Food and Nutrition Sciences, **2012, 3, 1615-1618**
http://dx.doi.org/10.4236/fns.2012.312211 Published Online December 2012 (http://www.SciRP.org/journal/fns)

Effects of Fructooligofructoses Chain Length on the Bifidobacteria of the Human Colon: A Pilot Study

Krista Mendlik, Julie A. Albrecht, Marilynn Schnepf

Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, USA.
Email: jalbrecht1@unl.edu

Received August 24th, 2012; revised October 31st, 2012; accepted November 7th, 2012

ABSTRACT

Human gastrointestinal health may be improved by the consumption of prebiotic food ingredients such as fructooligofructoses. A study was initiated to determine the effect of fructooligofructoses of different chain lengths on gastrointestinal parameters. Nineteen healthy subjects aged 20 - 57 y took part in a 10-week cross-over designed study. Subjects consumed either inulin or oligofructose for 3 weeks followed by a 2-week washout period between treatments. Stool samples were collected five times (baseline, 2 treatments, 2 washout) and analyzed for bifidobacteria. Daily records were kept for stool frequency, stool consistency and flatulence frequency. Bifidobacteria counts (cfu/ml) were higher (trending toward significance) during inulin and oligofructose intakes ($1.2 \times 10^7 \pm 4.8 \times 10^7$ and $2.0 \times 10^8 \pm 4.7 \times 10^8$) and washout periods ($2.9 \times 10^6 \pm 6.5 \times 10^6$ and $1.1 \times 10^7 \pm 1.6 \times 10^7$) than baseline counts ($2.2 \times 10^5 \pm 5.1 \times 10^5$ and $2.9 \times 10^6 \pm 6.5 \times 10^6$), respectively. Inulin and oligofructose treatment periods had a significant effect on stool consistency (watery/very hard) and flatulence frequency, but not stool frequency, when compared to baseline ($P < 0.05$). Further research is needed to confirm these results due to small sample size and the need for a longer washout period between treatments.

Keywords: Prebiotics; Fructooligofructoses; Inulin; Oligofructose; Bifidobacteria

1. Introduction

The health of the gastrointestinal tract is affected by many factors including pH, competition for nutrients, host conditions, metabolic interactions among bacteria and individual dietary intakes. Numerous studies have focused on prebiotic ingredients as functional foods and can impart a positive impact on the health of the gastrointestinal tract [1,2]. Prebiotics are defined as nondigestable food ingredients that beneficially affect the host by selectively stimulating the growth and or activity of beneficial bacterial species (such as *Bifidobacterium* and *Lactobacillus*) in the colon and thus improve host health [3]. Nondigestable fructooligofructoses are prebiotic ingredients that have been shown to have the positive effect on host health, reducing the risk of gastrointestinal diseases such as diverticulosis, diverticulitis and colon cancer [4-7]. The effects may be due to the decrease in pH which results from the production of short chained fatty acids such as butyrate by the Bifidobacterium.

Fructooligofructoses are digested by certain types of bacteria, *Bifidobacterium* and *Lactobacillus*. When Bifidobacteria is the predominant bacteria in the gut, such as the case when fructooligofructoses are ingested in the proper amounts, the number of pathogenic bacteria such as *E. coli* and *Clostridia* are decreased by competitive inhibition [5,8,9].

Fructooligofructoses are categorized by their degree of polymerization. Fructooligofructoses that have a degree of polymerization from 2 - 10 are named oligofructose and those with a degree of polymerization up to 60 are named inulin [9,10]. Fructooligofructoses are indigestible in the gastrointestinal tract, reach the colon intact and serve as a growth substrate for the gut microflora, which then break the fructooligofructose down [6]. Side effects from consuming fructooligofructoses can result in a higher level of flatulence and possible constipation and/or diarrhea [1]. The ability of bifidobacteria to ferment fructooligofructoses of different chain lengths has been proposed to occur at different rates [11]. The location of fermentation has been shown to correlate chain length of the fructooligofructoses. Longer chains, which are fermented more slowly, could be fermented in more distal regions and slower fermentation could lead to less side effects such as flatulence. A beneficial dose of 20 g per day has been shown to be effective in producing an increase in bifidobacteria, although considerable individual variation existed [6].

