



Colonization of Beneficial Microflora in the GUT of Indian Adult Females (35-50 yrs) as Affected by Dietary Fibre Intakes.

Short Running Title: Gut Health and Dietary Fiber

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ABSTRACT

BACKGROUND AND OBJECTIVES: Probiotics and prebiotics have recently been recognized as important functional foods that enhance the establishment of beneficial bacteria in the human gut which helps in the prevention of life threatening disease. In view of this, the present study was undertaken to study the consumption pattern of prebiotic and probiotic foods and determining the gut health of normal adult females (35-50 yrs) of Urban Vadodara.

METHODS: The consumption pattern of prebiotic and probiotic foods were studied using food frequency questionnaire and seven day estimated record method. Subjects were identified with frequent and least frequent intakes of these foods and their stool samples were analyzed and enumerated for beneficial micro organisms i.e. Bifidobacterium and Lactic acid bacteria and pathogenic micro organisms i.e. E. coli and Bacteriodes. **RESULTS:** Young adult females were found to have a frequent consumption of prebiotic and probiotic foods. Subjects taking curd more frequently reported lower occurrence of constipation and higher occurrence of flatulence. High consumption of dietary fibre also significantly increased the occurrence of flatulence. Results revealed that high consumption of onion and garlic did not significantly affect the occurrence of constipation and flatulence. Subjects consuming dietary fibre frequently showed higher counts of beneficial micro organisms and less of the pathogenic micro organisms. **CONCLUSION:** Consumption of more than 10 prebiotic and probiotic foods at least weekly helps to establish significantly higher number of beneficial micro organisms and decreased colonization of pathogenic micro organisms.

KEYWORDS: Constipation, Dietary fibre, Flatulence, Gut health, Probiotics, Prebiotics

INTRODUCTION:

Due to increasing urbanization globally, there has been an alarming "nutrition transition" with an increasing consumption of processed and refined foods and a clear shift from a diet rich in fiber, minerals and vitamins towards one rich in energy, saturated fats and cholesterol¹. This has led to an increase in the incidence of various chronic diseases of middle and later adult life. Several health benefits of dietary fibre many of which are prebiotics and probiotics have been documented in the literature such as improved lactose tolerance, supply of SCFA as energy substrates for the host bacteria, antitumor properties, neutralization of certain toxins, stimulation of the intestinal immune system and reduction of blood lipid levels^{2,3}. But little research investigating the frequency of its consumption in Indian population has been published. The aim of this study was to investigate the consumption pattern of probiotics and dietary fibre rich foods and determine the gut health of normal adult females (35-50 yrs) in terms of beneficial microflora namely the *Lactic acid bacteria* and *Bifidobacterium*.

METHODS AND MATERIALS:

STUDY DESIGN AND SELECTION OF SUBJECTS:

Hundred normal adult females (35-50 yrs) willing to participate in the study were selected from four different zones of urban Baroda using purposive sampling technique. Permission to undertake the study was obtained from the institute and approval was also sought from the institutional ethics committee of The M.S. University of Baroda (F.C.Sc./FND/ME 27) dated 25.10.07. Written informed consent was obtained from every subject who participated in this study.

BASELINE AND SES SURVEY:

A survey was carried out to obtain the data pertaining to socio-economic status (name, age, type of family, total number of family members, religion, total family monthly income, marital status), physical activity pattern, anthropometric measurements and past morbidity profile using a pretested semi structured questionnaire. The activity pattern was studied with the help of a checklist of light, moderate and heavy activities and the subjects were classified into sedentary, moderate and heavy workers⁴. For Past morbidity profiles, responses on the

illness suffered in the past one month was obtained from the subjects with respects to, gastrointestinal problems, cardiovascular problems, psychological problems, liver disease and general illness such as a cough, cold, fever and skin infection. Weights and heights of all the 100 subjects were taken and their BMI was calculated.

DIETARY INTAKES:

To study the consumption pattern of dietary fibre and probiotic foods, information was collected using a food frequency questionnaire. The dietary intake of 20 selected subjects was done using the seven day estimated record method⁵. The intakes of energy, protein, fat, crude and dietary fibre intake were calculated using the tables of NIN/ICMR (1999)⁶.

CLASSIFICATION OF SUBJECTS ON THE BASIS OF FOOD FREQUENCY METHOD AND THEIR SELECTION FOR DETERMINATION OF GUT MICROFLORA:

On the basis of the data obtained through the food frequency method questionnaire, the subjects were categorized into those consuming dietary fibre and probiotic foods most frequently (daily consumption of curd and 18-20 fibre rich foods at least weekly) and least frequently (twice a week consumption of curd and ≤ 10 fibre rich foods at least weekly). Ten subjects from these categories were subjected to determination of gut micro flora.

