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Research article

FRUCTOOLIGOSACCHARIDE (FOS) SUPPLEMENTATION IN TYPE 2 DIABETIC ADULTS IMPROVES SYSTOLIC BLOOD PRESSURE, SERUM LIPID, AND GUT MICROBIOTA

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ABSTRACT: Fructooligosaccharide (FOS) is a prebiotic, becoming apparent for its therapeutic role for diseased conditions like CHD, hypertension, diabetes and obesity. CHD and hypertension are one of the most prevalent NCD's encountered in diabetic adults. Present study aims to assess the effect of FOS supplementation in type 2 diabetic adults on their lipemic, biophysical parameters and gut microflora parameters. A cross-sectional study was designed with 65 adult type 2 diabetics enrolled from Health clinic of The M.S. University of Baroda, Gujarat. All the subjects were randomly divided into two groups control and experimental. The experimental group was given 10 g of fructooligosaccharide and compared with the controls for lipemic parameters, hypertension and fecal counts in terms of *lactic acid bacteria, bifidobacteria* and *enteric pathogen.* Eight week supplementation of FOS resulted in an appreciable reduction in serum TC, TG and LDL levels by 10%, 4.9% and 7.8% respectively. A significant reduction ($p<0.05$) in systolic blood pressure was also observed as a result of FOS supplementation in the experimental group. A decline was seen in TC/HDL, LDL/HDL and non-HDL by 10.4%, 7.6% and 6.6% respectively ($p<0.05$, $p<0.001$). Gut microbiota values exhibited a significant increment in fecal $\log_{10}$ counts of *Lactic acid bacteria* and *bifidobacteria* by 9.3% and 10.9% respectively ($p<0.001$) while a significant reduction by 4.8% ($p<0.001$) was observed for Enteric pathogen. These outcomes revealed an efficacy of FOS in reducing serum lipid parameters, systolic BP and improving the gut microbiota in type 2 diabetic adults.

Keywords: Lipemic, Hypertension, Lactobacillus, Bifido bacteria, Enteric pathogen, Diabetes mellitus, Fructooligosaccharide

INTRODUCTION

There is bountiful market and public attentiveness in the ability of functional foods in benefiting health and lowering the risk of allied diseases which are caused by diet and lifestyle. Plausible effects of prebiotics and probiotics outfitting such health benefits has been known way back. The recent use of fructooligosaccharide as a food ingredient has provoked a number of researches on their creditable health effects. However, since the concept of prebiotics and probiotics has been first evolved, its health related benefits have been shifted from hygiene related diseases to Non-communicable diseases like CHD, diabetes, hypertension, obesity etc. Cardiovascular diseases are one of the main causes of morbidity and mortality today. WHO has predicted that, by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the world (WHO, 2009).Many studies have shown lipid lowering effects of fructooligosaccharide (FOS). Because FOS is not hydrolyzed by enzymes in the small intestine of humans, they reach the colon intact where they are fermented by the colonic microflora (Alles. M.S, et.al., 1996). End products of this fermentation are some gases and short chain fatty acids such as acetate, propionate and butyrate. These SCFA's are readily absorbed by the colonic mucosa. It is known that butyrate act as an energy substrate for the mucosa and, whereas acetate and propionate enter the portal blood and may influence systemic carbohydrate and lipid metabolism (Cummings. J.H., et.al., 1987).

High production of SCFA is assumed to be beneficial in reducing hepatic glucose output and improving lipid homeostasis. This is known to be achieved by Glucose dependent insulinotropic polypeptide (GIP) and Glucagon like peptide -1 (GLP-1) known to be involved in glucose and fat metabolism (Heijnen. M.L., et.al., 1995). Along with the prevalence of increased CHD related problems there is an alarming rise in incidence of hypertension especially in developing countries like India. Prevalence of HTN globally is 972 million, and India accounts for 65.5 million cases. The coexistence of type 2 diabetes mellitus (T2DM) and essential HTN is common (Hazari. M.A., et.al., 2012).The prevalence of hypertension is 1.5–2.0 times more in those with diabetes than in those without diabetes, whereas almost one-third of the patients with hypertension develop diabetes later (Sahay. B.K., 2007).This coexistence presents an increased risk and can accelerate vascular complications (Williams. G., 1991).Few animals and human studies have reported a decrease in hypertension after ingestion of FOS (Namakamura. Y., et.al., 1995 and Streppel. M.T., et.al., 2004). However, they need to be validated in humans. Keeping the above literature in mind the present study was planned to understand the mechanisms involved through the use of FOS in combating hyperlipidemia in type 2 diabetic adults.

SUBJECTS AND METHODS

Study Design

The study was a cross-sectional study design which involved 65 known type 2 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 used to enroll subjects who were on oral drugs; metformin and sulfonylurea. Thereafter, total subjects were divided randomly into two groups, control (20) and experimental (45). The inclusion criteria included known type 2 diabetic subjects, Body mass index (BMI <35), non-smokers and non-alcoholic. Patients with very high blood glucose levels, total cholesterol (TC) >280 mg/dl, triglyceride (TG) > 300 mg/dl and severe forms of renal, hepatic, hematological or respiratory disorders were excluded from the study. No changes were made in the methods after trial commencement.

