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Utilization of [U-^{14}C]Fructooligosaccharides in Man as Energy Resources

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Summary Utilization of fructooligosaccharides, 1^F-(β-fructofuranosyl)$_{n-1}$-sucrose, which are not digestible in the small intestine but are fermentable by intestinal microorganisms in man, was investigated by a radiorespirometric study and an anaerobic incubation of [U-^{14}C]fructooligosaccharides with feces.

About 49% and 55% of the administered radioactivity were detected in expired $^{14}CO_2$ after 24 and 48 h, respectively.

In the anaerobic incubation, the saccharides were catabolized to $^{14}CO_2$ (9.6%), microbial cell constituents (10.4%), and ^{14}C-volatile fatty acid (acetic acid, 24.1%; propionic acid, 20.2%; butyric acid, 11.4% and valeric acid, 2.2%).

Complemental interpretation of the two studies allowed quantitation of the catabolic pathway of fructooligosaccharides in man, and utilization of the oligosaccharides was estimated to be 1.5 kcal/g.

Key Words: fructooligosaccharides, energy, nondigestible saccharide, radiorespirometric method, anaerobic fecal incubation

Fructooligosaccharides, 1^F-(β-fructofuranosyl)$_{n-1}$-sucrose in which n varies from 2 to 4 (e.g., GF_2, 1-kestose; GF_3, nystose; and GF_4, 1^F-β-fructofuranosyl nystose), occur in many kinds of plants [1]. A mixture of these saccharides is successfully produced from sucrose by the action of fungal β-fructofuranosidase [2]. There have been many reports on the physiological properties of fructooligosaccharides: non-digestibility [3], effects of chronic intake on the body weight and

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gastrointestinal tract [4], selective utilization by *Bifidobacterium* [5], and improvement of serum lipid levels in hyperlipidemic [6] and diabetic [7] patients.

Fructooligosaccharides are not hydrolyzed by digestive enzymes or the internal organs [3]. Therefore, fructooligosaccharides do not contribute to human subject as saccharides (in the common process of digestion and absorption in the small intestine).

Some investigators, however, have postulated that dietary fibers are partly able to contribute to human subject as energy resources after they are fermented to volatile fatty acids (VFAs) in the lower intestine [8, 9].

We attempted to elucidate the possibility of utilization of fructooligosaccharides for energy in man by a radiorespirometric method after ingestion of [^{14}C]fructooligosaccharides. Further observation was carried out by anaerobic incubation of [^{14}C]fructooligosaccharides with feces *in vitro* to obtain the evidence of their catabolism in the colon.

MATERIALS AND METHODS

Preparation of [^{14}C]fructooligosaccharides. [U-^{14}C]Fructooligosaccharides were prepared from [U-^{14}C]sucrose (NEN Research Product, Boston) by the action of purified β-fructofuranosidase (EC 3.2.1.26: obtained from *Aspergillus niger* ATCC 20611), followed by removal of monosaccharides and sucrose by gel permeation chromatography (TOYOPEARL gel HW-40S, Tosoh, Tokyo). Specific activity of the [^{14}C]fructooligosaccharides was 2 μCi/mg and the sugar composition was GF_2, 41.0%; GF_3, 51.5%; GF_4, 7.1%; and F_2 (fructofuranosylfructose, 0.4%) by analysis with thin-layer chromatography.

Experimental design. The protocol for the radiorespirometric experiment was approved by the Ethical Committee of the Faculty of Medicine Siriraj Hospital (Mahidol University, Bangkok, Thailand), and six healthy male volunteers (average age: 38.2$\pm$10.9 years, average body weight: 59.7$\pm$3.4 kg) participated in the study. After daily intake of 6.1 g of unlabeled fructooligosaccharides for 7 days to adapt the intestines 6.1 g of [^{14}C]fructooligosaccharides (66.4 μCi) was given after breakfast on the 8th day. The $^{14}CO_2$ contained in the breath and flatus and the radioactivities in the urine and feces collected for 48 h were determined.

In parallel with this metabolic experiment, anaerobic incubation of [^{14}C]-fructooligosaccharides with feces (collected just before ingestion of the labeled sample) was carried out in order to simulate the degradation of fructooligosaccharides in the colon.

