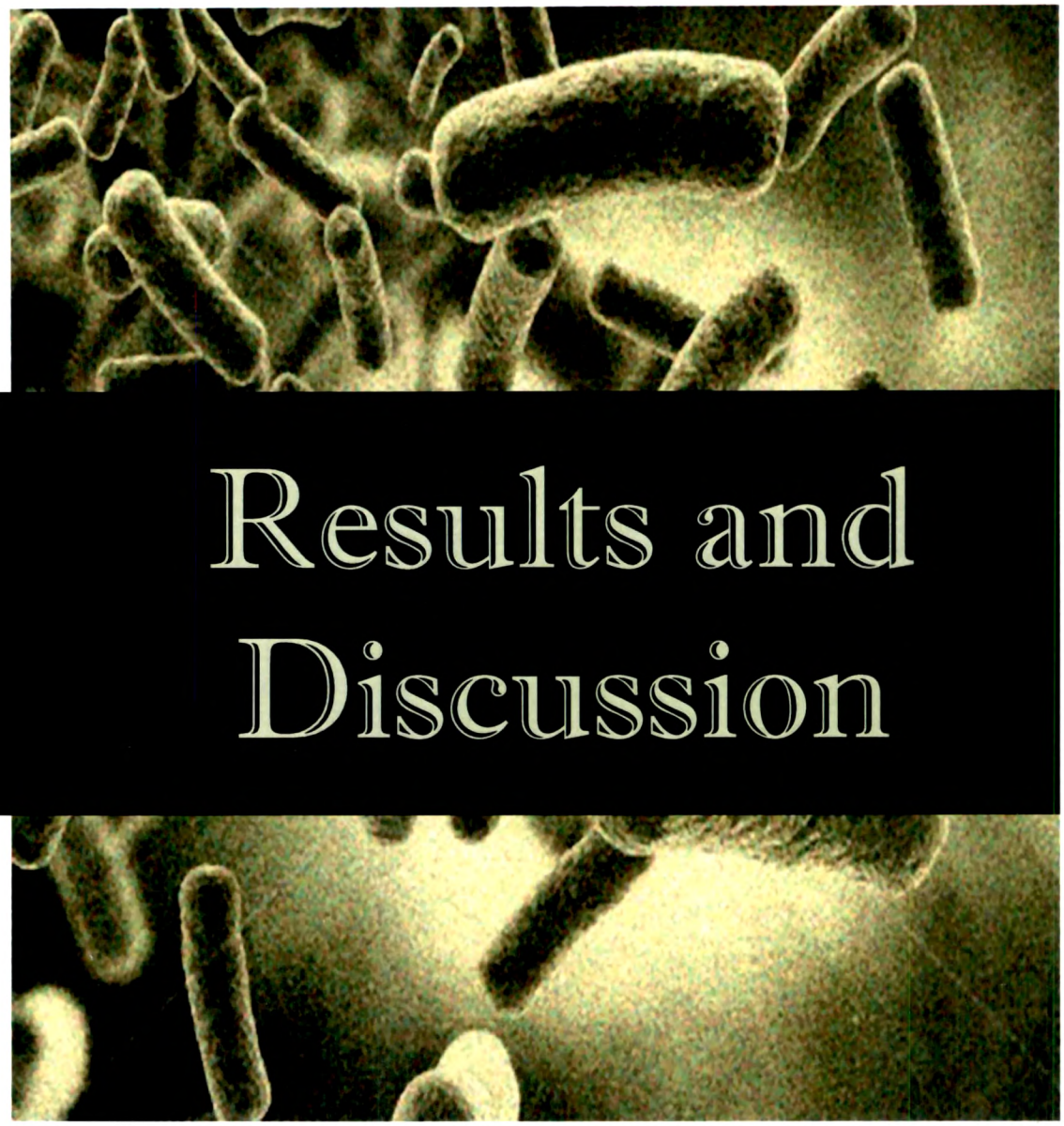


Results and Discussion

CHAPTER 5

RESULTS AND DISCUSSION

The results of the present study entitled **"Acceptability trials of fructooligosaccharide (FOS) substituted food products and impact evaluation of FOS supplementation in type 2 diabetic adults in terms of their glycemia, gut incretin (GLP-1) and gut microbiota"** are presented, discussed and interpreted in this chapter. These results are presented in to three main phases according to the objectives of the study.

PHASE I Development of FOS incorporated food products and studying their various organoleptic attributes, overall acceptability and the recovery of FOS during processing of these products using HPLC technique.

PHASE II Collection of baseline data of type 2 diabetic subjects attending health clinic of M.S. University of Baroda in terms of anthropometry, dietary, biophysical, glycemic, lipemic, GLP-1 and gut microbiota (*LAB, bifidobacteria* and enteric pathogen) and understanding the correlations between various parameters.

PHASE III Effect of Fructooligosaccharide (FOS) supplementation on Glycemic, lipemic parameters, Gut incretin (GLP-1) and Gut Microflora in type 2 diabetic adults.

PHASE I Development of FOS incorporated food products, studying their acceptability and the recovery of FOS during processing using HPLC technique

Functional foods have been defined as foods and food components that provide a health benefit beyond basic nutrition. These may include conventional foods; fortified, enriched or enhanced foods; and dietary supplements. Shortwhile, fructooligosaccharide (FOS) has been classified as functional food ingredients (Arai 2002), as it is a known to have prebiotic properties and exhibits many health benefits. Apart from health benefits the use of inulin or oligofructose as a fibre ingredient often leads to an improved taste and texture (Franck and Coussement 1997). Therefore, this phase of the research work was undertaken to study the acceptability trials of Fructooligosaccharide (FOS) incorporated food products *viz.* ***Chapati, Thepla, Dhokla*** and ***Patra*** at varying levels of substitution.

The results of this phase are divided into following sections-

Section 5.0 Effect of substitution of base material in food products *viz. chapati, thepla, dhokla and patra* with varying levels of FOS

Section 5.1 Recovery of FOS during processing

Section 5.0 Effect of substitution of base material in food products viz. *chapati, thepla, dhokla* and *patra* with varying levels of FOS

For this section of the phase, four food products for FOS substitution selected were *chapati thepla, dhokla* and *patra* and were assessed for their physical and organoleptic properties. Since these food products are the most commonly consumed in Gujarat region, they were considered as a vehicle for FOS substitution. Base material of *chapati* and *thepla* (wheat flour and bengal gram flour) were substituted with FOS at four levels (6%, 10%, 16% and 20%) and in case of *dhokla* and *patra* base material (Bengal gram flour and semolina mix in

case of *dhokla* and bengal gram flour in case of *patra*) was substituted with FOS at 3 levels (6%, 10% and 20%).

5.0.1 Effect of substitution of base material in chapati at varying level of FOS

The result of this subsection are presented in Table 5.0.1.1 to 5.0.1.3

a) Assessment of Physical properties of the *Chapati*

Table 5.0.1.1 presents physical properties of *chapati* substituted with varying levels of FOS in the base material. As the level of FOS substitution increased, total dough weight of *chapati* decreased from 200g (std.) to 175 g (20%). Added water had to be decreased with higher level of substitution with FOS as to obtain desirable dough consistency. Any additional water added to the dough increased its stickiness and lead to difficulty in its kneading. The dough became harder with increase in concentration of FOS in the flour. Time required for the cooking of *chapatis* also decreased at the higher percent substitution from 38.3 seconds to 31.3 seconds. The water absorption power (WAP) of the dough decreased from 92% (6% level of FOS incorporation) to 68% (20% level of FOS incorporation). The WAP upto 84% was suitable for *chapati* making. The *chapatis* prepared from flours with WAP less than 84% tended to be stiff and semi stiff and resulted in partial puffing. Puffing of *chapati* usually takes place depending upon several factors including water retention inside the dough. In present study, in order to prepare the dough with FOS for avoiding sticky consistency; water had to be minimized and therefore, puffing may have reduced.

b) Organoleptic evaluation of the *chapati*

The organoleptic scores of *chapati* prepared by substituting base flour with varying levels of FOS are presented graphically in Figure 5.0.1 (a-h) and tabulated in Table 5.0.1.2.

i) Color and Appearance: The color and appearance scores of *chapati* reduced significantly by 16.8% as the level of substitution increased ($p<0.01$). With the increase in level of FOS substitution, all samples exhibited an increase in whiteness. There was a significant reduction in the color and appearance scores even at 3% level of substitution (Figure 5.0.1 a).