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. Sitting blood pressure of subjects was measured using the standard electric sphygmomanometer on the right arm. Enzymatic colorimetric method (GPO/PAP) was used to measure serum total cholesterol (TC) and triglyceride (TG) procured from Transansia Bio Medicals Ltd, Vadodara. Serum HDL, LDL and VLDL fraction of cholesterol was determined using enzymatic, colorimetric method (CHOD/PAP) without sample pretreatment (Transansia Bio Medical Ltd.). Gut microbial counts levels was determined in terms of *lactobacilli, bifidobacteria* and enteric pathogen. The subjects were randomly assigned using computer generated random tables to either control or experimental group by the investigator (Online Random tables). Informed consent from the subjects was obtained prior to supplementation. The experimental group was asked to consume 10 grams of FOS (powder) per day for 8 weeks along with their meals. Generally changes in lipemic parameters and gut microflora may be observed within 8 weeks. Hence, the intervention was stopped after 8 weeks. Subjects were required to provide fecal samples and serum blood samples before and at the end of intervention. Fecal samples were collected for determining microbial counts in terms of *lactobacilli, bifidobacteria* and enteric pathogen and blood samples were collected in the fasted state for biochemical estimation. Subjects were advised not to alter their usual calorie intakes and physical activity pattern and were asked to document any unusual symptoms or side effects and to keep a diary of illness and medications. Subjects were asked to keep a record of intake of FOS in a compliance sheet. Subjects were followed up every week for compliance and felt side effects if any. Of the forty five subjects in the experimental group, five subjects did not comply fully as two of them left the city and discontinued taking the supplementations; other two had poor compliance and one subject showed poor health conditions.

Study food and mode of Intervention

The Fructooligosaccharide (FOS) used for the intervention wasfood gradefructooligosaccharide (Orafti., 1996)was procured in a pack of 25 kg from Brenntag Ingredients India Pvt. Ltd, Mumbai. FOS was given in powder form packed in 10g sachets. The subjects were asked to incorporate FOS in to water along with the lunch. According to FAO-WHO, FOS is regarded as safe when consumed upto 20 g (FAO/WHO., 2001).

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Test Methods

Blood collection and analysis

After the overnight fast, venous blood sample was collected in clean, sterilized vacuum containers and allowed to stand at room temperature for 15 minutes and serum was separated from total blood. Total cholesterol (TC) was estimated using end point enzymatic colorimetric technique. Cholesterol is cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine-imine dye. The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm (Richmand.W., 1973). Enzymatic colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone was used to assess triglycerides. Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK). The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H2O2). In the presence of peroxidase (POD), hydrogen peroxide affects the oxidative coupling of 4-chlorophenol and 4-aminophenazone to form red coloured quinoneimine dye, which is measured at 512 nm. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample (Fossati.P., and Prencipe.L., 1982). HDL, LDL and VLDL fraction of cholesterol was determined using enzymatic, colorimetric method (CHOD/PAP) without sample pretreatment. The principle of HDL- Cholesterol Direct is based on the absorption of synthetic polyanions to the surface of lipoproteins. LDL, VLDL and chylomicrons are thereby transformed into a detergent resistant form, where as HDL is not. Combined action of polyanions and detergent solubilises cholesterol from HDL, but not from LDL, VLDL and chylomicrons. Solubilized cholesterol is oxidized by the sequential enzymatic action of cholesterol esterase (CE) and cholesterol oxidase (CHOD). The hydrogen peroxide formed reacts with N, N-bis (4-sulfonyl)-m-toluidine (DSBmT) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) and forms a red quinoneimine dye. The colour intensity of the red quinoneimine dye formed is directly proportional to the HDL-Cholesterol concentration. It is determined by measuring the increase in absorbance at 552 nm (Sughichi. H., 1995). Blood pressure measurements were taken after the subject was made to sit down quietly for at least 5 minutes. The bare arm of the subject was supported and positioned at heart level. A cuff of suitable size was evenly applied to the exposed upper arm, with the bladder of the cuff positioned over the brachial artery. The bladder length was at least 80% and width at least 40% of the circumference of the arm. The cuff was snugly wrapped around the upper arm and inflated to 30 mmHg above the pressure at which the radial pulse disappeared. The cuff was deflated at rate greater than 2 mmHg/beat. If initial readings were high, several further readings were taken after 5 min. of rest. On each occasion two or more readings were averaged. For diastolic reading, the disappearance of sound was used; muffing of sound was used if sound continued towards zero (Thomas. G., et.al., 2005).

Fecal bacteria enumeration

Fecal samples were collected in an air tight sterile container kept with cold packs. The serial dilutions of 1 gram of fecal samples were made up from 10^{-2} to 10^{-8} dilutions (Ramona. R., et.al., 2000).The media for bifidobacteria was prepared in laboratory using the dehydrated *bifidobacterium* agar procured from Hi Media and was autoclaved at 121 degree C for 15 minutes (Nystrom. T., et.al., 2004). The prepared media were poured into sterile petri plates and was allowed to set. The enumeration of *Lactic acid bacteria* andenteric pathogen was done using ready-made HiTouchFlexi plates supplied by HiMedia. HiTouchFlexi plates have ready to use sterile media supplied in flexible disposable plates, 55mm in diameter.The Flexi plates were kept inside laminar flow under UV light before using them for inoculation and enumeration of bacteria. The samples were plated on the respective media as per the methods for the selective enumeration of *Lactobacilli, Bifidobacteria* andEnteric pathogen (Ramona. R., et.al., 2000; Nystrom. T., et.al., 2004 and De. Man. J. et.al., 1960).The plates of *Bifidobacterium* were incubated at 37ºC placed in the anaerobic jar with gas packs in the incubator and read after 48 hours. Flexi plates of *Lactic acid bacteria* were placed in a dessicator as it is a facultative anaerobe and those of enteric *pathogen* were directly placed in the incubator. After 48 hours of incubation the colonies were counted using a colony counter and were converted in to log counts after multiplying with their dilution factors (FAO/WHO., 2001).

