, zinc, and niacin. These same studies have reported mixed results regarding the statistical significance and direction of trend for vitamin A (4 studies), vitamin C (4 studies), iron (4 studies), calcium (4 studies), and thiamin (2 studies) (35–38). Our study found that vitamin A and vitamin C decreased significantly across quintiles of UPFDs, whereas decreasing trends for iron and thiamin, and increasing trends for calcium were not statistically significant. It is important to note that the mean dietary share of UPFDs has varied widely in the studies to date, including our own (9.5 to 57.5% total energy intake) (35–38). These studies have also utilized different populations, and have not adjusted crude models for the same confounding variables, all of which could contribute to differences in mean nutrient intake trends across the dietary share of UPFDs.

Highly processed foods are likely to contribute the highest density of fat, saturated fat, *trans* fat, carbohydrates (due to added sugar not measured separately), and sodium to diets. We therefore expected intakes of these nutrients to be higher across increasing PFDI and NOVA score quintiles. However, we did not consistently observe these trends. Total fat intake increased across quintiles of both PFDI and NOVA scores. Carbohydrate intake similarly increased across quintiles of NOVA scores, however it decreased across quintiles of PFDI scores. The observed trends for saturated fat, trans fat, and sodium were not statistically significant. Saturated fat intake decreased across PFDI quintiles and increased across NOVA quintiles; *trans* fat increased

across PFDI and NOVA quintiles whereas sodium decreased across PFDI and NOVA quintiles. Observing the trend opposite as expected for carbohydrates across PFDI quintiles and the lack of association with saturated fat and *trans* fat intakes may be attributed to the consumption trends of food groups within the processing level groups. For example, consumption of traditional beverages, teas, fresh juices, and coffee with added sugar contributed 17.7% of total energy intake whereas UPFDs that are high in sugar contributed less than 9.5% of total energy intake (percentages derived from **Table 2.2**). Ingredients in beverages were often classified differently across the PFDI and NOVA systems resulting in lower PFDI scores than NOVA scores, potentially resulting in observed trends that were not aligned with our hypotheses. The lack of association with saturated and *trans* fat intake across quintiles of the PFDI and NOVA scores may be attributed to the higher intake of red meats in minimally and moderately processed groups than in highly and ultra-processed groups (i.e., minimally processed (cooked beef: 1.7% total energy intake), moderately processed (pan-fried/grilled/barbecued beef: 4.6% total energy intake), highly processed (cured and/or dried meats: 0.6%) ultra-processed (processed meats: 0.3%)), as well as the recent decrease in availability of *trans* fat in the food supply. Surprisingly, the lowest intakes of sodium observed were among individuals in the fourth and fifth quintiles of the PFDI and NOVA indices. This finding contrasted with expectations and may be due to the addition of table salt in mixed dishes as compared to the relatively low intake of UPFDs that are high in sodium (~.5% of total energy intake), as well as inaccurate food composition tables.

In comparing the macronutrient and sodium intakes from the previously mentioned studies across increasing dietary share of UPFDs, we found that protein (4 studies) was consistently significantly lower and saturated fat (4 studies) was consistently significantly higher across increasing quintiles of shares of UPFDs (35–38). There were mixed results regarding significance and direction of trend for carbohydrates (4 studies), total fat (4 studies), and sodium (3 studies) (35–38). Our findings also showed that intake of protein was significantly lower and saturated fat was significantly higher across increased dietary share of UPFDs. *Trans* fat was not included as a nutrient indicator in three of the four other studies.

Study participants consistently had a lower average PFDI score than NOVA score. This disparity was likely due to the way in which foods and beverages were classified according to their

respective processing level groups. For example, foods and beverages considered "unprocessed" scored a "0" utilizing the PFDI but scored a "1" utilizing the NOVA system. However, the consistently lower PFDI averages were not due to consumption of a higher number of "unprocessed" foods as classified by the PFDI, as evidenced by raw fruits and vegetables making up only 1.9% of total energy intake. The majority of foods and beverages consumed in this population were considered "moderately processed" utilizing the PFDI classification system (37%), scoring a "2"; however, utilizing the NOVA system, these foods scored a "3", and contributed the most to differences in PFDI and NOVA scores. The dietary pattern and availability of specific commodities for this particular population did not allow us to analyze how the reclassification of a number of food groups, notably flours/pastas and canned goods, affected PFDI and NOVA scores. The main sources of complex carbohydrates in this population were from rice (5.9% of total energy intake), starchy vegetables (10.2% of total energy intake), and fresh breads (10.7% of total energy intake); the use of flours in mixed dishes and the consumption of pasta was minimal (3.3% of total energy intake). The consumption of canned goods was also minimal, contributing to only 1.4% of energy intake.

The key strength of this study was utilizing a 24-hr dietary recall instrument, repeated three times per individual, that was designed to record the specific details needed to accurately classify foods and beverages according to their respective processing level groups within the PFDI and NOVA classification systems. Therefore, we expect that misclassification of foods according to their processing level was limited. Yet, this study had several limitations. First, the small sample size provided us with limited statistical power to observe some relationships, and likely prevented a comprehensive comparison of classification systems. Second, intakes of added sugars were not analyzed in this study given that they were not included in the nutrient composition tables that were utilized. Third, despite rigorous training of diet assessment enumerators and strict protocols for implementing the multiple pass interview method with probes for snacks and all foods and beverages consumed, it is likely that energy and nutrient intakes were underestimated, a common source of measurement error with dietary recall (39). Finally, though not a concern for the internal validity of the study, the findings are not generalizable to the larger Bolivian population given our focus on women in eastern Bolivia and how participants were selected on the extent of processing in their diet.

Conclusion

Overall, we found that the expected nutrient intake trends were not always consistent with the extent of processing as defined by the PFDI and NOVA classification systems; for example, a more highly processed diet was not necessarily higher in saturated fat, *trans* fat, and sodium. In theory, using nutrients as the main construct to distinguish levels of processing is sound, based on what we know regarding the impact of various methods of food processing on the loss and/or gain of nutrient values. However, enrichment and/or fortification of foods at various levels of processing and the formulation of ultra-processed foods with ingredients often meant to add an otherwise absent dietary benefit likely attenuates expected trends of nutrients across levels of processing. Our findings were consistent with other studies that also did not show expected nutrient trends with increased intakes of UPFDs. Inconsistencies in the direction and significance of nutrient trends were also found between these studies. Our findings suggest that using nutrients to validate classification systems based on processing, such as NOVA and the PFDI, requires further research. Furthermore, to the best of our knowledge, NOVA, the most widely-used classification system based on processing, has not been validated using any metric and should be applied with caution. As the field of nutritional epidemiology continues to investigate the utilization of classification systems based on the extent of processing, it should also cautiously explore translating processing level groups into dietary guidelines that are easy for the public to understand and adopt into practice.

Another important consideration from our dietary analysis is that our sample population consumed a very low percentage of UPFDs (9.5%) as part of their overall diet, making it difficult to determine whether differences in nutrient values across UPFD quintiles were objectively meaningful **(Table 2.8)**. With the majority of research utilizing NOVA solely focusing on the role of UPFDs, it's important recognize that it may not be an appropriate or useful tool to use in certain contexts, particularly in a cross-sectional setting among low- and middle-income populations where UPFD intake may not be a substantial part of the diet.

Finally, an ancillary objective of this research was to compare the nutrient value trends across a spectrum of processing derived using the PFDI and NOVA, to evaluate the effect of classifying certain food groups in different processing level groups. Notably, there were very few

differences in the direction and significance of trend between the PFDI and NOVA quintiles **(Tables 2.6** and **2.7)**, most likely attributable to the dietary pattern and size of the sample population. It would be judicious to compare the nutrient trends across processing utilizing NOVA and the PFDI using dietary data from a much larger, and perhaps, more industrialized population. Regardless, we hope that the authors of NOVA consider the suggestions regarding the system's processing groups' **(Appendix A)**, especially if NOVA is meant to be a tool applicable to the global food system.

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Table 2.1 Distribution of FFQ minimally processed group nutrient means across PFDI quintiles. Montero, Bolivian women of child-bearing age, 2016; n=80.

			PFDI quintiles (FFQ Minimally Processed Group)					Standardized regression coefficient[a]	
Dietary content	Mean	Interquartile range	1 (n=16)	2 (n=16)	3 (n=16)	4 (n=16)	5 (n=16)	Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	50.6-63.5	59.4	58.2	54.3	53.1	56.2	-0.17	-0.03
Protein	15.9	13.3-18.1	15.1	17.3	16.7	15.9	14.4	-0.06	0.02
Total fat	30.0	23.3-34.5	27.4	26.1	30.6	32.4	29.4	0.16	-0.00*
Saturated fat	8.4	6.3-10.0	8.4	7.5	9.7	8.5	8.0	0.01	-0.09
Polyunsaturated fat	6.7	4.6-8.8	6.0	6.7	7.1	7.8	6.0	0.07	-0.06
Monounsaturated fat	11.1	8.8-12.5	10.4	9.4	10.8	13.0	12.2	0.19*	0.10
Trans fat	1.8	0.3-1.8	1.2	1.6	1.6	2.6	2.5	0.11	0.15
Dietary fiber (g/1000 kcal)	10.9	8.0-13.2	12.4	12.4	11.0	8.9	8.5	-0.32***	-0.27*
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	583.8	324.8-762.9	685.9	669.0	539.1	572.2	338.1	-0.21*	-0.32**
Vitamin C (mg/1000 kcal)	66.0	39.6-83.6	83.6	60.5	72.4	53.9	48.7	-0.21*	-0.09**
Thiamin (mg/1000 kcal)	0.9	0.7-1.0	0.9	0.9	0.9	0.8	0.7	-0.04**	-0.03
Riboflavin (mg/1000 kcal)	0.9	0.7-1.0	0.8	1.0	1.0	0.9	0.9	0.01	-0.01
Niacin (mg/1000 kcal)	34.9	15.8-45.0	37.8	36.2	38.9	30.5	29.3	-0.09	-0.02
Calcium (mg/1000 kcal)	254.7	152.9-298.3	226.7	273.3	287.1	231.3	279.8	0.06	-0.02
Iron (mg/1000 kcal)	7.1	5.8-8.1	7.4	7.6	7.2	6.8	6.3	-0.12*	-0.08
Magnesium (mg/1000 kcal)	125.4	94.0-147.2	131.5	145.0	137.3	106.6	100.0	-0.23*	-0.23
Phosphorus (mg/1000 kcal)	529.8	431.1-618.3	509.4	577.9	557.8	504.4	508.5	-0.04	-0.06
Sodium (mg/1000 kcal)	1893.0	1362.8-2106.7	2054.6	1518.7	2560.4	1669.7	1663.2	-0.06	-0.10
Potassium (mg/1000 kcal)	1342.1	1034.1-1541.1	1562.3	1391.4	1394.5	1129.7	1080.9	-0.32**	-0.25**
Zinc (mg/1000 kcal)	22.8	14.0-26.9	19.0	22.0	25.3	24.6	26.2	0.14	0.25*

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.2 Distribution of FFQ highly processed group nutrient means across PFDI quintiles. Montero, Bolivian women of child-bearing age, 2016; n=80.

Dietary content	Mean	Interquartile range	PFDI quintiles (FFQ Highly Processed Group) 1 (n=16)	2 (n=16)	3 (n=16)	4 (n=16)	5 (n=16)	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.3	51.3-60.8	57.3	59.2	54.7	54.9	56.3	-0.08	0.02
Protein	14.9	13.2-16.7	15.3	15.4	16.0	14.3	13.9	-0.20	-0.23
Total fat	29.7	25.1-33.6	30.1	27.2	29.7	30.9	30.5	0.08	0.05
Saturated fat	8.4	6.6-9.7	8.2	7.6	8.1	9.1	8.6	0.08	0.10
Polyunsaturated fat	6.8	5.1-7.7	8.4	6.3	6.8	7.1	6.3	-0.09	-0.05
Monounsaturated fat	11.8	9.2-13.3	12.9	11.7	11.4	11.6	11.9	-0.04	-0.01
Trans fat	2.0	0.3-1.1	1.4	3.1	1.6	3.5	0.5	-0.09	-0.08
Dietary fiber (g/1000 kcal)	9.2	7.4-11.1	11.6	10.7	9.3	8.6	7.3	-0.31***	-0.32**
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	419.0	234.3-536.1	680.2	464.4	454.8	323.6	307.6	-0.23**	-0.19**
Vitamin C (mg/1000 kcal)	53.9	33.2-67.9	74.2	62.4	66.7	33.6	41.9	-0.25**	-0.22
Thiamin (mg/1000 kcal)	1.0	0.7-0.9	2.0	1.6	0.8	0.8	0.7	0.26*	-0.08*
Riboflavin (mg/1000 kcal)	1.1	0.6-0.9	2.0	1.6	0.8	0.8	1.0	-0.19	-0.03*
Niacin (mg/1000 kcal)	27.8	16.7-33.9	30.4	36.6	28.1	23.7	22.4	-0.14*	-0.14
Calcium (mg/1000 kcal)	243.5	159.4-302.4	199.7	289.0	259.1	235.1	218.7	-0.06	-0.00
Iron (mg/1000 kcal)	7.6	5.8-8.1	8.1	9.3	7.5	7.6	6.0	-0.31**	-0.27
Magnesium (mg/1000 kcal)	99.2	80.7-111.0	106.2	104.5	100.5	107.1	84.0	-0.12*	-0.14
Phosphorus (mg/1000 kcal)	461.5	392.5-506.7	438.3	483.5	478.8	455.4	442.8	-0.05	-0.03
Sodium (mg/1000 kcal)	1608.7	1344.7-1776.9	1813.6	1724.4	1666.4	1367.9	1561.6	-0.07*	-0.06
Potassium (mg/1000 kcal)	1034.6	863.3-1223.1	1177.9	1064.0	1146.7	962.6	897.8	-0.18**	-0.20**
Zinc (mg/1000 kcal)	22.9	13.3-28.2	25.4	27.8	20.6	22.1	20.6	-0.12	-0.02

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.3 Differences in mean nutrient intake between FFQ groups. Montero, Bolivian women of child-bearing age, 2016; n=160.