Assay procedures. The samples of breath and flatus were collected in gas sampling bags for just 4 min, and the $^{14}CO_2$ in 5 liters of each was trapped with a CO_2-absorbent (OXISORB-CO_2, NEN Research Product) after measuring the total gas volume with a gas meter. The radioactivities in the feces were measured after homogenization and solubilization feces with SOLUENE-350 (Packard Instrument), and decolorization with H_2O_2.

Anaerobic incubation of [^{14}C]fructooligosaccharides (2 μCi, 300 mg) with a fecal homogenate (10 g in 40 ml of 0.1 M $NaHCO_3$ solution, pH 7.5) was carried out under an N_2 gas stream for 8 h at 37°C, and the $^{14}CO_2$ production, conversion to [^{14}C]VFAs and ^{14}C incorporation into bacteria were assayed. The $^{14}CO_2$ in the exhausted N_2 gas was trapped with 10 ml of OXISORB-CO_2. After extraction with ethyl ether and condensation in the form of Na salts, [^{14}C]VFAs were separated on a thin-layer chromatoplate (HPTLC-NH_2 $_{F254S}$, Merck, Darmstadt) with a developing solvent of acetone : *n*-butanol : *t*-butanol : 1.5 N NH_4OH=2 : 1 : 1 : 1 and detected by spraying with phenolphthalein. The ^{14}C-compound at each spot was scraped off the plate and then extracted with 0.5 ml of H_2O.

RESULTS

Radiorespirometric study in man

Throughout the study, the gastrointestinal condition of each subject was stable, and neither diarrhea nor soft stool was observed. The recovery of radioactivity during the initial 48 h after oral administration of [^{14}C]fructooligosaccharides to six subjects is summarized in Table 1. The respirometric patterns of $^{14}CO_2$ expiration are presented in Fig. 1.

The total recovery of radioactivity was 58.2±1.4% for 24 h and 67.2±1.5% for 48 h. The total $^{14}CO_2$ expiration was 48.9±1.3% for 24 h and 55.2±1.6% for 48 h, and the maximum $^{14}CO_2$ expiration rate was found at 7 h, after which the rate decreased rapidly until the 14 h. The recovery of radioactivity in the feces was 10.1±1.5% for 48 h, and four of the six subjects evacuated more than 80% of the fecal radioactivity in the first 24 h.

The urinary radioactivity was 1.9±0.2% and most of it was excreted in the first 12 h. The amount of $^{14}CO_2$ in the flatus was very little, so most of the $^{14}CO_2$ produced from [^{14}C]fructooligosaccharides in the colon must have been absorbed from the colon and expired in the breath.

Table 1. Recovery of radioactivity after oral administration of [^{14}C]fructooligosaccharides to six subjects.

	0–12	12–24	24–36	36–48	0–48
			(h)		
					(%)
Respiratory $^{14}CO_2$	40.3±1.2	8.6±0.5	3.9±0.2	2.4±0.3	55.2±1.6
Urinal ^{14}C	0.9±0.0	0.5±0.1	0.3±0.0	0.3±0.0	1.9±0.1
	(0–24 h)		(24–48 h)		
Fecal ^{14}C	7.9±1.9		2.2±1.0		10.1±1.5
Flatus $^{14}CO_2$	less than 0.05		not detected		less than 0.05
Total recovery					67.2±1.5

Mean±SE of six subjects.