In vitro studies [9,11-13] demonstrated that fermenta-

tion and growth rates of bifidobacteria increase when short chain oligofructose is the carbon source and that the chain length affects the microflora composition and activity. Numerous human studies have been conducted which demonstrate the effect of consumption of bifidobacteria on increasing the colonic bifidobactiera and subsequent return to baseline within days of discontinued consumption of bifidobateria [8,14,15].

Mixed findings have been reported for the consumption of inulin or oligofructoses [6,16-18]. Results depended on the amount and type of fructooligofructoses consumed, length of time consumed, and wash out periods between treatments. Side effects (abdominal pain, distention, flatulence, constipation or diarrhea) were dependent on these same factors. Kruse, *et al*. [18] concluded that long term inulin supplementation was practicable and can positively change bifidobacteria with out major gastrointestinal discomfort.

The objectives of this research project were to determine if the chain length of fructooligofructoses had an impact on flatulence frequency, stool frequency and consistency when incorporated into the diet and to determine if chain length affected bifidobacteria growth.

2. Subjects and Methods

2.1. Subjects

After approval was obtained from Institutional Review Board, participants were recruited from a local organization and the university. Potential subjects were screened based on their past history of colon diseases, diarrhea, constipation, diverticulosis and diverticulitis to allow those whith abnormalities in the digestive tract to be omitted from the study. When subjects passed the screening, they were given a questionnaire to determine their current dietary fiber intake.

The study consisted of a 10-week blind-cross over design, which included 2, 3-week ingestion periods and 2, 2-week wash out periods. Subjects were given 20 g of fructooligofructoses (inulin or oligofructose) baked in a muffin by the researchers or as powder to mix with a beverage of the participants' choice and instructed to consume the muffin or the pre-measured powder daily. Subjects were instructed to evaluate four side effect variables (scale of zero to 10) daily into a log book provided by the researchers during the 2 - 3 week ingestion periods. The four variables were: stool frequency, flatulence frequency, stool consistency and amount of powder/muffin consumed. Subjects were encouraged to write subjective comments related to side effects associated with the inges- tion of fructooligofructoses. Subjects provided five stool samples; prior to beginning the study, during each ingestion period and during each wash-out period. Subjects provided three days of food intake records during each wash-out period.

2.2. Materials

The two commercial products used in this study were Raftiline HP and Raftilose P95 (Orafti Active Food Ingredients, Malvern, PA). Raftiline HP is 100% chicory inulin, with a degree of polymerization of fructose units from 2 - 60. Each unit is terminated by a glucose molecule. Raftilose P95 is 95% oligofructose with a degree of polymerization from 2 - 8 units. The units may or may not have a terminal glucose unit. It also contains 5% glucose (fructose and sucrose). Raftilose P95 is produced through enzymatic hydrolysis of chicory inulin.

2.3. Specimen Collection and Microbiological Analysis

Subjects were instructed on proper stool collection techniques. Stools were collected in plastic containers (Cardinal Health, Omaha, NE) and analyzed within 60 minutes of defecation. Analysis was conducted according to Munoa *et al*. [19]. Stools were weighted and 9 g was extracted and placed in a 909 ml saline solution. Samples were homogenized using a stomacher (Interscience) and the homogenates were serially diluted and plated for isolation of bifidobacteria on BIM-25 media [19]. BIM-25 media consisted of reinforced clostridial agar (RCA), 51 g/l; nalidixic acid, 0.02 g/l; polymixin B sulfate, 0.0085 g/l; kanamycin sulfate, 0.05 g/l; iodoacedtic acid, 0.025 g/l; and 2,3,5-triphenyltatrazolium chloride (TTC), 0.025 g/l. Plates were incubated at 37˚C for 48 hr using anaerobic atmosphere generating sachets (Oxiod Aerogen, Mitsubishi Gas Chemical Co.). Colonies were counted and recorded.