	FFQ Minimally Processed Group (n=80)		**FFQ Highly Processed Group (n=80)**		
	Mean	*Range*	*Mean*	*Range*	*p-value**
PFDI score	1.46	0.53-2.63	1.66	0.78-2.97	0.008
Energy (kcals)	1624.7	646.5-3464.2	1713.5	545.6-3940.7	0.31
Macronutrients (% of total energy intake)	*Mean*	*Interquartile range*	*Mean*	*Interquartile range*	
Carbohydrates	56.5	50.6-63.5	56.3	51.3-60.8	0.83
Protein	15.9	13.3-18.1	14.9	13.2-16.7	0.08
Total fat	30.0	23.3-34.5	29.7	25.1-33.6	0.52
Saturated fat	8.4	6.3-10.0	8.4	6.6-9.7	0.88
Polyunsaturated fat	6.7	4.6-8.8	6.8	5.1-7.7	0.75
Monounsaturated fat	11.1	8.8-12.5	11.8	9.2-13.3	0.19
Trans fat	1.8	0.3-1.8	2.0	0.3-1.1	0.73
Dietary fiber (g/1000 kcal)	10.9	8.0-13.2	9.2	7.4-11.1	0.002
Micronutrients (mean density)					
Vitamin A (µg/1000 kcal)	583.8	324.8-762.9	419.0	234.3-536.1	0.003
Vitamin C (mg/1000 kcal)	66.0	39.6-83.6	53.9	33.2-67.9	0.04
Thiamin (mg/1000 kcal)	0.9	0.7-1.0	1.0	0.7-0.9	0.35
Riboflavin (mg/1000 kcal)	0.9	0.7-1.0	1.1	0.6-0.9	0.25
Niacin (mg/1000 kcal)	34.9	15.8-45.0	27.8	16.7-33.9	0.05
Calcium (mg/1000 kcal)	254.7	152.9-298.3	243.5	159.4-302.4	0.58
Iron (mg/1000 kcal)	7.1	5.8-8.1	7.6	5.8-8.1	0.23
Magnesium (mg/1000 kcal)	125.4	94.0-147.2	99.2	80.7-111.0	<.0001
Phosphorus (mg/1000 kcal)	529.8	431.1-618.3	461.5	392.5-506.7	0.0001
Sodium (mg/1000 kcal)	1893.0	1362.8-2106.7	1608.7	1344.7-1776.9	0.12
Potassium (mg/1000 kcal)	1342.1	1034.1-1541.1	1034.6	863.3-1223.1	<.0001
Zinc (mg/1000 kcal)	22.8	14.0-26.9	22.9	13.3-28.2	0.98

*p-values from two-sample t-test of FFQ group means

Table 2.4 Distribution of total energy intake by PFDI food processing groups across PFDI quintiles and NOVA food processing groups across NOVA quintiles.

	Mean (SD)	1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	p-value*
PFDI food processing groups		PFDI quintiles					
Group 1: Unprocessed foods, %	1.9 (2.5)	2.4 (2.3)	2.2 (2.7)	2.1 (3.2)	1.4 (1.5)	1.3 (2.5)	0.0443
Group 2: Minimally processed foods, %	19.9 (10.8)	21.7 (10.7)	20.1 (10.6)	21.5 (10.0)	19.0 (11.8)	16.9 (10.8)	0.0256
Group 3: Moderately processed foods, %	37.0 (18.3)	46.4 (18.6)	37.8 (19.4)	31.0 (17.3)	37.7 (17.0)	31.8 (15.6)	0.0056
Group 4: Highly processed foods, %	31.8 (16.1)	23.2 (12.3)	32.6 (18.5)	36.7 (14.1)	31.1 (16.9)	35.4 (15.6)	0.0082
Group 5: Ultra-processed foods, %	9.5 (9.3)	6.1 (8.8)	7.3 (6.7)	8.6 (9.3)	10.8 (8.0)	14.5 (11.0)	< .0001
NOVA food processing groups		NOVA quintiles					
Group 1: Unprocessed or minimally processed foods, %	22.6 (11.4)	24.2 (10.3)	24.9 (12.0)	25.9 (12.0)	19.7 (9.8)	18.3 (11.2)	0.0065
Group 2: Culinary processed ingredients, %	27.9 (19.3)	42.7 (17.8)	29.0 (19.3)	18.7 (16.2)	25.9 (19.3)	23.1 (15.5)	0.0006
Group 3: Processed foods, %	40.1 (17.6)	27.3 (14.1)	39.8 (17.1)	46.4 (15.9)	42.6 (18.6)	44.3 (16.2)	0.0007
Group 4: Ultra-processed foods, %	9.5 (9.3)	5.8 (8.8)	6.3 (6.8)	9.0 (8.8)	11.8 (7.5)	14.3 (11.3)	< .0001

*p-values for linear regression of crude models

Table 2.5 Distribution of total energy intake by PFDI food processing groups. Montero, Bolivian women of child-bearing age; 2016.

Food group	kcal/day	% of total energy intake
Unprocessed foods	**31.7**	**1.9**
Raw fruits	21.7	1.3
Raw vegetables	10.0	0.6
Minimally processed foods	**332.1**	**19.9**
100% fruit juice	4.5	0.3
Milk	17.7	1.1
Vegetables		
Starchy, cooked	72.5	4.3
Non-starchy, cooked	10.4	0.6
Dried spices/herbs	0.2	0.0
Legumes	2.3	0.1
Grains		
Rice	98.7	5.9
Other (oats, quinoa, wheat)	16.0	1.0
Cooked (e.g., simmered, boiled, roasted) meats	106.9	6.4
Eggs, boiled	3.1	0.2
Moderately processed foods	**617.6**	**37.0**
Traditional beverages[a] w/ added sugar	96.5	5.8
Tea w/ added sugar	79.0	4.7
Freshly squeezed juices w/ added sugar	72.6	4.3
Coffee w/ added sugar	49.1	2.9
Pan-fried/grilled/barbecued meats	216.5	12.9
Flours and pastas	54.6	3.3
Pan-fried/scrambled eggs	25.9	1.6
Canned fruit (heavy syrup)	17.3	1.0
Canned fish (packed in oil)	6.2	0.4
Highly processed foods	**530.8**	**31.8**
Fresh breads, tortillas	178.6	10.7
Home-made savory or sweet pastries, may be fried[b]	159.4	9.5
Shallow or deep-fried starchy vegetables/fruits		
French fries	70.7	4.2
Other (cassava, plantains)	28.7	1.7
Fried chicken	60.5	3.6
Cheese	11.5	0.7
Cured and/or dried meats	10.0	0.6
Alcoholic beverages (beer, wine, brandy)	8.2	0.5
Condiments (mustard, mayonnaise)	3.2	0.2
Ultra-processed foods	**158.6**	**9.5**
Soft drinks	61.0	3.7
Flour-based confections[c]	33.4	2.0
Dairy-based confections[d]	12.7	0.8
Instant cocoa beverages	12.7	0.8
Self-contained sugar sweetened beverages[e]	12.1	0.7
Flavored yogurt, yogurt drinks	12.1	0.7
Processed meats (hotdogs, sausages, pepperoni)	5.0	0.3
Crackers, popcorn, plantain chips	3.1	0.2
Ultra-processed breads	2.5	0.1
Chocolate candies	1.2	0.1
Other[f]	2.9	0.1

a Refresco de mocochinchi, refresco de linaza, tujure, api de maiz morado, refresco de tamarindo;
b Empanadas, salteñas, fritos, tamales, sonso, cuñapé; c Donuts, cakes, cookies, sweet breads;
d Pudding, flan, dulce de leche, ice cream, milkshakes; e Juice drinks, chocolate milk, Powerade;
f Jell-O, flavoring/coloring additives, soy-based products, cereal/cereal bars

Table 2.6 Distribution of nutrient means across PFDI quintiles. Montero, Bolivian women of child-bearing age, 2016; n=160.

Dietary content	Mean	Interquartile range	PFDI quintiles 1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	51.0–62.1	58.8	58.7	54.6	54.0	56.3	-0.12*	-0.02*
Protein	15.4	13.2–17.5	15.2	16.3	16.3	15.1	14.1	-0.14	-0.13
Total fat	29.3	24.1–34.3	28.1	26.7	30.1	31.6	30.1	0.12*	0.03**
Saturated fat	8.4	6.6–9.8	8.4	7.6	8.8	8.8	8.4	0.04	-0.01
Polyunsaturated fat	6.7	5.0–8.0	6.7	6.5	6.9	7.4	6.2	-0.00	-0.05
Monounsaturated fat	11.4	9.2–13.1	11.1	10.6	11.2	12.3	12.0	0.10	0.06
Trans fat	1.9	0.3–1.3	1.2	2.3	1.6	3.1	1.3	0.02	0.06
Dietary fiber (g/1000 kcal)	10.1	7.5–11.8	12.2	11.6	10.0	8.8	7.8	-0.34***	-0.31***
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	501.4	281.1–615.0	684.3	566.7	489.1	447.9	319.0	-0.24***	-0.37***
Vitamin C (mg/1000 kcal)	59.9	34.5–75.9	80.9	61.5	69.0	43.8	44.5	-0.24***	-0.15**
Thiamin (mg/1000 kcal)	1.0	0.6–1.0	1.2	1.3	0.8	0.8	0.7	-0.13*	-0.07
Riboflavin (mg/1000 kcal)	1.0	0.6–1.0	1.1	1.3	0.9	0.8	0.9	-0.06	-0.02*
Niacin (mg/1000 kcal)	31.3	16.2–39.2	35.7	36.4	32.5	27.1	25.0	-0.13*	-0.10
Calcium (mg/1000 kcal)	249.1	155.2–299.1	219.1	281.1	270.5	233.20	241.6	-0.00	-0.02
Iron (mg/1000 kcal)	7.3	5.8–8.1	7.6	8.5	7.4	7.2	6.1	-0.19**	-0.16
Magnesium (mg/1000 kcal)	112.3	87.4–126.3	124.4	124.8	115.5	106.9	90.0	-0.22***	-0.22*
Phosphorus (mg/1000 kcal)	495.7	409.2–547.3	489.4	530.7	510.9	479.9	467.4	-0.08	-0.08
Sodium (mg/1000 kcal)	1751.3	1347.0–1936.6	1986.8	1621.5	2029.6	1518.8	1599.7	-0.08	-0.10
Potassium (mg/1000 kcal)	1188.4	925.9–1365.6	1454.2	1227.7	1247.3	1046.1	966.5	-0.30***	-0.27***
Zinc (mg/1000 kcal)	22.8	13.9–27.8	20.8	24.9	22.5	23.4	22.7	0.02	0.11

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.7 Distribution of nutrient means across NOVA quintiles. Montero, Bolivian women of child-bearing age, 2016; n=160.

Dietary content	Mean	Interquartile range	NOVA quintiles 1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	51.0–62.1	58.6	57.7	55.5	54.6	56.0	-0.10	0.02*
Protein	15.4	13.2–17.5	15.7	16.5	16.1	14.6	14.2	-0.21**	-0.21
Total fat	29.3	24.1–34.3	27.5	27.2	30.0	31.6	30.3	0.14*	0.03**
Saturated fat	8.4	6.6–9.8	7.8	7.8	8.8	9.0	8.6	0.09	0.03
Polyunsaturated fat	6.7	5.0–8.0	6.4	6.9	7.0	7.2	6.3	0.00	-0.07
Monounsaturated fat	11.4	9.2–13.1	10.7	10.6	11.6	12.4	11.8	0.11	0.04
Trans fat	1.9	0.3–1.3	1.2	2.1	2.2	2.5	1.5	0.03	0.07
Dietary fiber (g/1000 kcal)	10.1	7.5–11.8	12.6	11.5	10.1	8.3	7.7	-0.38***	-0.37***
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	501.4	281.1–615.0	692.9	564.5	526.9	416.5	306.3	-0.26***	-0.21***
Vitamin C (mg/1000 kcal)	59.9	34.5–75.9	83.8	67.1	60.1	47.8	40.9	-0.28***	-0.19**
Thiamin (mg/1000 kcal)	1.0	0.6–1.0	0.9	1.2	1.2	0.8	0.7	-0.08*	-0.05
Riboflavin (mg/1000 kcal)	1.0	0.6–1.0	0.8	1.2	1.2	0.9	0.9	-0.01	0.02*
Niacin (mg/1000 kcal)	31.3	16.2–39.2	41.2	31.7	33.3	24.1	26.4	-0.16*	-0.13
Calcium (mg/1000 kcal)	249.1	155.2–299.1	237.7	261.1	275.0	214.5	257.2	-0.01	-0.04
Iron (mg/1000 kcal)	7.3	5.8–8.1	8.0	8.3	7.3	7.1	6.1	-0.22**	-0.22
Magnesium (mg/1000 kcal)	112.3	87.4–126.3	134.5	127.5	107.7	100.5	91.2	-0.29***	-0.29*
Phosphorus (mg/1000 kcal)	495.7	409.2–547.3	516.3	522.1	512.2	447.6	480.2	-0.13	-0.13
Sodium (mg/1000 kcal)	1751.3	1347.0–1936.6	2059.6	1628.3	1961.2	1563.8	1543.4	-0.10	-0.13
Potassium (mg/1000 kcal)	1188.4	925.9–1365.6	1507.6	1288.7	1182.6	963.1	999.7	-0.35***	-0.31***
Zinc (mg/1000 kcal)	22.8	13.9–27.8	18.8	22.4	27.1	23.2	22.6	0.06	0.17

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.8 Distribution of nutrient means across quintiles of ultra-processed foods. Montero, Bolivian women of child-bearing age, 2016; n=160.

Dietary content	Mean	Interquartile range	Quintiles of ultra-processed foods and drinks (UPFDs) 1 n=32 0-0.95%	2 n=32 0.96-5.6%	3 n=32 5.7-9.4%	4 n=32 9.5-15.0%	5 n=32 15.1-51.1%	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	51.0–62.1	56.7	59.2	55.0	55.3	56.1	-0.06	0.34*
Protein	15.4	13.2–17.5	17.4	15.2	15.7	14.9	13.6	-0.34***	-0.34**
Total fat	29.3	24.1–34.3	27.7	27.0	30.9	30.6	30.3	0.13*	0.05**
Saturated fat	8.4	6.6–9.8	7.8	7.3	8.9	9.1	8.9	0.13*	0.12*
Polyunsaturated fat	6.7	5.0–8.0	6.7	6.2	7.4	6.6	6.7	0.06	-0.02
Monounsaturated fat	11.4	9.2–13.1	10.7	11.6	12.0	11.5	11.4	0.04	-0.02
Trans fat	1.9	0.3–1.3	1.6	2.6	3.0	1.4	0.8	-0.08	-0.06
Dietary fiber (g/1000 kcal)	10.1	7.5–11.8	12.8	10.4	10.0	8.8	8.3	-0.31***	-0.29***
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	501.4	281.1–615.0	719.6	562.8	423.3	415.8	385.4	-0.23***	-0.19**
Vitamin C (mg/1000 kcal)	59.9	34.5–75.9	87.6	65.7	51.4	48.1	46.9	-0.26***	-0.20***
Thiamin (mg/1000kcal)	1.0	0.6–1.0	0.9	1.1	1.2	0.8	0.7	-0.07	-0.01
Riboflavin (mg/1000 kcal)	1.0	0.6–1.0	0.9	1.2	1.3	0.9	0.8	-0.03	0.01*
Niacin (mg/1000 kcal)	31.3	16.2–39.2	42.1	27.4	28.1	33.6	25.4	-0.12*	-0.09
Calcium (mg/1000 kcal)	249.1	155.2–299.1	243.4	235.2	258.3	229.9	278.8	0.05	0.06
Iron (mg/1000 kcal)	7.3	5.8–8.1	8.0	8.1	7.2	6.9	6.5	-0.18**	-0.16
Magnesium (mg/1000 kcal)	112.3	87.4–126.3	142.7	102.6	111.6	104.4	100.2	-0.21**	-0.19
Phosphorus (mg/1000 kcal)	495.7	409.2–547.3	555.2	472.6	493.4	462.0	495.1	-0.11*	-0.09
Sodium (mg/1000 kcal)	1751.3	1347.0–1936.6	1892.9	1713.2	1704.9	1938.1	1507.4	-0.05	-0.04
Potassium (mg/1000 kcal)	1188.4	925.9–1365.6	1541.9	1123.9	1162.0	1081.9	1032.0	-0.28***	-0.24***
Zinc (mg/1000 kcal)	22.8	13.9–27.8	24.1	25.2	21.7	21.4	21.7	-0.06	0.01

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.9 Distribution of nutrient means across quintiles of unprocessed and minimally processed foods according to PFDI indices. Montero, Bolivian women of child-bearing age, 2016; n=160.