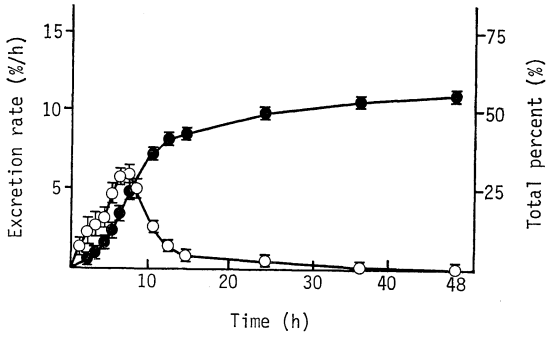

Fig. 1. $^{14}CO_2$ Expiration after ingestion of [^{14}C]fructooligosaccharides in six subjects. The total breath expired per a 4 min period was collected in a Douglas bag at intervals of 1 h during the initial 8 h, then, every 2 or more hours until 48 h after ingestion of 6.1 g of [^{14}C]fructooligosaccharides (66.4 μCi). The $^{14}CO_2$ in 5 liters of sample was trapped in 10 ml of CO_2 absorbent (OXISORB-CO_2) after measuring the total gas volume with a gas meter. The open circles indicate the $^{14}CO_2$ expiration rate (% of ingested radioactivity per hour), and the closed circles show the cumulative amount of $^{14}CO_2$. The circles and vertical bars represent the mean of 6 subjects and the standard error of the mean, respectively.

Table 2. Degradation of [^{14}C]fructooligosaccharides during anaerobic incubation with feces of subjects.

	Average±SE (%)
$^{14}CO_2$ production	9.6±1.2
^{14}C incorporated in cells	10.4±1.4
^{14}C in supernatant	68.6±2.7
total VFAs	57.9±1.7
acetate	24.1±1.8
propionate	20.2±1.4
butyrate	11.4±0.6
valeriate	2.2±0.3
Total recovery	88.6±3.1

Respective radioactive ratio of VFA is as follows, acetate, 0.415; propionate, 0.350; butyrate, 0.197; and valeriate, 0.039.

Anaerobic incubation of [^{14}C]fructooligosaccharides with feces

The mean results of anaerobic incubation of [^{14}C]fructooligosaccharides *in vitro* with each subject's feces for 8 h are summarized in Table 2. Direct $^{14}CO_2$ production from [^{14}C]fructooligosaccharides was about 10% (range: 7 to 13%), and most of it was produced within 4 h. More than half of the [^{14}C]fructooligosaccharides was converted to ether-extractable compounds, and they were identified as VFAs by thin-layer chromatography. The total amount of VFAs was almost the same in each subject, and about 40% of the [^{14}C]VFAs was identified as acetic acid.

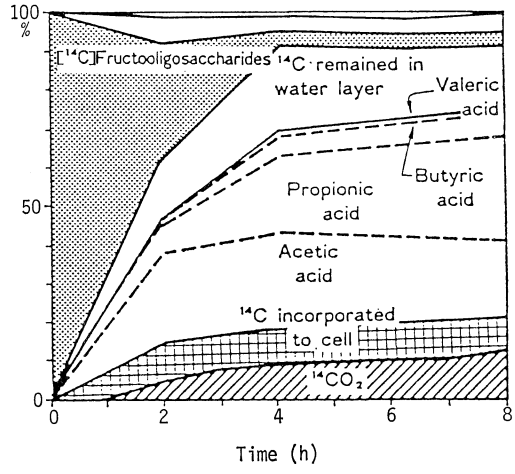

Fig. 2. Degradation of [^{14}C]fructooligosaccharides during anaerobic incubation with feces. [^{14}C]Fructooligosaccharides (300 mg) were anaerobically incubated with 10 g of fresh feces under an N_2 gas stream. The $^{14}CO_2$ in exausted N_2 gas was trapped in 10 ml of CO_2 absorbent (OXISORB-CO_2) throughout the experiment. The amounts of [^{14}C]VFAs were assayed by thin-layer chromatography as described in the Methods. The residual [^{14}C]fructooligosaccharides were estimated by colorimetric measurement of fructose by the resorcinol-HCl method.

The degradation pattern of [^{14}C]fructooligosaccharides in anaerobic incubation with feces *in vitro* is presented in Fig. 2. More than 90% of the [^{14}C]fructooligosaccharides was converted to $^{14}CO_2$, [^{14}C]VFAs and ^{14}C-bacterial constituents within 4 h. Acetic acid was a major product early in the degradation (72.5% of the total of [^{14}C]VFAs), but later propionic acid increased.

DISCUSSION

Although fructooligosaccharides are not digested in the intestines, more than half of the administered radioactivity was recovered as expired $^{14}CO_2$ during 48 h. This result can be analyzed by hypothesizing that fructooligosaccharides are partly available as an energy source in human subjects, not by digestion, but by hind-gut fermentation and subsequent utilization of their catabolites in the body.

