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Inability of the human fecal microflora to metabolize the nonabsorbable fat substitute, olestra

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SUMMARY

Olestra is a non-caloric fat substitute under review by the Food and Drug Administration. It consists of a mixture of octa-, hepta- and hexaesters of sucrose formed with long chain fatty acids. Previous studies showed olestra is not hydrolyzed by mammalian lipases and is not absorbed. The objective of this study was to evaluate the potential for colonic microflora to metabolize olestra after continued exposure. Neat and emulsified ^{14}C-[fatty acid] olestra was incubated for 72 h in both minimal and organically-enriched anaerobic media inoculated with feces from seven subjects who had consumed olestra (9 g per day) over a 3–4 week period. ^{14}C-sucrose and ^{14}C-glucose served as positive controls. Production of $^{14}CO_2$, $^{14}CH_4$, ^{14}C-volatile fatty acids (VFAs) and ^{14}C-long chain fatty acids (LCFAs) was determined. In addition, the ester distribution and fatty acid composition of olestra were examined before and after incubation. Significant quantities of $^{14}CO_2$ and ^{14}C-VFAs were generated from the ^{14}C-sugars, indicating that the microflora were active under the incubation conditions. Furthermore, free oleic acid was extensively hydroxylated and hydrogenated. In contrast, no degradation products (gas, VFAs, LCFAs) or changes in the olestra resulting from bacterial activity were detected. These results indicate that under anaerobic conditions the colonic microflora of the humans, consuming olestra, did not metabolize olestra.

INTRODUCTION

Obesity, coronary artery disease and some cancers have been linked with excessive intake of dietary fat [12]. As a consequence, public health organizations have recommended that people limit their intake of fats from a current level of approximately 36% of calories to no more than 30% of calories. One alternative is to replace a portion of natural fat in foods with nonabsorbable substitutes. Olestra is one such substitute that is currently under review as a food additive by the US Food and Drug Administration. Olestra is a mixture of octa-, hepta-, and hexaesters of sucrose formed with long-chain fatty acids derived from edible oils. Olestra has physical and organoleptic properties similar to triglycerides and can function as a replacement for fat in a wide variety of foods, including those that are cooked, baked, or fried.

Olestra is not hydrolyzed by mammalian lipases and is not absorbed from the GI tract [3,7,8,9]. Little, however, is known about its potential transformation by colonic bacteria. These transformations could include hydrolysis yielding lower-ester olestra as well as free fatty acids and ultimately sucrose, which could be metabolized to VFAs, methane and carbon dioxide. Liberated unsaturated fatty acids alternatively could be hydroxylated or hydrogenated. In a like manner, it is possible that unsaturated fatty acids in intact olestra could be hydroxylated or reduced resulting in a more polar or saturated olestra. The objective of this study was to determine whether any of these reactions are catalyzed by the colonic microflora of subjects, who have consumed olestra for an extended period.

Several aproaches exist for examining the metabolism of ingested substances by the microflora of humans. These include in vivo studies that attempt to balance the amounts ingested and egested as well as characterize the identity of the egested materials [3]. This approach is not particularly sensitive, and it is difficult to isolate the activities of the microflora from those of the host. Another approach involves in vitro incubation of the substance with human feces or cultures of intestinal bacteria and monitoring the formation of products. This approach is more sensitive, particularly if radiolabeled substances are utilized, and makes it possible to test several inocula with replication. Such incubations, however, are temporally limited and do not provide an opportunity for the organisms to adapt. Alternatively, longer term in vitro studies can be performed utilizing semi-continuous and continuous culture systems [6]. Such systems provide an opportunity for adaptation but require a great deal of maintenance. Thus, only one or two inocula can be tested and one needs to be concerned whether the community established in vitro remains comparable to that in vivo. In the present study, an attempt was made to exploit the advantages of short-term in vitro incubations, while providing

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an opportunity for adaptation to occur in vivo. Fecal samples were obtained from several individuals who had consumed olestra for an extended period. They were incubated in vitro with radiolabeled olestra under low and high nutrient conditions, and the formation of radiolabeled methane, carbon dioxide and volatile fatty acids was monitored. In addition, microbially-incurred changes in the ester distribution and fatty acid composition of olestra were examined.