Dietary content	Mean	Interquartile range	Quintiles of unprocessed/minimally-processed foods (PFDI) 1 (n=32) 1.2-13.6%	2 (n=32) 13.7-18.4%	3 (n=32) 18.5-23.6%	4 (n=32) 23.7-30.3%	5 (n=32) 30.4-64.0%	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	51.0–62.1	56.5	55.3	57.2	55.6	57.7	0.03	-0.02*
Protein	15.4	13.2–17.5	13.8	15.5	15.4	16.1	16.2	0.23**	0.23
Total fat	29.3	24.1–34.3	30.2	31.0	28.8	29.2	27.4	-0.11**	-0.06**
Saturated fat	8.4	6.6–9.8	8.1	9.1	8.7	8.2	7.7	-0.06	-0.03
Polyunsaturated fat	6.7	5.0–8.0	7.2	7.1	6.7	6.9	5.9	-0.10	-0.07
Monounsaturated fat	11.4	9.2–13.1	12.0	12.4	11.1	11.2	10.4	-0.12*	-0.09
Trans fat	1.9	0.3–1.3	0.7	3.4	2.2	1.8	1.5	-0.00	0.03
Dietary fiber (g/1000 kcal)	10.1	7.5–11.8	9.2	9.6	10.3	10.0	11.3	0.13*	0.13*
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	501.4	281.1–615.0	501.3	506.7	459.4	449.2	590.4	0.03	0.01*
Vitamin C (mg/1000 kcal)	59.9	34.5–75.9	40.1	68.4	53.3	59.3	78.6	0.18**	0.15**
Thiamin (mg/1000 kcal)	1.0	0.6–1.0	0.8	1.2	1.2	0.8	0.8	-0.03	-0.04
Riboflavin (mg/1000 kcal)	1.0	0.6–1.0	0.8	1.3	1.2	0.8	1.0	0.06	-0.01*
Niacin (mg/1000 kcal)	31.3	16.2–39.2	33.1	26.4	29.1	36.2	31.9	0.03	0.03
Calcium (mg/1000 kcal)	249.1	155.2–299.1	240.7	238.3	247.1	254.6	264.8	0.05	0.00
Iron (mg/1000 kcal)	7.3	5.8–8.1	6.8	7.9	8.0	6.9	7.1	-0.02	-0.02
Magnesium (mg/1000 kcal)	112.3	87.4–126.3	101.7	106.2	107.2	114.7	131.8	0.17**	0.14
Phosphorus (mg/1000 kcal)	495.7	409.2–547.3	462.2	482.9	483.9	523.6	525.7	0.05**	0.12
Sodium (mg/1000 kcal)	1751.3	1347.0–1936.6	1624.3	1899.5	1655.3	1601.4	1975.9	0.04	0.06
Potassium (mg/1000 kcal)	1188.4	925.9–1365.6	1006.8	1141.0	1123.2	1252.3	1418.5	0.24***	0.22***
Zinc (mg/1000 kcal)	22.8	13.9–27.8	18.0	26.9	20.5	20.0	28.7	0.11	0.13

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.

[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).

p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2.10 Distribution of nutrient means across quintiles of unprocessed and minimally processed foods according to NOVA indices. Montero, Bolivian women of child-bearing age, 2016; n=160.

Dietary content	Mean	Interquartile range	Quintiles of unprocessed/minimally-processed foods (NOVA) 1 (n=32) 1.2-13.6%	2 (n=32) 13.7-18.4%	3 (n=32) 18.5-23.6%	4 (n=32) 23.7-30.3%	5 (n=32) 30.4-64.0%	Standardized regression coefficient[a] Crude	Adjusted[b]
Macronutrients (% of total energy intake)									
Carbohydrates	56.5	51.0–62.1	56.3	55.5	56.8	57.0	56.7	0.03	-0.01*
Protein	15.4	13.2–17.5	14.2	15.2	15.5	15.7	16.4	0.21**	0.20
Total fat	29.3	24.1–34.3	30.1	30.3	29.7	28.4	28.0	-0.09	-0.05**
Saturated fat	8.4	6.6–9.8	8.5	8.4	8.8	8.2	7.9	-0.05	-0.04
Polyunsaturated fat	6.7	5.0–8.0	6.6	7.2	7.2	6.5	6.2	-0.05	-0.04
Monounsaturated fat	11.4	9.2–13.1	12.0	11.7	12.0	10.9	10.5	-0.11	-0.09
Trans fat	1.9	0.3–1.3	1.0	2.9	2.2	1.9	1.4	-0.00	0.01
Dietary fiber (g/1000 kcal)	10.1	7.5–11.8	8.6	9.8	10.4	10.6	10.8	0.15**	0.15*
Micronutrients (mean density)									
Vitamin A (µg/1000 kcal)	501.4	281.1–615.0	544.5	438.8	483.3	471.9	568.5	0.02	-0.00*
Vitamin C (mg/1000 kcal)	59.9	34.5–75.9	45.7	60.5	56.4	65.1	72.1	0.15**	0.12**
Thiamin (mg/1000 kcal)	1.0	0.6–1.0	0.8	0.9	1.5	0.9	0.8	0.00	-0.03
Riboflavin (mg/1000 kcal)	1.0	0.6–1.0	0.8	0.9	1.6	0.8	1.0	0.01	-0.02*
Niacin (mg/1000 kcal)	31.3	16.2–39.2	29.3	28.3	30.1	37.2	31.7	0.06	0.06
Calcium (mg/1000 kcal)	249.1	155.2–299.1	244.1	238.3	250.7	244.5	268.0	0.05	-0.01
Iron (mg/1000 kcal)	7.3	5.8–8.1	6.6	7.8	7.9	7.5	7.0	0.02	0.00
Magnesium (mg/1000 kcal)	112.3	87.4–126.3	94.3	110.6	107.2	118.7	130.7	0.20**	0.18
Phosphorus (mg/1000 kcal)	495.7	409.2–547.3	468.9	481.7	484.1	512.7	531.0	0.14*	0.10
Sodium (mg/1000 kcal)	1751.3	1347.0–1936.6	1762.4	1721.4	1728.1	1663.4	1881.1	0.02	0.04
Potassium (mg/1000 kcal)	1188.4	925.9–1365.6	1016.9	1095.7	1191.0	1259.4	1378.8	0.23***	0.21***
Zinc (mg/1000 kcal)	22.8	13.9–27.8	19.4	24.1	20.6	22.0	28.0	0.11*	0.12***

[a]The standardized regression coefficients were derived by standardizing all nutrient variables to have a mean of 0 and an SD of 1.
[b]Adjusted for age (categorical variable: 18-29, 30-39, 40-49), urbanicity (categorical variable: urban, peri-urban, rural), educational attainment (categorical variable: some primary, completed primary, completed secondary, some post-secondary); caloric intake (continuous), wealth quintiles (categorical variable: lowest, low, middle, high, highest), household food security (categorical: food secure, food insecure, moderately food insecure, extremely food insecure) and physical activity (continuous variable: total MET-minutes/week).
p-values for linear trend: *p < 0.05; **p < 0.01; ***p < 0.001

Chapter 3

Neither Processing Level of the Diet nor Consumption of Ultra-Processed Foods is Associated with Obesity Among Women of Reproductive Age in Eastern Bolivia

Introduction

The nutrition transition, characterized by rapid shifts in dietary and physical activity patterns (1) due to increasing national income and urbanization (2), is especially prominent in Latin America (3). The principal defining characteristic of dietary change has been a shift in consumption of more minimally processed, traditional foods to highly processed foods that are abundant in a Western diet (1). This dietary transition has been implicated in the rising prevalence of obesity in many low- and middle-income countries (LMICs), particularly in Latin America (3–5). Bolivia, a lower middle-income country in this region, has experienced tremendous economic growth and urbanization in the last 25 years (6,7). At the same time, the national prevalence of obesity more than tripled from 7.8% in 1994 (8) to 24.5% in 2013 (9).

Increasingly, *a posteriori* and *a priori* dietary patterns have been utilized to examine the relationship between the human diet and obesity. *A posteriori* patterns are derived from dietary data utilizing multivariate methods such as factor and cluster analysis (10) and reduced ranked regression (11), whereas *a priori* dietary patterns are frequently derived from pre-defined indices measuring diet quality (e.g., Healthy Eating Index (HEI) (12)) or adherence to specific dietary patterns (e.g., Mediterranean dietary pattern) or recommendations (e.g., Dietary Approaches to Stop Hypertension DASH diet) (13). A meta-analysis of 39 studies examining the association between *a posteriori* dietary patterns and risk of obesity in adults found a significant inverse association between "healthy/prudent dietary patterns" and risk of obesity, but no significant association between "unhealthy/Western dietary patterns" and risk of obesity (14). Additionally, a recent systematic review of studies (n=34) that utilized of a variety of diet indices to assess *a priori* dietary patterns found mixed results regarding the association of dietary indices with obesity in adults (15). Of note, the Healthy Eating Index (HEI) and its alternate versions were the

most frequently utilized index (n=13) (15). Among these particular studies, 10 demonstrated an inverse relationship with obesity related measures such that a healthier diet according to HEI indices was associated with a lower risk of obesity (15).

Recently, the examination of dietary patterns has focused on the role of food processing utilizing the NOVA classification system (16). Researchers have utilized NOVA as a "scoring system" to examine *a priori* dietary patterns based on the extent of processing in the diet and various chronic disease outcomes. Ultra-processed foods and drink products (UPFDs), defined by NOVA as industrial formulations of food and drink products that undergo processes with no domestic equivalents to create products that are ready to heat, eat, or drink (16), are a particular focus of the NOVA classification system. A number of observational studies, using heterogeneous study designs and analytical approaches, have observed a positive association between the household purchase or consumption of UPFDs (the highest level of processing as defined by NOVA) and indicators of obesity (17–23). Two well-conducted Brazilian cross-sectional studies found that, on average, UPFDs contributed an average of 25.5% (18) to 29.6% (19) of total energy intake. In the first of these studies, in analyses adjusting for sociodemographic characteristics, smoking, and physical activity, a statistically significant positive association was found between the household availability of UPFDs and BMI, as well as a 37.4% higher likelihood of obesity among those in the highest quartile of household availability of UPFDs compared to those in the lowest quartile (18). In the second of these studies, in analyses adjusted solely for sociodemographic characteristics, authors similarly found that participants that consumed the greatest proportion of UPFDs (≥ 44%) had a significantly higher BMI (0.94 kg/m^2 (95% CI=0.42,1.47)) and higher odds of obesity (1.98 (95% CI=1.23,3.12) than those that consumed the lowest proportion of UPFDs (≤ 13%) (19). Recently, evidence from a cross-sectional study of US adults adjusted for sociodemographic characteristics, smoking, and physical activity found that consuming ≥ 74.2% versus ≤ 36.5% of UPFDs was associated with 53% higher odds of obesity (95% CI=1.29, 1.81); UPFDs ≥contributed 58% of total energy intake among Americans (23). In addition to these cross-sectional studies, three ecological studies examined the trends of UPFDs and obesity in Latin American, Sweden, and Europe. In the first, a time-series study adjusting for social and economic factors, an association between increasing annual sales of UPFDs and increasing adult

BMI from 2000 to 2009 was observed in 12 Latin American countries (24). In the second, sales of UPFDs in Sweden were observed to increase 142% from 1960 to 2010 in parallel with an estimated 15% increase in energy intake and the doubling of adult obesity prevalence (5 to 11%) from 1980 to 2010 (20). The third study among 19 European countries found the median household availability of UPFDs contribute 26.4% of total energy intake; after adjustment for national income, prevalence of physical inactivity, prevalence of smoking, obesity, and time lag between obesity and food budget surveys, every percentage point increase in household availability of UPFDs was associated with a 0.25 percentage point increase in obesity prevalence among adults (22). The only prospective study to examine the relationship between processing level of the diet and obesity, a prospective cohort study in Spain adjusting for sociodemographic characteristics, physical activity, smoking, BMI at baseline, and specific dietary behaviors, found that adults in the highest quartile of UPFD consumption were at a higher risk of developing overweight or obesity (1.26 (95% CI=1.10, 1.45) compared to those in the lowest quartile (21). However, a large cross-sectional study in the UK, also accounting for known confounders of obesity, observed no association between the intake of UPFDs and measures of obesity despite UPFDs contributing 53% of energy intake (25). To the author's knowledge, despite increasing evidence of the association between consumption of UPFDs and obesity, no studies to date have examined the direct association of NOVA scores with obesity. Therefore, our understanding of how the NOVA, utilized as a single measure of overall diet quality based on processing, relates to obesity is not well understood.

Limitations of NOVA related to how processing groups were defined and food groups were categorized, described previously **(Appendix A)**, led to the development of the Processed Food Dietary Index (PFDI) **(Appendix B)**. Building from the NOVA food classification system, the PFDI is a food processing-based index that incrementally classifies distinct food groups based on the various methods of processing different food groups endure. Differences in the number of processing groups and the incremental of nature of the PFDI led to a number of fundamental differences of how specific food groups were classified in the PFDI as compared to the NOVA system (i.e., distinguishing between unprocessed and minimally processed foods; recategorizing canned and packaged fruits, vegetables, legumes, meats by their packing solution; and redefining

flour as a processed culinary ingredient). However, the PFDI retained the NOVA definition of UPFDs; as such, UPFDs are classified the same according to both indices.

The objective of this study was to determine the relationship between the processing level of diets using both the PFDI and NOVA classification systems and measures of obesity among a sample of women of reproductive age in Montero, Bolivia, a city located on the outskirts of Santa Cruz, one of the fastest growing metropolitan areas in Bolivia (7). We hypothesized that more highly processed diets among women would be associated with a higher BMI, waist circumference (WC), and waist-to-hip ratio (WHR) and higher odds of obesity.

Methods

Study design and participant selection

The study design and sampling methods utilized for this cross-sectional sub-study have been described previously (Manuscript 1). Briefly, baseline participants of a three-year longitudinal cohort study in Montero, Bolivia, which examined regional changes in food environments, diets, and nutritional status of women of reproductive age (18-49 years) (26), were eligible for recruitment based on the extent of processing in their diet as determined from dietary data collected between August and December 2015 during the baseline of the longitudinal cohort study. In total, 160 women aged 18-49 years were randomly selected from cumulative food frequency scores; 80 representing minimally processed diets and 80 representing highly processed diets (n=160). Women with these food frequency scores were excluded from eligibility if they were known to be currently pregnant (n=7) or taking antibiotics (n=24). Between August and October 2016, trained enumerators conducted in-person interviews with selected participants collecting dietary and anthropometric data.