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 (SPSS 19.0 version) (SPSS 19.0 version). Paired t test was performed to observe the effect of FOS supplementation. The significance levels were set at 5% by two sided tests. Student t test was performed for the comparison between control and experimental group. Pearson correlation was calculated between lipemic and biophysical parameters and gut microflora counts. For further prediction of indirect variables on dependent variables, linear multiple regression was performed.

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

Medical and lifestyle history

The mean age of the subjects was 55.4 years. No prevalence of substance abuse (tobacco or alcohol) was observed amongst subjects. All the subjects used oral hypoglycemic agents (OHA) to control hyperglycemia.

Subject compliance

FOS was well tolerated by all the subjects who completed the study and no intolerance or adverse events were reported. Volunteer compliance was assessed by return of used sachets and by self-reported FOS intake (by means of compliance sheet), which indicated good compliance.

Effect of FOS supplementation on blood pressure and Lipemic levels

A significant reduction ($p<0.05$) in systolic blood pressure was observed as a result of FOS supplementation in the experimental group. However, diastolic blood pressure remains unchanged. FOS supplementation resulted in a significant reduction in serum TC, TG and LDL levels by 10%, 5.4% and 6.8% respectively. However, no significant reduction was observed for HDL and VLDL levels (Table 1). Subjects were analyzed gender wise and the results reveled that FOS supplementation resulted in 11.9% and 8.13% reduction in TC in males and females respectively. About 8.9% and 12% reduction was observed for LDL in both the groups. In terms of TG only females had significant percent reduction (8.5%) after supplementation. However, no significant reduction was seen in HDL for both males and females (Table 2). A significant reduction was observed in TC/HDL, LDL/HDL and non-HDL by 10.4%, 7.6% and 13% respectively ($p<0.05$, $p<0.001$). However, no significant changes were seen for TG/HDL after FOS supplementation (Table 3).

Effect of FOS supplementation on human fecal microbiota

Table 4 summarizes the effect of FOS intervention on gut microbial counts. The fecal log10 counts of *Lactic acid bacteria* and *bifidobacteria* showed a significant increase by 9.3% and 10.9% respectively ($p<0.001$). While a significant reduction by 4.8% ($p<0.001$) was observed for Enteric pathogen.

Association of Lipemic parameters and hypertension with gut microflora

An association was determined amongst biophysical, lipemic parameters and gut microflora as shown in Table 5, *Lactobacillus* was significant negatively associated with VLDL-C ($p<0.05$). A non-significant negative association was also found amongst *LAB*, SBP and LDL-C. *Bifidobacteria* was significant negatively correlated with SBP and TC ($p<0.05$). Enteric pathogenwas significant positively associated with DBP ($p<0.001$) and SBP ($p<0.05$).

Relationship of total cholesterol and systolic blood pressure with microbial parameters

To further assess the relationship between lipemic parameters and blood pressure with microbial parameters, linear multiple regression analysis was performed as it is a strong tool to predict the most affecting predictor or independent variable on dependent or criterion variables. The Standardized Beta Coefficients give a measure of the contribution of each variable to the model. A large value indicates that a unit change in this predictor variable has a large effect on the criterion variable. As shown in Figure 1.a, reduction in systolic blood pressure is maximum affected by LDL-C followed by TG and *bifidobacteria*and reduction in total cholesterol values were most affected by *Bifidobacteria* followed byenteric pathogen (1.b).

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Table 1: Lipemic response and Blood pressure values of the subjects before and after FOS supplementation

Parameters		Control (n = 20)	Experimental (n = 40)	*t* test
TC	**Pre**	209.8±35.35	200.5±34.35	**1.71 NS**
	Post	205.3±32.11	180.2±30.65	**2.09***
	Paired t test	**0.80 NS**	**2.71****	
	% difference	↓4.54	↓ 10.00	
TG	**Pre**	145.5±49.5	146.5±39.03	**0.95 NS**
	Post	139.7±43.03	137.7±30.59	**1.69 NS**
	Paired t test	**0.51 NS**	**0.70***	
	% difference	↓ 4.53	↓ 5.46	
LDL	**Pre**	138.0±28.46	131.6±36.87	**0.00 NS**
	Post	135.1±28.46	122.1±25.34	**1.15 NS**
	Paired t test	**0.00 NS**	**1.31***	
	% difference	↓ 2.17	↓ 6.89	
HDL	**Pre**	42.3±3.08	42±3.50	**1.31 NS**
	Post	42.1±3.16	42.2±2.9	**0.50 NS**
	Paired t test	**1.20 NS**	**0.33 NS**	
	% difference	↓ 0.47	↑ 0.47	
VLDL	**Pre**	29±6.82	27.5±11.72	**0.23 NS**
	Post	28.6±6.27	26±6.21	**1.56 NS**
	Paired t test	**0.05 NS**	**1.11 NS**	
	% difference	↓ 1.68	↓ 5.45	
Diastolic BP	**Pre**	82.2±5.41	83.9±7.66	**0.20 NS**
	Post	83.5±7.11	81.7±6.1	**1.58 NS**
	Paired t test	**0.02 NS**	**1.50 NS**	
	% difference	↑ 1.58	↓ 2.62	
Systolic BP	**Pre**	134.7±13.21	137.9±13.90	**0.20 NS**
	Post	134.1±13.95	132.9±12.45	**1.37 NS**
	Paired t test	**0.90 NS**	**2.40***	
	% difference	↓ 0.44	↓ 3.62	