MATERIALS AND METHODS

Chemicals

[1-^{14}C-fatty acid] Olestra with a specific activity of 1.21 mCi g^{-1} was synthesized at The Procter & Gamble Co. (Cincinnati, OH, USA). Its ester distribution and fatty acid composition are shown in Fig. 1. [U-^{14}C] Sucrose and [U-^{14}C] glucose with respective specific activities of 1.5 mCi mg^{-1} and 1.3 mCi mg^{-1} were purchased from Amersham Corp. (Arlington Heights, IL, USA). The purity of sucrose was 97.0% based on analysis by HPLC, and the purity of glucose was 98.0% based on analysis by paper chromatography. [1-^{14}C] Oleic acid with a specific activity of 184 μCi mg^{-1} and purity of 97.8% was also obtained from Amersham Corp. Scintillation cocktail consisted of RPI Scintillator (Research Products International Inc., Mount Prospect, IL, USA) in toluene (15:1 v:v). Flow Scint I and Flow Scint II were purchased from Radiomatic Instruments Inc. (Tampa, FL, USA).

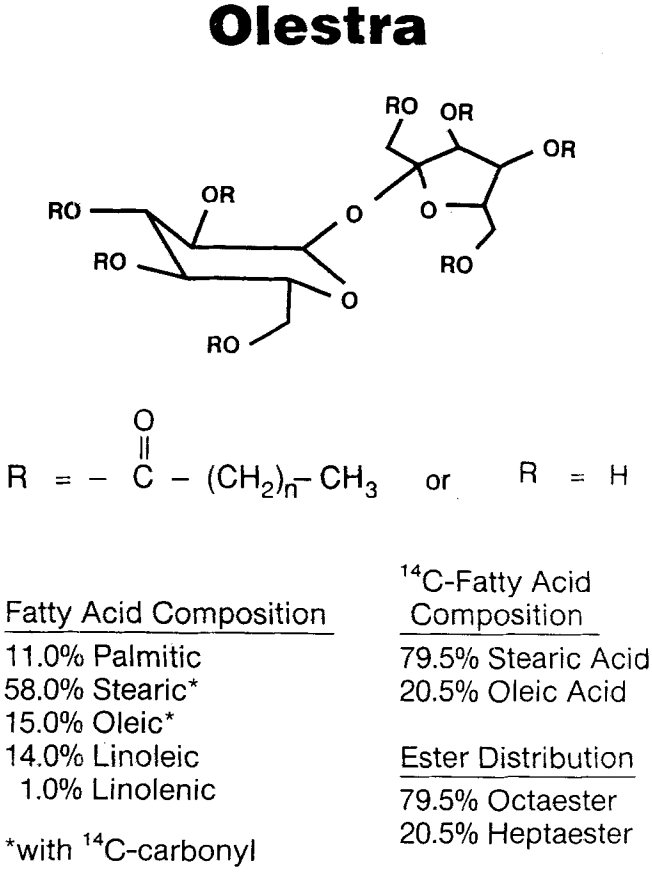

Fig. 1. Structure and characteristics of olestra used in this study.

High purity solvents were obtained from Burdick & Jackson (Muskegon, MI, USA). Unlabeled olestra was synthesized at Procter & Gamble Co. (Cincinnati, OH, USA). Oleic acid (99% free acid) and lecithin (99% type III-E from frozen egg yolk) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Nicotinamide, thiamine-HCl, riboflavin, pyridoxine-HCl, calcium D-pantothenate, cyanocobalamin, biotin, *p*-aminobenzoic acid, folic acid, casein hydrolysate and hemin were obtained from Sigma Chemical Co. Reagent grade sucrose, glucose, mercuric chloride, urea, sodium bicarbonate, potassium phosphate, sodium phosphate dibasic heptahydrate and sodium phosphate monobasic were obtained from Fisher Scientific Co. (Itasca, IL, USA). Sodium chloride was obtained from Baker (Phillipsburg, NJ, USA).