Measurement of variables

Dietary assessment

During a one-week period on non-consecutive days, recruited participants completed three in-person 24-hour dietary recalls on two weekdays and one weekend day using the standard multiple pass method (27). Information regarding the types and amounts of foods and beverages consumed, the methods used for preparation, and time and place of consumption were recorded

by the enumerators. Labels of consumed commercial food and beverage products were photographed when available and with permission from the participant. Food portion tables compiled from previous work in Bolivia and Peru were used to convert reported amounts of foods and beverages to grams or milliliters. Bolivian (28) and Peruvian (29) food composition tables, supplemented by the USDA National Nutrient Database for Standard Reference, Release 28 (SR28) (30), were used to estimate dietary energy, total fat, carbohydrates, protein, dietary fiber intake, and 12 micronutrients.

The PFDI and NOVA classification systems were used to classify reported food and beverage items on a scale from 0 to 4 and 1 to 4, respectively, as previously described (Manuscript 1). Culinary dishes prepared in the home were disaggregated into their constituent ingredients and scored individually when possible. When recipes were not available for such dishes, mean scores from existing recipes were utilized. The scores were then weighted by calculating each respective PFDI and NOVA score by the amount, in grams or milliliters, of each food and beverage item. Using the respective weighted PFDI and NOVA scores for each item consumed, we calculated average PFDI and NOVA scores for each participant. The distribution of PFDI and NOVA scores, respectively, were divided into five equal parts to determine quintiles.

Anthropometric assessment

The height (cm) and weight (kg) of each participant were measured in triplicate during the first home visit and recorded to the nearest 0.1 cm and 0.1 kg, respectively, using regularly calibrated, portable, digital floor scales (Seca® 874) and stadiometers (Seca® 213). Height and weight were used to calculate body mass index (BMI). Waist and hip circumference (cm) were also measured in triplicate using an ergonomic circumference measuring tape (Seca® 201) and recorded to the nearest 0.1cm. Waist circumference was measured at the iliac waist; hip circumference was measured at the broadest hip location. Waist-to-hip ratio was calculated as the waist measure relative to the hip measure. Waist circumference was utilized as a measure of abdominal obesity; waist-to-hip ratio as an additional measure of body fat distribution.

Information regarding potential confounding covariates, including age, educational attainment, physical activity level, household food insecurity, wealth status, and urban residence (i.e., rural,

peri-urban, and urban) were obtained from the baseline longitudinal cohort data. Smoking was also examined as a potential confounder; no association was found due to the small number of smokers in this sample (n=4); therefore, this variable was excluded from analyses. The date of birth of each participant was confirmed upon enrollment. Physical activity level was calculated using WHO's Global Physical Activity Questionnaire (GPAQ) by calculating the total time spent in physical activity during a typical week by the intensity of the physical activity for a total metabolic equivalent (MET) minutes per week (31). The WHO recommends ≥ 600 metabolic equivalent (MET) minutes of physical activity per week (31). The Latin American and Caribbean Household Food Security Measurement Scale (ELCSA) instrument (32) was used to measure household food insecurity (food secure, or mildly, moderately, severely food insecure). Standardized household asset scores generated from a principal component analysis were used to create an index of household wealth that was subsequently categorized into quintiles (33).

Statistical analyses

All analyses were performed using the statistical software package SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Counts and means were calculated for anthropometric measures, dietary indicators, and sociodemographic characteristics, as well as across quintiles of PFDI and NOVA scores. Pearson's chi-squared test statistics were calculated to test for differences in proportions of categorical characteristics across quintiles of PFDI and NOVA scores. Due to small cell values (< 5), Fisher's exact tests were used to calculate exact p-values to determine associations between categorical covariates across PFDI and NOVA quintiles. When unable to compute a Fisher's exact test p-value, a Monte Carlo approximation (10,000 simulation replicates) was implemented to obtain an accurate p-value without cell-count constraint. F-statistics from one-way ANOVA were calculated to test for differences in means of covariates across PFDI, NOVA, and UPFD quintiles. One-way ANOVA was also used to calculate F-statistics to test for differences in crude and adjusted mean BMI, waist circumference, and waist-to-hip ratio across PFDI, NOVA, and UPFD quintiles. Post-hoc analyses were conducted using the Tukey procedure to test differences in means between pairs of quintiles. Estimated crude and adjusted odds ratios (ORs) of obesity were calculated using logistic regression. Adjusted analyses controlled for total energy intake, age, urban residence, educational attainment, physical activity,

household wealth, and food insecurity. Associations were considered consistent with random variation at $p>0.05$.

Results

Two of the 160 women enrolled in the study initiated antibiotic treatment following enrollment and were replaced with a randomly selected eligible replacement participant. The total analytical sample was 160 women. Mean BMI among women in the sample was 29.6; more than three quarters of the women were overweight or obese (76.9%) (Table 3.1). The average energy intake of the women was 1,669 kcal/day with 29.3% of energy from total fat, 56.5% from carbohydrates, and 15.4% from protein. Differences in proportions and means of anthropometric measures, dietary indicators, and sociodemographic characteristics across quintiles of PFDI and NOVA scores are outlined in Table 3.2 and Table 3.3, respectively. Mean BMI and categories of BMI were not statistically significantly different across PFDI or NOVA quintiles. Mean caloric intake was statistically significantly different across PFDI and NOVA quintiles (caloric intake: $p < .0001$). We observed no differences across quintiles of PFDI and NOVA scores in the proportion of participants meeting the WHO recommendation for minimum physical activity level. A larger proportion of participants in the highest quintiles of PFDI and NOVA scores lived in urban areas as compared to lower quintiles ($p=0.05$).

Using ANOVA, we observed differences in mean BMI, WC, and WHR across quintiles of PFDI scores (BMI: $p=0.0002$; WC: $p=0.0004$; WHR: $p=0.001$), as well as differences in mean BMI and WC across quintiles of NOVA scores (BMI: $p=0.001$; WC: $p=0.0001$) (Table 3.4). Mean BMI and WC were higher in the first quintile (BMI: 33.1 [95% CI: 30.9, 35.3]); WC: 93.9 cm [95% CI: 88.6, 99.2]) as compared to the fifth quintile (BMI: 30.2 [95% CI: 28.1, 32.3]); WC: 88.5 cm [95% CI: 83.5, 93.5]) of PFDI scores. This trend was also observed for NOVA scores, with higher BMI and WC values in the first quintile (BMI: 31.9 [95% CI: 29.7, 34.1]); WC: 93.6 cm [95% CI: 88.5, 98.8]) as compared to the fifth quintile (BMI: 30.5 [95% CI: 28.4, 32.6]); WC: 89.7 cm [95% CI: 84.7, 94.7]). We had hypothesized that we would observe a positive linear relationship between mean BMI, WC, and WHR measurements with quintiles of PFDI and NOVA scores. However, when examining pairwise comparisons of these outcomes using a post

hoc Tukey test, we observed no differences in mean BMI, WC, or WHR between any pairs of PFDI and NOVA quintiles.

We also utilized ANOVA to examine differences in BMI, WC, and WHR across quintiles of UPFDs (BMI: p=0.0007; WC: p=0.0002; WHR: p=0.0374) (Table 3.5), the highest level of processing according to both PFDI and NOVA classification systems, with the hypothesis that increasing intake of UPFDs would be positively associated with measures of obesity. Mean BMI and WC were actually highest in second quintile of UPFD intake (BMI: 31.6 [95% CI: 29.5, 33.7]); WC: 92.6 cm [95% CI: 87.7, 97.5]) and lowest in the fifth quintile (BMI: 29.2 [95% CI: 27.0, 31.4]); WC: 86.7 cm [95% CI: 81.5, 91.8]). Post hoc Tukey analysis showed no differences in mean BMI, WC, or WHR between any pairs of UPFD quintiles.

Finally, we used logistic regression to calculate the odds of obesity based on the processing level of the diet and proportion of total energy from UPFDs (Table 3.6). Neither the PFDI (OR=0.55; 95% CI: 0.23, 1.35; p=0.19), NOVA (OR=0.77; 95% CI: 0.28, 2.13; p=0.62), nor energy intake from UPFDs (OR=0.98; 95% CI: 0.94, 1.02; p=0.38) were associated with obesity among women in this sample.

Discussion

In this cross-sectional study we investigated the relationship between the processing level of diet and obesity among a population of Bolivian women of child-bearing age. We utilized an average PFDI and NOVA score as a single measure of overall diet quality to examine the extent to which an overall processed diet was associated with BMI, WC, and WHR. We also examined whether the share of UPFDs in the diet was associated with obesity in these women. Adjusting for covariates, we detected no differences in mean anthropometric outcomes (BMI, WC, and WHR) between any pairs of PFDI, NOVA, or UPFD quintiles. Unexpectedly, mean BMI was highest in both the first PFDI and NOVA quintiles and lowest in the second PFDI quintile and third NOVA quintiles. WC displayed a similar trend, with mean WC the highest in both the first PFDI and NOVA quintiles and lowest in the third PFDI and NOVA quintiles.

We observed that caloric intake was higher across increasing quintiles of the PFDI and NOVA. Utilizing the PFDI, caloric intake monotonically increased from 1359 kcal/day (quintile 1) to 1951 kcal/day (quintile 5) (Table 3.2). Similarly, caloric intake increased from 1470 to 1997 kcal/day between quintiles 1 and 5 of NOVA scores (Table 3.3). This may be due to the substitution of more energy-dense, processed foods for less energy-dense, less processed foods. Highly processed foods are generally more refined, lower in water content, and contain larger quantities of fats and/or sugars than more minimally processed foods, contributing to increased caloric load. People tend to consume a constant weight or volume of food rather than a constant quantity of energy (34–39). Therefore, substituting the consumption of more highly processed foods for more minimally processed foods could result in the consumption of a greater amount of energy. For example, 100 grams of potato chips contain 2.4 grams of water and contribute 545 kcal (30), whereas 100 grams of baked potato contain 74.9 grams of water and contribute 93 kcal (30). An increase in adiposity and weight could result if excessive caloric intake is not offset with increased physical activity.

We hypothesized that more highly processed diets would be associated with higher BMI, WC, and WHR. A substantial body of research has analyzed the relationship between the macronutrient composition of various dietary patterns (e.g., low-carbohydrate, low-fat, Mediterranean, low-glycemic load) and body weight regulation or weight loss (40). These studies have comprehensively demonstrated that caloric restriction, not macronutrient composition, is the key determinant of long-term weight loss (40). Thus, excessive caloric intake, not macronutrient composition, is likely the key dietary determinant of long-term weight gain that leads to obesity. Therefore, we expected to observe an association between measures of obesity and differences in caloric intake associated with more highly processed diets. However, despite higher caloric intakes among women with more highly processed diets, neither PFDI nor NOVA quintiles were associated with differences in BMI, WC, and WHR.

There are a number of reasons why we may not have observed an association between the processing level of diet and the anthropometric measures we assessed. First, both NOVA and the PFDI are *a priori* dietary patterns and there have not been consistent results regarding the association of dietary indices and obesity in adults (15). Second, it is evident from the higher

caloric intake across increasing quintiles of PFDI and NOVA scores and the low energy intake from UPFDs in this population, that caloric displacement may not be driven solely by increased consumption of UPFDs, but by other degrees of processing as well (e.g., adding sugar to beverages, eating fried chicken in place of grilled chicken, consuming shallow or pan-fried starchy vegetables in place of cooked or raw vegetables). Therefore, it is important to examine how all levels of processing comprehensively contribute to obesity by using a single measure of diet quality based on processing, such as average PFDI or NOVA scores. Third, we have a very low mean proportion of UPFD consumed in this population (9.5%) compared to other studies that found an association between UPFD intake and measures of obesity (18,22,23,41). This difference is likely attributable to the more limited availability of UPFDs in lower-middle income countries such as Bolivia, as evidenced by lower (but rapidly increasing) per capita sales of UPFDs as compared to higher income countries (24,42). At the population level, low intake of UPFDs, a very large and wide-ranging food group, narrows the range of average PFDI and NOVA scores as well as the range of proportions of UPFDs consumed. This makes it particularly difficult to distinguish if, for example, an average PFDI or NOVA score of 2.3 is representative of a more minimally processed diet than an average score of 2.6 or if consuming 5% of energy intake from UPFDs is healthier than consuming 15%. Furthermore, these small differences at the population level make it even more difficult to detect if they are associated with differences in nutritional status, especially when such a large proportion of the population is obese.

This study had several strengths including the application of a 24-hr dietary recall instrument designed to capture the details needed to accurately classify foods and beverages according to their respective processing level groups with the PFDI and NOVA classification systems. In addition, to the author's knowledge, this study for the first time examines the association between the scores of *a priori* processed based dietary pattern indices and obesity. However, the study has several limitations. First, the study is cross-sectional and therefore the causal nature of the observed associations cannot be established. Furthermore, though we collected three days of dietary recall, these data may not reflect long-term dietary habits and usual food intakes. Despite rigorous training of survey enumerators on the dietary recall method employed, estimates of dietary energy intake were also likely underestimated—a common source of measurement error with dietary recall (43). Nonetheless, given the adaptation of our dietary recall method to

accurately classify foods and beverages according to their respective processing level groups, we expect that misclassification of foods and beverages according to processing level was limited. Finally, the sample size of the study may have provided limited statistical power to observe some relationships despite our sample size calculation suggesting sufficient power due to UPFDs not being as prevalent in the diet as expected.

Conclusion

Despite a high prevalence of excess weight among the Bolivian women sampled in this study (76.9% overweight or obese), the processing level of the diet and/or the proportion of UPFDs consumed was not associated with BMI or WC. Furthermore, the results indicate that caloric displacement in the diet may not be driven solely by increased consumption of UPFDs, but by other degrees of processing as well (e.g., adding sugar to beverages). This finding supports the importance of examining how all levels of processing, not only UPFDs, impact the diet and may contribute to obesity.

While more highly processed diets are associated with increased caloric intake and potential weight gain, the types of foods and dietary patterns from which calories originate must also be considered. For example, fruits, vegetables, nuts, and whole grains can protect against chronic disease while consumption of refined grains and sugar-sweetened beverages can increase chronic disease risk (44). There is also evidence that these same foods can both aid in weight control as well as contribute to weight gain (44). Therefore, utilizing the PFDI or NOVA as a single measure of diet quality is as important as examining the proportion of UPFDs in the diet.

The development, application, and validation of a tool (i.e., food-based index) that best measures overall diet quality across global food patterns is essential to understanding how to best prevent future chronic disease and obesity, particularly in LMICs. Whether that tool is solely based on processing (e.g., the PFDI or NOVA), or incorporates measures of processing with other dietary attributes, (e.g., components of the HEI), also warrants further consideration.

