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A COMPARATIVE STUDY OF SYNBIOTIC AND PREBIOTIC SUPPLEMENTATION ON GUT HEALTH, SCFA, HS-CRP AND LIPID PROFILE OF TYPE 2 DIABETIC SUBJECTS WITH PRE HYPERTENSION.

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ABSTRACT
Objective: The study was designed to determine the impact of synbiotic and prebiotic supplementation on gut health, hs-CRP, lipid profile and short chain fatty acids (SCFA) of type 2 diabetic subjects with pre hypertension. **Methods:** 60 pre hypertensive with type 2 NIDDM subjects were selected purposively from Sun Valley Hospital, Assam, India. The subjects were randomly divided into Control (N=10), Synbiotic group (N=25) and Prebiotic group (N=25) based on their informed consent. The synbiotic group was supplemented with 1 gm of freeze dried synbiotic product and the prebiotic group with 10 ml of fructo oligosaccharide (FOS) daily to be taken along with meals, for 45 days. All the parameters were determined using standard methods. **Results:** Synbiotic supplementation showed a significant improvement in the gut health of the subjects by increase in LAB and Bifidobacteria by 32.6% and 131.6% respectively and prebiotic supplementation resulted in a significant increase in LAB and Bifidobacteria by 77.6% and 29.7 % respectively. Synbiotic supplementation and prebiotic resulted in a significant reduction on hs-CRP and in TG, TC, LDL, VLDL and a significant increase in HDL and prebiotic supplementation showed a significant reduction in the TG, TC, LDL, VLDL and a significant increase in HDL. Synbiotic and prebiotic resulted in an increase in fecal butyrate levels and fecal propionate levels. **Conclusion:** Daily intake of 1 g synbiotic product and 10 ml FOS improves gut health, hs-CRP, lipid profile and short chain fatty acids (SCFA) of the subjects which may be due to increased production of SCFA.

KEYWORDS: Diabetes, SCFA, synbiotic, prebiotic.

INTRODUCTION
India currently faces the dual burden of communicable diseases and chronic non communicable diseases (NCDs) such as cardiovascular disease (CVD), diabetes and cancer. Moreover, diabetes and hypertension is an important risk factor for CVD and CVD is the major cause of death and disability in persons with diabetes. Science based strategies have been established to prevent Cardiovascular disease (CVDs) using functional foods. The functional foods which are less explored include the probiotics, prebiotics and the synbiotics. Synbiotic is the marriage of the concepts of probiotics and prebiotics. Fructooligosaccharide (FOS) and synbiotic has been recognized for their great potential which is becoming apparent as a considerable food element in increasing the bacterial bionomics which might have a concrete role in reducing the burden of CVDs. They have the potential to regulate lipemic control, reduce the expression of hs-CRP levels, modulate the gut health by increasing the colonization of gut microbiota and also increase the production of SCFA.

MATERIALS AND METHODS
Sixty pre hypertensive subjects with type 2 NIDDM aged 35-55yrs were selected purposively from Sun Valley Hospital, Guwahati, Assam, India, and based on their informed consent, they were randomly divided into Control (N=10), synbiotic group (N=25) and prebiotic group (N=25). The synbiotic group was supplemented with 1 gm of freeze dried synbiotic product (2 species of *Lactobacillus*, *Bifidobacterium* each, one species of *Streptococcus*, one species of yeast along with 300 mg Fructo oligosaccharide) and the prebiotic group was supplemented with 10 ml of fructo oligosaccharide (FOS) for 45 days to be taken daily along with meals. From the stool samples DNA was isolated and was

quantified for determination of LAB, Bifidobacteria and Enteric pathogens using PCR techniques. hs-CRP was estimated by using automatic chemistry analyzer manufactured by **Siemens and Model: Advia 2400.** Lipid profile was determined by using enzymatic colorimetric method and using Gas Chromatography Mass Spectro Photometer (GCMS). SCFA was determined.

Determination of the gut microbiota

The gut microbiota was determined in terms of the microorganisms-*Lactic acid bacteria*, *Bifidobacterium* and Enteric pathogen.