Media

Two media were utilized: a minimal medium (phosphate-buffered saline) and an organically-enriched medium described by Miller and Wolin [11]. The phosphate-buffered saline (PBS) consisted of 0.15 M sodium chloride buffered with 0.02 M sodium phosphate adjusted to pH 7.4. The enriched medium consisted of 50 g L^{-1} each of comminuted lettuce, celery, carrots and unsweetened applesauce in a bicarbonate-buffered (pH 7.2) salts medium amended with casein hydrolysate (2 g L^{-1}), urea (500 mg L^{-1}), sodium deoxycholate (350 mg L^{-1}), hemin (50 μg L^{-1}) and vitamins. Media were autoclaved and allowed to cool in an anaerobic chamber containing an atmosphere of 5% hydrogen, 10% carbon dioxide and 85% nitrogen. Prior to initiation of the experiments, the radiolabeled test materials were dissolved or dispersed into the media. Olestra and oleic acid were dispersed into the media at 50 °C by mixing liquified radiolabeled and unlabeled material with media and homogenizing the mixture with a Tekmar Model TDT Tissuemizer (Cincinnati, OH, USA) at high speed for 30 s. The test media consisted of: i) 50 mg sucrose in 100 ml PBS with 0.5 μCi ^{14}C-sucrose; ii) 50 mg glucose in 100 ml PBS with 0.5 μCi ^{14}C-glucose; iii) 30 mg oleic acid in 100 ml PBS with 3 μCi ^{14}C-oleic acid; iv) 100 mg olestra in 100 ml PBS with 3 μCi ^{14}C-olestra; v) 100 mg olestra in 100 ml enriched media with 3 μCi ^{14}C-olestra; vi) 90 mg olestra with 10 mg lecithin in 100 ml PBS with 3 μCi ^{14}C-olestra; and vii) 90 mg olestra with 10 mg lecithin in 100 ml enriched media with 3 μCi ^{14}C-olestra.

Inocula and incubation conditions

Seven healthy male humans consumed cookies containing olestra (9 g) daily for 21–31 days. At the end of this period, each subject provided a fecal sample. The entire stool was passed into a sterile plastic bag purged continuously with argon. The sample was immediately transferred to a Forma Scientific Model 1024 anaerobic chamber (Marietta, OH, USA), where all subsequent manipulations were performed. The feces were mixed with PBS at a ratio of 1 g wet feces

to 4 ml buffer and homogenized with a stomacher (Tekmar, Cincinnati, OH, USA).

Fifteen-milliliter samples of media containing the test substances described above were mixed with 5 ml of inoculum and placed into 60-ml serum bottles that were sealed with neoprene septa. These samples were incubated at 37 °C for 72 h. Each treatment was prepared in triplicate. In addition, an abiotic control, which was prepared as described above but also amended with mercuric chloride (2 mg per 20 ml) and autoclaved, was included for each treatment.

Analytical scheme

Figure 2 is a flowchart describing how the formation of potential biotransformation products was assessed. At the termination of the incubation, the contents of each serum bottle were acidified to pH 2 with 2 N sulfuric acid injected through the septum, and radiolabeled carbon dioxide and methane in the head space were quantified. A subsample (5 ml) of the incubation mixture was recovered for volatile fatty acids (VFA) analysis. Another subsample (15 ml) was extracted in a separatory funnel with three volumes of hexane:ethyl ether (1:1 v:v) six times. The combined extract was concentrated under vacuum and stored under nitrogen at −80 °C. Subsamples of this extract were analyzed by HPLC to determine the presence of ^{14}C-long chain fatty acids (LCFA) as well as to detect any changes in the ester distribution and fatty acid composition of the olestra.

