

In: Oligosaccharides

ISBN: 978-1-62948-328-3

Editors: L. S. Schweizer, S. J. Krebs

© 2014 Nova Science Publishers, Inc.

No part of this digital document may be reproduced, stored in a retrieval system or transmitted commercially in any form or by any means. The publisher has taken reasonable care in the preparation of this digital document, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained herein. This digital document is sold with the clear understanding that the publisher is not engaged in rendering legal, medical or any other professional services.

Chapter II

Prebiotic Oligosaccharides, Resistant Starch, and Sugar Alcohols in Lentils: Implications for Obesity

Casey Johnson¹, Dil Thavarajah^{1},
Pushparajah Thavarajah¹ and Shiv Kumar²*

¹School of Food Systems, North Dakota State University,
Fargo, ND, US

²ICARDA/Rabat Office, Rabat-Institutes, Rabat, Morocco

Abstract

Imbalanced energy intake, coupled with low concentrations of bioactive compounds in foods, has fueled the rising epidemic of obesity and related non-communicable diseases. Currently, over 35% of American adults are obese, including nearly 17% of children and adolescents. In relation, chronic non-communicable diseases result in an estimated 36 million deaths globally each year. To combat these diet-related disorders, support health, and contribute to overall sustainability of health care, it is important to develop foods that supply proper amounts of energy and nutrients. Lentil (*Lens culinaris* L.), a cool season food legume, is a good source of protein (20-30%), essential fatty acids, micronutrients, and prebiotic carbohydrates and elicits a low glycemic

index. Carbohydrate profiles of lentil that contribute to healthful effects include prebiotics: raffinose-family oligosaccharides, fructooligosaccharides, sugar alcohols, and resistant starch. The book chapter will provide insights on prebiotic carbohydrates in lentil, including genetic diversity and growing environmental and processing effects. Additionally, the chapter will include a synopsis of health consequences associated with consumption of these important prebiotic carbohydrates, and, in closure, will discuss avenues of further research.

1. Introduction

While for centuries, the greatest disease threats facing humanity were infectious, now, chronic non-communicable diseases (obesity, type 2 diabetes, cardiovascular diseases, hypertension, etc.) account for an unprecedented 63% of global disease burden (United Nations, 2012). Dietary behaviors contribute to the etiology and prognosis of such disorders (Singh et al., 1992). Increased caloric intake and altered diet composition, e.g., refined sugar and vegetable oils, are associated with a drastic increase in the prevalence of obesity and related non-communicable diseases (Austin, Ogden, & Hill, 2011). Furthermore, along with the addition of refined, high-energy foods to the typical diet, traditional foods with natural protective agents have been displaced (Kearney, 2010). A variety of bioactive compounds that exist in traditional foods are now being realized for their capacity to reduce risk factors of obesity and its comorbidities. As the burden of disease escalates, demand for these traditional staple crops – previously a pillar of the food system – will increase.

Lentil (*Lens culinaris* L.), a cool-season food legume and a staple in many Eastern diets, is an important component of a sustainable food system. Lentil is a good source of protein (20-30%), carbohydrates (~60%), essential fatty acids, and a range of vitamins and minerals (Bhatty, 1988; Thavarajah et al., 2011). Contributing to its low glycemic index (Jenkins et al., 1981), lentil also has a unique profile of carbohydrates including several healthful prebiotic compounds: raffinose-family oligosaccharides (RFO), fructooligosaccharides (FOS), sugar alcohols, and resistant starch (RS) (Bhatty, 1988; Wang, Hatcher, Toews, & Gawalko, 2009).

A prebiotic is a component of food which is neither digested nor absorbed in the small intestine, is passed to the large intestine and fermented, and elicits its effects via interactions with the microbial flora (Gibson & Roberfroid, 1995). Diet rich in prebiotics contributes to human health and well-being

through multiple facets, both physiological and pathophysiological, including reduction of risk factors for obesity and non-communicable diseases (Roberfroid et al., 2010). These attributes make prebiotics and prebiotic-rich foods such as lentil an interesting research topic for prevention of obesity.

The objectives of this book chapter are to (1) overview the current obesity epidemic, and (2) discuss those prebiotics which are present in lentil and their human health consequences. We will also review the need for producing appropriate crops for human nutrition and address lentil and its importance in healthy food systems. The remainder of the chapter will focus on prebiotic oligosaccharides, polysaccharides, and sugar alcohols in lentil, and the effects of genetics, growing environment, and cooking and processing on their concentration.

2. Obesity

Obesity is simply defined as having excess body fat (CDC). The most commonly used measurement of excess body fat is body mass index (BMI), a calculation from a person's height and weight (m^2/kg). In the US, BMI is used to characterize overweight (BMI >25) and obese (BMI >30) individuals (Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults). Essentially, BMI is a tool to be used in epidemiological studies that lead to a better understanding of the accumulation of body fat and the development of obesity.

The accumulation of excess body fat is the result of a metabolic imbalance of energy, *i.e.*, more energy is consumed than is utilized (Horton et al., 1995). The human body naturally stores any available energy exceeding its requirements, most notably in the forms of glycogen and lipid, the latter being preferred for long-term storage (Horton et al., 1995). Lipids are energy-dense and require no water to store, making them ideal as an energy reserve. Fat storage in the body is a survival mechanism: when food is unavailable the body utilizes stored fat reserves to maintain function for extended periods of time (Cahill, 1970). Moreover, adipocytes, or lipid depot cells, are responsible for sequestering circulating glucose and triglycerides and for maintaining plasma insulin concentrations (Gavrilova et al., 2000; Seip & Trygstad, 1996). So, if our body fat is so important for metabolism and general well-being, why is obesity of so much concern?

The death toll associated with obesity is over 300,000 each year in the US (U.S. Department of Health & Human Services). Obesity greatly increases the

risk of a long list of health consequences – heart disease, type-2 diabetes, and certain types of cancer, stroke, arthritis, breathing problems, and psychological disorders, such as depression (Popkin, Kim, Rusev, Du, & Zizza, 2006). Globally, over 500 million people are obese (Finucane et al., 2011); this includes about 36% of US adults (Flegal, Carroll, Kit, & Ogden, 2012). In recent years, the percentage of obese adults (over 20 years) in the US increased by 8% in men and 3% in women (Table 1). The combined prevalence of overweight and obesity has reached about 70% in the US (Flegal et al., 2012). In addition to morbidity and mortality concerns, the estimated economic cost of obesity in the US was \$117 billion in the year 2000, seen in medical services and loss of worker income and productivity (U.S. Department of Health & Human Services). To put the consequences of obesity in perspective, in addition to causing a drastic reduction in quality of life and disability-free life years, obesity and related comorbidities account for 63% of global deaths (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006; United Nations, 2012). The situation deserves global attention.

3. Obesity and the Current Food System: Are They Related?

The question of how obesity and non-communicable diseases came to be a global problem and how they progressed cannot be easily answered. However, it will be valuable to consider a few key elements in order to create a logical framework to solve this global health problem. Two factors that contributed to the high prevalence of obesity will be briefly discussed: (1) increased production and consumption of foods that are energy-dense and deficient in bioactive compounds, and (2) decreased production and consumption of traditional pulse crops.

A significant change in the global food system can be dated back to the start of the agricultural, or so-called ‘green’, revolution (Welch & Graham, 1999). In an effort to preclude impending famine and starvation, technologically-advanced agricultural practices were implemented in many regions to increase productivity (Evenson & Gollin, 2003). The program successfully increased land productivity and food availability per person. Since then, food availability per person increased by about 350 kcal per capita per day, a 15% increase in energy within 30 years (FAOSTAT).

**Table 1. Trends in the age-adjusted and age-specific prevalence of obesity (BMI ≥ 30)
in US adults aged 20 years or older for 1999-2008**

	(% of Adults)							
	Age ≥ 20 y ^a	(%) Change Over 10 y	Ages 20-39 y	(%) Change Over 10 y	Ages 40-59 y	(%) Change Over 10 y	Age ≥ 60 y	(%) Change Over 10 y
Men^b								
1999-2000	28		24		29		32	
2001-2002	28		22		32		30	
2003-2004	31		28		35		30	
2005-2006	33		28		40		32	
2007-2008	32		28		34		37	
2009-2010	36	29	33	38	37	28	37	16
Women								
1999-2000	33		28		38		35	
2001-2002	33		30		36		35	
2003-2004	33		29		39		32	
2005-2006	35		31		41		34	
2007-2008	36		34		38		34	
2009-2010	36	9	32	14	36	-5	42	20

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared). (Continued on next page)
Sources: Adapted from (1) Flegal, K; Carroll, M; Ogden, C; Curtin, L. (2010) Prevalence and trends in obesity among US adults, 1999-2008. JAMA, 303(3):235-241[20] and (2) Flegal, K; Carroll, M; Kit, B; Ogden, C. (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA, 307(5):491-497.

^aAge adjusted by the direct method to the year 2000 Census population using the age groups 20-39 years, 40-59 years, and 60 years or older.

^bIncludes racial and ethnic groups not shown separately.