Table 5.0.1.1: Physical properties of FOS substituted *chapati*

Characteristics	Level of substitution				
	Std.	**6%**	**10%**	**16%**	**20%**
Dough weight (g)	200 ±0.5	196 ±0.5	184 ±1.0	180 ±0.1	175 ±0.5
Cooked weight (g)	140 ±0.5	130 ±0.0	121 ±0.5	112 ±0.5	104 ±0.5
WAP %	100	92	84	76	68
Time for cooking (sec)	38.3 ±2.8	33.3 ±1.5	33.6 ±1.1	33.3 ±2.3	31.3 ±1.1
Puffing	Full	Full	Full	Partial	Partial

ii) Burn spots: There was appearance of more number of brownish and black spots on the surface of *chapatis* with the increase in concentration on FOS. There was almost significant reduction in the burn spots of *chapati* with varying scores of 7.4 (std.) to 5.9 due to FOS substitution ($p<0.001$) (Figure 5.0.1 b).

iii) **Texture:** The texture scores for *chapati* reduced due to the hard feel against normally accepted soft feel, when touched or folded. There was 21% decrease in the texture scores at 20% FOS substitution ($p<0.001$) (Figure 5.0.1 c).

iv) Breakability: Breakability is another attribute that was used for studying the organoleptic attribute of *chapatis*. Normally *chapatis* should be foldable have and should have soft texture. In the present study *chapatis* made from wheat flour substituted with FOS became brittle when folded and the brittleness increased significantly ($p<0.01$) by 24% (Figure 5.0.1 d).

v) Taste and Mouthfeel: Reduction in the taste and mouthfeel scores decreased significantly ($p<0.01$) by 20.7% upon substitution of wheat flour upto 6% of FOS. (Figure 5.0.1 e).

vi) After taste: Mean scores for after taste of *chapati* ranged from 6.6 (6% level of FOS incorporated *chapati*) to 5.8 (20% level of FOS incorporated *chapati*) as against 7.3 scored by the standard sample. The reductions in the scores for after taste of *chapati* were not statistically significantly upto 10% of FOS substitution. At higher levels the sweet taste of *chapati* increased which is not a desirable character of *chapati*. There was 20.5% decrease in the after taste scores of *chapati* after 10% substitution ($p<0.01$) (Figure 5.0.1 f).

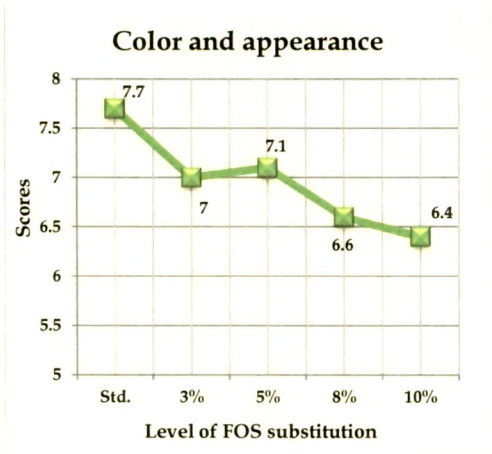
vii) Chewability: Chewiness is the length of time required to masticate the sample at constant rate of force application to reduce it to a consistency suitable for swallowing. *Chapatis* were fairly chewable upto 6% FOS incorporation, after which chewability scores decreased significantly ($p<0.001$). This may be because increase in hardness with increase in FOS in *chapatis* (Figure 5.0.1 g).

viii) Overall acceptability: With the increase in FOS, overall scores of *chapatis*, were greatly affected because of the reducing scores of organoleptic attributes such as texture, taste and mouthfeel. The scores for color and taste of *chapati* reduced significantly ($p<0.01$) at all the four levels of FOS substitution. The most affected attributes for *chapati* were chewability and breakability (Figure 5.0.1 h).

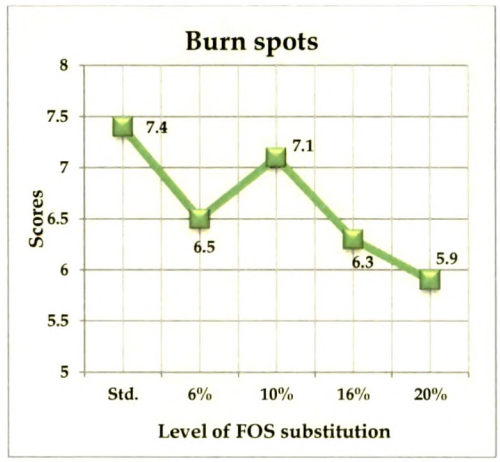
Table 5.0.1.2: Effect of varying levels of FOS substitution on the organoleptic qualities of *chapati*

Level of Substitution		Organoleptic Attributes							
		Color & Appearance	Burn Spots	Texture	Breakability	Taste & Mouthfeel	After taste	Chewability	OA
Std.	Mean	7.7^{ab}	7.4^{abc}	7.4^{a}	7.3^{a}	7.4^{a}	7.3^{abc}	7.1^{a}	7.6^{a}
	SD	± 1.4	± 1.3	± 1.4	± 1.8	± 1.4	± 1.7	± 1.8	± 1.5
6 %	Mean	7.0^{db}	6.5^{abd}	6.6^{abc}	6.4^{b}	6.6^{b}	6.6^{abc}	6.3^{a}	6.3^{b}
	SD	± 1.2	± 1.2	± 1.4	± 1.3	± 1.2	± 1.1	± 1.2	± 1.2
10 %	Mean	7.1^{db}	7.1^{abc}	6.7^{abc}	6.1^{bc}	6.5^{b}	6.5^{abc}	6.0^{b}	6.2^{b}
	SD	± 1.5	± 1.0	± 1.5	± 1.4	± 1.4	± 1.5	± 1.4	± 1.3
16 %	Mean	6.6^{db}	6.3^{abc}	6.1^{bc}	5.8^{bc}	6.0^{b}	6.2^{ba}	5.8^{b}	5.7^{b}
	SD	± 1.4	± 1.3	± 1.2	± 1.3	± 1.2	± 1.1	± 1.0	± 1.1
20 %	Mean	6.4^{cdb}	5.9^{abc}	5.8^{bc}	5.5^{bc}	5.9^{b}	5.8^{b}	5.3^{b}	5.8^{b}
	SD	± 1.1	± 1.2	± 1.4	± 1.5	± 1.5	±1.6	± 1.7	± 1.1
	Percent increase/ decrease	16.8 ↓	20.27 ↓	21.6 ↓	24.65 ↓	20.27 ↓	20.54 ↓	25.35 ↓	23.68 ↓
	ANOVA	4.47^{**}	7.11^{***}	5.84^{***}	6.32^{***}	5.78^{***}	4.33^{**}	6.19^{***}	10.01^{***}

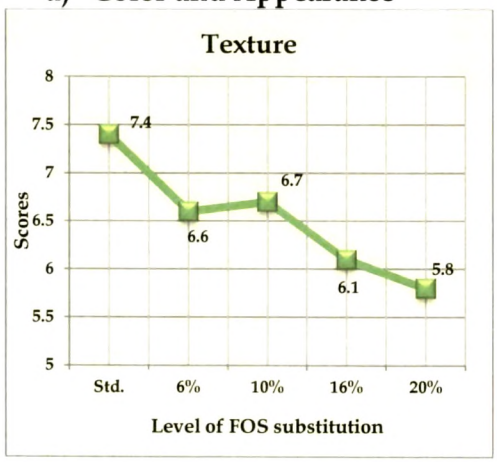
- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non-identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.