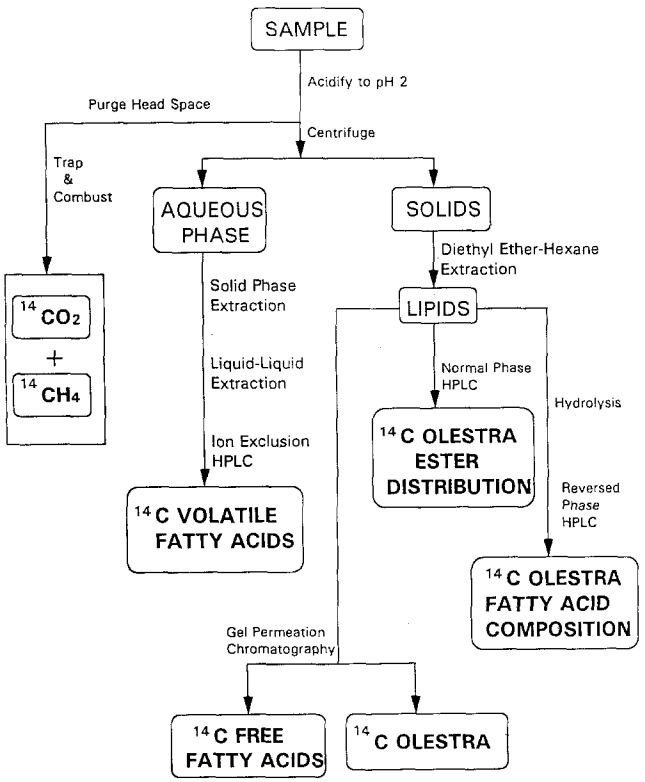

Fig. 2. Outline describing the scheme for analyzing olestra and biotransformation products following incubation with human fecal microflora.

Gas analysis

To measure the formation of $^{14}CO_2$ and $^{14}CH_4$ from the test substances, the head space of each bottle was purged with nitrogen via syringe needles inserted through the septa. The effluent gas was passed through two carbon dioxide traps in series then through a Vydac column packed with cupric oxide and heated to 800 °C in a tube oven to combust methane to carbon dioxide. The effluent gas was passed through two additional carbon dioxide traps. The carbon dioxide traps consisted of sealed gas collection tubes containing 20 ml of ethylene glycol monomethyl ether and ethanolamine (7:1; v:v). Ten milliliters from each trap was mixed with 10 ml of scintillation cocktail, and radioactivity was quantified by liquid scintillation counting.

Volatile fatty acid (VFA) analysis

A subsample of the incubation mixture was centrifuged, and the supernatant medium was analyzed for radioactivity by liquid scintillation counting. If radioactivity was present in this fraction, 1 ml of the supernatant was loaded onto a ChemElut column (Analytichem International, Inc., Harbor City, CA, USA), and the VFAs were eluted with 9 ml of ethyl ether. The VFAs were extracted from the ether with 0.2 N NaOH, which was acidified and analyzed by HPLC using an on-line radioactivity detector. The HPLC consisted of Waters Model 600E System Controller and Pumps (Milford, MA, USA), a Waters Model 700 Satellite WISP autosampler and tandem UV detector (214 nm) and Radiomatic Flo/One Beta radioactivity detector with a 2.5-ml flow-through liquid scintillation cell (Tampa, FL, USA). Separation was achieved with a Bio-Rad Aminex HPX-87H column with a HPX-85H guard column (Richmond, CA, USA) using 0.013 N H_2SO_4 with 5% acetonitrile as the mobile phase at a flow rate of 0.8 ml min^{-1}. Flow Scint II was used as the scintillation cocktail for the radioactivity detector and was mixed with the mobile phase at a 3:1 ratio. Typically, acetic acid eluted in approximately 11.0 min, while propionic and butyric acids eluted after 12.5 and 15.0 min, respectively.