Table 2. Calories from major commodities (kcal per capita per day) in developing countries, North America, and the World

	Year	Pulses	% Change Three Decades	Starchy Roots	% Change Three Decades	Cereals	% Change Three Decades	Vegetable Oils	% Change Three Decades	Sugar & Sweeteners	% Change Three Decades
Developing Countries	1970	119		131		1288		104		147	
	1985	91		122		1316		137		173	
	2000	84	-29	145	11	1355	5	178	71	168	14
North America	1970	28		99		592		347		561	
	1985	29		100		682		523		539	
	2000	43	54	110	11	872	47	627	81	626	12
World	1970	72		178		1188		142		222	
	1985	60		131		1309		200		239	
	2000	56	-22	141	-21	1306	10	247	74	228	3

Data from: FAOSTAT (<http://faostat.fao.org/site/368/Desktop.Default.aspx?PageID=368#ancor>).

Cereal crops including wheat (*Triticum* spp.), rice (*Oryza sativa* L.), and corn (*Zea mays* L.) were primary contributors to this energy boost (Table 2). The green revolution is commended for preventing food shortages in many regions.

The next agricultural revolution, however, will need to address hidden consequences of the last revolution – malnutrition and obesity.

Swinburn et al. (2011) emphasize that a global energy overbalance increases the obesity epidemic. As opposed to previous generations where energy expenditure determined energy intake, currently, energy intake is driving energy expenditure (Figure 1). As food availability increased, the world prevalence of obesity surged. Globally, the average BMI has increased significantly since 1980 (Finucane et al., 2011), and the prevalence of overweight and obesity among children in many countries has more than doubled since the 1970s (Figure 2). While excess food availability is certainly a large contributor to obesity, the question remains, “Does all food contribute to obesity equally?”

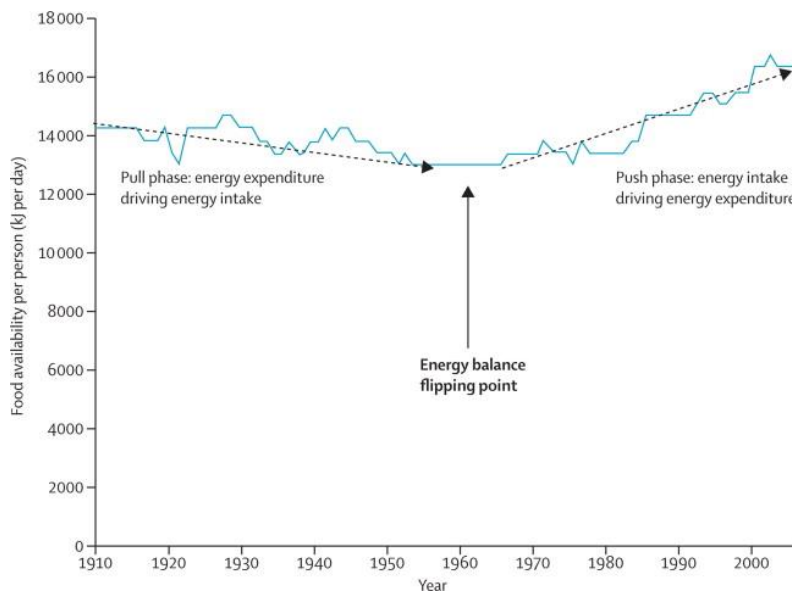


Figure 1. Food availability for the USA, 1910–2006. There are two distinct phases: a decrease in food energy supply (postulated to be pulled down by reduced energy expenditure requirements for daily living), followed by an increase in food energy supply (postulated to be pushed up by increasing food access). An energy balance flipping point is proposed, marking the change in how the US population generally achieved energy balance. Reproduced with permission from Swinburn et al. 2011.

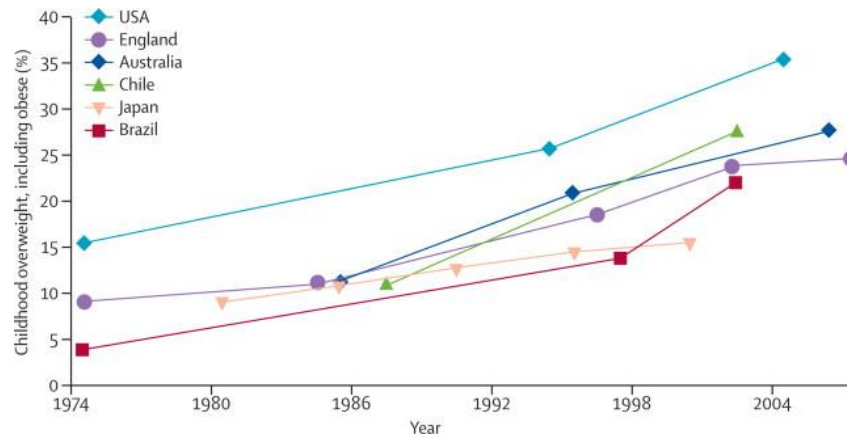


Figure 2. Estimates of percentage of childhood population overweight, including obese (with use of International Obesity Taskforce cutoffs) in a selection of countries. Reproduced with permission from (Swinburn et al., 2011).

During the green revolution, little attention was given to the nutritional quality of the food system as a whole.

Certain crops were produced disproportionately: an over-abundance of high yielding cereals and a displacement of micronutrient-rich crops, especially pulses. The world transitioned from traditional food staples – pulses, roots, and tubers – to processed cereal-based foods and foods rich in added fats, vegetable oils, and sugars (Table 2). Over the three decades between the 1970s and 2000s, availability of high-energy food products (cereals, vegetable oils, sugar, etc.) increased in North America and developing countries. Meanwhile, pulses, roots, and tubers availability decreased globally. In North America, although the amount of pulses, roots, and tubers per person increased, availability of these crops remained quite low as the major part of the pulses produced in North America is exported to developing countries.

The associations of increased prevalence of obesity with dietary patterns raised many questions. How much of the obesity epidemic can be attributed to the overall increase in calorie consumption? How much to the displacement of traditional foods with their diverse protective agents from the diet? Also, what caused the increase in calorie consumption? Did excess food availability lead to excess consumption, or did altered diet composition lead to excess consumption, or both? These are difficult questions which cannot be fully answered by any single group. Nevertheless, agricultural, nutrition, and food scientists can logically gather some helpful cues on which to focus attention:

(1) understanding of the chemistry of healthful bioactive compounds in foods and (2) production of foods that provide appropriate energy and nutrition. Thus, having reviewed the problems to be addressed, we will discuss an important element of the first of these objectives – prebiotics.

4. Gut Microbiota and Prebiotics

Prebiotics emerged in the literature in 1995 with the discovery that certain oligosaccharides could provide host benefits by altering the microbial ecology in the gut (Gibson & Roberfroid, 1995). Soon after, the gut microbiota and prebiotics were hot topics in the area of human nutrition including nutrient absorption, immunology, evolution, and epidemiology (Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012; Backhed et al., 2004; Lee & Mazmanian, 2010; Turnbaugh et al., 2006; Roberfroid et al., 2010; Rowland, 2009). Prior to these advances, surprisingly little was known about the complex relationship between the gut microbiota, its substrates, and the gastrointestinal tract.

The large intestine was, for many years, thought to have only two main functions: (1) waste excretion and (2) water absorption (Welch, 1936). We now understand that without the cooperative role of the gut microbiota in the large intestine human hosts are incapable of performing several vital physiological, metabolic, and immunological functions (Turnbaugh et al., 2007; Gill et al., 2006; Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005). The gastrointestinal microbiota is also involved in the development of miscellaneous human pathophysiological conditions (Rowland, 2009; Turnbaugh et al., 2006; Rabot et al., 2010). The intestinal epithelium and the gut microbial community function interdependently, cooperatively forming an intricate organ system – a partially external and partially “inhuman” organ system (Backhed et al., 2005). Thus with the wealth of recently generated information, the microbiota is now recognized as a key player in health and well-being.

The human gastrointestinal tract hosts about 10^{12} - 10^{14} microorganisms, varying greatly in composition, function, and location of colonization between individuals (Savage, 1977). The concentration of live microorganisms in the stomach is about 10^3 CFU/mL of contents, in the small intestine about 10^4 - 10^6 CFU/mL of contents, and in the large intestine about 10^{12} CFU/g of contents (Holzapfel, Haberer, Snel, & Schillinger, 1998). Over 1000 commensal species in the human hindgut were identified in a cohort of 124 individuals, with each individual being host to approximately 160 different species (Qin et

al., 2010). The dominant phyla present are the Firmicutes and Bacteroidetes, followed by Actinobacteria, and Proteobacteria (Table 3). These dominant groups are comprised of various genera, some potentially beneficial, some potentially harmful, and others have the potential to be either harmful or beneficial.

For example, many species within these phyla provide energy to the colonocytes in the form of short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate (Table 3). Additionally, certain species can produce essential vitamins (e.g., vitamins K₂ and B₁₂) and other beneficial metabolites; other species, however, can produce toxic, genotoxic, or carcinogenic metabolites (Pandeya et al., 2012).

