Dose-Response Effects of Inulin and Oligofructose on Intestinal Bifidogenesis Effects

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ABSTRACT Recent studies have identified several beneficial attributes of inulin (I) and oligofructose (OF) in human health. However, most of the studies pertaining to the physiologic role of these compounds have been conducted at higher concentrations (8–40 g/d) as a source of dietary fiber. There is growing interest in using I and OF as a substrate for the selective growth of beneficial gastrointestinal bacteria such as the bifidobacteria. In vitro fermentation studies using fecal inoculums have shown that I and OF are utilized rapidly and completely by intestinal microflora and that the degree of polymerization of the substrate influenced its rate of disappearance. In these and other studies, I and OF were shown to be efficient substrates for the growth of most strains of bifidobacteria compared with glucose. In vivo studies have also shown that when human volunteers ingested I or OF, the number of fecal bifidobacteria increased. However, when results from the reported studies are combined and analyzed, a dose-response relationship in terms of log increases in the count of bifidobacteria cannot be demonstrated. Initial numbers of bifidobacteria in the feces, independent of the dose of the fructo-oligosaccharides, seem to influence the results. Future investigations should consider this relationship carefully. J. Nutr. 129: 1442S–1445S, 1999.

KEY WORDS: • prebiotics • bifidobacteria • intestinal microflora • oligofructose • chicory oligofructose

The intestinal microbial flora of humans represent a rich ecosystem composed of a wide range of metabolically active microorganisms that play an important role in influencing the health of the host. Of the several hundred species of bacteria that colonize the large intestine, bifidobacteria are generally considered to be health promoting and beneficial. Predominance of bifidobacteria is now recognized as being essential for the prevention of diseases and maintaining good health (Mitsuoka 1990). Accordingly, considerable research is being directed at promoting the growth of bifidobacteria in the large intestine. One main strategy being employed is the use of selective carbohydrate substrates for the growth of indigenous bifidobacteria, the “prebiotic” approach (Gibson and Roberfroid 1995). To be effective, these carbohydrates must reach the colon undigested and unabsorbed in the upper gastrointestinal tract and be selectively utilized by the resident bifidobacteria. Inulin type fructans are examples of such carbohydrates. They consist of 2–60 fructose units linked by a β-(2→1)-glycosidic linkage often with a terminal glucose unit (Clevenger et al. 1988, Roberfroid et al. 1998). These fructans are not hydrolyzed by the digestive enzymes in the small intestine; they reach the colon unabsorbed and are utilized selectively as a substrate for the growth of bifidobacteria. They are present in many widely consumed fruits and vegetables. Commonly, they are either synthesized from sucrose through the action of fungal-fructofuranosidase (SF) or extracted from chicory root in the form of inulin (I) and enzymatically hydrolyzed under controlled conditions to oligofructose (OF). Several dietary intervention studies have been conducted in humans using fructans; these have been reviewed recently by Roberfroid et al. (1998). Collectively, there is convincing evidence to indicate that inulin-type fructans selectively stimulate the growth of bifidobacteria both in vitro and in vivo.

In vitro fermentation of inulin and oligofructose

The growth rates (per hour) of various pure cultures of bifidobacteria grown on either inulin, oligofructose or glucose were compared (Wang 1993). Results indicated that all strains of bifidobacteria tested grew better on chicory oligofructose than on glucose. Chicory inulin gave higher growth rates in the case of Bifidobacterium infantis, B. pseudo-longum, B. angulatum and B. breve (Fig. 1). In another study, human fecal slurries were incubated in batch anaerobic cultures (Wang and Gibson 1993) in the presence of 7 g/L fructose, starch, inulin or oligofructose. At the end of 12 h, both inulin and oligofructose showed significant increases in the number of bifidobacteria (Fig. 2). Chicory fructans were also shown to selectively stimulate the growth of bifidobacteria when continuous chemostat cultures were inoculated with 160 g/L fecal...
slurry (Gibson and Wang 1994). Glucose, on the other hand, failed to stimulate the growth of these bacteria. In another recent study, Reading et al. (1998) undertook an in vitro study to investigate the minimum level of oligofructose required to obtain significant growth stimulation of bifidobacteria. They tested five oligofructose doses, equivalent to daily doses of 1, 2, 4, 6 and 8 g, using 50-mL batch culture fermentations. Fecal samples obtained from six healthy human subjects were homogenized and used after dilution to inoculate the batch cultures. Microbial populations present in each batch culture after 0, 6 and 24 h of anaerobic incubation at 37°C were enumerated. Results indicated a bifidogenic effect at all oligofructose dosages tested. Although the number of bifidobacteria increased even at a dosage of 1 g/d equivalent of oligofructose, other bacteria including Bacteroides, coliforms and gram-positive cocci also grew well at this dose. However, an oligofructose dosage equivalent to 2 g/d increased bifidobacterial numbers more selectively. The biofidogenic effect of oligofructose was optimized at the 4 g/d level.

