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Research Article

GUT MICROFLORA AFFECTS GLYCEMIC RESPONSE OF TYPE 2 DIABETIC ADULTS: A CROSS-SECTIONAL STUDY

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ABSTARCT
Over the past decade the number of people in India with diabetes has increased dramatically. However, scattered information is available on association between gut microflora and glycemic response of diabetics. A cross-sectional study involving 120 known diabetic human subjects was conducted in health clinic of the Maharaja Sayajirao University of Baroda at Vadodara. Baseline information of subjects was collected on socio economic status, anthropometric measurements, biophysical, biochemical, dietary, physical activity and microbial parameters. Study outcome indicated that 89% of diabetic subjects had family history of diabetes and 37% had diabetes for less than 10 years. Almost 81% subjects were overweight and obese with poor control of mean fasting blood sugar (143.5 mg/dl), mean post prandial blood glucose (219.8 mg/dl) and mean $A1_c$levels (9.0). Physical activity level (PAL) revealed sedentary lifestyle of subjects with very low fiber intake averaging 12 g/day. The mean log values in terms of CFU/g for *Lactobacillus, Bifidobacteria* and *Enteric pathogen* were 6.34, 6.34 and 4.47 respectively. A positive correlation existed between FBS and family history of diabetes ($p<0.01$). Diabetic subjects with good control of diabetes ($A1_C$, 7-8) had better establishment of *Bifidobacteria* and *Lactobacillus* ($p<0.05$). However colonization of *LAB* and *bifidobacteria* reduced with poor control of diabetes.

Keywords: *Diabetes, Gut Microflora, Anthropometric Measurements, Lactobacillus, Bifidobacteria and EntericPathogen*

INTRODUCTION

In recent years, India has witnessed a rapidly exploding epidemic of Diabetes. Indeed, India today leads the world with its largest number of diabetic people in any given country. According to International Diabetes Federation (IDF) 2010, 50.8 million people have diabetes in India which will sharply rise to 87 million in year 2030. Many studies indicated that 50% people with diabetes have poor glycemic control, uncontrolled hypertension, cardiovascular problems, and obesity and lead a sedentary lifestyle (Hanson *et al.*, 1995; Colditz *et al.*, 1990).

Though there is sufficient literature available on diabetes and its association with other non-communicable diseases but scattered information is available on association between gutmicroflora and type 2 diabetes. To date, many of the mechanistic studies have mainly focused in the biology of relationships between various human organs and cell systems. However, there is increasing focus on relationship between microbiota of gut and diabetes. Amongst many bacteria of the intestinal microbiota that are considered to be beneficial, *Lactic acid bacteria* and *Bifidobacteria*, normal inhabitants of human gastro intestinal (GI) tract have raised great interest for their potential health benefits (Gibson & Roberfroid 1995; Delzenne, 2000).

Recent evidence suggests that a particular gut microbial community may favor occurrence of the metabolic diseases. Diet also seems to have an effect on the establishment of type of microflora in human intestine. Recently it was reported that high fat diet (HF) feeding was associated with higher endotoxaemia and lowered *bifidobacterium* species as observed in caecal content of mice (Cani *et al.*, 2007). In a high fat diet fed mice, the modulation of gut microbiota was associated with an increased intestinal per me ability that precedes the development of metabolic endotoxaemia, inflammation and associated disorders (Cani *et al.*, 2008). Animal models of obesity connect an altered microbiota

composition to the development of obesity, insulin resistance and diabetes in the host through a gut incretin known as Glucagon like peptide (GLP-1). GLP-1 is also known to be involved in glucose and fat metabolism under the impulse of altered SCFA composition of gut (Musso, Gambino & Cassader, 2010). These SCFA helps to stimulate (GLP 1) and Glucose induced polypeptide (GIP) which is known to be most potent enhancer of insulin secretion in the body. An animal study showed that short chain fatty acids (SCFA's) and especially butyrate have been proposed to have positive effect on intestinal GLP 1 secretion (Sajilata, Singhal and Kulkarni, 2006)

Furthermore, dietary fibers which reduce the impact of high fat diet on the occurrence of the metabolic diseases normalized the gram –ve to gram +ve ratio and plasma endotoxemia. These data strongly suggest that intestinal microbiota could be responsible for changes of metabolic disorders (Cani, 2008).