Long-chain fatty acids (LCFA) analysis

The presence of free long-chain fatty acids that could result from the hydrolysis of olestra was determined by gel-permeation HPLC using on-line radioactivity detection. Olestra and fatty acids were separated based upon their molecular weights. The hexane/ether extracts of the olestra treatments were reconstituted in tetrahydrofuran and filtered (ARCO LC13; 0.45 μm pore size). The LC system consisted of a Varian Aerograph with a Rheodyne 7125 injector, a refractive index detector (Anspec) and a Flow/One Beta radioactive flow detector with a 2.5-ml cell. The separation was achieved with a PL-Gel column (60 × 0.75 cm; 5 μm, 100 A) and a 5-μm PL-Gel precolumn (Polymer Laboratories, Inc., Amherst, MA, USA). The mobile phase was tetrahydrofuran at 1 ml min^{-1}. Flo Scint I with a flow rate of 1 ml min^{-1} was the scintillation cocktail used with the detector. Olestra eluted as a single peak after 11.1 min, while free fatty acids eluted as a single peak after 14.1 min.

Ester distribution of olestra

A normal phase HPLC method was used to determine the ester distribution of the olestra. The extracts from the olestra treatments were reconstituted in hexane or chloroform, filtered (ARCO LC13; 0.45 μm pore size), and analyzed by HPLC. The HPLC system consisted of a Waters 600E System Controller and Pumps, and a Waters 700 Satellite WISP and an on-line Flo/One Beta radioactivity detector with a 2.5-ml flow-through cell. Separation of octa- and heptaester olestra components was achieved using a Zorbax Reliance 3 Silica column (4 × 0.6 cm; 3 μm) with a 3-μm guard column (Mac-Mod Analytical, Inc., Chadds Ford, PA, USA). The mobile phase was hexane with increasing amounts of methyl-*t*-butyl ether. The percent methyl-*t*-butyl ether in the mobile phase was 0% initially, increased to 5% after 5 min, 16% after 8 min, 25% after 10 min, 50% after 12 min, followed by a linear increase to 100% between 14 and 16 min. The flow rate was 3 ml min^{-1}. Flow Scint I with a flow rate of 3 ml min^{-1} was the scintillation cocktail used with the detector. Typical elution times were 8.5 min for the octaester and 10.5 min for the heptaester.

Fatty acid composition of olestra

Samples of the hexane/ether extracts from the olestra treatments were subjected to alkaline hydrolysis to cleave the fatty acids from the sucrose backbone. Samples were refluxed with 90% methanolic sodium hydroxide (3 N) at 100 °C for 2.5 h. After cooling, the samples were acidified with 6 N HCl and 5 ml each of water and chloroform were added. The aqueous-methanol phase was discarded and the chloroform phase containing the free fatty acids was recovered and dried under nitrogen and stored at −80 °C. This fraction was reconstituted in 1 ml of tetrahydrofuran and analyzed using a Waters 600E controller and pump system, a Waters 700 WISP and a Flo/One Beta radioactive flow detector. Separation was achieved with a Beckman Ultrasphere Octyl column (150 × 4.6 mm; 5 μm) (San Ramon, CA, USA) with a Brownlee Spheri-5 RP-8S guard column (30 × 4.6 mm; 5 μm) (Rainin Instrument Co., Inc., Woburn, MA, USA). The mobile phase was acetonitrile:tetrahydrofuran:0.1 N H_3PO_4 (50.4:21.6:28) at a flow rate of 1.5 ml min^{-1}. Flo Scint I with a flow rate of 3 ml min^{-1} was the scintillation cocktail used with the radioactivity detector. Oleic acid eluted after approximately 14.5 min, while stearic acid eluted after 21 min.

Hexane/ether extracts from the oleic acid treatment group also were analyzed using this HPLC methodology. Hydroxy stearic acid eluted at 3.8 min.

RESULTS

Table 1 shows the level of free ^{14}C-fatty acids and the ester distribution of olestra recovered following incubation with human fecal microflora. Analysis of the lipids extracted from the incubation mixtures indicated a complete absence of free ^{14}C-fatty acids in any olestra sample. No hydrolysis was observed when olestra was the sole carbon source or when it was in a nutritionally rich environment. Furthermore, emulsification of the olestra to increase its bioavailability did not result in metabolism. Hence, the fecal microflora of all seven subjects did not hydrolyze the sucrose–fatty acid ester bonds of olestra to release free fatty acids. This finding is confirmed by the analyses of the ester distribution of olestra recovered from the incubation mixtures. Overall, the relative abundance of octa- and hepta-esters was similar in the biologically-active treatments, abiotic treatments and starting materials.

