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Effect of a High-Beef Diet on the Fecal Bacterial Flora of Humans[1]

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SUMMARY

Ten human volunteers completed a 4-month diet series consisting of 1 month each of a control diet, a meatless diet, a high-beef diet, and the same control diet. Fat and fiber contents were essentially the same in all four diets, but protein content was doubled during the high-beef diet. During the 4th week on each diet, three stool specimens collected from each volunteer were analyzed for chemical composition and content of facultative, aerobic, and anaerobic bacteria. The bacteriological data are presented in this paper.

High beef protein consumption had little effect on the composition of the intestinal flora. There were no significant differences in total counts of facultative and aerobic or anaerobic organisms in the feces when volunteers were on meatless or high-beef diets. At the species level, when counts during the two control diets were comparable, in only three instances did the change from the meatless to a high-beef diet significantly influence the bacterial numbers. The ratio of mean counts of anaerobic to facultative and aerobic organisms was approximately 15:1 during the meatless diet and 34:1 during the high-meat diet. The data indicate that animal protein consumption has little effect on the fecal bacterial profile in humans.

INTRODUCTION

A number of observations indicate that the incidence of colon cancer in a population may be related to dietary habits (7, 8, 19). In the industrialized countries of northwest Europe and North America, where a great deal of animal fat and protein are consumed, the incidence of colon cancer is much higher than in the developing countries of Africa, South America, and Asia where much less meat is consumed and the diet contains a higher proportion of vegetable fiber (3).

Hill *et al.* (11) examined the fecal flora of individuals living in areas at high risk and at low risk for colon cancer. The same broad groups of bacteria were found in the feces from all the populations studies. However, British and American subjects, representing high-risk populations, yielded many more anaerobes than did Ugandans, Indians, and Japanese, representing low-risk populations. Conversely, the Ugandans, Indians, and Japanese had many more facultative and aerobic bacteria in their feces than did the British and Americans. Thus, the ratio of anaerobes to aerobes was much higher in people living on a Western high-risk diet than in those subsisting on a vegetarian diet.

Similar observations were made by Reddy *et al.* (16) in a recent study with human volunteers on mixed Western, high-meat, high-fat, and nonmeat diets. The anaerobic microflora count in the feces of the volunteers was significantly greater during the mixed Western diet, when large quantities of animal fat were consumed, than during the nonmeat diet. The 2 diets contained nearly the same amount of protein.

As a result of these observations, it was proposed that the typical high-meat diet of Western peoples supports a microbial flora that is capable of converting food and intestinal secretions into carcinogenic or cocarcinogenic agents. A systematic study was initiated to examine specifically the effect of a diet high in beef protein on the bacterial and chemical composition of the feces of humans. The fat content of the test diet was essentially the same as that of the control diet. The bacteriological data from this study, the subject of this report, show that few changes in fecal flora composition occurred as a result of high beef protein consumption.

MATERIALS AND METHODS

Details of the procedures used in administration of the diets and determination of the fecal flora were published previously (14). In brief, 10 male graduate and medical student volunteers were placed on a 4-month diet series consisting of 1 month each of a control "typically American" diet, a meatless diet, a high-beef diet, and again the control diet. The diets were carefully formulated so that the fat and fiber contents were held essentially constant throughout but total protein was doubled during the high-beef diet. The composition of the diets is given in Table 1. Table 2 provides a comparison of the amounts of protein, carbohydrate, fat, fiber, calories, ash, calcium, and magnesium found in the 3 diets. This information also appears in another paper (5). During the 4th week of each diet, 3 fecal samples collected from each volunteer were homogenized and processed in the bacteriology laboratories. Each of the 120 samples obtained during the study was placed on an anaerobic glovebox isolator (1), and serial 10-fold dilutions were prepared. The dilutions were then plated on a battery of prereduced media. Colonies were counted and approximately 35 to 40 representative types were picked from the

