School of Public Health

University of São Paulo

Ultra-processed foods and the nutritional quality of US diets.

Eurídice Martínez Steele

PHD thesis presented to the Postgraduate Program in Nutrition and Public Health of the University of São Paulo as a partial requirement to obtain the title of Doctor in Sciences.

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DEDICATION

This dissertation is dedicated to all members of my family, who encouraged and inspired me in many different ways throughout the course of this research and life in general. And it is especially dedicated to my supportive and encouraging husband Kiko, daughter Yara and son Gabriel, to my parents, and family, whose faithful support during all stages of this Ph.D. was crucial.

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ABSTRACT

MARTÍNEZ STEELE, E. Ultra-processed foods and the nutritional quality of US diets. [Thesis]. University of São Paulo, School of Public Health, 2017.

Background: The introduction of agricultural and animal husbandry has not provided the human genome time enough to adapt, much less the advancing technology after Industrial Revolution. According to Cordain et al., displacement of minimally processed foods by post-agricultural and post-industrial food items adversely affected the following dietary indicators: glycemic load, fatty acid and macronutrient compositions, micronutrient density, acid-base balance, sodium-potassium ratio and fiber content. Many current diseases of civilization, in turn may be ascribable to those unbalanced dietary indicators. Indeed, Raubenheimer and Simpson have proposed the Protein Leverage Hypothesis (PLH) to explain how a drop in dietary protein content might lead to obesity and associated cardiometabolic disease. **Objective:** This thesis aims to study the effect of an increased consumption of ultra-processed foods on dietary indicators in the US population, including macronutrient composition, micronutrient and fiber densities, and urinary phytoestrogens. It also explores whether the dietary share of ultra-processed foods, expressed as a percentage of total energy intake, is a meaningful determinant of overall nutritional quality of contemporary diets. Lastly, it also looks into whether the association between ultra-processed food, protein and energy consumptions fit predictions of the PLH model. **Methods:** Participants from cross-sectional 2009-2010 National Health and Nutrition Examination Survey with at least one 24-hour dietary recall were evaluated. Food items were classified according to extent and purpose of industrial food processing as: unprocessed or minimally processed foods, processed culinary ingredients, processed foods and ultra-processed foods. Manuscript 1, examines the relationship between dietary contribution of ultra-processed foods and nutritional quality of US diet through the evaluation of dietary contents of critical nutrients individually and also overall, using Principal Component Analysis (PCA). Manuscript 2 studies the association between dietary contribution of ultra-processed foods and energy intake from added sugars. Manuscript 3 examines how consumption of ultra-processed food influences relative dietary protein content and,

absolute energy and protein intakes; it furthermore, tests whether the relationships fit PLH predictions. Manuscript 4 assesses the relationship between dietary contribution of ultra-processed foods and urinary levels of phytoestrogens. **Results:** The average content of protein, fiber, vitamins A, C, D and E, zinc, potassium, phosphorus, magnesium and calcium in US diet decreased significantly across quintiles of energy contribution of ultra-processed foods, while carbohydrate, added sugars and saturated fat contents increased. An inverse dose-response association was found between ultra-processed food consumption and overall dietary quality measured through a Nutrient balanced pattern PCA derived factor score. Consistent with PLH, dietary contribution of ultra-processed foods was inversely associated with protein density and directly associated with total energy intake, while absolute protein intake remained relatively constant with increases in ultra-processed food consumption. Average urinary mammal lignan levels decreased across quintiles of ultra-processed food consumption, while isoflavone levels remained unchanged. **Conclusions:** This study suggests that decreasing the dietary share of ultra-processed foods is a rational and effective way to improve the nutritional quality of US diets.

Keywords: US, NHANES, ultra-processed, dietary nutrient profile, principal component analysis, dietary patterns, diet quality, macronutrients, micronutrients, phytoestrogens, protein leverage hypothesis.

RESUMO

MARTÍNEZ STEELE, E. Alimentos ultraprocessados e a qualidade nutricional das dietas dos EUA. [Tese]. Universidade de São Paulo, Faculdade de Saúde Pública, 2017.

Introdução: A introdução da agricultura e pecuária foram muito recentes para que o genoma humano se adaptasse e a tecnologia avançada pós revolução Industrial foi ainda mais. Segundo Cordain, a substituição de alimentos minimamente processados por alimentos pós-agrícolas e pós-industriais influenciaram os indicadores nutricionais: carga glicêmica, composição de ácidos graxos e macronutrientes, densidade de micronutrientes, equilíbrio ácido-base, relação sódio/potássio e teor de fibras, levando a um desequilíbrio que é causa de várias doenças atuais da civilização. A Protein Leverage Hypothesis (PLH) propõe que a queda na ingestão de proteínas possa levar a obesidade e doenças cardiometabólicas associadas. **Objetivos:** Estudar o efeito do consumo de alimentos ultraprocessados nos indicadores nutricionais na população dos EUA, incluindo a composição de macronutrientes, densidade de fibras e micronutrientes e fitoestrógenos urinários; avaliar se a contribuição calórica de alimentos ultraprocessados é determinante para a qualidade nutricional das dietas contemporâneas; e finalmente estudar se a associação entre o consumo de alimentos ultraprocessados, proteína e energia correspondem às previsões do modelo PLH. **Métodos:** Foram avaliados os participantes do *National Health and Nutrition Examination Survey* 2009-2010, com pelo menos um recordatório alimentar de 24 horas. Os itens foram classificados em: alimentos in natura ou minimamente processados, processados, ultraprocessados e ingredientes de uso culinário. O manuscrito 1 examina a relação entre a contribuição calórica de alimentos ultraprocessados e qualidade nutricional da dieta, avaliando individual e globalmente a contribuição de cada ingrediente crítico, usando a análise de componentes principais (ACP). O manuscrito 2 estuda a associação entre a contribuição calórica dos alimentos ultraprocessados e consumo de açúcares de adição. O manuscrito 3 avalia como o consumo de alimentos ultraprocessados influencia o conteúdo proteico relativo da dieta e as ingestões absolutas de energia e proteína, e se essas relações se encaixam nas

previsões da PLH. O manuscrito 4 avalia a relação entre a contribuição calórica de alimentos ultraprocessados e níveis de fitoestrógenos urinários. **Resultados:** O teor médio de proteínas, fibras, vitaminas A, C, D e E, zinco, potássio, fósforo, magnésio e cálcio na dieta diminuiu ao longo dos quintis de contribuição calórica de alimentos ultraprocessados, enquanto o de carboidratos, açúcares de adição e gordura saturada aumentou. Uma associação inversa de dose-resposta foi encontrada entre o consumo de alimentos ultraprocessados e qualidade nutricional total, medida através de um escore de padrão balanceado de nutrientes derivado usando ACP. Consistente com a PLH, a contribuição calórica de alimentos ultraprocessados foi inversamente associada à densidade proteica e diretamente ao consumo energético total, enquanto a ingestão absoluta de proteínas permaneceu constante com aumento do consumo de alimentos ultraprocessados. Os níveis médios de enterolignanos urinários diminuíram ao longo dos quintis de consumo de alimentos ultraprocessados, enquanto os níveis de isoflavonas permaneceram inalterados. **Conclusões:** Este estudo mostra que a diminuição da contribuição calórica de alimentos ultraprocessados é um meio racional e eficaz de melhorar a qualidade nutricional das dietas dos EUA.

Palavras- chave: US, NHANES, ultraprocessados, perfil nutricional, análise de componentes principais, padrões nutricionais, qualidade da dieta, macronutrientes, micronutrientes, fitoestrógenos, protein leverage hypothesis.

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PRESENTATION

According to Cordain, the displacement of minimally processed foods composing hunter-gatherer diets by both post-agricultural and post-industrial food items, adversely affected the following dietary indicators: glycemic load, fatty acid composition, macronutrient composition, micronutrient density, acid-base balance, sodium-potassium ratio and fiber content. Many actual health conditions, in turn may be ascribable to those unbalanced dietary indicators. Indeed, Simpson and Raubenheimer have proposed the Protein Leverage Hypothesis to explain why a drop in dietary protein content might lead to obesity and associated cardiometabolic disease (OACD). Still, Cordain et al. indicate that it was multifactorial rather than single dietary elements, which were most likely affected by the Neolithic and Industrial era changes in diet, and thus, underlie the etiology of most diseases of civilization.

Though Cordain et al. were interested in studying the effects on human diet of all dietary changes which took place from the Neolithic onwards, referring to both the introduction of food staples during the Neolithic and food-processing procedures during Industrial Periods, this research is largely concerned with the latter. However, we consider that using Cordain's nutritional indicators to measure the impact of an increased consumption of industrial foods on human diet is valid.

Under the assumption that it is the increased consumption of ultra-processed foods that elicits unbalanced dietary indicators, the objective of this thesis is to explore the association between ultra-processed food consumption in the US today, and different dietary indicators including some of those suggested by Cordain. This thesis also explores whether the dietary share of ultra-processed foods, expressed as a percentage of total energy intake, is a meaningful determinant of the overall nutritional quality of contemporary diets. Lastly, it looks into whether the association between ultra-processed food, protein and energy consumptions fit the predictions of the Protein Leverage Hypothesis (PLH) model.

In the first chapter, Cordain's hypothesis (and his dietary indicators) and the Protein Leverage Hypothesis are outlined. Subsequently, the NOVA classification is briefly

explained and, finally, a justification is provided as to the importance of studying ultra-processed food consumption across the world as well as in the US population.

In chapter 2 the study objectives are provided.

In chapter 3 the four manuscripts which compose this thesis are included. Manuscript 1, examines the relationship between the dietary contribution of ultra-processed foods and the nutritional quality of the US diet through the evaluation of dietary contents of critical nutrients individually (including macronutrient composition, micronutrient and fiber densities) and also overall, using dietary pattern analysis. Manuscript 2 assesses the contribution of ultra-processed foods to both total energy intake and the energy intake from added sugars, and quantifies the relationship between their consumption and the total dietary content of added sugars. Manuscript 3 examines how the consumption of ultra-processed food influences the relative dietary protein content and the absolute energy and protein intakes and furthermore tests whether the relationships fit the predictions of the PLH model. Manuscript 4 assesses the relationship between the dietary contribution of ultra-processed foods and the urinary levels of phytoestrogens.

In chapter 4, the final considerations are discussed, including the study strengths and limitations, future studies which may be conducted to better understand the implications of ultra-processed food consumption on health outcomes, and final conclusions.

1. INTRODUCTION

Today's human nutritional needs emerged after a multimillion year evolutionary process in which the life circumstances of ancestral species molded the genome (Eaton & Konner, 1985). Humans, like any other species, are genetically adapted to the nutritional environment in which their ancestors survived because it was this environment that conditioned their genetic makeup (Nesse & Williams, 1994; Gould, 2002; Boaz, 2002; Cordain et al., 2005). If the introduction of agricultural and animal husbandry, approximately 10,000 years ago, occurred too recently to allow the human genome to adapt (Eaton & Konner, 1985; Eaton et al., 1988; Nesse & Williams, 1994; Boaz, 2002; Cordain et al., 2005), advancing technology following the Industrial Revolution is even more the case. The food-processing procedures developed, exposed humans to both quantitative and qualitative food and nutrient combinations not previously encountered throughout hominin evolution (Cordain et al., 2005).

Because we remain nearly genetically identical to our late Paleolithic ancestors (Tooby & Cosimides, 1990), some authors have defended the use of pre-agricultural diet as the gold standard of contemporary human nutrition (O'Dea & Sinclair, 1983; Eaton & Konner, 1985; Burkitt & Eaton, 1989; Eaton et al., 1997). It must be noted, however, that there is evidence of some physiological adaptation to the upsurge of a new nutritional environment since the Upper Paleolithic at least in some populations (Wells, 2010; Simpson & Raubenheimer, 2012). Some examples may be lactose tolerance in adults and the selection of genes that confer resistance to diabetes (Gibson, 2007; Simpson & Raubenheimer, 2012).

1.1 Characteristics of pre-agricultural diets

Despite the fact that during the 5-7 million year evolution period since the emergence of hominins, diets varied according to geographic locale, climate, and specific ecologic niche, some common characteristics did exist in pre-agricultural diets (Cordain et al., 2005). Understanding the changes in these universal characteristics may help explain why the post-agricultural, and especially the post-industrial revolution eras, predisposed populations to disease (Cordain et al., 2005).

Skeletal analyses carried out in pre-agricultural population remains indicate that individuals were most probably large (Walker, 1993; Roberts et al., 1994) and lean (Ruff et al., 1993; Bridges, 1996). Height, robusticity and physical activity were the cause of an increased caloric output, demanding in turn a greater caloric intake (Eaton et al., 1997). A diet based on game and wild plants guaranteed an abundant supply of protein, micronutrients and roughage (at least in comparison to contemporary supermarket diets), while keeping the consumption of simple sugar, fat, and starch down to a minimum (Eaton et al., 1997). Protein may have comprised up to 37% of total energy intake, whereas carbohydrate was 41% and fat was 22% (Eaton et al., 1997), which is strikingly different from current US intakes (approximately 15.5% protein, 50% carbs and 32.5% fat) (based on data from NHANES 2009-2010). The micronutrient density, on the other hand, was consistently higher in the former than in the latter (Cordain et al., 2005).

1.2 Consequences of post-agricultural and post-industrial diets

Pre-agricultural hominins were scarcely exposed to dairy products, cereals, refined sugars, refined vegetable oils and alcohol, all of which make up close to three quarters of US energy intake today (Cordain et al., 2000; Cordain et al., 2005). Hominins were also obviously not exposed to the post-industrial ubiquitous food mixtures making up processed foods such as cookies, cakes, bakery foods, breakfast cereals, bagels, rolls, muffins, crackers, chips, snack foods, pizza, soft drinks, candy, ice cream, condiments, and salad dressings (Cordain et al., 2005).

According to Cordain, displacement of minimally processed foods composing hunter-gatherer diets by post-agricultural and post-industrial food items adversely affected the following dietary indicators: 1) glycemic load, 2) fatty acid composition, 3) macronutrient composition, 4) micronutrient density, 5) acid-base balance, 6) sodium-potassium ratio, and 7) fiber content (Cordain et al., 2005). These seven dietary indicators along with an additional two are briefly discussed below.

Glycemic load

The glycemic index (GI) is a relative comparison of the blood glucose raising potential of the available carbohydrates in a food or combination of foods (expressed as a percentage of the area under the glucose response curve) compared to the effect of equal amounts of carbohydrate taken as glucose (GI=100) (Jenkins et al., 1981). The glycemic load (defined as glycemic index times the carbohydrate content per serving size), on the other hand, was introduced to assess the blood glucose raising potential of a food (Liu & Willet, 2002). Acute elevation in blood glucose concentrations along with an elevation in hormones secreted from the gut in turn cause an acute rise in blood insulin concentrations (Cordain et al., 2005). Repeated consumption of high glycemic index, mixed meals results in higher mean 24 h blood glucose and insulin concentrations when compared with low glycemic index, mixed meals (Jenkins et al., 1987; Miller, 1994). Long term consumption of high glycemic load carbohydrates may induce chronic hyperglycemia and hyperinsulinemia and subsequently promote insulin resistance (Liu & Willet, 2002; Ludwig, 2002; Cordain et al., 2003). Diseases of insulin resistance, practically absent in the Paleolithic, are frequently referred to as “diseases of civilization” (Eaton et al., 1988; Reaven, 1995; Cordain et al., 2003) and include: obesity, coronary heart disease (CHD), type 2 diabetes, hypertension, and dyslipidemia (Cordain et al., 2005).

Fatty acid composition

Dietary and storage fats are mainly triacylglycerols, compounds in which 3 fatty acid molecules are bound to a single glycerol molecule. Fatty acids can be saturated (SFAs), monounsaturated (MUFAs) or polyunsaturated (PUFAs), whereas PUFAs can be either n-6 PUFAs or n-3 PUFAs. Evidence indicates that the absolute amount of dietary fat is less important than the type of fat in preventing the risk of chronic disease (Institute of Medicine of the National Academies, 2002). Whereas MUFAs and some PUFAs are health beneficial, most SFAs and trans fatty acids are detrimental when consumed in excess, increasing the risk of cardiovascular disease (CVD) (Institute of Medicine of the National Academies, 2002). Five of the six major sources of SFAs in the United States diet today (fatty meats, baked goods, cheese, milk, margarine, and butter) were not part of hominin diets before the coming-in of animal husbandry (Subar et al., 1998).

Macronutrient composition

In the current US diet, approximately 15.5% of total calories are derived from protein, 50% from carbs and 32.5% from fat (based on data from NHANES 2009-2010). These values differ substantially from current hunter-gatherer intakes, in which dietary protein is characteristically elevated (19–35% of energy) at the expense of carbohydrate (22–40% of energy) (Cordain et al., 2000; Cordain et al., 2002). Data from skeletons also suggest that protein consumption was higher during the Neanderthal and Upper Paleolithic periods (Eaton et al., 1997; Richards et al., 2000a; Richards et al., 2000b). Some authors have argued that with the shift to agriculture, there was probably an increase in the dietary carbohydrate contribution (especially starch from grains), which associated with a drop in protein and micronutrient unbalance probably increased the risk of famine and burden of disease (also due to population concentration and sedentary life) (Prentice, 2001; Prentice et al., 2008; Wells, 2010). Consequently, populations grew smaller and less healthy than in the Upper Paleolithic (Simpson & Raubenheimer, 2012). Evidence exists that high-protein diets may improve blood lipid profiles (O'Dea, 1984; O'Dea et al., 1989; Wolfe & Giovannetti, 1991; Wolfe & Giovannetti, 1992; Wolfe & Piche, 1999) and moreover decrease the risk of CVD (Hu et al., 1999; Cordain et al., 2005).

Raubenheimer and Simpson have proposed the Protein Leverage Hypothesis to further explain why a drop in protein dietary content might lead to obesity and associated cardiometabolic disease (OACD) (Raubenheimer & Simpson, 2016). Their hypothesis suggests that the strong human appetite for protein along with a low food protein density is the main driver of the high fats and carbohydrate, and in turn, total energy intakes (Simpson & Raubenheimer, 2005). This hypothesis is explained in more detail in Appendix 1.

Micronutrient density

Wild plants and muscle meat of wild animals, known to be consumed by hunter-gatherers 10,000 years ago probably contained higher micronutrient concentrations than their domesticated counterparts (Eaton & Konner, 1985; Brand-Miller & Holt,

1998). Therefore, the average micronutrient content of the diet probably declined in the Neolithic as a consequence of the introduction of dairy foods and cereal grains as staples (Cordain et al., 2005). The dietary micronutrient content further declined during the Industrial era in parallel with the development of cereal milling techniques, which allowed for the production of bread flour devoid of the bran and the germ (Storck & Teague, 1952). The substitution of more nutrient-dense foods by less-dense foods and the subsequent decline in dietary vitamin and mineral density has far-reaching consequences not only in the development of vitamin- deficiency diseases, but also numerous infectious and chronic diseases (Cordain, 1999).

Acid-base balance

Nearly all foods, after metabolism release either acid or bicarbonate (base) into the systemic circulation (Frassetto et al., 1998; Sebastian et al., 2002). While fish, meat, poultry, eggs, shellfish, cheese, milk, and cereal grains are net acid producing, fresh fruit, vegetables, tubers, roots, and nuts are net base producing (Cordain et al., 2005). Legumes yield near-zero mean acid values, whereas energy-dense, nutrient-poor foods such as separated fats and refined sugars are neither acid nor base releasing (Cordain et al., 2005). Additionally, salt is net acid producing because of the chloride ion (Frassetto et al., 1998).

Some authors have argued that while throughout most of hominin evolution, diets were net base releasing, during the Neolithic and Industrial Eras diets became net acid-producing due to the displacement of base-yielding fruit and vegetables by cereals and energy-dense, nutrient poor foods (Sebastian et al., 2002). It has been estimated that Western diets yield a net acid load ranging from 10 to 150 mEq/d (diet potential bicarbonate yield) (Lennon et al. 1966; Kurtz et al., 1983; Frassetto et al., 1996; Lemann, 1999) with a mean value of 50 mEq/d (Lemann, 1999). Despite the multiple homeostatic mechanisms that operate in the human body to mitigate deviations in systemic acid-base equilibrium (through the lungs, the kidneys, and buffers in the blood), some studies have shown that contemporary diets lead to blood acidity increase and plasma bicarbonate concentration decrease (Kurtz et al., 1983; Frassetto et al.,

1996). At least one study has related a more alkaline diet, with higher fruit and vegetable and lower meat intake, with more alkaline urine (Welch et al., 2008).

Current net acid diets may be responsible for the chronic, low-grade pathogenic metabolic acidosis found in US adults consuming a standard US diet (Frassetto et al., 1998; Frassetto et al., 1996). A net base-yielding diet on the other hand, is known to prevent osteoporosis (Sebastian et al., 1994; Bushinsky, 1996), age-related muscle wasting (Frassetto et al., 1997), calcium kidney stones (Pak et al., 1985; Preminger et al., 1985), hypertension (Sharma et al., 1990; Morris et al., 1999), and exercise-induced asthma (Mickleborough et al., 2001) and furthermore, slow the progression of age- and disease-related chronic renal insufficiency (Alpern & Sakhaee, 1997).

Sodium-potassium ratio

In the US, the average sodium dietary intake is 3.4 g/day while the potassium intake is 2.6 g/day (according to data from NHANES 2009-2010). The dietary ratio of sodium to potassium is therefore above the 1.0 cut-off point recommended by Cordain (Cordain et al., 2005), and way above the 0.5 cut-off point, calculated as the ratio between the sodium upper limit and adequate potassium intake for both male and females of all age groups, as per American Dietary Guidelines (DGAC, 2015). These cut-off points are surpassed due to both excessive sodium intake and insufficient potassium intake (DGAC, 2015; Cordain et al., 2005). Because 90% of sodium intake comes from sodium chloride (manufactured salt), it is known that salt added to meals or industrial products is the main reason for excessive sodium intake (rather than the sodium naturally found in foods). The main cause of the low potassium intake, on the other hand, is the high consumption of potassium-devoid vegetable oils and refined sugars (which constitute up to 36% of total energy intake), and the displacement of vegetables and fruit rich in potassium by whole grains and milk products (First Data Bank, 2000).

It has been estimated that the addition of manufactured salt to the food supply and the displacement of traditional potassium-rich foods by those introduced during the Neolithic and Industrial periods combined, caused both a 400% drop in the potassium intake and a 400% increase in sodium ingestion (Eaton & Konner, 1985; Frassetto et al., 2001;

Cordain, 2002). A high sodium to potassium intake ratio may be associated with hypertension and stroke (Antonios & MacGregor, 1996), kidney stones (Massey & Whiting, 1995), bone density and osteoporosis (Devine et al., 1995), gastrointestinal tract cancers (Jansson, 1986; Tuyns, 1988), asthma (Carey et al., 1993), exercise-induced asthma (Gotshall et al., 2000), insomnia (Miller, 1945), air sickness (Lindseth & Lindseth, 1995), high-altitude sickness (Porcelli & Gugelchuk, 1995), and Meniere's Syndrome (ear ringing) (Thai-Van et al., 2001).

Fiber content

In the US (NHANES 2009-2010) more than 90% of the population consumed less fiber than recommended (USDA, 2015). Reasons for this are the high consumption of refined cereal grains, dairy products, refined oils and sugar, along with a low consumption of fruits and vegetables. For example, in the US 48% of energy intake comes from fiber-depleted refined sugars, vegetable oils, dairy products, and alcohol; furthermore, fiber-depleted, refined grains, with 400% less fiber than whole grains, constitute 85% of total grains (Gerrior & Bente, 2002; USDA, 1997; USDA 2002). Hominin diets prior to the Neolithic and Industrial periods contained significantly more fiber than after (42.5 g/d) (Cordain, 2002), both because of the consumption of plant foods instead of staples, and because wild plant foods are known to have more fiber than their domestic counterparts (Brand-Miller & Holt ,1998). A high dietary fiber intake may reduce total and LDL cholesterol concentrations, reduce appetite and caloric intake (Anderson et al., 1994), while also decreasing the risk of constipation, appendicitis, hemorrhoids, deep vein thrombosis, varicose veins, diverticulitis, hiatal hernia, and gastroesophageal reflux (Trowell, 1985).

Overall nutrient profile

After describing each dietary indicator, individually, Cordain (2005) suggests that it was multifactorial dietary elements rather than single dietary elements, which were most likely affected by the Neolithic and Industrial era changes in diet and hence, underlie the etiology of most diseases existing in our civilization. Moreover, studying nutrients one at a time has several drawbacks, which may be worked around by focusing on dietary

patterns (Hu, 2002; Kant, 2004; Kant, 1996; National Academy Press, 1989; Lee et al., 1988; Sacks et al., 1994; Farchi et al., 1989; Kant et al., 1991; Randall et al., 1990). Indeed, studying the effect of the displacement of minimally processed foods by more processed foods, the distinguishing character of western diets, on the overall nutrient profile of diets seems equally important.

Phytoestrogen intake

Even though foods contain thousands of different chemicals, Cordain (2005) largely focused on those which are better described and currently measurable. Yet there is evidence that many of the less described chemicals, such as phytoestrogens, may have important health benefits. Non-nutrient phytochemical intakes (i.e. protease inhibitors, organic isothiocyanates, organosulfur compounds, plant phenols, lignans and flavonoids) were probably higher in the Paleolithic period than nowadays, as their content in wild plant foods is probably high, though largely unknown (Eaton et al., 1997). The possible role of these phytoestrogens in protecting against diseases and dysfunctions related to aging, mental processes, metabolism, malignant transformation, cardiovascular diseases, breast and prostate cancers, menopausal symptoms, osteoporosis, atherosclerosis and stroke, and neurodegeneration is being actively researched (Sirotkin & Harrath, 2014; Paterni et al., 2016).

In summary, Cordain et al. sustained that in the past 10,000 years, two important revolutions occurred (the introduction of food staples during the Neolithic and food-processing during Industrial Periods) which had an overall impact on human nutrition. What they did not tackle however, is the degree to which industrial food processing alone, have an effect on nutrition. This is exactly what we strive to uncover in this thesis, using some of Cordain's dietary indicators along with others to measure the effect.

1.3 Defining and classifying foods based on the extent and purpose of industrial food processing

If it is the displacement of minimally processed foods by more processed foods, which has brought disease to populations via negative effects on dietary indicators (Cordain et al., 2005) or protein dilution (Simpson & Raubenheimer, 2012), reaching a consensus

and defining what processed foods are seems a warrant. In 2009, Monteiro et al. developed *NOVA* (a name, not an acronym), a food classification based on the extent and purpose of industrial food processing (Monteiro et al., 2010a; Monteiro et al., 2015). NOVA includes 4 groups: “unprocessed or minimally processed foods” (such as fresh, dry or frozen fruits or vegetables; packaged grains and pulses; grits, flakes or flours made from corn, wheat or cassava; pasta, fresh or dry, made from flours and water; eggs; fresh or frozen meat and fish and fresh or pasteurized milk); “processed culinary ingredients” (including sugar, oils, fats, salt, and other substances extracted from foods and used in kitchens to season and cook unprocessed or minimally processed foods and to make culinary preparations), “processed foods” (including canned foods, sugar-coated dry fruits, salted meat products, cheeses and freshly made unpackaged breads, and other ready-to-consume products manufactured with the addition of salt or sugar or other substances of culinary use to unprocessed or minimally processed foods), and “ultra-processed foods”.

Ultra-processed foods are formulations manufactured using several ingredients and a series of processes (hence ‘ultra-processed’). Most of their ingredients are lower cost industrial sources of dietary energy and nutrients, and additives used with the purpose of imitating sensorial qualities of minimally processed foods or of culinary preparations of these foods, or to disguise undesirable sensory qualities of the final product. They are made to be hyper-palatable and attractive by use of many additives, with long shelf life, and able to be consumed anywhere, anytime. Ultra-processed foods include but are not limited to soft drinks, sweet or savory snacks, reconstituted meat products and pre-prepared frozen dishes (Monteiro, 2009; Ludwig, 2011; Moodie et al., 2013; Monteiro et al., 2015; FAO, 2015; PAHO, 2015).

When Cordain et al. suggest that the displacement of minimally processed foods by more processed foods was the trigger for nutritional unbalances, they were referring to the substitution of unprocessed/minimally processed foods (excluding at least pasta, cereals, flour, and milk) by processed culinary ingredients + processed foods + ultra-processed foods + pasta + cereals + flour + milk (using NOVA terminology). We however, uphold that it is the displacement of minimally processed foods + processed

culinary ingredients + processed foods by ultra-processed foods that at least nowadays, unties the unbalances. For this reason, the focus of this thesis is on ultra-processed food consumption.

1.4 Studying the effect of ultra-processed food consumption on dietary indicators across the world

In order to understand the impact of the rise in ultra-processed food consumption (Moodie et al., 2013; PAHO 2015; Stuckler et al. 2012) on dietary indicators, performing studies in different populations across the world is essential. This is especially true because of the variation of the total and subgroup- specific contribution of ultra-processed foods in space and time.

Some studies using NOVA have been carried out in nationally representative samples of the Brazilian population showing that the group of ultra-processed foods have higher content of free sugars, total fats, saturated fats and trans fats and lower content of protein, fiber, and most micronutrients than the rest of the diet, and that high consumption of ultra-processed foods renders grossly nutritionally unbalanced diets (Monteiro et al., 2010a; Louzada et al., 2015a; Louzada et al., 2015b). In Canada, similar results have been documented regarding free sugars, total fats, protein and fiber (Moubarac et al., 2012). A US study, using a slightly different classification, found that highly processed barcoded consumer packaged foods and beverages, mostly ultra-processed products, are higher in saturated fat, sugar, and sodium contents compared to less-processed foods (Poti et al., 2015).

At present, studying the US population seems important because it is probably the nation with the highest ultra-processed food consumption in the world, and being so, these results will shed light on the effect of a high ultra-processed food consumption on the dietary quality. This may be especially relevant for nations with ultra-processed food consumption on the rise which have the chance to revert this situation on time, both through individual education intervention and food environment regulatory policies.

1.5 References

1. Alpern RJ, Sakhaee S. The clinical spectrum of chronic metabolic acidosis: homeostatic mechanisms produce significant morbidity. AmJ Kidney Dis 1997; 29: 291–302.
2. Anderson JW, Smith BM, Gustafson NJ. Health benefits and practical aspects of high-fiber diets. Am J Clin Nutr 1994; 59(suppl):1242S–7S.
3. Antonios TF, MacGregor GA. Salt—more adverse effects. Lancet 1996; 348:250 –1.
4. Boaz NT. Evolving health: the origins of illness and how the modern world is making us sick. New York: Wiley & Sons, Inc, 2002.
5. Brand-Miller JC, Holt SH. Australian aboriginal plant foods: a consideration of their nutritional composition and health implications. Nutr Res Rev 1998; 11:5–23.
6. Bridges PS. Skeletal biology and behavior in ancient humans. Evol. Anthropol. 1996; 5, 112±120.
7. Burkitt DP, Eaton SB. Putting the wrong fuel in the tank. Nutrition 1989; 5, 189±191.
8. Bushinsky DA. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol 1996; 271:F216 –22.
9. Carey OJ, Locke C, Cookson JB. Effect of alterations of dietary sodium on the severity of asthma in men. Thorax 1993; 48:714–8.
10. Cordain L. Cereal grains: humanity's double edged sword. World Rev Nutr Diet 1999; 84:19 –73.
11. Cordain L, Brand Miller J, Eaton SB, Mann N, Holt SHA, Speth JD. Plant to animal subsistence ratios and macronutrient energy estimations in world wide hunter-gatherer diets. Am J Clin Nutr 2000; 71: 682–92.
12. Cordain L. The nutritional characteristics of a contemporary diet based upon Paleolithic food groups. J Am Neutraceutical Assoc 2002; 5:15–24.
13. Cordain L, Eaton SB, Brand Miller J, Mann N, Hill K. The paradoxical nature of hunter-gatherer diets: meat based, yet non-atherogenic. Eur J Clin Nutr 2002; 56(suppl): S42–52.
14. Cordain L, Eades MR, Eades MD. Hyperinsulinemic diseases of civilization: more than just syndrome X. Comp Biochem Physiol Part A 2003; 136:95–112.

15. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, and Brand-Miller J. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 2005; 81: 341–54.
16. Devine A, Criddle RA, Dick IM, Kerr DA, Prince RL. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. Am J Clin Nutr 1995; 62:740 –5.
17. Diet and health: implication for reducing chronic disease risk. National Academy Press, Washington, DC; 1989.
18. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. N. Engl. J. Med. 1985, 312, 283±289.
19. Eaton SB, Konner M, Shostak M. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 1988; 84: 739–49.
20. Eaton SB, Eaton SB III, Konner MJ. Review. Paleolithic nutrition revisited: A twelve-year retrospective on its nature and implications. European Journal of Clinical Nutrition 1997; 51, 207±216.
21. FAO. Guidelines on the collection of information on food processing through food consumption surveys. FAO, Rome, 2015.
22. Farchi G, Mariotti S, Menotti A, Seccareccia F, Torsello S, Fidanza F. Diet and 20-y mortality in two rural population groups of middle-aged men in Italy. Am J Clin Nutr. 1989; 50:1095±1103.
23. First Data Bank. Nutritionist V nutrition software, version 2.3. San Bruno, CA: First Data Bank, 2000.
24. Frassetto L, Morris RC, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. Am J Physiol 1996; 271:1114 –22.
25. Frassetto L, Morris RC Jr, Sebastian A. Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. J Clin Endocrinol Metab 1997; 82: 254 –9.
26. Frassetto LA, Todd KM, Morris RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 1998; 68:576–83.

27. Frassetto L, Morris RC Jr, Sellmeyer DE, Todd K, Sebastian A. Diet, evolution and aging—the pathophysiologic effects of the postagricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. Eur J Nutr 2001; 40:200 –13.
28. Gerrior S, Bente L. Nutrient content of the U.S. food supply, 1909-99: a summary report. Washington, DC: US Department of Agriculture, Center for Nutrition Policy and Promotion, 2002. (Home Economics report no. 55.)
29. Gibson G. Human evolution: Thrifty genes and the Dairy Queen. Curr. Biol. 2007; 17: R295-R296.
30. Gotshall RW, Mickleborough TD, Cordain L. Dietary salt restriction improves pulmonary function in exercise-induced asthma. Med Sci Sports Exerc 2000; 32:1815–9.
31. Gould SJ. The structure of evolutionary theory. Cambridge, MA: Harvard University Press, 2002.
32. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999; 70:221–7.
33. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol. 2002 Feb; 13(1):3-9.
34. Institute of Medicine of the National Academies. Dietary fats: total fat and fatty acids. In: Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: The National Academy Press, 2002: 335–432.
35. Jansson B. Geographic cancer risk and intracellular potassium/sodium ratios. Cancer Detect Prev 1986; 9:171–94.
36. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34: 362– 6.
37. Jenkins DJ, Wolever TM, Collier GR, Ocana A, Rao AV, Buckley G, Lam Y, Mayer A, Thompson LU. Metabolic effects of a low-glycemic diet. Am J Clin Nutr 1987; 46: 968 –75.

38. Kant AK, Schatzkin A, Block G, Ziegler RG, Nestle M. Food group intake patterns and associated nutrient profiles of the US population. J Am Diet Assoc. 1991; 91:1532±1537.
39. Kant AK. Indexes of overall diet quality: a review. J Am Diet Assoc 1996(8): 785-791.
40. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc 2004; 104(4):615–635.
41. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A. Effect of diet on plasma acid-base composition in normal humans. Kidney Int 1983; 24:670–80.
42. Lee CN, Reed DM, MacLean CJ, Yano K, Chiu D. Dietary potassium and stroke. N Engl J Med. 1988; 318:995-6.
43. Lemann J. Relationship between urinary calcium and net acid excretion as determined by dietary protein and potassium: a review. Nephron 1999; 81(suppl 1):18 –25.
44. Lennon EJ, Lemann J Jr, Litzow JR. The effect of diet and stool composition on the net external acid balance of normal subjects. J Clin Invest 1966; 45:1601–7.
45. Lindseth G, Lindseth PD. The relationship of diet to airsickness. Aviat Space Environ Med 1995; 66:537– 41.
46. Liu S, Willett WC. Dietary glycemic load and atherothrombotic risk. Curr Atheroscler Rep 2002; 4:454–61.
47. Louzada ML, Martins AP, Canella DS, Baraldi LG, Bertazzi RL, Claro RM, Moubarac JC, Cannon G, Monteiro CA. Ultra-processed foods and the nutritional dietary profile in Brazil. Rev Saúde Pública 2015a; 49:38. DOI:10.1590/S0034-8910.2015049006132.
48. Louzada ML, Martins AP, Canella DS, Baraldi LG, Bertazzi RL, Claro RM, Moubarac JC, Cannon G, Monteiro CA. Impact of ultra-processed foods on micronutrient content in the Brazilian diet. Rev Saúde Pública 2015b; 49:45. DOI:10.1590/S0034-8910.2015049006211.
49. Ludwig DS. The glycemic index: physiological mechanisms relating obesity, diabetes, and cardiovascular disease. JAMA 2002; 287: 2414 –23.

50. Ludwig DS. Technology, diet, and the burden of chronic disease. JAMA 2011; 305:1352-1353.
51. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and kidney stone risk. Nutr Rev 1995; 53:131–9.
52. Mickleborough TD, Gotshall RW, Kluka EM, Miller CW, Cordain L. Dietary chloride as a possible determinant of the severity of exerciseinduced asthma. Eur J Appl Physiol 2001; 85:450–6.
53. Miller JC. Importance of glycemic index in diabetes. Am J Clin Nutr 1994; 59(suppl): 747S–52S.
54. Miller MM. Low sodium chloride intake in the treatment of insomnia and tension states. JAMA 1945; 129:262– 6.
55. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. Public Health Nutr. 2009; 12(5):729-731.
56. Monteiro CA, Levy RB, Claro RM, Castro IR, Cannon G. A new classification of foods based on the extent and purpose of their processing. Cad. Saúde Pública, Nov 2010a, vol.26, no.11, p.2039-2049.
57. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C. Ultra-processing and a new classification of foods. In: Neff R (ed) Introduction to U.S. Food System. Public Health, Environment, and Equity. San Francisco: Jossey Bass A Wiley Brand; 2015: 338-339.
58. Moodie R, Stuckler D, Monteiro C, Sheron N, Neal B, Thamarangsi T, Lincoln P, Casswell S. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. Lancet. 2013 Feb 23; 381(9867):670-9. doi: 10.1016/S0140-6736(12)62089-3.
59. Morris RC Jr, Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 1999; 33:18 –23.
60. Moubarac J-C, Martins APB, Claro RM, Levy RB, Cannon G, Monteiro CA. Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr, 2012; doi: 10.1017/S1368980012005009.
61. Nesse RM, Williams GC. Why we get sick. The new science of Darwinian medicine. New York: Times Books, 1994.

62. O'Dea K, Sinclair A. The modern western diet- the exception in man's evolution. In Agriculture and Human Evolution, eds KA Boundy & GH Smith, pp 56±61. Melbourne: Australian Institute Agricultural Science, 1983.
63. O'Dea K. Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to traditional lifestyle. Diabetes 1984; 33:596–603.
64. O'Dea K, Traianedes K, Ireland P, Niall M, Sadler J, Hopper J, De Luise M. The effects of diet differing in fat, carbohydrate, and fiber on carbohydrate and lipid metabolism in type II diabetes. J Am Diet Assoc. 1989; 89:1076–86.
65. Pak CY, Fuller C, Sakhaee K, Preminger GM, Britton F. Long-term treatment of calcium nephrolithiasis with potassium citrate. J Urol 1985; 134:11–9.
66. Paterni I, Granchi C, Minutolo F. Risks and Benefits Related to Alimentary Exposure to Xenoestrogens. Critical Reviews in Food Science and Nutrition 2016. DOI: 10.1080/10408398.2015.1126547
67. Porcelli MJ, Gugelchuk GM. A trek to the top: a review of acute mountain sickness. J Am Osteopath Assoc 1995; 95:718 –20.
68. Poti JM, Mendez MA, Ng SW, Popkin BM. Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households? Am J Clin Nutr. 2015 Jun; 101(6):1251-62. doi:10.3945/ajcn.114.100925.
69. Preminger GM, Sakhaee K, Skurla C, Pak CY. Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. J Urol 1985; 134: 20 –3.
70. Prentice AM. Fires of life: The struggles of an ancient metabolism in a modern world. BNF Nutr. Bull. 2001; 26: 13-27.
71. Prentice AM, Hennig BJ, Fulford AJ. Evolutionary origins of the obesity epidemic: Natural selection of thrifty genes or genetic drift following predation release? Int. J. Obes. 2008; 32: 1607- 1610.
72. Randall E, Marshall JR, Graham S, Brasure J. Patterns in food use and their associations with nutrient intakes. Am J Clin Nutr. 1990; 52:739±745.