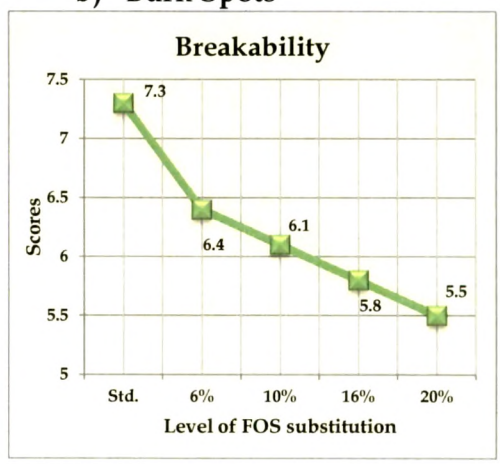
a) Color and Appearance



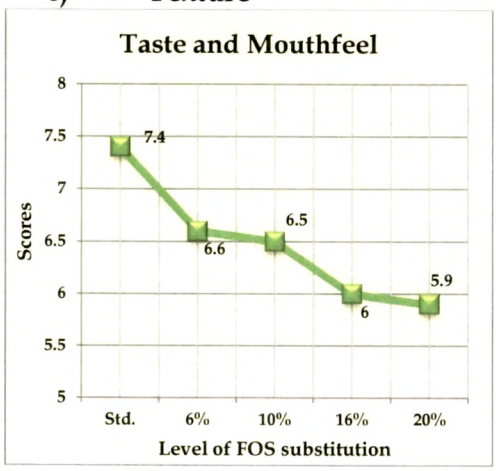
b) Burn Spots



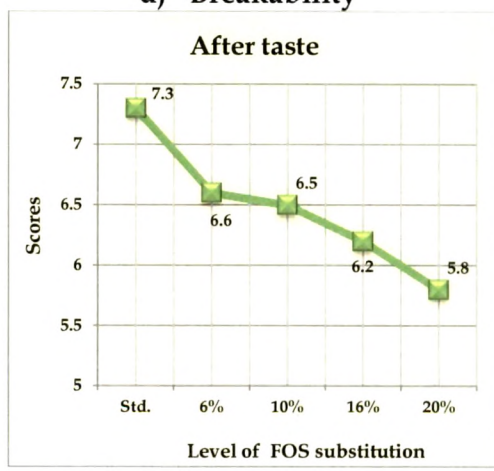
c) Texture



d) Breakability

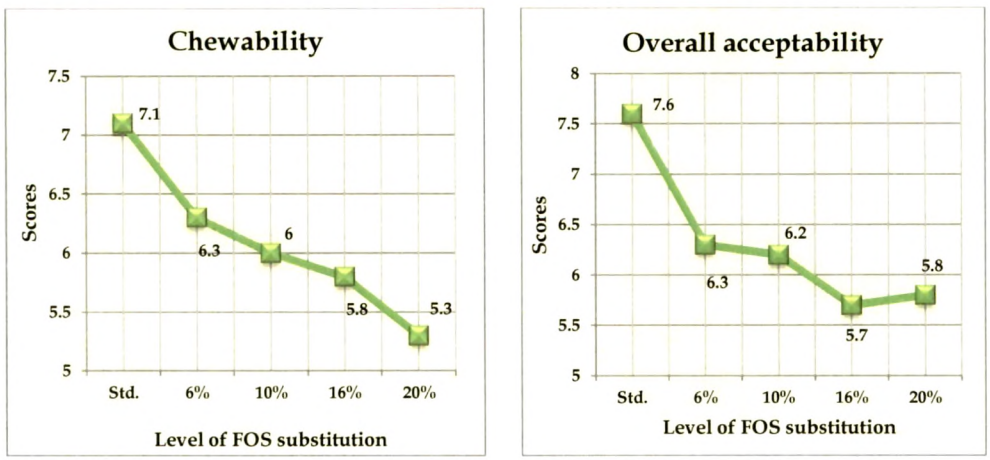


e) Taste and Mouthfeel



f) After Taste

Fig. 5.0.1 (a-f): Scores for organoleptic attributes of *chapati* substituted with varying levels of FOS



g) Chewability h) Overall acceptability

Fig. 5.0.1 (g-h): Scores for organoleptic attributes of *chapati* substituted with varying levels of FOS

c) Difference in the organoleptic attributes of individual test samples in comparison with the standard *chapati*

As can be seen in Table 5.0.1.3, a significant difference existed between 6-10% and 16-20% levels of FOS substitution for color and appearance scores ($p<0.01$). For taste scores, a significant difference was noted in the *chapati* after FOS incorporation at varying levels (6-10% and 16-20%) ($p<0.001$). However, 73% panel judges rated *chapatis* as equal and superior to the standard at 10% FOS substitution for overall acceptability. Almost 47% judges found *chapatis* upto FOS substitution as superior or equal to the standard.

Chapatis were well accepted up to 6% level of FOS substitution. As the level of substitution increased there was a gradual significant decrease in the organoleptic scores for all the attributes according to Numerical scoring test. However, _chapatis_ were acceptable by almost 47% panel members at 20% FOS substitution according to difference test

Table 5.0.1.3: Number of panel members indicating the difference in the organoleptic attributes of *chapati* in a difference test

Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
Chapati																
6-10%	17	36	7	12.5**	26	22	12	14.0***	16	28	16	1.3^{NS}	15	29	16	5.5^{NS}
16-20%	16	21	23		19	10	31		22	24	14		14	14	32	

Level of significance in increasing order- (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)
NS- Non significant

5.0.2 Effect of substitution of base material in *thepla* with varying levels of FOS

The result of this subsection are presented in Table 5.0.2.1 to 5.0.2.3

a) Assessment of Physical properties of the *Thepla*

Table 5.0.2.1 reveals physical properties of *thepla* substituted with varying levels of FOS in the base material. As the level of FOS substitution increased, total dough weight of *thepla* decreased from 250g (std.) to 200 g (20%). This might be due less absorption of water during preparation of dough making due to decreased solubility of FOS in water. The dough became harder with increase in concentration of FOS in the flour. Time required for the cooking of *thepla* also decreased at the higher percent substitution from 1.5 minute to 1 minute.

The water absorption power (WAP) varied from 92% (6% level of FOS incorporation) to 68% (20% level of FOS incorporation). The *thepla* prepared from flours with WAP less than 84% tended to be stiff and semi stiff.

Table 5.0.2.1: Physical properties of FOS substituted *thepla*

Characteristics	Level of substitution				
	Std.	6%	10%	16%	20%
Dough weight (g)	250 ±0.5	244 ±0.5	230 ±0.5	215 ±0.7	200 ±0.5
Cooked weight (g)	170 ±0.4	145 ±0.2	132 ±0.8	125 ±0.5	115 ±0.8
WAP %	100	92	84	76	68
Time for cooking (min)	1.5	1.3	1	1	1

b) Organoleptic evaluation of *thepla*

The organoleptic scores of *thepla* prepared by substituting base flour with varying levels of FOS are presented graphically in Figure 5.0.2 (a-h) and tabulated in Table 5.0.2.2.

i) Color and Appearance: The color and appearance scores of *thepla* reduced significantly by 11.6% as the level of FOS substitution increased ($p<0.001$). With the increase in level of FOS substitution, all samples exhibited an increase in whiteness. There was a significant difference in the color and appearance scores even at 6% level of substitution. After which the scores were stable (Figure 5.0.2 a).

ii) Burn spots: There was appearance of more number of brownish and black spots on the surface of *thepla* with the increase in substitution of FOS. There was almost significant reduction in the burn spots of *thepla* with varying scores of 8.3 (std.) to 7.6 due to FOS substitution ($p<0.05$). Although, there was no significant difference in the standard and 6% FOS substituted *theplas* (Figure 5.0.2 b).

iii) **Texture:** Reduction in the test scores of texture in the *thepla* was seen due to the hard feel against normally accepted soft feel, when touched of folded. Though there was 9.6% decrease in the texture scores of *thepla* after FOS substitution ($p<0.01$). Texture of *theplas* was fairly acceptable from 6% to 20% of FOS substitution (Figure 5.0.2 c).

iv) Breakability: In the present study *thepla* made from wheat flour substituted with FOS became brittle when folded and the brittleness increased significantly ($p<0.01$) upon and beyond addition of 6% of FOS. However, the scores did not alter significantly after 10% of FOS substitution (Figure 5.0.2 d).

v) Taste and Mouthfeel: Reduction in the taste and mouthfeel scores decreased significantly ($p<0.05$) by almost 6% upon substitution of base four with 6% of FOS, after which the taste scores were constant upto 10% (Figure 5.0.2 e).

vi) After taste: Mean scores for after taste of *thepla* ranged from 7.8 (6% level of FOS incorporated *thepla*) to 7.5 (20% level of FOS incorporated *thepla*) as against 8.3 scored by the standard sample. *Theplas* were well accepted upto 10% of FOS substitution and decreased significantly ($p<0.05$) at 16%, but constant scores was noted till 20% of substitution (Figure 5.0.2 f).

vii) Chewability: Chewiness is the length of time required to masticate the sample at constant rate of force application to reduce it to a consistency suitable for swallowing. *Thepla* were fairly chewable upto 10% level of substitution after which chewability scores decreased significantly ($p<0.05$) by 7.2%. This may be because increase in hardness with increase in FOS in *thepla* (Figure 5.0.2 g).

viii) Overall acceptability: Overall *thepla* was acceptable upto 6% of FOS substitution. After 6% of substitution, organoleptic scores of thepla reduced from 8.1 (10% of FOS substitution) to 7.9 (20% of substitution), although it was not significant (Figure 5.0.2 h). The most affected attribute for *thepla* were after taste and breakability. However, theplas were very well accepted at 10% of FOS substitution.

c) Difference in the organoleptic attributes of individual test samples in comparison with the standard *thepla*

As can be seen in Table 5.0.2.3, a significant difference existed between 6-10% and 16-20% levels of FOS substitution for color and appearance scores ($p<0.01$). However, for attributes like taste, after taste and overall acceptability no difference existed in the scores in the *thepla* after FOS substitution. Therefore, according to difference test almost 70% panel judges rated *theplas* as equal and superior to the standard upto 20% level of FOS substitution.