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

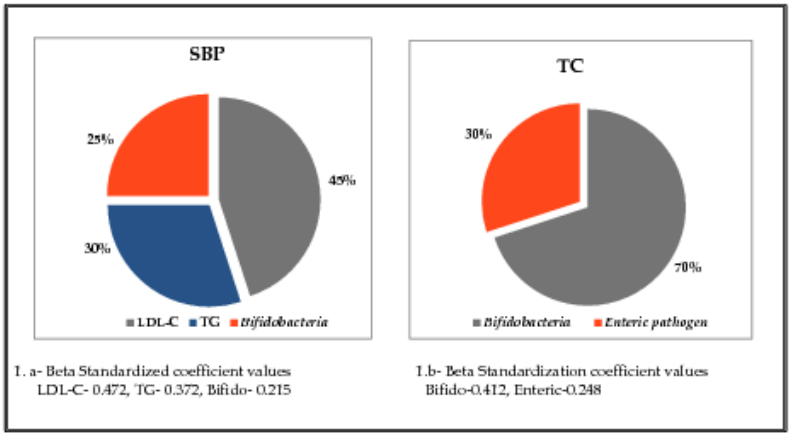

Figure I: Most affecting predictor variables in reduction in SBP, DBP and TC values (criterion variables) as per linear multiple regression

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Table 2: Lipemic response and blood pressure values of male and female subjects before and after FOS supplementation

PARAMETERS		CONTROL		EXPERIMENTAL	
		Males (N=12)	Females (N=8)	Males (N=21)	Females(N=19)
TC	**Pre**	198.68±15.66	223.44±20.18	193.18±34.99	209.53±32.23
	Post	186.18±24.30	220.61±25.87	170.54±31.53	192.65±25.66
	Paired t test	**1.32^{NS}**	**0.16^{NS}**	**2.25***	**2.10***
	% difference	6.05 ↓	1.34 ↓	11.91 ↓	8.13 ↓
TG	**Pre**	135.45±38.65	144.94±37.95	136.63±33.92	142.89±41.60
	Post	131.86±33.70	140.55±36.13	130.50±22.28	132.16±37.30
	Paired t test	**0.03^{NS}**	**0.77^{NS}**	**0.24^{NS}**	**1.80***
	% difference	3.47 ↓	2.08 ↓	4.4 ↓	8.57 ↓
LDL	**Pre**	128.27±29.19	140.94±23.61	110.56±30.74	139.52±38.46
	Post	128.95±30.13	139.88±25.22	98.89±23.16	122.54±20.55
	Paired t test	**0.07^{NS}**	**0.12^{NS}**	**2.41***	**2.61***
	% difference	0.69 ↑	1.11 ↓	10.90 ↓	11.87 ↓
HDL	**Pre**	41.95±2.98	42.83±3.22	41.22±2.79	42.94±4.09
	Post	41.81±7.27	42.61±3.32	41.86±2.91	42.72±3.02
	Paired t test	**0.14^{NS}**	**0.27^{NS}**	**0.73^{NS}**	**0.13^{NS}**
	% difference	0.33 ↓	0.51 ↓	1.55 ↑	0.51 ↑
Diastolic BP	**Pre**	81.63±4.50	82.88±6.40	83.18±7.74	84.88±7.67
	Post	83.72±7.64	83.22±6.64	81.18±5.36	82.33±7.07
Paired t test		**1.10^{NS}**	**1.00^{NS}**	**0.447^{NS}**	**1.03^{NS}**
% difference		2.09 ↑	0.34 ↑	2.40 ↓	3.00 ↓
Systolic BP	**Pre**	133.9±14.10	131.64±5.88	136.0±14.48	138.8±12.0
	Post	136.3±13.19	130.55±7.54	132.9±13.07	132.8±7.67
Paired t test		**0.56^{NS}**	**0.778^{NS}**	**2.22***	**2.06****
% difference		**1.83** ↑	**0.82** ↓	**2.27** ↓	**4.32** ↓

*****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 3: Atherogenic indices of the subjects before and after supplementation

Parameters		Control (n = 20)	Experimental(n = 40)	*t* test
TC / HDL	**Pre**	4.97±0.58	4.71±1.20	**2.49***
	Post	4.86±0.61	4.27±0.78	**1.23^{NS}**
	Paired t test	**0.12^{NS}** ↑	**2.87***** ↓	
	% difference	2.21	10.48	
LDL/HDL	**Pre**	3.28±0.67	3.13±10.53	**1.95^{NS}**
	Post	3.21±0.72	2.89±8.56	**2.55***
	Paired t test	**0.13^{NS}** ↑	**2.50*** ↓	
	% difference	2.13	7.6	
TG / HDL	**Pre**	3.45±0.89	3.48±8.56	**0.53^{NS}**
	Post	3.31±0.89	3.25±10.34	**0.03^{NS}**
	Paired t test	**0.03^{NS}** ↑	**0.93^{NS}** ↓	
	% difference	4.05	6.6	
Non HDL	**Pre**	167.52±52.68	158.52±30.85	**0.07^{NS}**
	Post	163.26±40.40	137.95±27.69	**0.37^{NS}**
	Paired t test	**1.33^{NS}**	3.34*	
	% difference	2.67 ↓	13.03 ↓	