ENUMERATION OF THE GUT MICRO FLORA:

The determination of the gut micro flora was carried out in terms of two beneficial probiotic organisms- *Lactic acid bacteria*, *Bifidobacterium*; and two pathogenic micro organisms such as *E. coli* and *Bacteriodes*⁷. The media used for the enumeration of *Bifidobacterium* and *Bacteriodes* were Hi media bifidobacterium agar and Hi media bacteriodes bile esculin agar respectively. The enumeration of *Lactic acid bacteria* and *E. coli* was done using ready made Hi media flexi plates which used the tomato juice agar and modified tergitol 7 agar respectively. The anaerobic conditions inside the anaerobic jar were ensured using an LE001B indicator tablet and LEO02F Hi media anaerogas pack 1.5 L.

RESULTS:

SOCIO ECONOMIC PROFILE AND ACTIVITY PATTERN:

Most subjects (41%) were in the age group of 46-50 years of age. Of the total subjects, 62% belonged to Hindu religion and the remaining were non Hindus. Majority belonged to nuclear families and the per capita

income of about 51% of the subjects ranged between Rs. 3,000 to Rs. 7,000 per month. The activity pattern of the subjects showed that almost 70% had a sedentary lifestyle.

DIETARY HABITS:

Seventy one of the subjects were vegetarians, 50% of the subjects observed 'fasts' ranging from once or twice a week to occasionally and took either nutrient supplements, herbal or a ayurvedic supplement.

CONSUMPTION PATTERN OF DIETARY FIBRE:

Forty six percent subjects consumed dietary fibre frequently (18-20 fibre rich foods at least weekly) while 28% had a least frequent consumption (≤ 10 fibre rich foods at least weekly). Subjects from joint families consumed dietary fibre more frequently than those nuclear families and this difference was not found statistically significant ($P \leq 0.05$).

DIETARY FIBRE CONSUMPTION AND REPORTED GI TRACT CHANGES:

Majority (76%) of the population suffering from constipation had a frequent consumption of dietary fibre. High consumption of dietary fibre significantly increased the occurrence of flatulence.

CONSUMPTION PATTERN OF CURD ONION, AND GARLIC:

As seen in Table I, II a & II b, 31-42% consumed curd, onion and garlic more frequently. The following trend was observed with regards to consumption of curd, onion and garlic on two GI problems i.e. constipation and flatulence. Constipation problem was reported to occur less in subjects (70%) who consumed curd more frequent to frequent (Table I) whereas excess consumption of curd caused flatulence. Onion consumption and reported GI tract changes: More frequent consumption of onion showed no occurrence of constipation in 45% subjects and among those who did not experience majority of them (76%) had frequent intake of onion. Garlic consumption and reported GI tract changes: Garlic consumption was negatively correlated to constipation. Majority of the subjects (78%) who suffered from flatulence had more frequent to frequent consumption of garlic.

However the association of consumption of curd, onion and garlic on the occurrence of constipation and flatulence was not statistically significant.

NUTRIENT INTAKE OF SUBJECTS:

As shown in Table III, the intakes of all the nutrients were higher in the group with a higher frequency of consumption of dietary fibre and probiotic foods. This

difference was statistically significant for intakes of energy, fat, protein, crude fiber and dietary fibre in groups

GUT MICRO FLORA OF SELECTED SUBJECTS AS AFFECTED BY DIETARY FIBRE AND PROBIOTIC FOOD INTAKES:

The counts for *Bifidobacterium* and *Lactic acid bacteria* were higher in the subjects with frequent consumption of prebiotic and probiotic foods, whereas counts for *E. coli* were higher in the group with the least frequent consumption of prebiotic and probiotic foods (Table IV). A positive correlation ($r=0.0215$; $r = 0.424$) between physical activity and the establishment of the beneficial bacteria i.e. *Bifidobacterium* and *Lactic acid bacteria* was seen, whereas for *E.coli* and *Bacteriodes*,

there was an inverse correlation ($r = -0.277$; $r = -0.328$) for physical activity respectively. There existed a positive correlation between the dietary fibre intake and the establishment of the beneficial bacteria i.e. *Bifidobacterium* and *Lactic acid bacteria*. The odds of having low counts of beneficial micro organisms i.e. *Bifidobacterium* and *Lactic acid bacteria* when the dietary fibre intake is low were found to be high (OR=2). The odd of having higher numbers of *bifidobacterium* and *bacteriodes* when the protein intake is more was high. In case of high fat intake the odds of having more amount of *bifidobacterium*, *Lactic acid bacteria* and *bacteriodes* was high