To examine other possible biotransformations of the olestra molecule, the olestra recovered from the incubations was hydrolyzed to determine the effect of incubation on the fatty acid composition of the olestra (Table 2). Potential biotransformations could have included hydroxylation or reduction of the unsaturated fatty acid moeities. The fatty acid composition of olestra was similar in all treatments and was the same as the starting material. Hence, the fecal microflora did not modify the constituent fatty acids of olestra.

Table 3 shows the percent of radioactivity derived from the ^{14}C-test substances recovered as gas and VFAs from the seven subjects. $^{14}CO_2$ was the only radioactive gas detected. With the abiotic controls, a small fraction of radioactivity was detected in the gas phase, but no radiolabeled VFAs were formed. No significant levels (<0.1%) of ^{14}C-gases or VFAs were generated from olestra, either when it was the sole carbon source or when it was in a nutritionally rich environment. Furthermore, emulsification to increase bioavailability did not result in increased metabolism. In general, the small amount of radioactivity recovered in the gas phase of the biologically-active treatments was equal to or less than that recovered from the abiotic treatments.

On average, 57–60% of radiolabeled sucrose and glucose were recovered as VFAs and gas in the biologically-active samples. The remaining radioactivity was associated with the solids and presumably was in biomass. ^{14}C-Acetic, ^{14}C-propionic and ^{14}C-butyric acids were formed. The average ratio of acetic to propionic to butyric was roughly 2:1:1 on a molar basis.

Little or no ^{14}C-gas or VFAs were formed from radiolabeled oleic acid. However, >70% of oleic acid was biotransformed to hydroxy stearic or stearic acid (Table 4). The fecal flora of all subjects converted oleic acid to hydroxy stearic. Additionally, the flora of six of the seven subjects converted the oleic to stearic acid with varying degrees of efficiency. With the abiotic samples, reduced levels of oleic acid conversion were observed.

DISCUSSION

The results of this study indicate that human intestinal microflora are not capable of metabolizing olestra. To maximize sensitivity, the olestra utilized for this study was radiolabeled, and the label was localized in the most labile carbons, the fatty acid carbonyls. This provided an opportunity to detect formation of small amounts of free fatty acids resulting from hydrolysis of olestra as well as

TABLE 1

Level of ^{14}C-free fatty acid and the ester distribution of olestra following incubation (72 h; 37 °C) of neat and emulsified ^{14}C-(fatty olestra) with feces from seven human subjects in phosphate-buffered saline (PBS) and enriched medium

Test substance (medium)	Treatment	Free fatty acid	Ester distribution % Octa	% Hepta
Starting neat olestra		NA[b]	79.5 ± 1.2	20.5 ± 1.2
Neat olestra	abiotic[a]	ND[c]	75.3 ± 3.2	24.7 ± 3.2
(PBS)	bioactive	ND	75.5 ± 5.6	24.5 ± 5.6
Neat olestra	abiotic	ND	77.4 ± 1.9	22.6 ± 1.9
(enriched)	bioactive	ND	78.7 ± 2.8	21.3 ± 2.8
Starting emulsified olestra		NA	79.5 ± 1.2	20.5 ± 1.2
Emulsified olestra	abiotic	ND	76.4 ± 5.6	23.6 ± 5.6
(PBS)	bioactive	ND	77.6 ± 6.4	22.4 ± 6.4
Emusified olestra	abiotic	ND	77.3 ± 5.2	22.7 ± 5.2
(enriched)	bioactive	ND	78.1 ± 7.2	21.9 ± 7.2

[a] Autoclaved and amended with mercuric chloride.
[b] Not applicable.
[c] Not detected (<2% of olestra radioactivity converted to free fatty acid).