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Table 3.1 Anthropometric measures, dietary indicators, and sociodemographic characteristics of women of child-bearing age in Montero, Bolivia (n=160).

	n	% or mean ± SD
Anthropometric measures		
BMI	160	29.6 ± 5.6
Under-[a] and normal weight (BMI < 25)	37	23.1
Overweight (≥ 25 BMI < 30)	53	33.1
Obese (BMI ≥ 30)	70	43.8
Dietary indicators		
Caloric intake, kcal/day	160	1669.1 ± 553.6
Total fat, % caloric intake	160	29.3 ± 7.1
Carbohydrates, % caloric intake	160	56.5 ± 8.0
Protein, % caloric intake	160	15.4 ± 3.3
Fiber, grams/day	160	16.4 ± 6.9
Sociodemographic characteristics		
Age (years)	160	32.6 ± 8.9
18-29	63	39.4
30-39	56	35.0
40-49	41	25.6
Parity	160	2.7 ± 2.0
Physical activity, meets WHO recommendation[a]	88	55.0
Highest attained education level (n=155)		
Attended primary	10	6.5
Completed primary	63	40.6
Completed secondary	66	42.6
Some post-secondary	16	10.3
Urbanicity		
Urban	71	44.4
Peri-urban	51	31.9
Rural	38	23.8
Household food security		
Food secure	64	40.0
Minimal food insecurity	50	31.3
Moderate food insecurity	28	17.5
Severe food insecurity	18	11.3
Wealth quintiles		
Lowest	30	18.8
Low	28	17.5
Middle	42	26.3
High	30	18.8
Highest	30	18.8

[a] Two participants had a BMI < 18.5

[b] The WHO recommends ≥ 600 metabolic equivalent (MET) minutes of physical activity per week as calculated using the Global Physical Activity Questionnaire (GPAQ)

Table 3.2 Distribution of anthropometric measures, dietary indicators, and sociodemographic characteristics by quintiles of PFDI scores.

		Quintiles of PFDI scores					
		1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	p-value
Anthropometric measures	*n*	% or mean ± SD					
BMI	**160**	32.2 ± 6.2	29.4 ± 5.7	28.7 ± 4.1	29.4 ± 29.2	28.2 ± 5.5	0.05[b]
Under-[a] and normal weight (BMI < 25)	37	5.4	21.6	21.6	24.3	27.0	
Overweight (≥ 25 BMI < 30)	53	22.6	22.6	18.9	17.0	18.9	0.37[c]
Obese (BMI ≥ 30)	70	25.7	17.1	20.0	20.0	17.1	
Dietary characteristics							
Caloric intake, kcal/day	**160**	1359.3 ± 360.7	1520.7 ± 467.8	1611.0 ± 407.1	1903.6 ± 541.6	1951.0 ± 707.0	< 0.0001[b]
Sociodemographic characteristics							
Age, years	**160**	35.0 ± 9.2	33.1 ± 8.2	33.7 ± 9.8	30.7 ± 8.4	30.4 ± 8.4	0.17[b]
18-29	63	17.5	17.5	19.0	25.4	20.6	
30-39	56	17.9	23.2	17.9	16.1	25.0	0.67[d]
40-49	41	26.8	19.5	24.4	17.1	12.2	
Parity	**160**	3.2 ± 2.0	2.4 ± 1.7	2.7 ± 2.0	3.2 ± 2.5	2.2 ± 1.5	0.16[b]
Physical activity, meets WHO guidance[d]	88	20.5	22.7	17.0	17.0	22.7	0.53[d]
Highest attained education level	**155**						
Attended primary	10	40.0	10.0	10.0	30.0	10.0	
Completed primary	63	27.0	20.6	17.5	19.0	15.9	0.42[e]
Completed secondary	66	12.1	19.7	22.7	21.2	24.2	
Some post-secondary	16	6.3	25.0	25.0	12.5	31.3	
Urbanicity	**160**						
Urban	71	9.9	18.3	23.9	22.5	25.4	
Peri-urban	51	23.5	21.6	19.6	13.7	21.6	0.05[c]
Rural	38	34.2	21.1	13.2	23.7	7.9	
Household food insecurity	**160**						
Food secure	64	26.6	17.2	12.5	21.9	21.9	
Minimally food insecure	50	20.0	24.0	26.0	16.0	14.0	0.48[e]
Moderately food insecure	28	14.3	21.4	17.9	25.0	21.4	
Severely food insecure	18	5.6	16.7	33.3	16.7	27.8	
Wealth quintiles	**160**						
Lowest	30	16.7	14.3	33.3	20.0	16.7	
Low	28	25.0	10.7	28.6	14.3	21.4	
Middle	42	23.8	19.0	16.7	21.4	19.0	0.15[e]
High	30	13.3	16.7	13.3	36.7	20.0	
Highest	19	31.6	5.3	15.8	10.5	36.8	

[a] Two participants had a BMI < 18.5 and were in the 4th and 5th quintiles of PFDI scores; [b] p-value for F-statistic of one-way ANOVA; [c] Exact p-value determined using Fisher's exact test; [d] p-value for chi-square test statistic; [e] p-value from Monte Carlo approach (10,000 simulated replications); [f] The WHO recommends ≥ 600 metabolic equivalent (MET) minutes of physical activity per week as calculated using the Global Physical Activity Questionnaire (GPAQ)

Table 3.3 Distribution of anthropometric measures, dietary indicators, and sociodemographic characteristics by quintiles of NOVA scores.

		Quintiles of NOVA scores					
		1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	p-value
Anthropometric measures	*n*	% or mean ± SD					
BMI	**160**	30.8 ± 6.0	30.6 ± 6.3	29.2 ± 4.8	28.9 ± 4.9	28.6 ± 5.7	0.39[b]
Under-[a] and normal weight (BMI < 25)	37	18.9	8.1	24.3	18.9	29.3	
Overweight (≥ 25 BMI < 30)	53	15.1	32.1	15.1	20.8	17.0	0.20[c]
Obese (BMI ≥ 30)	70	24.3	17.1	21.4	20.0	17.1	
Dietary characteristics							
Caloric intake, kcal/day	**160**	1469.9 ± 402.8	1416.2 ± 421.1	1554.8 ± 383.2	1908.0 ± 540.2	1996.7 ± 709.5	< 0.0001[b]
Sociodemographic characteristics							
Age, years	**160**	34.0 ± 8.1	34.8 ± 9.6	30.3 ± 9.1	29.9 ± 8.2	33.8 ± 8.9	0.08[b]
18-29	63	19.0	14.3	19.0	23.8	23.8	
30-39	56	19.6	25.0	14.3	17.9	23.2	0.40[c]
40-49	41	22.0	22.0	29.3	17.1	9.8	
Parity	**160**	2.5 ± 1.6	2.7 ± 2.1	3.3 ± 2.5	2.2 ± 1.5	3.0 ± 1.9	0.17[b]
Physical activity, meets WHO guidance[d]	**88**	20.5	23.9	15.9	18.2	21.6	0.45[d]
Highest attained education level	**155**						
Attended primary	10	50.0	0.0	20.0	20.0	10.0	
Completed primary	63	23.8	25.4	14.3	22.2	14.3	0.07[e]
Completed secondary	66	13.6	16.7	22.7	21.2	25.8	
Some post-secondary	16	6.3	18.8	37.5	6.3	31.3	
Urbanicity	**160**						
Urban	71	11.3	14.1	25.4	21.1	28.2	
Peri-urban	51	17.6	27.5	19.6	19.6	15.7	0.05[c]
Rural	38	39.5	21.1	10.5	18.4	10.5	
Household food insecurity	**160**						
Food secure	64	23.4	17.2	17.2	23.4	18.8	
Minimally food insecure	50	28.0	20.0	26.0	12.0	14.0	0.14[e]
Moderately food insecure	28	3.6	32.1	14.3	25.0	25.0	
Severely food insecure	18	11.1	11.1	22.2	22.2	33.3	
Wealth quintiles	**160**						
Lowest	30	6.7	30.0	23.3	23.3	16.7	
Low	28	17.9	25.0	14.3	28.6	14.3	
Middle	42	28.6	21.4	11.9	16.7	21.4	0.25[e]
High	30	16.7	10.0	23.3	26.7	23.3	
Highest	30	26.7	13.3	30.0	6.7	23.3	

[a] Two participants had a BMI < 18.5 and were in the 4th and 5th quintiles of PFDI scores; [b] p-value for F-statistic of one-way ANOVA; [c] Exact p-value determined using Fisher's exact test; [d] p-value for chi-square test statistic; [e] p-value from Monte Carlo approach (10,000 simulated replications); [f] The WHO recommends ≥ 600 metabolic equivalent (MET) minutes of physical activity per week as calculated using the Global Physical Activity Questionnaire (GPAQ)

Table 3.4 Difference in mean body mass index, waist circumference, and waist-to-hip ratio across quintiles of PFDI and NOVA scores.

		Mean (95% CI)	Quintiles of PFDI and NOVA scores					
			1 (n=32)	2 (n=32)	3 (n=32)	4 (n=32)	5 (n=32)	p-value[a]
Body mass index (kg/m^2)		29.6 (28.7, 30.5)						
Crude model	PFDI		32.2 (30.3–34.1)	29.2 (27.3–31.2)	28.7 (26.8–30.6)	29.2 (27.2–31.1)	28.6 (26.7–30.5)	0.0539[c]
	NOVA		30.8 (28.8–32.7)	30.6 (28.6–32.5)	29.2 (27.2–31.1)	28.9 (26.9–30.8)	28.6 (26.6–30.5)	0.3935[c]
Multivariate[b]	PFDI		33.1 (30.9–35.3)	29.8 (27.7–31.9)	30.2 (28.1–32.3)	30.4 (28.3–32.5)	30.2 (28.1–32.3)	0.0002[c]
	NOVA		31.9 (29.7–34.1)	31.4 (29.1–33.7)	29.7 (27.6–31.8)	30.4 (28.2–32.6)	30.5 (28.4–32.6)	0.0010[c]
Waist circumference (cm)		87.3 (85.2, 89.4)						
Crude model	PFDI		91.9 (87.2–96.5)	88.5 (83.9–93.1)	85.1 (80.4–89.7)	86.1 (81.5–90.8)	84.9 (80.3- 89.5)	0.1880[c]
	NOVA		90.6 (86.0–95.3)	90.3 (85.6–94.9)	84.5 (79.9–89.1)	85.6 (81.0–90.3)	85.5 (80.8–90.1)	0.1961[c]
Multivariate[b]	PFDI		93.9 (88.6–99.2)	90.2 (85.2–95.3)	87.8 (82.8–92.8)	88.6 (83.4–93.7)	88.5 (83.5–93.5)	0.0004[c]
	NOVA		93.6 (88.5–98.8)	92.5 (87.1–97.9)	85.6 (80.7–90.5)	88.3 (83.2–93.4)	89.7 (84.7–94.7)	0.0001[c]
Waist-to-hip ratio		0.83 (0.82, 0.85)						
Crude model	PFDI		0.82 (0.80–0.85)	0.86 (0.83–0.89)	0.83 (0.80–0.86)	0.83 (0.80–0.86)	0.83 (0.80–0.86)	0.4420[c]
	NOVA		0.83 (0.80–0.85)	0.86 (0.83–0.88)	0.84 (0.81–0.86)	0.83 (0.81–0.86)	0.82 (0.79–0.85)	0.5276[c]
Multivariate[b]	PFDI		0.82 (0.78–0.85)	0.87 (0.84–0.90)	0.84 (0.81–0.87)	0.83 (0.80–0.86)	0.85 (0.82–0.88)	0.0145[c]
	NOVA		0.83 (0.79–0.86)	0.86 (0.82–0.90)	0.84 (0.81–0.87)	0.84 (0.81–0.87)	0.84 (0.81–0.87)	0.0517[c]

[a] F-statistic

[b] Adjusted caloric intake (continuous), age (categorical), parity (continuous), physical activity (categorical), education (categorical), urbanicity (categorical), household food insecurity (categorical), and wealth (categorical)

[c] Post-hoc analysis using Tukey test revealed no statistically significant differences in mean BMI, waist circumference, or waist-to-hip ratio between pairs of PFDI or NOVA score quintiles

Table 3.5 Difference in mean body mass index, waist circumference, and waist-to-hip ratio across quintiles of UPFDs.

		Quintiles of UPFDs					
	Mean (95% CI)	1 (n=32) 0 – 0.95%	2 (n=32) 0.96 – 5.6%	3 (n=32) 5.7 – 9.4%	4 (n=32) 9.5 – 15.0%	5 (n=32) 15.1 – 51.1%	p-value[a]
Body mass index (kg/m^2)	29.6 (28.7, 30.5)						
Crude model	29.6 (27.7–31.6)	30.6 (28.6–32.5)	30.6 (28.7–32.5)	29.1 (27.1–31.0)	28.1 (26.2–30.1)	0.3509[c]
Multivariate[b]	30.8 (28.6–32.9)	31.6 (29.5–33.7)	31.3 (29.2–33.5)	30.2 (28.0–32.3)	29.2 (27.0–31.4)	0.0007[c]
Waist circumference (cm)	87.3 (85.2, 89.4)						
Crude model	87.2 (82.5–91.8)	89.3 (84.7–94.0)	90.8 (86.1–95.4)	85.8 (81.2–90.4)	83.4 (78.8-88.0)	0.1967[c]
Multivariate[b]	89.1 (84.0–94.1)	92.6 (87.7–97.5)	90.7 (85.7–95.8)	88.5 (83.5–93.5)	86.7 (81.5–91.8)	0.0002[c]
Waist-to-hip ratio	0.83 (0.82, 0.85)						
Crude model	0.83 (0.80–0.86)	0.84 (0.82–0.87)	0.83 (0.80–0.86)	0.84 (0.81–0.87)	0.83 (0.80–0.86)	0.9106[c]
Multivariate[b]	0.83 (0.80–0.86)	0.86 (0.83–0.89)	0.83 (0.80–0.86)	0.85 (0.82–0.88)	0.84 (0.81–0.88)	0.0374[c]

[a] F-statistic
[b] Adjusted for caloric intake (continuous), age (categorical), parity (continuous), physical activity (categorical), education (categorical), urbanicity (categorical), household food insecurity (categorical), and wealth (categorical)
[c] Post-hoc analysis using Tukey test revealed no statistically significant differences in mean BMI, waist circumference, or waist-to-hip ratio between pairs of UPFDs score quintiles

Table 3.6 Odds ratios of obesity by PFDI, NOVA scores, and UPFD intake.

	OR	95% CI	p-value[a]
PFDI			
Crude model	0.57	0.28–1.15	0.12
Multivariate[b]	0.55	0.23–1.35	0.19
NOVA			
Crude model	0.74	0.34–1.63	0.46
Multivariate[b]	0.77	0.28–2.13	0.62
UPFD			
Crude model	0.99	0.95–1.02	0.40
Multivariate[b]	0.98	0.94–1.02	0.38

[a] Z-statistic
[b] Adjusted for caloric intake (continuous), age (categorical), parity (continuous), physical activity (categorical), education (categorical), urbanicity (categorical), household food insecurity (categorical), and wealth (categorical)

Chapter 4

Processing Level of the Diet and Obesity Show Heterogenous Associations with Major Phyla and Diversity of the Gut Microbiome

Introduction

The gut microbiome – the trillions of bacteria, but also archaea, viruses, parasites, and fungi that reside in the digestive tract – is relatively stable (1) and diverse (2) within healthy adults. Its composition is influenced by many factors, including: genetics, age, hygiene, sanitation, geography, urbanicity, climate, antibiotic use, and most importantly, diet. Habitual diet is recognized as the key regulator of its composition (i.e., density and diversity of taxa) (3–8), which plays a critical role in modulating metabolism and energy balance.