In view of the research findings cited above, the present study was undertaken to further validate the existing information and determining the association between gut flora (*bifidobacteria, LAB and enteric pathogen*) and glycemic values of type 2 diabetic subjects.

MATERIALS AND METHODS

Study Design

The study was a cross-sectional design which involved 120 known diabetic male and female adults aged between 40-70 years, who attended the University health clinic of The Maharaja Sayajirao University of Baroda at Vadodara. The subjects were university staff members who voluntarily agreed to participate in the study. Purposive sampling method was done to enroll subjects who were on oral drugs; metformin and sulfonylurea. The inclusion criteria included their fasting blood sugar (FBS) and post prandial blood sugar (PP2BS) to be in the range of 120-200 mg/dl and 150-300mg/dl respectively, $A1_c \geq 7\%$, Body mass index (BMI <35), non-smokers and non-alcoholic. Patients with very high blood glucose levels, total cholesterol (TC) >280 mg, triglyceride (TG) > 300 mg and severe forms of renal, hepatic, hematological or respiratory disorders were excluded from the study.

Study Methodology

Relevant data was obtained through patient medical records, face to face interview and direct measurements. Information regarding age, gender, occupation, socio economic status, family history and medical history of subjects was elicited using a pre-tested semi-structured questionnaire. Anthropometric measurements like, height was measured using flexible measuring tape and weight of subjects was measured on a pre-standardized weighing scale nearest to 100 kg. BMI was calculated using the formulae weight (kg)/height (m^2). Waist circumference (WC) and hip circumference (HC) were measured using flexible measuring tape. To measure waist circumference, subjects were made to stand straight and with their stomach relaxed. The tape was positioned midway between the top of the hip bone and the bottom of the rib cage. Hip circumference was measured 24 inches below navel level, tape was wrapped and circumference measured. Blood pressure of the subjects was measured in a seating position after a five minute rest with standard clinical mercury sphygmomanometer. Blood glucose- FBS and PP2BS were estimated by GOD/POD method using enzymatic kit procured from Transansia Bio Medicals Ltd, Vadodara. Glycated hemoglobin ($A1_{c)}$ was estimated by ion exchange principle using high performance liquid chromatography (HPLC) (Transansia Bio Medicals Ltd, Vadodara). Glucagon like peptide 1 (7-36) amide (GLP-1) was estimated by antigen antibody reaction method using an ELISA technique (Epitome Diagnostic Pvt. Ltd, Mumbai). To record dietary intake of the subjects three consecutive day 24-hr-dietary recall method was used. Frequency of food consumed by the subjects was recorded by food frequency questionnaire (FFQ). Frequency of consumption of probiotic and prebiotic food was also recorded. Nutrients were calculated using Diet Soft software, developed by Gurdeep Kaur, AIIMS, Delhi. Physical activity pattern of the subjects was determined using was assessed using Global Physical Activity Questionnaire (GPAQ) given by the WHO 2008. It collects information on physical activity participation on three domains i.e. activity at work, travel to and from places; recreational activities and sedentary behavior. Physical activity level (PAL) was used as a composite index of physical activity

patterns and was calculated as, 24 hour energy expenditure/ Basal Metabolic Rate. Subjects who scored <1.4 PAL, were sedentary active. 1.55-1.60 PAL was considered as moderately active and >1.6 PAL as heavily active.

To determine the microbiota composition stool samples of the subjects were collected in sterilized stool sample containers. One gram stool sample was weighed and was serially diluted in sterilized atmosphere under laminar flow. Readymade flexi plates were used for growth of *Lactobacillus* and *Enteric pathogen* and for the growth of *Bifidobacteria,* bifidobacterium agar was used (Hi Media Pvt. Ltd, Mumbai). Each experiment was performed in triplicates. Sampled petri plates were kept under incubation for 48 hrs after which reading was noted.

Sample Size

The sample size estimates were based upon one-sided hypothesis. A 95% level of confidence and a power of 90% for primary variables using, Medical statistics online calculators developed by General Clinical Research Center Program, Massachusetts General Hospital and National Institutes of Health, 2010.