We have already reported the possibility of energy utilization of fructooligosaccharides with the aid of intestinal microorganisms in the rat. That is, oral administration of [^{14}C]fructooligosaccharides to conventional rats afforded a considerable amount of $^{14}CO_2$ expiration, but similar treatment of their germ-free counterpart resulted in little $^{14}CO_2$ [10]. Such results suggested that, in the rat, fructooligosaccharides are fermented by intestinal bacteria and their catabolites are absorbed and utilized.

In the present radiorespirometric study, it was found that orally administered

[^{14}C]fructooligosaccharides provided 48.9% of radioactivity in expired $^{14}CO_2$ within 24 h as the final metabolite (see Table 1). As the expired $^{14}CO_2$ was produced through the hind-gut fermentation of the saccharides by intestinal bacteria and subsequent utilization of the catabolite by man, it was essential to know how the fermentation was proceeding in human colon and what kind of catabolite was absorbed from the colon. An anaerobic incubation study using the subject's feces was applied to simulate the actual fermentation in human colon and it could clarify that the main catabolites in the colon were volatile fatty acids and intestinal gas (see Table 2). Complemental interpretation of two studies led us to the elucidation of catabolic pathway in which the [^{14}C]fructooligosaccharides were fermented by intestinal bacteria into $^{14}CO_2$ and [^{14}C]VFAs which were absorbed and utilized in man to give the respiratory $^{14}CO_2$. Therefore, caloric utilization of the saccharides could be quantified by estimating the amount of the [^{14}C]VFAs which were absorbed from the colon.

In order to accurately estimate the amount of absorbed [^{14}C]VFAs, we should consider the following two factors: (1) intestinal gaseous $^{14}CO_2$ which was included in the expired breath and (2) the rate of catabolic conversion of [^{14}C]VFAs to $^{14}CO_2$.

According to the anaerobic incubation study, about 10% of [^{14}C]fructooligosaccharides was converted to intestinal $^{14}CO_2$ directly by bacterial fermentation (see Table 2). If all of the intestinal CO_2 produced from the administered saccharides (6.1 g) were excreted in the flatus, the volume would be more than 1.5 liters because the normal CO_2 concentration in the flatus is 5 to 30% [11]. However, the volume of the flatus observed during the experiment was not only small but the radioactivity was also negligible (see Table 1). This fact suggests that most of the intestinal CO_2 liberated in the colon by fermentation was absorbed and expired in the breath. That is, it was necessary to subtract the intestinal $^{14}CO_2$ (9.6%) from the total respiratory $^{14}CO_2$ (48.9%) and the remainder (39.3%) was the actual respiratory $^{14}CO_2$ which represented the utilization of VFAs absorbed from the colon within 24 h.

During our experiment, amount of the absorbed [^{14}C]VFAs was not converted into $^{14}CO_2$, but a portion of them might be incorporated into tissue and endogenous metabolites. In order to include this incorporation in the estimation of caloric utilization, the respiratory $^{14}CO_2$ utilized within 24 h was corrected by the rate of catabolic conversion. Regarding the rate of VFAs, it was reported that about 55 to 70% of [^{14}C]VFAs instilled into human colon were metabolized to $^{14}CO_2$ within 24 h [12] and a value of 0.6 was used as the rate of acetate [13]. Similar rates were confirmed in our experiment: 70%, 64%, and 71% of the radioactivity were recovered as $^{14}CO_2$ when [^{14}C]acetate, [^{14}C]propionate, and [^{14}C]butyrate, respectively, were injected into the ceacum of rats. From these results, we expected that the rate of catabolic conversion of VFAs to CO_2 during the initial 24 h existed in range of 0.6–0.7. Dividing the actual respiratory $^{14}CO_2$ (39.3%) within 24 h with the rate of catabolic conversion (0.6), which was selected as an acceptable value to three kinds of the rate, gave total radioactive ratio (65%) of absorbed [^{14}C]VFAs

in man. By using the data of the total radioactive ratio of absorbed VFAs and respective radioactive ratio of VFA obtained by thin-layer chromatographic analysis (see Table 2), the absorbed VFAs produced from 1.0 g of fructooligosaccharides could be calculated as a mixture of following amount of VFA*; 0.29 g of acetate, 0.20 g of propionate, 0.10 g of butyrate and 0.02 g of valeriate. Then, the caloric utilization will be estimated by the following equation.

$$\text{Energy of fructooligosaccharides (1.0 g)} = \sum \text{each amount of absorbed VFA (g)} \times \text{energy of the VFA (kcal/g)}$$

The energy values of VFAs have not been reported except that of acetate which is defined as 2.4 kcal/g in Standard Tables of Food Composition in Japan (4th revised edition, 1982). When we use 2.4 kcal/g as the energy values of all of VFAs in the above equation, the caloric utilization of fructooligosaccharides in man would be 1.5 kcal/g.