*****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

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Table 4: Gut microbial counts of the subjects before and after supplementation

Parameters		Control (n = 20)	Experimental(n = 40)	*t* test
Lactic acid bacteria	**Pre**	6.43±1.21	6.33±0.20	**1.19** ^{NS}
	Post	6.31±1.18	7.13±0.49	**8.35*****
	Paired t test	**2.31***	**9.30*****	
	% difference	1.55 ↓	12.65 ↑	
Bifidobacteria	**Pre**	6.59±0.77	6.33±0.20	**0.30** ^{NS}
	Post	6.63±0.55	8.55±1.26	**6.75*****
	Paired t test	**0.26** ^{NS}	**10.9*****	
	% difference	0.60 ↑	34.75 ↑	
Enteric Pathogen	**Pre**	4.24±0.32	4.50±0.26	**0.98 NS**
	Post	4.51±0.21	3.95±0.66	**4.45*****
	Paired t test	**2.7****	**4.8*****	
	% difference	6.36 ↑	11.11 ↓	

*****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: Correlation amongst lipemic parameters, blood pressure and gut microflora of type 2 diabetic subjects (r value)

Variables	*Lactobacillus*	*Bifidobacteria*	*Enteric pathogen*
SBP	-0.215	-0.371*	0.328*
DBP	0.098	-0.199	0.595**
TC	-0.005	-0.298*	0.197
TG	0.10	0.157	-0.602
LDL-C	-0.228	0.026	0.163
HDL-C	-0.159	-0.006	0.116
VLDL-C	-0.266*	-0.233	-0.043

*****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

DISCUSSION

The present study demonstrated significant reductionin the lipemic parameters such as TC, TG and LDL by 10%, 5.4% and 6.8% respectively in the FOS supplemented group. A significant reduction by 10.4%, 7.6% and 13% in TC/HDL, LDL/HDL and non-HDL indices respectively was also observed. Studies with respect to effects of inulin and oligofructose on blood lipids in humans are inconsistent, with reports of both positive and negative outcomes. Brighenti et al observed significantly lower triglyceride and cholesterol concentrations in young male volunteers who consumed 9 g inulin added to the rice breakfast cereal for 4 weeks and levels significantly reduced forTC and LDL by 5% and 7% respectively after the end of intervention (Brigenti. F., et.al., 1999). A study conducted by Causey et al also observed a significant reduction in serum TG in subjects with moderate hyperlipidemia when given 18 g/d inulin for 3 weeks (Causey. J.L., et.al., 2000). Yamashita et al conducted a study on 8 and 10 diabetic male and female type 2 diabetic adults who were fed 8 g of FOS for 2 weeks in packed coffee drink showed reduction in TC and LDL-C levels (Yamashita. K., et.al., 1984). Reduction in Serum LDL and TC levels were observed in a study where 18 g of inulin was fed to 21 hyperlipidemic subjects for 6 weeks (Davidson. M.H., et.al., 1998). A study conducted on 40 institutionalized elderly hyperlipidemic subjects (>60 years) revealed significant 7.4% reduction in mean total cholesterol values and increased counts of fecal Bifidobacteria and Lactobacillus after supplementing probiotic curd for 6 weeks (Parnami. S. and Sheth M., 2011). The present study also elicits a significant inverse correlation between TC and *Bifidobacteria* (p<0.05) and VLDL and *Lactobacillus* (p<0.05) after FOS supplementation in the supplemented group.