Studies examining the impact of long-term dietary patterns on the gut microbiome have detected significant differences in taxonomy (i.e., ratio of prominent phyla *Firmicutes/Bacteroidetes*), diversity (i.e., number of distinct species), and complexity (i.e., gene richness) in gut microbiota related to the composition of the diet between Western and non-Western populations (9–11). From a nutritional standpoint, "Western" diets are typically described as higher in total fat, saturated fat, animal protein, and added sugar and lower in complex carbohydrates and dietary fiber than non-Western diets. This generally translates to a diet higher in animal products and more highly processed food and beverages, and lower in fruits, vegetables, and whole grains. Various methods and degrees of food processing – from home preparation to industrial manufacturing – are also known to influence the structure of the gut microbiome. This includes heat processing (i.e., cooking) (8,12), frying (12), fermentation (13,14), the refinement of grains (15,16), and the use of food preservatives and additives (e.g., emulsifiers) (6,8,17–24). Therefore, not only is the composition of the gut microbiome impacted by the proportions of macronutrients in the diet, but it is also affected by various food preparation, processing, and preservation practices.

The transition to a "Western" diet has been implicated in the rising prevalence of obesity in low- and middle-income countries (LMICs) (25). Dysbiosis of the gut microbiome – disruptions to the relative abundance and diversity of distal gut bacteria – has also been linked to obesity (26–29). A shift in the ratio of bacterial flora belonging to the *Firmicutes* and *Bacteroidetes* phyla, which jointly comprise over 90% of the adult gut microbiota, is frequently referenced as a key factor that differentiates obese and lean individuals, with a higher abundance of *Firmicutes* and a lower abundance of *Bacteroidetes* in those who are obese (26,27,30,31). However, some studies have failed to find statistically significant differences in the *Firmicutes to Bacteroidetes* (*F/B*) ratio between obese and lean individuals (32–37) or have found a predominance of *Bacteroidetes* in overweight and obese individuals (38). Low diversity of gut bacterial flora has also been linked to obesity (39,40).

Nutrition researchers have recently begun utilizing the NOVA classification system (41) – a pre-defined measure of diet quality based on food processing – to examine the role of food processing on health-related outcomes **(Appendix A)**. In response to a number of limitations of how processing levels were defined using NOVA, the Processed Food Dietary Index (PFDI) was developed as an alternate schema **(Appendix B)**. Both indices define a range of processing groups, from "unprocessed/minimally processed" to "ultra-processed", without consideration of whether a food or beverage is originally from an animal or plant source. Ultra-processed foods and drink products (UPFDs), the highest level of processing in both the NOVA and PFDI classification systems, are defined as industrial formulations of food and drink products that undergo processes with no domestic equivalents to create products that are ready to heat, eat, or drink (41). UPFDs are especially unique in that these products often contain numerous types of additives (e.g., emulsifiers) that are known to impact the structure of the gut microbiome.

To the best of our knowledge, no studies have utilized NOVA or any other classification systems based on food processing to examine whether the processing level of the diet influences the taxonomy of the gut microbiome. We identified one study that utilized a *degree of difference line scale* to differentiate individual food items as "processed" or "fresh" to quantify the food quality and examine associations with obesity and gut microbiota among US residents (42). This study found that consumption of processed foods influenced the composition of the gut microbiome

more so than an overweight or obesity (42). While a number of studies have examined differences in Western and non-Western diets and the gut microbiome, we are also unaware of any studies that have examined these or similar differences within the same population or solely within a non-Western population that is undergoing a nutrition transition.

The objective of this study was to compare the gut microbiota among a population of Bolivian women of child-bearing age that: 1) consumed a highly processed vs. minimally processed diet (as measured using the PFDI classification system); 2) consumed a diet high in proportion of UPFDs vs. a diet in which no UPFDs were consumed, and 3) were obese vs. healthy weight. We hypothesized that for each comparison we would observe significant differences in the prominent phyla and in the amount of diversity found within the gut microbiome. Specifically, we hypothesized that a higher *F/B* ratio and less diversity in the gut microbiome would be observed among study participants who ate a more highly processed diet vs. a minimally processed diet, a high proportion of UPFDs vs. no UPFDs, and were obese vs. healthy weight.

Methods

Study design and participant selection

The study design and participant sampling methods employed for this comparative study have been described previously **(Chapter 2)**. Briefly, women of child-bearing age who were baseline participants of a three-year cohort study, which examined regional changes in food environments, diets, and nutritional status of women of child-bearing age in Montero, Bolivia (43), were eligible for recruitment based on the extent of processing in their diet as determined from dietary data. Eighty participants with minimally processed diets and 80 participants with highly processed diets were randomly selected from cumulative food frequency scores for a total of 160 women aged 18-49 years. At the time of recruitment, women who were known to be currently pregnant (n=7) were excluded from participation. Recognizing the short-term ability of antibiotic exposure to reduce the diversity of gut bacterial taxonomy in adults (44–49), women who took a course of antibiotics 30 days prior to or at the time of recruitment (n=24) were also excluded; women who began a course of antibiotics during their interview week (n=2) were replaced. During a 10-week period between August and October 2016, trained enumerators administered three in-person interviews during a one-week period with randomly selected

eligible participants to collect dietary recall data, anthropometric measurements, and fecal samples.

Measurement of variables

Dietary and anthropometric assessment

The dietary and anthropometric assessment have been described previously **(Chapter 3)**. Briefly, recruited participants completed three in-person non-consecutive 24-hour dietary recalls on two weekdays and one weekend day during a one-week period using the standard multiple pass method (50). Compiled food portion tables from previous work in Bolivia and Peru were used to convert reported amounts of foods and beverages to grams or milliliters. Dietary energy was estimated using Bolivian (51) and Peruvian (52) food composition tables, supplemented by the USDA National Nutrient Database for Standard Reference, Release 28 (SR28) (53). The PFDI and NOVA classification systems were then used to categorize reported food and beverage items, which were then weighted by their quantity, as described previously **(Chapter 2)**. An average PFDI and NOVA score were calculated for each participant using the respectively weighted food and beverage item scores. Due to a high correlation between PFDI and NOVA scores ($r=0.94$, $p<.0001$) we only used the PFDI scores in analyses.

Among the 160 participants, the average PFDI scores were normally distributed and ranged from 0.53 to 2.97. To examine differences in microbiota between processing levels of diet we selected participants with a PFDI score $>$ 2.0 to represent a more highly processed diet (n=25) and participants with a PFDI score $<$ 1.0 to represent a more minimally processed diet (n=20).

The percentage of energy intake from each of the PFDI processing level groups, including UPFDs, was calculated for each participant (n=160). The consumption of UPFDs was right-skewed with a range of 0 to 51.1% (i.e., proportional contribution of UPFDs to total energy intake). To examine differences in microbiota between ranges of UPFD intake, we selected participants who consumed $>$ 20% of energy intake from UPFDs (n=22) and participants who consumed 0% of energy intake from UPFDs (n=19).

Body mass index (BMI) (weight (kg)/height (m)^2) was calculated for each participant from height (cm) and weight (kg) measurements taken during the first in-person visit, as described previously **(Chapter 3)**. BMI was used to classify participants as underweight (BMI <18), healthy weight (≥18 BMI <25), overweight (≥25 BMI <30), or obese (BMI ≥30). Obese (n=70) and healthy weight (n=35) participants were retained for analyses.

Fecal sample collection and storage

Recruited participants were provided with instructions during the first in-person interview regarding the collection and storage of their fecal matter using the OMNIgene GUT® Kit/OMR-200 (DNA Genotek, Inc.; Ontario, Canada). A kit was left with each participant after the first in-person interview to collect a sample prior to the second in-person interview, when it subsequently was collected. Participants were then left with an additional kit, which was collected during the final in-person interview. A total of two (2) kits were collected from each participant. Upon collection, the kits were held in cold storage (-80°C) until they were promptly air-couriered frozen on dry ice to the University of Michigan where they continued to be held in cold storage (-80°C) until they were aliquoted three days later for analysis.

DNA isolation and amplification

DNA was isolated from 320 human fecal samples with a PowerMag Microbiome RNA/DNA Isolation Kit (Mo Bio Laboratories, Inc.) using an epMotion 5075 liquid handling system. The V4 region of the 16S rRNA gene was amplified from the fecal samples by standard PCR using 1 μl DNA as described by Seekatz et al (54). Concentrated samples that failed to amplify using standard PCR were diluted 1:10 (n=145), 1:50 (n=73), and 1:100 (n=30). A total of three (n=3) samples failed to amplify. Amplicons were then processed and sequenced on the Illumina MiSeq platform as described by Seekatz et al (54).

Analysis of microbiota community

The 16S rRNA gene sequence data was processed and analyzed using the software package mothur (m.1.40.2. and v.1.39.5) and the most recent Schloss MiSeq SOP (55,56) as of May 2018 (56). Upon sequencing and alignment to the SILVA reference alignment (release 128) (57,58), sequences were binned into operational taxonomic units (OTUs) based on 97% sequence

similarity using the OptiClust method (59). We sub-sampled 2271 sequences per sample; due to non-amplification or low sequence counts, 8 samples were excluded. Each pair of samples originating from the same participant were pooled, that is, their sequence counts were added together. For the 8 cases with only one sample per participant, the corresponding composite sample was extrapolated from the available data (i.e., the counts were doubled), creating 160 composite samples that were used in the subsequent analyses. θ_{YC} distances (a metric that takes relative abundances of both shared and non-shared OTUs into account) (60) were calculated between communities. We investigated the taxonomic composition of the bacterial communities by classifying sequences with mothur using a modified version of the Ribosomal Database Project (RDP) training set (version 16) (61,62).

Statistical analyses

Inverse Simpson Diversity Index (iSDI) analyses were performed using mothur; non-parametric Kruskal-Wallis analyses were performed with the SciPy library (63). Values for iSDI, a measure of diversity which considers the number of species present (i.e., richness), as well as the relative abundance of each species (the higher the number, the higher the diversity), were calculated for each comparison group. H-statistics from the nonparametric Kruskal-Wallis test were calculated to test for differences in median phyla abundance, *F/B* ratios, and median iSDI values within comparison groups. Differences in median phyla abundance, *F/B* ratios, and median iSDI were considered consistent with random variation at $p>0.05$.

Results

Our final analytical sample included participants in the following comparison groups: PFDI (PFDI score >2.0 (n=25) vs. PFDI score <1.0 (n=20)); UPFD (>20% of energy intake from UPFDs (n=22) vs. 0% of energy intake from UPFDs (n=19)); and BMI (obese BMI (n=70) vs. healthy BMI (n=35)). Some participants were in more than one comparison group. For example, a participant could have been in the group PFDI score >2.0, >20% of energy intake from UPFDs, and/or obese BMI (n=70), or any combination thereof. Differences between respective comparison groups in median abundance percentages of major phyla, *F/B* ratios, and median iSDI values are outlined in **Table 4.1**.

We utilized the Kruskal-Wallis test to examine differences in median phyla abundance and the *F/B* ratio. The relative abundance of *Firmicutes* was comparable across all comparison groups. The relative abundance of *Bacteroidetes* varied between PFDI score groups (median (interquartile range)): 33.4% (27.5-45.6) in highly processed diet group, 46.1% (36.9-55.0) in minimally processed diet group; p=0.04); and BMI groups: 42.7% (34.5-56.0) in obese group, 38% (29.0-49.9) in healthy BMI group **(Table 4.1)**. We hypothesized that we would observe a higher *F/B* ratio among participants with a PFDI score >2.0, consumed >20% of energy intake from UPFDs, and were obese. While *F/B* ratios varied between PFDI groups: median (interquartile range): 1.43 (0.83-1.68) in PFDI score >2.0 group; 0.87 (0.68-1.35) in PFDI score <1.0 group, the ratios between the UPFD (p=0.30) and BMI groups (p=0.18) were not statistically significantly different.

We also utilized the Kruskal-Wallis test to examine differences in median iSDI values **(Table 4.1)**. We observed a difference in median iSDI between participants in the UPFD groups (median (interquartile range): 13.8 (11.2-24.5) in >20% of energy intake from UPFDs group, 9.3 (6.2-12.7) in 0% of energy intake from UPFDs group. A difference between participants in the BMI comparison groups approached significance (median (interquartile range)): 12.3 (7.3-24.4) in the obese group, 17.5 (11.4-32.2) in the healthy BMI group. Median iSDI was not statistically significantly different between participants in the PFDI comparison groups (p=0.48). Interestingly, the least diverse samples belonged to participants who consumed 0% energy intake from UPFDs (median (interquartile range): 9.3 (6.2-12.7) (p=0.009); the most diverse belonged to participants with a healthy BMI: 17.5 (11.4-32.2) (p=0.07).

Discussion

In this cross-sectional comparative study, we investigated compositional differences in the gut microbiome among a population of Bolivian women of child-bearing age related to the processing level of the diet and BMI. Specifically, we examined differences in the relative median abundance of major microbial phyla, *F/B* ratio, and median diversity between groups of participants that represented consumption of a highly or minimally processed diet as calculated utilizing an average PFDI score as a single measure of diet quality, extremes of UPFD energy intake consumption, and obese and healthy BMI. We detected differences in the median

abundance of *Bacteroidetes* between PFDI groups, which drove the differences we then observed between their respective *F/B* ratios. No differences in *F/B* ratios were observed between the UPFD or BMI comparison groups, respectively. We detected a difference in diversity between UPFD comparison groups but no difference was observed between BMI or PFDI comparison groups.

While we observed a higher *F/B* ratio among participants with a PFDI score >2.0 and >20% of energy intake from UPFDs, as hypothesized, this observation only approached significance for participants with a PFDI score >2.0. This is intriguing because both a higher PFDI score and a large share of energy intake from UPFDs represent a higher level of processing in the diet. Participants with a PFDI score >2.0 represent those with the highest cumulative, single measure of processing in the diet while participants with >20% of energy intake from UPFDs represent those with the most ultra-processed foods in the diet. These results may imply that the overall level of processing in the diet more adequately reflects the macronutrient composition of the diet than the dietary share of UPFDs. The macronutrient composition of diets has been shown previously to be associated with significant differences in taxonomy and diversity in gut microbiota (9–11). Therefore, to the extent that these different indicators of processing level of the diet differentiate macronutrient intake, it is plausible that overall level of processing in the diet may be a greater influence on the composition of the gut microbiome than proportion of energy from UPFDs. It would be prudent to examine the relationship between UPFD intake and *F/B* ratio among participants along a gradient of higher percent of energy intake from UPFDs (e.g., >20%, >30%, >40%, etc.) to observe if and at what point the relationship becomes statistically significant. However, few participants in our sample had high levels of energy intake from UPFDs (mean UPFD energy intake was 9.5% among the entire sample) **(Chapter 2)**.

We also observed that the abundance of *Firmicutes* (p=0.32) and *F/B* ratios (p=0.18) between our BMI comparison groups were not statistically significantly different (p=0.18); there was not a higher abundance of *Firmicutes* among the obese study participants. A difference in the abundance of *Bacteroidetes* approached significance (p=0.08) between the BMI comparison groups, with a higher abundance among obese women, also in contrast to the widely accepted concept that obese individuals have a higher abundance of *Firmicutes* and lower abundance of

Bacteroidetes than those who are lean. These findings are supported by other studies that did not find statistically significant differences in the *F/B* ratio between obese and lean individuals (32–37). While controlled animal studies in mice have found consistent differences in *F/B* ratios between obese and lean mice such that obese mice had a higher proportion of Firmicutes and lower proportion of Bacteroidetes than lean mice (26,28,64–67) conflicting findings in *F/B* ratios among human subjects may be driven by insufficient control for as of yet unidentified confounding factors within field studies of free-living subjects (38).