When the composition of commensal groups and their metabolites exist in the right balance, or ‘normobiosis,’ that is the potentially health-promoting microorganisms predominate over potentially harmful microorganisms, the human host is benefitted (Gibson & Roberfroid, 1995; Cummings & Kong, 2004). On the other hand, an unbalance, or ‘dysbiosis,’ in the gut microbiota results in a harmful relationship, causing inflammation and disease. The concept of prebiotics is based in the coexistence of these beneficial and harmful bacterial genera. A dietary prebiotic provides the right microbial ‘food’ to selectively alter the concentrations and functions of the microbial populations leading to ‘normobiosis’. The most extensively researched genus that is stimulated by prebiotics and is an important part of the normobiotic phenomenon is *Bifidobacterium* (Roberfroid et al., 2010).

Lactobacillus is also recognized as a beneficial genus, and other genera will likely be included as more data accrue, e.g. *Eubacterium*, *Faecalibacterium*, and *Roseburia* (Roberfroid et al., 2010).

Prebiotics have attracted enormous attention (mostly for marketing purposes), and the need for strict criteria to define them became apparent, leading to the establishment of the following requirements to be classified as a prebiotic food ingredient:

- Resist degradation by processes in the upper gastrointestinal tract (acidity, pancreatic enzymes, brush boarder enzymes, etc.)
- Be fermented by intestinal microbiota
- Selectively alter the composition/activity of certain microbes resulting in health benefits to the host

Table 3. Composition and characteristics of dominant phyla of human gut microbiota and several subgroups of bacteria and their substrates and products

Phyla	Bacterial Subgroup	Approx. CFU/g of Feces	Approx. (%) of Microbiota	Mode of Action on Substrate(s)	Fermentation Product(s)
Firmicutes		$3\text{-}5.3 \times 10^{10}$	30-53%		
	Clostridia			saccharolytic, some aa-fermenting species	Ac, Pr, Bu, La, e
	Eubacteriaceae			saccharolytic, some aa-fermenting species	Ac, Bu, La
	Rumminococcus			saccharolytic	Ac
	Lactobacillus	1×10^8	1%	saccharolytic	La
	Streptococcus			carbohydrate and aa-fermentation	La, Ac
Bacteroidetes		$0.9\text{-}4.2 \times 10^{10}$	9-42%		
	Bacteroides			saccharolytic	Ac, Pr, Su
Actinobacteria		$0.2\text{-}2.5 \times 10^{10}$	2-25%		
	Bifidobacterium	$0.7\text{-}1.0 \times 10^{10}$	1-14%	saccharolytic	Ac, La, f, e
	Collinsella-Atopobium	$0.3\text{-}4.0 \times 10^9$	0.7-10%		
Proteobacteria		$0.7\text{-}4.0 \times 10^9$	1-10%		
	Escherichia			carbohydrate and aa-fermentation	Mixed acids
	Desulfovibrio			various	Ac

aa, amino acid; Ac, acetate; Pr, propionate; Su, succinate; Bu, butyrate; La, lactate; f, formate; e, ethanol.
 Soures: (Pandeya et al., 2012; Roberfroid et al., 2010; Roberfroid, 2008).

Table 4. Main areas of pathophysiological interest in which prebiotics have been investigated

Effects	Primary model	References
Functional effects		
Intestinal/colonic functions (e.g., fecal bulking, stool production)	Human	Causey et al. 2000
	Human	Cummings et al. 2002
Resistance to intestinal infections	Human	Gibson et al. 2005
	Human	Bosscher et al. 2006
Immunostimulation	Dog	Field et al. 1999
	Human	Guigoz et al. 2002
Satiety and appetite	Human	Cani et al. 2009
	Rat	Parnell et al. 2012
Influence on gastrointestinal peptides (e.g., glucagon-like peptide 1 (GLP-1) and ghrelin)	Human	Cani et al. 2009
	Rat	Parnell et al. 2012
Influence on serum lipids and glucose	Human	Delzenne et al. 2001
	Rat	Pereira et al. 2002
Bioavailability of minerals, especially Ca and Mg	Human	Bosscher et al. 2003
	Human	Franck 2006
Disease risk reduction		
Infectious diarrhea	Human	Chouraqui et al. 2008
	Human	Bosscher et al. 2006
Inflammatory bowel diseases	Human	Friedman et al. 2000
	Human	Furrie et al. 2005
	Human	Lindsay et al. 2006
Obesity	Rat/human	Daubioul et al. 2000
	Rat	Cani et al. 2007
	Rat/human	Delzenne et al. 2010
Metabolic syndrome	Rat/human	Delzenne et al. 2005
	Rat	Cani et al. 2007
	Rat/human	Delzenne et al. 2010
Osteoporosis	Rat	Roberfroid et al. 2002
	Human	Abrams et al. 2005
Colon cancer	Rat	Wollowski et al. 2001
	Rat	Le Leu et al. 2010
	Rat	Conlon et al. 2012

The most recent and widely-accepted definition of a dietary prebiotic is a “selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (ISAPP, 2008).

A key condition of this definition is ‘selectively’. There are many fibers and dietary components that are fermented by the microbiota, but only those which are selectively fermented by certain beneficial microbes are prebiotic. Therefore, though it is likely that more carbohydrates will be considered prebiotic in the future, only several currently have sufficient experimental support to meet the necessary requirements (Roberfroid et al., 2010). Those that have prebiotic ‘status’ are fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose (Kolida & Gibson, 2008).

Prebiotics are included under the broad category of low-digestible carbohydrates (Grabitske & Slavin, 2009). Low-digestible carbohydrates (LDC) are fermentable and are comprised of three groups of compounds: non-starch polysaccharides, sugar alcohols, and resistant starch (RS). Some examples of non-starch polysaccharides are RFO, FOS, and inulin. Sugar alcohols are collectively known as hydrogenated mono-, di-, or polysaccharides. Naturally occurring sugar alcohols include sorbitol and mannitol. The final subgroup, RS, occurs naturally in foods in two forms (RS₁ and RS₂), though other forms exist synthetically. The above mentioned LDCs (discussed in detail under subheading 6) are poorly digested by human enzymes and fermented in the large intestine (Grabitske & Slavin, 2009).

Fermentation of prebiotics and certain LDCs elicits a variety of health effects which can be subdivided into two main groups: functional effects and disease risk reduction (Table 4). Functional effects are physiological effects that can be measured relatively easily, including induction of satiety (Parnell & Reimer, 2012), reduction of caloric intake (Cani et al., 2009), and reduction of serum cholesterol, triglycerides, and glucose concentrations (Pereira & Gibson, 2002). Disease risk reduction, as implied by the name, is the compounding effect over time of one or more functional effects to reduce the risk/severity of chronic diseases. For example, prebiotic-induced satiety, reduced caloric intake, and improved serum lipid profile contribute to reducing the risk and severity of obesity and metabolic syndrome (Delzenne & Kok, 2001; Delzenne, Neyrinck, Backhed, & Cani, 2011; Delzenne, Neyrinck, & Cani, 2013).

5. Lentil

Lentil is highly nutritious food crop, often consumed either as a whole food or dehulled and split. The proximate composition of lentil is as follows: moisture (c.a. 10-12%), carbohydrate (c.a. 60-65%), starch (c.a. 40-55%), protein (c.a. 20-30%), ash (c.a. 3%), and lipid (c.a. 1 – 3%) (Bhatty, 1988). The seed consists of three parts: the seed coat, cotyledons, and embryo which account for 8%, 90%, and 2% of the seed weight, respectively (Singh, Singh, & Sikka, 1968). Each of these components has a different chemical composition and nutritional quality. The seed coat is formed mostly of fibers – cellulose, hemicellulose, and lignin (Bhatty, 1988). Many of the seed's minerals and free and fiber-bound polyphenolics, flavonoids, and tannins are also present in the seed coat (Duenas, Sun, Hernandez, Estrella, & Spranger, 2003; Xu & Chang, 2010). The cotyledons are the main energy store of the seed, containing the starch fraction and about 90% of the total protein and lipids (Singh et al., 1968). Various sugar alcohols and mono-, di-, and oligosaccharides are also present in the cotyledons (c.a. 5-10% of dry matter (DM)) including glucose, sucrose, RFO (raffinose, stachyose, and verbascose), FOS (nystose), and various others in lesser concentrations (Tahir, Vandenberg, & Chibbar, 2011; Biesiekierski et al., 2011; Bhatty, 1988). Minerals and water-soluble vitamins such as ascorbic acid are also concentrated in the cotyledons (Ekinici & Kadakal, 2005). Lipids and fat-soluble vitamins are contained in both the cotyledons and the embryo. The bulk of the lipids in lentil are triacylglycerides: three fatty acid residues bound with ester linkages to a glycerol backbone (Bhatty, 1988). The fatty acid profile is as follows: linoleic acid (37%), oleic acid (16%), palmitic acid (13%), linolenic acid (9%), and less than 1% of stearic, arachadonic, and eicosenoic acids (Salunkhe, Kadam, & Chavan, 1985).