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Table 1

Constant diets served 4 weeks each

Control	g	Meatless	g	High beef	g
		Morning			
Milk (2%)	240	Milk (2%)	120	Milk (2%)	240
Bread	50	Bread	50	Bread	50
Margarine	10	Margarine	10	Margarine	10
Orange juice	100	Orange juice	100	Orange juice	100
Cornflakes	30	Rice Krispies	30	Beef patty	80
Jelly	14	Jelly	14	Bran flakes	33
				Jelly	14
		Noon			
Milk (2%)	240	Bread	50	Milk (2%)	240
Cheese (American)	40	Margarine	15	Bread	25
Bread	50	Cottage cheese	120	Roast beef (cooked)	100
Peas	80	Egg (1)	50	Margarine	10
Pears	100	Peas	100	Beef patty	100
Margarine	20	Pears	100	Green beans	100
Angel cake	45	Peaches	50	Pears	80
Lemonade	30	Cranberry sauce	20	Peaches	80
		Angel cake	90	Sugar cookies	20
		Cream	15		
		Lemonade	30		
		Evening			
Milk (2%)	240	Milk (2%)	240	Milk (2%)	240
Bread	25	Bread	50	Roast beef	200
Margarine	20	Egg whites	100	Potato (dry)	25
Beef (roast)	80	Cheese	25	Peas	100
Potato (dry)	25	Potato (dry)	25	Angel cake	60
Green beans	100	Green beans	100		
Peaches	100	Ice cream (vanilla)	100		
Angel cake	45	Margarine	10		
Cranberry sauce	20				
		p.m. snack			
Vanilla wafers	33	Vanilla wafers	33	Graham crackers	20
7-Up	360	7-Up	360	7-Up	360

Table 2

Dietary nutrients determined by calculation and chemical analyses

	Calories	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Ash (g)	Calcium (mg)	Magnesium (mg)
Control diet								
Calculated	2560	86	81	373	6.3	20.0	1587	348
Analyzed	2385	80	61	380	6.4	20.0	1343	287
Meatless diet								
Calculated	2650	90	81	391	6.3	18.8	1180	294
Analyzed	2495	82	72	381	5.3	18.9	1101	265
High-beef diet								
Calculated	2680	176	87	299	6.3	20.3	1117	499
Analyzed	2560	179	78	286	7.0	20.2	1137	393

anaerobic plates and identified (12, 18). The dilutions were also plated for isolation, enumeration, and identification of facultative and aerobic organisms. From each sample, approximately 35 representative facultative or aerobic colony types were identified (6, 13).

Counts of each organism obtained from the 3 fecal specimens from each volunteer were averaged. These values were again averaged to obtain a mean ± S.E. for each organism for the 4 diets. The Wilcoxon sign-ranked test was used, at the 5% level, to determine the significance of the difference in counts between diets because of great variation in counts from specimen to specimen.

RESULTS

The mean counts ± S.E. for each of the major groups and genera of fecal organisms isolated from the volunteers under the different dietary conditions are given in Tables 3 and 4. There were great variations in counts obtained from the different individuals as is reflected by the large values of the standard error for the various organisms. Despite these variations, with the exception of clostridia and the filamentous fungi, the mean counts of the organisms were remarkably similar under all dietary conditions. Filamentous fungi were isolated from the volunteers early during the course of

Table 3

Effect of diet on counts of major facultative and aerobic organisms isolated from feces

Organism	Control 1	Meatless	High-beef	Control 2
Gram-negative rods	$1.60 \pm 0.70 \times 10^{8}$[a]	$4.11 \pm 2.56 \times 10^{8}$	$1.47 \pm 0.77 \times 10^{9}$	$9.61 \pm 3.48 \times 10^{7}$
Lactobacillus	$1.87 \pm 1.64 \times 10^{7}$	$2.32 \pm 1.88 \times 10^{7}$	$2.30 \pm 1.45 \times 10^{6}$	$1.07 \pm 0.62 \times 10^{7}$
Streptococcus	$4.34 \pm 2.21 \times 10^{7}$	$3.39 \pm 1.87 \times 10^{7}$	$1.09 \pm 0.58 \times 10^{8}$	$1.55 \pm 0.79 \times 10^{7}$
Bacillus	$1.82 \pm 0.40 \times 10^{5}$	$2.06 \pm 0.58 \times 10^{5}$	$2.93 \pm 1.06 \times 10^{5}$	$2.03 \pm 0.56 \times 10^{5}$
Staphylococcus	$6.64 \pm 2.06 \times 10^{3}$	$1.07 \pm 0.64 \times 10^{4}$	$1.09 \pm 0.63 \times 10^{4}$	$4.38 \pm 0.27 \times 10^{4}$
Yeasts	$3.12 \pm 1.51 \times 10^{3}$	$1.26 \pm 1.04 \times 10^{4}$	$3.53 \pm 2.07 \times 10^{3}$	$5.61 \pm 4.34 \times 10^{3}$
Filamentous fungi	$1.60 \pm 1.60 \times 10^{1}$	$4.76 \pm 2.06 \times 10^{3}$	$1.00 \pm 1.40 \times 10^{0}$	0
Total	$4.23 \pm 2.48 \times 10^{9}$	$8.26 \pm 2.73 \times 10^{9}$	$5.65 \pm 2.01 \times 10^{9}$	$3.94 \pm 1.82 \times 10^{9}$

[a] Mean ± S.E. for 10 subjects, expressed per g feces dry weight.