73. Raubenheimer D, Simpson S. Nutritional Ecology and Human Health. Annu. Rev. Nutr. 2016; 36:603–26.
74. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75:473– 86.
75. Richards MP, Pettitt PB, Trinkaus E, Smith FH, Paunovic M, Karavanic I. Neanderthal diet at Vindija and Neanderthal predation: the evidence from stable isotopes. Proc Natl Acad Sci USA 2000a; 97: 7663–6.
76. Richards MP, Hedges RM. Focus: Gough's Cave and Sun Hole Cave human stable isotope values indicate a high animal protein diet in the British Upper Palaeolithic. J Archaeol Sci 2000b; 27:1–3.
77. Roberts MB, Stringer CB, Par®tt SA. A hominid tibia from middle pleistocene sediments at Boxgrove, UK. Nature 1994; 369, 311±313.
78. Ruff CB, Trinkhaus E, Walker A, Larsen CS. Postcranial robusticity in Homo. 1: Temporal trends and mechanical interpretation. Am. J. Physical Anthropol. 1993; 91, 21±53.
79. Sacks FM, Obarzanek E, Windhauser MM, for the DASH investigators. Rational and design of the Dietary Approaches to Stop Hypertension Trial (DASH): a multicenter controlled-feeding study of dietary patterns to lower blood pressure. Ann Epidemiol 1994; 5:108±118.
80. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris RC Jr. Improved mineral balance and skeletal metabolism in post-menopausal women treated with potassium bicarbonate. N Engl J Med 1994; 330: 1776–81.
81. Sebastian A, Frassetto LA, Sellmeyer DE, Merriam RL, Morris RC. Estimation of the net acid load of the diet of ancestral preagricultural Homo sapiens and their hominid ancestors. Am J Clin Nutr 2002; 76: 1308–16.
82. Sharma AM, Kribben A, Schattenfroh S, Cetto C, Distler A. Salt sensitivity in humans is associated with abnormal acid-base regulation. Hypertension 1990; 16:407–13.
83. Simpson S, Raubenheimer D. Obesity: the protein leverage hypothesis. Obes. Rev. 2005 6(2):133–42.
84. Simpson S, Raubenheimer D. The nature of nutrition: a unifying framework from animal adaptation to human obesity. Princeton University Press, NJ, US, 2012.

85. Sirotkin AV, Harrath AH. Phytoestrogens and their effects. Eur J Pharmacol. 2014 Oct 15; 741:230-6. doi: 10.1016/j.ejphar.2014.07.057.
86. Storck J, Teague WD. Flour for man's bread, a history of milling. Minneapolis: University of Minnesota Press, 1952.
87. Subar AF, Krebs-Smith SM, Cook A, Kahle LL. Dietary sources of nutrients among US adults, 1989 to 1991. J Am Diet Assoc 1998; 98: 537–47.
88. Thai-Van H, Bounaix MJ, Fraysse B. Meniere's disease: pathophysiology and treatment. Drugs 2001; 61:1089 –102.
89. Tooby J, Cosimides L. The past explains the present. Emotional adaptations and the structure of ancestral environments. Ethology and Sociobiology 1990; 11, 375±424.
90. Tuyns AJ. Salt and gastrointestinal cancer. Nutr Cancer 1988; 11: 229 –32.
91. Trowell H. Dietary fiber: a paradigm. In: Trowell H, Burkitt D, Heaton K, Doll R, eds. Dietary fibre, fibre-depleted foods and disease. New York: Academic Press, 1985:1–20.
92. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at http://health.gov/dietaryguidelines/2015/guidelines/.
93. Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications. Washington D.C.: Panamerican Health Organization, 2015.
94. US Department of Agriculture, Agricultural Research Service. Data tables: results from USDA's 1994-96 Continuing Survey of Food Int**a**kes by Individuals and 1994-96 Diet and Health Knowledge Survey. ARS Food Surveys Research Group, 1997. Internet: (available under "Releases"): http://www.barc.usda.gov/bhnrc/ foodsurvey/ home.htm.
95. US Department of Agriculture, Economic Research Service. Food Consumption (per capita) data system, sugars/sweeteners. 2002. Internet: http://www.ers.usda.gov/Data/foodconsumption/datasystem.asp.
96. Walker W. Perspectives on the Nariokotome discovery. In The Nariokotome Homo Erectus Skeleton, eds A Walker & R Leakey, pp 411±430. Cambridge, Mass.: Harvard Univ Press, 1993.

97. Welch AA, Mulligan A, Bingham SA, Khaw KT. Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. Br J Nutr. 2008 Jun; 99(6):1335-43. Epub 2007 Nov 28.
98. Wells JCK. The evolutionary biology of human body fatness. Cambridge: Cambridge University Press, 2010.
99. Wolfe BM, Giovannetti PM. Short term effects of substituting protein for carbohydrate in the diets of moderately hypercholesterolemic human subjects. Metabolism 1991; 40:338–43.
100. Wolfe BM, Giovannetti PM. High protein diet complements resin therapy of familial hypercholesterolemia. Clin Invest Med 1992; 15: 349 –59.
101. Wolfe BM, Piche LA. Replacement of carbohydrate by protein in a conventional-fat diet reduces cholesterol and triglyceride concentrations in healthy normolipidemic subjects. Clin Invest Med 1999; 22: 140–8.

2. OBJECTIVES

Under the assumption that it is the increase in ultra-processed food consumption that elicits unbalanced dietary indicators, the objective of this thesis is to explore the association between the dietary share of ultra-processed foods, expressed as a percentage of total energy intake, and different dietary intake indicators in the US population. Moreover, this thesis also explores whether the dietary share of ultra-processed foods determines the overall nutritional quality of the US diets as has been proposed (Monteiro, 2009; Monteiro et al., 2015; Monteiro et al., 2010a) and further recognized by the United Nations Food and Agriculture Organization (FAO, 2015), the Pan-American Health Organization (PAHO, 2015) and INFORMAS (International Network for Food and Obesity/non-communicable diseases Research, Monitoring and Action Support) (Vandevijvere et al., 2013). Lastly, this thesis also looks into whether the association between ultra-processed food, protein and energy consumptions fit the predictions of the Protein Leverage Hypothesis model.

Specific Objectives

1. Examine the relationship between dietary contribution of ultra-processed foods and the nutritional quality of the US diet through the evaluation of dietary contents of critical nutrients individually and also overall, using dietary pattern analysis (Manuscript 1).
2. Assess the contribution of ultra-processed foods to both total energy intake and the energy intake from added sugars, and, to quantify the relationship between their consumption and the total dietary content of added sugars (Manuscript 2).
3. Examine how the consumption of ultra-processed food influences the relative dietary protein content and the absolute energy and protein intakes of the US diet, and test whether the relationships fit the predictions of the PLH model (Manuscript 3).
4. Evaluate the relationship between the dietary contribution of ultra-processed foods and the urinary levels of phytoestrogens in the US population (Manuscript 4).

2.1 References

1. FAO. Guidelines on the collection of information on food processing through food consumption surveys. FAO, Rome, 2015.
2. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. Public Health Nutr. 2009; 12(5):729-731.
3. Monteiro CA, Levy RB, Claro RM, Castro IR, Cannon G. A new classification of foods based on the extent and purpose of their processing. Cad. Saúde Pública, Nov 2010a, vol.26, no.11, p.2039-2049.
4. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C. Ultra-processing and a new classification of foods. In: Neff R (ed) Introduction to U.S. Food System. Public Health, Environment, and Equity. San Francisco: Jossey Bass A Wiley Brand; 2015: 338-339.
5. Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications. Washington D.C.: Panamerican Health Organization, 2015.
6. Vandevijvere S, Monteiro C, Krebs-Smith SM, Lee A, Swinburn B, Kelly B, Neal B, Snowdon W, Sacks G; INFORMAS. Monitoring and benchmarking population diet quality globally: a step-wise approach. Obes Rev. 2013 Oct; 14 Suppl 1:135-49. doi: 10.1111/obr.12082.

3. MANUSCRIPTS

3.1 MANUSCRIPT 1

Accepted in Population Health Metrics

Title: The share of ultra-processed foods and the overall nutritional quality of diets in the US: evidence from a nationally representative cross-sectional study.

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Abstract

Background: Recent population dietary studies indicate that diets rich in ultra-processed foods, increasingly frequent worldwide, are grossly nutritionally unbalanced, suggesting that the dietary contribution of these foods largely determines the overall nutritional quality of contemporaneous diets. Yet, these studies have focused on individual nutrients (one at a time) rather than the overall nutritional quality of the diets. Here we investigate the relationship between the energy contribution of ultra-processed foods in the US diet and its content of critical nutrients, individually and overall.

Methods: We evaluated dietary intakes of 9,317 participants from 2009 to 2010 NHANES aged 1+ years. Food items were classified into unprocessed or minimally processed foods, processed culinary ingredients, processed foods, and ultra-processed foods. First, we examined the average dietary content of macronutrients, micronutrients, and fiber across quintiles of the energy contribution of ultra-processed foods. Then, we used Principal Component Analysis (PCA) to identify a nutrient-balanced dietary pattern to enable the assessment of the overall nutritional quality of the diet. Linear regression was used to explore the association between the dietary share of ultraprocessed foods and the balanced-pattern PCA factor score. The scores were thereafter categorized into tertiles, and their distribution was examined across ultra-processed food quintiles. All models incorporated survey sample weights and were adjusted for age, sex, race/ethnicity, family income, and educational attainment.

Results: The average content of protein, fiber, vitamins A, C, D, and E, zinc, potassium, phosphorus, magnesium, and calcium in the US diet decreased significantly across quintiles of the energy contribution of ultra-processed foods, while carbohydrate, added sugar, and saturated fat contents increased. An inverse dose–response association was found between ultra-processed food quintiles and overall dietary quality measured through a nutrient balanced-pattern PCA-derived factor score characterized by being richer in fiber, potassium, magnesium and vitamin C, and having less saturated fat and added sugars.

Conclusions: This study suggests that decreasing the dietary share of ultra-processed foods is a rational and effective way to improve the nutritional quality of US diets.

Keywords: NHANES, Ultra-processed, Dietary nutrient profile, PCA, Dietary patterns, Diet quality, Macronutrients, Micronutrients

Introduction

Ultra-processed foods are formulations manufactured using several ingredients and a series of processes (hence “ultra-processed”). Most of their ingredients are lower cost industrial sources of dietary energy and nutrients, and additives used for the purpose of imitating sensorial qualities of minimally processed foods or of culinary preparations of these foods, or to disguise undesirable sensory qualities of the final product. They are made to be hyper-palatable and attractive by the use of many additives, with long shelf life, and are able to be consumed anywhere, anytime. Ultra-processed foods include but are not limited to soft drinks, sweet or savory snacks, reconstituted meat products, and pre-prepared frozen dishes [1–6].

In studies carried out in nationally representative samples of the Brazilian population it has been shown that the group of ultra-processed foods have higher content of free sugars, total fats, saturated fats, and trans fats, and lower content of protein, fiber, and most micronutrients than the rest of the diet, and that high consumption of ultra-processed foods renders grossly nutritionally unbalanced diets [7–9]. In Canada, similar results have been documented regarding free sugars, total fats, protein, and fiber [10]. In the US, using 2009–2010 National Health and Nutrition Examination Survey (NHANES) day 1 data, a positive association was found between the dietary contribution of ultra-processed foods and the dietary content of added sugars [11]. Another US study found that highly processed barcoded consumer packaged foods and beverages, mostly ultra-processed products, are higher in saturated fat, sugar, and sodium contents compared to less-processed foods [12].

Based on the detrimental effects of ultra-processed foods on the dietary content of critical nutrients and taking into account their increasing predominance in global food supplies [3, 6, 13–16], the dietary share of ultra-processed foods, expressed as a percentage of total energy intake, has been proposed [1, 4, 17] and further recognized by the United Nations Food and Agriculture Organization [5], the Pan-American Health Organization [6], and INFORMAS (International Network for Food and Obesity/non-

communicable diseases Research, Monitoring and Action Support) [18] as a potentially meaningful determinant of the overall nutritional quality of contemporaneous diets.

In order to further evaluate the influence of the dietary share of ultra-processed foods on the nutritional dietary quality we need to study its relationship with the overall nutrient profile of diets. As several authors have pointed out [19–22], studying nutrients one at a time has a number of drawbacks, which may be overcome by focusing on dietary patterns [19, 23–30]. Yet, to date, population studies assessing the impact of ultra-processed food consumption on the nutritional quality of diets have focused on the dietary content of individual nutrients.

Dietary patterns can be derived using two approaches: a priori or a posteriori [31]. A priori techniques use scoring systems or overall measures of dietary quality based on nutritional variables, generally foods and/or nutrients, in order to assess the degree to which a participant complies with a predefined theoretical dietary pattern, created based on current nutrition knowledge. Empirically derived dietary patterns, on the other hand, are patterns derived a posteriori based on observed dietary intake of the various foods and/or nutrients. While a posteriori derived patterns may not necessarily represent optimal dietary patterns, as they are outcome independent, a priori techniques are limited by the current knowledge which may generate uncertainty regarding which nutrients and cutoff points to use when generating scores [19].

The objective of this study was to examine the relationship between dietary contribution of ultra-processed foods and the nutritional quality of the US diet through the evaluation of dietary contents of critical nutrients individually and also overall, using dietary pattern analysis.

Methods

Data source, population and sampling

We utilized nationally representative data from the 2009–2010 National Health and Nutrition Examination Survey (NHANES), a continuous, nationally representative, cross-sectional survey of non-institutionalized, civilian US residents [32].

The survey included an interview conducted in the home and a subsequent health examination performed at a mobile examination center (MEC). All NHANES examinees were eligible for two 24-h dietary recall interviews. The first dietary recall interview was collected in-person in the MEC while the second was collected by telephone three to ten days later. Dietary interviews were conducted by trained interviewers using the validated [33–35] US Department of Agriculture Automated Multiple-Pass Method. Among the 13,272 people screened in NHANES in 2009–2010, 10,537 (79.4%) participated in the household interview and 10,253 (77.3%) also participated in the MEC health examination. Of these, 9,754 individuals provided one day of complete dietary intakes, and 8406 provided two days' worth.

We evaluated 9,317 survey participants aged 1 year and above who had at least one day of 24-h dietary recall data and had not been breast-fed on either of the two days. Data for two recall days were used when available, and one day otherwise. These 9,317 individuals had similar sociodemographic characteristics (gender, age, race/ethnicity, family income, and educational attainment) to the full sample of 10,109 interviewed participants aged 1 year and above (**Additional file 1: Table S1**).

Food classification according to processing

We classified all recorded food items (N=280,132 Food Codes) according to Nova, a food classification based on the extent and purpose of industrial food processing [4, 17]. Nova includes four groups: "unprocessed or minimally processed foods" (such as fresh, dry, or frozen fruits or vegetables; packaged grains and pulses; grits, flakes, or flours made from corn, wheat, or cassava; pasta, fresh or dry, made from flour and water;

eggs; fresh or frozen meat and fish and fresh or pasteurized milk); “processed culinary ingredients” (including salt, vinegar, oils, fats, sugar, and other substances extracted from foods and used in kitchens to season and cook unprocessed or minimally processed foods and to make culinary preparations), “processed foods” (including pickled vegetables, fruit preserves, salted meat products, canned fish in water or oil, cheeses, artisan-style breads (no additives), and other ready-to-consume products manufactured with the addition of salt, vinegar, sugar, oil, or other substances of culinary use to unprocessed or minimally processed foods), and “ultra-processed foods.”

The Nova group of ultra-processed foods, of particular interest in this study, includes soft drinks, sweet or savory packaged snacks, confectionery and industrialized desserts, mass-produced packaged breads and buns, poultry and fish nuggets and other reconstituted meat products, instant noodles and soups, and many other ready-to-consume formulations of several ingredients. Besides salt, sugar, oils, and fats, ultra-processed foods ingredients include food substances not commonly used in culinary preparations, and this is what distinguishes them from processed foods. These ingredients include modified starches, hydrogenated oils, protein isolates, and additives whose purpose is to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations, or to disguise undesirable qualities of the inal product, such as colorants, flavorings, non-sugar sweeteners, emulsifiers, humectants, sequestrants, and firming, bulking, de-foaming, anti-caking, and glazing agents. Unprocessed or minimally processed foods represent a small proportion of, or are even absent from, the list of ingredients of ultra-processed products. A detailed definition of each Nova food group and examples of food items classified in each group are shown elsewhere [11]. The rationale underlying the classification is also explained elsewhere [1–3, 36, 37].

For all food items (Food Codes) judged to be a handmade recipe (prepared from fresh or minimally processed foods and processed culinary ingredients), the classification was applied to the underlying ingredients (Standard Reference Codes -SR Codes-) obtained

from the USDA Food and Nutrient Database for Dietary Studies (FNDDS) 5.0 [38]. More details in this regard have been previously published [11].

Assessing energy and nutrient contents

For this study, we used Food Code nutrient values as provided by NHANES.

For handmade recipes, we calculated the underlying ingredient (SR Code) nutrient values using variables from both FNDDS 5.0 [38] and USDA National Nutrient Database for Standard Reference, Release 24 (SR24) [39].

The following nutrients were considered in this study: protein, carbohydrates, added sugars, fats, saturated fats, sodium, vitamins A (as retinol activity equivalents), C, D, and E (as alpha-tocopherol), iron, zinc, potassium, phosphorus, magnesium, calcium, and fiber. These included most under consumed (vitamins A, C, D, and E, calcium, magnesium, potassium, and fiber) and all overconsumed (sodium, added sugar, and saturated fat) nutrients in the US population [40].

Data on added sugars per Food Code and per SR Code were obtained by merging the Food Patterns Equivalents Database (FPED) 2009–2010 and Food Patterns Equivalents Ingredients Database (FPID) 2009–2010 [41].

We used the following conversion factors: 4 kcal/g for carbohydrates and protein, 9 kcal/g for fat and 7 kcal/g for alcohol. Total energy intake was calculated as the sum of calories from carbohydrates, proteins, fat, and alcohol.

Data Analysis

We utilized all available dietary intake data for each participant, using means of both recall days when available (86% of participants) and one day otherwise.

Food items were sorted into mutually exclusive food subgroups within each of the four Nova groups, as shown in **Table 1**. First, we evaluated the contributions of each food group and subgroup to total energy intake and across quintiles of the dietary energy contribution of ultra-processed foods (henceforth “dietary share of ultra-processed

foods"). The group of unprocessed or minimally processed foods was also combined with the group of processed culinary ingredients, as foods belonging to these two groups are usually combined together in culinary preparations and therefore consumed together.

We then compared the average dietary content of macronutrients (expressed as percent of total energy) and of micronutrients and fiber (both expressed as g/1,000 kcal) across quintiles of dietary share of ultra-processed foods.

Principal Component Analysis (PCA) is one of the methods that can be used to empirically derive dietary patterns. This is a mathematical technique that allows reducing the complexity of interrelationships among observed variables into a smaller number of uncorrelated linear combinations of them referred to as "components" and which maximize the explained variance [19, 42].

Using PCA, through the correlation matrix applied to the dietary content of macronutrients, micronutrients, and fiber, we identified four nutrient dietary patterns in the sample (Vitamin E was excluded because it loaded on all main extracted components). The four patterns were selected based on the Kaiser criterion (eigenvalue > 1.0), scree plot, and PCA components interpretability. The components were rotated using the varimax procedure and a factor score was calculated for each of the four patterns.

PCA was conducted in the whole sample and stratifying by age (1–5, 6–11, 12–19, 20–39, 40–59, 60+ years), sex, race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race), ratio of family income to poverty line (0.00–1.30, >1.30–3.50, and >3.50) [32] and educational attainment of respondents aged 20+ years or of household reference person otherwise (<12, 12 years, and >12 years). Final PCA results are presented for all strata combined because, despite some variations, comparable patterns were observed across sociodemographic strata.

We used Gaussian regression to estimate the association between the dietary share of ultra-processed foods and the four component factor scores. To relax the linearity

assumption of the association, the dietary contribution of ultra-processed foods variable was transformed using restricted cubic splines with five knots. The model was also fit using z-standardized scores. The factor scores were then regressed on the quintiles of the dietary share of ultra-processed foods. Finally, factor scores were categorized into tertiles to express *low*, *middle*, and *high* adherence to the dietary pattern in order to examine the category distribution across quintiles of the dietary share of ultra-processed foods.

All regression models were adjusted for age, sex, race/ ethnicity, family income [32], and educational attainment. As 908 participants had missing values on family income and/or educational attainment, adjusted analyses included 8,409 individuals.

NHANES survey sample weights were used in all analyses except the PCA correlation matrix, to account for differential probabilities of selection for the individual domains, nonresponse to survey instruments, and differences between the final sample and the total US population. The Taylor series linearization variance approximation procedure was used to account for complex sample design and sample weights [32]. Tests of linear trend were performed to evaluate the effect of quintiles as a single continuous variable.

To minimize chance findings from multiple comparisons, statistical hypotheses were tested using a two tailed $p \leq 0.001$ level of significance. Data were analyzed using Stata version 12.1.

Results

Distribution of total energy intake according to food groups and across quintiles of dietary share of ultra-processed foods

The average US daily energy intake in 2009–2010 was 2,069.9 kcal, 57.5% of calories coming from ultra-processed foods, 30.2% from unprocessed or minimally processed foods, 9.3% from processed foods and 2.9% from processed culinary ingredients (**Table 1**). The energy contribution of most subgroups belonging to ultra-processed foods increased monotonically from the first to the last quintile of the dietary share of ultra-processed foods, with a few exceptions that showed a slight decrease between the fourth and fifth quintiles. An opposite trend was observed among subgroups from all three remaining groups.

Nutrient dietary contents according to dietary share of ultra-processed foods

The average dietary protein content decreased significantly and monotonically across quintiles of the dietary share of ultra-processed foods (from 17.9% of total energy intake in the lowest quintile to 13.1% in the highest). The content of alcohol evolved in a similar way (from 4.1% to 0.9% of total energy intake). In contrast, across the same quintiles, there were significant increases in the content of carbohydrates (from 46.5% to 53.4%), added sugars (7.7% to 19.2%), and saturated fats (10.1% to 10.9%) (**Table 2**).

The average dietary content of fiber and of all micronutrients except iron and sodium decreased significantly and monotonically across quintiles of the dietary share of ultra-processed foods: fiber (from 9.6 in the lowest quintile to 6.7 g/1,000 kcal in the highest), vitamin A (377.5 to 272.3 µg/1,000 kcal), vitamin C (58.2 to 32.4 mg/1,000 kcal), vitamin D (3.3 to 2.0 µg/1,000 kcal) and vitamin E (4.1 to 3.3 mg/1,000 kcal), zinc (6.3 to 4.9 mg/1,000 kcal), potassium (1.6 to 1.0 g/1,000 kcal), phosphorus (728.9 to 605.9 mg/1,000 kcal), magnesium (173.3 to 117.3 mg/1,000 kcal), and calcium (531.1 to 464.7 mg/1,000 kcal). The sodium dietary content decreased non-significantly across quintiles of the dietary share of ultra-processed foods (from 1.74 to 1.63 g/1,000 kcal),

while the iron content increased between the first and third quintiles and decreased thereafter.

Nutrient dietary patterns obtained through PCA

Through PCA, four of 15 components had an eigenvalue >1.0 and explained 67% of the variance, and all four were retained. The rotated factor loadings of these four components are displayed in **Table 3** (factor loadings above 0.20 and below -0.20 have been highlighted).

The first component was characterized by being richer in fiber, potassium, magnesium, and vitamin C, and having less saturated fat and added sugars (variables with factor loadings above 0.20 or below -0.20). The factor loading for sodium was close to zero in this first component (0.04). This component, called *nutrient balanced pattern*, was selected as an instrument to measure the quality of the diet overall.

Each of the three remaining components mixed healthy and unhealthy features regarding dietary nutrient contents. The second component indicated higher content in both saturated fat and micronutrients such as calcium, vitamin D, phosphorus, and vitamin A and lower content in sodium. The third showed higher content in protein, saturated fat, and sodium and phosphorus, and lower content in carbohydrates and added sugars. The fourth presented higher content in iron, zinc, vitamin A and sodium, and lower in vitamin C.

Comparable PCA patterns were observed across sociodemographic strata. This was especially true for the *Nutrient balanced pattern* as illustrated for race/ethnicity strata in **Additional file 1: Table S2**.

Association between the dietary share of ultra-processed foods and the nutrient balanced pattern

In unadjusted restricted cubic splines Gaussian regression analysis, a strong linear association was identified between the dietary share of ultra-processed foods and the *nutrient balanced pattern* factor score (coefficient for linear term = -0.03, 95% CI: -0.04

to -0.02) (**Fig. 1**). There was little evidence of nonlinearity in the restricted cubic spline model (Wald test for linear term $p < 0.001$; Wald test for all non-linear terms $p = 0.16$). The strength of the association remained nearly the same after adjusting for sex, age, race/ethnicity, family income, and educational attainment (coefficient for linear term = -0.04, 95% CI: -0.05 to -0.03). According to the adjusted model, one standard deviation increase in the dietary share of ultra-processed foods leads to a 0.38 standard deviation decrease in the *nutrient balanced pattern* factor score.

Across quintiles of the dietary share of ultra-processed foods, the adjusted mean *nutrient balanced pattern* factor score decreased monotonically, from 1.1 in the lowest quintile to -0.9 in the highest (**Table 4**). Across the same quintiles, the proportion of individuals with high adherence to the *nutrient balanced pattern* decreased monotonically from 58.4% in the lowest quintile of the dietary share of ultra-processed foods to 11.0% in the highest. Inversely, the proportion of individuals with *low* adherence increased from 13.3% in the lowest quintile to 61.7% in the highest (overall Chi square test $p < 0.001$).

The dietary share of ultra-processed foods also presented an inverse association with the remaining three components (**Additional file 1: Figure S1**). The mean factor scores of these three remaining components also decreased across the dietary share of ultra-processed foods (**Additional file 1: Table S3**).

Discussion

In this analysis of US nationally representative data, we show that a significant linear inverse relationship exists between the dietary contribution of ultra-processed foods and the dietary content of protein, fiber, vitamins A, C, D, and E, zinc, potassium, phosphorus, magnesium, and calcium. On the other hand, carbohydrate, saturated fat, and added sugar contents increased significantly with the dietary contribution of ultra-processed foods. Only diets in the lowest quintile of ultra-processed consumption had the average added sugar content below the upper limit recommended by the 2015–2020 Dietary Guidelines for Americans [40], while the average saturated fat content exceeded the same limit in all quintiles, with the lowest quintile moving closest to the recommendation.

We also found an inverse dose–response association between ultra-processed food dietary contribution and the overall dietary quality measured through a *nutrient balanced pattern* PCA-derived factor score characterized by being richer in fiber, potassium, magnesium, and vitamin C, and having less saturated fat and added sugars. Furthermore, we found substantially higher adherence to the *nutrient balanced pattern* in lower quintiles of ultra-processed food dietary contribution than in higher ones. These results are relevant because both individual education interventions and food environment regulatory policies have the potential to modify the dietary content of ultra-processed foods. To our knowledge, this is the first study to evaluate the association between the dietary contribution of ultra-processed foods and the overall nutritional quality of diets in the US.

The non-significant but somewhat unexpected sodium content decrease across quintiles of the dietary share of ultra-processed foods may be partly explained by the fact that in the US processed foods include basically “salty products” – such as cheese, ham, or vegetables in brine – while most ultra-processed foods are either “sweet products” (soft, fruit, and milk drinks, cakes, cookies, breakfast cereals, ice cream, sweet snacks, industrialized desserts) or products containing both salt and sugar (breads, sauces, canned soups, dressings, gravies, dips, spreads, mustard,

catsup). Still, the sodium dietary content was above the Tolerable Upper Intake Level for any sex-age group [40] regardless of the share of ultra-processed foods.

The not uncommon iron fortification of ultra-processed foods or their ingredients may explain why the iron content does not show the reverse gradient across quintiles of ultra-processed food consumption seen among other micronutrients.

Few studies have assessed the impact of levels of food processing on the nutrient contents of the US diet. One study [43] that applied a food-industry-supported classification system [44] to NHANES 2003–2008 food intake data found that, together, “mixtures of combined ingredients” and “ready-to-eat,” which are mostly ultra-processed foods, contributed to 51% of total energy intake in the US diet but to only 37% of the protein intake and to 73% of the added sugar intake. These two food groups also contributed to 37% of the fiber intake and to between 30% and 60% of the intake of micronutrients [43]. Analyses of the same data restricted to children and adolescents [45] and to adults [46] showed similar results. Unfortunately, these studies on data from NHANES 2003–2008 failed to explore whether the dietary content of critical nutrients actually differed between high and low consumers of “mixtures of combined ingredients” plus “ready-to-eat.”

Another study evaluated US household barcoded purchasing data from 2000 to 2012 using a classification system guided by the one used in our study [12]. In 2012, the mean per capita purchase of “highly processed foods,” a category similar to ultra-processed foods, had higher adjusted median saturated fat, total sugar, and sodium content than “less processed foods.” This report did not capture non-barcoded items such as unpackaged fresh fruit, vegetables, and meat, or highly processed foods such as ready-to-eat store-prepared items, and did not explore whether the dietary content of critical nutrients actually differed between high and low consumers of “highly processed foods".

Consistent with our results, an investigation in Canada using 2001 household purchasing data found a decrease in protein content and fiber density across quintiles of

the energy share of ultra-processed foods, together with an increase in the content of free sugars and total fats [10].

A study carried out in Brazil using 2008–2009 national food intake data found that protein, fiber, sodium, and potassium decreased significantly across quintiles of the dietary contribution of ultra-processed foods, while free sugars, total fats, and saturated fats increased [8]. After adjusting for family income, there was a significant drop in the dietary content of vitamin D, vitamin E, phosphorus, magnesium, and zinc, and an increase in calcium [9].

Our study has several strengths. We studied a large, nationally representative sample of the US population, increasing generalizability. Our investigation was based on total effective individual consumption data, rather than on household purchasing data [7, 10, 47], which do not evaluate the fraction of wasted food or purchases at restaurants.

Potential limitations should be considered. As with most population measures, dietary data obtained by 24-h recalls are imperfect. However, 24-h recalls are the least-biased self-report instrument available. Also, standardized methods and approach of NHANES have been shown to produce accurate intake estimates [33–35], and will therefore be suitable for assessing food group contributions and nutrient densities in the overall diet. Although NHANES collects limited information indicative of food processing (i.e., place of meals, product brands), these data are not consistently determined for all food items and this may lead to groups classification errors. Also, as some authors have highlighted, the number of food items reported in NHANES is smaller than the number available in the marketplace, and national food composition data are not updated as required to include all brand-specific products and to examine dietary profiles sensitive to brand preferences [48]. The PCA method also has limitations such as subjective decisions regarding the number of extracted components, method of rotation, naming of components, and cutoffs for factor loadings [23, 31, 49].

Conclusions

This study suggests that decreasing the dietary share of ultra-processed foods is a rational and effective way to substantially improve dietary quality in the US.

Additional file

Additional file 1: Table S1. Characteristics of study participants and of the full sample of interviewed participants aged 1 year and above, US population aged 1+ years (NHANES 2009–2010). **Table S2.** Rotated factor loadings for the first four components from principal component analysis using nutrients, across race/ethnicity strata, US population aged 1+ years (NHANES 2009–2010) (N=9,317). **Table S3.** PC2-PC4 score means and adherence according to the dietary share of ultra-processed foods, US population aged 1+ years (NHANES 2009–2010). **Figure S1.** PC2-PC4 factor scores regressed on the dietary share of ultra-processed foods evaluated by restricted cubic splines, US population aged 1+ years (NHANES 2009–2010) (N=9,317). (DOCX 1047 kb).

Abbreviations

FNDDS: USDA food and nutrient database for dietary studies; FPED: Food patterns equivalents database; FPID: Food patterns equivalents ingredients database; INFORMAS: International network for food and obesity/non-communicable diseases research, monitoring and action support; NHANES: National health and nutrition examination survey; PCA: Principal component analysis; SR Codes: Standard reference codes; USDA: National nutrient database for standard reference, release 24 (SR24).

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Availability of data and materials

Publicly available datasets have been used for this study.

Authors' contributions

CAM and EMS designed research; CAM and EMS analyzed data and performed statistical analysis; CAM, EMS, BP, and BS wrote the paper and CAM and EMS had primary responsibility for final content. All authors read and approved the final manuscript.

Competing interests

The authors declare having no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Secondary publicly available data were used in this study.

References

1. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. Public Health Nutr. 2009;12(5):729–31.
2. Ludwig DS. Technology, diet, and the burden of chronic disease. JAMA. 2011;305:1352–3.
3. Moodie R, Stuckler D, Monteiro C, Sheron N, Neal B, Thamarangsi T, Lincoln P, Casswell S. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. Lancet. 2013;381(9867):670–9. doi:10.1016/S0140-6736(12)62089-3.
4. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C. Ultra-processing and a new classification of foods. In: Neff R, editor. Introduction to U.S. Food System. Public Health, Environment, and Equity. Jossey Bass A Wiley Brand: San Francisco; 2015. p. 338–9.
5. FAO. Guidelines on the collection of information on food processing through food consumption surveys. Rome: FAO; 2015.
6. Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications. Washington D.C.: Panamerican Health Organization, 2015.
7. Monteiro CA, Levy RB, Claro RM, de Castro IR, Cannon G. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. Public Health Nutr. 2010a;14(1):5–13. doi:10.1017/S1368980010003241.
8. Louzada ML, Martins AP, Canella DS, Baraldi LG, Bertazzi RL, Claro RM, Moubarac JC, Cannon G, Monteiro CA. Ultra-processed foods and the nutritional dietary profile in Brazil. Rev Saude Publica. 2015a;49:38. doi:10.1590/S0034-8910.2015049006132.
9. Louzada ML, Martins AP, Canella DS, Baraldi LG, Bertazzi RL, Claro RM, Moubarac JC, Cannon G, Monteiro CA. Impact of ultra-processed foods on micronutrient content in the Brazilian diet. Rev Saude Publica. 2015b;49:45. doi:10.1590/S0034-8910.2015049006211.
10. Moubarac J-C, Martins APB, Claro RM, Levy RB, Cannon G, Monteiro CA.

Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr, 2012; doi:10.1017/S1368980012005009.

11. Martinez Steele E, Baraldi LG, Louzada ML, Moubarac JC, Mozaffarian D, Monteiro CA. Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. BMJ Open. 2016;6:e009892. doi:10.1136/bmjopen-2015-009892.

12. Poti JM, Mendez MA, Ng SW, Popkin BM. Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households? Am J Clin Nutr. 2015;101(6):1251–62. doi: 10.3945/ajcn.114.100925.

13. Stuckler D, McKee M, Ebrahim S, Basu S. Manufacturing epidemics: the role of global producers in increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. PLoS Med. 2012;9, e1001235.

14. Monteiro CA, Cannon G. The impact of transnational 'Big Food' companies on the South: a view from Brazil. PLoS Med. 2012a;9:e1001252.

15. Monteiro CA, Moubarac JC, Cannon G, Popkin BM. Ultra-processed products are becoming dominant in the global food system. Obes Rev. 2013;14 Suppl 2:21–8. doi:10.1111/obr.12107.

16. Juul F, Hemmingsson E. Trends in consumption of ultra-processed foods and obesity in Sweden between 1960 and 2010. Public Health Nutr. 2015. doi:10.1017/S1368980015000506.

17. Monteiro CA, Levy RB, Claro RM, Castro IR, Cannon G. A new classification of foods based on the extent and purpose of their processing. Cad Saúde Pública. 2010;26(11):2039–49.

18. Vandevijvere S, Monteiro C, Krebs-Smith SM, Lee A, Swinburn B, Kelly B, Neal B, Snowdon W, Sacks G. INFORMAS. Monitoring and benchmarking population diet quality globally: a step-wise approach. Obes Rev. 2013;Suppl 1:135–49. doi:10.1111/obr.12082.

19. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol. 2002;13(1):3–9.

20. Reedy J, Wirfalt E, Flood A, Mitrou PN, Krebs-Smith SM, Kipnis V, Midthune D, Leitzmann M, Hollenbeck A, Schatzkin A, Subar AF. Comparing 3 dietary pattern methods–cluster analysis, factor analysis, and index analysis–With colorectal cancer risk: The NIH-AARP Diet and Health Study. Am J Epidemiol. 2010;171(4):479–87.
21. Akin JS, Guilkey DK, Popkin BM, Fanelli MT. Cluster analysis of food consumption patterns of older Americans. J Am Diet Assoc. 1986;5:616–24.
22. Patterson RE, Haines PS, Popkin BM. Diet quality index: capturing a multidimensional behavior. J Am Diet Assoc. 1994;1:57–64.
23. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc. 2004; 104(4):615–35.
24. Kant AK. Indexes of overall diet quality: a review. J Am Diet Assoc. 1996;8:785–91.
25. Diet and health: implication for reducing chronic disease risk. National Academy Press, Washington, DC; 1989.
26. Lee CN, Reed DM, MacLean CJ, Yano K, Chiu D. Dietary potassium and stroke. N Engl J Med. 1988;318:995–6.
27. Sacks FM, Obarzanek E, Windhauser MM. for the DASH investigators. Rational and design of the Dietary Approaches to Stop Hypertension Trial (DASH): a multicenter controlled-feeding study of dietary patterns to lower blood pressure. Ann Epidemiol. 1994;5:108–18.
28. Farchi G, Mariotti S, Menotti A, Seccareccia F, Torsello S, Fidanza F. Diet and 20-y mortality in two rural population groups of middle-aged men in Italy. Am J Clin Nutr. 1989;50:1095–103.
29. Kant AK, Schatzkin A, Block G, Ziegler RG, Nestle M. Food group intake patterns and associated nutrient profiles of the US population. J Am Diet Assoc. 1991;91:1532–7.
30. Randall E, Marshall JR, Graham S, Brasure J. Patterns in food use and their associations with nutrient intakes. Am J Clin Nutr. 1990;52:739–45.
31. Moeller SM, Reedy J, Millen AE, Dixon LB, Newby PK, Tucker KL, Krebs-Smith SM, Guenther PM. Dietary patterns: challenges and opportunities in dietary patterns research an Experimental Biology workshop, April 1, 2006. J Am

Diet Assoc. 2007;107:1233–9.
32. Johnson CL, Paulose-Ram R, Ogden CL. National Health and Nutrition Examination Survey: Analytic guidelines, 1999–2010. National Center for Health Statistics. Vital Health Stat 2 (161). 2013.
33. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, Paul DR, Sebastian RS, Kuczynski KC, Ingwersen LA, Staples RC, The CLC, USDA. Automated Multiple-Pass Method reduces bias in the collection of energy intakes. Am J Clin Nutr. 2008;88:324–32.
34. Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. J Nutr. 2006;136(10):2594–9.
35. Rumpler WV, Kramer M, Rhodes DG, Moshfegh AJ, Paul DR, Kramer M. Identifying sources of reporting error using measured food intake. Eur J Clin Nutr. 2008;62:544–52.
36. Moubarac JC, Parra DC, Cannon G, Monteiro CA. Food Classification Systems Based on Food Processing: significance and implications for policies and actions: a systematic literature review and assessment. Curr Obes Rep. 2014;3:256–72.
37. Monteiro CA, Cannon G, Levy RB, Claro R, Moubarac JC, Martins AP, Louzada ML, Baraldi L, Canella D. The Food System. Processing. The big issue for disease, good health, well-being. World Nutr. 2012;3:527–69. www.wphna.org (accessed Jan 2017).
38. Ahuja JKA, Montville JB, Omolewa-Tomobi G, Heendeniya KY, Martin CL, Steinfeldt LC, Anand J, Adler ME, LaComb RP, and Moshfegh AJ. 2012. USDA Food and Nutrient Database for Dietary Studies, 5.0. U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group, Beltsville, MD.
39. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 24. 2011. Nutrient Data Laboratory Home Page, available at: http://www.ars.usda.gov/ba/bhnrc/ndl (accessed Jan 2017).
40. U.S. Department of Health and Human Services and U.S. Department of

Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at http://health.gov/dietaryguidelines/2015/guidelines/ (accessed Jan 2017).
41. Bowman SA, Clemens JC, Thoerig RC, Friday JE, Shimizu M, Moshfegh AJ. Food Surveys Research Group, Beltsville Human Nutrition Research Center, Agricultural Research Service. U.S.: Department of Agriculture, Beltsville, Maryland; 2013. Food Patterns Equivalents Database 2009–10: Methodology and User Guide [Online], <http://www.ars.usda.gov/ba/bhnrc/fsrg> (accessed June 2013).
42. Raykov T, Marcoulides GA. An Introduction to Applied Multivariate Analysis, 2008. ISBN-13: 978-0-8058-6375-8 (hardcover) (page 211–238).
43. Eicher-Miller HA, Fulgoni III VL, Keast DR. Contributions of Processed Foods to Dietary Intake in the US from 2003–2008: A Report of the Food and Nutrition Science Solutions Joint Task Force of the Academy of Nutrition and Dietetics, American Society for Nutrition, Institute of Food Technologists, and International Food Information Council. J Nutr. 2012;142: 2065S–72S. doi:10.3945/jn.112.164442.
44. International Food Information Council Foundation. Understanding our food communications tool kit, 2010. http://www.foodinsight.org/For-Professionals/Understanding-Our-Food/tabid/1398/Default.aspx (accessed Jan 2017).
45. Eicher-Miller HA, Fulgoni VL, Keast DR. Processed Food Contributions to Energy and Nutrient Intake Differ among US Children by Race/Ethnicity. Nutrients. 2015;7(12):10076–88. doi:10.3390/nu7125503.
46. Eicher-Miller HA, Fulgoni VL, Keast DR. Energy and Nutrient Intakes from Processed Foods Differ by Sex, Income Status, and Race/Ethnicity of US Adults. J Acad Nutr Diet. 2015;115(6):907–18. doi:10.1016/j.jand.2014.11.004. e6, Epub 2015 Jan 8.
47. Crovetto MM, Uauy R, Martins AP, Moubarac JC, Monteiro C. Household availability of ready-to-consume food and drink products in Chile: impact on nutritional quality of the diet. Rev Med Chil. 2014;142(7):850–8. doi:10.