Statistical methods

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS17.0 version, SPSS Inc., Chicago, IL, USA). Continuous variables have been described by summary statistics such as mean and standard deviation. Frequency and percentages were calculated for background information.'T' test of unequal variance was used to observe the difference that exists between male and female subjects. Pearson's correlation was used between biochemical parameters, microbial parameters, dietary, physical activity and lifestyle factors.

Statutory clearances

The Medical Ethics committee of the Foods and Nutrition Department, The M.S. University of Baroda approved the study proposal and provided the Medical ethics approval number (F.C.Sc/FND/ME/56). Written consent was obtained from the participants who agreed to give baseline information through questionnaire and give sample of blood and stool for biochemical and microbiological analysis respectively.

RESULTS

Baseline Characteristics

Socio economic data of type 2 diabetic subjects revealed that majority of the subjects surveyed in the study were Hindus (95%) with 43% male and 57% female subjects. All the subjects were literate. More than 45% subjects lived in nuclear family. Table 1 shows that most subjects (89%) had family history of diabetes. In 37% of subjects type 2 diabetes was detected since 10 years. With regards to associated diseases, 70% subjects suffered from hypertension, obesity (62%) and CHD (24%).

Anthropometric, Biochemical and Physical activity profile

According to International Obesity Taskforce IOTF, 2004 classification, the mean BMI of type 2 diabetic subjects was 26.4 (Table 3). About 40% and 27% subjects fell under the category of overweight and obese respectively, with poor control of FBS (143.5 mg/dl), PP2BS (219.8 mg/dl) and $A1_c$ (9.0%) (Table 4). Their mean GLP-1 values (0.36 p mol) were on the lower side of the normal range (0.3-16 p mol). Physical activity level (PAL) revealed that most subjects lead a sedentary lifestyle.

Dietary Intake Assessment

Nutrient analysis from 24 hr dietary recall revealed that on an average, subjects consumed a diet that was low in energy (1396-1551 kcals). 51-53% energy was contributed through carbohydrates, 11-12% energy through protein and 33-37% energy through fats (Table 6), indicating that the subjects were consuming an unbalanced diet, that was high in fat. Intake of almost all the micronutrients was less than the recommended dietary allowances (RDA), except calcium and vitamin C. Intake of calcium and iron was higher in male than female subjects. Fibre intake contributed only 45% of the daily requirement. Analysis of FFQ questionnaires revealed that almost all the food groups were consumed on frequent basis except

green leafy vegetables. Sweets were avoided by most of the subjects. Only 12% participants consumed GLV's frequently. About 33% subjects consumed fried snacks on daily basis.

Table 1: Medical history of type 2 Diabetic subjects

Category	Percent Subjects N=120 N (%)	Male (n=52) n (%)	Female (n=68) n (%)
DM			
1-5 y	27 (22.5)	10(19.2)	17(25)
>5-10 y	18 (15)	7(13.4)	11(16.1)
>10-15 y	8 (6.5)	3(5.7)	5(7.3)
>15 y	67 (56)	32(38.3)	35(48.4)
DM+Hypertension			
1-5 y	42(35)	12(23)	30(44)
>5-10 y	18(15)	8(15.3)	10(14.7)
>10-15 y	18(15)	9(17.3)	9(13.2)
>15 y	8(6)	3(5.7)	5(7.3)
No HT	34(29)	15(28)	19(27.9)
DM+CHD			
1-5 y	15(12.5)	10(19.2)	5(7.3)
>5-10 y	12(10)	9(17.3)	3(4.4)
>10-15 y	3(2.5)	3(5.7)	0(0)
>15 y	0(0)	0(0)	0(0)
No HT	90(75)	30(57.6)	60(88.2)
DM+Obesity			
1-5 y	17(14.1)	8(15.3)	9(13.2)
>5-10 y	27(22.5)	10(19.2)	17(25)
>10-15 y	19(15.8)	12(23)	7(10.2)
>15 y	11(9.1)	7(13.4)	4(5.8)
No obesity	46(38.5)	15(28.8)	31(45.5)

Table 2: Family history of NCD's among type 2 diabetic subjects

Category	Percent subjects N=120 N (%)
Diabetes Mellitus	
Both parents	12(10)
Single parent	68(58)
Sibling	25(21)
No family history	13(11)
Hypertension	
Both parents	10(8.3)
Single parent	72(60)
Sibling	9(7.5)
No family history	29(24.1)
CHD	
Both parents	2(1.6)
Single parent	39(32.5)
Sibling	12(10)
No family history	67(55.9)