The steps involved while determining the fecal flora were:

Fecal sample collection and DNA isolation for enumeration of the micro-organisms

In air tight sterile containers, the stool samples from the subjects were collected, kept with dry ice n stor t-8 until the samples were analysed.

Extraction of DNA using QIAagen kit

In 2 ml tube sterile tube, 180-220 mg of stool was weighed and placed in 2 ml tube (placed in ice) for thawing. Th r ft r, 1.4 µl of ASL buff r w s to th stool samples and vortexed for 1 minute until it got thoroughly homogenized. The suspension was then heated for 5 mins. t 7 95 for r m pos t v b t r n vort x for 15 seconds and the samples were centrifuged at 14,000rpm for 1 minute to pellet the stool sample. 1.2 ml of the supernatant was pipetted into a new 2 ml micro centrifuge tube and the pellet was discarded. 1 inhibit Ex tablet was added to each sample and vortexed for 1 minute to allow inhibitors to absorb to the inhibitor Ex motrix. Then the sample was centrifuged at 14000rpm for 3 minutes to pellet inhibitors bound to inhibitors Ex. Supernatant was taken into a new tube and centrifuged t 14 rpm for 3 m nut s. 15 µl prot n s K w s t k n nto n w tub n 4 µl of sup rn t nt to t. 4 µl of AL buff r w s n vort x for 15 secs. 4 µl of th nol w s to th lysate and mixed by vortexing. Complete lysate was added from the previous step to the QIAamp column and centrifuged at 14000rpm for 1 minute and placed the QIAamp spin column in a new 2 ml collection tube and the filtrate w s s r . 5 µl of AW1 buff r w s to th sp n olumn n ntr fu d at 14000rpm for 1 minute. The QIAamp column was placed in a new 2 ml collection tube and the filtrate was d s r . 5 µl AW1 buff r w s added to the QIAamp spin column and centrifuged for 3 minutes and the filtrate was discarded. The QIAamp spin column was tr nsf rr nto n w tub n 1 µl AE buff r w s on to th QIA mp m mbr n . In ub t on w s on t room temperature for 1 minute centrifuged at 14000rpm for 1 minute and the DNA was eluted.

RNase treatment protocol: The precipitate was re suspende n r nom NA n 5 µl of 1 TE buff r. 15 µl of 1µ µl RN s w s n n ub t n 65 w t r b th for 1 m nut s. .4 volum of 7.5M mmon um t t w s dded to precipitate the RNase, the tube was flicked several times to mix. Let the sample stand for 3 m nut t 4 , sp n t 13, rpm for 3 m nut , t 4 n r fully r mov sup rn t nt to th n w tub n th sup rn t nt w s s v . NA w s pr p t t with 1 ml of cold 100% ethanol, gently mixed for 1 minute and let it stand at - for 3 m nut . Sp n t 13, rpm for m nut t 4 nt n s r th th nol n th s mpl was w sh w th 7 % th nol. 5 µl of 7 % ethanol was added and centrifuged for 1 m nut . P ll t w s r mov n 5 µl of 7 % th nol w s ed in the same test tube. Again the pellet was removed and dried in laminar flow to get the DNA. All samples w r put n th r fr r tor t - for 48 hours.

Gel formation procedure

Stock solution of TAE buffer was 50X TAE and working concentration was 1X. To the electrophoresis tank 1lt. of the total solution was transferred. 1 ml of 50X TAE buffer was Pipetted out to 100 ml of the above solution. 0.8g of ros w s w th 1 µl of Eth um brom n ssolv n w s pour to th st n tr y and was allowed to cool. Using a pipette 1 µl of the DNA samples were loaded into the wells of an agarose gel. After 30 minutes DNA formation was visualized using **bioDoc-Itusing** a **TM**Imaging system.