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In contrast with the findings of the present study LuoR and co-workers, investigated effects of oligofructose (20g/d) fed as 100 g cookies a day in a randomized cross-over design with treatment periods of 4 weeks. No changes in serum triglyceride and cholesterol were observed in either treatment or placebo groups (Luo.Rizkalla. S.W., et.al., 1996). This could be because subjects were given FOS in the form of cookies which already have about 40% fat. Therefore, the chances of cholesterol reduction were less likely. Studies also show that high fat diet is correlated to endotoxaemia which in known to cause detrimental effect on lipid profile. Pedersen et al reported no effect on blood lipids of a daily intake of 14 g inulin added to a low fat spread for a period of 4 weeks. The study was double blind randomized cross over design conducted in sixty six young healthy women, where HDL cholesterol and the LDL: HDL ratio was lower at the end of the study (Pedersen. A., et.al., 1997). In a study conducted on fifty-eight middle aged subjects with moderately raised blood lipid concentrations, subjects consumed 10 g/d of inulin in a powdered form which was added to beverages, soups cereals etc. There were no significant changes in total, LDL and HDL cholesterol over the 8-week intervention. However, serum TG levels were 19% lower after intervention in the inulin treated group (Jackson. K.G., et.al., 1999). A number of possible mechanisms have been proposed for reducing the lipid levels in FOS supplemented group. During the fermentation process, short chain fatty acids are produced which enter the portal blood stream where they are utilized by the liver. Acetate is converted to acetyl CoA in the liver and act as a lipogenic substrate for lipogenesis, whereas propionate has been reported to inhibit synthesis of lipid. Butyrate on the other hand, is taken up by the large intestinal cells (Wolver. T.M.S., et.al., 1989 and Demigne. C., et.al., 1995). Oligofructose has been studied to determine the mechanism of action of prebiotics in animals. *In vitro* studies using isolated rat hepatocytes suggested that the hypolipidemic action of OFS was associated with an inhibition of cholesterol synthesis by propionate, following impairment of acetate utilization by the liver for lipogenesis (Demigne. C., et.al., 1995). Evidence suggests that TG lowering effect of prebiotic occurs via a reduction in VLDL TG secretion from the liver due to a reduction in the activity of all lipogenic enzymes (acetyl coA, carboxylase, fatty acid synthase, malic enzymes, ATP citatelyase and glucose-6 phosphate dehydrogenase) and, in the case of fatty acid synthase, via modification of lipogenic gene expression (Delzenne. N.M., and Kok. N., 1998; Rebecca. W., et.al., 2012). Another mechanism proposed is, deconjugation reaction which is catalyzed by a conjugated bile acid hydrolase enzyme, produced exclusively by bacteria. Deconjugation is widely seen in many intestinal bacteria including genera such as *Enterococcus, Bifidobacterium, Fusabaterium, Clostridium* and *Lactobacillus*(Hylemond.P.B., 2004). This reaction liberates an amino acid moiety and a deconjugated bile acid, thereby reducing cholesterol re-absorption and increases fecal excretion of the deconjugated bile acids. Many *in vitro* studies have investigated the ability of various bacteria to deconjugate a variety of bile acids (Grill. J.P., et.al., 1995).There is also *in vitro* evidence to support the hypothesis that some bacteria can assimilate cholesterol. It has been reported that *lactobacillus acidophilus* and *Bifidobacterium bifidum* have the ability to assimilate cholesterol during *in vitro* studies, but only in the presence of bile salts and under anaerobic conditions (Rasic. I.J., 1992). Cholesterol binding to bacterial cell walls has also been suggested by a possible mechanism for the hypocholesterolemic effect of probiotics. Honsono and Tono-oka reported that *Lactococcuslactis* had the highest bonding capacity for cholesterol for a range of bacteria tested (Hosono. A., and Tono-oka. T., 1991). It was speculated that differences in binding of the bacteria were due to chemical and structural properties of their cell walls (Kumar. M., et.al., 2012) .The mechanisms of action of prebiotics on lipid reduction include one or all the above mechanisms, with an ability of different bacterial species to have varying effects. The present study also revealed a significant reduction in systolic blood pressure by 3.6% in the supplemented group as a result of FOS supplementation. Diabetes has also been commonly associated with the over activity of the sympathetic nervous system where long-term sympatho-activation could raise arterial pressure by causing peripheral vasoconstriction and by increasing renal tubular sodium reabsorption (.Rahmouni. K., et.al., 2005). Preliminary evidence indicates that synbiotic or their fermented products may also play a role in blood pressure control, with animal and clinical studies documenting antihypertensive effects of synbiotic consumption (Namakamura. Y., et.al., 1995). Various mechanisms have been postulated to explain the ability of prebiotics to reduce the risk of hypertension. One of the possible mechanisms is via the lowering of blood lipid and cholesterol. This cholesterol lowering effect could reduce the stiffness of large arteries and thus could potentially reduce blood pressure (Ferrier. K.E., et.al., 2002). In another study, Lairon et al suggested that the reduction of obesity upon consumption of prebiotics such as fiber could prevent the elevation of blood pressure (Lairon. D., et.al., 2005). Additionally, inulin has also been reported to reduce the risk of hypertension by improving the absorption of mineral such as calcium in the gastrointestinal tract (Streppel. M.T., et.al., 2005). Past studies have shown promising evidence on the correlation of dietary calcium and hypertension. Allender et alconducted a meta-analysis of randomized clinical trials on the correlation of dietary calcium and blood pressure and had found that median intake of calcium by 1 g/day could significantly decrease the systolic blood pressure by 1 mm Hg to 2 mm Hg (Allender. P.S., et.al., 2004).

Diets high in calcium have been found to reduce peripheral vascular resistance and blood pressure leading to a reduced risk of hypertension (Zemel. M.B., 2005). Increased calcium intake due to intake of fermented milk could have served as an additional factor for reducing hypertension. Present study also demonstrated a significant negative correlation between systolic and diastolic blood pressure and *Bifidobacteria* and a positive correlation with *enteric pathogen* in supplemented group. Fermentation of prebiotics enhances the number of bacteria in the gut which secretes various bioactive peptides with many other metabolites. These peptides inhibits angiotensin I-converting enzyme (ACE), the key enzyme responsible for the regulation of blood pressure via the renin-angiotensin system. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor; ACE also hydrolyzes and inactivates bradykinin, a potent vasodilator. Therefore, excessive activity of ACE leads to an increased rate of vasoconstriction and development of high blood pressure. Inhibitory peptides block ACE-mediated production of angiotensin II, and the reduction in ACE activity results in enhanced levels of bradykinin, resulting in overall antihypertensive effect (Shah. N.P., 2000; Donkor. O.N., et.al., 2005 and Qaisar. M., et.al., 2012). A theory proposes the underlying mechanismfor antihypertensive effects of incretins is the relaxant effect of GLP-1 in vasodilation of the brachial artery (Nystrom. T., et.al., 2005). In rodents, Golpon et al demonstrated that GLP-1 relaxed pulmonary artery rings in a dose- and time-dependent manner, presumably due to the synthesis of NO by the endothelium (Golpon. H.A., et.al., 2001). Moreover, another study reported that GLP-1(9–36), the rapidly produced metabolite of GLP-1, induces vasodilatation via an NO⁄ cGMP-associated mechanism without involvement of the GLP-1R (Ban K., et.al., 2008).