Table 4

Effect of diet on counts of major anaerobic organisms isolated from feces

Organism	Control 1	Meatless	High-beef	Control 2
Bacteroides	$3.91 \pm 1.62 \times 10^{10}$[a]	$4.47 \pm 1.52 \times 10^{10}$	$1.06 \pm 0.30 \times 10^{11}$[b]	$1.30 \pm 0.35 \times 10^{11}$[c]
Eubacterium	$8.85 \pm 5.94 \times 10^{9}$	$3.08 \pm 1.37 \times 10^{10}$	$3.48 \pm 0.99 \times 10^{10}$	$2.36 \pm 0.74 \times 10^{10}$[c]
Propionibacterium	$3.15 \pm 2.32 \times 10^{8}$	$2.65 \pm 1.61 \times 10^{10}$	$2.12 \pm 0.46 \times 10^{10}$	$1.85 \pm 1.63 \times 10^{10}$
Bifidobacterium	$1.35 \pm 0.35 \times 10^{10}$	$1.98 \pm 0.76 \times 10^{10}$	$1.05 \pm 0.49 \times 10^{10}$	$1.63 \pm 0.58 \times 10^{10}$
Fusobacterium	$1.08 \pm 0.71 \times 10^{9}$	$2.85 \pm 1.31 \times 10^{9}$	$8.01 \pm 3.72 \times 10^{9}$	$4.04 \pm 3.22 \times 10^{9}$
Clostridium	$8.38 \pm 3.00 \times 10^{4}$	$3.41 \pm 3.40 \times 10^{7}$	$1.43 \pm 1.14 \times 10^{6}$	$7.41 \pm 7.41 \times 10^{8}$[c]
Peptostreptococcus	$2.55 \pm 1.51 \times 10^{9}$	$6.64 \pm 3.28 \times 10^{9}$	$3.92 \pm 2.05 \times 10^{9}$	$1.01 \pm 0.70 \times 10^{9}$
Total	$7.39 \pm 1.94 \times 10^{10}$	$1.21 \pm 0.15 \times 10^{11}$	$1.94 \pm 0.29 \times 10^{11}$	$2.17 \pm 0.45 \times 10^{11}$[c]

[a] Mean ± S.E. for 10 subjects, expressed per g feces dry weight.
[b] Significantly different from value obtained during the meatless diet.
[c] Significantly different from value obtained during Control 1 diet.

the study but were rarely found during the high-beef diet and were not isolated from any of the volunteers during the 2nd control diet. The values for the clostridial counts were erratic. The organisms were occasionally isolated from a few of the volunteers in very high numbers, but this occurred irregularly. When values obtained during the meatless and high-beef diets were compared, few major differences could be found. The *Bacteroides* count was significantly greater during the high-beef diet than during the meatless diet ($p < 0.01$), but the importance of this observation must be questioned since an even greater increase was noted between the initial and final control diets (Table 3). Significantly greater counts during the final control diet compared with the initial control diet were also observed with the genera *Eubacterium* ($p = 0.01$) and *Clostridium* ($p = 0.02$) and with the total anaerobic count ($p = 0.01$). The gradual increase in the mean anaerobic count that occurred as the volunteers progressed through the diet series cannot be explained. It was observed twice when the diets were administered at different times to 2 groups of 5 volunteers. The ratio of mean total anaerobic organisms to mean total facultative and aerobic organisms in the feces of the volunteers was approximately 15:1 during the meatless diet and 34:1 during the high-beef diet.

Dietary change had little effect on the counts of the 84 species and subspecies of fecal organisms identified. Where tests for significance could be performed, differences in counts between meatless and high-beef diets were observed in only 5 instances. The organisms that were affected are listed in Table 5. With *Bacteroides fragilis* ss. *fragilis* and *Clostridium*, species unidentified, the differences in counts observed between the 2 control diets were greater than the differences in counts between the meatless and high-beef diets. The importance of these observations is therefore questionable. With the other 3 organisms, there was no significant difference in counts between the 2 control diets. Total counts of *B. fragilis*, ss. *vulgatus*, and *B. fragilis*, subspecies undetermined, were significantly higher ($p < 0.05$) in the feces of the volunteers during the high-beef diet than during the meatless diet. By contrast, counts of *Bifidobacterium adolescentis* var. A were significantly lower ($p < 0.01$) during the high-beef diet than during the meatless diet. In most cases, however, tests for significance could not be performed on the individual species and subspecies because of their irregular presence in the feces of the volunteers.