The Thermo Scientific Nano Drop™ 1000 Spectrophotometer measures 1 ul samples with high accuracy and reproducibility. To hold the sample in place, the full spectrum (220 nm-750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone. 1 ul sample was pipetted onto the end of a fiber optic cable (the receiving fiber). A second fiber optic cable (the source fiber) was brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. The gap was controlled to both 1 mm and 0.2 mm paths. The PC based software controlled the instrument and the data was logged in an archive file on the PC. With the sampling arm open, the sample was pipetted onto the lower measurement pedestal. The sampling arm was closed and a spectral measurement was initiated using the operating software on the PC. The sample column was automatically drawn between the upper and lower measurement pedestals and the spectral measurement was made. When the measurement got completed, the sampling arm was opened and the sample from both the upper and lower pedestals was wiped using a soft laboratory wipe. And RNase impurity was calculated in the 260/280 ratio.

Selection of appropriate primers for maximal efficiency and specificity is a critical factor in PCR. A number of factors, including sequence, primer location, and the PCR system used affects the primer specificity. To avoid mispriming and primer-dimer formation general primer-design rules for PCR are applicable. Therefore, the primers used for the study are as follows:

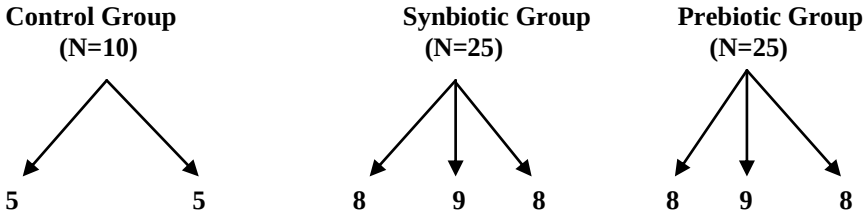
Primer	Primer sequence (5'-3')	Annealing temp
Lac-F	AGCAGTAGGGAATCTTCCA	55◦C
Lac-R	CACCGCTACACATGGAG	
Bifido-F	CTCCTGGAAACGGGTGG	60◦C
Bifido-R	GGTGTTCTTCCCGATATCTACA	
Uni 331F	TCCTACGGGAGGCAGCAGT	60◦C
Uni 797R	GGACTACCAGGGTATCTATCCTGTT	
Enter F	TGCCGTAACTTCGGGAGAAGGCA	60◦C
Enter R	TCAAGGACCAGTGTTCAGTGTC	

The samples were run in the Master cycler nexus gradient model PCR. The concentration of the bacteria was determined.

Procedure of PCR technique

Extraction of metagenomic DNA was done from the stool samples using the **Qiagen Kit**. PCR amplification of V6-V8 region of bacterial 16S rDNA was carried out using the genus specific primer for Bifidobacterium, Lactobacillus, Enterococcus and an universal primer for overall bacteria. PCR reaction was performed in a 10 µl reaction volume in a thermal cycler (**Mastercycler Nexus gradient, Eppendorf, Germany**). Each PCR reaction constituted a final concentration of 1x standard Taq buffer, 1.75 mM of $MgCl_2$, 200µM of dNTPs, 0.2µM of each primer, 0.5U of Taq DNA polymerase (Sigma Aldrich, USA) and 25ng of template DNA. PCR conditions were, initial denaturation at 94˚C for 5 min. followed by 25 cycles of denaturation at 94˚C for 30 sec., annealing for 30 sec., extension at 72˚C for 30 sec. and a final extension at 72 ˚C for 7 min. PCR products were separated in a 1.5% agarose gel along with 100 bp DNA size and 50 bp DNA mass ladder for size and mass calculation, respectively and visualized under **BioDoc-It Imaging System (UVP, USA).** Band quantification was performed using Image J software comparing with the mass ladder.

Determination of Short-chain fatty acid; Sample preparation: The samples were pooled in the following way