We hypothesized that we would observe a less diverse gut microbiome among participants that consumed a more highly processed diet (PFDI score > 2.0) and a higher proportion of UPFDs based on previous research that found lower diversity in the gut microbiome among European populations consuming a Western diet as compared to African populations consuming a traditional diet (9,11), as well as macronutrient similarities between a "Western" diet and a "highly processed diet". However, we did not observe any difference in gut microbiota diversity between participants in the PFDI comparison groups (p=0.48). Furthermore, the differences we observed between UPFD energy intake groups were the reverse of what we had hypothesized – participants that consumed 0% UPFDs had a less diverse microbiome than their respective counterparts. This difference in microbiota diversity observed between UPFD consumption groups was surprising, but not unprecedented. A study comparing the gut microbiota of rural Africans and U.S.-based African Americans found that the microbiota of African Americans was more diverse (10). The authors theorized that African Americans may have a more diversified diet (10). This theory could be extended to our study as well. A diet that contains > 20% of energy intake from UPFDs may reflect greater diversity in the diet than one that contains few or no UPFDs. Such a diet might therefore contribute to greater gut microbial diversity. We had also hypothesized that we would observe a less diverse gut microbiome among obese participants as compared to healthy weight participants. Indeed, obese participants had a less diverse gut microbiome (median (interquartile range)): 12.3 (7.3-24.4) in the obese group, 17.5 (11.4-32.2) in the healthy BMI group. This difference approached statistical significance (p=0.07). These results are supported by previous human gut microbial composition exploratory studies comparing obese and lean individuals that found reduced phylogenetic diversity (39) and lower bacterial richness (40) in obese individuals.

Upon analyzing the preceding results, there was an apparent disconnect between the findings. Based on the association between the transition to a 'Western' or more highly processed diet and obesity in LMICs, we had hypothesized that we would observe a higher *F/B* ratio and less diversity in the gut microbiome among participants who consumed more highly processed diets and were obese. However, this was not the case and could be related to a finding in a previous study **(Chapter 3)** which found no association between the processing level of the diet, dietary share of UPFDs, and obesity in this study population. It may be possible that the etiology of obesity in this population is being driven by non-diet related factors, such as epigenetics and changes to physical activity patterns.

This study had several strengths including the application of a repeat 24-hr dietary recall instrument to collect detailed dietary data, the collection of two fecal samples from each participant to account for variability in the diet, and robust comparison groups for which to examine differences in the gut microbiota. To the author's knowledge, this study for the first time characterizes the gut microbiome of a population of women in Bolivia, a country undergoing the nutrition transition, and is the first to examine differences in gut microbiota related to diet and obesity within a non-Western population. A limitation of this study includes the multiple freeze thaws the fecal samples underwent during their storage and transport between Bolivia and the US. These freeze thaws, while minimized to the extent possible, may have contributed to DNA degradation and altered the detection of bacterial taxa in our samples (68).

Conclusion

Our findings from a sample of Bolivian women of child-bearing age indicate that the processing level of the diet may influence the proportions of *Firmicutes* and *Bacteroidetes* at the phylum level present in the human gut. In addition, consistent with previous evidence, obesity is associated with a less diverse gut microbiome. We also found evidence that the proportion of UPFD intake in the diet is associated with the diversity of the gut microbiome, possibly by influencing the diet diversity. Overall, our results raise further questions regarding the relationship between processing level of the diet and gut microbiota, as well as questions regarding how to best measure processing - as a single measure of diet quality, or as proportion of UPFD intake – to analyze this relationship.

As obesity continues to rise globally and we advance our understanding of how the diet drives the composition of the gut microbiota, it will be important for researchers to have access to food-based measurement tools in which to examine differences in dietary patterns and how each relates to obesity and the gut microbiome. Utilizing the PFDI or NOVA to measure processing in the diet, or other tools that measure diet quality, rather than comparing "Western" dietary patterns to "non-Western" dietary patterns, which are often not defined similarly across studies, will be important for advancing this research.

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Table 4.1 Median and interquartile range of major gut microbial phyla and iSDI values across comparison groups of women of child-bearing age in Montero, Bolivia.

	PFDI >2.0 (n=25)	PFDI <1.0 (n=20)	p-value[a]	> 20% energy intake UPFDs (n=22)	0% energy intake UPFDs (n=19)	p-value[a]	Obese (BMI ≥30) (n=70)	Healthy weight (≥18 BMI <25) (n=35)	p-value[a]
Firmicutes	47.8 (38.9–54.0)	40.3 (34.0–48.0)	0.12	44.4 (38.0–51.4)	39.8 (33.6–46.4)	0.22	44.0 (35.2–51.7)	43.9 (37.8–56.7)	0.32
Bacteroidetes	33.4 (27.5–45.6)	46.1 (36.9–55.0)	0.04	42.8 (32.9–54.1)	48.6 (37.5–55.1)	0.42	42.7 (34.5–56.0)	38.0 (29.0–49.9)	0.08
F/B ratio[b]	1.43 (0.83–1.68)	0.87 (0.68–1.35)	0.06	1.04 (0.74–1.59)	0.82 (0.64–1.18)	0.30	1.03 (0.62–1.52)	1.16 (0.80–1.72)	0.18
iSDI[c]	14.3 (9.9–23.1)	9.7 (6.9–24.1)	0.48	13.8 (11.2–24.5)	9.3 (6.2–12.7)	0.009	12.3 (7.3–24.4)	17.5 (11.4–32.3)	0.07

[a] H-statistic from Kruskal-Wallis test
[b] F/B ratio: proportion of major phyla *Firmicutes* to *Bacteroidetes*
[c] iSDI: values calculated using inverse Simpson's Diversity Index

Chapter 5

Conclusion

This dissertation examined the extent to which the processing level of diets of women of reproductive age (18-49 years) in Bolivia, a country undergoing the nutrition transition, is associated with obesity and gut microbiome composition. Evidence linking obesity to the nutrition transition in low-and middle-income countries (LMICs) is best established in women (1–5) and the gut microbiome is relatively stable in adults whereas it is still developing in young children (6).

Chapter 2 assessed the nutrient adequacy of both NOVA and the Processed Food Dietary Index (PFDI), *a priori* food processing classification systems, and characterized the processing level of diets among our study population. Chapter 3 examined the association between the processing level of the diet and obesity, using both NOVA and the PFDI as a single measure of overall dietary quality. Chapter 4 assessed the association between the PFDI, obesity, and gut microbiome composition.

Chapter 2

In Chapter 2, we described the four processing level groups of the NOVA classification system (Group 1: Unprocessed or minimally processed foods; Group 2: Processed culinary ingredients; Group 3: Processed foods; Group 4: Ultra-processed food and drink products (UPFDs)) (7), its limitations, and its current application in research. To address the limitations of the NOVA classification system we subsequently developed the Processed Food Dietary Index (PFDI), a food processing-based classification system built on the foundation of NOVA. The PFDI defines foods and beverages into one of five processing level groups – “unprocessed”, “minimally processed”, “moderately processed”, “highly processed”, and “ultra-processed foods and drinks”.

Upon distinguishing differences among the lower processing level groups and discussing how the PFDI and NOVA defined the highest level of food processing, ultra-processed foods and drinks (UPFDs), equivalently, we utilized both the PFDI and NOVA to examine and compare the relationships between energy and nutrient intakes and the dietary share of processing level groups. Our research was unique in that it was the first to utilize both the PFDI and NOVA classification systems as a single measure of diet quality. The higher the PFDI or NOVA score, the greater degree of processing in the diet.

Examining the distribution of total energy intake by PFDI food processing groups, we found that as the PFDI diet quality score increased, fewer calories were derived from less processed foods and more calories were derived from foods that were more highly processed. We observed a similar trend using the NOVA classification system. As the NOVA diet quality score increased, fewer calories were derived from unprocessed and minimally processed foods and culinary processed ingredients and more calories were derived from processed foods and UPFDs.

Unprocessed foods and minimally processed foods contributed 1.9% and 19.9% of total energy intake, respectively. Moderately processed foods contributed the most energy (37%) out of the five PFDI food processing groups; whereas highly processed foods only contributed 31.8% of total energy. Unexpectedly, despite an increase in energy intake from UPFDs across both PFDI and NOVA quintiles, we found that the mean proportion of energy intake from UPFDs in this population (9.5%) was relatively small, especially when compared to other Latin American middle-income countries. Studies in Brazil, Chile, and Mexico estimate that UPFDs contribute 21.5 to 29.8% of total energy intake (8–10). Although per capita sales of UPFDs are rapidly increasing in Bolivia, the availability of UPFDs is still lower compared to higher income countries (11), contributing to the relatively low intake in our study population.

Utilizing both the PFDI and NOVA classification systems as single measures of diet quality, we examined the distribution of nutrient means across PFDI and NOVA quintiles, adjusted for age, educational attainment, physical activity, household wealth, food insecurity, urban residence, and total energy intake. We hypothesized that a more highly processed diet would contribute the highest density of fat, saturated fat, *trans* fat, carbohydrates, and sodium; and

conversely, the lowest density of vitamins, minerals (excluding sodium), and dietary fiber. In short, we observed heterogenous associations with macro- and micronutrients across quintiles of both PFDI and NOVA sores. Observed trends were not always in the expected direction; if they were, there were often not statistically significant. These trends were observed to be in the direction expected and statistically significant across quintiles of PFDI and NOVA scores for carbohydrates (NOVA scores only), total fat, dietary fiber, vitamin A, vitamin C, riboflavin, magnesium, and potassium.

Similarly, we also examined the distribution of nutrient means across quintiles of the dietary share of UPFDs and unprocessed and minimally processed foods, adjusting for the same potential confounders from the previous analyses. Inverse directional trends of association were observed for a number of nutrients across both PFDI and NOVA quintiles representing the extremes of consumption of processed foods; these trends were observed to be in the expected direction and statistically significant for total fat, dietary fiber, vitamin C, and potassium.

A number of previous studies also examined the distribution of nutrient means across the dietary share of UPFDs (12–15); in comparing these studies we found mixed results regarding the significance and direction of expected trend for a number of nutrients. However, the mean dietary share of UPFDs varied widely (9.5 to 57.5% of total energy intake) (12–15), the study populations were different, and crude models were not adjusted for the same confounding variables within these studies, all of which may have contributed to differences in mean nutrient intake trends.

We attributed trends observed in an unexpected direction and with lack of statistical significance to the consumption trends of particular food groups within the processing level groups unique to this study population. For example, while soft drinks were the most popular UPFD consumed, they only contributed 3.7% of total energy intake as compared to homemade beverages (traditional drinks, teas, coffees, freshly squeezed juices) in which sugar was added contributing 17.7% of total energy intake. We also attributed unexpected trends to a decrease of *trans* fat from the food supply, inaccurate food composition tables, and the enrichment and fortification of foods across all levels of processing. While there is uncertainty regarding how widespread and to

what degree food products in Bolivia are enriched or fortified (16), enrichment – replacing vitamins and minerals lost during processing – would attenuate expected trends of micronutrients in the middle and higher levels of processing (e.g., moderately processed flours enriched with B vitamins, highly processed products made from enriched flours), and fortification – adding extra nutrients to foods – would attenuate expected nutrient trends across the spectrum of processing (e.g., minimally processed milk fortified with vitamin D, 100% fruit juice fortified with calcium; UPFDs fortified with dietary fiber and a variety of micronutrients). UPFDs are often fortified or modified at the macronutrient level as well, as these products are often fortified with protein and "low-, reduced-fat, and fat-free" versions of foods are often manufactured using carbohydrates and additives as fillers.

In conclusion, while expected energy intake trends correlated with the processing levels as defined by the PFDI and NOVA, expected nutrient intake trends did not. However, the low percentage UPFD consumption (9.5% of total energy intake) in this population brings into question the relevance of the calculated nutrient values across PFDI, NOVA, and UPFD quintiles. Utilizing the PFDI, NOVA, or any other food processing classification system with UPFDs, as the processing group is currently defined, may not be appropriate to use with low- and middle-income populations in which a UPFDs do not constitute a large share of dietary intake. The minimum mean threshold of UPFD intake as a proportion of the overall diet for nutrient intake trends to be considered relevant is undetermined.

We also found that despite identifying a number of limitations regarding how processing groups were defined and food groups were classified according the NOVA classification system with the development of the PFDI, there were very few differences in the direction and significance of nutrient trends between the two systems, presumably due to the dietary pattern and size of the study population. Future research in this field should consider comparing nutrient trends utilizing both the PFDI and NOVA using dietary data from a much larger and Westernized population where the diet is more varied and a larger proportion of the diet is derived from UPFDs.

Our findings, in conjunction with other studies examining nutrient intake trends across quintiles of UPFD intake (12–15), suggest that nutrients cannot be used at this time to validate

classification systems or indices based on processing, despite our knowledge of how various methods of processing effect the loss or addition of nutrients to foods.

Chapter 3

In Chapter 3, we utilized the PFDI and NOVA classification systems as a single measure of overall dietary quality and the dietary share of UPFDs to analyze the association between the processing level of the diet and measures of obesity. We hypothesized that with an increase in the processing level of the diet, BMI, waist circumference (WC), and waist-to-hip ratio (WHR) would also increase.

Upon examining the distribution of mean BMI, WC, and WHR across PFDI, NOVA, and UPFD quintiles, adjusting for age, educational attainment, physical activity, household wealth, food insecurity, urban residence, and total energy intake, we detected no differences in mean anthropometric outcomes between any pairs of PFDI, NOVA, or UPFD quintiles. Notably, mean BMI was highest in the first PFDI and NOVA quintiles and second UPFD quintile and lowest in the second PFDI quintile, third NOVA quintile and fifth UPFD quintile. WC presented a similar trend, with mean WC the highest in both the first PFDI and NOVA quintiles and second UPFD quintile and lowest in the third PFDI and NOVA quintiles and fifth UPFD quintiles. In summary, values of BMI and WC were higher at the lower quintiles of processing and lower in the middle and higher quintiles of processing – BMI and WC did not increase with an increase in the processing level of the diet.

Regardless of neither detecting differences in mean anthropometric outcomes between pairs of PFDI, NOVA, or UPFD quintiles, nor observing the expected direction of trend, we recognized that caloric intake was higher across increasing PFDI (quintile 1: 1359 kcal/day; quintile 5: 1951 kcal/day) and NOVA quintiles (quintile 1: 1470 kcal/day; quintile 5: 1997 kcal/day). This finding is consistent with the substitution of minimally processed, less energy-dense foods with more highly processed, more energy-dense foods as the processing level of the diet increases.

Numerous studies have demonstrated that caloric restriction is the key determinant of long-term weight loss (17); therefore, excessive caloric intake is likely the key determinant of long-term weight gain that leads to obesity. Correspondingly, as caloric intake was higher across increasing quintiles of the PFDI and NOVA indices, we expected to observe an association between measures of obesity and differences in caloric intake associated with more highly processed diets. However, despite higher caloric intakes among women with more highly processed diets, the differences were not significant enough between PFDI or NOVA quintiles to be associated with differences in BMI, WC, or WHR. It may also be that diet is not the key factor driving obesity in this study population; the interaction of other genetic, environmental, and socioeconomic factors, as well as individual behaviors such as physical activity, may have a greater influence on the development of excess body weight in this population (18).