Problem	More frequent consumption (28)		Frequent consumption (46)		Least Frequent consumption (46)		Chi-square χ^2
	N	%	N	%	N	%	
Constipation (24)	8	33.33	9	37.5	7	29.16	0.306
No Constipation (76)	30	39.47	23	30.26	23	30.26	N.S.
Flatulence (36)	14	38.88	13	36.11	9	25	0.706
No Flatulence(64)	24	37.50	19	29.68	21	32.81	N.S

Table I: Consumption pattern of Curd and occurrence of Constipation and Flatulence among the subjects (adult females)

* - Significant at $p<0.25$, N.S. – Not significant.

Problem	More frequent consumption (28)		Frequent consumption (46)		Least Frequent consumption (46)		Chi-square χ^2
	N	%	N	%	N	%	
Constipation (24)	8	33.33	9	37.5	7	29.16	0.911
No Constipation (76)	34	44.73	26	34.21	16	21.05	N.S.
Flatulence (36)	15	41.66	10	27.77	7	19.44	1.281
No Flatulence(64)	27	39.70	25	36.76	16	23.52	N.S

Table II. a: Consumption pattern of Onion and occurrence of Constipation and Flatulence among the subjects (adult females)

* - Significant at $p<0.25$, N.S. – Not significant.

Problem	More frequent consumption (28)		Frequent consumption (46)		Least Frequent consumption (46)		Chi-square χ^2
	N	%	N	%	N	%	
Constipation (24)	6	25.11	10	41.66	8	33.33	2.158
No Constipation (76)	32	42.10	22	28.94	22	28.94	N.S.
Flatulence (36)	7	33.33	16	44.44	8	22.22	3.85*
No Flatulence(64)	21	40.62	16	25	22	34.37	

Table II.b: Consumption pattern of Garlic and occurrence of Constipation and Flatulence among the subjects (adult females)

* - Significant at $p<0.25$, N.S. – Not significant.

Nutrients	Frequent to less Frequent consumption (n=10)	Least Frequent consumption (n=10)	't' value
Energy (Kcal)	1721± 209.88	1486.3±188.94	2.631**
Protein (g)	54.43±6.29	46.42±7.07	2.610**
Fat (g)	49.4±5.88	42.44±4.82	2.90***
Crude Fiber (g)	10.42±1.70	8.78±0.96	2.72**
Dietary Fiber(g)	45.32±6.65	38.98±3.95	2.615**

Table III: Nutrient intake of subjects consuming Prebiotic and Probiotic foods frequently and least frequency.

Significant at p<0.005, *- Significant at p<0.01

Micro organisms	Frequent consumption (n=10)	Least Frequent consumption (n=10)	't' value
Bifidobacterium bifidum	4.7001±23	4.3659±0.29	2.141**
Lactic acid bacteria	4.4245±0.44	4.2707±0.40	0.816N.S.
E.coli	4.2446±0.36	4.5774±0.38	1.88*
Bacteriodes	3.2655±0.35	3.2832±0.19	0.166 NS

Table IV: Gut Micro flora of the subject consuming Prebiotic and Probiotic foods frequently and least frequently.

*Significant at p<0.05, **- Significant at p<0.1, N.S. – Not significant.

DISCUSSION:

In this study, the dietary fibre intake among women ranged from 38.9-45.2 gms/day, whereas the crude fibre intake was found to be around 8.7-10.4 gms per day. However, higher intakes (55-120 g) have been reported in the habitual Indian diets⁸. This shows that there has been a decline in the fibre intake over a period of time. According to NIN/ ICMR (1999), the desirable level of daily dietary fibre intake by an adult is generally believed to be around 40g which shows that the fibre intake in the Indian diets are still within the normal range as compared to the fibre content of diets in western countries which varies from 5-25 gm/day. Fiber intake in the UK is about 14 g a day⁹ and in the USA it is about 13 g/day¹⁰. Relationship of meal pattern with the consumption pattern of dietary fibre among subjects showed that the vegetarians consumed dietary fibre more frequently than the non vegetarians, though the difference was statistically insignificant. A study by Alexander, Ball and Mann (1994) showed that the dietary fibre intake was higher in the vegetarians as compared to omnivore adults residing in New Zealand¹¹. The effect of per capita income on the consumption pattern of dietary fibre showed that the majority of the subjects had their capita income ranging from Rs 3000 - Rs.7000. There was a difference seen in the consumption pattern in various income groups. Due to high purchasing power, there is a more frequent consumption of dietary fibre (59-61 g/d) among high income and middle income. The BMI of the subjects and consumption pattern of dietary fibre showed that the odds of having low dietary fibre intake when the BMI of the subjects is low are high. A