The unique food matrix of lentil leads to a number of desirable nutritional responses (Jenkins et al., 1981; Abeysekara, Chilibeck, Vatanparast, & Zello, 2012). Lentil has a low glycemic index (Jenkins et al., 1981). In other words, after a lentil meal the concentration of glucose in the serum does not increase greatly or rapidly. Jenkins et al. (1980) found that the glycemic responses to pulses in general was about 45% lower than to cereal grains, biscuits, pasta, and tubers. Moreover, consumption of lentil induces a higher degree of satiety after a meal than most foods (McCrory, Hamaker, Lovejoy, & Eichelsdoerfer, 2010). Although it has been suggested that this is accomplished via modulation of gastrointestinal hormones such as cholecystokinin and also through short-chain fatty acid production in the large intestine, a direct causal

relationship to satiety has not been firmly established (Sufian, Hira, Asano, & Hara, 2007). Abeysekara et al. (2012) found that lentil diet reduced serum cholesterol in elderly by about 8% compared with regular diet. Comparing the physiological effects of lentil consumption to several effects of prebiotics (Table 4), directly or indirectly, prebiotic components likely play a role in low glycemic, satiating, and cholesterol-reducing responses in lentil (Cani et al., 2009).

Table 5. Nutrient concentration data in raw lentil and cereal grains

Proximates		Value per 100.0g			
		Lentil	Wheat, hard red spring	Brown rice, long-grain	White rice, long-grain, unenriched
Protein	g	26	15	8	7
Total lipid (fat)	g	1	2	3	1
Carbohydrate, by difference	g	60	68	77	80
Fiber, total dietary	g	31	12	4	1
Calcium, Ca	mg	56	25	23	28
Iron, Fe	mg	8	4	2	1
Potassium, K	mg	955	340	223	115
Zinc, Zn	mg	5	3	2	1
Vitamin C	mg	4	0	0	0
Riboflavin	mg	0.2	0.1	0.1	0.1
Niacin	mg	3	6	5	2
Folate, DFE	µg	479	43	20	8
Vitamin A, RAE	µg	2	0	0	0
Vitamin K (phylloquinone)	µg	5	2	2	0

Data obtained from the USDA Nutrient Database (USDA, 2012).

Compared with cereal grains, concentrations of vitamins, minerals, protein, and complex carbohydrates are comparatively greater in lentil (Table 5). Especially when consumed as a whole food, lentil is an excellent source of various nutrients; per 100 g, lentil contains ~31g dietary fiber, 8 mg iron, 5 mg zinc, 4 mg vitamin C, 479 µg folate, and 5 µg vitamin K. A ½-cup serving of cooked lentil can provide about one third of the recommended intake of dietary fiber (USDA, 2012). The large quantity of dietary fiber in lentil is a

desirable trait for several reasons. First, dietary fiber is associated with reduced incidence of heart diseases and certain types of cancer (Fuchs et al., 1999; Pietinen et al., 1996), and second, some of the components of lentil dietary fiber are prebiotic carbohydrates (Brown, 2004; Martínez -Villaluenga, Frias, Vidal-Valverde, & Gomez, 2005).

6. Lentil Prebiotics

A number of prebiotic carbohydrates are widespread in plant-derived foods in varied concentrations; vegetables, roots, tubers, and legumes in particular often contain high concentrations of the one or more prebiotics (Table 6). Concentration of prebiotics in foods ranges from trace amounts, as is the case in white rice, to relatively high amounts in other foods, such as Jerusalem artichoke (van Loo, Coussement, De Leenheer, Hoebregs, & Smits, 1995). In lentil, several groups of prebiotic carbohydrates have been indicated, including certain non-digestible oligosaccharides (FOS and RFO), RS, and sugar alcohols (Wang et al., 2009; Tahir et al., 2011; Bhatta, 1988). There are gaps in our knowledge of these important compounds, however. What is the profile of prebiotic carbohydrates in lentil? How much variation in their concentration exists between lentil genotypes? between growing environments? How much prebiotic carbohydrates are found in commercially available lentils? How does dehulling, cooking, and cooling affect those concentrations? These issues, when pertinent, will be presented for various carbohydrates in the following sections. Additionally, chemical structure of these compounds, as well as their concentration in foods and respective health consequences, will be discussed.

6.1. FOS

Often synonymously called oligofructose, FOS are by far the most famous family of prebiotic oligosaccharides. FOS consist of small chains of β (2 $\rightarrow$ 1) D-fructose residues of varying length with a terminal α (1 $\rightarrow$ 2)-linked D-glucose (Lewis, 1993). The DP of FOS is between 3 and 10 (Kolida & Gibson, 2008). Polymers of β -D-fructofuranosyl units – having a DP greater than 10 – are known as inulin (Roberfroid, 2007). The few investigations of FOS in legumes have focused on the shortest chain length compounds, kestose (β -D-fructofuranosyl-(2 $\rightarrow$ 1)- β -D-fructofuranosyl α -D-glucopyranoside) and

nystose (β -D- fructofuranosyl -(2 $\rightarrow$ 1)- β -D- fructofuranosyl -(2 $\rightarrow$ 1)- β -D- fructofuranosyl -(2 $\rightarrow$ 1)- α -D- glucopyranoside) (Biesiekierski et al., 2011).

The human small intestine lacks the necessary enzymes for degradation of FOS (Roberfroid, 1999). Case studies on patients with ileostomies have been the most important in confirming the non-digestibility of FOS (Kolida & Gibson, 2008). These non-digested compounds reach the large intestine intact, where they are fermented by the microbiota to produce SCFAs (Cummings, Macfarlane, & Englyst, 2001). The majority of this fermentation takes place in the cecum and ascending colon (Macfarlane, Gibson, & Cummings, 1992). The capacity of FOS to selectively stimulate microbial populations, especially bifidobacteria and lactobacilli, has been thoroughly demonstrated in recent years (Gibson & Roberfroid, 1995; Kruse, Kleessen, & Blaut, 1999). Supplementation of 15 g of FOS significantly increased counts of *Bifidobacterium* sp., while reducing counts of other prominent bacteria, including bacteroides, fusobacteria, and potentially pathogenic *Clostridium* sp. (Gibson & Roberfroid, 1995). The physiological and disease risk reduction effects of FOS have been widely examined, many of which are included in Table 4. In addition to maintenance of normal intestinal microbiota and prevention of pathogen colonization (Bouhnik et al., 1999), investigators demonstrated lower levels of circulating glucose and cholesterol in humans after ingestion of FOS (Pereira & Gibson, 2002).

Varying concentrations of FOS occur in over 36,000 plant species (Carpita, Kanabus, & Housley, 1989); high concentrations occur in chicory, Jerusalem artichoke, asparagus, garlic, and onion. Moderate concentrations of FOS have been observed in food legumes (Biesiekierski et al., 2011; Muir et al., 2009). In lentil, only small concentrations of FOS exist: ~100–200 mg/100g food weight (Biesiekierski et al., 2011). However, it may be possible to enhance this concentration, because FOS is already present in lentil seeds, suggesting that the genetic machinery leading to FOS accumulation, fructosyltransferase (Yun, 1996), is functional in lentil. To our knowledge, no studies have reported concentrations of FOS in lentil genotypes or taken into consideration changes in FOS concentration with growing environment.

6.2. RFO

The most well-known and studied prebiotics, such as FOS and GOS, are oligosaccharides. Not surprisingly, other oligosaccharides have attracted the attention of researchers for their potential to promote health. Oligosaccharides

that are common in legumes include the members of the raffinose family: raffinose, stachyose, and verbascose (Guillon & Champ, 2002). The basic structure of RFO contains a sucrose backbone and one or more α (1 $\rightarrow$ 6)-linked galactose residues with a DP of less than 10. This structure differs from trans-GOS in that trans-GOS have a lactose backbone instead of sucrose and β (1 $\rightarrow$ 4)-linked galactose residues instead of α (1 $\rightarrow$ 6)-linkages (Barreteau, Delattre, & Michaud, 2006). Raffinose, stachyose, and verbascose have chain lengths of 3, 4, and 5 saccharide residues, respectively (Guillon & Champ, 2002).

Due to a lack of α galactosidase activity in the small intestine, RFO are non-digestible (Smiricky et al., 2002). Once RFO reach the large intestine intact, they are fermented by the hindgut microbiota (Desjardins, Roy, & Goulet, 1990). Studies suggest that fermentation of RFO results in the selective increase of bifidobacteria in the large intestine, which is commonly associated with prebiotic compounds (Benno, 1987; Saito, Takano, & Rowland, 1992; Hayakawa et al., 1990). Supplementation of 15g/day raffinose to healthy subjects resulted in increased counts of bifidobacteria (Benno, 1987). Moreover, total bacterial counts remained stable, and *Bacteroides* spp. and *Clostridium* spp. were significantly lesser after raffinose administration than before (Benno, 1987). These observations were confirmed by a double-blind, placebo-controlled study in which 2.5 to 10 g/day raffinose significantly stimulated bifidogenesis (Bouhnik et al., 2004).