The third theory proposes that the incretin-induced reductions in BP occur as a result of inhibition of the renin–angiotensin–aldosterone system (RAAS) and an increase in urinary sodium excretion (Yu. M., et.al., 2003; Gutzwiller. J.P., et.al., 2006 and Liu. Q., et.al., 2010).

CONCLUSION

On the above grounds, it can be concluded by the various mechanisms postulated by researchers and results based on the present study that Fructooligosaccharide supplementation in diabetic adults with respect to borderline lipid parameters and borderline hypertension brought about changes in their lipemic and blood pressure values and a significant establishment of beneficial microbiota in the gut. Therefore, FOS is a promising remedy for reducing serum lipid parameters and systolic blood pressureand establishment of benefiting gut microflora in type 2 diabetic adults. Moreover, longitudinal studies are needed to resolve the complex relationships in this area.

REFERENCES

Allender PS, Cutler JA, Follmann D, Cappucio FP, Pryer J and Elliott P. (2204). Dietary Calcium and Blood Pressure: A Meta-Analysis of Randomized Clinical Trials. Ann Intern Med 124: 825-831.

Alles MS, Hautvast JGAJ, Nagengast FM, Hartemink R, Van Laere MKJ, Jansen JBMJ. (1996). Fate of fructo-oligosaccharide in the human intestine. British Journal of Nutrition 76:211-212.

Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ and Husain M. (2208). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and-independent pathways. Circulation 117:2340–50.

Brigenti F, Casirgahi M, Canzi E and Ferrari A. (1999). Effect of consumption of ready to eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. European Journal of Clinical Nutrition 53: 726-733.

Causey JL, Feirtag JM, Gallaher DD, Tungland BC and Salvin JL. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. Nutrition Research 20:191-201.

Cummings JH, Pomare EW, Branch WJ, Naylor CPE, MacFarlane GT. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28:1221-1227.

Davidson MH, Synecki C, Maki KC and Drennen KB. (1998). Effects of dietary inulin in serum lipids in men and women with hypercholesterolemia. Nutrition Research 3:503-517.

De Man J, Rogosa M, and Sharpe M. (1990). A Medium for the Cultivation of Lactobacilli. Journal of Applied Bacteriology 23:130.

Delzenne NM and Kok N. (1998). Effect of non-digestible fermented carbohydrates on hepatic fatty acid metabolism. Biochemical Society Transactions 26:228-230.

Demigne C, Ronald C, Levart MA, Bessen C, Moundras C and Remesey C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. British Journal of Nutrition 1995; 74:209-219.

Donkor ON, Henriksson A, Vasiljevic T and Shah NP. (2005). Probiotic strains as starter cultures improve angiotensin-converting enzyme inhibitory activity in soy yogurt. Journal of Food Science 70:M375-381.

FAO/WHO. (2001). Expert Consultation report, on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Argentina ;1-34

Ferrier KE, Muhlmann MH, Baguet JP, Cameron JD, Jennings GL, Dart AM and Kingwell BA. (2002). Intensive Cholesterol Reduction Lowers Blood Pressure and Large Artery Stiffness in Isolated Systolic Hypertension. J Am CollCardiol 39:1020-1025.

Fossati P and Prencipe L. (1982). Serum Triglycerides Determined Calorimetrically with an Enzyme That Produces Hydrogen Peroxide. Clinical Chemistry 28(10): 2077-2080.

Golpon HA, Puechner A, Welte T, Wichert PV, Feddersen CO. (2001). Vasorelaxant effect of glucagon-like peptide (7–36) amide and amylin on the pulmonary circulation of the rat. RegulPept 102: 81–6.

Grill JP, ManiginotDurr C, Schneidar F and Ballongue J. (1995). Bifidobacteria and probiotic effects; action of Bifidobacterium species on conjugated bile salts. Current Microbiology 31:23-27

Gutzwiller JP, Hruz P, Huber AR (2006). Glucagon-like peptide-1 is involved in sodium and water homeostasis in humans. Digestion 73: 142–50.

Hazari MA, Khan RT, Reddy BR and Hassan MA. (2012). Cardiovascular autonomic dysfunction in type 2 diabetes mellitus and essential hypertension in a South Indian population. Neurosciences 17(2): 173-175.

Heijnen ML, Van Amelsvoort JM and Westrate JA. (1995). Interaction between physical structure and amylose: amylopectin ratio of foods on postprandial glucose and insulin responses in healthy subjects. European J of Clinical Nutrition 49: 446–57.

Hosono A and Tono-oka T. (1991). Binding of cholesterol with lactic acid bacterial cells. Milchewissenschaft 50:556-560.

Hylemond PB. (2004). Metabolism of bile acids in intestinal microflora. In: Daniel H, Sjovall J editors. Sterols and Bile acids: 331-343. Elsevier Science Publishing Inc, New York.

Jackson KG, Taylor GRJ, Clohessy AM and Williams Cm. (1999). The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. British Journal of Nutrition 82:23-30

Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A et al. Cholesterol-Lowering Probiotics as Potential Bio-therapeutics for Metabolic Diseases. Diabetes Research 2012. doi:10.1155/2012/902917.