4067/S0034-98872014000700005.
48. Slining MM, Yoon EF, Davis J, Hollingsworth B, Miles D, Ng SW. An Approach to Monitor Food and Nutrition from “Factory to Fork”. J Acad Nutr Diet. 2015;115(1):40–9.
49. Martinez ME, Marshall JR, Sochrost L. Invited commentary: factor analysis and the search for objectivity. Am J Epidemiol. 1998;148:17–9.

Tables and figures

Table 1. Distribution (%) of the total daily per capita energy intake (kcal) according to NOVA food groups by quintiles of the dietary share of ultra-processed foods. US population aged 1 + years (NHANES 2009-2010) (N=9,317)

		Quintile of dietary share of ultra-processed foods (% of total energy intake)[a]				
	All quintiles (n=9,317) (2069.9 kcal)	Q1 (n=1,941) (1970.9 kcal)	Q2 (n=1,903) (2017.6 kcal)	Q3 (n=1,791) (2061.8 kcal)	Q4 (n=1,785) (2151.5 kcal)	Q5 (n=1,897) (2147.7 kcal)
Unprocessed or minimally processed foods	***30.2***	***48.3***	***36.7***	***29.4***	***23.3***	***13.2****
Meat (includes poultry)	8.0	11.6	9.6	8	6.7	4*
Fruit and freshly squeezed fruit juices	5.5	8.8	6.8	5.4	4.3	2.5*
Milk and plain yoghurt	5.1	6.4	6.1	5.3	4.8	2.9*
Grains	2.9	6.3	3.4	2.3	1.6	0.7*
Roots and tubers	1.7	2.6	2.3	1.7	1.2	0.7*
Eggs	1.5	2.1	1.8	1.4	1.2	0.7*
Pasta	1.4	2.4	1.6	1.4	1.1	0.5*
Legumes	0.9	1.8	1.1	0.8	0.5	0.2*
Fish and sea food	0.8	1.5	1	0.7	0.4	0.2*
Vegetables	0.9	1.5	1	0.8	0.6	0.4*
Other unprocessed or minimally processed foods[1]	1.7	3.2	2	1.5	1	0.5*
Processed culinary ingredients	***2.9***	***4.9***	***3.4***	***2.9***	***2.2***	***1.2****
Sugar[2]	1.1	1.6	1.3	1.1	0.9	0.6*
Plant oils	1.2	2.5	1.4	1.2	0.7	0.3*
Animal fats[3]	0.5	0.7	0.6	0.6	0.5	0.2*
Other processed culinary ingredients[4]	0.05	0.1	0.04	0.05	0.03	0.01
Unprocessed or minimally processed foods + Processed culinary ingredients	***33.1***	***53.2***	***40.1***	***32.4***	***25.4***	***14.5****

Processed foods	***9.3***	***14.1***	***11.2***	***9.2***	***7.2***	***4.8****
Cheese	3.6	4.1	4.1	3.9	3.4	2.5*
Ham and other salted, smoked or canned meat or fish	1.2	1.5	1.4	1.4	1.1	0.8
Vegetables and other plant foods preserved in brine	0.7	0.9	0.8	0.7	0.6	0.5*
Other processed foods[5]	3.7	7.6	4.8	3.2	2.1	1*
Ultra-processed foods	***57.5***	***32.6***	***48.6***	***58.4***	***67.3***	***80.7****
Breads	9.5	7.2	9.9	10.3	10.6	9.4*
Soft and Fruit drinks[6]	6.9	3	4.7	6.7	8.2	11.8*
Cakes, cookies and pies	5.5	2.6	4.6	5.5	6.8	7.9*
Salty-snacks	4.4	2.4	3.7	4.3	5.4	6.2*
Frozen and shelf-stable plate meals	3.9	1.3	2.2	3.7	5.2	7.3*
Pizza (ready-to-eat/heat)	3.3	0.5	1.4	2.6	4.1	7.8*
Breakfast cereals	3.1	2.2	3.2	3.6	3.5	3.1
Sauces, dressings and gravies	2.5	2.4	2.7	2.7	2.8	2.1
Reconstituted meat or fish products	2.3	0.9	2.1	2.4	2.9	2.9*
Ice cream and ice pops	2.3	1.1	1.9	2.4	2.9	3*
Sweet-snacks	2.3	1.1	2.1	2.4	2.7	3.4*
Milk-based drinks	1.9	1.1	1.7	1.9	2.1	2.6*
Desserts[7]	1.8	1.3	1.9	2.1	2.1	1.8*
French fries and other potatoe products	1.7	0.4	1.1	1.7	1.9	3.5*
Sandwiches and hamburgers on bun (ready-to-eat/heat)	1.4	0.2	0.5	1.2	1.5	3.5*
Instant and canned soups	0.9	0.7	0.8	0.9	0.9	1
Other ultra-processed foods[8]	3.8	3.9	4	3.9	3.7	3.2

Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

[a]Mean (range) dietary share of ultra-processed foods per quintile: 1st=32.6 (0 to 42.6); 2nd= 48.6 (42.6 to 54.0); 3rd= 58.4 (54.0 to 62.8); 4th= 67.3 (62.8 to 72.3); 5th= 80.7 (72.3 to 100)

[1]Including nuts and seeds (unsalted); yeast; dried fruits (without added sugars) and vegetables; non pre-sweetened, non-whitened, non-flavored coffee and tea; coconut water and meat; homemade soup and sauces; flours; tapioca

[2]Including honey, molasses, maple syrup (100%)

[3]Including butter, lard and cream

[4]Including starches; coconut and milk cream; unsweetened baking chocolate, cocoa powder and gelatin powder; vinegar; baking powder and baking soda

[5]Including salted or sugared nuts and seeds; peanut, sesame, cashew and almond butter or spread; beer and wine

[6]Including energy drinks, sports drinks, nonalcoholic wine

[7]Including ready-to-eat and dry-mix desserts such as pudding

[8]Including soy products such as meatless patties and fish sticks; babyfood and baby formula; dips, spreads, mustard and catsup; margarine; sugar substitutes, sweeteners and all syrups (excluding 100% maple syrup); distilled alcoholic drinks

*Significant linear trend across all quintiles ($p<0.001$), both in unadjusted and models adjusted for sex, age group (1-5, 6–11, 12–19, 20–39, 40–59, 60 + years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

Table 2. Indicators of the dietary content of macronutrients and micronutrients according to the dietary share of ultra-processed foods. US population aged 1 + years (NHANES 2009-2010) (N=9,317)

		Quintiles of dietary share of ultra-processed foods (% of total energy intake) [n][a]				
		Q1 [n=1941]	Q2 [n=1903]	Q3 [n=1791]	Q4 [n=1785]	Q5 [n=1897]
Macronutrient Indicators (mean % of total energy intake)	Protein	17.9	16.7	15.8	14.7	13.1*
	Total Carbohydrates	46.5	48.6	49.9	51.3	53.4*
	Added sugars	7.7	11	13.4	15.7	19.2*
	Total fats	31.4	32.2	32.5	32.6	32.5
	Saturated fats	10.1	10.7	10.9	10.9	10.9*
	Alcohol	4.1	2.4	1.8	1.4	0.9*
Micronutrient Indicators (mean density)	Fiber (g/ 1000 kcal)	9.6	8.9	8.2	7.4	6.7*
	Na (g/ 1000 kcal)	1.74	1.69	1.69	1.66	1.63
	Vit. A (μg/ 1000 kcal)	377.5	358.5	347.4	306.2	272.3*
	Vit. C (mg/ 1000 kcal)	58.2	51.4	42.9	40.3	32.4*
	Vit. D (μg/ 1000 kcal)	3.3	3.2	2.9	2.5	2.0*
	Vit. E (mg/ 1000 kcal)	4.1	3.8	3.6	3.5	3.3*
	Iron (mg/ 1000 kcal)	7.4	7.7	7.8	7.5	7.4
	Zinc (mg/ 1000 kcal)	6.3	6	5.8	5.4	4.9*
	Potassium (g/ 1000 kcal)	1.6	1.4	1.3	1.2	1.0*
	Phosphorus (mg/ 1000 kcal)	728.9	715.9	691.7	653.9	605.9*
	Magnesium (mg/1000 kcal)	173.3	156.6	144.3	130.6	117.3*
	Calcium (mg/ 1000 kcal)	531.1	539.6	532.2	507	464.7*

[a]Mean (range) dietary share of ultra-processed foods per quintile: 1st=32.6 (0 to 42.6); 2nd= 48.6 (42.6 to 54.0); 3rd= 58.4 (54.0 to 62.8); 4th= 67.3 (62.8 to 72.3); 5th= 80.7 (72.3 to 100)

*Significant linear trend across all quintiles (p<=0.001), both in unadjusted and models adjusted for sex, age group (1-5, 6–11, 12–19, 20–39, 40–59, 60 + years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

Table 1. Rotated factor loadings for the first 4 components from principal component analysis using nutrients. US population aged 1 + years (NHANES 2009-2010) (N=9,317)

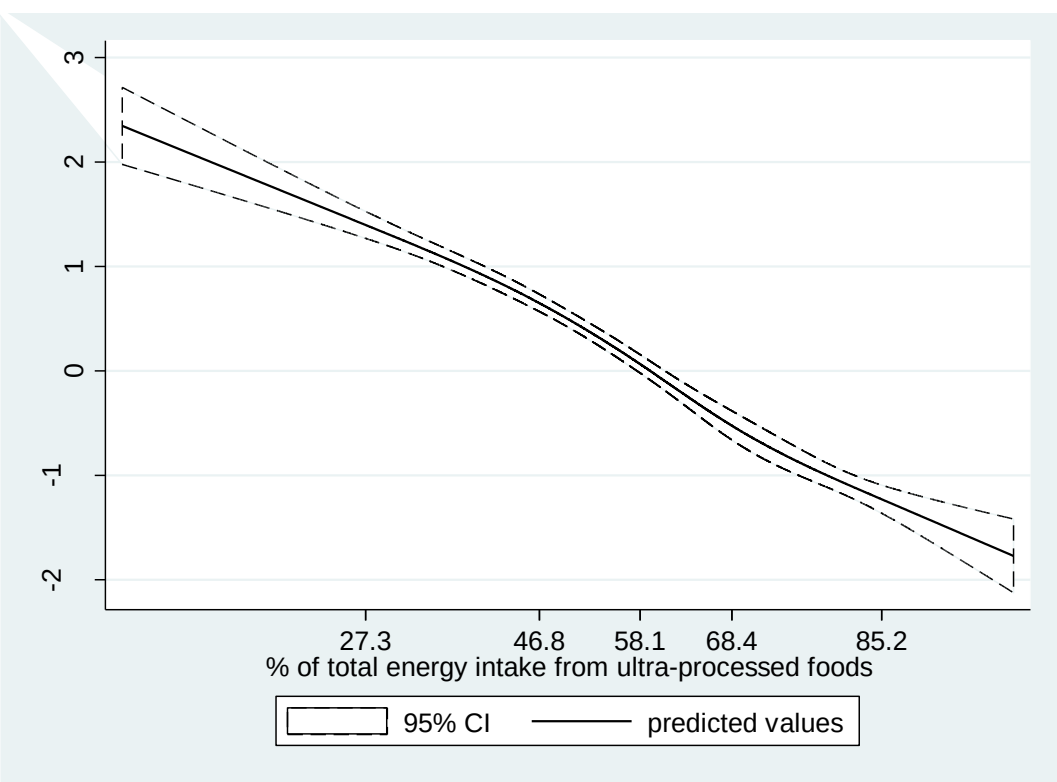
Indicator[1]	PC1 (% expl.[2]=20.4)	PC2 (% expl.=18.0)	PC3 (% expl.=17.7)	PC4 (% expl.=10.9)
Fiber density (g/1000 kcal)	**0.47**[3]	-0.12	0.00	0.09
Sodium density (g/1000 kcal)	0.04	**-0.22**	**0.39**	**0.20**
Potassium density (mg/1000 kcal)	**0.44**	0.15	0.10	-0.08
Iron density (mg/1000 kcal)	0.02	0.00	-0.09	**0.68**
Zinc density (mg/1000 kcal)	-0.08	0.06	0.16	**0.53**
Phosphorus density (mg/1000 kcal)	0.09	**0.38**	**0.21**	0.08
Magnesium density (mg/1000 kcal)	**0.44**	0.05	0.11	0.05
Calcium density (mg/1000 kcal)	0.02	**0.55**	-0.07	0.02
Vitamin A density (μg/ 1000 kcal)	0.06	**0.24**	-0.09	**0.24**
Vitamin C density (mg/1000 kcal)	**0.40**	0.07	-0.15	**-0.21**
Vitamin D density (μg/1000 kcal)	-0.02	**0.55**	-0.08	0.00
Protein (% of total energy)	0.05	0.03	**0.45**	0.14
Carbohydrate (% of total energy)	0.17	0.00	**-0.54**	0.17
Added sugars (% of total energy)	**-0.24**	-0.03	**-0.41**	0.14
Saturated fat (% of total energy)	**-0.34**	**0.30**	**0.22**	-0.16

[1]For details on Indicators, see Methods section

[2]Proportion of the variance explained by each factor after orthogonal varimax rotation (Kaiser on)

[3]Items with a factor loading above 0.20 or below -0.20 have been highlighted

Figure 1. "Nutrient balanced pattern" factor score regressed on the dietary share of ultra-processed foods evaluated by restricted cubic splines. US population aged 1 + years (NHANES 2009-2010) (N=9,317)



Legend: The values shown on the x-axis correspond to the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for percentage of total energy from ultra-processed foods (knots). Coefficient for linear term=-0.03, 95% CI: -0.04 to -0.02 (beta=-0.35). There was little evidence of nonlinearity in the restricted cubic spline model (Wald test for linear term p<0.0001; Wald test for all non-linear terms p=0.16).

Table 2. "Nutrient balanced pattern" factor score means and adherence according to the dietary share of ultra-processed foods. US population aged 1 + years (NHANES 2009-2010)

Dietary share of ultra-processed foods (% of total energy intake)		"Nutrient balanced pattern" factor score		Adherence to "Nutrient balanced pattern"[2]		
		Mean				
Quintiles	Mean (range)	unadj. (R2=0.18)	adj.[1] (R2=0.24)	Low (%)	Middle (%)	High (%)
Q1 (n=1941)	32.6 (0 to 42.6)	1.2*	1.1*	13.3	28.3	58.4
Q2 (n=1903)	48.6 (42.6 to 54.0)	0.6*	0.5*	19.6	35.0	45.5
Q3 (n=1791)	58.4 (54.0 to 62.8)	0.04	0.002	30.0	37.3	32.7
Q4 (n=1785)	67.3 (62.8 to 72.3)	-0.5*	-0.4*	42.2	38.8	19.0
Q5 (n=1897)	80.7 (72.3 to 100)	-1.0*¥	-0.9*¥	61.7	27.4	11.0

[1]Adjusted for sex, age group (1-5, 6–11, 12–19, 20–39, 40–59, 60 + years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

[2]"Nutrient balanced pattern" (PC1) factor score tertiles: T1 (-4.7 to -0.9 points); T2 (-0.9 to 0.6 points); T3 (0.6 to 9.9 points).

*Statistically significant p<=0.001

¥Significant linear trend across all quintiles (p<=0.001), both in unadjusted and models adjusted for sex, age group, race/ethnicity, ratio of family income to poverty and educational attainment.

Online Supporting Material

Additional file 1: Table S1: Characteristics of study participants and of the full sample of interviewed participants aged 1 year and above, US population aged 1+ years (NHANES 2009–2010)

		Study (N=9,317)	Full sample (N=10,109)
Gender	Men	49.0	48.9
	Women	50.9	51.0
Age (years)	1 to 5	6.7	7.1
	6 to 11	8.1	8.2
	12 to 19	11.3	11.1
	20 to 39	27.2	27.3
	40 to 59	28.4	28.2
	60 and over	18.3	18.2
Race/ethnicity	Mexican American	10.4	10.4
	Other Hispanic	5.4	5.4
	Non-Hispanic White	65.2	64.8
	Non-Hispanic Black	11.9	12.0
	Other Race (including Multi-Racial)	7.2	7.4
Income to poverty	0.00–1.30	24.7	24.8
	>1.30–3.50	36.8	36.7
	>3.50 and above	38.5	38.5
Educational attainment	<12 years	19.2	19.4
	12 years	22.6	22.7
	>12 years	58.2	58.0

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Additional file 1: Table S2: Rotated factor loadings for the first four components from principal component analysis using nutrients, across race/ethnicity strata, US population aged 1+ years (NHANES 2009–2010) (N=9,317)

	Mexican-American (n=2,064)				**Other Hispanic** (n=988)			
Indicator[1]	PC1 (% expl.[2]=21.7)	PC2 (% expl.=18.2)	PC3 (% expl.=17.6)	PC4 (% expl.=10.2)	PC1 (% expl.=21.2)	PC2 (% expl.=19.4)	PC3 (% expl.=16.6)	PC4 (% expl.=11.0)
Fiber density (g/1,000 kcal)	**0.49**[3]	-0.18	0.00	0.04	**0.45**	-0.12	-0.01	0.11
Sodium density (g/1,000 kcal)	0.00	**-0.21**	**0.41**	0.09	0.05	**-0.24**	**0.31**	**0.26**
Potassium density (mg/1,000 kcal)	**0.43**	0.18	0.05	-0.05	**0.46**	0.13	0.11	-0.14
Iron density (mg/1,000 kcal)	-0.01	-0.08	-0.05	**0.70**	0.05	0.05	-0.18	**0.67**
Zinc density (mg/1,000 kcal)	-0.03	0.03	**0.24**	**0.43**	-0.03	0.11	0.13	**0.49**
Phosphorus density (mg/1,000 kcal)	0.17	**0.32**	**0.21**	0.09	0.07	**0.36**	**0.22**	0.12
Magnesium density (mg/1,000 kcal)	**0.48**	0.00	0.10	0.01	**0.42**	0.09	0.09	0.05
Calcium density (mg/1,000 kcal)	0.05	**0.54**	-0.09	0.03	0.00	**0.53**	-0.04	0.02
Vitamin A density (μg/1,000 kcal)	-0.03	0.16	-0.07	**0.45**	0.12	**0.31**	-0.12	0.13
Vitamin C density (mg/1,000 kcal)	**0.34**	0.13	**-0.23**	-0.12	**0.39**	0.06	-0.10	**-0.31**
Vitamin D density (μg/1,000 kcal)	-0.02	**0.54**	-0.08	0.08	-0.03	**0.53**	-0.05	-0.05
Protein (% of total energy)	0.10	0.00	**0.47**	0.11	0.08	-0.02	**0.46**	**0.20**
Carbohydrate (% of total energy)	0.18	-0.06	**-0.52**	0.19	**0.20**	0.02	**-0.55**	0.11
Added sugars (% of total energy)	**-0.25**	-0.10	**-0.33**	0.13	**-0.24**	-0.01	**-0.44**	0.12
Saturated fat (% of total energy)	**-0.32**	**0.37**	0.19	-0.12	**-0.36**	**0.30**	**0.22**	-0.14

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	Non-Hispanic White (n=3,984)				**Non-Hispanic Black** (n=1,726)			
Indicator[1]	PC1 (% expl.=20.1)	PC2 (% expl.=19.0)	PC3 (% expl.=18.0)	PC4 (% expl.=11.2)	PC1 (% expl.[2]=20.9)	PC2 (% expl.=18.5)	PC3 (% expl.=15.4)	PC4 (% expl.=11.4)
Fiber density (g/1,000 kcal)	**0.49**	-0.13	-0.01	0.08	**0.44**	0.00	-0.11	0.15
Sodium density (g/1,000 kcal)	0.04	**-0.17**	**0.37**	**0.23**	0.04	**0.39**	**-0.23**	**0.22**
Potassium density (mg/1,000 kcal)	**0.43**	0.14	0.12	-0.07	**0.46**	0.10	0.13	-0.08
Iron density (mg/1,000 kcal)	0.05	0.02	-0.11	**0.65**	0.02	-0.10	-0.01	**0.68**
Zinc density (mg/1,000 kcal)	-0.08	0.08	0.11	**0.59**	-0.05	0.14	0.03	**0.47**
Phosphorus density (mg/1,000 kcal)	0.04	**0.40**	**0.20**	0.07	0.08	**0.26**	**0.36**	0.10
Magnesium density (mg/1,000 kcal)	**0.43**	0.05	0.09	0.06	**0.44**	0.13	0.04	0.03
Calcium density (mg/1,000 kcal)	-0.02	**0.55**	-0.04	0.01	0.03	-0.07	**0.57**	0.04
Vitamin A density (µg/1,000 kcal)	0.16	**0.31**	-0.09	0.07	0.04	-0.07	0.12	**0.34**
Vitamin C density (mg/1,000 kcal)	**0.42**	0.06	-0.12	**-0.20**	**0.38**	-0.14	0.12	**-0.26**
Vitamin D density (µg/1,000 kcal)	-0.05	**0.55**	-0.07	-0.04	0.01	-0.08	**0.57**	0.05
Protein (% of total energy)	0.02	0.08	**0.41**	0.19	0.06	**0.49**	-0.02	0.07
Carbohydrate (% of total energy)	0.10	0.07	**-0.57**	0.14	**0.23**	**-0.51**	-0.03	0.16
Added sugars (% of total energy)	**-0.24**	0.01	**-0.43**	0.12	**-0.20**	**-0.40**	-0.06	0.12
Saturated fat (% of total energy)	**-0.31**	**0.23**	**0.28**	**-0.20**	**-0.38**	0.18	**0.33**	-0.05

	Other Race - Including Multi-Racial (n=555)			
Indicator[1]	PC1 (% expl.=20.5)	PC2 (% expl.=18.9)	PC3 (% expl.=17.9)	PC4 (% expl.=11.6)
Fiber density (g/1,000 kcal)	**0.41**	-0.08	-0.07	0.12
Sodium density (g/1,000 kcal)	0.08	**-0.24**	**0.40**	0.13
Potassium density (mg/1,000 kcal)	**0.47**	0.11	0.14	-0.08
Iron density (mg/1,000 kcal)	-0.02	0.00	-0.05	**0.70**
Zinc density (mg/1,000 kcal)	**-0.23**	-0.01	**0.30**	**0.46**
Phosphorus density (mg/1,000 kcal)	0.08	**0.39**	**0.25**	0.03
Magnesium density (mg/1,000 kcal)	**0.43**	0.02	0.14	0.04
Calcium density (mg/1,000 kcal)	0.03	**0.53**	-0.11	0.14
Vitamin A density (µg/1,000 kcal)	0.16	**0.26**	-0.10	**0.33**
Vitamin C density (mg/1,000 kcal)	**0.40**	0.01	-0.18	-0.12
Vitamin D density (µg/1,000 kcal)	0.02	**0.51**	-0.02	-0.02
Protein (% of total energy)	0.02	-0.01	**0.54**	0.04
Carbohydrate (% of total energy)	0.18	-0.13	**-0.43**	**0.24**
Added sugars (% of total energy)	**-0.27**	-0.03	**-0.33**	0.11
Saturated fat (% of total energy)	**-0.26**	**0.39**	0.03	**-0.20**

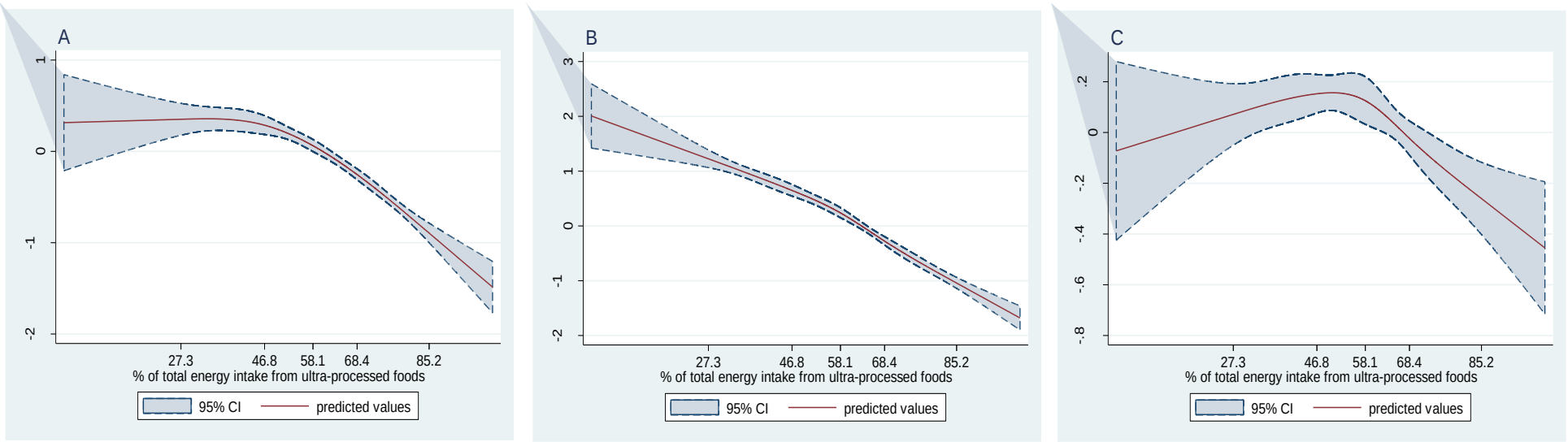
[1]For details on Indicators, see Methods section

[2]Proportion of the variance explained by each factor after orthogonal varimax rotation (Kaiser on)

[3]Items with a factor loading above 0.20 or below -0.20 have been highlighted

Manuscript 1. Online Supporting Material

Additional file 1: Figure S1: PC2-PC4 factor scores regressed on the dietary share of ultra-processed foods evaluated by restricted cubic splines, US population aged 1+ years (NHANES 2009–2010) (N=9,317)



A. Unadjusted model: There was little evidence of linearity in the restricted cubic spline model (Wald test for linear term p=0.8; Wald test for all non-linear terms p<0.001). Adjusted model: Wald test for linear term p=0.3; Wald test for all non-linear terms p=0.003.

B. Coefficient for linear term = -0.03, 95% CI: -0.04 to -0.01. There was little evidence of nonlinearity in the restricted cubic spline model (Wald test for linear term p=0.002; Wald test for all non-linear terms p=0.03). Adjusted model: Coefficient for linear term = -0.03, 95% CI: -0.04 to -0.01; Wald test for linear term p=0.001; Wald test for all non-linear terms p=0.02.

C. Unadjusted model: There was little evidence of linearity in the restricted cubic spline model (Wald test for linear term p=0.3; Wald test for all non-linear terms p=0.03). Adjusted model: Wald test for linear term p=0.1; Wald test for all non-linear terms p=0.02.

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Additional file 1: Table S3: PC2-PC4 score means and adherence according to the dietary share of ultra-processed foods, US population aged 1+ years (NHANES 2009–2010)

Dietary share of ultra-processed foods (% of total energy intake)		"Calcium and Vitamin D driven pattern" factor score		Adherence to "calcium and Vitamin D driven pattern"[2]			"Protein driven pattern" factor score		Adherence to "protein driven pattern"[3]			"Iron and zinc driven pattern" factor score		Adherence to "iron and zinc driven pattern"[4]		
		Mean					Mean					Mean				
Quintiles	Mean (range)	unadj. (R2=0.06)	adj.[1] (R2=0.18)	Low (%)	Middle (%)	High (%)	unadj. (R2=0.16)	adj.[1] (R2=0.20)	Low (%)	Middle (%)	High (%)	unadj. (R2=0.01)	adj.[1] (R2=0.02)	Low (%)	Middle (%)	High (%)
Q1 (n=1941)	32.6 (0 to 42.6)	0.32*	0.41*	26.4	32.0	41.6	1.08*	1.08*	15.2	29.1	55.8	0.09	0.06	30.5	32.5	37.0
Q2 (n=1903)	48.6 (42.6 to 54.0)	0.27*	0.26*	24.7	33.1	42.1	0.57*	0.57*	22.5	33.7	43.8	0.14	0.13	29.5	31.9	38.7
Q3 (n=1791)	58.4 (54.0 to 62.8)	0.06	0.03	29.9	32.3	37.8	0.22	0.23	31.1	37.3	31.6	0.14	0.13	29.8	34.0	36.2
Q4 (n=1785)	67.3 (62.8 to 72.3)	-0.24*	-0.26*	35.2	36.3	28.6	-0.23*	-0.17	39.6	39.2	21.2	-0.06	-0.04	33.8	36.7	29.5
Q5 (n=1897)	80.7 (72.3 to 100)	-0.69*¥	-0.71*¥	50.5	32.9	16.6	-0.83*¥	-0.76*¥	58.3	27.5	14.2	-0.16¥	-0.12	43.2	31.6	25.2

[1]Adjusted for sex, age group (1-5, 6–11, 12–19, 20–39, 40–59, 60+ years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Race – Including Multi-Racial), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over), and educational attainment (<12, 12 years, and >12 years).

[2]"Calcium and Vitamin D driven pattern" (PC1) factor score tertiles: T1 (-4.6 to -0.8 points); T2 (-0.8 to 0.3 points); T3 (0.3 to 9.8 points).

[3]"Protein driven pattern" (PC1) factor score tertiles: T1 (-8.9 to -0.5 points); T2 (-0.5 to 0.8 points); T3 (0.8 to 8.4 points).

[4]"Iron and zinc driven pattern" (PC1) factor score tertiles: T1 (-4.5 to -0.6 points); T2 (-0.6 to 0.3 points); T3 (0.3 to 12.9 points).

*Statistically significant p<=0.001

¥Significant linear trend across all quintiles (p<=0.001), both in unadjusted and models adjusted for sex, age group, race/ethnicity, ratio of family income to poverty, and educational attainment.

3.2 MANUSCRIPT 2

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Title: Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study.

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ABSTRACT

Objectives: To investigate the contribution of ultra- processed foods to the intake of added sugars in the USA. Ultra-processed foods were defined as industrial formulations which, besides salt, sugar, oils and fats, include substances not used in culinary preparations, in particular additives used to imitate sensorial qualities of minimally processed foods and their culinary preparations.

Design: Cross-sectional study.

Setting: National Health and Nutrition Examination Survey 2009–2010.

Participants: We evaluated 9317 participants aged 1+ years with at least one 24 h dietary recall.

Main outcome measures: Average dietary content of added sugars and proportion of individuals consuming more than 10% of total energy from added sugars.

Data analysis: Gaussian and Poisson regressions estimated the association between consumption of ultra-processed foods and intake of added sugars. All models incorporated survey sample weights and adjusted for age, sex, race/ethnicity, family income and educational attainment.

Results: Ultra-processed foods comprised 57.9% of energy intake, and contributed 89.7% of the energy intake from added sugars. The content of added sugars in ultra-processed foods (21.1% of calories) was eightfold higher than in processed foods (2.4%) and fivefold higher than in unprocessed or minimally processed foods and processed culinary ingredients grouped together (3.7%). Both in unadjusted and adjusted models, each increase of 5 percentage points in proportional energy intake from ultra-processed foods increased the proportional energy intake from added sugars by 1 percentage point. Consumption of added sugars increased linearly across quintiles of ultra-processed food consumption: from 7.5% of total energy in the lowest quintile to 19.5% in the highest. A total of 82.1% of Americans in the highest quintile exceeded the recommended limit of 10% energy from added sugars, compared with 26.4% in the lowest.

Conclusions: Decreasing the consumption of ultra- processed foods could be an effective way of reducing the excessive intake of added sugars in the USA.

INTRODUCTION

Increasing policy attention has focused on added sugars, including by the WHO,[1] the UK National Health System,[2] the Canadian Heart and Stroke Foundation,[3] the American Heart Association (AHA)[4] and the US Dietary Guidelines Advisory Committee (USDGAC).[5]

These reports concluded that a high intake of added sugars increases the risk of weight gain,[1 4 5] excess body weight[5] and obesity;[3 5] type 2 diabetes mellitus;[3 5] higher serum triglycerides[5] and high blood cholesterol;[3] higher blood pressure[5] and hypertension;[5] stroke;[3 5] coronary heart disease;[3 5] cancer;[3] and dental caries.[1 3 5] Moreover, foods higher in added sugars are often a source of empty calories with minimum essential nutrients or dietary fibre,[6–8] which displace more nutrient-dense foods[9] and lead, in turn, to simultaneously overfed and undernourished individuals.

All reports recommended limiting intake of added sugars.[1 3–5] In the USA, the USDGAC recommended limiting added sugars to no more than 10% of total calories. This is a challenge, as recent consumption of added sugars in the USA amounted to almost 15% of total calories in 2005–2010.[10 11]

To design and implement effective measures to reduce added sugars, their dietary sources must be clearly identified. Added sugars can be consumed either as ingredients of dishes or drinks prepared from scratch by consumers or a cook, or as ingredients of food products manufactured by the food industry. According to market disappearance data from 2014, more than three quarters of the sugar and high fructose corn syrup available for human consumption in the USA were used by the food industry.[12] This suggests that food products manufactured by the industry could have an important role in the excess added sugars consumption in the USA. However, to assess this role, it is essential to consider the contribution of manufactured food products to both total energy intake and the energy intake from added sugars, and, more relevantly, to quantify the relationship between their consumption and the total dietary content of added sugars. To address these questions, we performed an investigation utilising the 2009–2010 National Health and Nutrition Examination Survey (NHANES).

SUBJECTS AND METHODS

Data source, population and sampling

We utilised nationally representative data from the 2009–2010 NHANES, specifically the dietary component What we eat in America (WWEIA).[13]

NHANES is a continuous, nationally representative, cross-sectional survey of the non-institutionalised, civilian US residents.[14] The NHANES sample was obtained by using a complex, stratified, multistage probability cluster sampling design based on the selection of counties, blocks, households and the number of people within households.[14] In order to improve the estimate precision and reliability, NHANES 2009–2010 oversampled the following subgroups: Hispanic, Non-Hispanic black, Non-Hispanic white and Other persons at or below 130% of the federal poverty level and Non-Hispanic white and Other persons aged 80+ years.[14]

The survey included an interview conducted in the home and a subsequent health examination performed at a mobile examination centre (MEC). All NHANES examinees were eligible for two 24 h dietary recall interviews. The first dietary recall interview was collected in-person in the MEC[15] while the second was collected by telephone 3–10 days later but never on the same day of the week as the MEC interview.[16] Dietary interviews were conducted by trained interviewers using the validated[17–19] US Department of Agriculture Automated Multiple-Pass Method (AMPM).[20] For children under 9 years of age, the interview was conducted with a proxy; for children between 6 and 8 years of age, in the presence of the child. Children aged 9–11 years provided their own data assisted by an adult household member (assistant). The preferred proxy/assistant was the most knowledgeable person about the child's consumption on the day before the interview. If the child had more than one caregiver, several individuals could contribute to the intake data.[15 16]

Among the 13 272 people screened in NHANES 2009–2010, 10 537 (79.4%) participated in the household interview and 10 253 (77.3%) also participated in the MEC health examination.[21] Of these, 9754 individuals provided 1 day of complete dietary intakes, of which 8406 provided 2 days.[22]

We evaluated 9317 survey participants aged 1 year and above who had 1 day 24 h dietary recall data and had not been breast fed on either of the 2 days. These individuals had similar sociodemographic characteristics (gender, age, race/ethnicity, family income and educational attainment) to the full sample of 10 109 interviewed participants aged 1 year and above.

Food classification according to processing

We classified all recorded food items (N=280 132 Food Codes for both recall days) according to NOVA, a food classification based on the extent and purpose of industrial food processing.[23–25] This classification includes four groups: 'unprocessed or minimally processed foods' (such as fresh, dry or frozen fruits or vegetables, grains, legumes, meat, fish and milk); 'processed culinary ingredients' (including table sugar, oils, fats, salt, and other substances extracted from foods or from nature, and used in kitchens to make culinary preparations); 'processed foods' (foods manufactured with the addition of salt or sugar or other substances of culinary use to unprocessed or minimally processed foods, such as canned food and simple breads and cheese) and 'ultraprocessed foods' (formulations of several ingredients which, besides salt, sugar, oils and fats, include food substances not used in culinary preparations, in particular, flavours, colours, sweeteners, emulsifiers and other additives used to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations or to disguise undesirable qualities of the final product). A detailed definition of each food group and examples of food items classified in each group are shown in online supplementary table S1. The rationale underlying the classification is described elsewhere.[26–29]

For all food items (Food Codes) judged to be a handmade recipe, the classification was applied to the underlying ingredients (Standard Reference Codes –SR Codes-) obtained from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS) 5.0.[30] Refer to online supplementary material (OSM) for further details.

Assessing energy and added sugar contents

For this study, we used Food Code energy values as provided by NHANES.

For handmade recipes, we calculated the underlying ingredient (SR Code) energy values using variables from both FNDDS 5.0[30] and USDA National Nutrient Database for Standard Reference, Release 24 (SR24).[31] Refer to OSM for further details.

Data on added sugars per Food Code and per SR Code were obtained by merging the Food Patterns Equivalents Database (FPED) 2009–2010 and the Food Patterns Equivalents Ingredients Database (FPID) 2009–2010.[32] Added sugars are defined in these databases as "sugars that are added to foods as an ingredient during preparation, processing, or at the table. Added sugars do not include naturally occurring sugars (eg, lactose in milk, fructose in fruits). Examples of added sugars include brown sugar, cane sugar, confectioners' sugar, granulated sugar, dextrose, white sugar, corn syrup and corn syrup solids, molasses, honey, and all types of syrups such as maple syrup, table syrups, and pancake syrup."[32] These two databases express the content of added sugars in teaspoons per 100 g. Teaspoons were converted into grams using the factor 4.2 g/teaspoon and into kcal using the factor 3.87 kcal/g.

Data analysis

We utilised all available day 1 dietary data for each participant. Food items were sorted into mutually exclusive food subgroups within unprocessed or minimally processed foods (n=11), processed culinary ingredients (n=4), processed foods (n=4) and ultra-processed foods (n=18), as shown in **table 1**. First, we evaluated the contributions of each of the NOVA food groups and subgroups to total energy and to the energy from added sugars. Next, we calculated the average content of added sugars in the overall US diet and in fractions of this diet composed by each of the NOVA food groups and subgroups. We also calculated the dietary content of added sugars in the group of unprocessed or minimally processed foods combined with the group of processed culinary ingredients, as foods belonging to these two groups are usually combined together in culinary preparations and therefore consumed together.

We used Gaussian regression to estimate the association between the dietary contribution of ultraprocessed foods and the dietary content of added sugars, each expressed as proportions of total energy. This association was also explored after adjusting for the proportion of added sugars in non-ultra-processed energy intake. The dietary contribution of ultraprocessed foods was transformed using restricted cubic spline functions to allow for non-linearity.

The average content of added sugars in the overall diet was compared across quintiles of the dietary contribution of ultra-processed foods. Poisson regression was used to assess whether the percentage of diets with more than 10% or 20% of total energy from added sugars increased across quintiles. This increase was also evaluated across demographic subgroups in stratified analysis. Tests of linear trend were performed in order to evaluate the effect of quintiles as a single continuous variable.

All regression models were adjusted for age (1–5, 6–11, 12–19, 20–39, 40–59, 60+ years), sex, race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic white, Non-Hispanic black, Other race including Multi-racial), ratio of family income to poverty (categorized on the basis of Supplemental Nutrition Assistance Program (SNAP) eligibility as 0.00–1.30, >1.30–3.50 and >3.50 and above)[14] and educational attainment of respondents, for participants aged 20+ years, and of household reference persons otherwise (<12, 12 and >12 years). Since 908 participants had missing values on family income and/or educational attainment, multivariable-adjusted analysis included 8409 individuals. The analysis which also adjusted for the added sugar content of all non-ultra-processed foods grouped together included 8335 individuals.

The NHANES sample weights were used in all analyses to account for differential probabilities of selection for the individual domains, non-response to survey instruments, and differences between the final sample and the total US population. The Taylor series linearization variance approximation procedure was used for variance estimation in all analysis in order to account for the complex sample design and the sample weights.[14]

To minimise chance findings from multiple comparisons, statistical hypotheses were tested using a two tailed $p<0.001$ level of significance. Data were analysed using Stata statistical software package V.12.1.

RESULTS

Distribution of total energy intake by food groups

The average US daily energy intake in 2009–2010 was 2069.5 kcal, and nearly three in five calories (57.9%) came from ultra-processed foods (**table 1**).

Unprocessed or minimally processed foods contributed 29.6% of total calories, processed foods an additional 9.4%, and processed culinary ingredients the remaining 2.9%. The most common ultra-processed foods in terms of energy contribution were breads; soft drinks, fruit drinks and milk-based drinks; cakes, cookies and pies; salty snacks; frozen and shelf-stable plates; pizza and breakfast cereals. Meat, fruit and milk provided the most calories among unprocessed or minimally processed foods; ham and cheese, the most calories among processed foods; and table sugar and plant oils, the most calories among processed culinary ingredients.

Distribution of energy intake from added sugars by food groups

The average US daily intake of added sugars was 292.2 kcal (**table 1**). Notably, almost 90% of this (89.7%) came from ultra-processed foods. The main sources of added sugars among ultra-processed foods were: soft drinks (17.1% of US intake of added sugars); fruit drinks (13.9%); milk-based drinks (4.6%); cakes, cookies and pies (11.2%); breads (7.6%); desserts (7.3%); sweet snacks (7.1%); breakfast cereals (6.4%); and ice creams and ice pops (5.9%). In contrast, only 8.7% of the added sugars in the US diet came from processed culinary ingredients (table sugar consumed as part of dishes or drinks prepared from scratch by consumers or a cook), and only 1.6% from processed foods.

The average content of added sugars in ultra-processed foods (21.1% of calories) was eightfold higher than in processed foods (2.4%) and fivefold higher than in unprocessed or minimally processed foods and processed culinary ingredients grouped together (3.7%) (**table 1**).