Some of the first reports of RFO in lentil appeared in the late 1970s; total RFO concentrations ranged from 2.5 to 7.2% (Bhatty, 1988; Wang et al., 2009). The profile of individual RFO concentrations has also been reported in lentil [raffinose, 0.1–1.0 g ; stachyose, 1.1–4.0 g; and verbascose, not detectable–6.4 g per 100g DM] (Martinez-Villaluenga, Frias, & Vidal-Valverde, 2008). The majority of RFO are concentrated in the cotyledons of lentil; however, multiple investigations have observed significantly higher raffinose concentrations in the seed coat, but not stachyose or verbascose (Wang et al., 2009; Wang, Hatcher, & Gawalko, 2008). This factor results in raffinose concentration decrease with dehulling (Wang et al., 2009). Owing to the water soluble nature of RFO, boiling results in significant leaching into the cooking water. Discarding cooking water therefore results in significant decreases in RFO concentrations in food (Vidal-Valverde et al., 1994). Onigbinde & Akinyele (1983) observed another interesting effect of cooking – RFO in African legumes were partially hydrolyzed leading to lesser concentrations of higher degree of polymerization (DP) oligosaccharides and greater

concentrations of short-chain oligosaccharides and sucrose. The authors attributed this to heat hydrolysis of the α (1→6)-linkages during cooking.

6.2.1. Sugar Alcohols

Sugar alcohols are low-digestible, hydrogenated monosaccharides, otherwise known as polyols (Grabitske & Slavin, 2009). They are neither sugars nor alcohols and have a representatively lower energy contribution compared with carbohydrates: sorbitol, 2.6 kcal/g; mannitol, 1.6 kcal/g; and carbohydrates, 4.0 kcal/g (Wolever, Piekarz, Hollands, & Younker, 2002). Sugar alcohols are found naturally in berries, mushrooms, and many higher plants (Makinen & Soderling, 1980), and are used extensively as artificial sweeteners for their low-calorie properties (Beards, Tuohy, & Gibson, 2010). In addition to the low glycemic index of sugar alcohols (Wolever et al., 2002), research suggests they may also have prebiotic action (de Vaux, Morrison, & Hutkins, 2002). In a mixed bacteria culture, addition of sorbitol to media resulted in the displacement of pathogenic bacteria, *Escherichia coli* O157:H7. Beards *et al.* (2010) assessed the prebiotic capacity of sugar alcohol and other confectionary sweeteners in a human trial and reported beneficial changes in the microflora, based on predominant prebiotic markers: bifidobacteria, lactobacilli, and SCFAs.

6.2.2. RS

Native starch is made up of two polysaccharides, amylose and amylopectin (Tester, Karkalas, & Qi, 2004). These polymers are acted upon within the upper gastrointestinal tract by a cohort of digestive enzymes (e.g., α -amylase, β -amylase, and amyloglucosidase) (Gray, 1992). Amylose is linear, consisting of α (1→4) linked glucose moieties, and is hydrolyzed by exo- and endo-enzymes (Tester et al., 2004). The average DP of amylose varies among food sources (Zobel, 1988). Amylopectin is highly branched and therefore additionally requires debranching enzymes such as amyloglucosidase for complete hydrolysis (Gray, 1992). The average molecular weight and DP of branches also varies among starch sources (Zobel, 1988).

Resistant starch, as its name suggests, is resistant to hydrolysis by human digestive enzymes. There are a myriad of factors that contribute to this non-digestibility (Hoover & Zhou, 2003). To name but a few, differences in *in vitro* starch digestibility have been attributed to the following: amylose/amylopectin ratio (Hoover & Sosulski, 1985), starch granule size (Snow & O'Dea, 1981), degree of starch crystallinity (Hoover & Sosulski, 1985), starch with B-type crystallinity (Englyst & Macfarlane, 1986),

amylose-lipid complexes (Guraya, Kadan, & Champagne, 1997; Nebesny, Rosicka, & Tkaczyk, 2002), enzyme inhibitors (Lajolo, Finardi Filho, & Menezes, 1991), protein and dietary fiber matrix (Dreher, Dreher, Berry, & Fleming, 1984), physical entrapment in cell structures (Wursch, Del Vedovo, & Koellreutter, 1986), and interactions of starch molecules (Dreher et al., 1984). The high resistance to hydrolysis of legume starch is a cumulative effect of high concentration of amylose, extensive physical entrapment by fibers and other food matrix factors, antinutrients, and strong interactions between amylose chains (Tovar, Francisco, Bjorek, & Asp, 1991; Hoover & Zhou, 2003; Deshpande & Cheryan, 1984).

There are five main types of RS which vary in structure and source (Bird, Conlon, Christophersen, & Topping, 2010). Current categorizations of RS are based on its source or derivation (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). RS1 refers to starch that is physically encapsulated in food, for example, in a fiber mesh or thick cell wall, and is therefore unavailable to enzymes. RS2 is naturally resistant starch due to crystallinity or tightly-packed and unhydrated nature. RS3 is derived from heating and cooling of gelatinized starch. RS4 has been modified chemically, which may include the formation of cross-linkages and esterification. RS5 is resistant to hydrolysis because of complexation with lipids (Bird et al., 2010).

By definition, RS is “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Asp, 1992). Thus RS can come from any food containing starch, limiting the presence of RS to any starchy food, but its concentration varies greatly (Murphy, Douglass, & Birkett, 2008). The estimated consumption of RS in the United States is 4.9 grams per person per day, on average (Murphy et al., 2008). Even though relative concentrations of RS are low, bread, pasta, and non-legume vegetables are the major contributors to RS consumption because they are widely eaten.

There has been enormous interest and research emphasis on RS in recent decades because of prebiotic responses and its putative therapeutic and preventative role in obesity and NCDs (Cummings et al., 2001; Johnston, Thomas, Bell, Frost, & Robertson, 2010; Conlon et al., 2012). Highlighted responses to administration of RS include reducing glycemic response, reducing caloric intake, improving bowel health (Brown, 2004), increasing absorption of micronutrients (Scholz-Ahrens et al., 2007), preventing colorectal cancer (Conlon et al., 2012), and improving insulin sensitivity (Johnston et al., 2010). These and other responses related to reducing risk factors of obesity and NCDs have marked it as a target for therapeutic and food applications (Brown, 2004).

Table 6. Various prebiotics in common foods

Food	Prebiotics ^a	g per 100 g Food			Reference
		Mean	Min.	Max.	
Lentil (boiled, drained)	RS	3.4	1.6	9.1	<i>Murphy et al. 2008*</i>
	RFO	0.4	0.2	0.5	<i>Biesiekierski et al. 2011</i>
	FOS	0.2	0.1	0.2	<i>Biesiekierski et al. 2011</i>
	SA	TR			<i>Biesiekierski et al. 2011</i>
Common bean (pinto, boiled, drained)	RS	1.9	1.8	2	<i>Murphy et al. 2008</i>
	RFO	1.4			<i>Biesiekierski et al. 2011</i>
	FOS	0.5			<i>Biesiekierski et al. 2011</i>
(red kidney, boiled, drained)					
(red kidney, boiled, drained)					
Chickpea (cooked/canned)	RS	2.6	0.8	4.3	<i>Murphy et al. 2008</i>
	RFO	0.2			<i>Biesiekierski et al. 2011</i>
	FOS	0.2			<i>Biesiekierski et al. 2011</i>
White rice (long grain, cooked)	RS	1.2	0	3.7	<i>Murphy et al. 2008</i>
	RFO	ND			<i>Biesiekierski et al. 2011</i>
	FOS	ND			<i>Biesiekierski et al. 2011</i>
White bread	RS	1.2	0.1	4.4	<i>Murphy et al. 2008</i>
	RFO	0.2			<i>Biesiekierski et al. 2011</i>
	FOS	0.7			<i>Biesiekierski et al. 2011</i>
Potato (boiled)	RS	1.3	0.3	4.5	<i>Murphy et al. 2008</i>
		~3 × greater			<i>Englyst et al. 1987</i>
					<i>Muir et al. 1992</i>
Jerusalem artichoke	FOS	12.2			<i>Muir et al. 2007</i>

RS, resistant starch; RFO, raffinose-family oligosaccharides; FOS, fructooligosaccharides; and SA, sugar alcohols; TR, trace amounts detected only; ND, not detected.

^aAdditional prebiotics may be present in selected foods. *Indicates individual reference is a comprehensive review.

Reported concentrations of RS in lentil range widely (Table 6). This may be dependent upon lentil cultivars, growing location, and whether or not the material was processed or analyzed as eaten or freeze dried (Skrabanja, Liljeberg, Hedley, Kreft, & Björck, 1999; Wang et al., 2009). Literature reports of RS concentration in cooked lentils have ranged from 1.6 to 5.2% (dry weight) (Chung et al., 2008; Wang et al., 2009) and from 1.6 to 9.1% (food weight) (Murphy et al., 2008).

Only limited data is available however on the effect of genotype of RS concentration in the lentil seed. In field pea RS concentrations were found to vary with genotype (Skrabanja et al., 1999). Processing also plays a large role in RS concentration in food. Mishra et al. (2008) nicely demonstrated that cooling of cooked potato increases the RS concentration by a factor of 2 or 3. Yadav et al. (2009) also reported increased RS concentrations in legumes with heating and cooling cycles. To date, demonstrations of changes in RS concentrations in commercially available lentils from the United States have not been reported.