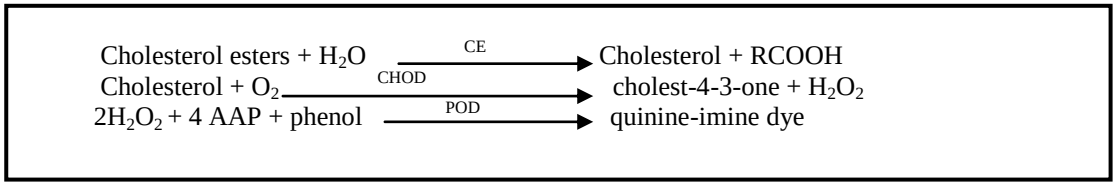
GC-MS was performed on the Shimadzu **GC 2010 plus with triple quadrupole MS (TP-8030)** fitted with EB-5MS column (length-30 m, thickness-0.25 µm, ID-0.25 mm). Temperature programme was started at 60 °C, held for 2 mins and then raised finally to 250 °C @ 4 °C/min at which it was held for 15 mins. 250 °C was the injection temperature and column flow rate was 1.0 ml/min with He as carrier gas. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV with an ion source temperature of 230 °C and a continuous scan from 45 to 800 m/z. The peaks were identified by matching the mass spectra with the National Institute of Standards and Technology (NIST) library.

Determination of hs-CRP: hs-CRP was estimated by using automatic chemistry analyzer manufactured by **Siemens and Model: Advia 2400.** 10 µl of blood serum of each subject was loaded in the smart card of the machine and the sample was analyzed with the help of the smart card technology and the results were automatically generated by the machine.

Determination of the lipid profile

Total Cholesterol (TC)

Total cholesterol was estimated using end point enzymatic colorimetric technique. Cholesterol esters are 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 colour intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm (Richmond W 1973)



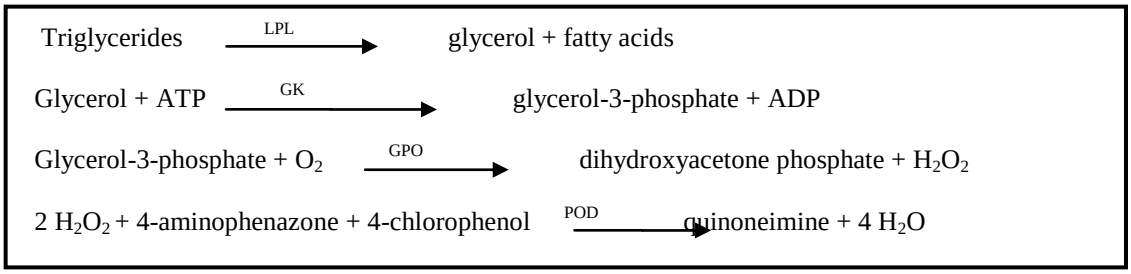
Cut offs for Serum Cholesterol

Classification	Cholesterol Value (mg/dl)
Desirable	< 200
Borderline High	200-239
High	>240

NCEP-Adult Treatment Panel (NCEP-ATP3) guidelines, 2002

Triglycerides (TG)

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 (H_2O_2). 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).



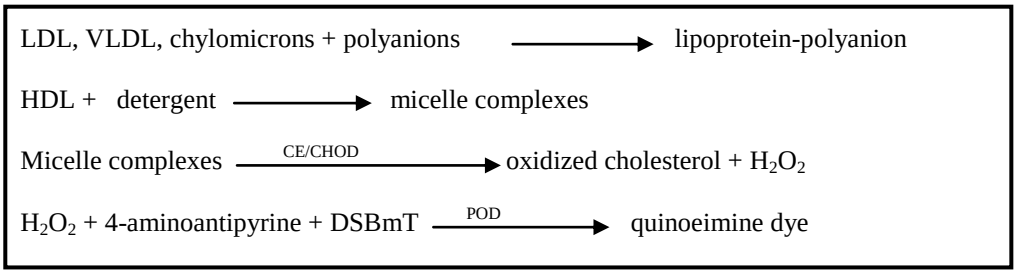
Cut offs for Triglycerides

Classification	Triglycerides Value (mg/dl)
Desirable	< 150
Borderline High	150-199
High	200-499
Very High	≥500

NCEP-Adult Treatment Panel (NCEP-ATP3) guidelines 2002

HDL Cholesterol (HDL–C)

HDL 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 color 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 (Sugiuchi J et al 1995).