Association between consumption of ultra-processed foods and added sugar intake

In unadjusted restricted cubic splines Gaussian regression analysis, a strong linear association was identified between the dietary contribution (percentage of calories) of ultra-processed foods and the dietary content (percentage of calories) in added sugars (coefficient for linear term=0.20, 95% CI 0.17 to 0.23) (**figure 1**).

There was little evidence of non-linearity in the restricted cubic spline model (Wald test for linear term p<0.0001; Wald test for all non-linear terms p=0.27). The strength of the association remained fairly the same after adjusting for age, sex, race/ethnicity, family income, educational attainment and proportion of added sugars in non-ultra-processed energy intake (coefficient for linear term=0.19, 95% CI 0.17 to 0.22). Overall, each increase in 5 percentage points of energy in consumption of ultra-processed foods was associated with 1 higher percentage point of energy in the consumption of added sugars.

Across quintiles of energy-adjusted ultra-processed food consumption, the intake of added sugars increased substantially and monotonically from 7.5% of total calories in the lowest quintile to 19.5% in the highest quintile. Across the same quintiles, the proportion of individuals consuming more than 10% of total energy from added sugars (59.6% in the total population) increased from 26.4% to 82.1%, respectively. An even more pronounced increase was seen in the proportion of individuals consuming more than 20% of their total energy from added sugars: from 4.7% in the lowest quintile to 41.2% in the highest quintile (**table 2**). Similar increases were seen in stratified analysis by major demographic subgroups (see online supplementary table S2). The magnitude and the statistical significance of the association between the dietary contribution of ultra-processed foods and the dietary content in added sugars did not change with adjustment for sex, age, race/ethnicity, family income and educational attainment.

DISCUSSION

In this analysis of nationally representative data, we confirmed the excessive consumption of added sugars in the USA.[10 11] We also provide new evidence that ultraprocessed foods represent more than half of all calories in the US diet, and contribute nearly 90% of all added sugars. Added sugars represented 1 of every 5 calories in the average ultra-processed food (21.1%), far higher than the content of added sugars in processed foods (2.4%) and in unprocessed or minimally processed foods, and processed culinary ingredients grouped together (3.7%). A strong linear relationship was found between the dietary contribution of ultra-processed foods and the dietary content of added sugars. Moreover, the risk of exceeding the recommended upper limit of 10% energy from added sugars was far higher when ultra-processed food consumption was high, and risk differences were even more pronounced for exceeding a limit of 20% energy. Notably, only those Americans in the lowest quintile of ultra-processed food consumption met the recommended limit of <10% energy from added sugars. To the best of our knowledge, this is the first study to assess the consumption of ultra-processed foods and establish its relationship with excessive added sugar intake in the USA.

The high consumption of added sugars in the USA is most likely contributing to excess obesity, type 2diabetes, dyslipidaemia, hypertension and coronary heart disease.[1 3–5] Consequently, most dietary guidelines now recommend limiting added sugar consumption. However, such guidelines are not always clear on how to put this recommendation into practice. Our study suggests that in the USA, limiting the consumption of ultraprocessed foods may be a highly effective way to decrease added sugars. A reduction in ultra-processed foods should also increase the intake of more healthful, minimally processed foods such as milk, fruits and nuts, and freshly prepared dishes based on whole grains and vegetables, which would produce additional health benefits beyond the reduction in added sugar. Consistent with this approach, in Brazil, where the consumption of added sugars is as high as in the USA,[33] the new dietary guidelines launched in 2014 emphasise the importance of not replacing unprocessed or minimally processed foods and freshly prepared dishes by ultra-processed foods.[34]

Few studies have assessed the impact of levels of food processing on the nutrient profile of the US diet. One analysis using data from NHANES 2003–2008[35] used a food classification system[36] including 'Mixtures of combined Ingredients' and 'Ready-to-eat', which are mostly ultra-processed foods, and together contributed to about half of the total energy intake and three-quarters of energy intake from added sugars. Another study evaluated household barcoded purchasing data from 2000 to 2012 using a classification system guided by the one used in our study.[37] In 2012, the mean per capita purchase of 'highly processed foods', a category similar to ultra-processed foods, corresponded to 61.0% of all calories and had higher adjusted median total sugar content than 'less processed foods'. This report did not evaluate added sugars nor the contribution of processed foods to sugar intake. It also did not capture non-barcoded items such as unpackaged fresh fruit, vegetables and meat, or highly processed foods such as ready-to-eat store-prepared items. An investigation in Canada, using 2001 household purchasing data, found that ultra-processed foods are high in free sugars and that only households in the lowest quintile of ultra-processed food purchasing might have met the recommended limit of <10% energy from free sugars (9.2%).[38] Being based on household purchasing data, these two prior studies and others based on the NOVA classification system[23 39–42] could not evaluate the fraction of wasted food nor purchases at restaurants, which represent a substantial proportion of US calories. Our findings build on and considerably extend these prior reports by evaluating food processing and added sugar intake using contemporary, nationally representative dietary intake data in the USA.

Our study has several strengths. We studied a large, nationally representative sample of the US population, increasing generalisability. Use of data on added sugars, rather than total sugars or sugar-sweetened beverages, corresponds to the relevant area of prioritisation of recent national and international guidelines. Our investigation was based on individual consumption data, rather than market disappearance or household purchasing data which cannot account for differences between amounts purchased and amounts actually consumed.

Potential limitations should be considered. As with most population measures, dietary data obtained by 24 h recalls are imperfect. However, the standardized methods and approach of NHANES minimise potential error and bias, particularly for assessing population averages as focused on in the present study. Previous studies suggest that people with obesity may underreport consumption of foods with caloric sweeteners[43] such as desserts and sweet baked goods.[44 45] If so, these biases may lead to an underestimation of the dietary contribution of ultra-processed foods and the overall intake of added sugars, but should have much less effect on the association between these. Although NHANES collects some information indicative of food processing (ie, place of meals, product brands), these data are not consistently determined for all food items, which could lead to modest overestimation or underestimation of the consumption of ultra-processed foods.

In conclusion, we found that ultra-processed foods contribute almost 60% of calories and 90% of added sugars consumed in the USA. Only Americans in the lowest quintile of ultra-processed food consumption met the recommended guidelines for intake of added sugars. Decreasing the consumption of ultra-processed foods could be an effective way of reducing the excessive intake of added sugars in the USA.

Contributors: CAM, EMS and DM designed the research. EMS, LGB and MLdCL took care of data management. EMS, J-CM and MLdCL analysed the data. EMS, DM and CAM wrote the paper. CAM and EMS had primary responsibility for the final content. All the authors read and approved the final manuscript. All the authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. *Guideline: sugars intake for adults and children.* Geneva: World Health Organization, 2015.

2. NHS Health Choices. Eight tips for healthy eating. http://www.nhs.uk/Livewell/Goodfood/Pages/eight-tips-healthy-eating.aspx (accessed Aug 2015).

3. Canadian Heart and Stroke Foundation Position Statement, August 2014. http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.9201361/k.47CB/Sugar_heart_disease_and_stroke.htm (accessed Aug 2015).

4. Johnson RK, Appel LJ, Brands M, et al. American Heart Association Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism and the Council on Epidemiology and Prevention. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. *Circulation* 2009;120:1011–20.

5. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. http://www.health.gov/dietaryguidelines/2015-scientificreport/PDFs/Scientific-Report-of-the-2015-Dietary-Guidelines-Advisory-Committee.pdf

6. US Department of Agriculture and US *Department of health and human services. Dietary guidelines for Americans, 2010.* 7th edn, Washington DC: US Government Printing Office, December, 2010.

7. Marriott BP, Olsho L, Hadden L, et al. Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003–2006. *Crit Rev Food Sci Nutr* 2010;50:228–58.

8. Bowman SA. Diets of individuals based on energy intakes from added sugars. Fam *Econ Nutr Rev* 1999;12:31–8.

9. Murphy S, Johnson R. The scientific basis of recent US guidance on sugars intake. *Am J Clin Nutr* 2003;78:827S–33S.

10. Welsh JA, Sharma AJ, Grellinger L, et al. Consumption of added sugars is decreasing in the United States. *Am J Clin Nutr* 2011;94:726–34.

11. Yang Q, Zhang Z, Gregg EW, et al. Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA Intern Med* 2014;174:516–24.

12. United States Department of Agriculture. Economic Research Service. Sugar and Sweeteners Yearbook Tables. http://www.ers.usda.gov/data-products/sugar-and-sweeteners-yearbook-tables.aspx (accessed Aug 2015).

13. National Health and Nutrition Examination Survey http://wwwn.cdc.gov/nchs/nhanes/search/nhanes09_10.aspx (accessed Aug 2015).

14. Johnson CL, Paulose-Ram R, Ogden CL, et al. National health and nutrition examination survey: analytic guidelines, 1999–2010. *Vital Health Stat 2*. 2013;1–24. http://www.cdc.gov/nchs/data/series/sr_02/sr02_161.pdf (accessed Aug 2015).

15. NHANES. MEC In-Person Dietary Interviewers Procedures Manual. January 2009. http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/DietaryInterviewers_Inperson.pdf (accessed Aug 2015).

16. NHANES. Phone Follow-Up Dietary Interviewer Procedures Manual. September 2009. http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/Dietary_PFU_09.pdf (accessed Aug 2015).

17. Moshfegh AJ, Rhodes DG, Baer DJ, et al. The USDA Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *Am J Clin Nutr* 2008;88:324–32.

18. Blanton CA, Moshfegh AJ, Baer DJ, et al. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. *J Nutr* 2006;136:2594–9.

19. Rumpler WV, Kramer M, Rhodes DG, et al. Identifying sources of reporting error using measured food intake. *Eur J Clin Nutr* 2008;62:544–52.

20. Automated Multiple-Pass Method. United States Department of Agriculture. Agriculture Research Service. http://www.ars.usda.gov/ba/bhnrc/fsrg (accessed Aug 2015).

21. National Health and Nutrition Examination Survey. NHANES Response Rates and Population Totals. Response Rates. http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm (accessed Aug 2015).

22. National Health and Nutrition Examination Survey. 2009–2010 Data Documentation, Codebook, and Frequencies. http://www.cdc.gov/nchs/nhanes/nhanes2009–2010/DR1IFF_F.htm (accessed Aug 2015).

23. Monteiro CA, Levy RB, Claro RM, et al. A new classification of foods based on the extent and purpose of their processing. *Cad Saúde Pública* 2010;26:2039–49.

24. Moubarac JC, Parra DC, Cannon G, et al. Food Classification Systems Based on Food Processing: significance and implications for policies and actions: a systematic literature review and assessment. *Curr Obes Rep* 2014;3:256–72.

25. Monteiro CA, Cannon G, Levy RB, et al. Ultra-processing and a new classification of foods. In: Neff R, ed. Introduction to US food system. Public health, environment, and equity. San Francisco: Jossey Bass AWiley Brand, 2015:338–9.

26. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. *Public Health Nutr* 2009;12:729–31.

27. Ludwig DS. Technology, diet, and the burden of chronic disease. *JAMA* 2011;305:1352–3.

28. Monteiro CA, Cannon G, Levy RB, et al. The Food System. Processing. The big issue for disease, good health, well-being. *World Nutr* 2012;3:527–69. http://www.wphna.org (accessed Aug 2015).

29. Moodie R, Stuckler D, Monteiro C, et al. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. *Lancet* 2013; 381:670–9.

30. Ahuja JKA, Montville JB, Omolewa-Tomobi G, et al. *USDA food and nutrient database for dietary studies, 5.0.* US Beltsville, MD: Department of Agriculture, Agricultural Research Service, Food Surveys Research Group, 2012.

31. US Department of Agriculture, Agricultural Research Service. 2011. USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl (accessed Aug 2015).

32. Bowman SA, Clemens JC, Thoerig RC, et al. *Food patterns equivalents database 2009–10: methodology and user guide[Online]. Food surveys research group, beltsville human nutrition research center, agricultural research service, US Beltsville*, Maryland: Department of Agriculture, 2013. http://www.ars.usda.gov/ba/bhnrc/fsrg (accessed Jun 2013).

33. da Costa Louzada ML, Bortoletto Martins AP, Silva Canella D, et al. Ultra-processed foods and dietary quality in Brazil. *Rev Saude Publica* 2015;49:38.

34. *Dietary Guidelines for the Brazilian Population*. 2nd edn. Ministry of Health of Brazil, 2014.

35. Eicher-Miller HA, Fulgoni VL III, Keast DR. Contributions of processed foods to dietary intake in the US from 2003–2008: a report of the Food and Nutrition Science Solutions Joint Task Force of the Academy of Nutrition and Dietetics, American Society for Nutrition, Institute of Food Technologists, and International Food Information Council. *J. Nutr* 2012;142:2065S–72S.

36. International Food Information Council Foundation. Understanding our food communications tool kit, 2010. http://www.foodinsight.org/ For-Professionals/Understanding-Our-Food/tabid/1398/Default.aspx (accessed Aug 2015).

37. Poti JM, Mendez MA, Ng SW, et al. Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households? *Am J Clin Nutr* 2015;101:1251–62.

38. Moubarac JC, Martins AP, Claro RM, et al. Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. *Public Health Nutr* 2013;16:2240–8.

39. Monteiro CA, Levy RB, Claro RM, et al. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. *Public Health Nutr* 2011;14:5–13.

40. Crovetto MM, Uauy R, Martins AP, et al. [Household availability of ready-to-consume food and drink products in Chile: impact on nutritional quality of the diet]. *Rev Med Chil* 2014;142:850–8.

41. Monteiro CA, Moubarac JC, Cannon G, et al. Ultra-processed products are becoming dominant in the global food system. *Obes Rev* 2013;14(Suppl 2):21–8.

42. Canella DS, Levy RB, Martins AP, et al. Ultra-processed food products and obesity in Brazilian households (2008–2009). *PLoS ONE* 2014;9:e92752.

43. Bingham S, Luben R, Welch A, et al. Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and nonobese individuals in the European prospective investigation of cancer Norfolk. *Cancer Epidemiol Biomarkers Prev* 2007;16: 1651–4.

44. Lafay L, Mennen L, Basdevant A, et al. Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Santé (FLVS) study. *Int J Obes Relat Metab Disord* 2000;24:1500–6.

45. Pryer JA, Vrijheid M, Nichols R, et al. Who are the ‘‘low energy reporters’’ in the dietary and nutritional survey of British adults? *Int J Epidemiol* 1997;26:146–54.

TABLES AND FIGURES

Table 1. Distribution of the total energy intake and of the energy intake from added sugars according to food groups, and the mean content of added sugars of each food group

Food groups	Mean energy intake		Mean energy intake from added sugars		Mean content of added sugars
	Absolute (kcal/day)	Relative (% of total energy intake)	Absolute (kcal/day)	Relative (% of total energy intake from added sugars)	% of energy from added sugars
Unprocessed or minimally processed foods	***585.5***	***29.6***	***0.0***	***0.0***	**0.0**
Meat (includes poultry)	165.3	7.9	0.0	0.0	0.0
Fruit*	97.5	5.2	0.0	0.0	0.0
Milk and plain yoghurt	96.4	5.1	0.0	0.0	0.0
Grains	53.3	2.8	0.0	0.0	0.0
Roots and tubers	32.2	1.6	0.0	0.0	0.0
Eggs	28.8	1.4	0.0	0.0	0.0
Pasta	28.4	1.4	0.0	0.0	0.0
Legumes	16.2	0.8	0.0	0.0	0.0
Fish and sea food	17.2	0.8	0.0	0.0	0.0
Vegetables	13.5	0.7	0.0	0.0	0.0
Other unprocessed or minimally processed foods†	36.7	1.8	0.0	0.0	0.0
Processed culinary ingredients	***64.3***	***2.9***	**24.4**	***8.7***	***38.8***
Table sugar‡	24.7	1.1	24.4	8.7	98.5
Plant oils	27.5	1.3	0.0	0.0	0.0

Animal fats§	11.2	0.5	0.0	0.0	0.0
Other processed culinary ingredients¶	0.9	0.04	0.0	0.0	0.0
Unprocessed or minimally processed foods + Processed culinary ingredients	***649.8***	***32.6***	***24.4***	***8.7***	***3.7***
Processed foods	***209.7***	***9.4***	***2.5***	***1.6***	**2.4**
Cheese	80.1	3.7	0.0	0.0	0.0
Ham and other salted, smoked or canned meat or fish	26.4	1.2	0.3	0.2	1.4
Vegetables and other plant foods preserved in brine	13.4	0.7	1.6	0.9	13.7
Other processed foods**	89.8	3.8	0.6	0.5	1.2
Ultra-processed foods	***1209.8***	***57.9***	***265.2***	***89.7***	**21.1**
Breads	191.6	9.5	10.6	7.6	5.7
Cakes, cookies and pies	122.8	5.7	29.8	11.2	24.2
Salty-snacks	93.2	4.6	1.2	0.7	1.4
Frozen and shelf-stable plate meals	80.6	4.02	1.1	0.7	1.6
Soft drinks, carbonated	81.8	3.7	75.2	17.1	69.9
Pizza (ready-to-eat/heat)	81.8	3.5	2.4	1.4	2.9
Fruit drinks‡‡	69.2	3.3	55.7	13.9	67.5
Breakfast cereals	50.9	2.8	12.4	6.4	23.3
Sauces, dressings and gravies	49.8	2.4	4.4	2.8	10.0
Reconstituted meat or fish products	51.5	2.4	0.7	0.6	2.0
Sweet-snacks	50.9	2.4	19.4	7.1	38.9

Ice cream and ice pops	48.7	2.3	18.3	5.9	36.9
Milk-based drinks§§	34.6	1.8	10.8	4.6	34.1
Desserts¶¶	36.4	1.8	18.5	7.3	48.5
French fries and other potatoe products[11]	37.8	1.7	0.0	0.0	0.0
Sandwiches and hamburgers on bun (ready-to-eat/heat)	32.5	1.4	1.3	0.6	4.4
Instant and canned soups	14.3	0.8	0.1	0.1	0.7
Other ultra-processed foods†††	81.5	3.8	3.1	1.5	7.8
Total	**2069.5**	**100.0**	**292.2**	**100.0**	**13.8**

US population aged 1+ years (National Health and Nutrition Examination Survey 2009–2010) (N=9317).

*Including freshly squeezed juices.

†Including nuts and seeds (unsalted); yeast; dried fruits (without added sugars) and vegetables; non presweetened, non-whitened, non-flavoured coffee and tea; coconut water and meat; homemade soup and sauces; flours; tapioca.

‡Including honey, molasses, maple syrup (100%).

§Including butter, lard and cream.

¶Including starches; coconut and milk cream; unsweetened baking chocolate, cocoa powder and gelatin powder; vinegar; baking powder and baking soda.

**Including salted or sugared nuts and seeds; peanut, sesame, cashew and almond butter or spread; beer and wine.

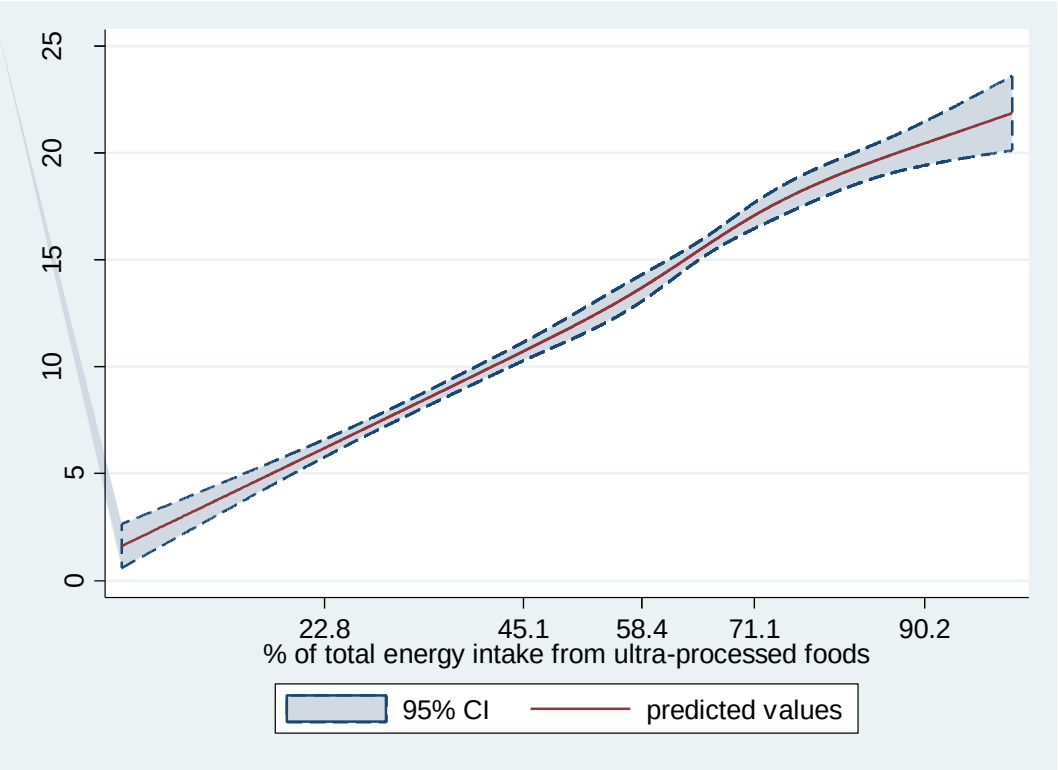
‡‡Including fruit and fruit-flavoured, non-carbonated and other sweetened drinks, including presweetened tea and coffee, energy drinks, sports drinks with no milk added, non-alcoholic wine.

§§Including flavoured yogurt sweetened with sugar or with low-calorie sweetener, milkshake.

¶¶Including ready-to-eat and dry-mix desserts such as pudding.

†††Including soya products such as meatless patties and fish sticks; baby food and baby formula; dips, spreads, mustard and catsup; margarine; sugar substitutes, sweeteners and all syrups (excluding 100% maple syrup); distilled alcoholic drinks.

Figure 1. The dietary content in added sugars regressed on the dietary contribution of ultra-processed foods evaluated by restricted cubic splines. US population aged 1+ years (National Health and Nutrition Examination Survey 2009–2010) (N=9317)



Legend: The values shown on the x-axis correspond to the 5th, 27.5th, 50th, 72.5th, and 95th centiles for percentage of total energy from ultra-processed foods (knots). Coefficient for linear term=0.20 (95% CI 0.17 to 0.23). There was little evidence of non-linearity in the restricted cubic spline model (Wald test for linear term p<0.0001; Wald test for all non-linear terms p=0.27).

Table 2. Indicators of the dietary content in added sugars according to the dietary contribution of ultra-processed foods

Dietary contribution of ultra-processed foods (% of total energy intake)		**Indicators**						
		% of total energy intake from added sugars	Participants with more than 10% of total energy intake from added sugars			Participants with more than 20% of total energy intake from added sugars		
Quintiles	Mean (range)	Mean	%	PR*	PRadj†	%	PR*	PRadj†
1st (n=1,937)	28.9 (0 to 40.1)	7.5	26.4	1	1	4.7	1	1
2nd (n=1,888)	47.3 (40.1 to 53.3)	11.1	50	1.9	1.9	10.5	2.2	2.2
3rd (n=1,814)	58.7 (53.3 to 64.1)	13.8	62.7	2.4	2.3	21.1	4.5	4.3
4th (n=1,779)	69.7 (64.1 to 75.7)	16.9	76.6	2.9	2.8	29.9	6.4	5.9
5th (n=1,899)	85.1 (75.7 to 100)	19.5‡	82.1	3.1‡	2.9‡	41.2	8.8‡	7.9‡
Total (n=9,317)	57.9 (0 to 100.0)	13.8	59.6	_	_	21.5	_	_

US population aged 1+ years (National Health and Nutrition Examination Survey 2009–2010).

*PR=Prevalence ratios estimated using Poisson regression (N=9317).

†PRadj=Prevalence ratios adjusted for sex, age groups, race/ethnicity, ratio of family income to poverty and educational attainment, as above (N=8409).

‡Significant linear trend across all quintiles (p≤0.001), both in unadjusted models and models adjusted for sex, age group (1–5, 6–11, 12–19, 20–39, 40–59, 60+ years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic white, Non-Hispanic black and Other race— including

Multiracial), ratio of family income to poverty (Supplemental Nutrition Assistance Program 0.00–1.30, >1.30–3.50 and >3.50 and over) and educational attainment (<12, 12 and >12 years).

ONLINE SUPPLEMENTARY MATERIAL

Food classification according to processing

Food items were initially classified into four groups shown in Table 1. This was accomplished by taking into account, the following three variables from the NHANES recall databases: “Main Food Description”, “Additional Food Description” and “SR Code Description”. Thereafter, the food item classification was modified, if necessary, taking two variables into account: “Combination Food Type” and “Source of food”. Thus, most “Frozen meals" or "Lunchables" or food items consumed in “Restaurant fast food/pizza” or acquired at a “Vending machine", were classified as ultra-processed foods.

As explained in the Subjects and Methods section, when Food Codes were judged to be a handmade recipe, the classification was applied to the underlying ingredients (SR Codes), to enable a more precise food item classification (1).

It must be noted, however, that SR Codes and their proportions are not necessarily the ingredients and proportions consumed by the participant. One of the reasons is that links between FNDDS 5.0 and SR24 were developed to estimate the nutrient content of a Food Code and not the ingredient intake (2). Furthermore, when assigning SR Codes to a Food Code the individual-specific variable “Modification Code” (“adjustments to predefined recipe ingredients that reflect more closely the food as described by the respondent” (2)) was not taken into account, as manual changes would have had been necessary to do so.

Absence of data or discrepancies regarding degree of processing were solved opting for the lesser degree of processing (conservative criterion), which could have led to a slight underestimation of ultra-processed food consumption.

We classified homemade recipes with unknown ingredients based on expected principal ingredients, which could slightly underestimate ultra-processed food consumption.

Regarding bread, the classification distinguishes between handmade bread (either homemade or made in restaurants or artisanal bakeries), and industrial bread (made in industrial bakeries or factories), either processed (when manufactured with ingredients

used in culinary preparations) or ultra-processed (when manufactured with food substances not used in culinary preparations). In our study, because of the large amount of industrial breads with unknown ingredients (approximately 3.7% of all industrial bread had fully known ingredients) and the very low consumption of processed breads when ingredients were reported (approximately 2.3% of industrial breads were processed), we ended up classifying all industrial bread as ultra-processed foods. This could slightly overestimate ultra-processed food consumption.

Assessing energy and added sugar contents

For some handmade recipes, the sum of the “calorie intake per SR Code" (calculated by us) of all underlying SR Codes did not add up exactly to the "calorie intake per Food Code" (provided by NHANES). In these cases, the “final calorie intake per SR code" was calculated as follows:

$$\text{Final calorie intake per SR code} = \text{NHANES Calorie intake per Food Code} * \left(\frac{\text{Calculated Calorie intake per SR code}}{\sum_{n=1}^{\infty} \text{Calculated Calorie intake per SR Code}}\right)$$

The same was done for added sugars:

$$\text{Final added sugars intake per SR code} = \text{Added sugars intake per Food Code} * \left(\frac{\text{Added sugars intake per SR code}}{\sum_{n=1}^{\infty} \text{Added sugars intake per SR code}}\right)$$

where n = each of the Food Code underlying SR Codes

Supplementary Table 1. NOVA food classification based on the extent and purpose of industrial processing (adapted from 3,4)

Food groups and definition	Examples
1 Unprocessed or minimally processed foods Unprocessed foods are those obtained directly from plants or animals (such as green leaves and fruits, or eggs and milk) and purchased for consumption without having undergone any alteration following their removal from nature. Minimally processed foods are natural foods that have been submitted to cleaning, removal of inedible or unwanted parts, fractioning, grinding, drying, fermentation, pasteurisation, cooling, freezing, or other processes which do not add substances to the original food. Purpose of minimum processes is to preserve foods and make it possible to store them and, sometimes, also to decrease stages of food preparation (cleaning and removing inedible parts) or facilitate their digestion, or render them more palatable (grinding or fermentation).	Natural, packaged, cut, chilled or frozen vegetables, fruits, potatoes, cassava, and other roots and tubers; bulk or packaged white, parboiled and wholegrain rice; whole or separated corn; grains of wheat and other cereals that are dried, polished, or ground as grits or flour; dried or fresh pasta made from wheat flour and water; all types of beans; lentils, chickpeas, and other legumes; dried fruits, fruit juices fresh or pasteurized without added sugar or other substances; nuts, peanuts, and other oilseeds without salt or sugar; fresh and dried mushrooms and other fungi; fresh and dried herbs and spices; fresh, frozen, dried beef, pork, poultry and other meat and fish; pasteurized, 'long-life' and powdered milk; fresh and dried eggs, yoghurt without sugar; and tea, herbal infusions, coffee, and tap, spring and mineral water.
2 Processed culinary ingredients These are substances extracted from natural foods or from nature itself by processes such as pressing, grinding, crushing, pulverising, and refining. Purpose of processing here is to obtain ingredients used in homes and restaurants kitchens to season and cook natural or minimally processed foods and to create with them varied and enjoyable dishes such as soups and broths, salads, rice and beans dishes, grilled or roasted vegetables and meat, and homemade breads, pies, cakes, and desserts.	Plant oils; coconut and animal fats (including butter and lard); table sugar, maple syrup (100%), molasses and honey; and table salt.
3 Processed foods These are relatively simple products manufactured essentially with the addition of salt or sugar or other substance of common culinary use, such as oil or vinegar, to natural or minimally processed foods. Purpose here is to prolong duration of foods and modify their palatability. If alcoholic beverages should be classified, drinks produced by the fermentation of group 1 food items such as wine, beer and cider will be classified in this group.	Canned and bottled vegetables, legumes or fruits; salted nuts or seeds; salted, smoked or cured meat or fish; canned sardine and tuna; cheeses, and breads made of ingredients used in culinary preparations (i.e. wheat flour, yeast, water, salt, butter or sugar).
4 Ultra-processed foods	Confectionery, soft drinks, sweetened juices and dairy drinks, powders for juices, sausages, chicken and fish

These are food and drink products whose manufacture involves several stages and various processing techniques and ingredients, many of which are used exclusively by industry. Purpose of processing here is to create durable, accessible, convenient, and highly palatable, ready-to-drink, ready-to-eat, or ready-to-heat products typically consumed as snacks or desserts or as fast meals which replace dishes prepared from scratch. If alcoholic beverages should be classified, drinks produced by fermentation of group 1 food items followed by distillation and eventual addition of sugars or other substances, such as rum, whiskey, vodka, gin, and liqueurs, will be classified in this group.	nuggets or sticks and other pre-prepared frozen dishes, dried products such as cake mix, powdered soup, instant noodles, ready-seasonings, and an infinity of new products including packaged snacks, morning cereals, cereal bars, and 'energy' drinks. Sugar substitutes, sweeteners and all syrups (excluding 100% maple syrup). Breads and baked goods become ultra-processed products when, in addition to wheat flour, yeast, water, and salt, their ingredients include substances nut used in culinary preparations such as hydrogenated vegetable fat, whey, emulsifiers, and other additives.

Supplementary Table 2. Percentage of participants with more than 10% of total energy intake from added sugars, by demographic subgroups, according to quintiles of the dietary contribution of ultra-processed foods. US population aged 1 + years (NHANES 2009-2010)

		Quintiles of the dietary contribution of ultra-processed foods (% of total energy intake)				
		1st (n=1,937)	2nd (n=1,888)	3rd (n=1,814)	4th (n=1,779)	5th (n=1,899)
Gender	Men (n=4,634)	24.5	48.6	61.7	78.1	78.5*
	Women (n=4,683)	28.3	51.4	63.7	75.3	85.6*
Age (years)	1 to 5 (n=1,136)	17.0	45.5	61.3	71.0	84.3*
	6 to 11 (n=1,154)	33.1	54.0	76.5	82.4	90.0*
	12 to 19 (n=1,265)	39.9	62.8	66.2	83.0	87.1*
	20 to 39 (n=1,928)	28.8	53.4	64.1	79.7	82.7*
	40 to 59 (n=1,935)	26.0	49.1	59.6	71.7	76.7*
	60 and over (n=1,899)	22.8	43.6	58.6	71.9	71.1*
Race/ethnicity	Mexican American (n=2,064)	28.5	52.8	64.5	79.4	84.7*
	Other Hispanic (n=988)	41.7	59.4	62.2	80.1	85.2*
	Non-Hispanic White (n=3,984)	22.9	47.3	60.1	75.3	80.4*
	Non-Hispanic Black	33.0	60.3	76.5	82.1	89.0*

	(n=1,726)					
	Other Race (including Multi-Racial) (n=555)	25.8	45.0	64.5	73.0	79.2*
Income to poverty*	0.00–1.30 (n=3,322)	31.1	58.8	72.3	81.0	86.5*
	>1.30–3.50 (n=3,062)	26.4	50.0	67.1	77.4	84.9*
	>3.50 and above (n=2,100)	23.0	46.1	52.0	72.8	75.2*
Educational attainment	<12 years (n=2,669)	32.9	50.6	68.7	76.8	86.4*
	12 years (n=2,136)	29.3	56.2	66.0	81.8	83.7*
	>12 years (n=4,398)	23.4	47.7	59.1	74.2	79.9*

*Significant linear trend across quintiles (P<=0.001), both in unadjusted and Poisson models adjusted for sex, age group (1-5, 6–11, 12–19, 20–39, 40–59, 60 + years), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

References (for Online Supplementary Material).

1. Fitt E, Mak TN, Stephen AM, Prynne C, Roberts C, Swan G and Farron-Wilson M. Disaggregating composite food codes in the UK National Diet and Nutrition Survey food composition databank. European Journal of Clinical Nutrition (2010) 64, S32–S36.
2. Ahuja JKA, Montville JB, Omolewa-Tomobi G, Heendeniya KY, Martin CL, Steinfeldt LC, Anand J, Adler ME, LaComb RP, and Moshfegh AJ. 2012. USDA Food and Nutrient Database for Dietary Studies, 5.0. U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group, Beltsville, MD.
3. Moubarac JC, Parra DC, Cannon G, Monteiro CA. Food Classification Systems Based on Food Processing: significance and implications for policies and actions: a systematic literature review and assessment. Curr Obes Rep 2014; 3: 256-272.
4. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C. Ultra-processing and a new classification of foods. In: Neff R (ed) Introduction to U.S. Food System. Public Health, Environment, and Equity. San Francisco: Jossey Bass A Wiley Brand; 2015.

3.3 MANUSCRIPT 3

Title: Ultra-processed foods, protein leverage and energy intake in the US.

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Running title: Ultra-processed foods and protein leverage hypothesis

Manuscript 3

ABSTRACT

Experimental studies have shown that human macronutrient regulation minimizes variation in absolute protein intake and consequently energy intake varies passively with dietary protein density ("protein leverage"). According to the "Protein Leverage Hypothesis" (PLH), protein leverage interacts with a reduction in dietary protein density to drive energy over-consumption and obesity. A worldwide increase in consumption of ultra-processed foods has been hypothesized to be an important determinant of dietary protein dilution, and consequently an ecological driving force of energy over-consumption and the obesity pandemic.

Objective: To examine the relationships between dietary contribution of ultra-processed foods, dietary proportional protein content and the absolute intakes of protein and energy.

Design: National representative cross-sectional study.

Setting: National Health and Nutrition Examination Survey 2009–2010.

Subjects: 9,042 participants aged 2+ years with at least one day 24-hour dietary recall data.

Results: We found a strong inverse relationship between consumption of ultra-processed foods and dietary protein density, with mean protein contents dropping from 18.2 to 13.3% between lowest and highest quintiles of dietary contribution of ultra-processed foods. Consistent with PLH, increases in dietary contribution of ultra-processed foods (previously shown to be inversely associated with protein density) were also associated with a rise in total energy intake, while absolute protein intake remained relatively constant.

Conclusions: The protein-diluting effect of ultra-processed foods might be one mechanism accounting for their association with excess energy intake. Reducing the contribution of ultra-processed foods in US diet may be an effective way to increase its dietary protein concentration and prevent excessive energy intake.

Keywords: NHANES, ultra-processed foods, dietary protein content, protein leverage hypothesis

Manuscript 3

Main Text:

Introduction

For many decades, beginning with the altercation between Ancel Keys and John Yudkin [(1)], the scientific community has debated whether the principal driver of obesity is the excessive consumption of fats or carbohydrates [(2-5)]. Historically, the role of protein has received comparatively little attention, both because it is proportionally the minor part of the energy content in the diet and because its absolute and relative intake has remained more constant over time and across populations than either fats or carbohydrates [(5-7)]. More recently, however, attention has turned to dietary protein, particularly in the context of body weight management, with specific emphasis on the role of this macronutrient in appetite regulation [(8, 9)].

The fact that all three macronutrients have now been implicated in obesity suggests that there is a need to broaden the focus from specific nutrients, to questions of how each contributes both individually and in interaction with others to drive energy over-consumption. In 2005, Simpson & Raubenheimer postulated the Protein Leverage Hypothesis (PLH) to address this issue. Like numerous other animal species [(10-14)], human macronutrient regulation minimizes variation in absolute protein intake. Consequently, any factor that causes a decrease in the dietary proportion of protein energy will also result in increased absolute intakes of fats and/or carbohydrates, and total energy; conversely, increased dietary protein will result in decreased energy intake ("protein leverage") [(5, 15-18)]. The PLH proposes that this characteristic of human macronutrient regulation has interacted with extrinsic cause of dietary protein dilution, for example increased availability of cheap low-protein foods, to drive energy over-consumption and the rise in obesity [(5, 14)].

In humans, protein leverage is supported by a recently published compendium of experimental studies [(15)], which shows how decreases in percent protein across the range typically seen in human populations with adequate food supply (10-25% protein of total energy) resulted in increased intake of energy in the form of either carbohydrates or fat [(5)]. Increases above 20% protein yielded a lesser rate of decline in

energy intake than from 10 to 20% [15], as was also found in controlled trials with diets of disguised macronutrient content [16]. A recent experimental study, also using diets of disguised macronutrient content, showed an approximately linear increase in both energy intake and body weight change as dietary protein decreased from 30% to 15% and 10% protein [17]. Another experimental study found that relative to 15% energy from protein, energy intakes were reduced on a diet of 30% protein, as predicted by PLH, but not increased on a diet of 5% protein [18]. A likely explanation for this is that 5% protein is lower than seen naturally in human populations with food sufficiency, and outside of the range of diets to which the species is evolutionarily adapted [14, 15]. Population level data also support PLH. In the US, according to FAOSTAT nutrient availability estimates [6], the dietary protein content gradually decreased from 14% of total calories in 1961 to 12.5% in 2000. In this situation, maintaining the absolute protein intake fairly constant required a 14% total increase in fat and carbohydrate consumption [5]. Another study carried out in the US with data from NHANES, estimated that the drop of relative dietary protein content in normal weight men from 15.9% in 1971-1975 to 15.4% in 2005-2006, was associated with a 10% increase in total daily energy intake [19].

Evidence exists that global food supplies are increasingly becoming dominated by ultra-processed food and drink products [20-24]. These are industrial formulations manufactured mostly or entirely from ingredients derived from constituents of foods and additives used to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations [25-27]. Given the disparity in the cost of macronutrients, it makes economic sense for food processors to replace expensive protein with cheaper carbohydrates and fats [28]. Ultra-processed products could therefore play an important role in determining the relative dietary protein content (% of total energy intake), trapping people in a suboptimal diet in which attempting to maintain absolute protein intake drives an increase in energy intake [5].

In the present study, we examine how the consumption of ultra-processed food and drink products (hereafter denominated simply as ultra-processed foods) is associated with the relative dietary protein content and the absolute energy and protein intakes of the US diet, and test whether the relationships fit the predictions of the PLH model.

Subjects and Methods

Data source, population and sampling

We used nationally representative data from the 2009-2010 *National Health and Nutrition Examination Survey (NHANES),* specifically the dietary component *What we eat in America (WWEIA)* [29].

NHANES is a continuous, nationally representative, cross-sectional survey of the non-institutionalized, civilian US residents [30]. NHANES sample was obtained by using a complex, stratified, multi-stage probability cluster sampling design, based on the selection of counties, blocks, households, and the number of people within households [30]. In order to improve the estimate precision and reliability, NHANES 2009-2010 oversampled the following subgroups: Hispanic, Non-Hispanic black, Non-Hispanic white and Other persons at or below 130% of the federal poverty level and Non-Hispanic white and Other persons aged 80 + years [30].

The survey included an interview conducted in the home and a subsequent health examination performed at a mobile examination center (MEC). All NHANES examinees were eligible for two 24-hour dietary recall interviews. The first dietary recall interview was collected in-person in the MEC [31] while the second was collected by telephone 3 to 10 days later [32]. Dietary interviews were conducted by trained interviewers using the validated [33-35] US Department of Agriculture Automated Multiple-Pass Method (AMPM) [36]. For children under 9 years of age, the interview was conducted with a proxy; for children between 6 and 8 years of age, in the presence of the child. Children 9 to 11 years old provided their own data assisted by an adult household member (assistant). The preferred proxy/assistant was the most knowledgeable person about the child's consumption the day before the interview. If the child had more than one caregiver, several individuals could contribute to the intake data [31,32].

Among the 13,272 people screened in NHANES 2009-2010, 10,537 (79.4%) participated in the household interview and 10,253 (77.3%) also participated in the MEC health examination [37]. Of these, 9,754 individuals provided one day of complete dietary intakes and 8,406 provided two days [38].

We evaluated 9,042 survey participants aged 2 years and above who had at least one day 24-hour dietary recall data and had not been breast-fed on either of the two days. These individuals had similar socio-demographic characteristics (in terms of gender, age, race/ ethnicity, family income and education) to the full sample of 9,787 interviewed participants aged 2 years and above.