Table 5.0.2.2: Effect of varying levels of FOS substitution on the organoleptic qualities of *thepla*

Level of Substitution		Organoleptic Attributes							
		Color & Appearance	Burn Spots	Texture	Breakability	Taste & Mouthfeel	After taste	Chewability	OA
Std.	Mean	8.6	8.3	8.3	8.5	8.2	8.3	8.3	8.6
	SD	± 0.8	± 1.2	± 1.0	± 0.8	± 0.9	± 0.9	± 0.9	± 0.6
6%	Mean	7.8	7.8	7.7	7.9	7.7	7.8	7.8	7.9
	SD	± 1.0	± 1.0	± 0.9	± 1.0	± 1.1	± 1.0	± 1.1	± 1.0
10%	Mean	8.1	8.2	7.7	8.0	8.0	8.0	8.2	8.1
	SD	± 0.9	± 0.9	± 0.9	± 0.9	± 0.9	± 0.9	± 0.9	± 0.8
16%	Mean	7.6	7.5	7.2	7.4	7.3	7.4	7.3	7.5
	SD	± 1.0	± 1.2	± 1.3	± 1.1	± 1.0	± 1.2	± 1.2	± 1.2
20%	Mean	7.6	7.6	7.5	7.7	7.7	7.5	7.7	7.9
	SD	± 1.0	± 1.1	± 0.9	± 1.2	± 1.2	± 1.1	± 1.1	± 1.0
	Percent increase/ decrease	11.6 ↓	8.43 ↓	9.63 ↓	9.41 ↓	6.09 ↓	9.63 ↓	7.22 ↓	8.13 ↓
	ANOVA	5.58***	2.68*	4.57**	4.45**	2.79*	3.30*	4.13**	4.27**

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non-identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.
- Level of significance in increasing order- (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Table 5.0.2.3: Number of panel members indicating the difference in the organoleptic attributes of *thepla* in a difference test

Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
Thepla																
6-10%	8	47	5	12.8**	14	39	7	3.4NS	15	41	4	3.56NS	5	44	11	4.26NS
16-20%	20	28	12		21	29	10		17	33	10		12	30	18	

Level of significance in increasing order- (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)
NS- Non significant

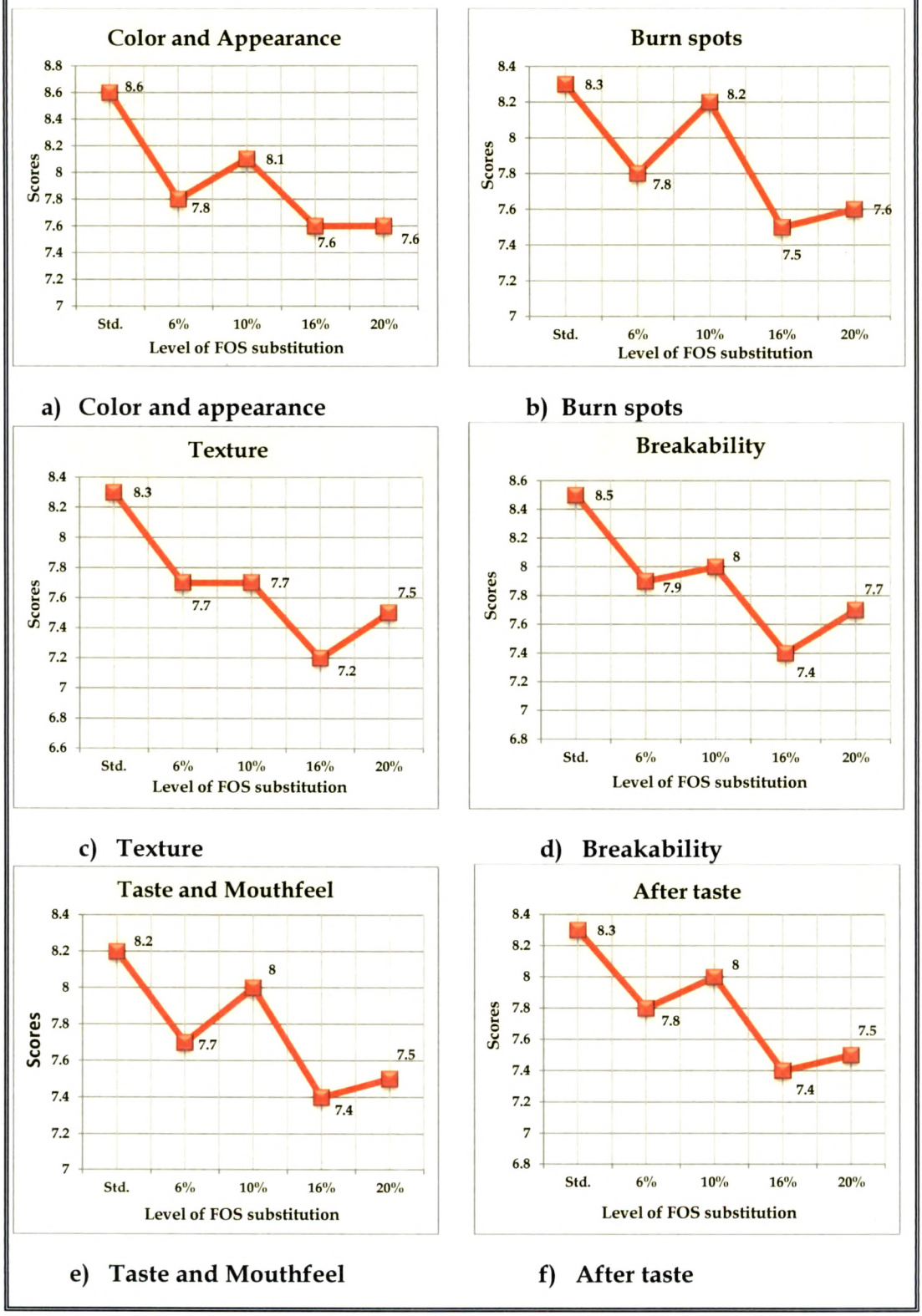
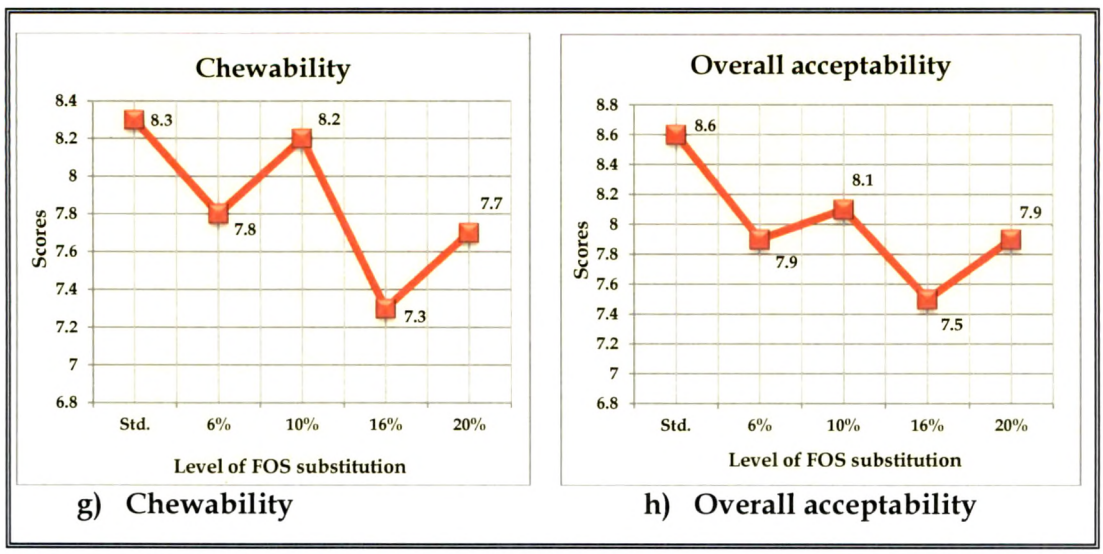