In vivo fermentation of inulin and oligofructose

Most of the studies pertaining to the physiologic role of fructans in human health have been conducted at higher concentrations as a source of dietary fiber. Hidaka et al. (1986) studied the effect of administering 8 g/d of oligofructose (Neosugar) per day in the form of a drink or jelly to humans for a period of 2 wk and found a 10-fold average increase in bifidobacteria colony counts and a significant decrease in Clostridium perfringens (pathogenic bacteria) count. Oligofructose had also normalized the stool consistency in subjects who had moderate/severe constipation or diarrhea. In another study (Hidaka et al. 1991), hyperlipidemic out-patients were randomly divided into either a placebo group (8 g/d sucrose) or an oligofructose group (8 g/d oligofructose) and maintained on their respective treatment for a period of 5 wk. Fecal bifidobacteria in those receiving oligofructose were significantly greater compared with those given sucrose. From an initial 9.8% of the total fecal flora, bifidobacteria accounted for 50.1% by the end of 5 wk. A simultaneous reduction in Bacteroidaceae and Eubacterium was also observed in this study (Fig. 3). (Williams

![FIGURE 1](https://academic.oup.com/jn/article-abstract/129/7/1442S/4722587)  
**FIGURE 1** Effect of fructans on growth of bifidobacteria (Wang 1993).

![FIGURE 2](https://academic.oup.com/jn/article-abstract/129/7/1442S/4722587)  
**FIGURE 2** Effect of different sugars on the in-vitro growth of human fecal bacteria (Wang and Gibson 1993).

![FIGURE 3](https://academic.oup.com/jn/article-abstract/129/7/1442S/4722587)  
**FIGURE 3** Effects of fructans on predominant intestinal microflora (Hidaka et al. 1991).

![FIGURE 4](https://academic.oup.com/jn/article-abstract/129/7/1442S/4722587)  
**FIGURE 4** In vivo effects of inulin on fecal microflora (Gibson et al. 1995).
et al. (1993) showed a significant increase in bifidobacteria with 4 g/d of oligofructose ingestion by healthy human subjects. Both chicory inulin and oligofructose when added as supplements to strictly controlled diets at the rate of 15 g/d for 15 d produced significant modifications in the composition of the fecal microflora (Gibson et al. 1995). The most striking effect was an increase in the number of bifidobacteria of equal magnitude for both fructan products. In this study, a significant reduction in bacteroides, fusobacteria and clostridia was also observed (Figs. 4 and 5). Ingesting the chicory fructans resulted in a major shift toward fecal bifidobacteria that became numerically the most predominant bacterial group. Kleessen et al. (1997) fed inulin to constipated elderly subjects and demonstrated a significant increase in the bifidobacteria count in the feces by more than one log cycle, at the same time improving their constipation. Bouhnik et al. (1996) compared the effect on fecal bifidobacteria of prolonged ingestion of Bifidobacteria sp. fermented milk (BFM) with or without the addition of inulin at the rate of 18 g/d. They observed that the ingestion of BFM itself, without the addition of inulin, significantly increased fecal bifidobacteria (Fig. 6). As a result, no further increase was shown with the addition of inulin. However, in this study, 2 wk after stopping the ingestion of BFM products, subjects who consumed inulin-supplemented BFM had significantly higher fecal bifidobacteria compared with subjects ingesting BFM that was not supplemented with inulin. These results did support the conclusion that inulin was able to sustain a higher level of bifidobacteria for longer periods of time. More recently, Menne et al. (1997) studied the effect of feeding Fm-type oligofructose on fecal bifidobacteria. After a run-in period of 2 wk of consuming a controlled diet, eight healthy volunteers were administered a supplemented controlled diet for 2 wk followed by 3 wk of a supplement-free diet. Both diets were supplemented with 8 g/d of chicory oligofructose. At the end of the two feeding periods, a significant increase in the fecal bifidobacteria counts compared with the placebo period was observed. In this study, a concomitant reduction in Bacteroides spp. was also observed. In a recent article, Roberfroid et al. (1998) summarized the published data showing an in vivo increase in the counts of bifidobacteria in human feces after consumption of various doses of oligofructose added to the usual diet (Table 1). The dose of oligofructose ranged from 4 to 20 g/d. In all cases, significant increases in fecal bifidobacteria at the end of the trial were reported. However, as the author notes, levels of other fecal bacteria were not always reported in these studies.

**Dose-effect relationship between inulin-type fructans and human fecal bifidobacteria**

Published results from various human feeding studies clearly demonstrate the bifidogenic nature of inulin and oligofructose. However, the question of a dose-effect relationship remains unanswered. Roberfroid et al. (1998), in their review article, present an interesting comparison between the ingested doses of fructans and the log 10 increase in bifidobacteria. They conclude that there is no correlation between the two variables of 4 and 20 g/d. However, they go on to suggest that the initial number of fecal bifidobacteria, independent of the dose of the fructan, may correlate with the increases observed. If the levels of fecal bifidobacteria are sufficiently high to start with, then a further increase in their numbers may not be demon-
strable. Perhaps the does-effect relationship between the ingestion of fructans and fecal bifidobacteria should be evaluated in terms of both the levels and sustainability.

CONCLUSION

There is convincing evidence that both the chicory inulin and its hydrolysate product, the oligofructose, stimulate the growth of bifidobacteria in humans. A recent in vitro study has indicated that an oligofructose dosage equivalent to a daily dose of 4 g increased bifidobacterial numbers, whereas the number of bacteroides, coliform and gram-positive cocci decreased. At present, there is considerable interest from the pharmaceutical industries as well as the food industry to take advantage of the beneficial properties of chicory inulin and oligofructose in their products.

LITERATURE CITED