Food classification according to processing

We classified all recorded food items (N=4,981 different Food Codes consumed in cycle 2009-2010) according to NOVA, a food classification based on the extent and purpose of industrial food processing [25, 26, 39]. This classification includes 4 groups: "unprocessed or minimally processed foods" (such as fresh, dry or frozen fruits and vegetables; packaged grains and pulses; grits, flakes or flours made from corn, wheat or cassava; pasta, fresh or dry, made from flours and water; eggs; fresh or frozen meat and fish and fresh or pasteurized milk); "processed culinary ingredients" (including sugar, oils, fats, salt, and other substances extracted from foods and used in kitchens to season and cook unprocessed or minimally processed foods and to make culinary preparations), "processed foods" (including canned foods, sugar-coated dry fruits, salted meat products, cheeses and freshly made unpackaged breads, and other ready-to-consume products manufactured with the addition of salt or sugar or other substances of culinary use to unprocessed or minimally processed foods), and "ultra-processed foods".

The group of ultra-processed foods, of particular interest in this study, includes soft drinks, sweet or savory packaged snacks, confectionery and industrialized desserts, mass-produced packaged breads and buns, poultry and fish nuggets and other reconstituted meat products, instant noodles and soups, and many other ready-to-consume formulations of several ingredients. Besides salt, sugar, oils, and fats, these ingredients include food substances not commonly used in culinary preparations, such as modified starches, hydrogenated oils, protein isolates, and additives whose purpose is to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations, or to disguise undesirable qualities of the final product, such as

colorants, flavorings, non-sugar sweeteners, emulsifiers, humectants, sequestrants, and firming, bulking, de-foaming, anti-caking and glazing agents. A detailed definition of each NOVA food group and examples of food items classified in each group are shown elsewhere [40]. The rationale underlying the classification is described elsewhere [22, 41-43].

For all food items (Food Codes) judged to be a handmade recipe, the classification was applied to the underlying ingredients (Standard Reference Codes -SR Codes-) obtained from the USDA Food and Nutrient Database for Dietary Studies (FNDDS) 5.0 [44]. Further details have been previously published [40].

Assessing energy and protein contents

For this study, we used Food Code energy values as provided by NHANES.

For handmade recipes, we calculated the underlying ingredient (SR Code) energy and protein values using variables from both *FNDDS 5.0* [44] and *USDA National Nutrient Database for Standard Reference, Release 24 (SR24)* [45].

Protein intake was converted into MJ using conversion factor 0.016736 MJ/ g.

Data Analysis

We used all available dietary data for each participant, using means of both recall days when available (86% of participants). Food items were sorted into mutually exclusive food subgroups within Unprocessed or minimally processed foods (n= 11), Processed culinary ingredients (n=4), Processed foods (n=4) and Ultra-processed foods (n=17), as shown in Table 1. First, we evaluated the contributions of each of the NOVA food groups and subgroups to total energy and protein intakes. Thereafter, we calculated the average relative protein content, expressed as proportion of total energy intake, in the overall US diet and in fractions of this diet composed by each of the NOVA food groups and subgroups. We also calculated the relative protein content of the group of unprocessed or minimally processed foods combined with the group of processed

culinary ingredients, as the items belonging to these two groups are usually combined in culinary preparations and therefore consumed together.

We used Gaussian regression to estimate the association of the dietary contribution of ultra-processed foods with the relative dietary protein content (% of total energy intake) and the absolute energy (MJ) and protein intakes (MJ). Dietary contribution of ultra-processed foods was transformed using restricted cubic spline functions to allow for nonlinearity.

Crude and adjusted average dietary protein content and absolute energy and protein intakes were compared across quintiles of the dietary contribution of ultra-processed foods. Poisson regression was used to evaluate whether the percentage of individuals with relative dietary protein contents lower than 20%, 15% or 10% of total energy intake decreased across quintiles [46]. This decrease was also assessed across demographic subgroups in stratified analysis. Tests of linear trend were performed in order to evaluate the effect of quintiles as a single continuous variable.

All regression models were adjusted for race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race including Multi-Racial), ratio of family income to poverty (categorized based on Supplemental Nutrition Assistance Program (SNAP) eligibility as 0.00–1.30, >1.30–3.50, and >3.50 and above) (29) and educational attainment of respondents for participants aged 20 + years and of household reference person otherwise (<12, 12 years and >12 years). In order to take into account different dietary protein content requirements according to age groups (5-20, 10-20 or 10-35% of total energy), different daily protein intake requirements according to sex-age groups (0.2176, 0.3179, 0.569, 0.7699, 0.8703, 0.9372 or 1.1883 MJ) and different daily energy intake requirements according to sex-age-physical activity levels groups (4.184, 5.0208, 5.8576, 6.6944, 7.5312, 8.368, 9.2048, 10.0416, 10.8784, 11.7152, 12.552 or 13.3888 MJ) [47], analyses using relative dietary protein content, protein daily intake or energy daily intake as outcomes were accordingly adjusted for these requirements (as dummy variables). As 886 participants had missing values on family income and/or education, adjusted analyses included 8,156 individuals.

Analysis also adjusting for daily energy intake requirements included 8,128 because 31 had missing values for physical activity. As the included sample differs across models results may not always be comparable.

NHANES survey sample weights were used in all analyses to account for differential probabilities of selection for the individual domains, nonresponse to survey instruments, and differences between the final sample and the total US population. The Taylor series linearization was used for variance estimation in order to account for the complex sample design and the sample weights [30].

Statistical hypotheses were tested using a two-tailed $p<0.01$ level of significance. Data were analyzed using Stata statistical software package version 12.1.

Results

Distribution of total energy intake by food groups

The average US daily energy intake in 2009-2010 was 8.6 MJ, and nearly 3 in 5 MJ (57.6%) came from ultra-processed foods (**Table 1**). Unprocessed or minimally processed foods contributed 30.1% of total energy intake, processed foods an additional 9.4%, and processed culinary ingredients the remaining 2.9%. The most common ultra-processed foods in terms of energy contribution were breads; soft and fruit drinks; cakes, cookies, and pies; salty-snacks; frozen and shelf-stable plates; pizza; and breakfast cereals. Meat, fruit, and milk provided the most energy among unprocessed or minimally processed foods; ham and cheese, the most energy among processed foods; and table sugar and plant oils, the most energy among processed culinary ingredients.

Distribution of total protein intake by food groups

The average US daily protein intake corresponded to 1.3 MJ (Table 1) and almost half (48.7%) of this intake came from unprocessed or minimally processed foods. Ultra-processed foods contributed 38.3%, processed foods an additional 12.8% and the remaining 0.2% of the protein intake came from processed culinary ingredients. The main sources of protein among unprocessed or minimally processed foods were meat, milk, eggs, and fish, while among processed foods were cheeses and ham and among ultra-processed foods were breads and frozen and shelf-stable plate meals.

The average protein content in ultra-processed foods (9.5% of total energy intake) was less than half of that in both processed foods (24.3%) and unprocessed or minimally processed foods and processed culinary ingredients grouped together (25.3%) (Table 1).

Association between dietary contribution of ultra-processed foods and overall dietary protein content

The unadjusted restricted cubic splines (RCS) Gaussian regression analysis showed an inverse linear association between the dietary contribution of ultra-processed foods and

the overall dietary protein content (both expressed as % of total energy intake) (coefficient for linear term=-0.08, 95% CI: -0.13 to -0.03) **(Figure 1)**. The strength of the association remained similar after adjusting for the sex-age dummies corresponding to groups with different protein content requirements, race/ethnicity, family income and education (coefficient for linear term=-0.07, 95% CI: -0.12 to -0.02; Wald test for linear term p=0.008; Wald test for all non-linear terms p=0.05). Overall, each increase in 14 percentage points in dietary contribution of ultra-processed foods was associated with 1 lower percentage point in the relative dietary protein content.

Consistent with the spline models, across quintiles of the dietary contribution of ultra-processed foods, unadjusted mean dietary protein content decreased substantially and monotonically, from 18.2% of total energy intake in the lowest quintile to 13.3% in the highest **(Table 2)**. Across the same quintiles, the proportion of individuals consuming less than 15% of total energy from protein increased from 25.1% to 73.3%, respectively. An even more pronounced increase was seen in the proportion of individuals consuming less than 10% of energy from protein, rising from 2.8% to 14.9%, respectively. The increases cut across demographic subgroups though they varied somewhat by subgroup **(Table S1)**. The magnitude and the statistical significance of the association between the dietary contribution of ultra-processed foods and the overall dietary protein content did not change with adjustment for age groups with different dietary protein content requirements, race/ethnicity, family income and education.

Association between dietary contribution of ultra-processed foods and absolute energy and protein intakes

RCS Gaussian analysis suggested a non-linear association of dietary contribution of ultra-processed foods with absolute energy intake (**Figure 2A**), especially the adjusted model (coefficient for linear term=0.044, 95% CI: 0.012 to 0.076; Wald test for linear term p=0.01; Wald test for all non-linear terms p=0.009). Total energy intake rose with increases in the dietary contribution of ultra-processed foods up until this contribution reached nearly 70% of total energy intake. Further increases in the contribution of ultra-processed foods were associated with slight reductions in total energy intake. These

results were consistent with the analysis across quintiles of the dietary contribution of ultra-processed foods (**Table 3**) which showed that adjusted mean energy intake increased between the first (8.2 MJ) and the fourth quintiles (8.9 MJ) while slightly decreasing in quintile 5 (8.8 MJ). Despite the non-statistically significant slight decrease in energy intake between quintiles 4 and 5 (adjusted Wald test p=0.5), there was an overall positive significant linear trend across all quintiles (p<0.001).

In combination with falling relative dietary protein content and rising total energy intake, total protein intake remained much the same with increases in the dietary contribution of ultra-processed foods up until this contribution reached nearly 40% of total energy intake. Further increases in the contribution of ultra-processed foods were first associated with slight declines in total protein intake (up to the point that ultra-processed foods represented near 70% of total energy intake) and then with greater declines as ultra-processed energy intakes fell away (**Figure 2B**). Similar results were obtained in adjusted models (coefficient for linear term=0.002, 95% CI:-0.005 to +0.008; Wald test for linear term p=0.5; Wald test for all non-linear terms p<0.001). Adjusted mean total protein intakes changed little from the first to the third quintiles of dietary contribution of ultra-processed foods while a slight decline was observed from the third to the fifth quintile (Table 3).

The protein leverage hypothesis

Figure 3 presents a synthesis of our results which addresses the protein leverage model. The vertical black line represents the prediction under 100% protein leverage (i.e. constant absolute protein intake), assuming a target protein intake of 1.3 MJ (determined as the adjusted mean across all ultra-processed food quintiles, Table 3). The black negative diagonal line represents the alternative model of 0% protein leverage (i.e. constant energy intake regardless of the relative dietary protein content). The positively sloped radials show the proportional contribution of protein to dietary energy, and the negative diagonals show the energy intakes associated with each quintile of the dietary contribution of ultra-processed foods (UPF). The data are strongly consistent with the protein leverage hypothesis, as demonstrated in experimental

studies [15-18]. Firstly, absolute protein intakes are relatively constant (vertical black line) across UPF quintiles, consistent with the strong human protein appetite. Secondly, as the percentage of dietary energy from protein decreases (positive radials, 18.2-13.3%), in this case corresponding with increasing UPF consumption (UPF quintiles 1 – 5), the intake of non-protein energy and consequently total energy (negative diagonals, 8.2- 8.8 MJ) increases. There is, by contrast, no correspondence to the prediction if energy intake was independent of macronutrient ratios, in which case the points for UPF quintiles would align along the black negative diagonal.

Discussion

In this analysis of nationally representative data, we provide evidence that ultra-processed foods represent almost 60% of all energy intake in the US diet, and contribute less than 40% of all protein. Proteins represented 1 of every 10 MJ in the average ultra-processed food (9.5%), far lower than the protein content in either processed foods (24.3%) or unprocessed or minimally processed foods, and processed culinary ingredients grouped together (25.3%). A strong inverse relationship was found between the dietary contribution of ultra-processed foods and the overall dietary protein content. Moreover, the probability of dietary protein contents below 15% increased three times from the lowest to the highest quintile of the dietary contribution of ultra-processed foods and six times for dietary protein contents below 10%. Consistent with the Protein Leverage Hypothesis (PLH), we observed that increases in the dietary contribution of ultra-processed foods were associated with a dilution of dietary protein density and with a rise in total energy intake, while absolute protein intake remained relatively constant. This was seen for increases in ultra-processed consumption up to values corresponding to approximately 70% of total energy intake, or up to values found among four fifths of the US population. Beyond this point, rises in dietary contribution of ultra-processed foods yielded slight drops in both total energy and protein intakes. A possible explanation for the latter result may be limits in increasing energy intake after a certain level (e.g. as discussed in reference 15) and other mechanisms triggered by consequences of excessive energy intake such as the accumulation of adipose tissue.

To our knowledge, this is the first study to establish the relationship of the dietary contribution of ultra-processed foods with the overall dietary protein content and the absolute energy and protein intakes in the US.

As a solution to the pandemic of excessive energy intake, Simpson and Raubenheimer proposed following diets with a moderate intake of high quality protein-rich foods [(5)]. Our study shows that both processed foods and unprocessed or minimally processed foods combined with processed culinary ingredients provide more than twice as much protein per unit of energy than ultra-processed foods. A reduction in ultra-processed foods should also increase the intake of more healthful, minimally processed foods such as

milk, fruits and nuts, and freshly-prepared dishes based on whole grains and vegetables, which would provide additional health benefits [48].

Few studies have assessed the impact of food processing levels on the protein nutrient profile of the US diet. One study [49] based on NHANES 2003-2008 data employed a food classification system [50] including two food groups which are mostly ultra-processed foods ("Mixtures of combined Ingredients" and "Ready-to-eat"). That study showed that these two food groups together, contributed about half of total energy intake and 40% of energy intake from protein. An investigation in Canada, using 2001 household purchasing data, found that ultra-processed foods are low in protein and that the relative dietary protein content also linearly decreased across the quintiles of the dietary contribution from ultra-processed foods (from 14.9 to 11.6% of total energy) [51]. Being based on household purchasing data, this prior study and others based on the NOVA classification system [23, 25, 52-54] could not evaluate fraction of wasted food nor purchases at restaurants, which represent a substantial proportion of US energy intake. A study carried out in Brazil with dietary consumption data from 2008-2009, also found a linear drop in protein content across quintiles of ultra-processed food consumption from 19.3 to 14.8% [55]. Our findings build upon and considerably extend these prior reports by evaluating food processing and protein intake using contemporary, nationally representative dietary intake data in the US.

Our study has several strengths. We tested an a-priori prediction of the PLH, using data for a large, nationally representative sample of the US population, increasing generalizability. Our investigation was based on individual consumption data, rather than household purchasing data which do not evaluate the fraction of wasted food nor purchases at restaurants.

Potential limitations should be considered. As with most population dietary measures, data obtained by 24-hour recalls are imperfect [56], although the standardized methods and approach of NHANES and use of two recalls per person minimize potential error and bias. Even though some authors have recommended not using self-reported energy intake as a measure of true energy intake [56, 57], it must be noted that the primary aim of

the present study was not to estimate true energy intakes but rather differences in energy intakes across levels of ultra-processed food consumption. Some people (for example obese) may underreport food intake [58, 59] and more specifically fat intake [33, 60] or consumption of foods with caloric sweeteners [61] such as desserts and sweet baked goods [62, 63], which may lead to an underestimation of total energy intake or an overestimation of the percentage of energy contributed by protein. This might lead us to underestimate the association of the dietary contribution of ultra-processed foods and energy intake if high consumers of ultra-processed foods tend to be overweight [64] and these, in turn, tend to be under-reporters. If so, this sort of bias would only work against the Protein Leverage Hypothesis. Although NHANES collects some information indicative of food processing (i.e. place of meals, product brands), these data are not consistently determined for all food items what may result in errors in food group classification. Also, the number of food items reported in NHANES is smaller than the number available in the marketplace, and national food composition data are imprecise and not updated as required [65].

In conclusion, we found that ultra-processed foods have lower protein content when compared with both processed foods and unprocessed or minimally processed foods combined with processed culinary ingredients and, also, that their dietary contribution is associated with reduced relative protein content in the diet. Consistent with the protein leverage hypothesis, the dilution of the overall dietary protein content in the US diet by ultra-processed foods is associated with higher total energy intake, while the absolute protein intake remains relatively constant. Therefore, reducing the contribution of ultra-processed foods in the US diet may be an effective way to increase its dietary protein concentration and prevent excessive energy intake.

References

1. Scrinis G. 2013. Nutritionism: The Science and Politics of Dietary Advice. New York: Columbia University Press
2. Bray GA, Popkin BM. Dietary fat intake does affect obesity! Am J Clin Nutr 1998; 68: 1157–1173.
3. Stubbs RJ, Mazlan N, Whybrow S. Carbohydrates, appetite and feeding behavior in humans. J Nutr 2001; 131: 2775S–2781S.
4. Willet WC. Dietary fat and obesity: an unconvincing relation. Am J Clin Nutr 1998; 68: 1149–1150.
5. Simpson SJ, Raubenheimer D. Obesity: the protein leverage hypothesis. Obes Rev 2005; 6: 133–142.
6. FAOSTAT database. 2002 [WWW document]. URL http://faostat.fao.org/faostat/collections; accessed Dezember 2015.
7. Westerterp-Plantenga MS. Nutrient utilization and energy balance. In: Westerterp-Platenga MS, Fredrix EWHM, Steffens AB, Kissileff HR (eds). Food Intake and Energy Expenditure. CRC Press: Boca Raton, 1994, pp. 311–319.
8. Weigle DS, Breen PA, Matthys CC et al. (2005). A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. In: American Journal of Clinical Nutrition, 82, 41-48.
9. Astrup A, Raben A & Geiker N. The role of higher protein diets in weight control and obesity-related comorbidities. International Journal of Obesity 2015, 39, 721-726.
10. Raubenheimer D, Simpson SJ. Integrative models of nutrient balancing: application to insects and vertebrates. Nutr Res Rev 1997; 10: 151–179.
11. Kyriazakis I, Emmans GC, Whittemore CT. The ability of pigs to control their protein intake when fed in three different ways. Physiol Behav 1991; 50: 1197–1203.
12. Webster AJ. Energy partitioning, tissue growth and appetite control. Proc Nutr Soc 1993; 52: 69–76.

13. Simpson SJ & Raubenheimer D. The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity (2012)
14. Raubenheimer D, Machovsky-Capuska GE, Gosby AK and Simpson S. Nutritional ecology of obesity: from humans to companion animals. British Journal of Nutrition (2014), page 1 of 14. doi:10.1017/S0007114514002323.
15. Gosby AK, Conigrave AD, Raubenheimer D, Simpson SJ. Protein leverage and energy intake. Obes Rev. 2014 Mar;15(3):183-91. doi: 10.1111/obr.12131. Epub 2013 Oct 28.
16. Gosby AK, Conigrave AD, Lau NS et al. Testing protein leverage in lean humans: a randomised controlled experimental study. PLoS ONE 2011; 6: e25929.
17. Campbell CP, Raubenheimer D, Badaloo AV et al. Developmental contributions to macronutrient selection: A randomized controlled trial in adult survivors of malnutrition. Evolution, Medicine, and Public Health, in press.
18. Martens EA, Lemmens SG & Westerterp-Plantenga MS. Protein leverage affects energy intake of high-protein diets in humans. American Journal of Clinical Nutrition 2013, 97, 86-93.
19. Austin GL, Ogden LG, Hill JO. Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal weight, overweight, and obese individuals: 1971–2006. Am J Clin Nutr 2011; 93: 836–843.
20. Stuckler D, McKee M, Ebrahim S, Basu S. Manufacturing epidemics: the role of global producers in increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. PLoS Med 2012; 9: e1001235.
21. Monteiro CA, Cannon G. The impact of transnational 'Big Food' companies on the South: a view from Brazil. PLoS Med 2012a; 9: e1001252.
22. Moodie R, Stuckler D, Monteiro C et al; Lancet NCD Action Group. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. Lancet. 2013 Feb 23;381(9867):670-9. doi: 10.1016/S0140-6736(12)62089-3.
23. Monteiro CA, Moubarac JC, Cannon G, Popkin BM. Ultra-processed products are becoming dominant in the global food system. Obesity Reviews 2013; 14 (Suppl. 2): 21-28; doi:10.1111/obr.12107.

24. Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications. Washington D.C.: Panamerican Health Organization, 2015.
25. Monteiro CA, Levy RB, Claro RM, de Castro IRR, Cannon G. A new classification of foods based on the extent and purpose of their processing. Cad. Saúde Pública, Nov 2010a, vol.26, no.11, p.2039-2049.
26. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C. Ultra-processing and a new classification of foods. In: Neff R (ed) Introduction to U.S. Food System. Public Health, Environment, and Equity. San Francisco: Jossey Bass A Wiley Brand; 2015.
27. FAO. 2015. Guidelines on the collection of information on food processing through food consumption surveys. FAO, Rome.
28. Brooks RC, Simpson SJ, and Raubenheimer D. The price of protein: combining evolutionary and economic analysis to understand excessive energy consumption. Obesity Reviews 2010, 11, 887–894.
29. National Health and Nutrition Examination Survey <*http://wwwn.cdc.gov/nchs/nhanes/search/nhanes09_10.aspx*> (accessed August 2015).
30. Johnson CL, Paulose-Ram R, Ogden CL, et al. National Health and Nutrition Examination Survey: Analytic guidelines, 1999–2010. National Center for Health Statistics. Vital Health Stat 2 (161). 2013.
31. NHANES. MEC In-Person Dietary Interviewers Procedures Manual. January 2009a. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/DietaryInterviewers_Inperson.pdf
32. NHANES. Phone Follow-Up Dietary Interviewer Procedures Manual. September 2009b. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/Dietary_PFU_09.pdf.
33. Moshfegh AJ, Rhodes DG, Baer DJ et al. The USDA Automated Multiple-Pass Method reduces bias in the collection of energy intakes. Am J Clin Nutr 2008; 88:324-332.

34. Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. J Nutr 2006 Oct; 136(10):2594-9.
35. Rumpler WV, Kramer M, Rhodes DG, Moshfegh AJ, Paul DR, Kramer M. Identifying sources of reporting error using measured food intake. Eur J Clin Nutr 2008; 62:544-52.
36. Automated Multiple-Pass Method. United States Department of Agriculture. Agriculture Research Service. *http://www.ars.usda.gov/ba/bhnrc/fsrg* (accessed August 2015).
37. National Health and Nutrition Examination Survey. NHANES Response Rates and Population Totals. Response Rates. <*http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm*> (accessed August 2015).
38. National Health and Nutrition Examination Survey. 2009 - 2010 Data Documentation, Codebook, and Frequencies. http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/DR1IFF_F.htm (accessed August 2015).
39. Moubarac JC, Parra DC, Cannon G, Monteiro CA. Food Classification Systems Based on Food Processing: significance and implications for policies and actions: a systematic literature review and assessment. Curr Obes Rep 2014; 3: 256-272.
40. Martinez Steele E, Baraldi LG, Louzada ML, Moubarac JC, Mozaffarian D, Monteiro CA. Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. BMJ Open 2016;6: e009892. doi:10.1136/bmjopen-2015-009892.
41. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. Public Health Nutr. 2009;12(5):729-731.
42. Ludwig DS. Technology, diet, and the burden of chronic disease. JAMA 2011; 305:1352-1353.
43. Monteiro CA, Cannon G, Levy RB, Claro RM, Moubarac J-C (2012b).The Food System. Processing. The big issue for disease, good health, well-being. World Nutrition 3: 527–569. Available: www.wphna.org.

44. Ahuja JKA, Montville JB, Omolewa-Tomobi G et al. 2012. USDA Food and Nutrient Database for Dietary Studies, 5.0. U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group, Beltsville, MD.
45. U.S. Department of Agriculture, Agricultural Research Service. 2011. USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl.
46. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol. 2003 Oct 20;3:21.
47. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at http://health.gov/dietaryguidelines/2015/guidelines/.
48. Solon-Biet SM, Mitchell SJ, de Cabo R, Raubenheimer D, Le Couteur DG, and Simpson SJ.. Macronutrients and caloric intake in health and longevity. Journal of Endocrinology 2015, 226: R17-R28.
49. Eicher-Miller HA, Fulgoni III VL, Keast DR. Contributions of Processed Foods to Dietary Intake in the US from 2003–2008: A Report of the Food and Nutrition Science Solutions Joint Task Force of the Academy of Nutrition and Dietetics, American Society for Nutrition, Institute of Food Technologists, and International Food Information Council. J. Nutr. 142: 2065S–2072S, 2012.
50. International Food Information Council Foundation. Understanding our food communications tool kit, 2010 Sept [cited 2014 Aug 31]. Available from: http://www.foodinsight.org/For-Professionals/Understanding-Our-Food/tabid/1398/Default.aspx.
51. Moubarac J-C, Martins APB, Claro RM, Levy RB, Cannon G, Monteiro CA. Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr, 2012; DOI: http://dx.doi.org/10.1017/S1368980012005009.
52. Monteiro CA, Levy RB, Claro RM, de Castro IRR, Cannon G. Increasing consumption of ultra-processed foods and likely impact on human health:

evidence from Brazil. Public Health Nutrition 2010b; 14(1): 5-13; doi:10.1017/S1368980010003241.

53. Crovetto M M, Uauy R, Martins AP, Moubarac JC, Monteiro C. Household availability of ready-to-consume food and drink products in Chile: impact on nutritional quality of the diet. Rev Med Chil. 2014 Jul; 142(7):850-8. doi: 10.4067/S0034-98872014000700005.
54. Canella DS, Levy RB, Martins APB et al. Ultra-processed food products and obesity in Brazilian households (2008-2009). Plos One 2014; 9(3): e92752.
55. Louzada ML, Martins AP, Canella DS et al. Ultra-processed foods and the nutritional dietary profile in Brazil. Rev Saúde Pública 2015; 49:38. DOI:10.1590/S0034-8910.2015049006132.
56. Subar AF, Freedman LS, Tooze JA et al. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. The Journal of Nutrition. First published online October 14, 2015; doi:10.3945/jn.115.219634.
57. Dhurandhar NV, Schoeller D, Brown AW. Energy Balance Measurement: When Something is Not Better than Nothing. Int J Obes (Lond). 2015 July ; 39(7): 1109–1113. doi:10.1038/ijo.2014.199.
58. Murakami K, Livingstone MBE. Prevalence and characteristics of misreporting of energy intake in US adults: NHANES 2003-2012. British Journal of Nutrition, 2015 (114) 1294-1303.
59. Murakami K, Livingstone MBE. Prevalence and characteristics of misreporting of energy intake in US children and adolescents: National Health and Nutrition Examination Survey (NHANES) 2003-2012. British Journal of Nutrition, 2016 (115) 294-304.
60. Livingstone MB, Black AE. Markers of the validity of reported energy intake. J Nutr 2003;133:895S–920S.
61. Bingham S, Luben R, Welch A, Tasevska N, Wareham N, Khaw KT. Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and non-obese individuals in the European Prospective Investigation of Cancer Norfolk. Cancer Epidemiol Biomarkers Prev 2007; 16:1651–4.

62. Lafay L, Mennen L, Basdevant A, et al. Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Sante (FLVS) study. Int J Obes Relat Metab Disord. 2000; 24:1500–6.
63. Pryer JA, Vrijheid M, Nichols R, Kiggins M, Elliott P. Who are the ‘‘low energy reporters’’ in the dietary and nutritional survey of British adults? Int J Epidemiol. 1997; 26: 146–54.
64. Louzada ML, Baraldi LG, Steele EM et al. Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults. Prev Med. 2015 Dec;81:9-15. doi: 10.1016/j.ypmed.2015.07.018. Epub 2015 Jul 29.
65. Slining M M, Yoon EF, Davis J, Hollingsworth B., Miles D. and Ng SW. "An Approach to Monitor Food and Nutrition from “Factory to Fork”." Journal of the Academy of Nutrition and Dietetics 2015, 115(1): 40-49.

TABLES AND FIGURES

Table 1. Distribution of total energy and protein intakes according to Nova food groups, and mean protein content of each food group. US population aged 2 + years (NHANES 2009-2010) (N=9,042)

Food groups	Mean energy intake		Mean protein intake		Mean protein content
	Absolute (MJ/day)	Relative (% of total energy intake)	Absolute (MJ/day)	Relative (% of total energy intake from protein)	(% of energy from protein)
Unprocessed or minimally processed foods	***2.5***	***30.1***	***0.7***	***48.7***	**27.6**
Meat (includes poultry)	0.7	8.4	0.4	23.7	52.5
Fruit and freshly squeezed fruit juices	0.4	5.2	0.0	1.7	4.9
Milk and plain yoghurt	0.4	4.9	0.1	8.6	28.4
Grains	0.2	2.9	0.0	1.9	10.5
Roots and tubers	0.1	1.6	0.0	1.0	10.8
Eggs	0.1	1.5	0.0	3.2	36.6
Pasta	0.1	1.4	0.0	1.4	14.2
Legumes	0.1	0.9	0.0	1.4	25.6
Fish and sea food	0.1	0.8	0.0	2.9	68.3
Vegetables	0.1	0.8	0.0	1.2	24.9
Other unprocessed or minimally processed foods[1]	0.2	1.7	0.0	1.7	30.6
Processed culinary ingredients	***0.3***	***2.9***	**0.0**	***0.2***	***0.8***
Sugar[2]	0.1	1.1	0.0	0.0	0.0
Plant oils	0.1	1.3	0.0	0.0	0.0
Animal fats[3]	0.0	0.5	0.0	0.2	3.8
Other processed culinary ingredients[4]	0.0	0.04	0.0	0.02	3.4

Unprocessed or minimally processed foods + Processed culinary ingredients	***2.7***	***33***	***0.7***	***48.9***	***25.3***
Processed foods	***0.9***	***9.4***	***0.2***	***12.8***	***24.3***
Cheese	0.3	3.7	0.1	6.2	26.7
Ham and other salted, smoked or canned meat or fish	0.1	1.3	0.1	3.7	49.9
Vegetables and other plant foods preserved in brine	0.1	0.7	0.0	0.8	16.1
Other processed foods[5]	0.4	3.7	0.0	2.1	9.3
Ultra-processed foods	***5.0***	***57.6***	***0.5***	***38.3***	***9.5***
Breads	0.8	9.7	0.1	8.6	13.6
Soft and Fruit drinks[6]	0.6	6.8	0.0	0.7	5.4
Cakes, cookies and pies	0.5	5.5	0.0	2.5	5.8
Salty-snacks	0.4	4.4	0.0	2.3	7.2
Frozen and shelf-stable plate meals	0.3	3.9	0.1	5.2	18.4
Pizza (ready-to-eat/heat)	0.3	3.3	0.1	3.9	16.6
Breakfast cereals	0.2	3	0.0	1.7	8.6
Sauces, dressings and gravies	0.2	2.6	0.0	0.5	8.5
Reconstituted meat or fish products	0.2	2.3	0.1	3.9	31.7
Ice cream and ice pops	0.2	2.3	0.0	1.2	6.9
Sweet-snacks	0.2	2.3	0.0	0.9	4.6
Milk-based drinks	0.1	1.9	0.0	2.1	18.2
Desserts[7]	0.1	1.8	0.0	0.3	2.7
French fries and other potatoe products	0.2	1.7	0.0	0.7	5.1
Sandwiches and hamburgers on bun (ready-to-	0.1	1.4	0.0	1.9	19.2

eat/heat)					
Instant and canned soups	0.1	0.9	0.0	1.1	32.3
Other ultra-processed foods[8]	0.3	3.7	0.0	0.6	2.9
Total	**8.6**	**100.0**	**1.3**	**100.0**	**15.8**

[1]Including nuts and seeds (unsalted); yeast; dried fruits (without added sugars) and vegetables; non pre-sweetened, non-whitened, non-flavored coffee and tea; coconut water and meat; homemade soup and sauces; flours; tapioca

[2]Including honey, molasses, maple syrup (100%)

[3]Including butter, lard and cream

[4]Including starches; coconut and milk cream; baking chocolate, cocoa powder and gelatin powder; vinegar; baking powder and baking soda

[5]Including salted or sugared nuts and seeds; peanut, sesame, cashew and almond butter or spread; beer and wine

[6]Including energy drinks, sports drinks, nonalcoholic wine

[7]Including ready-to-eat and dry-mix desserts such as pudding

[8]Including soy products such as meatless patties and fish sticks; babyfood and baby formula; dips, spreads, mustard and catsup; margarine; sugar substitutes, sweeteners and all syrups (excluding 100% maple syrup); distilled alcoholic drinks

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Table 2. Indicators of the dietary protein content according to the dietary contribution of ultra-processed foods. US population aged 2 + years (NHANES 2009-2010)

Dietary contribution of ultra-processed foods (% of total energy intake)		Indicators										
		% of total energy intake from protein		Diets with less than 20% of total energy intake from protein			Diets with less than 15% of total energy intake from protein			Diets with less than 10% of total energy intake from protein		
Quintiles	Mean (range)	Mean	Adj. Mean[1]	%	PR[2]	PRadj[3]	%	PR[2]	PRadj[3]	%	PR[2]	PRadj[3]
1st (n=1,852)	32.5 (0 to 42.8)	18.1	18.2	70.4	1	1	25.1	1	1	2.8	1	1
2nd (n=1,846)	48.6 (42.8 to 54.2)	16.9	16.9	80.9	1.1	1.1	32.1	1.3	1.3	1.8	0.6	0.8
3rd (n=1,736)	58.3 (54.2 to 62.8)	16	16	89.1	1.3	1.3	41.7	1.7	1.7	2.2	0.8	0.9
4th (n=1,733)	67.2 (62.8 to 72.2)	14.8	14.8	95.6	1.4	1.4	56.0	2.2	2.3	3.5	1.3	1.3
5th (n=1,875)	80.7 (72.2 to 100)	13.2*	13.3*	97.2	1.4*	1.4*	73.3	2.9*	2.9*	14.9	5.4*	6.1*
Total (n=9,042)	57.5 (0 to 100.0)	15.8	15.8	86.6	__-_	-_	45.6	_-	_-	5	_-	-_

[1]adjusted for protein % requirements (dummy variables), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

[2]PR=Prevalence ratios estimated using Poisson regression

[3]PRadj=Prevalence ratios adjusted for protein % requirements (dummy variables), race/ethnicity, ratio of family income to poverty and educational attainment as above (N=8,156).

*Significant linear trend across all quintiles (p<=0.001).

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Table 3. Total energy and protein intakes according to the dietary contribution of ultra-processed foods. US population aged 2 + years (NHANES 2009-2010)

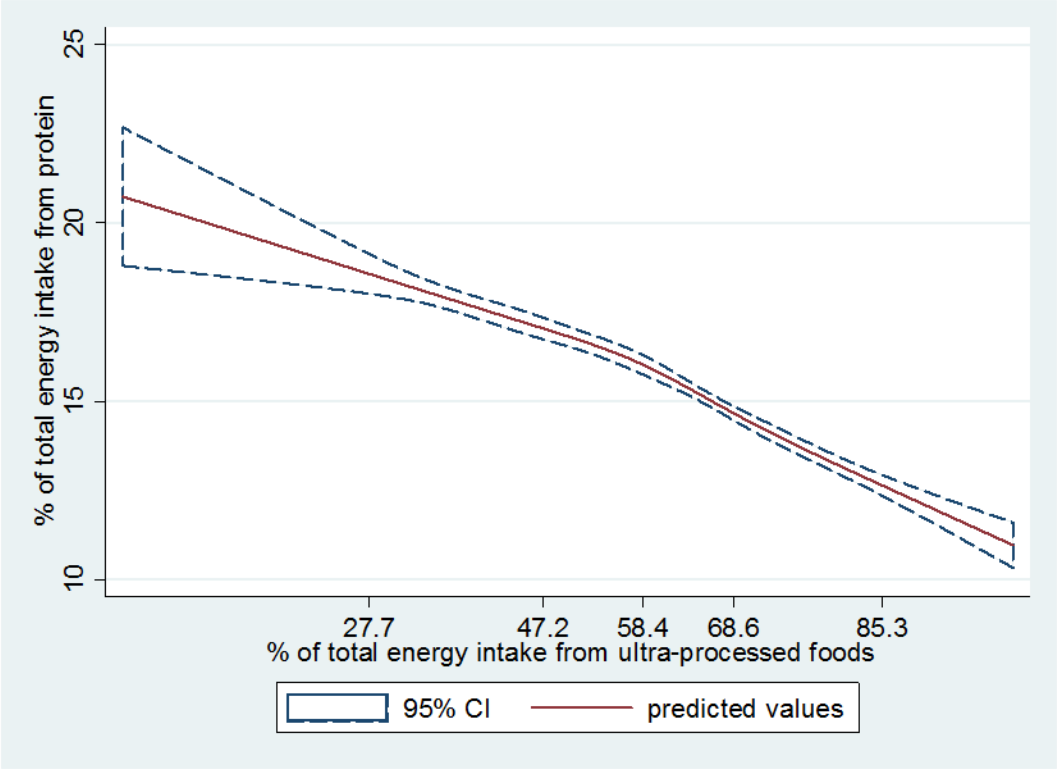
Dietary contribution of ultra-processed foods (% of total energy intake)	Total energy intake (MJ)		Total protein intake (MJ)	
Quintiles	Mean	Adj.[1] Mean	Mean	Adj.[2] Mean
1st (n=1,852)	8.2	8.2	1.5	1.4
2nd (n=1,846)	8.4	8.5	1.4	1.4
3rd (n=1,736)	8.5	8.6	1.3	1.4
4th (n=1,733)	8.9	8.9	1.3	1.3
5th (n=1,875)	8.9*	8.8*	1.2*	1.2*
Total (n=9,042)	8.6	8.6	1.3	1.3

[1]adjusted for energy requirements (dummy variables), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

[2]adjusted for absolute protein requirements (dummy variables), race/ethnicity, ratio of family income to poverty and educational attainment.

*Significant linear trend across all quintiles (p<=0.001).

Figure 1. Dietary protein content regressed on the dietary contribution of ultra-processed foods evaluated by restricted cubic splines. US population aged 2 + years (NHANES 2009-2010) (N=9,042)

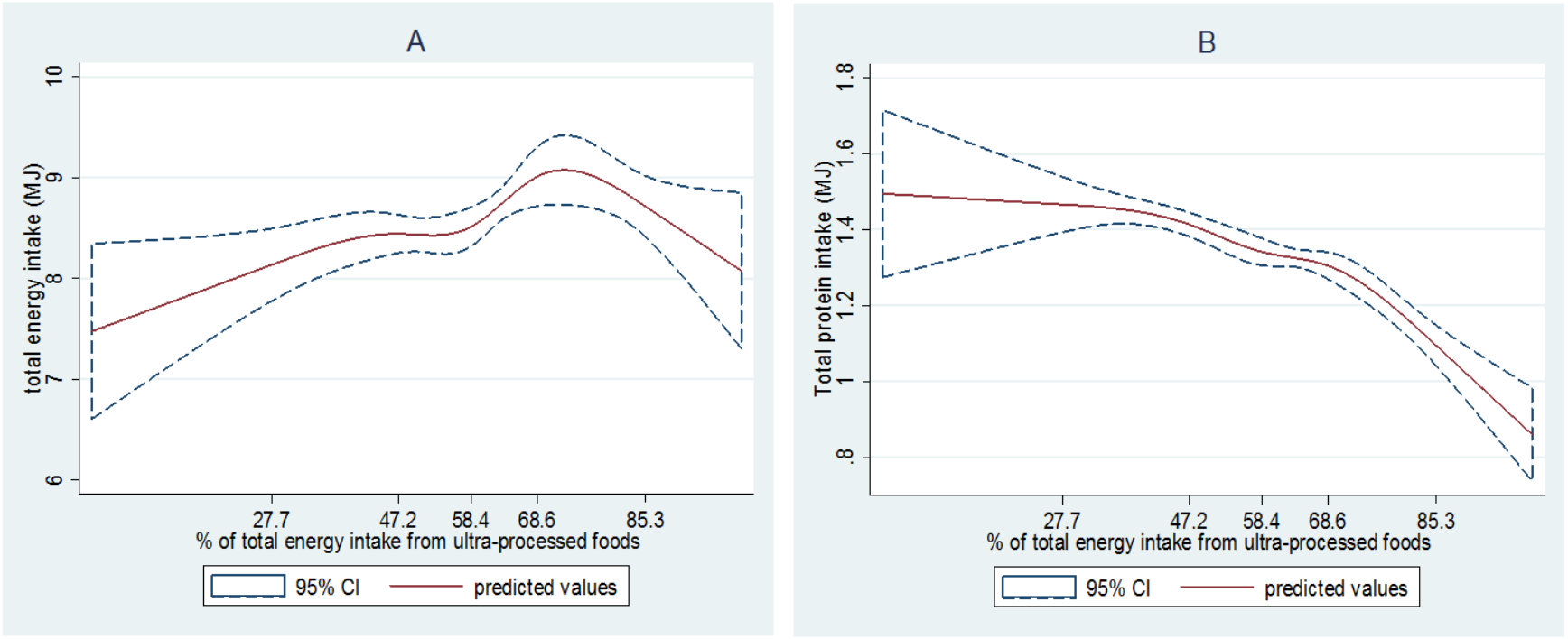


Legend:

The values shown on the x-axis correspond to the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for percentage of total energy from ultra-processed foods (knots).