Lairon D, Arnault N, Bertrais S, Planells R, Clero E, Hercberg S and Boutron RMC.(2202). Dietary Fiber Intake and Risk Factors for Cardiovascular Disease in French Adults. Am J ClinNutr 82:1185-1194.

Liu Q, Adams L, Broyde A, Fernandez R, Baron AD and Parkes DG. (2005). The exenatide analogue AC3174 attenuates hypertension, insulin resistance, and renal dysfunction in Dahl salt-sensitive rats. CardiovascDiabetol 2010; 9: 32.

LuoRizkalla SW, Alamowitch C, Boussairi A, Blayo A, Barry JL, Laffifle A, Ouyon F, Bornet FRJ and Salma VS. (1996). Chronic consumption of short chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin stimulated glucose metabolism. American journal of Clinical Nutrition 63:939-945.

Namakamura Y, Yamamoto N, Sakal K, Takano T. (1995). Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin-1-converting enzyme. J Dairy Sci 78:1253–1257

Nystrom T, Gonon AT, Sjo holm A, Pernow J. (2005). Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism. RegulPept 125: 173–7.

Nystrom T, Gutniak MK, Zhang Q (2004). Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. Am J Physiol Endocrinol Metab; 287:E1209–15.

Online Random tables, STAT TREK, (1996). http://stattrek.com/tables/random.aspx.Orafti Raftilose and Raftiline. Product book. Orafti, Belgium.

Parnami S and Sheth M. (2011). Indian Fermented Milk (Dahi) Fortified with Probiotic Bacteria and Inulin Improves Serum Lipid, Blood Glucose Levels and Gut Microflora. Journal of the Indian Academy of Geriatrics 7:1-5.

Pedersen A, Sandstrom B and Van Amlesvobrt JMM. (1997). The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. British Journal of Nutrition 78:215-222.

Qaisar M, Amara J, Nighat B and Muhammad I. (2012). Angiotensin-converting enzyme (ACE) gene II genotype protects against the development of diabetic peripheral neuropathy in type 2 diabetes mellitus. Journal of Diabetes 4(3):257–261.

Rahmouni K, Correia MLG, Haynes WG and Mark AL. (2005). Obesity-Associated Hypertension: New Insights into Mechanisms. Hypertension 45:9-14.

Ramona R, Robinson MS, Joellen F and Joanne L. (2000). Department of Food Science & Nutrition, University of Minnesota, St. Paul, Minnesota. Journal of American College of Nutrition 20(4): 279-285.

Rasic IJ, Vujieie LF, Skrinjar M and Vulie M. (1992). Assimilation of cholesterol by some cultures of Lactic acid bacteria and Bifidobacteria. Biotechnology letters 14:39-44.

Rebecca Wall, Paul RR, Fergus S, Eamonn M Q, Timothy GD, John F, Cryan (2012). Influence of gut microbiota and manipulation by probiotics and prebiotics on host tissue fat: Potential clinical implications. Lipid Technology 24(10): 227-229.

Richmand W. (1993). Preparation and Properties of a Cholesterol Oxidase from Nocardia sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. Clinical Chemistry 19(12): 1350-1356.

Sahay BK: API-ICP guidelines on diabetes (2007). J Assoc Physicians India 2007; 55:1–50.

Shah NP. (2000). Natural bioactive substances in milk and colostrum. British Journal of Nutrition 84(S1):S3-S10.

SPSS19.0 version, SPSS Inc., Chicago, IL, USA

Streppel MT, Arends LR, Vant Veer P, Grobbee DE and Geleijnse JM. (2005). Dietary Fiber and Blood Pressure: A Meta-Analysis of Randomized Placebo-Controlled Trials. Arch Intern Med 165:150-156.

Sughichi H, Uji O, Okabe H, Irie T, Uekama K, Kayahara N and Miyauchi K. (1995). Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with Polyethylene Glycol-Modified Enzymes and Sulfated a-Cyclodextrin. Clinical Chemistry 41(5): 717-723.

Thomas G, Hall JE, Appel JL, Falkner BE, Graves J, Hill MN (2005). Recommendations for Blood Pressure Measurement in Humans and Experimental Animals. Circulation 111: 697-716.

Transansia Bio Medical Ltd., Vadodara

WHO, "Cardiovascular Disease," Fact sheet no. 317, WHO, Geneva, Switzerland, 2009, http://www.who.int/mediacentre/ factsheets/fs317/en/print.html.

Williams G. Hypertension in diabetes. In: Pickup J, Williams G, eds. (1991). Textbook of Diabetes. London: Blackwell Scientific Publications, 719–732.

Wolver TMS, Brighenti F, Royalle D, Jenkins AL and Jenkins DJA. (1989). Effect of rectal infusion of short chain fatty acids in human subjects. American Journal of Gastroenterology 84:1027-1033

Yamashita K, Kawai K and Itakura K. (1984). Effect of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutr Res 4: 961-966.

Yu M, Moreno C, Hoagland KM (2003). Antihypertensive effect of glucagon- like peptide 1 in Dahl salt-sensitive rats. J Hypertens; 21: 1125–35.

Zemel MB. (2005). Calcium Modulation of Hypertension and Obesity: Mechanism and Implication. J Am CollNutr 20:428S-435S.