study by Cani et al 2006 showed that the intake oligofructose increased the satiety value thus reducing the food intake which ultimately lowered the BMI in humans¹². When consumption of dietary fibre was more only 13% subjects suffered from constipation. However study by Hongisto et al (2006) showed that high consumption of fibre rich foods could alleviate the problem of constipation¹³. Thus it can be said that dietary fibre alone may not play a role in the occurrence of constipation, there may be other factors responsible for the occurrence of constipation such as low physical activity, advancing age, decreased fluid intake etc. Johanson, Sonnenberg and Koch (1989) stated that the occurrence of constipation increased with advancing age, showing an exponential increase in its prevalence after the age of 65¹⁴.

A study by Bolin et al (1998) showed that Flatus emissions significantly correlated with dietary fibre intake when the fibre intake was 24±3 g for women¹⁵. The present study revealed that among subjects who did not experience flatulence consumed dietary fibre more frequently to frequently. High intake of dietary fibre resulted in less occurrence of flatulence. Also the present study showed that high intakes of curd and garlic resulted in high occurrence of flatulence. As more subjects under the study were Gujarati's, their consumption of curd and garlic was high, therefore it can be said dietary fibre alone may not necessarily be responsible for the occurrence of flatulence but the consumption of curd and garlic may affect its occurrence. Among subjects who did not suffer from constipation around 45% subjects had more frequent consumption of onion compared to 21% who had least

frequent consumption of onion. A study by Hidaka et al (1991) and Kleesen et al (1997) showed that because inulin and FOS have somewhat laxative effects that might be helpful in reducing constipation¹⁶⁻¹⁷. Among subjects who did not experience flatulence, majority of them (76%) had frequent consumption of onion. Thus it can be said when the consumption of onion is high the occurrence of constipation and flatulence is less. However, some human studies have also shown that intestinal discomfort is high (particularly flatulence) when consumption of prebiotic foods is high¹⁸⁻¹⁹.

It was seen in the present study that curd had beneficial effect on constipation and flatulence. The present study shows that an increased consumption of garlic resulted in less occurrence of constipation and a high occurrence of flatulence. Rumessen et al (1990) said that since oligosaccharides reach the large intestine largely intact, the possibilities exists that gastrointestinal discomfort may be experienced²⁰.

The present study shows that a frequent consumption of prebiotic and probiotic foods helped in the establishment of the beneficial bacteria i.e. *Bifidobacteria* and *Lactic acid bacteria* and lowered the counts of micro organism such as *E. coli* when compared to those with the least frequent consumption. Studies also show that oligo-fructose and inulin (Sources: onion, garlic etc) significantly modify in vivo composition of the microbiota by stimulating the growth of *bifidobacteria*²¹. An in vitro study by Bouhnik et al (1999) showed that prebiotics stimulate the growth of *Bifidobacterium*²². Another study by Rowland, Tanaka (1993) showed that diet supplemented with prebiotics foods provide an effective means to promote growth of *Bifidobacterium* and *lactobacilli*, while selectively reducing the growth of pathogenic microorganisms and potentially treating intestinal dysfunctions²³. The result of the present study reveals that the type of physical activity did not strongly correlate with the establishment of gut microflora. Although various studies have shown the beneficial effect of the physical activity on conditions such as constipation, colon, cancer, IBS etc no such study is seen which shows the direct relation of physical activity with the establishment of beneficial micro flora²⁴. Another study by Meshkinpour H and Kempe et al (1989) showed that differences in the physical activity levels might also affect micro flora composition. Although moderate exercise has not been shown to decrease transit time through the intestinal tract, increased activity levels might change other aspects of intestinal physiology and thus the conditions for microbial growth. Studies have also shown that differences in

physical activity levels might affect micro flora composition²⁵.

CONCLUSION:

The Indian normal adult female population consumes a fairly moderate amount of dietary fibre (33-40 gms/day) and crude fibre (8 gms\ day). The consumption of prebiotic and probiotic foods such as onion, garlic and curd was also found to be frequent.

The frequent consumption of dietary fibre does help in the establishment of the beneficial micro organisms in the gut of normal adult females as compared to those with least frequent in takes of dietary fibre. Frequent consumption of onion did not result flatulence but the subjects experienced constipation, however frequent consumption of garlic showed opposite effect on constipation and flatulence.

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