Coefficient for linear term=-0.08, 95% CI: -0.13 to -0.03. There was little evidence of nonlinearity in the restricted cubic spline model (Wald test for linear term p=0.006; Wald test for all non-linear terms p=0.07)

Figure 2. Total energy intake and total protein intake regressed on the dietary contribution of ultra-processed foods evaluated by restricted cubic splines. US population aged 2 + years (NHANES 2009-2010) (N=9,042)

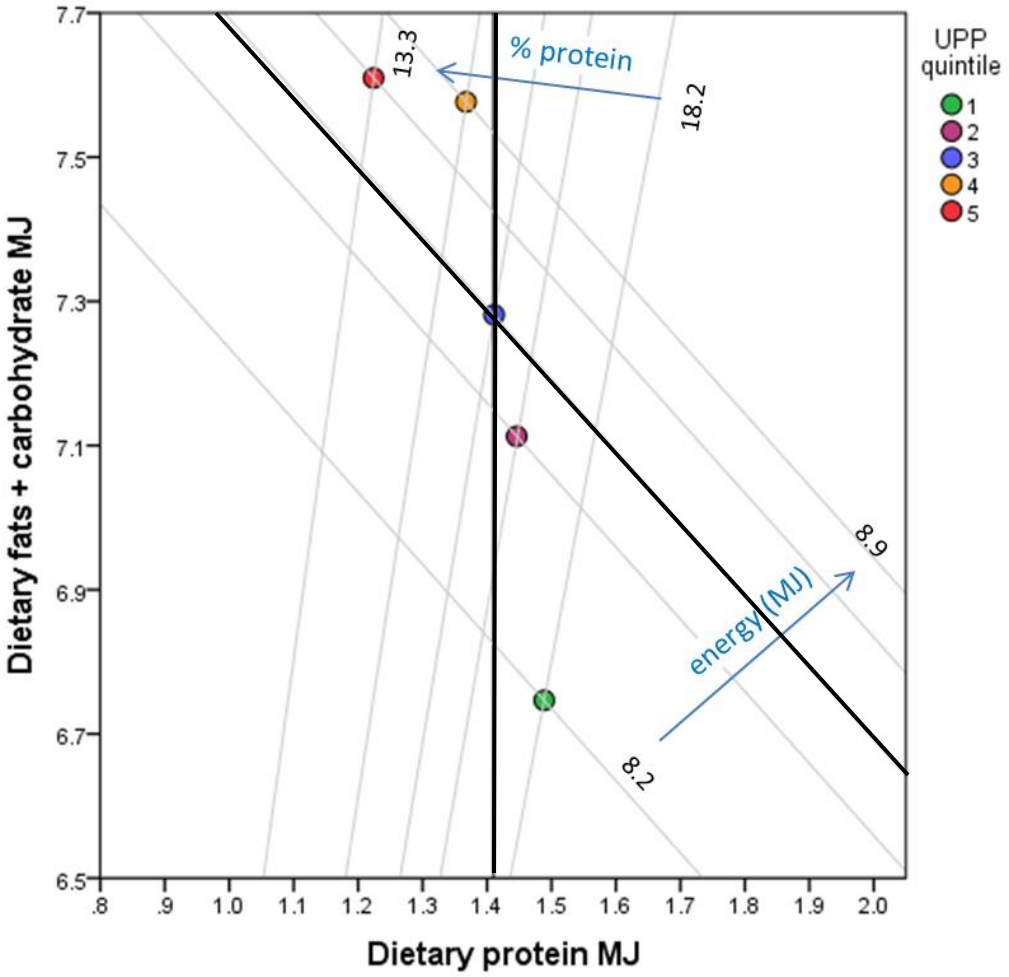


Legend:

Figure 2A. The values shown on the x-axis correspond to the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for percentage of total energy from ultra-processed foods (knots). Coefficient for linear term=0.024, 95% CI: 0.002 to 0.046. There was little evidence of linearity in the restricted cubic spline model (Wald test for linear term p=0.035; Wald test for all non-linear terms p=0.049).

Figure 2B. The values shown on the x-axis correspond to the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for percentage of total energy from ultra-processed foods (knots). Coefficient for linear term=-0.001, 95% CI: -0.007 to 0.004. There was little evidence of linearity in the restricted cubic spline model (Wald test for linear term p=0.7; Wald test for all non-linear terms p=0.0009; Wald test for all terms p<0.001).

Figure 3. Macronutrient and energy correlates of dietary contribution of ultra-processed foods (UPF, discretised into quintiles).



Legend:

The negatively-sloped diagonals represent daily total energy intakes (calculated as the sum of X + Y), and the positive radials represent the dietary ratio of protein: total energy (X/X+Y). The dark vertical line represents perfect protein leverage, in which protein intake remains constant with decreasing dietary % protein (upper blue arrow) and consequently total energy intake increases (lower blue arrow). The dark negative diagonal represents the 0% protein leverage alternative, in which dietary % protein has no effect on total energy intake. The data show that protein intake varied little while energy intake increased with the reduction of dietary % protein associated with increasing contribution of processed foods to the diet, as predicted by the protein leverage hypothesis.

ONLINE SUPPLEMENTARY MATERIAL

Table S1. Percentage of diets with less than 15% of total energy intake from protein, by demographic subgroups, according to quintiles of the dietary contribution of ultra-processed foods. US population aged 2 + years (NHANES 2009-2010)

		Quintiles of the dietary contribution of ultra-processed foods (% of total energy intake)				
		1st (n=1,852)	2nd (n=1,846)	3rd (n=1,736)	4th (n=1,733)	5th (n=1,875)
Gender	Men (n=4,501)	23.1	31.6	39.0	52.2	70.8*
	Women (n=4,541)	27.2	32.6	44.1	59.5	75.7*
Age	2 to 5 (n=861)	24.7	46.4	52.7	65.0	85.7*
	6 to 11 (n=1,154)	23.7	23.9	41.6	64.1	81.5*
	12 to 19 (n=1,265)	35.3	25.6	39.2	47.4	74.9*
	20 to 39 (n=1,928)	24.5	35.8	41.0	52.4	74.6*
	40 to 59 (n=1,935)	25.7	29.3	39.9	55.7	63.2*
	60 and over (n=1,899)	23.1	32.7	43.2	61.8	68.8*
Race/ethnicity	Mexican American (n=1,976)	18.2	36.0	41.5	53.6	76.8*
	Other Hispanic (n=959)	19.7	35.9	55.8	51.6	73.2*
	Non-Hispanic White (n=3,887)	25.8	30.8	39.8	56.1	72.8*
	Non-Hispanic Black (n=1,679)	29.7	30.8	47.6	58.4	73.9*

	Other Race (including Multi-Racial) (n=541)	27.3	38.1	42.1	56.7	73.0*
Income to poverty	0.00–1.30 (n=3,185)	26.3	34.2	47.1	55.2	76.9*
	>1.30–3.50 (n=2,992)	23.5	30.5	38.6	59.6	77.2*
	>3.50 and above (n=2,051)	22.7	31.5	38.8	52.2	64.4*
Educational attainment	<12 years (n=2,600)	24.8	35.1	47.1	57.9	76.5*
	12 years (n=2,054)	21.0	31.5	42.7	53.8	77.5*
	>12 years (n=4,279)	26.4	31.6	40.0	56.4	69.9*

*Significant linear trend across quintiles (P<=0.001), both in unadjusted and Poisson models adjusted for % protein requirements (dummy variables), race/ethnicity (Mexican-American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race - Including Multi-Racial-), ratio of family income to poverty (SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

3.4 MANUSCRIPT 4

Accepted in Nutrients

Title: Association between Dietary Share of Ultra-Processed Foods and Urinary Concentrations of Phytoestrogens in the US

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Running title: Ultra-processed food and phytoestrogens in US

Abstract

The aim of this study was to examine the relationship between dietary contribution of ultra-processed foods and urinary phytoestrogen concentrations in the US. Participants from cross-sectional 2009–2010 National Health and Nutrition Examination Survey aged 6+ years, selected to measure urinary phytoestrogens and with one 24-h dietary recall were evaluated (2,692 participants). Food items were classified according to NOVA (a name, not an acronym), a four-group food classification based on the extent and purpose of industrial food processing. Ultra-processed foods are formulations manufactured using several ingredients and a series of processes (hence “ultra-processed”). Most of their ingredients are lower-cost industrial sources of dietary energy and nutrients, with additives used for the purpose of imitating sensorial qualities of minimally processed foods or of culinary preparations of these foods. Studied phytoestrogens included lignans (enterolactone and enterodiol) and isoflavones (genistein, daidzein, O-desmethylangolensin and equol). Gaussian regression was used to compare average urinary phytoestrogen concentrations (normalized by creatinine) across quintiles of energy share of ultra-processed foods. Models incorporated survey sample weights and were adjusted for age, sex, race/ethnicity, family income, and education, among other factors. Adjusted enterodiol geometric means decreased monotonically from 60.6 in the lowest quintile to 35.1 µg/g creatinine in the highest, while adjusted enterolactone geometric means dropped from 281.1 to 200.1 across the same quintiles, respectively. No significant linear trend was observed in the association between these quintiles and isoflavone concentrations. This finding reinforces the existing evidence regarding the negative impact of ultra-processed food consumption on the overall quality of the diet and expands it to include non-nutrients such as lignans.

Keywords: national health and nutrition examination survey (NHANES); ultra-processed foods; phytoestrogens; lignans; isoflavones; enterolignans

1. Introduction

Phytoestrogens are the most abundant class of natural xenoestrogens, a group of estrogen-mimicking compounds structurally or functionally related to the human sex hormone 17β-estradiol with the capacity of binding to estrogen receptors [1]. Phytoestrogens may also modulate the concentration of endogenous estrogens by inducing sex hormone binding globulin or through the inhibition of enzymes such as aromatase. In addition to tissue-specific hormonal effects and estrogen receptor-specific effects, phytoestrogens may also exert other biological effects via antioxidant mechanisms [2,3]. In fact, studies have shown that consumption of foods rich in phytoestrogens may protect against diseases and dysfunctions related to aging, mental processes, metabolism, malignant transformation, cardiovascular diseases, breast and prostate cancers, menopausal symptoms, osteoporosis, atherosclerosis and stroke, and neurodegeneration [3–6]. Yet, more research is needed in order to fully understand the mechanisms of phytoestrogen action. Indeed, even though studies have reported that isoflavone (a type of phytoestrogen) intake has the potential benefit of preventing colon, endometrial and ovarian cancer, the effects on breast cancer risk are more controversial [3]. On the other hand, some authors do not exclude their negative effect on reproductive disorders, even though no adverse events have been reported in humans [3]. Further details on the effects of each type of phytoestrogen have been described elsewhere [1-3].

Based on their chemical structure and biosynthesis patterns, phytoestrogens have been divided into chalcones, flavonoids (flavones, flavonols, flavanones, isoflavonoids), lignans, stilbenoids, and miscellaneous classes. Lignans and flavonoids are the two main forms [3]. Lignans are polyphenolic components of plant cell walls found in berries, seeds, grains, nuts, fruits, cruciferous vegetables and red wine, with flaxseed being one of the major sources [1]. Flavonoids can be found in berries, wine, grains, nuts, legumes, and especially soybeans and soy-based products which contain relevant amounts of isoflavones genistein and daidzein [1].

Dietary phytoestrogens are first metabolized by intestinal bacteria, then absorbed, conjugated in the liver, circulated in plasma and lastly excreted in urine [4]. Gut

metabolism is, apparently, key in determining the biological effects of dietary phytoestrogens [2,6]. For example, mammal lignans enterolactone and enterodiol are produced from plant lignans matairesinol, secoisolariciresinol, lariciresinol and pinoresinol, their glycosides, and other precursors in the diet by the microflora in the proximal colon [2]. Bioavailability of isoflavones, on the other hand, depends on the initial hydrolysis of glucose-conjugated isoflavones to corresponding aglycones by colon microbial families [7] to allow the subsequent uptake by enterocytes and the flow through the peripheral circulation [3].

During the past decades, analyses of lignan and isoflavone food contents have allowed the compilation of databases to estimate intakes of these compounds, such as the food composition database for isoflavones from The US Department of Agriculture [8,9]. However, accurate measurement intake is limited both because of intake measurement methodology constraints and difficulties with establishing the phytoestrogen content of foods [10]. Furthermore, the lignan or isoflavone concentration within a food varies substantially according to variety, crop season, location and processing methods [11–15]. Also, the fast-growing list of pre-prepared foods, functional foods and dietary supplements available to consumers makes it difficult to have an updated food-intake instrument which fully captures the intake of these phytoestrogens [16]. An alternative approach is estimating human exposure to lignans and isoflavones through the use of biologic samples such as urine [16]. Even though using these types of measurements has its own limitations, several studies have shown that urinary concentrations of phytoestrogens are reliable biomarkers of phytoestrogen intake in both Asian and western populations [16–21] and at least one study found a strong correlation between spot urine and serum phytoestrogen concentrations [10].

Ultra-processed foods include sweet or savory snacks, soft drinks, ready meals and other formulations manufactured using several ingredients and a series of processes (hence ‘“ultra-processed”). Most of their ingredients are lower-cost industrial sources of dietary energy. Nutrients and additives are used with the purpose of imitating sensorial qualities of minimally processed foods or of culinary preparations of these foods, or to disguise undesirable sensory qualities of the final product [22–27]. Evidence exists that

global food supplies are increasingly becoming dominated by these foods [24,27–31] and that consumption of ultra-processed food is associated with excess weight, obesity [32–34], and other diet-related non-communicable diseases (NCDs) [35,36]. Also, nationally representative studies carried out in the US [37,38] and other countries [39–42] have shown that a high dietary contribution of ultra-processed foods renders grossly nutritionally unbalanced diets. Yet, studies carried out to date have focused mainly on nutrients, and did not evaluate the association between ultra-processed food consumption and phytoestrogens in the diet.

This study aims to expand the knowledge on the impact of ultra-processed food consumption on dietary quality by assessing its relationship with urinary concentrations of phytoestrogens in the US population.

2. Subjects and Methods

2.1. Data Source, Population and Sampling

Nationally representative data from the 2009–2010 National Health and Nutrition Examination Survey (NHANES), specifically the dietary component What we eat in America (WWEIA) was utilized. NHANES is a continuous, nationally representative, cross-sectional survey of the non-institutionalized, civilian US residents [43].

The survey included an interview conducted in the home and a subsequent health examination performed at a mobile examination center (MEC), including blood and urine collection. All NHANES examinees were eligible for two 24-h dietary recall interviews. The first dietary recall interview was collected in-person in the MEC [44] while the second was collected by telephone 3–10 days later [45]. Dietary interviews were conducted by trained interviewers using the validated [46–48] US Department of Agriculture Automated Multiple-Pass Method [49].

Of the 13,272 people screened in NHANES 2009–2010, 10,537 (79.4%) participated in the household interview and 10,253 (77.3%) also participated in the MEC health examination [50]. A one-third subsample of 2,941 participants 6 years and over (8,591 individuals) was selected to measure urinary phytoestrogens.

After excluding participants with missing dietary data (129) and an additional 120 with missing urinary phytoestrogens data, 2,692 participants who provided one day of complete dietary intakes were evaluated, 2,411 of which provided two days. The final sample had similar socio-demographic characteristics (gender, age, race/ethnicity, family income and educational attainment) to the full subsample of 2,941 participants selected to measure urinary phytoestrogens (**Table S1**).

2.2. Urinary Phytoestrogen Measurement

Studied phytoestrogens were measured in spot urine samples and included lignans (enterolactone and enterodiol) and isoflavones (genistein, daidzein, O-desmethylangolensin, and equol).

Urine specimens were collected the morning after a recommended fast at the MEC, and processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. Vials were stored under appropriate frozen (–20 °C) conditions until they were shipped to National Center for Environmental Health for testing.

The test principle for the quantitative detection of genistein, daidzein, equol,

O-desmethylangolensin (O-DMA), enterodiol, and enterolactone utilized high performance liquid chromatography–atmospheric pressure photoionization–tandem mass spectrometry (HPLC–APPI–MS/MS). Human urine samples were processed using enzymatic deconjugation of the glucuronidated phytoestrogens followed by size-exclusion filtration. Phytoestrogens were then separated from other urine components by reversed-phase HPLC, detected by APPI–MS/MS, and quantified by isotope dilution. Assay precision was improved by incorporating carbon-13 labeled internal standards for each of the analytes, as well as a 4-methylumbelliferyl glucuronide and

4-methylumbelliferyl sulfate standards to monitor deconjugation efficiency (further details are provided in NHANES Laboratory Procedure Manual) [51].

In order to correct for urine dilution, urinary phytoestrogen concentrations were normalized by urinary creatinine (expressed in μg/g creatinine). This was done by dividing each individual's phytoestrogen concentration value (expressed in ng/mL) by the corresponding urinary creatinine value (expressed in mg/dL). Creatinine was measured using Roche/Hitachi Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA) at the University of Minnesota [52].

For the sample of 2,692, 12 individuals were below the lower detection limit for enterodiol (0.04 ng/mL), 0 for enterolactone (0.1 ng/mL), 2 for daidzein (0.4 ng/mL), 3 for equol (0.06 ng/mL), 0 for genistein (0.2 ng/mL), and 129 for O-desmethylangolensin (0.2 ng/mL) [51]. Several approaches exist to handle left-handed censored data. In NHANES, urinary phytoestrogen measurements below the limits of detection of the used method [51] were replaced with $1/\sqrt{2}$ fraction of the detection limit. Treating these

left-handed censored values incorrectly may introduce bias when estimating the point and confidence interval of distributions [53]. Still, censoring should not affect estimate reliability in this study, as it has been shown that little bias is introduced by any of the replacement techniques if only a small percentage of the values have been censored (i.e., less than 5%) [54].

2.3. Food Classification According to Processing

All recorded food items (n = 238,239 Food Codes) were classified according to NOVA (a name, not an acronym), a food classification based on the extent and purpose of industrial food processing [25,55]. NOVA includes four groups: “unprocessed or minimally processed foods” (such as fresh, dry or frozen fruits or vegetables; packaged grains and pulses; grits, flakes or flours made from corn, wheat or cassava; pasta, fresh or dry, made from flours and water; eggs; fresh or frozen meat and fish and fresh or pasteurized milk); “processed culinary ingredients” (including sugar, oils, fats, salt, and other substances extracted from foods and used in kitchens to season and cook unprocessed or minimally processed foods and to make culinary preparations), “processed foods” (including canned foods, sugar-coated dry fruits, salted meat products, cheeses and freshly made unpackaged breads, and other ready-to-consume products manufactured with the addition of salt or sugar or other substances of culinary use to unprocessed or minimally processed foods), and “ultra-processed foods”.

The NOVA group of ultra-processed foods, of particular interest in this study, includes soft drinks, sweet or savory packaged snacks, confectionery and industrialized desserts, mass-produced packaged breads and buns, poultry and fish nuggets and other reconstituted meat products, instant noodles and soups, and many other ready-to-consume formulations of several ingredients. Besides salt, sugar, oils, and fats, these ingredients include food substances not commonly used in culinary preparations, such as modified starches, hydrogenated oils, protein isolates and classes of additives whose purpose is to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations, or to disguise undesirable qualities of the final product. These additives include colorants, flavorings, non-sugar sweeteners, emulsifiers,

humectants, sequestrants, and firming, bulking, de-foaming, anti-caking and glazing agents. Unprocessed or minimally-processed foods represent a small proportion of or are even absent from the list of ingredients of ultra-processed products. A detailed definition of each NOVA food group and examples of food items classified in each group has been previously published [37]. The rationale underlying the classification is also shown elsewhere [22–24,56,57].

For all food items (Food codes) judged to be a handmade recipe, the classification was applied to the underlying ingredients (Standard Reference codes or SR codes) obtained from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS) 5.0 [58] as further explained in a previously published paper [37].

2.4. Assessing Energy Content

For this study, Food code energy values as provided by NHANES were used.

On the other hand, for handmade recipes, the underlying ingredient (SR code) energy values were calculated using variables from both FNDDS 5.0 [58] and USDA National Nutrient Database for Standard Reference, Release 24 (SR24) [59].

2.5. Data Analysis

All available day 1 dietary intake data for each participant were utilized.

Food items were sorted into mutually exclusive food subgroups within unprocessed or minimally processed foods (n = 11), processed culinary ingredients (n = 4), processed foods (n = 4) and ultra-processed foods (n = 17), as shown in **Table 1**. First, the contributions of each of the NOVA food groups and subgroups to total energy intake and across quintiles of the dietary energy contribution of ultra-processed foods (henceforth ‘dietary share of ultra-processed foods’) were evaluated. The group of unprocessed or minimally processed foods was also combined with the group of processed culinary ingredients, as foods belonging to these two groups are usually combined together in culinary preparations and therefore consumed together.

As urinary phytoestrogen concentrations (both in ng/mL and normalized by creatinine) had skewed distributions, these variables were log transformed (using natural logarithms) and geometric means were presented.

The average phytoestrogen urinary concentrations were compared across quintiles of the dietary share of ultra-processed foods using Gaussian regression. Tests of linear trend were performed in order to evaluate the effect of quintiles as a single continuous variable. For each phytoestrogen, four models were explored: (1) crude (in ng/mL); (2) normalized by creatinine (µg/g); (3) normalized by creatinine and adjusted for socio-demographic variables: sex, age group (6–11 years, 12–19 years, 20–39 years, 40–59 years, 60+ years), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race including multi-racial), ratio of family income to poverty (categorized based on Supplemental Nutrition Assistance Program (SNAP) eligibility as 0.00–1.30, >1.30–3.50, and >3.50 and above) [43], and educational attainment of respondents for participants aged 20+ years and of household reference person otherwise (<12, 12 years and >12 years); and (4) normalized by creatinine and adjusted for socio-demographic + other variables: socio-demographic variables, difference between recommended and actual energy intake (z-score), BMI (body weight divided by height squared, kg/m2: z-score for age if <20 years; z-score if ≥20 years old), minutes per week of physical activity (z-score; estimated separately in <12 and ≥12 years old) and current smoking (yes, no).

As 264 participants had missing values on family income and/or educational attainment, adjusted analysis included 2,428 individuals. Analyses which also adjusted for difference between recommended and actual energy intake, BMI and minutes per week of physical activity included 2,403 individuals.

NHANES survey sample weights were used in all analyses to account for differential probabilities of selection for the individual domains, nonresponse to survey instruments, and differences between the final sample and the total US population. The Taylor series linearization variance approximation procedure was used for variance estimation in all

analysis in order to account for the complex sample design and the sample weights [43].

Statistical hypotheses were tested using a two-tailed $p < 0.01$ level of significance. Data were analyzed using Stata statistical software package version 12.1 (StataCorp LP, College Station, Texas, USA).

3. Results

3.1. Contribution of Nova food Groups to Total Energy Intake

Table 1 presents estimates for the daily energy intake of the US population 6 years and over, the distribution of this intake according to the four NOVA food groups, and the NOVA group distribution across quintiles of the energy share of ultra-processed foods. The population average daily energy intake was 2,153 kcal. Most calories (57.8%) came from ultra-processed foods, 29.2% came from unprocessed or minimally processed foods, 9.8% from processed foods and 3.2% from processed culinary ingredients. An inverse linear trend ($p < 0.01$) was observed in the relationship between the share of ultra-processed foods (quintiles) and the dietary contribution of unprocessed or minimally processed foods, as well as processed culinary ingredients and processed foods. The same applies to individual sub-groups within these three NOVA groups including (but not limited to) lignan and isoflavone food sources such as fruit, grains, roots and tubers, legumes, vegetables, other unprocessed (including nuts and seeds) and other processed (including wine) foods. The energy contribution of most subgroups belonging to ultra-processed foods, many of which are also potential sources of isoflavones[60], increased monotonically from the first to the last quintile of the dietary share of ultra-processed foods, with a few exceptions which showed a slight decrease between the fourth and fifth quintiles (i.e., breakfast cereals or sauces).

3.2. Association between Consumption of Ultra-Processed Foods and Urinary Phytoestrogen Concentrations

Table 2 presents the average concentrations of urinary phytoestrogens across quintiles of the dietary share of ultra-processed foods. An inverse linear trend ($p < 0.01$) was observed in the association between quintiles of the dietary share of ultra-processed foods and enterolignan concentration averages (geometric mean). Indeed, adjusted enterodiol concentrations decreased monotonically from 60.6 in the lowest quintile to 35.1 µg/g creatinine in the highest quintile, while enterolactone concentrations dropped from 281.1 to 200.1 µg/g creatinine across the same quintiles.

In the association between quintiles of the dietary share of ultra-processed foods and each of the four urinary isoflavone concentrations (genistein, daidzein, O-desmethylangolensin, and equol) no significant linear trend was observed.

4. Discussion

In this analysis of US nationally representative data, a strong inverse linear trend was observed in the association between the quintiles of the dietary contribution of ultra-processed foods and urinary concentrations of enterolignans, one of the two main groups of dietary phytoestrogens. Indeed, adjusted enterodiol concentrations decreased monotonically from 60.6 in the lowest quintile to 35.1 µg/g creatinine in the highest quintile, while enterolactone concentrations dropped from 281.1 to 200.1 µg/g creatinine across the same quintiles. This is consistent with the fact that the main dietary sources of lignans are whole foods (berries, seeds, grains, nuts, fruits, and cruciferous vegetables [1]), which are absent from most ultra-processed foods or present in very small amounts.

Studies have found statistically significant correlations between urine and serum concentrations for enterodiol (r = 0.83; r = 0.62) [10,61] and enterolactone (r = 0.94; r = 0.84) [10,61], while several intervention studies found that dietary intake of lignan-containing foods leads to increased enterolignan blood concentrations in nearly all individuals [62]. Several studies have also shown that urinary concentrations of enterolignans are reliable biomarkers of lignan intake [16,18,21]. Based on this evidence, we may also conclude that diets rich in ultra-processed foods lead to a reduced lignan bioavailability, either because of low lignan intake, low conversion to enterolignans or low absorption at the colon. Any component altering intestinal flora or its environment, such as diet, may potentially affect the degree to which precursors are converted to enterolignans and absorbed.

Decreased urinary enterolactone concentrations as observed in high ultra-processed food consumers may have serious health consequences if, as was shown in a study carried out with US NHANES 2003–2010 survey, urinary enterolactone concentrations are inversely associated with serum g-glutamyl transaminase (GGT) levels in adult males and females, with alkaline phosphatase (ALP) in females, and with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in adult males as well [63]. Also, another study carried with US NHANES 2001–2010, found an inverse association between urinary enterolactone levels and obesity, waist circumference,

serum triacylglycerols (TAG) levels, fasting glucose levels, fasting insulin levels and metabolic syndrome in adult males, and a direct association with high-density lipoprotein HDL-cholesterol levels [64]. Another study found an inverse association between urinary concentrations of total enterolignans and cardiovascular mortality and between urinary concentrations of both total enterolignans and enterolactone and all-cause mortality [65]. Interestingly, this study also found a direct association between urinary concentration of total isoflavones and daidzein and risk of death from cardiovascular disease and all causes [65].

The somewhat unexpected absence of association between the dietary contribution of ultra-processed foods and the other important group of dietary phytoestrogens—isoflavones—may be explained by the widespread presence of soy in ultra-processed foods [60], in the form of soybean oil, soy flour, soy protein concentrates, soy protein isolates, textured soy protein or soymilk among others [60]. The presence of these ingredients in ultra-processed foods, probably compensate the absence of other food sources of isoflavones in diets rich in ultra-processed foods.

This study has several strengths. A large, nationally representative sample of the US population was used, increasing generalizability. This investigation was based on individual consumption data, rather than market disappearance or household purchasing data which cannot account for differences between amounts purchased and amounts actually consumed.

Exposure to individual phytoestrogens was assessed by measuring their urinary concentrations, the only option at present to assess dietary intake of lignans as these are not captured by the US Department of Agriculture food composition database (isoflavones are not fully captured either). The advantage of urinary measurements is that they are free of recall bias inherent in self-reported dietary data and represent phytoestrogen intakes from all sources, including those that may be inadequately captured in food composition databases [10,16,65]. An additional advantage of using urinary phytoestrogen testing is that it also captures phytoestrogen metabolites (such as equol and O-desmethylangolensin) produced by intestinal bacteria [66].

Potential limitations should be considered. As with most population measures, dietary data obtained by 24-h recalls is imperfect [67]. However, 24-h recalls are the least-biased self-report instrument available. Also, standardized methods and approach of NHANES have been shown to produce accurate intake estimates [46–48], and will therefore be suitable for assessing population averages. Previous studies suggest that people with obesity may underreport consumption of foods with caloric sweeteners [68] such as desserts and sweet baked goods [69,70], which may lead to an underestimation of the dietary contribution of ultra-processed foods or a dilution of the association between ultra-processed food consumption and enterodiol and enterolactone urinary concentrations. Although NHANES collects limited information indicative of food processing (i.e., place of meals, product brands), these data are not consistently determined for all food items and may also not provide updated, market-representative nutrient information [71], which could lead to modest over-or underestimation of the dietary contribution of ultra-processed foods.

A further limitation arises from the use of spot urine samples for the determination of phytoestrogen concentrations as these measurements might be different from those obtained using 24-h urine due to potential circadian rhythm [65]. Even though there have been no studies examining the correlation between spot and 24-h urinary phytoestrogen concentrations, the concentrations of phytoestrogens in spot urine have been reported to be statistically significantly correlated with their concentrations measured in serum [10,61] and with their dietary intake [16–21]. Also, even though single measurements in spot urines might not accurately reflect individual usual dietary intake due to within-person variation, in this study we were more interested in the urinary measurements actually reflecting the individual's dietary intake during the previous 24 h. Taking into account that isoflavone metabolites, like equol, are excreted in the urine within 24 h after exposure [66] and, that peak serum daidzein and genistein concentrations are attained 4–8 h after ingestion while elimination half-lives are in the range of 8–10 h, respectively [72], we may assume this is the case.

5. Conclusions

This study shows there is a strong inverse linear trend in the association between the dietary share of ultra-processed foods and urinary concentrations of enterodiol and enterolactone in the US, which probably reflects a lower intake and/or bioavailability of lignans among high consumers of ultra-processed foods. This finding reinforces the existing evidence regarding the negative impact of ultra-processed food consumption on the overall quality of the diet [37–42], and expands it to include non-nutrients such as lignans.

Supplementary Materials: The following are available online at www.mdpi.com/link, Table S1. Characteristics of study participants and full subsample of participants selected to measure urinary phytoestrogens. Subsample of US population aged 6+ years (NHANES 2009–2010).

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References

1. Paterni, I.; Granchi, C.; Minutolo, F. Risks and Benefits Related to Alimentary Exposure to Xenoestrogens. Crit. Rev. Food Sci. Nutr. 2016, doi:10.1080/10408398.2015.1126547.
2. Wang, L.Q. Mammalian phytoestrogens: Enterodiol and enterolactone. J. Chromatogr. B 2002, 777, 289–309.
3. Sirotkin, A.V.; Harrath, A.H. Phytoestrogens and their effects. Eur. J. Pharmacol. 2014, 741, 230–236.
4. Cassidy, A. Potential risks and benefits of phytoestrogen-rich diets. Int. J. Vitam. Nutr. Res. 2003, 73, 120–126.
5. Tuohy, P.G. Soy infant formula and phytoestrogens. J. Paediatr. Child Health 2003, 39, 401–405.
6. Branca, F.; Lorenzetti, S. Health effects of phytoestrogens. Forum Nutr. 2005, 57, 100–111.
7. Vitale, D.C.; Piazza, C.; Melilli, B.; Drago, F.; Salomone, S. Isoflavones: Estrogenic activity, biological effect and bioavailability. Eur. J. Drug Metab. Pharmacokinet. 2013, 38, 15–25.
8. Bhagwat, S., Haytowitz, DB, and Holden, JM. 2008. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav (accessed on 12 February 2017).
9. U.S. Department of Agriculture, Agricultural Research Service. 2011. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav (accessed on 12 February 2017).
10. Grace, P.B.; Taylor, J.I.; Low, Y.L.; Luben, R.N.; Mulligan, A.A.; Botting, N.P.; Dowsett, N.; Welch, A.A.; Khaw, K.T.; Wareham, N.J.; et al. Phytoestrogen Concentrations in Serum and Spot Urine as Biomarkers for Dietary Phytoestrogen Intake and Their Relation to Breast Cancer Risk in European Prospective Investigation of Cancer and Nutrition-Norfolk. Cancer Epidemiol. Biomark. Prev. 2004, 13, 698–708.

11. Eldrige, A.; Kwolek, W. Soybean isoflavones: Effect of environment and variety on composition. J. Agric. Food Chem. 1983, 31, 394–396.
12. Wang, H.-J.; Murphy, P.A. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year and location. J. Agric. Food Chem. 1994, 42, 1674–1677.
13. Wang, H.-J.; Murphy, P.A. Isoflavone content in commercial soybean foods. J. Agric. Food Chem. 1994, 42, 1666–1673.
14. Thompson, L.U.; Rickard, S.E.; Cheung, F.; Kenaschuk, E.O.; Obermeyer, W.R. Variability in anticancer lignan levels in flaxseed. Nutr. Cancer 1997, 27, 26–30.
15. Setchell, K.D.; Cole, S.J. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. J. Agric. Food Chem. 2003, 51, 4146–4155.
16. Lampe, J.W. Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J. Nutr. 2003, 133, 956S–964S.
17. Seow, A.; Shi, C.Y.; Franke, A.A.; Hankin, J.H.; Lee, H.P.; Yu, M.C. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle aged and older Chinese in Singapore. Cancer Epidemiol. Biomark. Prev. 1998, 7, 135–140.
18. French, M.R.; Thompson, L.U.; Hawker, G.A. Validation of a phytoestrogen food frequency questionnaire with urinary concentrations of isoflavones and lignan metabolites in premenopausal women. J. Am. Coll. Nutr. 2007, 26, 76–82.
19. Maskarinec, G.; Singh, S.; Meng, L.; Franke, A.A. Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. Cancer Epidemiol. Biomark. Prev. 1998, 7, 613–619.
20. Jaceldo-Siegl, K.; Fraser, G.E.; Chan, J.; Franke, A.; Sabate, J. Validation of soy protein estimates from a food-frequency questionnaire with repeated 24-h recalls and isoflavonoid excretion in overnight urine in a western population with a wide range of soy intakes. Am. J. Clin. Nutr. 2008, 87, 1422–1427.
21. Lampe, J.W.; Gustafson, D.R.; Hutchins, A.M.; Martini, M.C.; Li, S.; Wahala, K.; Grandits, G.A.; Potter, J.D.; Slavin, J.L. Urinary isoflavonoid and lignan excretion on

a western diet: Relation to soy, vegetable, and fruit intake. Cancer Epidemiol. Biomark. Prev. 1999, 8, 699–707.

22. Monteiro, C.A. Nutrition and health. The issue is not food, nor nutrients, so much as processing. Public Health Nutr. 2009, 12, 729–731.
23. Ludwig, D.S. Technology, diet, and the burden of chronic disease. JAMA 2011, 305, 1352–1353.
24. Moodie, R.; Stuckler, D.; Monteiro, C.; Sheron, N.; Neal, B.; Thamarangsi, T.; Lincoln, P.; Casswell, S.; Lancet NCD Action Group. Profits and pandemics: Prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. Lancet 2013, 381, 670–679.
25. Monteiro, C.A.; Cannon, G.; Levy, R.B.; Claro R.M,; Moubarac J-C. Ultra-processing and a new classification of foods. In Introduction to U.S. Food System. Public Health, Environment, and Equity; Neff, R., Ed.; Jossey Bass A Wiley Brand: San Francisco, CA, USA, 2015.
26. Food and Agriculture Organization. Guidelines on the Collection of Information on Food Processing through Food Consumption Surveys; FAO: Rome, Italy, 2015.
27. Ultra-Processed Food and Drink Products in Latin America: Trends, Impact on Obesity, Policy Implications; Panamerican Health Organization: Washington, DC, USA, 2015.
28. Stuckler, D.; McKee, M.; Ebrahim, S.; Basu, S. Manufacturing epidemics: The role of global producers in increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. PLoS Med. 2012, 9, e1001235.
29. Monteiro, C.A.; Cannon, G. The impact of transnational ‘Big Food’ companies on the South: A view from Brazil. PLoS Med. 2012, 9, e1001252.
30. Monteiro, C.A.; Moubarac, J.C.; Cannon, G.; Popkin, B.M. Ultra-processed products are becoming dominant in the global food system. Obes. Rev. 2013, 14 (Suppl. S2), 21–28.
31. Juul, F.; Hemmingsson, E. Trends in consumption of ultra-processed foods and obesity in Sweden between 1960 and 2010. Public Health Nutr. 2015, 18, 3096–3107.

32. Mendonça, R.D.; Pimenta, A.M.; Gea, A.; de la Fuente-Arrillaga, C.; Martinez-Gonzalez, M.A.; Lopes, A.C.; Bes-Rastrollo, M. Ultraprocessed food consumption and risk of overweight and obesity: The University of Navarra Follow-Up (SUN) cohort study. Am. J. Clin. Nutr. 2016, 104, 1433–1440.
33. Louzada, M.L.; Baraldi, L.G.; Steele, E.M.; Martins, A.P.; Canella, D.S.; Moubarac, J.C.; Levy, R.B.; Cannon, G.; Afshin, A.; Imamura, F.; et al. Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults. Prev. Med. 2015, 81, 9–15.
34. Canella, D.S.; Levy, R.B.; Martins, A.P.; Claro, R.M.; Moubarac, J.C.; Baraldi, L.G.; Cannon, G.; Monteiro, C.A. Ultra-processed food products and obesity in Brazilian households (2008–2009). PLoS ONE 2014, 9, e92752.
35. Rauber, F.; Campagnolo, P.D.; Hoffman, D.J.; Vitolo, M.R. Consumption of ultra-processed food products and its effects on children's lipid profiles: A longitudinal study. Nutr. Metab. Cardiovasc. Dis. 2015, 25, 116–122.
36. Tavares, L.F.; Fonseca, S.C.; Garcia Rosa, M.L.; Yokoo, E.M. Relationship between ultra-processed foods and metabolic syndrome in adolescents from a Brazilian Family Doctor Program. Public Health Nutr. 2012, 15, 82–87.
37. Martinez Steele, E.; Baraldi, L.G.; Louzada, M.L.; Moubarac, J.C.; Mozaffarian, D.; Monteiro, C.A. Ultra-processed foods and added sugars in the US diet: Evidence from a nationally representative cross-sectional study. BMJ Open 2016, 6, e009892.
38. Martinez Steele, E.; Popkin, B.M.; Swinburn, B.; Monteiro, C.A. The share of ultra-processed foods and the overall nutritional quality of diets in the US: evidence from a nationally representative cross-sectional study. Population Health Metrics 2017 (accepted for publication). doi: 10.1186/s12963-017-0119-3.
39. Monteiro, C.A.; Levy, R.B.; Claro, R.M.; de Castro, I.R.; Cannon, G. Increasing consumption of ultra-processed foods and likely impact on human health: Evidence from Brazil. Public Health Nutr. 2010, 14, 5–13.
40. Louzada, M.L.; Martins, A.P.; Canella, D.S.; Baraldi, L.G.; Bertazzi, R.L.; Claro, R.M.; Moubarac, J.C.; Cannon, G.; Monteiro, C.A. Ultra-processed foods and the nutritional dietary profile in Brazil. Rev. Saúde Pública 2015, 49, doi:10.1590/S0034-8910.2015049006132.

41. Louzada, M.L.; Martins, A.P.; Canella, D.S.; Baraldi, L.G.; Bertazzi, R.L.; Claro, R.M.; Moubarac, J.C.; Cannon, G.; Monteiro, C.A. Impact of ultra-processed foods on micronutrient content in the Brazilian diet. Rev. Saúde Pública 2015, 49, doi:10.1590/S0034-8910.2015049006211.
42. Moubarac, J.-C.; Martins, A.P.B.; Claro, R.M.; Levy, R.B.; Cannon, G.; Monteiro, C.A. Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr. 2012, 16, 1–9.
43. Johnson, C.L.; Paulose-Ram, R.; Ogden, C.L.; Carroll, M.D.; Kruszon-Moran, D.; Dohrmann, S.M.; Curtin, L.R. National Health and Nutrition Examination Survey: Analytic guidelines, 1999–2010; Vital Health Stat 2; National Center for Health Statistics: Hyattsville, Maryland, USA, 2013; Volume 161.
44. NHANES. MEC In-Person Dietary Interviewers Procedures Manual. 2009. Available online: https://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/mec_in_person_dietary_procedures_manual_mar_2010.pdf (accessed on 12 February 2017).
45. NHANES. Phone Follow-Up Dietary Interviewer Procedures Manual. 2009. Available online: https://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/phone_follow_up_dietary_procedures_manual_mar_2010.pdf (accessed on 12 February 2017).
46. Moshfegh, A.J.; Rhodes, D.G.; Baer, D.J.; Murayi, T.; Clemens, J.C.; Rumpler, W.V.; Paul, D.R.; Sebastian, R.S.; Kuczynski, K.C.; Ingwersen, L.A.; et al. The USDA Automated Multiple-Pass Method reduces bias in the collection of energy intakes. Am. J. Clin. Nutr. 2008, 88, 324–332.
47. Blanton, C.A.; Moshfegh, A.J.; Baer, D.J.; Kretsch, M.J. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. J. Nutr. 2006, 136, 2594–2599.
48. Rumpler, W.V.; Kramer, M.; Rhodes, D.G.; Moshfegh, A.J.; Paul, D.R.; Kramer, M. Identifying sources of reporting error using measured food intake. Eur. J. Clin. Nutr. 2008, 62, 544–552.