DISCUSSION

The results of this study differed from those reported earlier by Hill *et al.* (11) and Reddy *et al.* (16). We varied the dietary protein and held the fat constant, while Reddy *et al.* (16) varied the fat and held the protein constant. We found no significant differences in total numbers of anaerobic organisms or facultative and aerobic organisms during the meatless and high-beef diets, suggesting that high animal protein intake does not appreciably affect the composition of the intestinal flora. When counts during the 2 control diets were similar, in only 3 instances, at the species level, were there significant differences during the meatless and high-beef diets. However, the importance of this finding must be interpreted cautiously. The irregular presence of the organisms in the feces at the species level precluded uniform testing for significance. A small increase in the ratio of anaerobic bacteria to facultative and aerobic bacteria occurred during the high-beef diet as compared with the meatless diet, but the increase was not of the magnitude reported by Hill et al. (11). Our data lend support to the view that animal protein consumption has little effect on the fecal bacterial profile in humans.

However, we examined only the effect of high-meat protein consumption on the composition of the feces of hu-

Table 5

Effect of diet on counts of selected species isolated from feces

Organism	Control 1	Meatless	High-beef	Control 2
B. fragilis, other	$1.12 \pm 0.43 \times 10^{10}$[a]	$4.59 \pm 1.98 \times 10^{9}$	$1.49 \pm 0.96 \times 10^{10}$[b]	$9.81 \pm 2.43 \times 10^{9}$
B. fragilis vulgatus	$2.55 \pm 1.83 \times 10^{9}$	$2.01 \pm 0.81 \times 10^{9}$	$3.09 \pm 0.84 \times 10^{10}$[b]	$3.39 \pm 1.73 \times 10^{10}$
B. fragilis fragilis	$1.83 \pm 1.66 \times 10^{8}$	$5.48 \pm 2.58 \times 10^{9}$	$2.52 \pm 1.27 \times 10^{10}$[b]	$4.62 \pm 2.90 \times 10^{10}$[c]
B. adolescentis A	$5.85 \pm 3.05 \times 10^{9}$	$1.21 \pm 0.42 \times 10^{10}$	$2.29 \pm 1.73 \times 10^{9}$[b]	$6.95 \pm 2.75 \times 10^{9}$
Clostridium, unidentified	$8.38 \pm 3.00 \times 10^{4}$	$1.59 \pm 0.60 \times 10^{5}$	$1.38 \pm 1.12 \times 10^{8}$[b]	$7.41 \pm 7.41 \times 10^{8}$[c]

[a] Mean ± S.E. for 10 subjects, expressed per g feces dry weight.
[b] Significantly different from value obtained during the meatless diet.
[c] Significantly different from value obtained during Control 1 diet.

mans. In contrast, some studies indicate that animal fat consumption influences the composition of the flora and the concentration of steroids in the feces (9). High fecal acid steroid concentration and large bowel cancer risk in a population appear to be related (10, 17). Hill *et al.* (11) and Reddy and Wynder (17) have speculated that high fat intake changes the composition of fecal steroids and modifies the bowel flora, which in turn produces carcinogenic substances from the steroids. It appears from these studies that animal fat rather than animal protein is the dietary component involved in the induction of colon cancer.

Attempts to demonstrate differences in flora composition between individuals at high and low risk for colon cancer have not been uniformly successful. Recent studies (2, 4, 15) suggest that an examination of the metabolic activities of the intestinal bacteria of individuals at different risks for colon cancer might prove to be more fruitful. The composition of the intestinal flora of high-risk populations, including polyp patients, North Americans, and Japanese-Americans on Western type diets, was compared with the composition flora of the low-risk populations, including rural Japanese, Africans of the Tswana tribe, and Japanese-Americans eating a traditional Japanese diet (4, 15). There were no striking differences in the types of intestinal bacteria present in the different populations. Although there were some minor quantitative variations (the low-risk groups tended to maintain a higher concentration of a few species of bacteria in their feces than did the high-risk groups), no organisms characteristic of high or low risk groups were detected. Therefore, taxonomic grouping of bacteria may be inadequate when measuring the effect of diet on the intestinal flora. It appears that the metabolic activities of the bacteria, regardless of species, need to be examined under different dietary conditions in order to understand the role of bacteria in the etiology of colon cancer.

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