7. Measurement of Prebiotics

Identification and quantification of prebiotic carbohydrates require different approaches. Oligosaccharides can be easily analyzed with simple instrument procedures. High performance anion-exchange chromatography with pulsed electrochemical detection (HPAEC-PED), high performance liquid chromatography- refractive index (HPLC-RI), capillary zone electrophoresis (CZE), gas chromatography with flame ionization detection (GC-FID), Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and nuclear magnetic resonance (NMR) spectroscopy are the most used instrumental procedures to analyze any non-polysaccharide carbohydrate including RFO, FOS, and sugar alcohols. Identification and quantification of RS, on the other hand, require enzymatic and/or chemical/physical treatment prior to analysis of resulting carbohydrates. RS, by definition, is the starch fraction that escapes hydrolysis by human digestive enzymes, does require prior enzyme treatment.

The HPAEC-PED could be considered as the most widely used accurate method to quantify RFO. This is mainly due the excellent chromatographic resolution of target compounds from each other and from other compounds. Furthermore, greater detection sensitivity provides advantage over other methods. The GC-FID and CZE also provide similar advantages as HPAEC-

PED. Survey of literature indicates HPAEC-PED is widely used procedure due to greater analytical accuracy and versatility in oligosaccharide separation. The MALDI-TOF-MS and NMR techniques are also powerful analytical chemistry techniques to determine molecular masses and chemical structural details of the carbohydrates. However, these instruments are relatively expensive and require highly skilled personal to operate and interpretate data.

Many carbohydrates are weak acids. At high pH, hydroxyl groups of carbohydrates are partially or totally transformed to oxyanions depending on the pKa values of those hydroxyl groups. High pH resistant strong anion-exchange columns with sodium hydroxide and/or sodium acetate mobile phases provide selective elution of carbohydrates based on their number of hydroxyl groups, isomerism, and degree of polymerization (DP). The eluted carbohydrates are then detected by PED. Therefore, HPAEC-PED is a versatile technique to analyze large number of carbohydrates in a single run. The difficulties in analysis of prebiotic carbohydrates with high DP could be overcome by comparison to commercial standards. For those carbohydrate with no commercial standards, those ones could be isolated employing anion exchange chromatography, and then selective acid/enzyme hydrolysing to determine their monosaccharide compositions.

Regarding quantification of RS in foods, one of the greatest obstacles has been validation of data (Champ, Kozlowski, & Lecannu, 2001). This difficulty is largely attributed to the vast amount of factors that lead to resistance to starch hydrolysis mentioned in the previous section. Major advances in RS determination were made with the use of *in vivo* comparisons obtained from ileostomy patients (Muir & O'Dea, 1993). Researchers continued to improve the existing methods, even developing standard reference material of known RS concentrations (Megazyme, 2012).

There are many ways of preparing foods. For example, lentil may be sprouted, boiled, boiled and cooled, and ground into a flour. Furthermore, analysis can be done with fresh samples, oven-dried samples, or freeze-dried samples. Changes in RS concentration may accompany any of these changes (Mishra, Monro, & Hedderley, 2008). Therefore, different RS values can be achieved for the same lentil genotype depending on the preparation. To assess RS in lentil, understanding of both native starch resistance and resistance after cooking or processing is informative, but the two may or may not be related, stressing the need for further development of the RS analytical procedures.

8. Future Directions

Understanding the problem is hard, how much more so the solution? Einstein is quoted saying “We cannot solve our problems with the same thinking we used when we created them.”

The challenges we face in food security, nutrition, and obesity and its comorbidities are indeed difficult to understand and approach. Taken as a whole, the situation is overwhelming. However, focused efforts from many cooperative disciplines will yield results.

For the food scientist working with lentil, this requires answering several important questions. What is profile of these various prebiotic and low-digestible carbohydrates in lentil genotypes? Are variations in those traits heritable? How much prebiotics are contained in different lentil market classes? Additionally, how are dehulling, cooking, and refrigeration going to affect those concentrations? Furthermore, while it has been demonstrated that both lentil as a whole food and individual carbohydrates found in lentil contribute to reducing risk factors for obesity and NCDs, a causal link still remains to be established using animal and human trials.

In conclusion, although much remains to be elucidated and understood, lentil is a prime candidate as a dietary source of prebiotics and as a potential functional food. Lentil is a popular food in many countries, circumventing the problem of social or cultural rejection associated with many foods. Also, it can also be grown successfully in many regions of the world, so availability (at least under present circumstances) will not be an issue. Finally, prebiotic carbohydrates that are found in lentil have been repeatedly shown *in vitro* and *in vivo* to have beneficial health effects (Brown, 2004; Johnston et al., 2010; Conlon et al., 2012; Koo & Rao, 1991; Benno, 1987). With the necessary questions answered, lentil may be a useful tool in reducing obesity and NCDs (Hermsdorff, Zulet, Abete, & Martínez, 2011).

References

- Abeysekara, S., Chilibeck, P. D., Vatanparast, H., & Zello, G. A. (2012). A pulse-based diet is effective for reducing total and LDL-cholesterol in older adults. *British Journal of Nutrition*, 108, S103.
- Asp, N. G. (1992). Preface: resistant starch, proceedings from the second plenary meeting of Eureka: European FLAIR Concerted Action N11 on

- physiological implications of the consumption of resistant starch in man. *European Journal of Clinical Nutrition*, 46, S1.
- Austin, G. L., Ogden, L. G., & Hill, J. O. (2011). Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal-weight, overweight, and obese individuals: 1971–2006. *The American Journal of Clinical Nutrition*, 93, 836-843.
- Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, 307, 1915-1920.
- Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A. et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15718-15723.
- Barreteau, H., Delattre, C., & Michaud, P. (2006). Production of oligosaccharides as promising new food additive generation. *Food Technology and Biotechnology*, 44, 323.
- Beards, E., Tuohy, K., & Gibson, G. (2010). A human volunteer study to assess the impact of confectionery sweeteners on the gut microbiota composition. *104*, 701-708.
- Benno, Y. (1987). Effect of raffinose intake on human fecal microflora. *Bifidobacteria Microflora*, 6, 59-63.
- Bhatty, R. S. (1988). Composition and Quality of Lentil (*Lens-Culinaris* Medik) - A Review. *Canadian Institute of Food Science and Technology Journal*, 21, 144-160.
- Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J. et al. (2011). Quantification of fructans, galacto-oligosaccharides and other short-chain carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics*, 24, 154-176.
- Bird, A. R., Conlon, M. A., Christophersen, C. T., & Topping, D. L. (2010). Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. *Beneficial Microbes*, 1, 423-431.
- Bouhnik, Y., Attar, A., Joly, F. A., Riottot, M., Dyard, F., & Flourie, B. (2004). Lactulose ingestion increases faecal bifidobacterial counts: a randomised double-blind study in healthy humans. *European Journal of Clinical Nutrition*, 58, 462-466.
- Bouhnik, Y., Vahedi, K., Achour, L., Attar, A., Salfati, J., Pochart, P. et al. (1999). Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria. *The Journal of Nutrition*, 129, 2286.

- Brown, I. L. (2004). Applications and uses of resistant starch. *Journal of AOAC International*, 87, 727-732.
- Cahill, G. F. (1970). Starvation in Man. *New England Journal of Medicine*, 282, 668-675.
- Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D. et al. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *American Journal of Clinical Nutrition*, 90, 1236-1243.
- Carpita, N. C., Kanabus, J., & Housley, T. L. (1989). Linkage Structure of Fructans and Fructan Oligomers from *Triticum aestivum* and *Festuca arundinacea* Leaves. *Journal of Plant Physiology*, 134, 162-168.
- Center for Disease Control and Prevention (CDC). (2010). Adult Obesity: Obesity Rises Among Adults. *Vital Signs*
- Champ, M., Kozlowski, F., & Lecannu, G. (2001). In vivo and in vitro methods for resistant starch measurement. *Advanced Dietary Fibre Technology*, 106-120.
- Chung, H. J., Liu, Q., Dormer, E., Hoover, R., Warkentin, T. D., & Vandenberg, B. (2008). Composition, molecular structure, properties, and in vitro digestibility of starches from newly released Canadian pulse cultivars. *Cereal Chemistry*, 85, 473-481.
- Conlon, M. A., Kerr, C. A., McSweeney, C. S., Dunne, R. A., Shaw, J. M., Kang, S. et al. (2012). Resistant Starches Protect against Colonic DNA Damage and Alter Microbiota and Gene Expression in Rats Fed a Western Diet. *The Journal of Nutrition*, 142, 832-840.
- Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A., & Englyst, H. N. (1996). Digestion and physiological properties of resistant starch in the human large bowel. *British Journal of Nutrition*, 75, 733-747.
- Cummings, J. H., Macfarlane, G. T., & Englyst, H. N. (2001). Prebiotic digestion and fermentation. *The American Journal of Clinical Nutrition*, 73, 415S-420S.
- Cummings, J. H. & Kong, S. C. (2004). Probiotics, prebiotics and antibiotics in inflammatory bowel disease. In D. Chadwick & J. Goode (Eds.), *Inflammatory Bowel Disease: Crossroads of Microbes, Epithelium and Immune Systems* (pp. 99-114). Wiley Online Library.
- de Vaux, A., Morrison, M., & Hutkins, R. W. (2002). Displacement of *Escherichia coli* O157:H7 from Rumen Medium Containing Prebiotic Sugars. 68, 519-524.