49. Automated Multiple-Pass Method. United States Department of Agriculture. Agriculture Research Service. Available online: http://www.ars.usda.gov/ba/bhnrc/fsrg (accessed on12 February 2017).
50. National Health and Nutrition Examination Survey. NHANES Response Rates and Population Totals. Response Rates. Available online: http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm (accessed on12 February 2017).
51. Laboratory Procedure Manual. Phytoestrogens in Urine NHANES 2009–2010. Bioactive Dietary Compounds Laboratory (BDCL); Nutritional Biomarkers Branch (NBB); Division of Laboratory Sciences (DLS); National Center for Environmental Health (NCEH). Available online: https://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/Phyto_F_met_phytoestrogens.pdf (accessed on 12 February 2017).
52. Laboratory Procedure Manual. Urinary Creatinine. University of Minnesota; January 2011. Available online: https://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/ALB_CR_F_met_creatinine.pdf (accessed on 12 February 2017).
53. Lyles, R.H.; Fan, D.; Chuachoowong, R. Correlation coefficient estimation involving a left censored laboratory assay variable. Statist. Med. 2001, 20, 2921–2933.
54. Croghan, C.; Egeghy, P.P. Methods of Dealing with Values below the Limit of Detection Using SAS. Presented at Southeastern SAS User Group, St. Petersburg, FL, USA, 22–24 September 2003.
55. Monteiro, C.A.; Levy, R.B.; Claro, R.M.; de Castro, I.R.R.; Cannon, G. A new classification of foods based on the extent and purpose of their processing. Cad. Saúde Pública 2010, 26, 2039–2049.
56. Moubarac, J.C.; Parra, D.C.; Cannon, G.; Monteiro, C.A. Food Classification Systems Based on Food Processing: Significance and implications for policies and actions: A systematic literature review and assessment. Curr. Obes. Rep. 2014, 3, 256–272.

57. Monteiro, C.A.; Cannon, G.; Levy, R.B.; Claro, R.M.; Moubarac, J.C. The Food System. Processing. The big issue for disease, good health, well-being. World Nutr. 2012, 3, 527–569.
58. Ahuja, J.K.A.; Montville, J.B.; Omolewa-Tomobi, G.; Heendeniya, K.Y.; Martin, C.L.; Steinfeldt, L.C.; Anand, J.; Adler, M.E.; LaComb, R.P.; Moshfegh, A.J. USDA Food and Nutrient Database for Dietary Studies, 5.0; U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group: Beltsville, MD, USA, 2012.
59. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page. 2011. Available online: http://www.ars.usda.gov/ba/bhnrc/ndl (accessed on 12 February 2017).
60. Barnes, S. The biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. Lymphat. Res. Biol. 2010, 8, 89–98.
61. Valentin-Blasini, L.; Blount, B.C.; Caudill, S.P.; Needham, L.L. Urinary and serum concentrations of seven phytoestrogens in a human reference population subset. J. Expo. Anal. Environ. Epidemiol. 2003, 13, 276–282.
62. Clavel, T.; Doré, J.; Blaut, M. Bioavailability of lignans in human subjects. Nutr. Res. Rev. 2006, 19, 187–196.
63. Xu, C.; Liu, Q.; Zhang, Q.; Jiang, Z.Y.; Gu, A. Urinary enterolactone associated with liver enzyme levels in US adults: National Health and Nutrition Examination Survey (NHANES). Br. J. Nutr. 2015, 114, 91–97.
64. Xu, C.; Liu, Q.; Zhang, Q.; Gu, A.; Jiang, Z.Y. Urinary enterolactone is associated with obesity and metabolic alteration in men in the US National Health and Nutrition Examination Survey 2001–10. Br. J. Nutr. 2015, 113, 683–690.
65. Reger, M.K.; Zollinger, T.W.; Liu, Z.; Jones, J.; Zhang, J. Urinary phytoestrogens and cancer, cardiovascular, and all‐cause mortality in the continuous National Health and Nutrition Examination Survey. Eur. J. Nutr. 2016, 55, 1029–1040.
66. Rowland, I.; Faughnan, M.; Hoey, L.; Wahala, K.; Williamson, G.; Cassidy, A. Bioavailability of phyto-oestrogens. Br. J. Nutr. 2003, 89, S45–S58.

67. Subar, A.F.; Freedman, L.S.; Tooze, J.A.; Kirkpatrick, S.I.; Boushey, C.; Neuhouser, M.L.; Thompson, F.E.; Potischman, N.; Guenther, P.M.; Tarasuk, V.; et al. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. J. Nutr. 2015, 145, 2639–2645.
68. Bingham, S.; Luben, R.; Welch, A.; Tasevska, N.; Wareham, N.; Khaw, K.T. Epidemiologic assessment of sugars consumption using biomarkers: Comparisons of obese and nonobese individuals in the European Prospective Investigation of Cancer Norfolk. Cancer Epidemiol. Biomark. Prev. 2007, 16, 1651–1654.
69. Lafay, L.; Mennen, L.; Basdevant, A.; Charles, M.A.; Borys, J.M.; Eschwège, E.; Romon, M. Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Sante (FLVS) study. Int. J. Obes. Relat. Metab. Disord. 2000, 24, 1500–1506.
70. Pryer, J.A.; Vrijheid, M.; Nichols, R.; Kiggins, M.; Elliott, P. Who are the ‘‘low energy reporters’’ in the dietary and nutritional survey of British adults? Int. J. Epidemiol. 1997, 26, 146–154.
71. Slining, M.M.; Yoon, E.F.; Davis, J.; Hollingsworth, B.; Miles, D.; Ng, S.W. An Approach to Monitor Food and Nutrition from “Factory to Fork”. J. Acad. Nutr. Diet. 2015, 115, 40–49.
72. Setchell, K.D.R.; Brown, N.M.; Desai, P.; Zimmer-Nechimias, L.; Wolfe, B.; Jakate, A.S.; Creutzinger, V.; Heubi, J.E. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J. Nutr. 2003, 133, 1027–1035.

Tables

Table 1. Distribution (%) of the total daily per capita energy intake (kcal) according to NOVA food groups by quintiles of the dietary share of ultra-processed foods[a].

		Quintile of Dietary Share of Ultra-Processed Foods (% of Total Energy Intake) [b]				
	All Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
	(*n* = 2,692)	**(*n* = 539)**	**(*n* = 530)**	**(*n* = 521)**	**(*n* = 540)**	**(*n* = 562)**
	(2,153 kcal)	**(2,040.5 kcal)**	**(2,212.1 kcal)**	**(2,143.0 kcal)**	**(2,143.9 kcal)**	**(2,227.6 kcal)**
Unprocessed or minimally processed foods	***29.2***	***50.7***	***35.7***	***29.5***	***20.8***	***9.4 ****
Meat (includes poultry)	8.2	13.2	10.5	8.7	6.3	2.4 *
Fruit and freshly squeezed fruit juices	4.7	7.7	5.1	5.2	3.6	2.0 *
Milk and plain yoghurt	4.3	5.6	4.6	5	4.1	2.2 *
Grains	3	7.4	3.9	1.9	1.4	0.4 *
Roots and tubers	1.4	2.3	1.9	1.8	0.9	0.4 *
Eggs	1.5	2.1	2.1	1.7	1.3	0.5 *
Pasta	1.3	2.8	1.8	0.9	0.9	0.2 *
Fish and sea food	1	1.9	1.3	0.9	0.5	0.4 *
Legumes	0.9	2	1.1	0.7	0.3	0.1 *
Vegetables	0.7	1.3	0.7	0.7	0.5	0.3 *
Other unprocessed or minimally processed foods [1]	2	4.4	2.5	1.9	0.9	0.4 *

Processed culinary ingredients	***3.2***	***5.6***	***4.1***	***3.1***	***2.1***	***1.0 ****
Sugar [2]	1.3	1.9	1.7	1.5	0.9	0.5 *
Plant oils	1.3	2.7	1.7	0.9	0.7	0.3 *
Animal fats [3]	0.5	0.7	0.6	0.6	0.5	0.2 *
Other processed culinary ingredients [4]	0.04	0.12	0.04	0.03	0.01	0.01
Unprocessed or minimally processed foods + Processed culinary ingredients	***32.4***	***56.2***	***39.8***	***32.6***	***22.9***	***10.4 ****
Processed foods	***9.8***	***15.3***	***13.2***	***9.2***	***7.5***	***3.9 ****
Cheese	3.5	4	4.6	3.8	3.3	2.0 *
Ham and other salted, smoked or canned meat or fish	1.3	1.4	1.6	1.7	1.5	0.6
Vegetables and other plant foods preserved in brine	0.8	0.8	0.9	0.7	0.7	0.3 *
Other processed foods [5]	4.2	9.1	6.1	2.9	2.1	0.9 *
Ultra-processed foods	***57.8***	***28.5***	***47***	***58.2***	***69.6***	***85.6 ****
Breads	9.8	6.9	9.8	11.5	11.5	9.4 *
Soft and fruit drinks [6]	7.3	3.1	5.3	7	8.9	11.9 *
Cakes, cookies and pies	5.7	2	4.3	6.7	7.7	7.6 *
Salty-snacks	4.5	1.6	3.9	4.2	5.5	7.4 *
Frozen and shelf-stable plate meals	3.6	0.6	2.1	2.6	4.6	7.9 *
Pizza (ready-to-eat/heat)	3.7	0.2	1.5	2.7	4.6	9.8 *

Breakfast cereals	2.5	1.7	2.6	2.9	2.8	2.7
Sauces, dressings and gravies	2.5	2	2.4	2.8	3.4	1.9
Reconstituted meat or fish products	2.5	0.6	2.6	2.3	3.1	3.9 *
Sweet-snacks	2.4	1.3	1.9	2.1	3.1	3.8 *
Ice cream and ice pops	2.1	0.8	1.4	2.1	2.6	3.7 *
Desserts [7]	1.7	1.5	1.4	1.6	1.9	1.9
French fries and other potato products	1.7	0.4	0.9	1.7	2	3.6 *
Sandwiches and hamburgers on bun (ready-to-eat/heat)	1.5	0.1	0.6	0.9	1.7	3.9 *
Milk-based drinks	1.4	0.8	1.3	1.3	1.4	2
Instant and canned soups	0.8	0.7	0.5	1	0.9	0.9
Other ultra-processed foods [8]	3.9	4	4.4	4.6	3.7	2.9
Total	**100**	**100**	**100**	**100**	**100**	**100**

[a] Subsample of US population aged 6+ years (National Health and Nutrition Examination Survey, NHANES 2009–2010); [b] Mean (range) dietary share of ultra-processed foods per quintile: first = 28.5 (1.6–39.5); second = 47.0 (39.5–52.9); third = 58.2 (52.9–63.5); fourth = 69.6 (63.5–75.9); fifth = 85.6 (76.0–100); [1] Including nuts and seeds (unsalted); yeast; dried fruits (without added sugars) and vegetables; non pre-sweetened, non-whitened, non-flavored coffee and tea; coconut water and meat; homemade soup and sauces; flours; tapioca; [2] Including honey, molasses, maple syrup (100%); [3] Including butter, lard and cream; [4] Including starches; coconut and milk cream; unsweetened baking chocolate, cocoa powder and gelatin powder; vinegar; baking powder and baking soda; [5] Including salted or sugared nuts and seeds; peanut, sesame, cashew and almond butter or spread; beer and wine; [6] Including energy drinks, sports drinks, nonalcoholic wine; [7] Including ready-to-eat and dry-mix desserts such as pudding; [8] Including soy products such as meatless patties and fish sticks; baby food and baby formula; dips, spreads, mustard and catsup; margarine; sugar substitutes, sweeteners and all syrups (excluding 100% maple syrup); distilled alcoholic drinks; * Significant linear trend across all quintiles ($p < 0.01$), both in unadjusted and models adjusted for sex, age group (6–11, 12–19, 20–39, 40–59, 60+ years), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic White, non-Hispanic Black and other race—including multi-racial) ratio of family income to poverty (Supplemental Nutrition Assistance Program, SNAP 0.00–1.30, >1.30–3.50, and >3.50 and over) and educational attainment (<12, 12 years and >12 years).

Table 2. Phyto-estrogen concentrations according to the quintiles of the dietary share of ultra-processed foods [a].

			Quintile of Dietary Share of Ultra-Processed Foods (% of Total Energy Intake) [b]					
			Q1	**Q2**	**Q3**	**Q4**	**Q5**	**All Quintiles**
Enterolignans (GM [c])	Enterodiol	Crude (ng/mL) (*n* = 2,692)	52.7	42.9	38.4	35.8	33.1 *	40.05
		Normalized by creatinine (μg/g) (*n* = 2,692)	61.2	49.2	39.8	38.3	31.6 *	
		Normalized and adjusted for socio-demographic variables [d] (*n* = 2,428)	60.8	51.9	38.3	39.3	33.6 *	
		Normalized and adjusted for socio-demographic + other variables (*n* = 2,403) [e]	60.6	50.7	38.5	40.0	35.1 *	
	Enterolactone	Crude (ng/mL)	255.6	224.4	226.2	209.7	176.4	216.9
		Normalized by creatinine (μg/g)	297.1	257.2	234.4	224.3	168.4 *	
		Normalized and adjusted for socio-demographic variables [d]	291.8	261.2	219.0	237.5	186.9 *	
		Normalized and adjusted for socio-demographic + other variables [e]	281.1	258.0	222.8	245.1	200.1	
Isoflavones (GM)	Daidzein	Crude (ng/mL)	57.3	66.8	70.9	70.0	82.3	69.0
		Normalized by creatinine (μg/g)	66.6	76.6	73.5	74.9	78.6	
		Normalized and adjusted for socio-demographic variables [d]	67.7	79.9	72.1	74.3	71.7	
		Normalized and adjusted for socio-	68.9	79.8	72.5	74.9	71.6	

	demographic + other variables [e]						
O-Desmethylangolensin (*O*-DMA)	Crude (ng/mL)	4.2	4.9	4.3	4.9	5.5	4.7
	Normalized by creatinine (μg/g)	4.9	5.6	4.4	5.2	5.2	
	Normalized and adjusted for socio-demographic variables [d]	5.0	5.8	4.3	5.2	5.1	
	Normalized and adjusted for socio-demographic + other variables [e]	5.1	5.7	4.2	5.3	5.2	
Equol	Crude (ng/mL)	6.8	7.6	8.5	7.8	9.0	7.9
	Normalized by creatinine (μg/g)	7.9	8.7	8.8	8.4	8.6	
	Normalized and adjusted for socio-demographic variables [d]	8.9	8.9	8.7	8.2	7.9	
	Normalized and adjusted for socio-demographic + other variables [e]	8.8	8.9	8.8	8.2	7.9	
Genistein	Crude (ng/mL)	27.9	29.4	35.6	31.5	38.8	32.4
	Normalized by creatinine (μg/g)	32.5	33.7	36.9	33.7	37.1	
	Normalized and adjusted for socio-demographic variables [d]	32.4	34.8	35.9	32.7	34.6	
	Normalized and adjusted for socio-demographic + other variables [e]	32.6	34.8	36.4	32.8	34.4	

[a] Subsample of US population aged 6+ years (NHANES 2009–2010); [b] Mean (range) dietary share of ultra-processed foods per quintile: first = 28.5 (1.6–39.5); second = 47.0 (39.5–52.9); third = 58.2 (52.9–63.5); fourth = 69.6 (63.5–75.9); fifth = 85.6 (76.0–100); [c] GM = geometric mean; [d] Normalized by creatinine (μg/g) and adjusted for sex, age group, race/ethnicity, ratio of family income to poverty and educational attainment; [e] Normalized by creatinine (μg/g) and adjusted for all socio-demographic variables + difference between recommended and actual energy intake (*z*-score), BMI (body weight divided by height squared, kg/m2: *z*-score), minutes per week of physical activity (*z*-score) and current smoking; *Significant linear trend across all quintiles ($p < 0.01$).

Supplementary Materials

Table S1. Characteristics of study participants and full subsample of participants selected to measure urinary phytoestrogens. Subsample of US population aged 6+ years (NHANES 2009–2010).

		Full Subsample (*n* = 2,941)	**Study Sample (*n* = 2,692)**
Gender	Men	48.8	49.1
	Women	51.2	50.9
Age	6 to 11	8.8	8.5
	12 to 19	12.0	12.4
	20 to 39	29.3	29.8
	40 to 59	30.3	29.6
	60 and over	19.6	19.7
Race/ethnicity	Mexican American	9.9	10.3
	Other Hispanic	5.1	5.2
	Non-Hispanic White	65.0	64.8
	Non-Hispanic Black	11.9	12.0
	Other Race (including Multi-Racial)	8.0	7.7
Income to poverty [a]	0.00–1.30	22.9	23.2
	>1.30–3.50	38.2	37.8
	>3.50 and above	38.8	39.1
Educational attainment [b]	<12 years	19.1	19.0
	12 years	22.9	23.2
	>12 years	58.0	57.7

[a] Full subsample: 269 with missing income values; study sample: 241 with missing income values; [b] Full subsample: 36 with missing education values; study sample: 32 missing education values.

4. FINAL CONSIDERATIONS

In this thesis we were able to confirm that in the US population an increased consumption of ultra-processed foods is inversely associated with protein, fiber, vitamins A, C, D and E, zinc, potassium, phosphorus, magnesium and calcium dietary contents, while being directly associated with carbohydrate, added sugars and saturated fat contents (Manuscripts 1 and 2). We also confirmed that ultra-processed food consumption is inversely associated with urinary enterolignan levels, while isoflavone levels remained unchanged (Manuscript 4). These results fit Cordain's predictions that the displacement of minimally processed foods by more processed foods negatively affects the following dietary indicators: 1) macronutrient composition, 2) fatty acid composition, 3) micronutrient density and 4) fiber content (Cordain et al., 2005). We did not find an association between ultra-processed food and fat consumption, probably because fat rich processed foods and processed culinary ingredients are also important sources of fat in today's diets. On the other hand, we did find a direct association with saturated fat intake, an important discriminator of dietary quality.

As previously explained, we must bear in mind that there is a theoretical gap between the hypothesis of Cordain et al. and our own. In effect, they suggest that the displacement of minimally processed foods by more processed foods elicits unbalanced dietary indicators (referring to all foods introduced during the Neolithic and Industrial Periods, meaning, processed culinary ingredients + processed foods + ultra-processed foods + flour, milk, cereals, pasta). In this study instead we tested, whether the displacement of minimally processed foods + processed culinary ingredients + processed foods, by ultra-processed foods is the trigger for unbalances in contemporary diets.

Ultra-processed food consumption was also directly associated with the sodium/ potassium ratio (though results are not presented in this thesis), as was foreseen by Cordain et al. Indeed, even though both sodium and potassium densities decreased

with the rise in ultra-processed food consumption (linear trend in the association was only statistically significant for potassium) (Manuscript 1), the fall in potassium density was greater. Nevertheless, the sodium/potassium ratio was above 1.0 (maximum recommended value according to Cordain) and way above 0.5 (ratio between sodium and potassium values recommended by the American Dietary Guidelines for all stratum) (DGAC, 2015) in all quintiles of ultra-processed food consumption.

Despite not having explored the association between the dietary contribution of ultra-processed foods and the dietary intake glycemic load, Cordain's prediction may also be met, because of the confirmed direct association with added sugars intake (Manuscript 2). We were also unable to evaluate whether ultra-processed food consumption yields net acid diets.

This thesis also confirmed that ultra-processed food consumption is a meaningful determinant of the overall nutritional quality of contemporary diets (manuscript 1). This also lines up with Cordain's idea that it was multifactorial rather than single dietary elements which were most likely affected by the Neolithic and Industrial era changes in diet, and therefore underlie the etiology of most diseases of civilization (Cordain et al., 2005). The United Nations Food and Agriculture Organization (FAO, 2015), the Pan-American Health Organization (PAHO, 2015) and INFORMAS (International Network for Food and Obesity/non-communicable diseases Research, Monitoring and Action Support) (Vandevijvere et al., 2013) also uphold that the dietary share of ultra-processed foods largely determines the overall nutritional quality of the diets.

In this thesis, we confirmed that increases in dietary contribution of ultra-processed foods (shown to be inversely associated with protein density) were also associated with a rise in total energy intake, while absolute protein intake remained relatively constant (Manuscript 3). This is consistent with the Protein Leverage Hypothesis model, which suggests that a low food protein density (characteristic of diets high in ultra-processed foods) together with the strong human appetite for protein is the main driver of the high fats and carbohydrate intakes, and in turn, total energy intakes.

4.1 Strengths and limitations

Being a population-based study will most likely free it from selection bias, maximizing external validity and generalizability of the obtained results.

An additional strength is that our investigation was based on total, effective individual consumption data, rather than on household purchasing data (Monteiro et al., 2010b; Moubarac et al., 2012; Crovetto et al., 2014) which do not account for the fraction of wasted food or for purchases at restaurants.

Nutrients versus foods

Human diet is so complex that representing diet is a daunting challenge (Willet, 1998). In this thesis, we opted to describe diet both in terms of nutrients and of food/ food subgroup contents, even though we largely focused on the former.

Focusing on food intakes makes sense if we consider that foods are not fully represented by their nutrient composition (i.e. milk ad yogurt have similar nutrient contents but different physiological effects). Indeed, as foods and ultimately diets are a complex mixture of compounds that may compete, antagonize or alter the bioavailability of any single nutrient, employing food intakes or food intake patterns may better capture the potential health effect than the dietary content of individual nutrients (Willet, 1998). Another disadvantage of using nutrients or other compounds is that nutrient contents are calculated only indirectly, based on food composition tables which not always fully capture nutrient content variability and are only available for a limited number of nutrients or compounds. Using food intakes also makes sense when a specific hypothesis has not yet been formulated regarding the chemical substance which constitutes the risk/ protective factor (Willet, 1998). One drawback of using foods to represent diet, however, is their large number and reciprocal interrelations; using food intake patterns is one way to overcome this limitation. An alternative approach is to use nutrient intakes (or nutrient intake patterns) that summarize the contribution of all foods or compute the contribution of nutrient intake from various food groups individually (i.e. fiber intake from fruits or vegetables and from grains) (Willet, 1998).

Despite the somewhat reductionist approach, focusing on nutrients has several other advantages (Willet, 1998) such as that the nutrient contents can be directly related to fundamental knowledge of biology. Besides, because the food intake variability is greater than the nutrient intake variability when using 24-hour recall data, assessing the association with total nutrient intake (i.e. fat intake) may provide a more powerful test of a hypothesis as opposed to the association with the contribution of any one food at a time (especially if the food is only modestly consumed).

As discussed by Willet (1998), employing both nutrients and food (or food groups) to represent diet may be the optimal approach, as each has its own strengths and limitations.

Dimension of Time

Owing to the cross-sectional design of this study, the dimension of time is somewhat lost. When exploring the association between ultra-processed food consumption on a given day and nutrient or food intake, the dimension of time will most likely be unimportant (manuscripts 1 through 3). However, when studying the association between ultra-processed food consumption and phytoestrogen urinary indicators (manuscript 4), the dimension of time may gain importance (more on this is discussed below).

Reverse causality should also not be a problem when exploring the association between ultra-processed food consumption and dietary indicators or urinary phytoestrogens (Manuscripts 1 through 4).

Validity of biochemical indicators (i.e. urine measurements) of diet

Because of the lack of updated and complete phytoestrogen food composition databases we chose to use urinary spot measurements of phytoestrogens as indicators of dietary intake. Although urinary spot measurements may be imprecise measures of dietary intake since they are influenced by differences in absorption and metabolism, short-term biological variation, and laboratory measurement error they offer the benefit that they do not depend on the subjects' knowledge or memory (Willet, 1998). An

alternative may have been to use serum phytoestrogens but these measurements were not available for cycle 2009-2010.

In NHANES 2009-2010 only one dietary intake measurement was performed the day before urinary measurement (and one after) and only one spot urinary measurement was obtained. With these data, to understand the association between ultra-processed food intake and urinary measurements of phytoestrogens, we would need to establish not only how well ultra-processed food intake the day before correlates with the spot urine measurement but, also, how well does ultra-processed food consumption the day prior to urine measurement reflect diet during the period of most influence on these urinary indicators. Considering that phytoestrogens are excreted in the urine within 24 h after exposure (Rowland et al. 2003), we may assume that diet during the previous 24 hours corresponds to the period of most influence on these indicators.

Usual intake

The most serious limitation of the 24-hour-recall method is that dietary intake highly varies from day to day, so the estimated intake on that given day may not represent the usual intake, especially for some nutrients or foods (Willet, 1998). For episodically-consumed foods, usual food intake may be more appropriate to evaluate compliance with food-based dietary recommendations and to relate food intake to health outcomes (Tooze et al. 2006).

In this thesis, we have focused on the study of the association between ultra-processed food consumption on a given day (or on the mean of two given days) and different dietary indicators. Even though ultra-processed foods are not episodically-consumed foods, it would be interesting to explore these same associations using usual ultra-processed food consumption instead of on a given day.

On the other hand, depending on how well ultra-processed food intake data on the given day represent diet during the period of most influence on phytoestrogen urinary indicators, it may be more appropriate to estimate the association between usual ultra-processed food consumption and urinary phytoestrogens.

Recall and information bias

As noted by Willet (1998), dietary data obtained through 24-hour recalls are subjected to recall biases as subjects erroneously recall foods that were not eaten and omit foods that were eaten. Standardized methods and approach used by NHANES should minimize this sort of non-differential recall bias. On the other hand, if people conceive ultra-processed foods as unhealthy, they might underreport their consumption leading, in turn, to an underestimation of ultra-processed food consumption (differential bias). This sort of information bias may less likely affect the association between ultra-processed food consumption and dietary quality, added sugars and protein content but may dilute the association between ultra-processed food consumption and phyto-estrogen urinary levels.

Food processing classification

As NHANES was not designed to estimate ultra-processed food consumption, limited information indicative of food processing (i.e. place of meals, product brands) is collected, and these data are not consistently determined for all food items what may originate errors in food groups classification. Also, as some authors have highlighted, the number of food items reported in NHANES is smaller than the number available in the marketplace, and national food composition data are not updated as required to include all brand-specific products and to examine dietary profiles sensitive to brand preferences (Slining et al., 2015). All these issues may lead to either under- or over-estimation of ultra-processed food consumption. Future studies should consider performing sensitivity analysis after excluding food items with an uncertain NOVA classification or after classifying these uncertain food items under their alternative group classifications.

Residual confounding

All analyses were adjusted for age, gender, race/ethnicity, education and income. In order to control for variation in total energy intake as a potential confounder, we chose to use as our main risk factor, the energy contribution of ultra-processed foods rather

than the absolute ultra-processed food energy intakes. Also, nutrient contributions and densities rather than absolute values were used as outcomes.

Also, when assessing the association between ultra-processed food consumption and total energy intake (in manuscript 3) we adjusted for energy intake requirements in order to control for energy intake variations secondary to differences in body size and physical activity (Willet, 1998).

Any residual confounding, although unlikely, is expected to be partial and not total.

Comparison between models

It must be noted that analyses carried throughout the thesis were not always performed on the same study samples (different age groups, full sample or subsample, under fasting conditions or not, using day 1 dietary intake or both day 1 and day 2 dietary intake data when available) so results from different models may not always be directly comparable.

Between-person variation in dietary intake

In order to study the association between ultra-processed food consumption and outcomes, we need not only a precise method to estimate dietary intake but an adequate amount of variation in diet and ultra-processed food contribution; otherwise, no association will be observed (Willet, 1998). According to Willet (1998), despite the fact that some authors have argued that diets in the US are too homogeneous to allow the study of relations with diet, evidence exists of sufficient variation to do so (Willet, 1998). Still, findings must be interpreted in the context of that variation. In our study, 60% of the population consumed between 40 and 70% of ultra-processed foods (total range from 0 to 100%), apparently providing sufficient variation to test associations. In fact, we chose to use ultra-processed energy intake contributions as our main risk factor rather than gram contributions because a variable based on the latter would probably capture less consumption variation because ultra-processed foods are energy dense by definition.

4.2 Exploring associations between ultra-processed food consumption and two of Cordain's additional dietary indicators

Acid-base balance

In order to test whether an increased consumption of ultra-processed foods yields a net acid dietary balance, bicarbonate in plasma (mmol/L) could be used (in the absence of potential net acid loads of NHANES Food Codes). In NHANES, bicarbonate measurements (measured in participants aged 12+ years in NHANES) together with pH determination, are used in the diagnosis and treatment of numerous potentially serious disorders associated with acid-base unbalance in the respiratory and metabolic systems. We must bear in mind, however, that a lack in association may not necessarily mean that diets rich in ultra-processed foods are not net acid-producing. Indeed, bicarbonate measurement in plasma may not be the best indicator of dietary acid-base balance as a healthy body uses all sorts of mechanisms in order to buffer changes in bicarbonate in plasma, triggered by an overload of acid foods, making any sort of association unlikely. Still, some studies have shown that contemporary diets lead to blood acidity increase and plasma bicarbonate concentration decrease (Kurtz et al., 1983; Frassetto et al., 1996; Welch et al., 2008). Urine ph may be an alternative indicator, but, unfortunately, NHANES did not collect urine PH measurements in cycle 2009-2010.

Glycemic load

In the absence of glycemic loads for each of the different NHANES food items, we may explored instead the association between ultra-processed food consumption and each of the four following blood indicators used in NHANES to assess Diabetes Mellitus: fasting serum insulin and plasma glucose concentrations; two-hour glucose concentration and glycohemoglobin. Indeed, according to Cordain et al. (2005), high glycemic loads lead to acute elevation in blood glucose concentrations and, in turn, acute rise in blood insulin concentrations. Long term consumption of high glycemic load carbohydrates may induce chronic hyperglycemia and hyperinsulinemia and promote, in turn, insulin resistance (Liu & Willet, 2002; Ludwig, 2002; Cordain et al., 2003).

Nevertheless, all caution is not enough when using blood measurements as dietary indicators, as many other factors may have an impact on these variables. Also, if it is long term ultra-processed food consumption that leads to acidosis or, chronic hyperglycemia and hyperinsulinemia and insulin resistance, it may make more sense to study these associations using a cohort study or at least usual ultra-processed food consumption rather than consumption on a given day (or any two given days). Also, any sort of information bias may dilute the association between ultra-processed food consumption and any of these blood measurements. In order to fully understand the association between diet and biochemical indicators, we need to establish not only how diet during the previous 24 hours correlates with these indicators but, also, how diet during the previous 24 hours reflects diet during the period of most influence on these blood indicators. For nutrients whose blood levels substantially fluctuate over time, one blood measurement may not reliably reflect long-term intake (Willet, 1998). When exploring the association with blood measurement outcomes, tighter inclusion and exclusion criteria, more rigorous adjustment for potential confounders and effect-modifiers, will be needed. We must also take into account that results may be distorted by reverse causality if individuals change their diet as a consequence of their condition (i.e. acidosis, hyperinsulinemia or hyperglycemia).

4.3 Exploring associations between ultra-processed food consumption and short- and long-term outcomes ascribable to dietary indicator unbalances

Once the effect of ultra-processed food consumption on Cordain's dietary indicators are confirmed, the next step would be to explore the association with short- or long-term health-related outcomes ascribable to those dietary indicator unbalances and which, non-coincidentally, present a low prevalence in non-westernized populations. Some of these outcomes are briefly discussed below.

Cardiovascular, diabetes and obesity-related outcomes (Diseases of insulin resistance)

Association between ultra-processed food consumption and all the following conditions should be explored as they are rare or absent in hunter-gatherer and less westernized societies living in a traditional manner (Eaton et al., 1988; Cordain et al., 2002;

Schaeffer, 1971; Trowell, 1980): blood pressure, including diastolic blood pressure and hypertension (Morris et al.,1999; Sharma et al., 1990); blood lipid profiles (O'Dea, 1984; O'Dea et al., 1989; Wolfe & Giovannetti, 1991; Wolfe & Giovannetti, 1992; Wolfe & Piche, 1999), including total, HDL and LDL cholesterol and serum triglycerides; serum insulin, leptin levels, caloric intake and appetite (Anderson et al., 1994); chronic serum hyperglycemia and hyperinsulinemia and insulin resistance (Liu & Willet, 2002; Ludwig, 2002; Cordain et al., 2003); metabolic syndrome (Reaven, 1995); cardiovascular disease (including stroke, ischaemic heart disease and congestive heart failure) (Hu et al., 1999); type-2 diabetes and obesity (also weight gain and excess body weight) (Spreadbury, 2012).

Muscular, skeletal and dental- health related outcomes

Evidence exists that the transition to agriculture was accompanied by a decrease in stature and bone mineral disorders, and an increase in tooth decay and dental caries, dental crowding, malocclusion and underdevelopment of dental arches (Cordain, 1999; Lieberman et al., 2004; von Cramon-Taubadel, 2011). While tooth decay can be attributed to a high intake of added sugars (Touger-Decker & van Loveren, 2003) the lack of chewing and mastication can lead to underdevelopment of dental arches, malocclusion and crowded teeth (Lieberman et al., 2004; von Cramon-Taubadel, 2011). Based on this evidence, assessing the association between ultra-processed food consumption and bone and dental- health related outcomes such as dental caries and tooth decay, dental crowding and malocclusion and underdevelopment of dental arches seems important.

It may be interesting to explore outcomes such as osteoporosis shown to be associated with acid-yielding diets (Sebastian et al., 1994; Bushinsky, 1996), high sodium-potassium ratio (Devine et al., 1995), and low phytoestrogen intake (Sirotkin & Harrath, 2014; Paterni et al., 2016). Largely prevalent osteopenia (Siris et al., 2001), hip or bone fractures and age-related muscle wasting (Frassetto et al., 1997) would be additional outcomes to be explored.

Renal diseases

Assessing the association between ultra-processed food consumption and both calcium kidney stones and progression of age- and disease-related chronic renal insufficiency seems relevant, as calcium kidney stones have been associated with both net-acid yielding diets (Pak et al., 1985; Preminger et al., 1985) and high sodium-potassium ratio (Massey & Whiting, 1995), while progression of age- and disease-related chronic renal insufficiency have been associated with net-acid diets (Alpern & Sakhaee, 1997).

Association with urine osmolality (mmol/Kg), a measure of the amount of solute particles contained in urine that is used as an indicator of hydration status or impaired renal function, can also be assessed.

Gastrointestinal diseases

The association with chronic, low-grade pathogenic metabolic acidosis which may be elicited by a net-acid diet (Frassetto et al., 1996; Frassetto et al., 1998) or with gastrointestinal tract cancer attributed to a high sodium-potassium ratio (Jansson, 1986; Tuyns, 1988) should be explored. Risks of constipation, appendicitis, hemorrhoids, deep vein thrombosis, varicose veins, diverticulitis, hiatal hernia, and gastroesophageal reflux (Trowell, 1985) attributed to low fiber intake should also be assessed.

Respiratory diseases

The association with exercise-induced asthma (Mickleborough et al., 2001), attributed both to net-acid diets (Mickleborough et al., 2001) and to a high sodium-potassium ratio (Gotshall et al., 2000), and with asthma, triggered by high sodium-potassium ratio (Carey et al., 1993), should also be evaluated.

Systemic chronic inflammation

Evidence exists that consumption of Western diets may lead to a suboptimal gene expression with an upregulation of genes involved in pro-inflammatory (Bouchard-Mercier et al., 2013; Myles, 2014; Ruiz-Nunez et al., 2013) and/ or immune responses, cancer and cardiovascular diseases (Bouchard-Mercier et al., 2013). Some authors have suggested that many of the typically Western chronic diseases may be traced

back to the impact of diet on the immune system (Myles, 2014). Indeed, high intake of saturated and trans fatty acids, a high omega-6/omega-3 ratio, a low status of vitamin D and certain other micronutrients, the low intake of dietary fiber, fruits, and vegetables, and the consumption of carbohydrates with a high glycemic index, have been shown to collectively cause systemic chronic inflammation (Galland, 2010; Myles, 2014; Ruiz-Nunez et al., 2013) which is also a common denominator of most chronic Western diseases (Ruiz-Nunez et al., 2013). Based on this evidence studying the association between ultra-processed food consumption and pro-inflammatory compounds such as interleukin-6 or C-reactive protein (or even rheumatoid arthritis) would also be important.

Human microbiome

While the effects of Western diets on the expression of genes in our eukaryotic human cells have been known for some time, the effects on the human microbiome are only now becoming clear (Hold, 2014; Voreades et al., 2014). Recent studies have shown that western diets may induce gut dysbiosis, impairment of intestinal barrier function, and increased intestinal permeability of bacterial endotoxins (Brown et al., 2012; Martinez-Medina et al., 2014). For example, the consumption of highly processed foods, as opposed to the minimally processed reduces the intake of various food-associated microbes, some of which may be beneficial to human health (Alcock et al., 2014; Leff & Fierer, 2013; Rook, 2010). Gut dysbiosis have been associated with many diseases including obesity, type-2 diabetes, and cardiovascular disease (Fujimura et al., 2010; Guinane & Cotter, 2013). Also, recent studies have shown that gut microbiota may impact our dietary preferences and appetite and that a consumption of a highly processed diet may start a vicious cycle in which certain foods may promote the growth of a specific set of microbes that in turn may increase the desire for these foods (Alcock et al. 2014; Norris et al. 2013). Based on this evidence, the association between ultra-processed food consumption and gut microbiota should also be explored. This may have to be done through intervention studies.

Other health outcomes

The association with other health outcomes such as insomnia (Miller, 1945), air sickness (Lindseth & Lindseth, 1995), high-altitude sickness (Porcelli & Gugelchuk, 1995), and Meniere's Syndrome (ear ringing) (Thai-Van et al., 2001) might also be explored.

Alternative study designs

In order to study the association between ultra-processed food consumption and these additional outcomes, the dimension of time becomes relevant. We would need to obtain the date of onset of the outcomes and establish the critical period prior to diagnosis (and duration of exposure) for which ultra-processed food consumption is relevant (i.e. during childhood, intake up to just before the diagnosis), in order to decide how to estimate consumption at that point in time. According to Willet (1998), eating patterns evolve over periods of years, so individuals' diets tend to be correlated from year to year, with a reasonable reproducibility during the period of 1 to 10 years and with lower reproducibility over longer intervals.

Some nutrient and food intakes are characterized by a marked day to day variation. If this should be the case for ultra-processed food consumption, assessing current long-term ultra-processed average intake would be more appropriate than intake over a small number of days, even though this will depend on how well current average ultra-processed food consumption correlates with intake at the critical period prior to the onset of the outcome.

When exploring the association with these outcomes, tighter inclusion and exclusion criteria will be needed, more rigorous adjustment for potential confounders and effect-modifiers, and possibly alternative study designs such as cohort studies to discard reverse causality. Results may be distorted by reverse causality if individuals change their diet as a consequence of their outcome/condition (i.e. acidosis, hyperinsulinemia or hyperglycemia).

Cross-sectional studies (which guarantee the maximum external validity if population based) can be used to study some of these short-term outcomes. More long-term

outcomes can also be studied through this type of study design as has been done in the past with obesity (Louzada et al., 2015c) and metabolic syndrome (Tavares et al., 2012). In this case, reverse causality may disguise the association if for example obese people decrease the consumption of ultra-processed foods as a consequence of their condition.

Cohort studies may be used to study long-term outcomes prone to reverse causality, as has been done in a study in Spain which found a positive association between ultra-processed food consumption and the incidence of overweight and obesity (Mendonça et al., 2016). Because dietary habits may change though out the course of life, we also need to understand if the effects of ultra-processed food consumption depend on the age these started to be consumed, and the total time of exposure. More short-term outcomes may also be studied as was done in a study in Brasil finding that ultra-processed product consumption plays a role in altering lipoprotein profiles in children (Rauber et al., 2015).

In order to fully control for residual confounding, experimental studies would need to be carried out, including normo- weight individuals (probably volunteers) exposed to diets with different dietary shares of ultra-processed foods. For ethical reasons, these studies should be of short duration, restricting the options to short-term outcomes such as serum triglycerides, cholesterol, glucose or insulin; blood pressure; or urine sodium or gut microbiota.

Regular sun exposure, sleeping pattern synchronization with the natural variation in light and dark, levels of chronic stress, regular physical activity and exposure to pollutants are some alternative risk factors which may act as confounders or effect-modifiers. Still, diet is considered to be one of the main risk factors (Cordain et al., 2005) as supported by recent clinical trials (Jonsson et al., 2009; Jonsson et al., 2010; Lindeberg et al., 2007; Mellberg et al., 2014).

4.4 Final conclusions

In conclusion, decreasing the consumption of ultra-processed foods could be an effective way to substantially improve the dietary quality in the US by increasing protein, fiber, vitamins A, C, D and E, zinc, potassium, phosphorus, magnesium, calcium and lignan contents, while also reducing carbohydrate, saturated fat and added sugars intake. Being thus, this thesis confirms the association between ultra-processed food consumption and most of Cordain's dietary indicators. Finally, this research also proves that the consumption of ultra-processed foods is an important determinant of the overall nutritional quality of contemporary diets.

This thesis also suggests that NOVA classification may be used in the design of policies, interventions and guidelines aimed at promoting healthy eating and prevention of chronic non-communicable diseases instead of the frequently used long list of foods and complicated recommendations, not necessarily supported by scientific evidence. Furthermore, this thesis proves that the Brazilian Dietary Guidelines based on the NOVA classification, are on the right track when they recommend limiting ultra-processed food consumption (among others) as a way to promote healthier diets.