Cut offs for HDL-Cholesterol

Classification	HDL Cholesterol Value (mg/dl) Female	HDL Cholesterol Value (mg/dl)Female
Low	<45	< 35
Optimal	45-55	45-55
High	>55	>45

NCEP-Adult Treatment Panel (NCEP-ATP3) guidelines, 2002

LDL Cholesterol (LDL-C)

Enzymatic colorimetric method (CHOD/PAD) was used for the direct estimation of LDL, HDL, VLDL and chylomicrons are specifically hydrolyzed by a detergent. The released cholesterol content in these lipoproteins reacts immediately in the enzymatic action of cholesterol esterase (CE) and cholesterol oxidase (CHOD) generating hydrogen peroxide. The latter is consumed by a peroxidase (POD) in the presence of 4-aminoantipyrine to generate a colorless product. During this first step, LDL particles remain intact. The reaction of LDL cholesterol is initiated by the addition of another detergent together with a coupler, N,N-bis(4-sulfonyl)-m-toluidine (DSBmT). The second detergent releases cholesterol in the LDL particles which are subjected to the enzymztic reaction in the presence of coupler to produce a coloured product. The colour intensity of the red quinoneimine dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 520nm (Sugiuchi J et al 1995).

Step 1: LDL, VLDL, chylomicrons + detergents $\longrightarrow$ release cholesterol

Cholesterol $\xrightarrow{CE/CHOD}$ oxidized cholesterol + H_2O_2

H_2O_2 + 4-aminoantipyrine + DSBmT $\xrightarrow{POD}$ colorless product

Step 2: LDL + detergent $\longrightarrow$ released cholesterol

Cholesterol $\xrightarrow{CE/CHOD}$ oxidized cholesterol + H_2O_2

H_2O_2 + 4-aminoantipyrine + DSBmT $\xrightarrow{POD}$ quinoeimine dye

Cut offs for LDL-Cholesterol

Classification	LDL Cholesterol Value (mg/dl)
Optimal	< 100
Near optimal/Above optimal	100-129
Borderline High	130-159
High	160-189
Very high	>190

NCEP-Adult Treatment Panel (NCEP-ATP3) guidelines 2002

RESULTS AND DISCUSSION

The present study on supplementation of FOS (10 ml) and synbiotic product (1 gm) to pre hypertensive type 2 diabetic subjects for a period of 45 days resulted in a significant reduction in hs-CRP and TG, TC, LDL-C, VLDL and with a significant increment in HDL-C, beneficial gut microbiota and fecal SCFA.

Table I: Impact of liquid FOS and synbiotic supplementation on LAB, Bifidobacteria, Enterobacteria, Butyrate and Propionate, hs-CRP and lipid profile.

Parameters	GROUPS	MEAN±SD	t stat	Paired two tail	% difference
Lactobacillus (ng)	Control Pre	176.85 ± 90.68	2.55	0.23 ^{NS}	23.19↓
	Control Post	135.83 ± 81.42			
	FOS Pre	265.80 ± 198.64	5.36	**0.00001*** **	**77.64**↑
	FOS Post	472.18 ± 240.43			
	Syn Pre	191.55± 183.7	7.63	**7.16*** **	**32.61**↑
	Syn Post	254.03 ± 231.52			
Bifidobacteria (ng)	Control Pre	177.64 ± 70.58	0.67	0.43 ^{NS}	3.52↓
	Control Post	171.40 ± 85.80			
	FOS Pre	362.98 ± 581.01	3.54	**0.001*** **	**29.71**↑