- Delzenne, N. M. & Kok, N. (2001). Effects of fructans-type prebiotics on lipid metabolism. *The American Journal of Clinical Nutrition*, 73, 456s-458s.
- Delzenne, N. M., Neyrinck, A. M., Backhed, F., & Cani, P. D. (2011). Targeting gut microbiota in obesity: effects of prebiotics and probiotics. *Nature Reviews Endocrinology*, 7, 639-646.
- Delzenne, N. M., Neyrinck, A. M., & Cani, P. D. (2013). Gut microbiota and metabolic disorders: how prebiotic can work? *British Journal of Nutrition*, 109, S81-S85.
- Deshpande, S. S. & Cheryan, M. (1984). Effects of phytic acid, divalent cations, and their interactions on α -amylase activity. *Journal of Food Science*, 49, 516-519.
- Desjardins, M. L., Roy, D., & Goulet, J. (1990). Growth of bifidobacteria and their enzyme profiles. *Journal of Dairy Science*, 73, 299-307.
- Dreher, M. L., Dreher, C. J., Berry, J. W., & Fleming, S. E. (1984). Starch digestibility of foods: a nutritional perspective. *Critical Reviews in Food Science & Nutrition*, 20, 47-71.
- Duenas, M., Sun, B., Hernandez, T., Estrella, I., & Spranger, M. I. (2003). Proanthocyanidin Composition in the Seed Coat of Lentils (*Lens culinaris* L.). *Journal of Agricultural and Food Chemistry*, 51, 7999-8004.
- Ekinci, R. & Kadakal, C. (2005). Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high-performance liquid chromatography. *ACTA Chromatographica*, 15, 289.
- Englyst, H. N., Trowell, H., Southgate, D. A., & Cummings, J. H. (1987). Dietary fiber and resistant starch. *The American Journal of Clinical Nutrition*, 46, 873-874.
- Englyst, H. N. & Macfarlane, G. T. (1986). Breakdown of resistant and readily digestible starch by human gut bacteria. *Journal of the Science of Food and Agriculture*, 37, 699-706.
- Evenson, R. E. & Gollin, D. (2003). Assessing the impact of the Green Revolution, 1960 to 2000. *Science*, 300, 758-762.
- Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. (1998). *The American Journal of Clinical Nutrition*, 68, 899-917.
- FAOSTAT. (2011). Food and Agricultural Commodities Production. Accessed June 5, 2013 from: <http://faostat.fao.org/site/368/Desktop.Default.aspx?PageID=368#ancor>

- Finucane, M. M., Stevens, G. A., Cowan, M. J., Danaei, G., Lin, J. K., Paciorek, C. J. et al. (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *The Lancet*, 377, 557-567.
- Flegal K. M., Carroll, M.D., Ogden, C.L., Curtin, L.R. (2010). Prevalence and trends in obesity among us adults, 1999-2008. *JAMA*, 303, 235-241.
- Flegal, K. M., Carroll, M. D., Kit, B. K., & Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*, 307, 491-497.
- Fuchs, C. S., Giovannucci, E. L., Colditz, G. A., Hunter, D. J., Stampfer, M. J., Rosner, B. et al. (1999). Dietary fiber and the risk of colorectal cancer and adenoma in women. *New England Journal of Medicine*, 340, 169-176.
- Gavrilova, O., Marcus-Samuels, B., Graham, D., Kim, J. K., Shulman, G. I., Castle, A. L. et al. (2000). Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *Journal of Clinical Investigation*, 105, 271-278.
- Gibson, G. R. & Roberfroid, M. B. (1995). Dietary Modulation of the Human Colonic Microbiota - Introducing the Concept of Prebiotics. *Pharmaceutiques*, 125, 1401-1412.
- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S. et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science*, 312, 1355-1359.
- Grabitske, H. A. & Slavin, J. L. (2009). Gastrointestinal effects of low-digestible carbohydrates. *Critical Reviews in Food Science and Nutrition*, 49, 327-360.
- Gray, G. M. (1992). Starch digestion and absorption in nonruminants. *The Journal of Nutrition*, 122, 172-177.
- Guillon, F. & Champ, M. M. J. (2002). Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition*, 88, S293-S306.
- Guraya, H. S., Kadan, R. S., & Champagne, E. T. (1997). Effect of rice starch-lipid complexes on in vitro digestibility, complexing index, and viscosity. *Cereal Chemistry*, 74, 561-565.
- Hayakawa, K., Mizutani, J., Wada, K., Masai, T., Yoshihara, I., & Mitsuoka, T. (1990). Effects of soybean oligosaccharides on human faecal flora. *Microbial Ecology in Health and Disease*, 3, 293-303.

- Hermisdorff, H. H., Zulet, M. Á., Abete, I., & Martínez, J. A. (2011). A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects. *European Journal of Nutrition*, 50, 61-69.
- Holzapfel, W. H., Haberer, P., Snel, J., & Schillinger, U. (1998). Overview of gut flora and probiotics. *International journal of food microbiology*, 41, 85-101.
- Hoover, R. & Sosulski, F. (1985). Studies on the functional characteristics and digestibility of starches from *Phaseolus vulgaris* biotypes. *Starch - Stärke*, 37, 181-191.
- Hoover, R. & Zhou, Y. (2003). In vitro and in vivo hydrolysis of legume starches by α -amylase and resistant starch formation in legumes - a review. *Carbohydrate Polymers*, 54, 401-417.
- Horton, T. J., Drougas, H., Brachey, A., Reed, G. W., Peters, J. C., & Hill, J. O. (1995). Fat and carbohydrate overfeeding in humans: different effects on energy storage. *The American Journal of Clinical Nutrition*, 62, 19-29.
- ISAPP (2008). 6th Meeting of the International Scientific Association of Probiotics and Prebiotics, London, Ontario. In.
- Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M. et al. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, 34, 362-366.
- Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H. M., & Fielden, H. (1980). Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *British Medical Journal*, 281, 578.
- Johnston, K. L., Thomas, E. L., Bell, J. D., Frost, G. S., & Robertson, M. D. (2010). Resistant starch improves insulin sensitivity in metabolic syndrome. *Diabetic Medicine*, 27, 391-397.
- Kearney, J. (2010). Food consumption trends and drivers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 2793-2807.
- Kolida, S. & Gibson, G. R. (2008). The Prebiotic Effect: Review of Experimental and Human Data. In G. R. Gibson & M. B. Roberfroid (Eds.), *Handbook of Prebiotics* (pp. 69-92). Boca Raton, FL: CRC Press.

- Koo, M. & Rao, A. V. (1991). Long-term effect of bifidobacteria and Neosugar on precursor lesions of colonic cancer in cf1 mice. *Nutrition and Cancer*, 16, 249-57.
- Krajmalnik-Brown, R., Ilhan, Z. E., Kang, D. W., & DiBaise, J. K. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *Nutrition in Clinical Practice*, 27, 201-214.
- Kruse, H. P., Kleessen, B., & Blaut, M. (1999). Effects of inulin on faecal bifidobacteria in human subjects. *British Journal of Nutrition*, 82, 375-382.
- Lajolo, F. M., Finardi Filho, F., & Menezes, E. W. (1991). Amylase inhibitors in Phaseolus vulgaris beans. *Food Technology*, 45, 119-121.
- Lee, Y. K. & Mazmanian, S. K. (2010). Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System? *Science*, 330, 1768-1773.
- Lewis, D. H. (1993). Nomenclature and diagrammatic representation of oligomeric fructans – a paper for discussion. *New Phytologist*, 124, 583-594.
- Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T., & Murray, C. J. (2006). Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *The Lancet*, 367, 1747-1757.
- Macfarlane, G. T., Gibson, G. R., & Cummings, J. H. (1992). Comparison of fermentation reactions in different regions of the human colon. *Journal of Applied Microbiology*, 72, 57-64.
- Makinen, K. K. & Soderling, E. (1980). A quantitative study of mannitol, sorbitol, xylitol, and xylose in wild berries and commercial fruits. *Journal of Food Science*, 45, 367-71, 374.
- Martínez-Villaluenga, C., Frias, J., & Vidal-Valverde, C. n. (2008). Alpha-galactosides: antinutritional factors or functional ingredients? *Critical Reviews in Food Science and Nutrition*, 48, 301-316.
- Martínez-Villaluenga, C., Frias, J., Vidal-Valverde, C., & Gomez, R. (2005). Raffinose family of oligosaccharides from lupin seeds as prebiotics: Application in dairy products. *Journal of Food Protection*, 68, 1246-1252.
- McCrory, M. A., Hamaker, B. R., Lovejoy, J. C., & Eichelsdoerfer, P. E. (2010). Pulse consumption, satiety, and weight management. *Advances in Nutrition*, 1, 17-30.