4.5 References

1. Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. Bioessays 2014, 36(10), 940-949. doi: 10.1002/bies.201400071.
2. Alpern RJ, Sakhaee S. The clinical spectrum of chronic metabolic acidosis: homeostatic mechanisms produce significant morbidity. AmJ Kidney Dis 1997; 29: 291–302.
3. Anderson JW, Smith BM, Gustafson NJ. Health benefits and practical aspects of high-fiber diets. Am J Clin Nutr 1994; 59(suppl):1242S–7S.
4. Bouchard-Mercier A, Paradis AM, Rudkowska I, Lemieux S, Couture P, Vohl MC. Associations between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study. Nutr J. 2013, 12, 24. doi: 10.1186/1475-2891-12-24.
5. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012, 4(8), 1095-1119. doi: 10.3390/nu4081095.
6. Bushinsky DA. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol 1996; 271:F216 –22.
7. Carey OJ, Locke C, Cookson JB. Effect of alterations of dietary sodium on the severity of asthma in men. Thorax 1993; 48:714–8.
8. Cordain L. Cereal grains: humanity's double edged sword. World Rev Nutr Diet 1999; 84:19 –73.
9. Cordain L, Eaton SB, Brand Miller J, Mann N, Hill K. The paradoxical nature of hunter-gatherer diets: meat based, yet non-atherogenic. Eur J Clin Nutr 2002; 56(suppl): S42–52.
10. Cordain L, Eades MR, Eades MD. Hyperinsulinemic diseases of civilization: more than just syndrome X. Comp Biochem Physiol Part A 2003; 136:95–112.
11. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, and Brand-Miller J. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 2005; 81: 341–54.

12. Crovetto M M, Uauy R, Martins AP, Moubarac JC, Monteiro C. Household availability of ready-to-consume food and drink products in Chile: impact on nutritional quality of the diet. Rev Med Chil. 2014 Jul; 142(7): 850-8. doi: 10.4067/S0034-98872014000700005.
13. Devine A, Criddle RA, Dick IM, Kerr DA, Prince RL. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. Am J Clin Nutr 1995; 62:740 –5.
14. Eaton SB, Konner M, Shostak M. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 1988; 84: 739–49.
15. FAO. Guidelines on the collection of information on food processing through food consumption surveys. FAO, Rome, 2015.
16. Frassetto L, Morris RC, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. Am J Physiol 1996; 271:1114 –22.
17. Frassetto L, Morris RC Jr, Sebastian A. Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. J Clin Endocrinol Metab 1997; 82: 254 –9.
18. Frassetto LA, Todd KM, Morris RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 1998; 68:576–83.
19. Fujimura KE, Slusher NA, Cabana MD, Lynch SV. Role of the gut microbiota in defining human health. Expert Rev Anti Infect Ther 2010, 8(4), 435-454. doi: 10.1586/eri.10.14.
20. Galland L. Diet and inflammation. Nutr Clin Pract. 2010, 25(6), 634-640. doi: 10.1177/0884533610385703.
21. Gotshall RW, Mickleborough TD, Cordain L. Dietary salt restriction improves pulmonary function in exercise-induced asthma. Med Sci Sports Exerc 2000; 32:1815–9.

22. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol 2013, 6(4), 295-308. doi: 10.1177/1756283x13482996.
23. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999; 70:221–7.
24. Jansson B. Geographic cancer risk and intracellular potassium/sodium ratios. Cancer Detect Prev 1986; 9:171–94.
25. Jonsson T, Granfeldt Y, Ahren B, Branell UC, Palsson G, Hansson A, Söderström M, Lindeberg S. Beneficial effects of a Paleolithic diet on cardiovascular risk factors in type 2 diabetes: a randomized cross-over pilot study. Cardiovasc Diabetol. 2009, 8, 35. doi: 10.1186/1475-2840-8-35.
26. Jonsson T, Granfeldt Y, Erlanson-Albertsson C, Ahren B, Lindeberg S. A paleolithic diet is more satiating per calorie than a mediterranean-like diet in individuals with ischemic heart disease. Nutr Metab (Lond) 2010, 7, 85. doi: 10.1186/1743-7075-7-85.
27. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A. Effect of diet on plasma acid-base composition in normal humans. Kidney Int 1983; 24:670–80.
28. Leff JW, Fierer N. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS One 2013, 8(3), e59310. doi: 10.1371/journal.pone.0059310.
29. Lieberman DE, Krovitz GE, Yates FW, Devlin M, St Claire M. Effects of food processing on masticatory strain and craniofacial growth in a retrognathic face. J Hum Evol. 2004 Jun; 46(6):655-77.
30. Lindeberg S, Jonsson T, Granfeldt Y, Borgstrand E, Soffman J, Sjostrom K, Ahren B. A Palaeolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischaemic heart disease. Diabetologia 2007, 50(9), 1795-1807. doi: 10.1007/s00125-007-0716-y.
31. Lindseth G, Lindseth PD. The relationship of diet to airsickness. Aviat Space Environ Med 1995; 66:537– 41.

32. Liu S, Willett WC. Dietary glycemic load and atherothrombotic risk. Curr Atheroscler Rep 2002; 4:454–61.
33. Louzada ML, Baraldi LG, Steele EM, Martins AP, Canella DS, Moubarac JC, Levy RB, Cannon G, Afshin A, Imamura F, Mozaffarian D, Monteiro CA. Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults. Prev Med. 2015c, 81, 9-15. doi:10.1016/j.ypmed.2015.07.018.
34. Ludwig DS. The glycemic index: physiological mechanisms relating obesity, diabetes, and cardiovascular disease. JAMA 2002; 287: 2414 –23.
35. Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R, Darfeuille-Michaud A, Barnich N. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. Gut 2014, 63(1), 116-124. doi: 10.1136/gutjnl-2012-304119.
36. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and kidney stone risk. Nutr Rev 1995; 53:131–9.
37. Mellberg C, Sandberg S, Ryberg M, Eriksson M, Brage S, Larsson C, . . . Lindahl B. Long-term effects of a Palaeolithic-type diet in obese postmenopausal women: a 2-year randomized trial. Eur J Clin Nutr 2014, 68(3), 350-357. doi: 10.1038/ejcn.2013.290.
38. Mendonca RD, Pimenta AM, Gea A, de la Fuente-Arrillaga C, Martinez-Gonzalez MA, Souza Lopes AC, Bes-Rastrollo M. Ultraprocessed food consumption and risk of overweight and obesity: the University of Navarra Follow-Up (SUN) cohort study. Am J Clin Nutr. 2016 Nov; 104(5):1433-1440. Epub 2016 Oct 12.
39. Mickleborough TD, Gotshall RW, Kluka EM, Miller CW, Cordain L. Dietary chloride as a possible determinant of the severity of exerciseinduced asthma. Eur J Appl Physiol 2001; 85:450–6.
40. Miller MM. Low sodium chloride intake in the treatment of insomnia and tension states. JAMA 1945; 129:262– 6.
41. Monteiro CA, Levy RB, Claro RM, de Castro IR, Cannon G. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. Public Health Nutr. 2010b; 14(1):5-13. doi:10.1017/S1368980010003241.

42. Morris RC Jr, Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 1999; 33:18 –23.
43. Moubarac J-C, Martins APB, Claro RM, Levy RB, Cannon G, Monteiro CA. Consumption of ultra-processed foods and likely impact on human health. Evidence from Canada. Public Health Nutr, 2012; doi: 10.1017/S1368980012005009.
44. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. Nutr J. 2014; 13, 61. doi: 10.1186/1475-2891-13-61.
45. Norris V, Molina F, Gewirtz AT. Hypothesis: bacteria control host appetites. J Bacteriol 2013, 195(3), 411-416. doi: 10.1128/jb.01384-12.
46. O'Dea K. Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to traditional lifestyle. Diabetes 1984; 33:596–603.
47. O'Dea K, Traianedes K, Ireland P, Niall M, Sadler J, Hopper J, De Luise M. The effects of diet differing in fat, carbohydrate, and fiber on carbohydrate and lipid metabolism in type II diabetes. J Am Diet Assoc. 1989; 89:1076–86.
48. Pak CY, Fuller C, Sakhaee K, Preminger GM, Britton F. Long-term treatment of calcium nephrolithiasis with potassium citrate. J Urol 1985; 134:11–9.
49. Paterni I, Granchi C, Minutolo F. Risks and Benefits Related to Alimentary Exposure to Xenoestrogens. Critical Reviews in Food Science and Nutrition 2016. DOI: 10.1080/10408398.2015.1126547
50. Porcelli MJ, Gugelchuk GM. A trek to the top: a review of acute mountain sickness. J Am Osteopath Assoc 1995; 95:718 –20.
51. Preminger GM, Sakhaee K, Skurla C, Pak CY. Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. J Urol 1985; 134: 20 –3.
52. Rauber F, Campagnolo PD, Hoffman DJ, Vitolo MR. Consumption of ultra-processed food products and its effects on children's lipid profiles: a longitudinal study. Nutr Metab Cardiovasc Dis. 2015; 25(1), 116-122. doi:10.1016/j.numecd.2014.08.001.

53. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75:473– 86.
54. Rook G A. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin Exp Immunol 2010, 160(1), 70-79. doi: 10.1111/j.1365-2249.2010.04133.x.
55. Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, Cassidy A. Bioavailability of phyto-oestrogens. Br J Nutr 2003; 89:S45–S58
56. Ruiz-Nunez B, Pruimboom L, Dijck-Brouwer DA, Muskiet FA. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. J Nutr Biochem. 2013; 24(7), 1183-1201. doi: 10.1016/j.jnutbio.2013.02.009.
57. Schaeffer O. When the Eskimo comes to town. Nutr Today 1971; 6:8 –16.
58. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris RC Jr. Improved mineral balance and skeletal metabolism in post-menopausal women treated with potassium bicarbonate. N Engl J Med 1994; 330: 1776–81.
59. Sharma AM, Kribben A, Schattenfroh S, Cetto C, Distler A. Salt sensitivity in humans is associated with abnormal acid-base regulation. Hypertension 1990; 16:407–13.
60. Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Berger ML, Santora AC, Sherwood LM. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women. Results from the National Osteoporosis Risk Assessment. JAMA 2001; 286:2815–22.
61. Sirotkin AV, Harrath AH. Phytoestrogens and their effects. Eur J Pharmacol. 2014 Oct 15; 741:230-6. doi: 10.1016/j.ejphar.2014.07.057.
62. Slining MM, Yoon EF, Davis J, Hollingsworth B, Miles D and Ng SW. An Approach to Monitor Food and Nutrition from “Factory to Fork”. Journal of the Academy of Nutrition and Dietetics 2015, 115(1): 40-49.
63. Spreadbury I. Comparison with ancestral diets suggests dense acellular carbohydrates promote an inflammatory microbiota, and may be the primary dietary

cause of leptin resistance and obesity. Diabetes Metab Syndr Obes. 2012; 5, 175-189. doi: 10.2147/dmso.s33473.

64. Tavares LF, Fonseca SC, Garcia Rosa ML, Yokoo EM. Relationship between ultra-processed foods and metabolic syndrome in adolescents from a Brazilian Family Doctor Program. Public Health Nutr 2012, 15(1), 82-87. doi:10.1017/S1368980011001571.
65. Thai-Van H, Bounaix MJ, Fraysse B. Meniere's disease: pathophysiology and treatment. Drugs 2001; 61:1089 –102.
66. Tooze JA, Midthune D, Dodd KW, Freedman LS, Krebs-Smith SM, Subar AF, Guenther PM, Carroll RJ, Kipnis V. A new method for estimating the usual intake of episodically consumed foods with application to their distribution. J Am Diet Assoc. 2006 October ; 106(10): 1575–1587.
67. Touger-Decker R, van Loveren C. Sugars and dental caries. Am J Clin Nutr, 78(4), 881s-892s, 2003.
68. Tuyns AJ. Salt and gastrointestinal cancer. Nutr Cancer 1988; 11: 229 –32.
69. Trowell H. Dietary fiber: a paradigm. In: Trowell H, Burkitt D, Heaton K, Doll R, eds. Dietary fibre, fibre-depleted foods and disease. New York: Academic Press, 1985:1–20.
70. Trowell HC. From normotension to hypertension in Kenyans and Ugandans 1928–1978. East Afr Med J 1980; 57:167–73.
71. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at http://health.gov/dietaryguidelines/2015/guidelines/.
72. Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications. Washington D.C.: Panamerican Health Organization, 2015.
73. Vandevijvere S, Monteiro C, Krebs-Smith SM, Lee A, Swinburn B, Kelly B, Neal B, Snowdon W, Sacks G; INFORMAS. Monitoring and benchmarking population diet quality globally: a step-wise approach. Obes Rev. 2013 Oct; 14 Suppl 1:135-49. doi: 10.1111/obr.12082.

74. von Cramon-Taubadel N. Global human mandibular variation reflects differences in agricultural and hunter-gatherer subsistence strategies. Proc Natl Acad Sci USA 2011; 108(49), 19546-19551. doi: 10.1073/pnas.1113050108.
75. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol. 2014, 5, 494. doi: 10.3389/fmicb.2014.00494.
76. Welch AA, Mulligan A, Bingham SA, Khaw KT. Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. Br J Nutr. 2008 Jun; 99(6):1335-43. Epub 2007 Nov 28.
77. Willet WC. Nutritional Epidemiology. In: Rothman KJ & Greenland S. Modern Epidemiology. Second Edition. Lippincott Williams & Wilkins, US, 1998.
78. Wolfe BM, Giovannetti PM. High protein diet complements resin therapy of familial hypercholesterolemia. Clin Invest Med 1992; 15: 349 –59.
79. Wolfe BM, Giovannetti PM. Short term effects of substituting protein for carbohydrate in the diets of moderately hypercholesterolemic human subjects. Metabolism 1991; 40:338–43.
80. Wolfe BM, Piche LA. Replacement of carbohydrate by protein in a conventional-fat diet reduces cholesterol and triglyceride concentrations in healthy normolipidemic subjects. Clin Invest Med 1999; 22: 140–8.

5. APPENDIXES

APPENDIX 1. Protein Leverage Hypothesis

As outlined in the main text, Raubenheimer and Simpson proposed the Protein Leverage Hypothesis to explain why a drop in protein dietary content might lead to obesity and associated cardiometabolic disease (OACD) (Raubenheimer & Simpson, 2016).

The Protein Leverage Hypothesis suggests that the strong human appetite for protein together with a low food protein density is the main driver of the high fats and carbohydrate, and in turn, total energy intakes (Simpson & Raubenheimer, 2005).

Raubenheimer and Simpson built their hypothesis based on evidences from difference species and using a different and more integrative analytical approach: the geometric framework (Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993b). The objective of this framework is to understand how the animal relates to the environment through nutrition. In order to be integrative it needs to take into account the animal, the environment, and the basis for the interaction between both (Raubenheimer et al., 2009). Several concepts are used to build up the geometric framework some of which are: *nutrient space*, *intake target*, *nutrient balance*, and *rule of balance* (Simpson & Raubenheimer, 2012). The *nutrient space* is a geometric space built of two or more axes in which each axis represents a food component (i.e. macronutrient) suspected to play a role in the animal's responses to the environment (Simpson & Raubenheimer, 2012). In the geometric framework, the optimal nutrient requirement of an animal is represented as a point in the nutrient space, called *intake target* (Simpson & Raubenheimer, 2012). The diet of an animal may comprise one or several foods and these may be modeled in the nutrient space according to the absolute amount or the balance of nutrients. The lines that represent the balance of nutrients in foods are called *nutritional rails* (Simpson & Raubenheimer, 2012). The animal's challenge when feeding is to choose foods which direct them to their intake target and to eat the correct amount to arrive there. The most obvious way to arrive at the intake target would be by selecting a food containing the same nutrient balance as needed (i.e. if the intake target were 4 g protein: 2 g carbohydrate, a direct way to achieve this target would be to eat 6 grams of

a nutritionally balanced food -nutrient balance 2 g protein: 1 g carbohydrate-). Alternatively, an animal may reach the intake target, indirectly, by feeding on several nutritionally imbalanced but complementary foods. For example, the animal may feed on 8 grams of food *a* (3 g protein: 1 g carbohydrate) and 4 grams of food *b* (1 g protein: 1 g carbohydrate) in order to reach the intake target of 8 g protein: 4 carbohydrate. If the animal should feed on nutritionally imbalanced foods which are non-complementary, it will never reach its intake target [i.e. by feeding on food *a* (3 g protein: 1 g carbohydrate) and food *c* (1 g protein: 8 g carbohydrate) it will never attain the intake target of 4 g protein: 2 carbohydrate]. In this case, the animal will presumably follow a *rule of compromise* (Simpson & Raubenheimer, 2012) between over-eating some nutrients and under-eating others. In order to find out what the rule of compromise is for a certain animal, we would have to measure and plot in the nutrient space (intake array) the response to the exposure to different types of diets (each one with a different nutrient balance). The rule of compromise each animal adopts will depend on the capacity of each species to deal with nutrient shortages and excesses (i.e. capacity to excrete or store the excesses of each nutrient). The *nutrient target* is described as the balance of nutrients that tissues need in order to carry out all their functions (i.e. growth, metabolism…). Due to the constrained nutrient losses (through feces, urine…), the intake target will always be greater than the nutrient target (Simpson & Raubenheimer, 2012).

Prior to feeding, the animal needs to assess both the composition of available foods and its nutritional requirements (Simpson & Raubenheimer, 2012). The presence in food of some nutrients may be detected through taste organs located in the mouth or along the gastrointestinal tract or by associating the features of the food with the consequences of eating it (learning process) (Simpson & Raubenheimer, 2012). The nutritional state, on the other hand, may be assessed through systemic nutrient-sensing mechanisms or hormonal feedbacks from body reserves (Simpson & Raubenheimer, 2012). The animal must then integrate the information about food composition and nutritional status which can be done both at the periphery (nutrient- specific modulation of taste receptors) or centrally, as signals converge onto neural circuits that control feeding behavior

(Simpson & Raubenheimer, 2012). Rebalancing of nutrient intake may occur after ingestion, thanks to the structural and functional plasticity of the gastrointestinal tract in response to the nutritional state (Simpson & Raubenheimer, 2012). Finally, a further rebalancing may take place after ingesting and absorbing nutrients across the gut, by voiding nutrient excesses and conserving nutrient in limited supply (Simpson & Raubenheimer, 2012).

Simpson and Raubenheimer argue that to understand how human nutrition is regulated three core questions must be answered: whether humans regulate different macronutrients to an intake target; what is the rule of compromise for humans; how do humans deal with nutrient excesses (Simpson & Raubenheimer, 2012).

Human intake target

Even though no properly controlled geometric experiments have been published on humans (Stubbs, 1998; Friedman, 2000; Berthoud & Seeley, 2000), according to Simpson and Raubenheimer the following evidence suggests that humans can regulate some nutrient intakes: data from rodents, studies on human macronutrient appetite and human population- level data (Simpson & Raubenheimer, 2012). Rodents, which are widely used as models for human nutritional physiology because they are both mammals and broad-scale food generalists like humans, have the capacity to regulate their protein and carbohydrate intakes (Simpson & Raubenheimer, 1997; Simpson & Raubenheimer, 2012). Indeed, this was confirmed after replotting data collected by Theall et al. (1984) on rats which had been provided one of eight different complementary food pairings, and all converged on the same protein and carb intake (Simpson & Raubenheimer, 2012). This was also confirmed in mice after conducting a full geometric analysis of protein and carbs regulation (Sorensen et al., 2008; Simpson & Raubenheimer, 2012).

Other studies carried out in humans have provided evidence that humans are able to regulate macronutrient intake, especially protein (Berthoud & Seeley, 2000; see Simpson et al. 2003; Simpson & Raubenheimer, 2012), and that macronutrient-specific feedbacks operate over a period of 1-2 days. Humans apparently also have the capacity

to associate foods with consequences of eating them (de Castro, 1999; Weigle et al., 2005; Simpson & Raubenheimer, 2012).

Studies across different populations and time seem to show that the proportion of dietary protein remains fairly constant, around 15% of total energy, whereas fat and carbs vary (Westerterp-Plantenga, 1994). The absolute protein intake is also consistent across some populations at least (Simpson & Raubenheimer, 2012). For example, according to UK nutrient supply FAOSTAT data (2010) between 1961 and 2000 protein, carbs and fat intakes have remained fairly constant despite perturbations in the nutritional environment. Simpson & Raubenheimer discuss that this is because compensatory changes took place to counterbalance the perturbations. For example, the drop in animal fat consumption in the 1980's was compensated by a rise in the vegetable fat intake, or the declining consumption of beef, pork and lamb was compensated by an increase in poultry consumption (Simpson & Raubenheimer, 2012). S & R discuss that the regulation of macronutrient intake is not always perfect, as in the US between 1961 and 2000 where protein intake rose less than carbs and fat intake resulting in a drop in protein content from 14% to 12.5% (FAOSTAT, 2010). This drop in protein content caused by increases in fat and carb intake has been confirmed in data from NHANES (Austin et al., 2011; Fulgoni, 2008; Swinburn et al., 2009).

Human rule of compromise

Simpson and Raubenheimer have used the Geometric framework to explore the human rule of compromise (Simpson et al., 2003). They performed an initial experiment with 10 volunteers housed together during 6 days in the Swiss Alps in which everything they ate was weighed. For the 2 first days they selected all meals from a buffet with a wide range of macronutrient compositions. For the next 2 days a group of subjects was offered high-protein and low-fat-carb food items while another one was offered low-protein and high-fat-carb food items. For the last 2 days, subjects were offered the same free choice as in the first 2 days. Authors arrived at the conclusion that when subjects were offered a diet on days 3-4 that contained a higher (treatment 1) or lower (treatment 2) ratio of protein/ fat+carbs than self-selected during the first 2 days, they maintained protein

intake at the expense of regulating carb and fat intake. This means that subjects from treatment 1 under ingested carbs + fat to not overeat protein, while subjects from treatment 2 overate carbs + fat to maintain protein intake. The fact that humans seem to prioritize protein intake was termed “protein leverage hypothesis” (Simpson & Raubenheimer, 2005).

One limitation of the previous experiment has to do with the potential confounding of palatability on the association of treatments with different protein contents and actual macronutrient intakes. In order to control for this confounder, an additional study was performed in which the macronutrient composition was disguised (10%, 15% or 25% protein; 30% fat was kept constant; 60%, 55% or 45% carbs adjusted accordingly) (Gosby et al., 2010; Gosby et al., 2011; Simpson & Raubenheimer, 2012). Recruited lean adult subjects spent three 4 –day periods in an apartment eating as much as they wished of foods containing 10%, 15% or 25% protein (i.e. each offered food from the first period contained 10% protein, 30% fat or 60% carbs). As predicted by the protein leverage hypothesis, subjects on a lower protein content diet increased their energy intake over the 4-day period in 12% (the increase was evident from the first day and was mainly due to eating between meals and with a predilection for savory over sweet-tasting meals). Foods were matched between treatment periods regarding palatability, availability, variety, and sensory aspects.

The plotting of 23 separate studies measuring ad libitum intake in diets with different macronutrient compositions for time periods from several days to 12 months confirmed the protein leverage hypothesis, as daily protein intake remained fairly more constant than that of the other 2 macronutrients (Simpson & Raubenheimer, 2012). In consistency with previous studies (Cheng et al., 2008), another finding was that subjects are more willing to over ingest total kcals when on low density protein diets to gain limiting protein, than to limit total energy intake to avoid over ingesting proteins and this. This asymmetry may have to do with the fact that overeating protein is less costly than undereating, even after taking into account the risks of excess protein consumption (Simpson & Raubenheimer, 2012). This is not the case for all species. For example, humans seem to be more willing to overconsume protein to gain limiting non-protein

energy intake than spider monkeys (Felton et al., 2009a) but less than mountain gorillas (Rothman et al., 2011).

According to Simpson and Raubenheimer, data from other omnivorous species, such as rodents, chickens and pigs, and from both herbivorous and omnivorous species, such as locusts and cockroaches, are also consistent with the protein leverage hypothesis (Simpson & Raubenheimer, 2012). Rodents, for example, on a diet with lower protein to carb ratio than the intake target maintained protein intake fairly constant at the expense of overeating carbs. Rodents on a higher protein to carb ratio than the intake target did not overeat protein to reach their carb target Simpson & Raubenheimer, 2012). The extreme example of protein leverage is what happens in spider monkeys in which protein intake is always maintained constant at the expense of free variation of non-protein intake (Felton et al., 2009a).

Simpson and Raubenheimer discuss in their book the implications of the protein leverages (Simpson & Raubenheimer, 2012). In order to do so they discuss 4 scenarios: 1) a shift to a diet containing a higher percentage of carbs and fat; 2) a shift to a diet containing a higher percentage of protein; 3) increase in protein requirements; 4) energy expenditure declines while diet remains unchanged.

We will focus here on the first and fourth scenarios as they seem to be the main problem after the industrial revolution. Nowadays, fat and/or carb- rich foods are more accessible, affordable, in greater variety, or more palatable than other alternatives (Hill et al., 2003, Brooks et al., 2010), trapping people in a suboptimal diet in which maintaining the amount of protein constant leads to the overconsumption of carbs + fat and the subsequent raise in body weight, predisposing people to obesity (Simpson & Raubenheimer, 2012). Reaching the protein target where protein to fat + carb ratio is lower than intake target ratio will depend on the nutrient density or physical bulk of foods (Rolls, 2000). However, modern diets with 24-hour access to food and based on energy-dense packaged and convenience foods will easily achieve the protein target (Simpson & Raubenheimer, 2012). If energy expenditure declines because of a decline in physical activity and thermoregulation, the fat and carb intake target will be lower. To

avoid an increase in weight gain, the solution will be to increase the percentage of dietary protein (Simpson & Raubenheimer, 2012).

Note: The content included under this Appendix 1 is largely based on Stephen J. Simpson and David Raubenheimer's book "The Nature of Nutrition. A unifying framework from animal adaptation to human obesity". Princeton University Press, 2012, USA (Simpson & Raubenheimer, 2012).

References- Appendix 1.

1. Austin GL, Ogden LG, Hill JO. Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal weight, overweight, and obese individuals: 1971–2006. Am J Clin Nutr 2011; 93: 836–843.
2. Berthoud HR & Seeley RJ (eds.). Neural and metabolic control of macronutrient intake. Boca Raton: CRC Press, 2000.
3. Brooks RC, Simpson SJ, Raubenheimer D. The price of protein: combining evolutionary and economic analysis to understand excessive energy consumption. Obes Rev. 2010 Dec; 11(12):887-94. doi: 10.1111/j.1467-789X.2010.00733.x.
4. Cheng K, Simpson SJ, Raubenheimer D. A geometry of regulatory scaling. Am Nat. 2008 Nov; 172(5):681-93. doi: 10.1086/591686.
5. de Castro JM. What are the major correlates of macronutrient selection in Western populations? Proc Nutr Soc. 1999 Nov; 58(4):755-63.
6. FAOSTAT database. 2010. Food Balance Sheets. http://faostat.fao.org/site/368/default.aspx#ancor (accessed 15 September 2011).
7. Felton AM, Felton A, Raubenheimer D, Simpson SJ, Foley WJ, Wood JT, Wallis IR and Lindenmayer DB. Protein content of diets dictates the daily energy intake of a free-ranging primate. Behav. Ecol. 2009; 20: 685-690.
8. Friedman MI. Too many choices? A critical essay on macronutrient selection. In *Neural and metabolic control of macronutrient intake*, ed. H.-R. Berthoud and R.J. Seeley, 11-18. Boca Raton: CRC Press, 2000.
9. Fulgoni VL 3rd. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003-2004. Am J Clin Nutr. 2008 May; 87(5):1554S-1557S.
10. Gosby AK, Soares-Wynter S, Campbell C, Badaloo A, Antonelli M, Hall RM, Martinez-Cordero C, Jebb SA, Brand-Miller J, Caterson ID, Conigrave AD, Forrester TG, Raubenheimer D, Simpson SJ. Design and testing of foods differing in protein to energy ratios. Appetite. 2010 Oct; 55(2):367-70. doi: 10.1016/j.appet.2010.06.009. Epub 2010 Jun 19.

11. Gosby AK, Conigrave AD, Lau NS, Iglesias MA, Hall RM, Jebb SA, Brand-Miller J, Caterson ID, Raubenheimer D, Simpson SJ. Testing protein leverage in lean humans: a randomised controlled experimental study. PLoS ONE 2011; 6: e25929.
12. Hill JO, Wyatt HR, Reed GW, Peters JC. Obesity and the environment: where do we go from here? Science. 2003 Feb 7; 299(5608):853-5.
13. Raubenheimer D & Simpson SJ. The geometry of compensatory feeding in the locust. Anim. Behav. 1993; 45: 953-964.
14. Raubenheimer D, Simpson SJ, Mayntz D. Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct. Ecol. 2009; 23(1):4–16.
15. Raubenheimer D & Simpson S. Nutritional Ecology and Human Health. Annu. Rev. Nutr. 2016; 36:603–26.
16. Rolls BJ. The role of energy density in the overconsumption of fat. J Nutr. 2000 Feb; 130(2S Suppl):268S-271S. Review.
17. Rothman JM, Raubenheimer D, Chapman CA. Nutritional geometry: gorillas prioritize non-protein energy while consuming surplus protein. Biol Lett. 2011 Dec 23;7(6):847-9. doi: 10.1098/rsbl.2011.0321. Epub 2011 Jun 1.
18. Simpson SJ and Raubenheimer D. A multi-level analysis of feeding behavior: The geometry of nutritional decisions. Phil. Trans. R. Soc. B 1993b; 342: 381-402.
19. Simpson SJ & Raubenheimer D. The geometric analysis of feeding and nutrition in the rat. Appetite 1997; 28: 201- 213.
20. Simpson SJ, Batley R, Raubenheimer D. Geometric analysis of macronutrient intake in humans: the power of protein? Appetite. 2003 Oct; 41(2):123-40.
21. Simpson SJ, Raubenheimer D. Obesity: the protein leverage hypothesis. Obes. Rev. 2005; 6(2):133–42.
22. Sørensen A, Mayntz D, Raubenheimer D, Simpson SJ. Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition. Obesity (Silver Spring). 2008 Mar; 16(3):566-71. doi: 10.1038/oby.2007.58.

23. Stephen JS & Raubenheimer D. The Nature of Nutrition. A unifying framework from animal adaptation to human obesity. Princeton University Press, USA, 2012.
24. Stubbs RJ. Appetite, feeding behavior and energy balance in human subjects. Proc. Nutr. Soc. 1998; 57: 141- 156.
25. Swinburn BA, Sacks G, Lo SK, Westerterp KR, Rush EC, Rosenbaum M, Luke A, Schoeller DA, DeLany JP, Butte NF, Ravussin E. Estimating the changes in energy flux that characterize the rise in obesity prevalence. Am J Clin Nutr. 2009 Jun;89(6):1723-8. doi: 10.3945/ajcn.2008.27061. Epub 2009 Apr 15.
26. Theall CL, Wurtman JJ, Wurtman RJ. Self-selection and regulation of protein: carbohydrate ratios in foods adult rats eat. J. Nutr. 1984; 114: 711-718.
27. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, Purnell JQ. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005 Jul; 82(1):41-8.
28. Westerterp-Plantenga MS. Nutrient utilization and energy balance. In: Westerterp-Platenga MS, Fredrix EWHM, Steffens AB, Kissileff HR (eds). Food Intake and Energy Expenditure. CRC Press: Boca Raton, pp. 311–319, 1994.

APPENDIX 2. Curriculum Lattes

Eurídice Martínez Steele

Curriculum Vitae
Personal Information

Full name Eurídice Martínez Steele

Birth information 16/09/1975 - East Orange, New Jersey/ - United States

CPF Number 233.935.878-70

Formal Education

2013 Doctorate in Programa Nutrição em Saude Pública.

Faculdade de Saúde Pública, USP, FSP%2C%20USP, Brazil

Title: Estudo sobre a associação entre o consumo de produtos ultraprocessados e a ocorrencia da Síndrome Metabólica na população estadunidense

Advisor: Carlos Augusto Monteiro Clique para abrir o CV do orientador

2002 - 2003 Professional Master's .

London School of Hygiene and Tropical Medicine, LSH%26TM, England

Title: Comparison of CD4 count at AIDS and Survival Time since AIDS between HIV-1 and HIV-2 in a Cohort of Incident Cases of AIDS, Year of degree: 2003

Advisor: Maarten F. Schim van der Loeff

1993 - 1999 Graduation in Biologia.

Universidad Autónoma de Madrid, UAM, Madrid, Spain

Complementary Education

2004 - 2004 Short Term Course in International Conference on Harmonization, GCP. (Carga horária: 24h).

SGS Tecnos- Life Science Services, SGS, Belgium

2004 - 2004 Short Term Course in Environmental Epidemiology and Health Policy. (Carga horária: 20h).

Annual Johns Hopkins Fall Institute in Health Policy, JHU, Spain

2000 - 2000 Short Term Course in Evaluation and application of scientific evidence. (Carga horária: 24h).

Universidad Complutense de Madrid, UCM, Spain

2000 - 2000 Short Term Course in Logistic regression. (Carga horária: 9h).

Hospital Universitario Ramon Y Cajal, H.U. RamonYCajal, Madrid, Spain

1999 - 2000 Social Anthropology. . (Carga horária: 200h).

Universidad Autónoma de Madrid, UAM, Madrid, Spain

2000 - 2000 Short Term Course in Linear regression. (Carga horária: 9h).

Hospital Ramón y Cajal, HRC, Spain

1999 - 1999 Short Term Course in Analysis and use of data in biosocial sciences. (Carga horária: 90h).

Universidad Autónoma de Madrid, UAM, Madrid, Spain

Professional Experience

1. NUPENS, Faculdade de Saúde Pública, USP - FSP

Contract institutional

2011 - Current Contract: Outro , Position: Colaboradora, Schemes of job: Part-time

Other information:

Colaboração nos projetos: 1) “Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico” (o artigo "Behavioral patterns of protective and risk factors for non- communicable diseases in Brazil" em revisão pela "Public Health Nutrition") 2) “Pesquisa de Orçamentos Familiares” (analise da evolução no consumo de sal na população Brasileira)

2. Instituto Valenciano de Infertilidade - IVI

Contract institutional

2006 - 2009 Contract: Consultor autônomo , Position: Epidemiologista , Working hours (weekly): 5, Schemes of job: Part-time

3. SGS Tecnos- Life Science Services - SGS

Contract institutional

2004 - 2009 Contract: Contratual , Position: Clinical Research Associate (CRA) , Working hours (weekly): 40, Schemes of job: Full-time

Other information:

Project responsible of "Expanded Access Programme to HIV drug in Spain (50 sites)" Site manager and Ethics Committee Submission in Phase II and Phase III, Multicentre, Double-Blind, Placebo-Controlled and Open label Randomised Clinical Trials in the following areas: 1.HIV-1 infected subjects (Spain & Portugal) 2.Adult Attention Deficit/Hyperactivity Disorder 3.Childhood Attention Deficit/Hyperactivity Disorder

4. Técnicas Avanzadas de Investigación en Servicios de Salud - TAISS

Contract institutional

2000 - 2002 Contract: Contratual , Position: Epidemiologista , Working hours (weekly): 40, Schemes of job: Full-time and exclusiveness

Other information:

Projects worked on (in study design, literature review, field- work, questionnaire development, data-management, statistical analysis, interpretation and report writing): 1. Study of the under- assessment of post- surgical pain 2. Study of social, economical and work-related factors in patients with muscular-skeletal disorders 3. Study of problems perceived by professionals working for a public hospital 4. Study of the motivation and satisfaction of physicians working for a private health insurer (leaded this project) 5. Validation of a questionnaire to measure the motivation of hospital nurses 6. Retrospective study of waiting time for coronary revascularization in comparison with standards for maximum waiting time developed using the Rand appropriateness method. 7. Development of new statistic to measure necessity and agreement from panel ratings using the Rand appropriateness method as a gold standard

5. Instituto Carlos III, Unidad de Investigación en Servicios de Salud - UISS

Contract institutional

2000 - 2000 Contract: Estagiária (com bolsa) , Position: Epidemiologista , Working hours (weekly): 20, Schemes of job: Part-time

Other information:

Comparison of European and Spanish criteria for the appropriateness and necessity of coronary revascularization. Grant from the European Social Foundation.

6. Universidad Autónoma de Madrid, Unidad de Antropología - UAM

Contract institutional

1998 - 2000 Contract: Estagiária , Position: Epidemiologista , Working hours (weekly): 15, Schemes of job: Part-time

Other information:

University Grant Projects worked on (in study- design, field- work, data management and statistical analysis): 1. Perception of self image, aesthetic models and nutritional habits in high school adolescents in Madrid 2. Aging of the female reproductive system and nutritional habits in populations of Madrid

7. Universidade do Vale do Rio dos Sinos - UNISINOS

Contract institutional

1998 - 1998 Contract: Estagiária , Position: Epidemiologista , Working hours (weekly): 20, Schemes of job: Part-time

Other information:

AECI grant Participation in Social Nutrition and HIV Prevention Programmes in low income communities

8. Instituto Butantan - IBU

Contract institutional

1997 - 1997 Contract: Estagiária , Position: Epidemiologista , Working hours (weekly): 20, Schemes of job: Part-time

Other information:

Training in Herpetology Laboratory and Vital Brazil Hospital for treatment of poisonous animal victims

S, T & A Production

Bibliographic Production

Articles Published in Scientific Journals

1. MARTÍNEZ STEELE, EURÍDICE; BARALDI, LARISSA GALASTRI; LOUZADA, MARIA LAURA DA COSTA; MOUBARAC, JEAN-CLAUDE; MOZAFFARIAN, DARIUSH; MONTEIRO, CARLOS AUGUSTO. Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. BMJ Open. Fator de Impacto(2015 JCR): 2,5620, v.6, p.e009892 - , 2016.
2. MARTÍNEZ STEELE, EURIDICE; POPKIN, BARRY M.; SWINBURN, BOYD; MONTEIRO, CARLOS A. The share of ultra-processed foods and the overall nutritional quality of diets in the US: evidence from a nationally representative cross-sectional study. Population Health Metrics. Fator de Impacto(2015 JCR): 2,7680, v.15, p.6 - , 2017.

3. MARTÍNEZ STEELE, EURÍDICE; MONTEIRO, CARLOS. Association between Dietary Share of Ultra-Processed Foods and Urinary Concentrations of Phytoestrogens in the US. Nutrients. Fator de Impacto(2015 JCR): 3,7590, v.9, p.209 - , 2017.
4. STEELE, EURÍDICE MARTÍNEZ; CLARO, RAFAEL MOREIRA; MONTEIRO, CARLOS AUGUSTO. Behavioural patterns of protective and risk factors for non-communicable diseases in Brazil. PUBLIC HEALTH NUTRITION. Fator de Impacto(2015 JCR): 2,4330, v.17, p.1 - 7, 2013.
5. MARTÍNEZ STEELE, E.; Awasana, A.A.; Corrah T.; Sabally S.; van der Sande M.; Jaye A.; T., T.; Sarge-Njie R; McConkey SJ; Whittle H; Schim van der Loeff MF. Is HIV-2- induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. AIDS. Fator de Impacto(2015 JCR): 4,4070, v.21, p.317 - 324, 2007.
6. DA COSTA LOUZADA, MARIA LAURA; BARALDI, LARISSA GALASTRI; STEELE, EURIDICE MARTINEZ; MARTINS, ANA PAULA BORTOLETTO; CANELLA, DANIELA SILVA; CLAUDE-MOUBARAC, JEAN; LEVY, RENATA BERTAZZI; CANNON, GEOFFREY; AFSHIN, ASHKAN; IMAMURA, FUMIAKI; MOZAFFARIAN, DARIUSH; MONTEIRO, CARLOS AUGUSTO. Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults. Preventive Medicine (1972. Print). Fator de Impacto(2015 JCR): 2,8930, v.81, p.9 - 15, 2015.
7. MOUBARAC, JEAN-CLAUDE; BATAL, M.; LOUZADA, M.L.; MARTINEZ STEELE, E.; MONTEIRO, C.A. Consumption of ultra-processed foods predicts diet quality in Canada. APPETITE. Fator de Impacto(2015 JCR): 3,1250, v.108, p.512 - 520, 2017.
8. SCHIM VAN DER LOEFF, MAARTEN F; MARTINEZ-STEELE, EURIDICE; CORRAH, TUMANI; AWASANA, AKUM A; VAN DER SANDE, MARIANE; SARGE-NJIE, RAMU; MCCONKEY, SAMUEL; JAYE, ASSAN; WHITTLE, HILTON. Is HIV-2-induced AIDS different from HIV-1-associated AIDS?. AIDS. Fator de Impacto(2015 JCR): 4,4070, v.22, p.791 - 792, 2008.

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Formal Education/Degree

1978 - 1979 Ph.D. in Saúde Pública .
Universidade de São Paulo, USP, Brasil. *Year of degree:* 1979.
Advisor: Yaro Ribeiro Gandra.
Keywords: Infância; Mortalidade.
Major Area: Health Sciences.
Activities Sectors: Nutrition and feeding.

1975 - 1977 Master´s in Medicina (Medicina Preventiva) .
Universidade de São Paulo, USP, Brasil. *Year of degree:* 1977.
Advisor: YARO RIBEIRO GANDRA.
Major Area: Health Sciences.

1976 - 1976 Specialization in Saude Publica .
Universidade de São Paulo, USP, Brasil.

1967 - 1972 Graduation in Medicina .
Universidade de São Paulo, USP, Brasil.

Postdoctorate and Habilitation

1982 Habilitation.
Universidade de São Paulo, USP, Brasil.
Title: , Year of degree: 1982.
Keywords: Estado Nutricional; Baixa Renda; Pre-Escolares.
Major Area: Health Sciences.
Activities Sectors: Human health.

1979 - 1981 Postdoctorate.
Columbia University, COLUMBIA, Estados Unidos.
Major Area: Health Sciences.