Table 3: Mean Values of Anthropometric Profile of Type 2 Diabetic Subjects

Variable	Range (max-min)	Percent subjects (N=120) Mean ±SD	Male (n=52) Mean ±SD	Female (n=68) Mean ±SD	't' test value
Height (cm)	180-147	160±8.4	166.9±7.1	155.3±5.2	9.8^{***}
Weight (kg)	103-40	68±14.2	76.6±14.8	61.5±9.5	6.4^{***}
Waist Circumference (cm)	45-30	37.5±4.1	39.9±3.8	35.6±3.2	6.5^{***}
Hip circumference (cm)	50-34	42.25±3.8	43.6±4	41.2±3.4	9.6^{***}
Waist to Hip ratio (cm)	1-0.6	0.85±0.06	0.89±0.06	0.83±0.05	4.8^{***}
Body mass index (BMI)	35.7-17.5	26.48±3.8	27.6±4.4	25.6±3.04	2.7^{**}

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001, NS - Non Significant

Table 4: Mean Values for Biochemical Profile and Physical Activity Levels of Type 2 Diabetic Subjects

Parameters	Total subjects (N=120)	Male (n=52)	Female (n=68)	't' test value
FBS (mg/dl)	143.5±35	144.9±33.5	142.4±31.2	0.3^{NS}
PP_2 (mg/dl)	219.8±36.6	233.6±36.8	209.3±36.7	2.3^{*}
HbA_{1c}	9.0±1.07	9.3±1.7	8.7±1.3	2.0^{**}
GLP-1 (p mol) (subsample)	0.368±0.32	0.433±0.38	0.288±0.18	1.6^{NS}
PAL (min/week)	571±155.5	644±186	503±71.2	3.8^{***}

*Significant from the baseline value at p<0.05, ** Significant from the baseline value at p<0.01, *** Significant from the baseline value at p<0.001, NS - Non Significant

Table 5: Mean Log Values For Microbial Parameters of Type 2 Diabetic Subjects

Parameters	Total subjects N=62 log_{10} cfu/g	Male n=32 log_{10} cfu/g	Female n=30 log_{10} cfu/g	't' test
Lactobacillus	6.3463±0.21	6.350±0.20	6.342±0.23	0.74^{NS}
Bifidobacteria	6.3408±0.21	6.343±0.22	6.337±0.21	0.30^{NS}
Enteric pathogen	4.4799±0.28	4.527±0.27	4.429±0.28	1.46^{NS}

Table 6: Mean Intake of Nutrients Of Type 2 Diabetic Subjects As Per 24 Hr Dietary Recall

Nutrients	Range	Total N=62	RDA for Female	% RDA	Female n=30	RDA for male	% RDA	Male n=32	't' test
Energy (Kcal)	716-2514	1471.8±374.8	1875	74.4	1396.9±332.1	2425	63.9	1551.6±406.0	1.45^{NS}
CHO (g)	102-339	193.2±51.0	-	-	179.6±42.0	-	-	207.8±56.1	1.94^{NS}
Protein (g)	25-72	43.3±11.6	50	80	40.6±11.5	60	76.6	46.1±11.5	1.74^{NS}
Fat (g)	23-93	58.4±17.4	20	290	58.1±17.0	20	290	58.8±18.2	0.05^{NS}
Calcium (mg)	279-1398	698.4 ±294.7	600	98	593.1 ±218.9	600	135	810.7 ±325.8	3.26**
Iron (mg)	5-26	13.8±±4.8	30	40	12.0±4.3	28	53.3	15.8±4.5	2.95**
Folic acid (µg)	69-357	172.0±65.4	100	61	161.6±59.2	100	83	183.1±70.8	1.38^{NS}
Sodium (mg)	73-726	285.9±143.9	-	-	267.0±117.0	-	-	306.1±167.7	0.83^{NS}
Magnesium (mg)	158-859	326.9±146.6	-	-	315.0±164.9	-	-	339.7±125.7	0.50^{NS}
Potassium (mg)	782-2457	1490.3±347.6	-	-	1416.8±315.5	-	-	1568.6±367.9	1.66^{NS}
Fiber (g)	4.2-27	13.5±5.6	30	45	10.4±5.7	30	49	4.7±5.7	1.60^{NS}
βCarotene (µg)	117-7260	3227±5146.0	2400		2367.7±4495.8	2400	-	4144.6±5693	1.60^{NS}
Vitamin A (µg)	148-457	163.3±97.2	600	25	152.9±82.9	600	29	174.4±110.8	0.70^{NS}
Vitamin C (mg)	16-361	119.4±78.9	40	262	105.8±57.8	40	332	133.9±95.4	1.19^{NS}
Vitamin B12 (µg)	0-1	0.3±0.2	1	30	0.3±0.1	1	40	0.4±0.2	0.92^{NS}
Vitamin B6 (mg)	0-0.2	0.10±0.0	2	5.5	0.11±0.0	2	4	0.08±0.0	3.53**
Riboflavin (mg)	0.4-2.3	1.0±0.4	1.1	64.2	0.9±0.3	1.4	100	1.1±0.4	0.92^{NS}