TABLE 2

The ^{14}C-fatty acid composition of neat and emulsified olestra incubated with feces from seven subjects in phosphate-buffered saline (PBS) and enriched medium

Test substance (media)	Treatment	Fatty acid composition % Oleic	% Stearic
Starting neat olestra		20.5 ± 2.7	79.5 ± 2.7
Neat olestra	abiotic	21.2 ± 2.0	78.8 ± 2.0
(PBS)	bioactive	20.6 ± 2.7	79.4 ± 2.7
Neat olestra	abiotic	20.2 ± 2.0	79.8 ± 2.0
(enriched)	bioactive	20.2 ± 2.0	79.8 ± 2.0
Starting emulsified olestra		18.9 ± 0.3	81.8 ± 0.3
Emulsified olestra	abiotic	19.4 ± 1.4	80.6 ± 1.4
(PBS)	bioactive	19.0 ± 0.9	81.0 ± 0.9
Emulsified olestra	abiotic	19.7 ± 1.6	80.3 ± 1.6
(enriched)	bioactive	19.4 ± 1.7	80.6 ± 1.7

changes in the fatty acid composition of olestra resulting from bacterial activity. Despite this sensitivity, no hydrolysis products or changes in olestra were detected. Although ester and glycosidic bonds are comparatively labile and amenable to enzymatic attack, those in olestra were not cleaved under the test conditions. Given the length and number of alkyl chains in the olestra molecule, it is likely that these bonds are sterically hindered and shielded from enzymatic attack. This finding is consistent with results obtained in vitro with mammalian lipases [8]. Thus, microbes would need to attack olestra in another manner. The alternative sites for attack are the alkyl portions of the fatty acid moieties. Since such an attack involves omega oxidation and requires molecular oxygen, it is unlikely to occur in the highly reduced environment of the human colon.

A very important factor affecting the metabolism of xenobiotic chemicals by microbial communities is adaptation, defined as a process whereby the rate or extent of metabolism is significantly increased as a result of prior exposure to the chemical [15]. Adaptation can result from derepression or induction of degradative enzymes not synthesized by the community prior to exposure, shifts in community structure in which populations with degradative abilities increase in dominance, and selection of organisms with new metabolic

TABLE 3

Recovery of ^{14}C-gases and ^{14}C-volatile fatty acids (VFAs) from ^{14}C-substrates incubated in phosphate-buffered saline (PBS) and enriched medium with fecal bacteria from seven human subjects

Substrate (media)	Treatment	Percent of substrate recovered as				Total percent metabolized
		Gas	Acetic acid	Propionic acid	Butyric acid	
Sucrose (PBS)	abiotic	1.10 ± 0.55[a]	ND[b]	ND	ND	1.10 ± 0.55
	bioactive	12.49 ± 3.02	20.33 ± 6.45	9.59 ± 3.98	13.11 ± 4.56	57.72 ± 15.25
Glucose (PBS)	abiotic	1.33 ± 0.92	ND	ND	ND	1.33 ± 0.92
	bioactive	12.39 ± 3.19	20.33 ± 6.45	11.62 ± 5.52	16.7 ± 5.13	59.85 ± 12.36
Oleic acid (PBS)	abiotic	0.18 ± 0.19	ND	ND	ND	0.18 ± 0.19
	bioactive	0.27 ± 0.25	ND	ND	ND	0.27 ± 0.25
Neat olestra (PBS)	abiotic	0.05 ± 0.04	ND	ND	ND	0.05 ± 0.04
	bioactive	0.08 ± 0.11	ND	ND	ND	0.08 ± 0.11
Neat olestra	abiotic	0.03 ± 0.03	ND	ND	ND	0.03 ± 0.03
(enriched medium)	bioactive	0.03 ± 0.04	ND	ND	ND	0.03 ± 0.04
Emulsified olestra	abiotic	0.02 ± 0.01	ND	ND	ND	0.02 ± 0.01
(PBS)	bioactive	0.01 ± 0.02	ND	ND	ND	0.01 ± 0.02
Emulsified olestra	abiotic	0.02 ± 0.01	ND	ND	ND	0.02 ± 0.01
(enriched medium)	bioactive	0.01 ± 0.01	ND	ND	ND	0.01 ± 0.01

[a] Mean ± standard deviation.