Fig. 5.0.2 (a-f): Scores for organoleptic attributes of *thepla* substituted with varying levels of FOS



g) Chewability h) Overall acceptability

Fig. 5.0.2 (g-h): Scores for organoleptic attributes of *thepla* substituted with varying levels of FOS

***Theplas* were well accepted at 6% of FOS substitution. As the level of substitution increased there was a gradual significant decrease in the organoleptic scores for all the attributes. According to difference test, apart from color and appearance *thepla* was accepted as high as 20% of FOS substitution in the base material.**

5.0.3 Effect of substitution of base material in *dhokla* with varying levels of FOS

The results for this sub-section are presented in Table 5.0.3.1 to 5.0.3.3 of FOS addition at varying levels on the physical and organoleptic properties of *Dhokla*.

a) Assessment of Physical properties of the *Dhokla*

As shown in Table 5.0.3.1, batter weight of dhokla reduced as the level of FOS substitution increased, as thinning of batter was perceived at higher levels of FOS incorporation. WAP% also reduced as the percent substitution increased. The bulk density of the prepared *dhokla* varied from 3.26 g/cc (std.) to 2.70 g/cc

(20% level of FOS substitution). The bulk density of the *dhokla* decreased with increasing levels of FOS substitution. Steaming was not altered, as 20 minutes were required to complete the steaming process of *dhoklas.*

Table 5.0.3.1: Physical properties of FOS substituted *dhokla*

Characteristics	Level of substitution			
	Std.	10%	16%	20%
Batter weight (g)	200 ±0.2	184 ±0.2	176 ±0.7	168 ±0.7
Cooked weight (g)	160 ±0.8	134 ±0.8	125 ±0.5	113 ±0.8
WAP %	100	84	76	68
Time for cooking (min)	20	20	20	20
Volume cc	8.27	8.35	8.42	8.68
Bulk density g/cc	3.26	2.75	2.80	2.70

b) Organoleptic evaluation of *dhokla*

The organoleptic scores of *dhokla* are linearly presented in Figure 5.0.3 (a-g) and in discreetly presented in Table 5.0.3.2.

i) Color and appearance: At all the levels of FOS incorporation, the color scores denoted that the substitution of FOS at varying levels brought about no significant changes in the color and appearance of *dhoklas.* Though, at higher level of substitution *dhoklas* became dark yellow in color. The mean score for

standard dhokla was 8.3 that continued to be almost similar with varying levels of FOS incorporation (Figure 5.0.3 a).

ii) Cell Size: Cell size in *dhokla* depicts the amount of air entered in the product. It is proportional to the softness of the product. It is a desirable quality for the softness of the *dhoklas*. At all the level of FOS substitution, scores for cell sized remained almost similar. No significant difference in the cell size was witnessed for varying levels of FOS substitution (Figure 5.0.3 b).

iii) Texture: No significant difference was noticed between all the FOS substituted *dhokla* and standard *dhokla* for the texture scores. Varying levels of FOS incorporation did not significantly affect texture scores (Figure 5.0.3 c).

iv) Taste and mouthfeel: Taste and mouthfeel scores of *dhokla* substituted with FOS, ranged from 7.9 (standard) to 7.2 (10% level of FOS incorporated *dhokla*). *Dhokla* prepared from 10%, 16% and 20% addition of FOS remained similar at varying levels of substitution. No significant differences in taste and mouthfeel scores were spotted amongst all the levels of FOS incorporated (Figure 5.0.3 d).

v) After taste: As illustrated in Figure 5.0.3 e, mean scores for after taste for *dhokla* ranged from 7.0 (10% level of FOS incorporated *dhokla*) to 7.2 (20% level of FOS incorporated *dhokla*) as against 7.8 scored by the standard sample. There was no significant difference between all the samples of *dhokla* within the levels of FOS enrichment. Twenty percent level of FOS substituted *dhokla* scored highest on organoleptic scale amongst varying level in the amount of FOS by the panel members for after taste.

vi) Softness: The softness scores for *dhokla* ranged between 7.7 (Standard *dhokla*) to 7.3 (20% level of FOS incorporated *dhokla*). There was no significant difference detected between all the samples of FOS fortified *dhoklas*. Organoleptic scores for *dhokla* for softness remained similar (7.3) at all the levels of FOS incorporation (Figure 5.0.3 f).

vii) Overall acceptability: As linearly sketched in Figure 5.0.3 g, overall organoleptic acceptability for *dhokla* ranged from 8.6 (standard *dhokla*) to 7.4 (20% level of FOS substituted *dhoklas*. As there was no significant difference witnessed in any of the organoleptic attributes, *dhokla* was accepted by the panel judges at all the levels of FOS incorporation.

c) Difference in the organoleptic attributes of individual test samples in comparison with the standard *dhokla*

As demonstrated in Table 5.0.3.3, chi square values depicts that, no significant difference was observed for all the organoleptic attributes of *dhokla* in terms of color, taste, after taste and overall acceptability. *Dhokla* were overall acceptable by almost 73% panel members as equal and superior to the standard upto 20% FOS substitution.

Table 5.0.3.2: Effect of varying levels of FOS substitution on the organoleptic qualities of *dhokla*

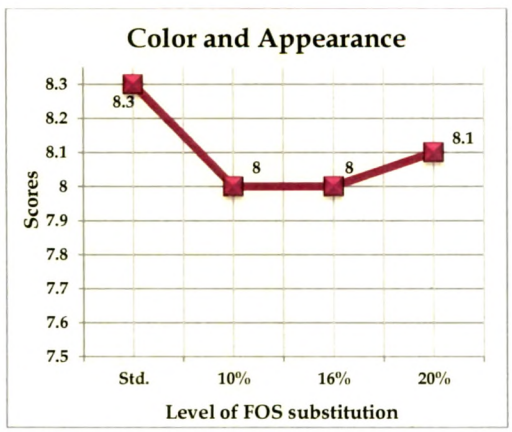
Level of Substitution		Organoleptic Attributes						
		Color & Appearance	Cell size	Texture	Taste & mouthfeel	After taste	Softness	OA
Std.	Mean	8.3^{NS}	7.9^{NS}	8.0^{NS}	7.9^{NS}	7.8^{NS}	7.7^{NS}	8.1^{NS}
	SD	± 1.2	± 1.0	± 1.1	± 1.3	± 1.3	± 1.4	± 1.1
10%	Mean	8.0^{NS}	7.5^{NS}	7.4^{NS}	7.2^{NS}	7.0^{NS}	7.3^{NS}	7.3^{NS}
	SD	± 1.3	± 1.1	± 1.3	± 1.5	± 1.5	± 1.5	± 1.3
16%	Mean	8.0^{NS}	7.7^{NS}	7.6^{NS}	7.2^{NS}	7.1^{NS}	7.3^{NS}	7.3^{NS}
	SD	± 1.1	± 1.1	± 1.0	± 1.1	± 7.3	± 1.2	± 1.1
20%	Mean	8.1^{NS}	7.7^{NS}	7.7^{NS}	7.2^{NS}	7.2^{NS}	7.3^{NS}	7.4^{NS}
	SD	± 1.2	± 1.1	± 1.3	± 1.5	± 1.5	± 1.5	± 1.3
	Percent increase/ decrease	2.40 ↓	2.53 ↓	3.75 ↓	8.86 ↓	7.69 ↓	5.19 ↓	8.64 ↓
	ANOVA	NS	NS	NS	NS	NS	NS	NS

- Note: NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

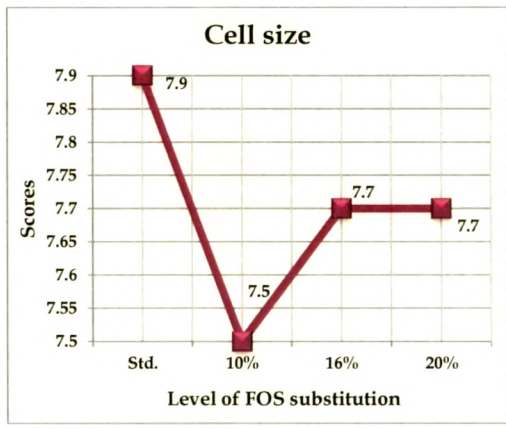
Table 5.0.3.3: Number of panel members indicating the difference in the organoleptic attributes of *dhokla* in a difference test

Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
***Dhokla* 10%**	8	48	4		16	38	6		18	38	4		10	36	14	
				0.1^{NS}				3.4^{NS}				3.8^{NS}				0.2^{NS}
16-20%	10	45	5		17	31	12		14	32	14		9	34	17	

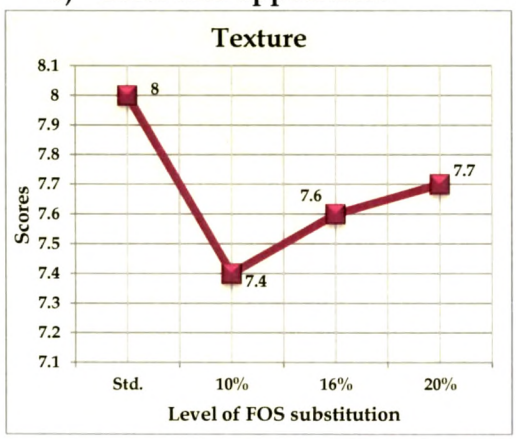
NS- Non significant



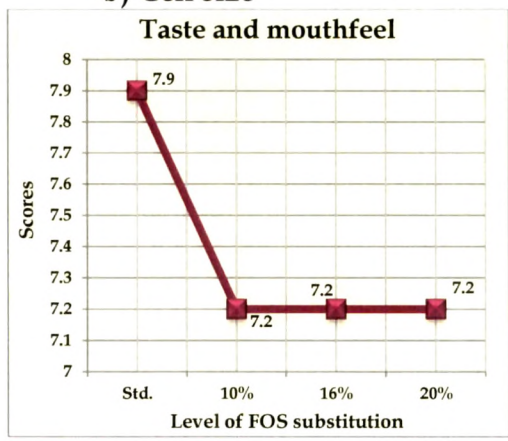
a) Color and appearance



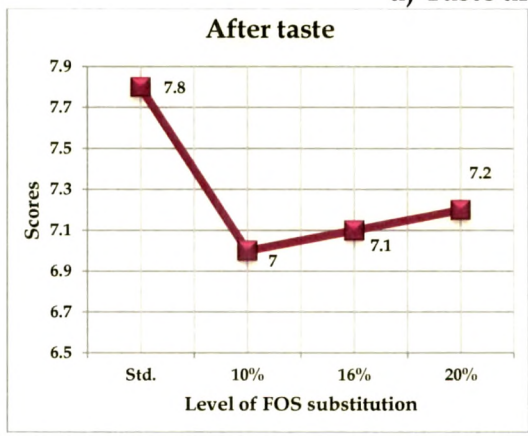
b) Cell size



c) Texture

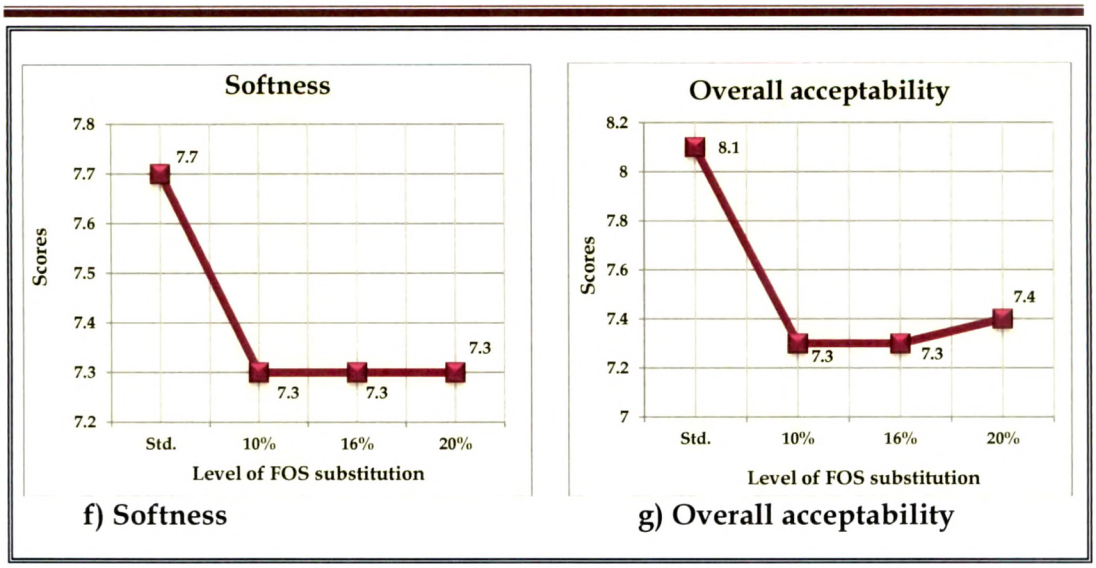


d) Taste and mouthfeel



e) After taste

Fig. 5.0.3 (a-e): Scores for organoleptic attributes of *dhokla* substituted with varying levels of FOS



f) Softness g) Overall acceptability

Fig. 5.0.3 (f-g): Scores for organoleptic attributes of *dhokla* substituted with varying levels of FOS

All the *dhokla*s were rated well within the acceptable limits. No significant difference was perceived in any of the organoleptic attributes of *dhokla*. For all the organoleptic attributes, FOS substituted *dhoklas* were acceptable up to 20% level of substitution according to Numerical scoring as well as Difference test

Section 5.0.4 Effect of substitution of base material in *patra* with varying levels of FOS

The results for this sub-section are presented in Table 5.0.4.1 to 5.0.4.3 of FOS addition at varying levels on the physical and organoleptic properties of *patra*.

a) Assessment of Physical properties of the *Patra*

The results of standard and experimental *patra* are presented in Table 5.0.4.1. Batter weight of *patra* ranged from 215g (std. *patra*) to 185g (20% FOS substituted *patra*). Raw batter weight and cooked weight both decreased gradually as the

level of FOS substitution was increased. Water absorption of *patra* also reduced as at higher level of substitution thinning of batter was observed. Time for steaming for patra remained constant for 20 minutes.

Table 5.0.4.1: Physical properties of FOS substituted *patra*

Characteristics	Level of substitution			
	Std.	10%	16%	20%
Batter weight (g)	215.6 ±5.1	201.6 ±2.8	192.3 ±2.5	185.5 ±3.2
Cooked weight (g)	160.6 ±5.1	152.3 ±4.0	143.4 ±2.8	135.0 ±5.0
WAP %	100	93.7	87.5	81.25
Time for cooking (min)	20	20	20	20

b) Organoleptic evaluation of *patra*

The organoleptic scores of *patra* prepared by substitution of base material with varying levels of FOS are presented graphically in Figure 5.0.4 (a-e) and tabulated in Table 5.0.4.2.

i) Color and appearance: At all the levels of FOS incorporation, the color scores denoted that the substitution of FOS at varying levels brought no significant difference in the color and appearance scores of *patra*. Mean scores ranged between 7.8 (std. *patra*) to 8.1 (20% level of FOS substitution). Values for FOS enriched *patra* for color and appearance increased by 6.4% (Figure 5.0.4 a).

ii) Texture: Mean scores for texture of *patra* ranged between 8.0 (std. *patra*) to 8.4 (20% level of FOS substitution). There was a gradual increase of 5% in the FOS substituted *patra* (Figure 5.0.4 b).

iii) Taste and mouthfeel: Taste and mouthfeel scores of *patra* substituted with FOS, ranged from 7.3 (standard) to 7.9 (20% level of FOS incorporated *patra*). Taste and mouthfeel scores increased as the level of substitution increased. Overall there was 8.4% increase in taste and mouthfeel scores of FOS enriched *patra* (Figure 5.0.4 c).

iv) After taste: There was almost 6% increase in the after taste mean scores of patra. At 10% and 20% level of FOS substituted *patra* scored highest on organoleptic scale amongst varying level in the amount of FOS by the panel members for after taste (Figure 5.0.4 d).

v) Overall acceptability: Overall acceptability of FOS incorporated *patra* increased by 8%. There was a gradual increase in overall acceptability which ranged from 7.9 (10% level of substitution) to 8.1 (20% level of substitution) against 7.5 (standard patra). The product was overall acceptable at all the varying level of substitution, and most acceptable at 20% level of substation with FOS.