REFERENCES

1. Shiomi, N., Yamada, J., and Izawa, M. (1976): Isolation and identification of fructooligosaccharides in roots of asparagus. *Agric. Biol. Chem.*, **40**, 567–575.
2. Hidaka, H., and Hirayama, M. (1985): Enzymatic glycosyl transferation and fructooligosaccharides. *Enzyme Eng. News*, **13**, 15–19 (in Japanese).
3. Oku, T., Tokunaga, T., and Hosoya, N. (1984): Nondigestibility of a new sweetener, "Neosugar" in the rat. *J. Nutr.*, **114**, 1574–1581.
4. Tokunaga, T., Oku, T., and Hosoya, N. (1986): Influence of chronic intake of new sweetener (Neosugar) on growth and gastrointestinal function of the rat. *J. Nutr. Sci. Vitaminol.*, **32**, 111–121.
5. Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T., and Tashiro, Y. (1986): Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora*, **5**, 37–50.
6. Hata, Y., Hara, T., Oikawa, T., Yamamoto, M., Hirose, N., Nagashima, T., Torihama, N., Nakajima, K., Watabe, A., and Yamashita, M. (1983): The effect of fructooligosaccharides (Neosugar) on hyperlipidemia. *Geriatric Med.*, **21**, 156–167 (in Japanese).

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*The average molecular formula ($C_{21.92}H_{38.53}O_{19.27}$; molecular weight, 609.72) of the administered fructooligosaccharides was given by their composition (see Materials and Methods).

As a catabolic equation of the saccharides to VFAs was shown by a Eq. (1),

$$C_{21.92}H_{38.53}O_{19.27} \longrightarrow \frac{21.92}{2+n} CH_3(CH_2)_nCOOH \qquad (1)$$

n=0 (acetate), n=1 (propionate), n=2 (butyrate), and n=3 (valeriate)

each amount of absorbed VFA could be calculated by the following Eq. (2).

$$\text{Each amount of absorbed VFA(g)} = \left(\frac{21.92}{2+n}\right) \times \left(\frac{60+14n}{609.72}\right) \times (\text{respective radioactive ratio of VFA}) \times (\text{total radioactive ratio of VFAs}) \qquad (2)$$

7. Yamashita, K., Kawai, K., and Itakura, M. (1984): Effects of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr. Res.*, **4**, 961–966.
8. Van Soest, P.J., and Robertson, J.B. (1977): What is fiber and fiber in food? *Nutr. Rev.*, **35**, 12–22.
9. Smith, C.J., and Bryant, M.P. (1979): Introduction to metabolic activities of intestinal bacteria. *Am. J. Clin. Nutr.*, **32**, 149–157.
10. Oku, T., Tokunaga, T., and Hosoya, N. (1982): Digestion, absorption and utilization of neosugar in rat, *in* Reports of Neosugar Conference (I), ed. by Hosoya, N., Meiji Seika Kaisha Ltd., Tokyo, pp. 49–62 (in Japanese).
11. Levitt, M.D., and Bond, H.J. (1970): Progress in gastroenterology, volume, composition, and source of intestinal gas. *Gastroenterology*, **59**, 921–929.
12. Høverstadt, T., Bøhmer, T., and Fausa, O. (1982): Absorption of short-chain fatty acids from the human colon measured by the $^{14}CO_2$ breath test. *Scand. J. Gastroenterol.*, **17**, 373–378.
13. Figdor, S.K., and Bianchine, J.R. (1983): Caloric utilization of [^{14}C]polydextrose in man. *J. Agric. Food Chem.*, **31**, 389–393.