Our analysis is among many *a priori* dietary pattern studies that failed to find associations between dietary indices and obesity related measures in adults (19). It may be that the current construct (e.g., number of processing groups, values/weights assigned to processing levels, averaged scores, etc.) of the PFDI and NOVA classification systems needs refinement for use as a single measure of dietary quality. In Chapter 2 we found that PFDI and NOVA scores in this population ranged from 0.53 to 2.97 and 1.17 to 3.02, respectively. While this was the first study to our knowledge that utilized NOVA and the PFDI as a single measure of dietary quality and we have no other basis on which to discern the range of scores, we were expecting a larger range, particularly extending on the higher end of processing (+3.0). Subsequently, we discovered that energy intake from UPFDs, a robust and wide-ranging processing group, was relatively low (9.5%) compared to other Latin American middle-income countries (8–10), but also other countries where studies found an association between UPFD intake and measures of obesity (20–22). The low intake of UPFDs most likely narrowed the range of PFDI and NOVA scores considerably in this population, making it difficult to distinguish differences between scores and to interpret their meaning, as well as to detect if they are associated with differences in nutritional status, especially in our study population with a large prevalence of excess weight (76.9% overweight or obese). However, by examining the dietary share of UPFDs in relationship to measures of obesity in this population, we realized that caloric displacement is not driven solely by UPFDs in this population, but by other degrees of processing as well. Reviewing the

distribution of total energy intake by PFDI food processing groups in Chapter 2, we found that moderately processed (37.0%) and highly processed foods (31.8%) contribute a much larger share of total dietary energy than UPFDs (9.5%). Therefore, while the current construct of the PFDI and NOVA classification systems may not lend themselves to accurately portraying the level of processing in the diet as a single measure of diet quality, we continue to recommend that future research considers a measure of food processing which comprehensively evaluates how each level of processing contributes to the health outcome in question.

Chapter 4

In Chapter 4, we utilized the PFDI as a single measure of overall dietary quality and the dietary share of UPFDs to analyze the association between the processing level of the diet, obesity, and gut microbiota composition. Specifically, we compared the abundance of phyla *Firmicutes* and *Bacteroidetes*, *Firmicutes/Bacteroidetes (F/B)* ratio, and diversity (measured using the inverse Simpson Diversity Index (iSDI)) of the gut microbiota across three comparison groups: PFDI (PFDI score >2.0 vs. PFDI score <1.0); UPFD (>20% of energy intake from UPFDs vs. 0% of energy intake from UPFDs); and BMI (obese BMI vs. healthy BMI). We hypothesized that we would observe a higher *F/B* ratio and less diversity in the gut microbiome among participants who consumed more highly processed diets (as measured by PFDI score > 2.0 and > 20% energy intake from UPFDs) and were obese.

Upon examination of the *F/B* ratios between processing groups, we observed a higher *F/B* ratio among participants with a PFDI score >2.0 and >20% of energy intake from UPFDs; however, this observation only approached significance for the PFDI score groups (p=0.06). While we are not confident in what these findings imply, these results may indicate that within this specific population, utilizing a single measure of diet quality (i.e., PFDI score) may more adequately reflect the macronutrient and dietary fiber composition of the diet – driving the composition of the gut microbiome – than the dietary share of UPFDs. By analyzing the distribution of macronutrient and dietary fiber proportions across levels of processing from Chapter 2, we observed that the value and differences between quintiles 1 and 5 across PFDI scores **(Table 2.6)**, representative of the PFDI comparison groups, (carbohydrates (Q1: 58.8%; Q5: 56.3%), dietary fiber (Q1: 12.2 g/1000 kcal; Q5: 7.8 g/1000 kcal), protein (Q1: 15.2%; Q5: 14.1%), and

total fat (Q1: 28.1%; Q5: 30.1%)) are very similar to the values and differences between quintiles 1 and 5 across dietary share of UPFDs **(Table 2.8)**, representative of the UPFD comparison groups (carbohydrates (Q1: 56.7%; Q5: 56.1%), dietary fiber (Q1: 12.8 g/1000 kcal; Q5: 8.3 g/1000 kcal), protein (17.4%; Q5: 13.6%) and total fat (Q1: 27.7%; Q5: 30.3%)). The PFDI comparison group has a larger difference in carbohydrate proportions between quintiles 1 and 5 than the UPFD comparison group; whether this is enough to drive the differences we observed in the PFDI comparison group, but not in the UPFD comparison group, remains unclear and requires further investigation.

We may have also not observed a difference in *F/B* ratios between UPFD comparison groups due to the threshold at which we set the upper limit of energy intake from UPFDs (>20%); the threshold may not have been high enough to represent a "highly processed" diet and subsequent macronutrient profile; however, the relatively low mean energy intake from UPFDs in this population (9.5%) limited our sample size. Upon examining the distribution of participants across of UPFD quintiles in Chapter 2, only 20% (n=32) of participants consumed greater than 15% of energy intake from UPFDs. This is a far from the average 58% of total energy intake UPFDs are estimated to contribute to the American diet (22), which is generally considered, along with other Westernized countries, highly processed. In fact, relatively low UPFD intake may have contributed to not observing statistically significant differences in both processing comparison groups but not driving differences in their macronutrient profiles.

In comparing the calculated *F/B* ratios between the BMI comparison groups, we observed they were not statistically different (p=0.18), in contrast to our hypothesis based on the widely accepted theory, promoted by controlled animal (23–28) and human (24,29–31) studies that obese individuals have a higher abundance of *Firmicutes* and lower abundance of *Bacteroidetes* than lean individuals. However, our findings were consistent with other human studies that did not find statistically significant differences in *F/B* ratios between obese and lean individuals (32–37). It has been proposed that the contradictory findings in *F/B* ratio among human studies may be influenced by the contrasting environments (controlled clinical studies vs. field studies with free-living subjects) in which human studies are conducted (38). This highlights the importance

of testing whether clinical results, particularly in animals, translate into real-world outcomes for humans.

Upon comparing diversity between processing groups, we found that median iSDI was not statistically different between participants in the PFDI comparison groups (p=0.48), but that it was between participants in UPFD comparison groups (p=0.009), in the direction opposite of which we had hypothesized. Our original theory, that a more highly processed diet would be associated with a less diverse microbiome was primarily based on information regarding the impact of various modern food production processes (e.g., heat processing, addition of preservatives) decreasing both pathogenic and beneficial bacteria (39), and a lack of whole grains and dietary fiber in the diet able to nourish and sustain butyrate-producing bacteria (40). As discussed earlier in this Chapter, the narrow range of PFDI scores influenced by the low intake of UPFDs in this population, which may have contributed to not detecting a difference in nutritional status between the PFDI comparison groups, may have also contributed to not detecting a difference in microbiota diversity between these same groups. However, based on the results of another study which found U.S.-based African Americans to have a more diverse microbiota than rural Africans (41), it may be that a diet with >20% of energy intake from UPFDs is more diversified than a diet without UPFDs within this population, and contribute to the greater gut microbial diversity that we observed. Prospective research should consider examining differences in gut microbial diversity in a Western population where the range of UPFD consumption is much greater. It may be that in populations with low UPFD intake, UPFDs contribute to diet diversity and higher gut microbiota diversity, but in populations with high UPFD intake, UPFDs contribute to a decrease in beneficial bacteria and lower gut microbiota diversity.

We also observed that differences in microbiota diversity between obese and healthy weight participants approached statistical significance (p=0.07), with obese participants exhibiting a less diverse gut microbiome than their lean counterparts. These results support those found in previous studies (40, 41); however, the mechanism(s) by which this occurs is not understood and requires further exploration.

Conclusion

The contribution of this exploratory research is significant because these studies utilized a new paradigm of dietary pattern analysis to investigate the extent to which the processing level of diets may be associated with obesity and the gut microbiome. The knowledge attained through this dissertation will inform future research regarding: 1) validation of food processing classification systems; 2) the construct and utilization of food processing classification systems as a single measure of diet quality; and, 3) associations between processing level of the diet, obesity, and the gut microbiome.

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Appendix A: NOVA classification system processing group descriptions and limitations

	NOVA Group 1: Unprocessed or minimally processed foods	NOVA Group 2: Processed culinary ingredients	NOVA Group 3: Processed foods	NOVA Group 4: Ultra-processed foods and drink products (UPFDs)
Brief description*	Unprocessed foods are edible parts of raw plants and animals. Minimally processed foods are edible parts of raw plants and animals that have been processed (e.g., boiled, roasted, ground, milled) to extend their life and diversify food production. No new substances, such as salt, sugar, oils or fats, are added. Examples include: fruits, leafy and root vegetables, legumes, fungi, 100% fruit or vegetable juice; meat, poultry, fish, seafood; eggs, milk, yogurt; grains, flours, flakes, grits, pasta; seeds, tree and ground nuts; spices and herbs; and tea, coffee, and drinking water.	Processed culinary ingredients are substances used to prepare, season, and cook Group 1 foods. Examples include: salt; sugar, molasses, and honey; vegetable oils; and, butter and lard.	Processed foods are made by adding Group 2 substances to Group 1 foods. Examples include: fresh breads; fresh cheeses; canned fruits, vegetables, and legumes; salted, cured, and smoked meats; and, canned fish.	Ultra-processed food and drink products are multi-ingredient industrial formulations that typically include methods, substances, and additives not used in culinary preparation. Examples include: Packaged snacks (chips, cookies, crackers, candy); mass produced bread products; breakfast cereals; pre-made mixes; ready-to-eat and ready-to-heat products; meat and poultry extract products; reconstituted meat products (e.g., hot dogs, "nuggets") carbonated drinks, energy drinks, milk drinks, cocoa drinks, fruit drinks, etc.
Limitations	NOVA Group 1 should be split into two separate groups. Thermal and mechanical processing of plants alters its' structure, nutrients, and other bioactive compounds (1). Therefore, it has been hypothesized that raw and minimally processed fruits and vegetables affect human physiology and health outcomes differently (2-6) Although these research findings have been inconclusive, distinguishing unprocessed and minimally processed foods will provide a better understanding of what people consume, help consumers understand the differences and benefits of both groups, as well as inform public health recommendations.	It is not logical for people to think of processed culinary ingredients as a level of processing from a food-based perspective. While processed culinary ingredients, as defined by NOVA, are derivatives of whole foods, they are typically not eaten in isolation, but are used as flavoring, a cooking medium, or as an ingredient in a dish. Rename this group "Moderately processed foods" to indicate a higher level of processing from minimally processed foods. Flours should be considered a culinary processed ingredient. Whole grains undergo significant processing and refinement resulting in the loss of vitamins, fiber, and other biological components to create flours; as such, there are epidemiological differences in those who consume whole versus refined grains (7). In this group include processed culinary ingredients and foods combined or cooked with these ingredients. For example, a boiled egg is considered a minimally processed food; with the use of a minimal amount of oil as a cooking medium, a fried egg is considered a moderately processed food.	Rename NOVA Group 3 "Highly processed foods" to indicate an incrementally higher level of processing from minimally processed foods. Differences in canned foods should be distinguished and reclassified accordingly. Canned foods are cooked foods (1), and therefore, at a minimum, should be considered a 'minimally processed food' as they have undergone thermal processing resulting in chemical changes and changes in nutrient composition (8-15). However, there are stark differences in the types of canned foods available in the market. Therefore, • Classify canned fruits packed in their own juice or 100% juice; canned vegetables and legumes with 'no salt added'; and canned fish packed in water as "minimally processed foods"; • Classify canned foods packed in syrup, oil, or with added salt should be classified as "moderately processed foods"; and, • Classify canned foods with additional ingredients other than sugar, salt, or oil as "highly processed foods".	There are countless examples in the global food system in which there are categories of ultra-processed foods that undergo a higher level of industrial processing than others. One such example is with breakfast cereals. While most people would agree that ready-to-eat breakfast cereals are ultra-processed in that they undergo industrial manufacturing processes for which there is no domestic equivalent, it can also be argued that not all breakfast cereals are nutritionally the same. For example, is Shredded Wheat nutritionally equivalent to Lucky Charms? Consider distinguishing ultra-processed food and drink products into at least two separate categories and further refining their definitions.

**Descriptions from Monteiro CA, Cannon G, Levy RB, et al. NOVA. The star shines bright. World Nutr. 2016;7(1–3):28–38.*

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Appendix B: The Processed Food Dietary Index (PFDI)

	PFDI Group 0: Unprocessed foods	PFDI Group 1: Minimally processed foods	PFDI Group 2: Moderately processed foods	PFDI Group 3: Highly processed foods	PFDI Group 4: Ultra-processed foods
Description	Includes edible parts of plants and animals[a] commonly known as "fresh", "raw", "natural"; also includes drinking water. These foods may be cleaned, sliced, diced, chopped, or peeled to enable consumption; may be refrigerated/cooled as a temporary means of storage.	Includes edible parts of plants and animals that have been altered thermally (with heat or water only), mechanically[b], or naturally (e.g., fermentation) without the addition of substances such as salt, sugar, oils, or fats. This group also includes canned and packaged fruits, vegetables, legumes, or fish, that are "packed in their own juice", "packed in 100% juice", or packed with "no salt added", respectfully.	Includes culinary processed ingredients (e.g., salt; sugar, molasses, and honey; vegetable oils; and, butter and lard; flours; and foods cooked or prepared with nominal amounts of these ingredients). Also includes canned and packaged minimally processed foods with added salt, sugar, or oil.	Includes food that undergo method(s) of processing that use a higher density (as compared to moderately processed foods) of culinary processed ingredients (e.g., natural cheeses, shallow or deep-fried foods, pickled foods) or a combination of processed culinary ingredients to create a food (e.g., pasta; culinary-prepared breads, muffins, cakes, pastries, etc.	Ultra-processed food and drink products – this group is currently the same as NOVA Group 4 and includes multi-ingredient industrial formulations of products that typically use methods, substances, and additives not used in culinary preparations. These products are typically ready-to-heat, ready-to-eat, or ready-to-drink or consist of "mixes" that enable shortcuts to traditional culinary preparation.
Examples	Fresh fruits and vegetables, mushrooms Raw nuts, seeds Fresh herbs Water	Spices, dried herbs Frozen, dried, vacuum-packed, canned, cooked fruits/ vegetables/mushrooms 100% fruit juice; unsweetened applesauce Nut/seed butters Canned fish packed in water Cooked grains (e.g., brown/white rice), legumes, starchy roots/tubers, meat, poultry, fish, seafood Poached/boiled eggs Pasteurized plain milk, yogurt	Culinary processed ingredients (see above) Canned vegetables, legumes, or fruit with added salt, sugar, or packed in syrup Canned fish packed in oil "Pickled" vegetables Pan fried vegetables, meats, eggs Nut/seed butters with added salt and/or sugar Beer, cider, wine	Pasta Shallow or deep-fried foods Deli meats, rotisserie chicken Cheeses (natural) Culinary prepared flour-based products Whisky, gin, rum, vodka	Packaged cookies/biscuits, pastries, cakes, cake mixes Breakfast cereals; cereal/granola/energy bars Mass-produced packaged breads and buns Meat and chicken extracts (e.g., hot dogs, chicken nuggets); instant sauces Powdered or 'fortified' meal/dish substitutes Processed cheeses; margarines and spreads Flavored yogurts/yogurt drinks