	FOS Post	470.78 ± 628.61			
	Syn Pre	131.14± 56.18	3.10	**0.004*** **	**131.61↑**
	Syn Post	303.75± 252.64			
Enterobacteria (ng)	Control Pre	223.82 ± 232.72	2.42	0.03 ^{NS}	59.1↑
	Control Post	356.01 ± 263.15			
	FOS Pre	349.27 ± 560.95	4.43	**0.01****	**44.63↓**
	FOS Post	201.81 ± 466.8			
	Syn Pre	248.77± 222.05	2.67	**0.0001*****	**49.6↓**
	Syn Post	125.33 ±130.90			
Butyrate (ppm)	Control Pre	49.25 ± 14.95	2.55	0.23^{NS}	64.24↓
	Control Post	17.61 ± 2.53			
	FOS Pre	34.13 ± 10.16	7.86	**0.01****	**661.91↑**
	FOS Post	260.04 ± 48.08			
	Syn Pre	37.73± 13.81	4.86	**0.03****	**547.41↑**
	Syn Post	244.27 ± 76.16			
Propionate (ppm)	Control Pre	58.36± 5.27	3.22	0.19^{NS}	41.79↓
	Control Post	33.97± 5.41			
	FOS Pre	37.48 ± 12.03	4.47	**0.04***	**525.1↑**
	FOS Post	234.29 ± 83.69			
	Syn Pre	46.04± 20.61	3.9	**0.05***	**310.03↑**
	Syn Post	188.77± 75.04			
hs-CRP (mg/L)	Control Pre	1.19 ± 0.33	0.14	0.89	0.25↓
	Control Post	1.19 ± 0.28			
	FOS Pre	0.97±0.26	6.81	**4.79*****	**27.25↓**
	FOS Post	0.71±0.11			
	Syn Pre	1.26± 0.20	14.38	**2.88*****	**48.78↓**
	Syn Post	0.63 ± 0.20			
TG(mg/dl)	Control Pre	197.5± 80.6	1.94	0.08^{NS}	1.06↑
	Control Post	199.6± 79.5			
	FOS Pre	160.12 ± 68.40	14.72	**1.63*****	**7.9↓**
	FOS Post	146.68 ± 69.88			
	Syn Pre	163.48± 60.14	11.02	**7.06*****	**3.9↓**
	Syn Post	155.64 ± 61.14			
TC (mg/dl)	Control Pre	181.7± 38.12	2.43	0.03^{NS}	5.2↑
	Control Post	184 ± 37.34			
	FOS Pre	166.16 ± 26.50	14.44	**2.47*****	**8.75↓**
	FOS Post	150.52 ± 27.45			
	Syn Pre	169.96 ± 39.78	11.9	**1.31*****	**6.77↓**
	Syn Post	158± 40.39			
HDL (md/dl)	Control Pre	38 ± 5.92	3.08	0.01^{NS}	3.15↓
	Control Post	36.8 ± 5.65			
	FOS Pre	35.84 ± 5.78	15.08	**9.63*****	**25.14↑**
	FOS Post	46.08 ± 6.43			
	Syn Pre	35.56±4.47	19.09	**5.12*****	**24.15↑**
	Syn Post	48.4±3.96			
LDL (mg/dl)	Control Pre	140.4 ± 10.87	2.75	0.02 ^{NS}	2.71↑
	Control Post	141.2 ± 11.94			
	FOS Pre	136.36 ± 14.44	12.4	**6.2*****	**8.03↓**
	FOS Post	119.32 ± 17.21			
	Syn Pre	141.2±11.94	10.86	**9.51*****	**8.22↓**
	Syn Post	127.12±13.74			
VLDL (mg/dl)	Control Pre	29.06 ± 10.89	1.78	0.10 ^{NS}	4.26↑
	Control Post	30.3 ± 10.47			
	FOS Pre	36.92 ± 9.86	5.11	**3.11*****	**9.63↓**
	FOS Post	33.4 ± 8.33			
	Syn Pre	33.84±9.59	10.18	**3.43*****	**15.11↓**
	Syn Post	28.26±7.55			

As shown in table 1, the present study revealed that supplementing the subjects with synbiotic resulted in a significant increase in LAB and *Bifidobacteria* by 32.6% and 131.6% respectively and supplementing the subjects with prebiotic resulted in a significant increase in LAB and *Bifidobacteria* by 77.6% and 29.7 % respectively. Prebiotic was two times more effective in increasing the LAB than synbiotic whereas, synbiotic proved to be more effective than prebiotic and increased Bifidobacteria, i.e. by more than 4 times. A study reported a significant increment in the colonization of *Bifidobacteria* (10.78%) and *Lactobacillus* (30.51%) at the end of the treatment period, after eating the prebiotic (20gm) for 90days.[1] Another study of FOS supplementation on diabetic subjects showed a significant increase by 9.3% and 10.9% on the fecal log counts of *Lactic acid bacteria* and *bifidobcantacteria* respectively along with a significant reduction by 4.8% of fecal log counts of Enteric pathogen.[2]