*Significant from the baseline value at $p<0.05$, ** Significant from the baseline value at $p<0.01$, *** Significant from the baseline value at $p<0.001$, NS - Non Significant

Table 7: Correlation values determining the degree of association amongst lifestyle factors, biochemical parameters and microbial parameters of type 2 diabetic subjects

Life style factors	FBS	PP2	HbA1c	GLP1	LAB	Bifido	E. Pathogen
Age	-0.12	0.03	-0.06	0.03	-0.19	0.05	-0.18
Family history	0.35**	-0.10	-0.09	-0.07	-0.01	0.19	0.06
Physical activity	-0.23	-0.21	-0.18	-0.14	-0.04	-0.05	0.17
BMI	0.08	-0.00	0.13	-0.05	0.03	0.07	-0.24
WC	-0.05	-0.16	-0.03	-0.03	0.09	0.13	-0.14
WHR	0.05	-0.00	-0.06	0.51*	0.21	0.22	-0.15
Calorie intake	-0.07	-0.04	-0.12	-0.06	-0.13	-0.11	-0.01
CHO intake	-0.04	-0.03	-0.11	-0.01	-0.08	-0.12	0.03
Protein intake	-0.27*	-0.09	-0.24	-0.21	-0.14	-0.07	-0.03
Fat intake	-0.08	-0.06	-0.10	-0.14	-0.15	-0.06	-0.09
Fiber intake	-0.02	-0.11	-0.05	-0.10	0.25*	0.26*	-0.21
Insoluble DF	-0.01	-0.01	-0.05	-0.16	-0.15	-0.11	-0.25*
Soluble DF	-0.08	-0.16	-0.10	0.11	-0.09	0.15	-0.25*
SFA	0.16	-0.05	0.08	-0.29	0.03	-0.06	-0.16
PUFA	-0.27*	-0.03	0.09	-0.25	-0.04	-0.08	-0.13
MUFA	0.14	0.10	0.05	-0.37	-0.12	-0.08	0.03
n-6	-0.26*	-0.04	0.09	-0.25	-0.03	-0.07	-0.13

** Correlation values are significant, p<0.05*

Table 8: Correlation values determining the degree of association amongst glucose parameters and microbial parameters of type 2 diabetic patients

Parameters	Lactobacillus	Bifidobacteria	Enteric pathogen
Mean $A1_c$	-0.4	-0.5	0.04
Mean FBS	-0.5	-0.5	0.05
Mean PP2	-0.6	0.4	0.03
A_{1c} 7-8	0.3	0.7*	-0.05
A_{1c} ≥9	0.4	0.5	0.04
FBS 80-110	0.7*	0.8*	0.08
FBS 110-126	0.3	0.4	0.04
FBS >126	0.3	-0.4	0.03
PP2 140-200	-0.5	0.9*	0.06
PP2 >200	-0.5	-0.5	0.05

** Correlation values are significant, p<0.05*

Microbial profile

The mean log values in terms of CFU/g of stool sample for *Lactobacillus, Bifidobacteria* and *Enteric pathogen* were 6.34, 6.34 and 4.47 respectively. There was no significant difference between the mean log counts of the gut microflora in male and female subjects (Table 5).