- Megazyme. (2012). Resistant Starch Assay Procedure. RSTAR 11/02. Megazyme International Ltd.
- Mishra, S., Monro, J., & Hedderley, D. (2008). Effect of processing on slowly digestible starch and resistant starch in potato. *Starch – Stärke*, 60, 500-507.
- Muir, J. G. & O'dea, K. (1992). Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. *The American Journal of Clinical Nutrition*, 56, 123-127.
- Muir, J. G. & O'Dea, K. (1993). Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *The American Journal of Clinical Nutrition*, 57, 540-546.
- Muir, J. G., Rose, R., Rosella, O., Liels, K., Barrett, J. S., Shepherd, S. J. et al. (2009). Measurement of Short-Chain Carbohydrates in Common Australian Vegetables and Fruits by High-Performance Liquid Chromatography (HPLC). *Journal of Agriculture and Food Chemistry*, 57, 554-565.
- Muir, J. G., Shepherd, S. J., Rosella, O., Rose, R., Barrett, J. S., & Gibson, P. R. (2007). Fructan and Free Fructose Content of Common Australian Vegetables and Fruit. *Journal of Agricultural and Food Chemistry*, 55, 6619-6627.
- Murphy, M. M., Douglass, J. S., & Birkett, A. (2008). Resistant starch intakes in the United States. *Journal of the American Dietetic Association*, 108, 67-78.
- Nebesny, E., Rosicka, J., & Tkaczyk, M. (2002). Effect of Enzymatic Hydrolysis of Wheat Starch on Amylose–Lipid Complexes Stability. *Starch - Stärke*, 54, 603-608.
- Onigbinde, A. O. & Akinyele, I. O. (1983). Oligosaccharide content of 20 varieties of cowpeas in Nigeria. *Journal of Food Science*, 48, 1250-1251.
- Pandeya, D. R., D'Souza, R., Rahman, M. M., Akhter, S., Kim, H. J., & Hong, S. T. (2012). Host-microbial interaction in the mammalian intestine and their metabolic role inside. *Biomedical Research*, 23, 9-21.
- Parnell, J. A. & Reimer, R. A. (2012). Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *British Journal of Nutrition*, 107, 601-613.
- Pereira, D. I. A. & Gibson, G. R. (2002). Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology*, 37, 259-281.

- Pietinen, P., Rimm, E. B., Korhonen, P., Hartman, A. M., Willett, W. C., Albanes, D. et al. (1996). Intake of Dietary Fiber and Risk of Coronary Heart Disease in a Cohort of Finnish Men The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Circulation*, 94, 2720-2727.
- Popkin, B. M., Kim, S., Rusev, E. R., Du, S., & Zizza, C. (2006). Measuring the full economic costs of diet, physical activity and obesity-related chronic diseases. *Obesity Reviews*, 7, 271-293.
- Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C. et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464, 59-65.
- Rabot, S., Membrez, M., Bruneau, A., Gerard, P., Harach, T., Moser, M. et al. (2010). Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *The FASEB Journal*, 24, 4948-4959, 10.
- Roberfroid, M. B., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I. et al. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, 104, S1-S63.
- Roberfroid, M. B. (2007). Prebiotics: The Concept Revisited. *The Journal of Nutrition*, 137, 830S-837S.
- Roberfroid, M. B. (1999). Caloric value of inulin and oligofructose. *The Journal of Nutrition*, 129, 1436S-1437s.
- Roberfroid, M. B. (2008). General Introduction: Prebiotics in Nutrition. In Glenn R Gibson & Marcel B Roberfroid (Eds.), *Handbook of Prebiotics* (pp. 1-11). Boca Raton, FL: CRC Press.
- Rowland, I. R. (2009). The role of the gastrointestinal microbiota in colorectal cancer. *Current Pharmaceutical Design* 15, 1524-1527.
- Saito, Y., Takano, T., & Rowland, I. (1992). Effects of soybean oligosaccharides on the human gut microflora in in vitro culture. *Microbial Ecology in Health and Disease*, 5, 105-110.
- Salunkhe, D. K., Kadam, S. S., & Chavan, J. K. (1985). *Postharvest biotechnology of food legumes*. (pp. 1-176). CRC Press.
- Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Reviews in Microbiology*, 31, 107-133.
- Scholz-Ahrens, K. E., Ade, P., Marten, B., Weber, P., Timm, W., Agil, Y. et al. (2007). Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *Journal of Nutrition*, 137, 838S-846S.

- Seip, M. & Trygstad, O. (1996). Generalized lipodystrophy, congenital and acquired (lipoatrophy). *Acta Paediatrica*, 85, 2-28.
- Singh, R. B., Rastogi, S. S., Verma, R., Laxmi, B., Singh, R., Ghosh, S. et al. (1992). Randomised controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. *BMJ: British Medical Journal*, 304, 1015.
- Singh, S., Singh, H. D., & Sikka, K. C. (1968). Distribution of nutrients in the anatomical parts of common Indian pulses. *Cereal Chemistry*, 45, 13-18.
- Skrabanja, V., Liljeberg, H. G., Hedley, C. L., Kreft, I., & Björck, I. M. (1999). Influence of genotype and processing on the in vitro rate of starch hydrolysis and resistant starch formation in peas (*Pisum sativum* L.). *Journal of Agricultural and Food Chemistry*, 47, 2033-2039.
- Smiricky, M. R., Grieshop, C. M., Albin, D. M., Wubben, J. E., Gabert, V. M., & Fahey, G. C. (2002). The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. *Journal of Animal Science*, 80, 2433-2441.
- Snow, P. & O'Dea, K. (1981). Factors affecting the rate of hydrolysis of starch in food. *The American Journal of Clinical Nutrition*, 34, 2721-2727.
- Sufian, M. K. N. B., Hira, T., Asano, K., & Hara, H. (2007). Peptides derived from dolicholin, a phaseolin-like protein in country beans (*Dolichos lablab*), potentially stimulate cholecystokinin secretion from enteroendocrine STC-1 cells. *Journal of Agricultural and Food Chemistry*, 55, 8980-8986.
- Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., Moodie, M. L. et al. (2011). The global obesity pandemic: shaped by global drivers and local environments. *The Lancet*, 378, 804-814.
- Tahir, M., Vandenberg, A., & Chibbar, R. N. (2011). Influence of environment on seed soluble carbohydrates in selected lentil cultivars. *Journal of Food Composition and Analysis*, 24, 596-602.
- Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch—composition, fine structure and architecture. *Journal of Cereal Science*, 39, 151-165.
- Thavarajah, D., Thavarajah, P., Wejesuriya, A., Rutzke, M., Glahn, R. P., Combs, G. F. et al. (2011). The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: preliminary results from a 3 year study. 180, 123-128.
- Tovar, J., Francisco, A. d., Bjorek, I., & Asp, N. G. (1991). Relationship between microstructure and in vitro digestibility of starch in precooked leguminous seed flours. *Food Structure*, 10.

- Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, 449, 804-810.
- Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444, 1027-1031.
- United Nations. (2012). Prevention and control of non-communicable diseases. Report of the Secretary General.
- U.S. Department of Health & Human Services. Overweight and Obesity: Health Consequences.
- Accessed July 16, 2013 from: http://www.surgeongeneral.gov/library/calls/obesity/fact_consequences.html.
- USDA (2012). Nutrient data for 16070, Lentils, mature seeds, cooked, boiled, without salt. <http://ndb.nal.usda.gov/ndb/foods/show/4684?fg=&man=&lfacet=&format=&count=&max=25&offset=&sort=&qlookup=lentil>
- van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., & Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Critical Reviews in Food Science and Nutrition*, 35, 525-552.
- Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M. J., Ruiz, R., & Bacon, J. (1994). Effect of processing on some antinutritional factors of lentils. *Journal of Agriculture and Food Chemistry*, 42, 2291-2295.
- Wang, N., Hatcher, D. W., Toews, R., & Gawalko, E. J. (2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). *LWT- Food Science and Technology*, 42, 842-848.
- Wang, N., Hatcher, D. W., & Gawalko, E. J. (2008). Effect of variety and processing on nutrients and certain anti-nutrients in field peas (*Pisum sativum*). *Food Chemistry*, 111, 132-138.
- Welch, C. (1936). Function of the large intestine of man in absorption and excretion: Study of a subject with an ileostomy stoma and an isolated colon. *Archives of Internal Medicine*, 58, 1095-1110.
- Welch, R. M. & Graham, R. D. (1999). A new paradigm for world agriculture: meeting human needs: productive, sustainable, nutritious. *Field Crops Research*, 60, 1-10.
- Wolever, T. M. S., Piekarz, A., Hollands, M., & Younker, K. (2002). Sugar alcohols and diabetes: a review. *Canadian Journal of Diabetes*, 26, 356-362.

- Wursch, P., Del Vedovo, S., & Koellreutter, B. (1986). Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *American Journal of Clinical Nutrition*, 43.
- Xu, B. & Chang, S. K. C. (2010). Phenolic Substance Characterization and Chemical and Cell-Based Antioxidant Activities of 11 Lentils Grown in the Northern United States. *Journal of Agriculture and Food Chemistry*, 58, 1509-1517.
- Yadav, B. S., Sharma, A., & Yadav, R. B. (2009). Studies on effect of multiple heating/cooling cycles on the resistant starch formation in cereals, legumes and tubers. *International Journal of Food Science and Nutrition*, 60, 258-272.
- Yun, J. W. (1996). Fructooligosaccharides—occurrence, preparation, and application. *Enzyme and Microbial Technology*, 19, 107-117.
- Zobel, H. F. (1988). Molecules to granules: a comprehensive starch review. *Starch - Stärke*, 40, 44-50.