Table 5.0.4.2: Effect of varying levels of FOS substitution on the organoleptic qualities of *patra*

Level of Substitution		Organoleptic Attributes				
		Color & Appearance	Texture	Taste & mouthfeel	After taste	OA
Std.	Mean	7.8^{NS}	8.0^{NS}	7.3^{NS}	7.3^{NS}	7.5^{NS}
	SD	± 0.8	± 0.9	± 1.1	± 1.1	± 1.0
10%	Mean	8.1^{NS}	8.1^{NS}	7.8^{NS}	7.8^{NS}	7.9^{NS}
	SD	± 1.0	± 0.8	± 0.8	± 1.0	± 0.9
16%	Mean	8.3^{NS}	8.1^{NS}	7.8^{NS}	7.7^{NS}	7.9^{NS}
	SD	± 0.8	± 0.8	± 1.0	± 1.0	± 0.8
20%	Mean	8.3^{NS}	8.4^{NS}	7.9^{NS}	7.8^{NS}	8.1^{NS}
	SD	± 0.9	± 0.9	± 1.1	± 1.2	± 1.2
	Percent increase/ decrease	6.41 ↑	5.0 ↑	8.21 ↑	6.84 ↑	8.0 ↑
	ANOVA	NS	NS	NS	NS	NS

- Note: NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

Table 5.0.4.3: Number of panel members indicating the difference in the organoleptic attributes of *patra* in a difference test

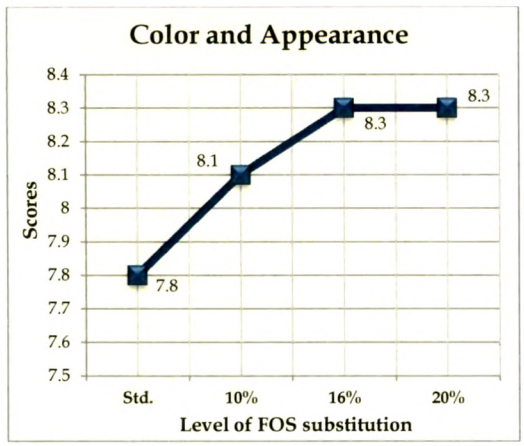
Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
Patra **10%**	12	46	2		18	34	8		20	34	6		22	30	8	
				0.1^{NS}				0.0^{NS}				3.8^{NS}				0.1^{NS}
16-20%	10	48	2		17	34	9		16	38	6		21	32	7	

NS- Non significant

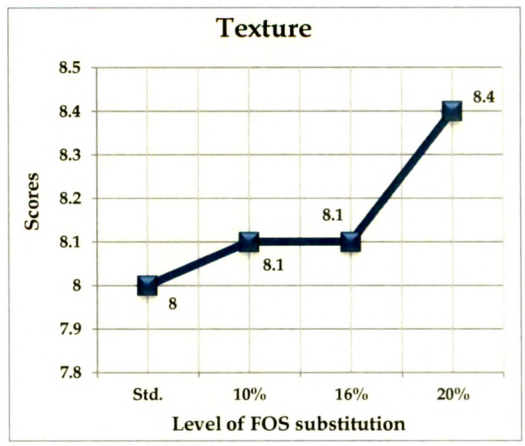
c) Difference test for the organoleptic attributes of individual test samples in comparison with the standard *patra*

As demonstrated in Table 5.0.4.3, chi square values depicts that, no significant difference was observed for all the organoleptic attributes of *patra* in terms of color, taste, after taste and overall acceptability. Overall 88% panel members rated *patras* as equal or superior to the standard at 20% level of FOS substitution. *Patra* was well accepted at varying levels of FOS substitution.

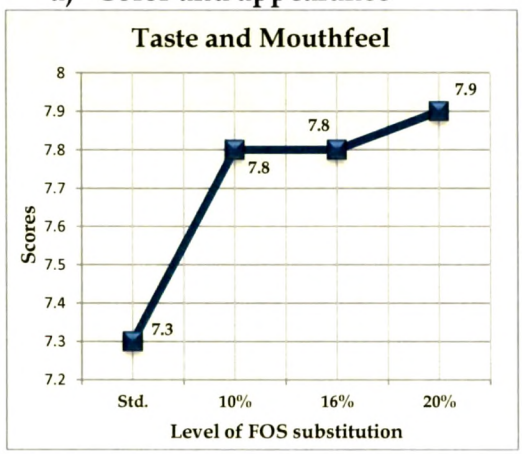
Patras were very well accepted at varying level of FOS substitution. Mean scores for *patra* increased for all the organoleptic attributes. It was most accepted at the maximum level of FOS substitution (20%). Difference test also indicated that *patras* were acceptable at all the 3 levels of FOS substitution



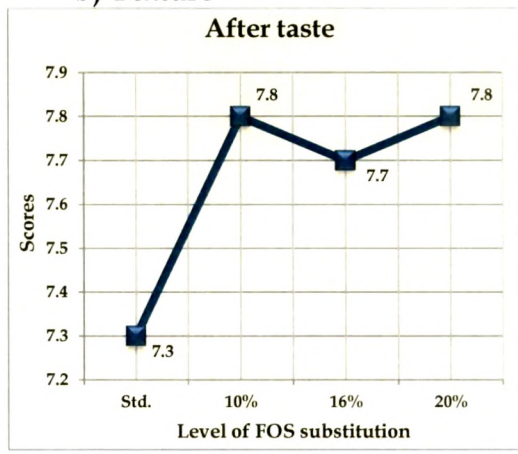
a) Color and appearance



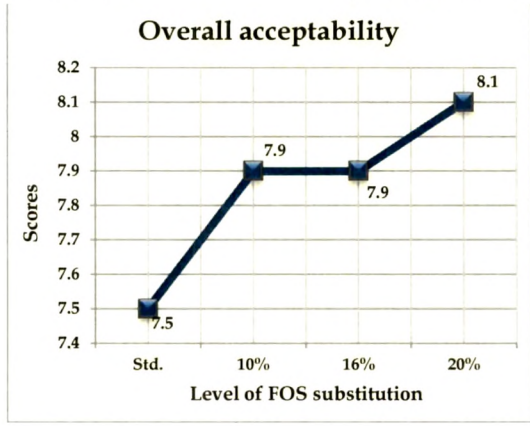
b) Texture



c) Taste and mouthfeel



d) After taste



e) Overall acceptability

Fig. 5.0.4 (a-e): Scores for organoleptic attributes of *patra* substituted with varying levels of FOS

Section 5.1: Recovery of FOS during processing in the food products *viz. chapati, thepla, dhokla* and *patra* using HPLC technique

This section of the study focuses on the recovery of FOS during different processing conditions i.e. *roasting, shallow frying* and *steaming* in four Gujarati food products *viz. chapati, thepla, dhokla* and *patra* using High Performance Liquid Chromatography (HPLC) technique (Details of the methodology outlined in chapter 4). The major findings of this section of the study are presented under following subsections.

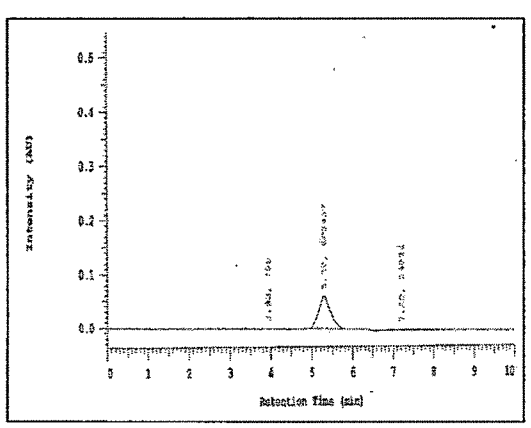
Section 5.1.1 Standardization of HPLC technique for FOS analysis

Section 5.1.2 Processing changes in the FOS content of the food products (*chapati, thepla, dhokla* and *patra*)

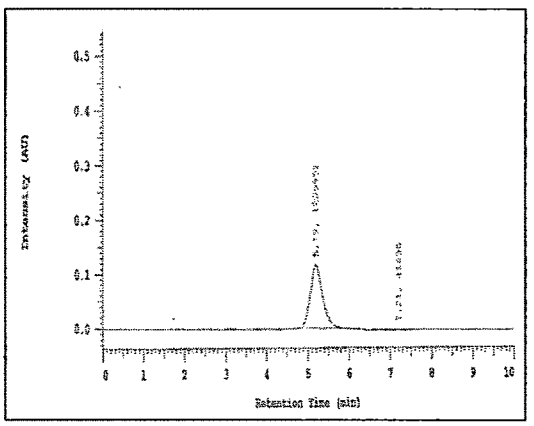
Section 5.1.1 Standardization of HPLC technique for FOS analysis