2.4. Dietary Analysis

The three day diet records were used to calculate normal dietary fiber intake during the wash-out periods. Nutrient calculations were performed using ESHA Food Processor for Windows (ESHA Research, Salem, OR).

2.5. Statistical Analysis

Statistical analysis included frequency distribution, mean comparisons (t-test, ANOVA, LSD and correlations using the Statistical Package for the Social Sciences [20].

3. Results

Nineteen subjects (age 20 - 57, mean 27 y; 12 females, 7 males) completed the study. Twenty subjects began the study with one subject dropping out after one week of inulin consumption due to increased flatulence. Subjects reported an average of 17 g/d of inulin and 16 g/d for oligofructose. Subjects consumed an average of 23 g/d fi-

ber during the inulin consumption period and 22 g/d fiber during the oligofructose consumption period. Gastrointestinal results including stool frequency, stool consistency and flatulence frequency for the subjects throughout the 10-week study are listed in **Table 1**.

Significant decreases ($p < 0.01$) were found for stool consistency from baseline to after inulin and oligofructose consumption. Significant increases ($p < 0.01$) were found for flatulence frequency from baseline to after inulin and oligofructose consumption. A significant decrease $p < 0.01$) in stool consistency occurred from baseline to after inulin and oligofructose consumption. The effect of the consumption of inulin and oligofructose on bifidobacteria is depicted in **Table 2**.

From baseline to consumption of either inulin or ogofructose, bifidobacteria significantly increases ($p < 0.01$). After the 14 day washout period for both inulin and oligofructose consumption, bifidobacteria dropped numerically after consumption but not significantly. Bifidobacteria did not return to baseline. Oligofructose consumption did significantly increase ($p < 0.01$) bifidobacteria when compared with inulin consumption.

4. Discussion

The chain lengths of inulin (>25 dp) and oligofructose (2 - 10 dp) did not affect the reported gastrointestinal effects of their consumption. However, within the group that consumed 16 - 20 g of inulin, 73% reported a stool frequency of one to two times per day compared to 46% of those consuming the same amount of oligofructose. However, 38% of those consuming oligofructose reported stool frequencies of 2.1 to 4 times per day compared to 26% of the inulin consuming group. Sixteen percent of the oligofructose consuming group reported a stool frequency greater than 4 times per day with no subjects in the inulin consuming group having this high of a stool frequency. These results demonstrate considerable variations among subjects with more subjects having greater sensitivity to oligofructose consumption. No significant relationship was found between the amount of fiber eaten and gastrointestinal effects which may indicate that fiber did not interfere with the changes in gastrointestinal effects or that the significant differences found in gastrointestinal effects are due to the consumption of the fructooligosaccharides.

To determine if there was an adaption period to consuming either fructooligosaccharides, the first five days of consumption were compared to the last five days of consumption and no statistically significant differences were found in gastrointestinal effects indicating no adaptations occurred. The 21 day consumption period of either fructooligosaccharide may have been too short to observe adaptation.

The consumption of the shorter chain length (oligofructose) did have a greater increase ($p < 0.01$) on bifidobacteria than the consumption of the longer chain length of inulin. Bifidobacteria growth was higher after ingestion of oligofructose but the bifidobacteria was higher for the baseline before consuming the oligofructose than the inulin. Although the baseline was higher when the three week oligofructose consumption began, the two baseline bafidobacteria amounts are not significant ($p < 0.05$). Adding either fructooligosaccharide to the diet did produce bifidobacteria levels that remained

Table 1. Effect of inulin and oligofructose ingestion on stool frequency, stool consistency and flatulence frequency from baseline to post ingestion.

Side-Effect Variable[1]	Baseline 1 (Beforeconsumption)	Inulin	Baseline 2 (Washout Period)	Oligofructose
	Times/Day	Times/Day	Times/Day	Times/Day
Stool Frequency Mean (Times/Day)	1.9 +/– 0.9	1.9 +/– 0.9	1.9 +/– 0.9	2.1 +/–1.3
Stool Consistency	5.5 +/– 1.6[a]	4.2 +/– 1.7[a]	5.5 +/– 1.6[c]	4.5 +/– 1.7[c]
Flatulence Frequency Mean (Times/Day)	3.1 +/– 1.8[b]	6.0 +/– 1.8[b]	3.1 +/– 1.8[d]	5.4 +/– 1.3[d]

[1]Based on a scale 0 - 10, with 0 = not frequent/liquid consistency and 10 = very frequent/very hard consistency; [a,b,c,d]Values in rows with the same superscripts are significantly different a $P < 0.01$.