Present study also revealed that by supplementing the subjects with synbiotic and prebiotic resulted in a significant increase in fecal butyrate levels by 547.4% and 661.9% respectively and a significant increase in fecal propionate levels by 310% and 525% respectively. Many studies have shown that supplementation of FOS, lead to increase in SCFA formation in the gut and related beneficial effects on the host metabolism like glucose tolerance.[3][4] SCFA, particularly acetate, propionate, and butyrate, are the dominating end-products of bacteria fermentation in the large bowel.[5]

A number of possible mechanisms have been proposed for the reduction in the glycemic and lipemic parameters when supplemented with FOS and synbiotic food. During the fermentation process, a number of short chain fatty acids are produced which enter the portal blood stream where they are utilized by the liver. Propionate has been reported to inhibit synthesis of lipid, thereby lowering the rates of triacylglycerol secretion while butyrate is taken up by the large intestinal cells and provide a source of energy for human colon epithelial cells.[6][7]

Prebiotic and synbiotic supplementation also revealed a significant reduction in hs-CRP by 27.2% and 49% respectively. Synbiotic was more effective on reducing the levels of hs-CRP almost by two folds than prebiotic. Similar results were found by several researchers. A double-blind placebo controlled trial was conducted among 44 type 2 diabetic patients. Patients in the synbiotic group received 1 synbiotic tablet daily whereas the placebo group received 1 placebo tablet. The hs-CRP levels decreased significantly in the synbiotic group at the end of 8 weeks compared to the baseline ($p<0.05$).[8]

Another study conducted on consumption of a synbiotic food for 6 weeks among diabetic patients, compared to the control, also resulted in a significant decrease on hs-CRP levels ($P=0.01$).[9]

The present study also reported that lipid levels reduced in synbiotic supplemented group for TG, TC, LDL, VLDL by 3.9%, 6.8%, 8.2%, 15.1%, respectively and a significant increase in HDL by 24.15%. A similar study on Prebiotic and synbiotic fermented milk supplementation on elderly for a period of six weeks revealed 3.57% and 6.9% reduction in TC and 0.75% and 4.1% increase in HDL levels respectively. A significant reduction in TG (4.3%) and LDL levels (8.3%) in synbiotic group was observed and a 5.5% reduction in LDL-C in male participants of synbiotic supplemented group respectively.[10]

A similar Randomized control trial was done for a period of 2 weeks to evaluate the effects of the daily consumption of synbiotic bread (L. sporogenes/inulin) on blood lipid profiles of patients with T2D which led to a significant reduction in serum TG ($P = 0.005$), VLDL-C ($P = 0.005$) and an increase in serum HDL-C ($P = 0.01$).[11] Hence, the combination of prebiotic and probiotic bacteria may have resulted in the improvement of the lipid profile.

The present study also revealed that supplementing the subjects with prebiotic resulted in a significant reduction in the TG, TC, LDL, and VLDL by 7.9%, 14.4%, 8%, 9.6% respectively and a significant increase in HDL by 25.1. A study conducted on FOS supplementation to type 2 diabetic adults resulted in a significant reduction in serum TC, TG and LDL levels by 10%, 5.4% and 6.8% respectively. However, no significant increase was observed for HDL and no significant reduction was seen in terms of VLDL.[12] Another study showed the effect of 6-week supplementation of a probiotic strain *Lactobacillus salivarius* UBL S22 with or without prebiotic fructo-oligosaccharide (FOS) on serum lipid profiles in 45 healthy young individuals. The patients were divided into 3 groups (15/group), that is, placebo, probiotic, and synbiotic. After 6 weeks, a significant reduction ($P < .05$) in total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides and increase in high-density lipoprotein cholesterol was observed in the probiotic as well as in the synbiotic group.[13]

CONCLUSION

Supplementation of 10 ml of FOS and 1 gm of synbiotic product can prove to be an attractive strategy for the management of pre hypertension and type 2 diabetes, as it not only improves the lipid profile and lowers hs-CRP levels but also helps in increasing the colonization of beneficial gut microbiota, and thereby reducing the risk of development of diabetes and other metabolic disorders.

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