Correlation amongst lifestyle factors, biochemical parameter and microbial parameters

As shown in Table 7, a positive correlation was observed between fasting blood sugar level and family history ($p<0.05$), and a negative correlation with dietary parameters like PUFA ($p<0.05$) and n6 fatty acids ($p<0.05$). Levels of PP2BS and A1c were not related with family history, anthropometry or dietary parameters. GLP-1 was positively correlated with WHR ($p<0.05$). *Bifidobacteria* and *LAB* were positively correlated with total fiber ($p<0.05$) whereas *enteric pathogen* showed a negative correlation with soluble and insoluble dietary fiber ($p<0.05$).

When association between blood glucose levels of the subjects and gut microflora of the subjects was examined by Pearson correlation, it showed that FBS, PP2BS and A1c was positively correlated with establishment of bacteria (bifidobacteria and LAB) ($p<0.05$) whereas higher levels of blood glucose parameters depicted negative correlation with establishment of beneficial bacteria (Table 8). Waist hip ratio (WHR) showed a positive correlation with LAB and bifidobacteria. However, WHR and BMI showed a non-significant negative correlation with enteric pathogen.

DISCUSSION

The present study was undertaken with a broad objective of determining gut microflora and glycemic response of type 2 diabetic adults attending university health clinic in Vadodara city. The role of heritability has long been known in diabetes. It has been shown that subjects with family history of diabetes develop diabetes earlier compared to subjects without family history. The study revealed that almost 89% of the diabetic subjects had family history of diabetes. However in a similar study conducted in the state of Kerala, India, demonstrated a lower percentage (25%) of subjects exhibiting family history of diabetes (Vijaykumar, Arun and Kutty, 2009).

In the present study both male and female subjects were predominantly overweight or obese (81%). Diabetes has been associated with obesity way back. In 1990's prevalence of obesity in India was less than 5% and prevalence of diabetes was 4.3% (WHO, 1997). One percent increase in the prevalence of obesity leads to 20 million additional cases of obesity. According to an ICMR-INDIAB study, there are 199 million and 61.3 million people with obesity and diabetes in India respectively (Mohan, 2011). This clearly confirms the association between diabetes and obesity. Evidence from several studies also indicates that overweight and obesity are associated with an increased risk of diabetes (Mokdad et.al, 2003). Obesity parameters like BMI and waist circumference were also significantly higher among subjects with family history of diabetes (Mohan *et al.*, 2007).

Most of the subjects had central obesity indicating a high risk for the development of NCD's. Asian Indians have increased visceral fat and central obesity and this is referred to as the Asian Indian phenotype (Joshi 2001; Snehlata et al 2003; Mehta, Kashyap and Das, 2009; Shetty, 2012). It has been reported in several studies that visceral fat is associated with abdominal obesity and type 2 diabetes mellitus. Hence, visceral fat is considered to be one of the links between intra-abdominal obesity and type 2 diabetes mellitus (Chen *et al.*, 2005; Sethi, Vidal-Puig, 2005; Ambady and Chamukuttans, 2009).

Present study also revealed that most subjects followed a sedentary lifestyle. The principal reason for escalating diabetes appears to be rapidly occurring socio-economic changes and affluence associated with dietary excess and reduced physical activity (Ramchandran *et al.*, 2001; Gupta and Misra, 2007; Sayeed *et al.*, 2007). The present study revealed a negative correlation between PAL and FBS ($p<0.05$). Studies conducted in animal models and human subjects have defined the importance of insulin stimulation of endogenous glucose production during light and moderate intensity exercise. Exercise increases both insulin dependent muscle glucose uptake and insulin sensitivity (Ronal and David *et al.*, 2004). This observation is similar to that reported in the National urban diabetes study, wherein the subjects with

sedentary lifestyle had higher prevalence of diabetes (Mohan *et al.,* 2001, 2005; Zimmet Paul, Alberti and Shaw, 2001).

Although type 2 diabetes is determined primarily by lifestyle and genes, dietary composition may affect both its development and complications.