Table 2. Effect of inulin and oligofructose ingestion on bifidobacteria from baseline to post ingestion.

Variable (Means)	Inulin (cfu/g)	Oligofructose (cfu/g)
Baseline	2.2×10^5 +/– 5.1×10^{5a}	2.9×10^6 +/– 6.5×10^{6b}
After 21 Days Ingestion	1.2×10^7 +/– 4.8×10^{7ac}	2.0×10^8 +/– 4.7×10^{8bc}
After 14 Days Washout	2.9×10^6 +/– 6.5×10^{6d}	1.1×10^7 +/– 1.6×10^{7d}

[a,b,c]Values with the same letter superscripts are significantly different a $P < 0.01$; [d]Values with this superscript are significantly different a $P < 0.10$.

higher than the baseline after supplementation ended indicating that these fructooligosaccharide may have a lasting effect on the microflora. The length of this effect is not known. A number of researchers [9,11,13] found that all strains of bifidobacteria grew better when oligofructose was a substrate versus inulin. Bouhnik and colleagues [21] found that when subjects consumed 20 g/d of oligofructose, bifidobacteria increased by 1.25 log cfu/ml whereas bifidobacteria in subjects in our study increased by 0.8 log cfu/ml for the three week oligofructose consumption period. Bifidobacteria may need a longer growth period with inulin consumption to reach the same levels. Some bifidobacteria strains do not use inulin as a substrate and bifidobacteria from our subjects were able to utilize oligofructose more efficiently than inulin.

5. Acknowledgements

A contribution of the University of Nebraska Agricultural Research Division, supported in part by funds provided through Hatch Act, USDA.

REFERENCES

[1] S. Kolida and G. R. Gibson, "Prebiotic Capacity of Inulin-Type Fructans," *Journal of Nutrition*, Vol. 137, No. S 11, 2007, pp. S2503-S2506.

[2] J. M. W. Wong and D. J. Jenkins, "Carbohydrate Digestibility and Metabolic Effects," *Journal of Nutrition*, Vol. 137, No. S11, 2007, pp. S2539-S2546.

[3] G. R. Gibson and X. Wang, "Regulatory Effects of Bifidobacteria on the Growth of Other Colonic Bacteria," *Journal of Applied Bacteriology*, Vol. 77, No. 4, 1994, pp. 412-420. doi:10.1111/j.1365-2672.1994.tb03443.x

[4] G. R. Gibson and A. L. McCartney, "Modification of the Gut Flora by Dietary Means," *Biochemical Society Transactions*, Vol. 26, No. 2, 1998, pp. 222-228.

[5] G. R. Gibson and M. R. Roberfroid, "Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics," *Journal of Nutrition*, Vol. 125, No. 6, 1995, pp. 1401-1412.

[6] B. Kleesen, B. Sykura, H. J. Zunft and M. Blaut, "Effects of Inulin and Lactose on Fecal Microflora, Microbial Activity, and Bowel Habit in Elderly Constipated Persons," *The American Journal of Clinical Nutrition*, Vol. 65, No. 5, 1997, pp. 1397-1402.

[7] M. B. Roberfroid, "Functional Effects of Food Components and the Gastrointestinal System: Chicory Froctoologosaccharides," *Nutrition Reviews*, Vol. 54, No. 11, 1996, pp. S38-S42. doi:10.1111/j.1753-4887.1996.tb03817.x

[8] M. M. Amann, M. J. Kullen, M. C. Martini, F. F. Busta and L. J. Brady, "Consumption of Exogenous Bifidobacteria does not Alter Fecal Bifidobacteria and Breath Hydrogen Excretion in Humans," *Journal of Nutrition*, Vol. 128, No. 6, 1998, pp. 996-1002.