[b] Not detected (<0.02% of test substance converted to VFAs).

TABLE 4

Relative concentrations of ^{14}C-oleic acid, hydroxy stearic acid and stearic acid following incubation of free ^{14}C-oleic acid with feces from seven human subjects

Treatment	% Oleic	% Hydroxy stearic	% Stearic
Abiotic	63.7 ± 7.6 (54.2–74.3)	35.3 ± 6.7 (25.7–43.0)	1.0 ± 2.4 (0–6.8)
Bioactive	17.7 ± 6.4 (7.0–28.6)	61.5 ± 14.7) (34.9–87.1)	20.9 ± 15.2 (0–52.6)

Mean ± standard deviation.
(Range).

activities acquired through gene transfer or mutation. Previous studies have shown adaptation of intestinal microflora to cyclamates [14], 5-fluorouracil [4] and 1-nitropyrene [5]. In the present study, the intestinal microflora of seven subjects were provided an in vivo opportunity to adapt to olestra. However, after 21 to 31 days, no olestra metabolism was observed in any incubation. Considering the very rapid growth rates and great size and diversity of the bacterial communities in the human colon, this period represents a substantial opportunity for adaptation to occur.

Two other factors that can affect the microbial metabolism of a chemical are its bioavailability and the nutritional environment of the microbes. To ensure that negative findings were not the result of olestra being unavailable for catabolism, olestra was presented to the fecal bacteria in a highly dispersed neat form and as an emulsion with phospholipid. Neither form of olestra was degraded or otherwise transformed. Biotransformation occurs when a material is utilized as a carbon and energy source or when it is gratuitously cometabolized during the catabolism of other substrates. The presence of other carbon sources in the media could provide the additional nutrients required to support growth of biotransforming populations as well as fuel cometabolism. Alternatively, they could prevent metabolism through catabolite repression and inhibition. As a consequence, both neat and emulsified olestra were tested in minimal and highly-enriched media to cover both contingencies. Again, no biotransformation was observed under any test condition.

This work is consistent with previous findings that metabolism of LCFAs by colonic bacteria is limited to hydroxylation and hydrogenation of unsaturated fatty acids [2,13,16]. Despite the existence of anaerobic consortia in nature that completely mineralize LCFAs [10,17], no such consortia appear to be present in human feces. This finding is somewhat surprising given the nutritional value of LCFAs and the diversity and size of the colonic bacterial community. However, it is consistent with previous in vitro studies as well as in vivo fat balance studies [1,2,13,16,18]. Based upon these data, one might conclude that the short residence time

for bacteria and LCFAs in the colon is insufficient for selection and establishment of LCFA-mineralizing consortia. If such was the case, these same conditions also would select against populations that degrade olestra.

In the future, other nonabsorbable food substitutes will likely be developed. While such materials may not be digested by humans, they may be susceptible to microbial transformation and represent a previously nonexistent nutrient for colonic bacteria. Hence, the toxicological as well as nutritional profile of such materials will depend upon knowing how they are metabolized and biotransformed by colonic bacteria. This study provides a model for how such information can be developed. Key considerations for such studies must include the use of several subjects, an opportunity for the microflora to adapt to the compound and the ability to detect low levels of transformation. The latter can be achieved by utilizing radiolabeled materials that are labeled in the most labile portions of the molecule.

In conclusion, extensive testing under low and highly-enriched nutrient conditions with acclimated intestinal bacteria from several subjects indicates that olestra is not metabolized by the microflora of the human intestine. This inability is probably related to the inaccessibility of the ester and glycosidic bonds for enzymatic hydrolysis rather than absence of degrader populations, nutritional conditions or low substrate bioavailability in the test systems.

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