The dietary pattern of the subjects under the study revealed that inspite of consuming total fewer calories than the prescribed RDA the total fat intake of the subjects was high. This could be attributed to increased consumption of fried snacks on daily basis. Evidence suggests that several dietary factors are associated with insulin resistance among the South Asians, that include high intake of carbohydrates, saturated fatty acids, Tran's fatty acids and low intake of n-3 PUFA and fiber. (Hu *et al.,* 1999; Misra *et al.,* 2009). Animal studies also suggest that the type of fat in the diet may affect insulin sensitivity by changing the fatty acid composition of membrane lipids. A higher proportion of unsaturated fat may improve insulin signaling by increasing membrane fluidity (Rob *et al.,* 2002). Fatty acids influence glucose metabolism by altering cell membrane function, enzyme activity, insulin signaling and gene expression (Uff Walter and Frank Hu, 2009). In the present study, a negative correlation was observed between PUFA, n-6 fatty acids and Fasting blood sugar. It has been shown that C20-C22 (PUFAs) in skeletal muscle membranes are associated with lower fasting insulin levels and enhanced insulin sensitivity which may be a result of changes in insulin receptors or glucose transporters (Borkman et al,1993).

The intake of total fiber by the subjects under the study was 55% less than the RDA. Frequency of consumption of fruits and green leafy vegetables was also very low. Dietary fiber is reported to improve the post prandial glycemic response and insulin concentrations, most likely by slowing the digestion and absorption of food and by regulating several hormones (Vinik, Jenkins, 1988). The dietary fiber has been related to number of disease conditions particulary the cardiovascular diseases, diabetes, obesity and cancer. Hence the synergistic effect of having predisposition to diabetic genes and increased intake of energy dense and low fiber foods along with sedentary lifestyle have led to alarming rise in the prevalence of diabetes.

The analysis of gut microflora of diabetic subjects indicated poor establishment of beneficial bacteria and higher establishment of harmful bacteria. There are reports demonstrating difference in bacterial composition of diabetic group with respect to genus *B. vulgatus* and *Bifidobacterium*, when compared to the healthy group. This suggests that the gut microbiota of diabetic patients changes with the development of diabetes (Xiaokang *et al.,* 2010; Larsen *et al.,* 2010; Esteve *et al.,* 2011). In the present study diabetic subjects with good control of diabetes (A1c, 7-8) had better establishment of *Bifidobacteria* and *Lactobacillus* ($p<0.05$), when compared with subjects with poor control of diabetes.

Low dietary fibre and high fat intake is known to affect gut health in terms of higher colonization of enteric pathogens resulting in endotoxemia which is now being revealed as a causative factor for several metabolic diseases. This is a link between gut microbiota and high-fat diet-induced inflammation, oxidative stress, metabolic disorders. A study demonstrated that after 4 weeks of high fat-feeding, mice exhibited an obese phenotype accompanied by a change in gut microbiota composition (the reduction of *Bifidobacteria* and *Eubacteria* spp). Gut microbiota contribute towards the pathophysiological regulation of endotoxaemia and set the tone of inflammation for occurrence of diabetes and/or obesity (Cani *et al.,* 2007).

Various prebiotic supplementation studies on animals and humans have revealed a time-dependent shift in fecal and large bowel short chain fatty acids (SCFA) profiles i.e. mainly acetic, propionic and butyric acid (Topping *et al.,* 2003; Cani *et al.,* 2006, 2007, Martin *et al.,* 2010; Diamant *et al.,* 2011). These SCFA help to stimulate gut incretins such as Glucagon like peptide 1 (GLP 1) and Glucose induced polypeptide (GIP) which are known to be most potent enhancer of insulin secretion in the body (Michael, 2012)

The present study also revealed low GLP-1 values in diabetic subjects (0.36 p mol). GLP-1 is a gastrointestinal hormone that is released in response to food intake from the distal small intestine. Its biological effects include a glucose dependent insulinotropic effect on the pancreatic β cell and inhibition of gastric emptying (Gutzwiller *et al.,* 1999). Present data on GLP-1 also emphasized a negative

correlation with WHR, indicating a relation between GLP-1 and energy homeostasis. Our findings are in agreement with a study where in GLP-1 infusion enhanced satiety and fullness compared with placebo and energy intake was reduced by 27%. (Gutzwiller *et al.,* 1999; Backhed *et al.,* 2004; Rabot *et al.,* 2010, Jumpertz *et al.,* 2011; Muegge *et al.,* 2011; Ravussin *et al.,* 2011).

In summary, we found that a relation exists between glycemia and gut microbiota. Thus, specific strategies focusing on colonization of beneficial gut microbiota could be useful for combating high glycemic status of type 2 diabetic adults.

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