[9] X. Wang and G. R. Gibson, "Effects of the *in-Vitro* Fermentation of Oligofructose and Inulin by Bacterial Growing in the Human Large Intestine," *Journal of Applied Bacteriology*, Vol. 75, No. 4, 1993, pp. 373-380. doi:10.1111/j.1365-2672.1993.tb02790.x

[10] M. B. Roberfriod, J. A. Van Loo and G. R. Gibson, "The Bifidogenic Nature of Chicory Inulin and Its Hydrolysis Products," *Journal of Nutrition*, Vol. 128, No. 1, 1998, pp. 11-19.

[11] S. Perrin, C. Foufnies, J. P. Grill, H. Jacobs and F. Schneider, "Fermentation of Chicory Fructo-Oligofructoses in Mixtures of Different Degrees of Polymerization by Three Strains of Bifidobactreria," *Canadian Journal of Microbiology*, Vol. 48, No. 8, 2002, pp. 759-763. doi:10.1139/w02-065

[12] G. R. Gibson and X. Wang, "Bifidogenic Properties of Different Types of Fructo-Oligofructoses," *Food Microbiology*, Vol. 11 No. 6, 1994. pp. 491-498. doi:10.1006/fmic.1994.1055

[13] M. Rossi, C. Corradini, A. Amaretti, M. Nicolini, A. Pompei, S. Zanoni and D. Matteuzzi, "Fermentation of Fructoologosaccharides and Inulin by Bifidobacteria: A Comparitive Study of Pure and Fecal Cultures," *Applied and Environmental Microbiology*, Vol. 71, No. 10, 2005, pp. 6150-6158. doi:10.1128/AEM.71.10.6150-6158.2005

[14] Y. Bouhnik, B. Flourie, C. Andrieux, N. Bisetti, F. Briet and J.-C. Rambaud, "Effects of Bifidobacterium sp Fermented Milk Ingested with or without Inulin Colonic Bifidobacteria and Enzymatic Activities in Healthy Humans," *European Journal of Clinical Nutrition*, Vol. 50, No. 10, 1996, pp. 267-273.

[15] Y. Bouhnik, B. Flourie, L. A'Gay-Abensour, P. Pochart, G. Gramlet, M. Durland and J.-C. Rambaud, "Administra- tion of Transgalacto-Oligofructoses Increased Fecal Bifi- dobacteria and Modifies Colonic Fermentation Metabo- lism in Health Humans," *Journal of Nutrition*, Vol. 127, No. 3, 1997, pp. 444-448.

[16] J. O. Hunter, Q. Tuffnell and A. J. Lee, "Controlled Trial of Oligofructose in the Management of Irritable Bowel Syndrome," *Journal of Nutrition*, Vol. 129, No. S7, 1999, pp. S1451-S1453.

[17] E. Menne, N. Guggenbuhl and M. Roberford, "Fn-Type Chicory Inulin Hydrolysate had a Prebiotic Effect in Humans," *Journal of Nutrition*, Vol. 130, No. 5, 2000, pp. 1197-1199.

[18] H.-P. Kruse, B. Kleesen and M. Blaut, "Effects of Inulin on Faecal Bifidobacteria in Human Subjects," *British Journal of Nutrition*, Vol. 82. No. 5, 1999, pp. 375-382.

[19] F. J. Munoa and R. Pares, "Selective Medium for Isolation and Enumeration of Bifidobacterium spp," *Applied and Environmental Microbiology*, Vol. 54, No. 7, 1988, pp. 1715-1718.

[20] SPSS, Version 14.0 for Windows, SPSS, Inc., Chicago.

[21] Y. Bouhnik, K. Vahedi, L. Achour, A. Attar, J. Salfati, P. Pochar, P. Marteau, B. Flourie, F. Bornet and J.-C. Rambaud, "Short-Chain Fructo-Oligosaccharide Administration Dose-Dependently Increases Fecal Bifidobacteria in Healthy Humans," *Journal of Nutrition*, Vol. 129, No. 1, 1999, pp. 113-116.