The peak areas of repetitive injections of either samples or standard sugars differ to some extent due to concentration variation. The errors associated with repetitive injection are: (a) the precise injection of small quantities of samples is very difficult, and (b) detector responses fluctuate with time. Water based standard solutions containing different concentrations 1000, 2000, 3000, 4000 and 5000 ppm of FOS were prepared and injected into HPLC with standard operating conditions. The chromatograms obtained for various concentrations of FOS and blank are depicted in Figure 5.1.1.1 (a-f)

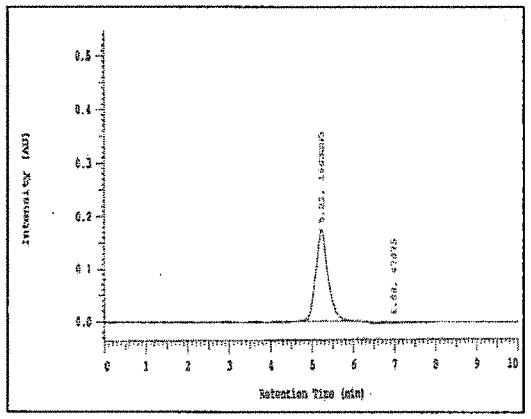
The standard curve plotted for various concentrations is depicted in Figure 5.1.1.2, which shows that there is no significant variation in the determined concentration, indicating that the detector response is valid at various concentrations of FOS. Such validation of detector response is essential to extrapolate the results in samples with different levels of FOS.



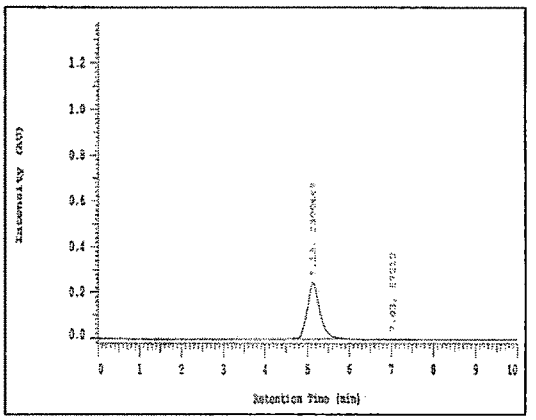
(a) 1000 ppm FOS



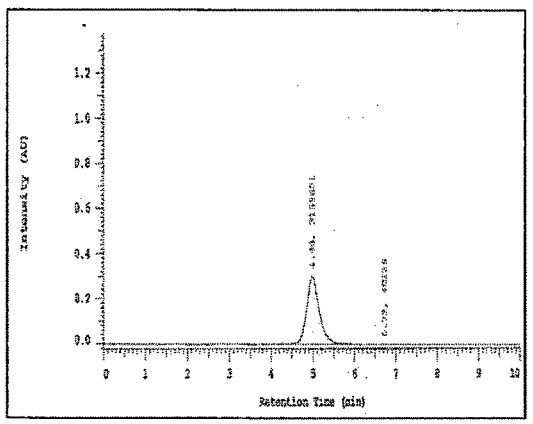
(b) 2000 ppm FOS



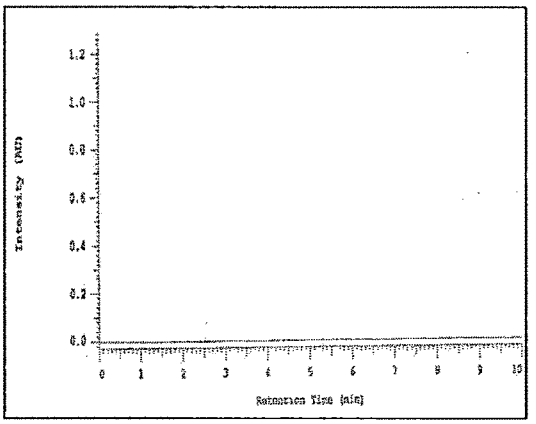
(c) 3000 ppm FOS



(d) 4000 ppm FOS



(e) 5000 ppm FOS



(f) Blank

Figure 5.1.1.1: Chromatograms depicting the FOS standard peak obtained at various concentrations (a-f)

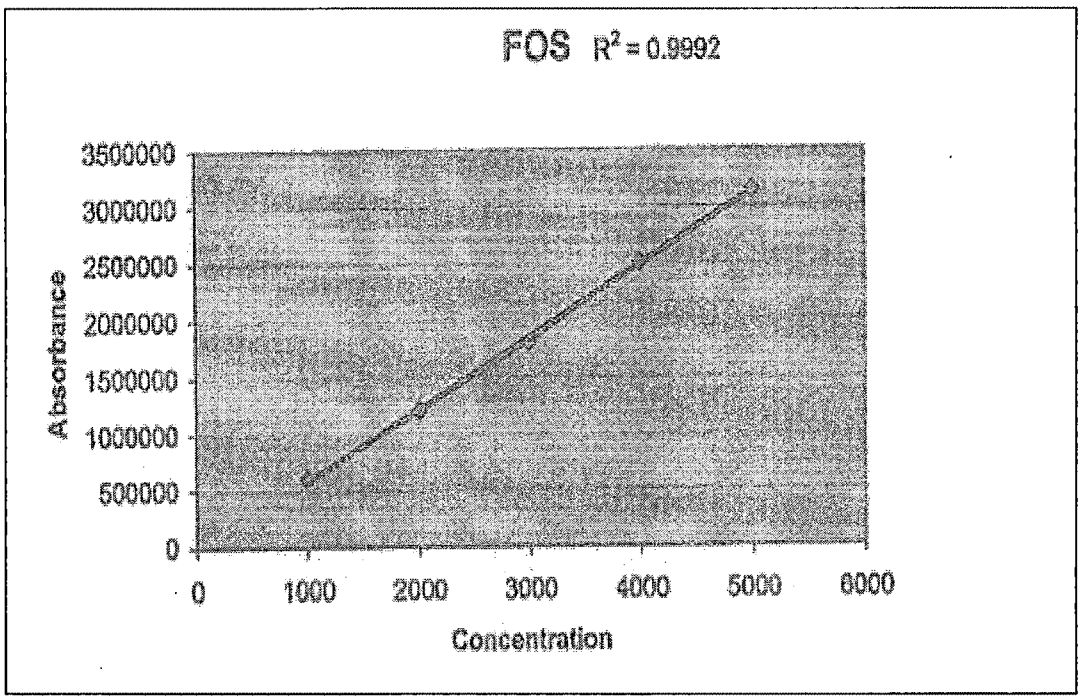
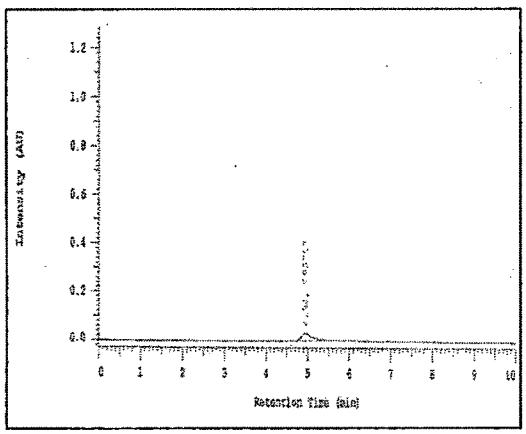
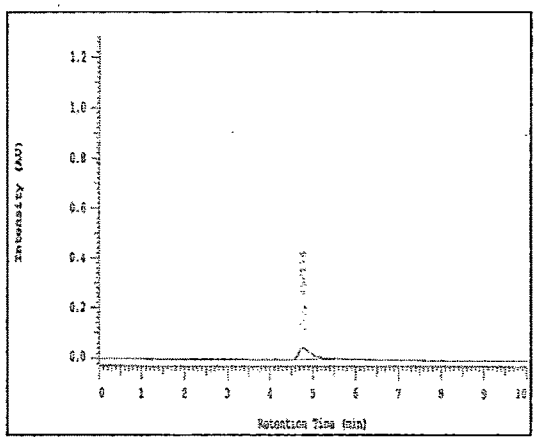


Figure 5.1.1.2: Standard calibration curve plotted for various concentrations of FOS

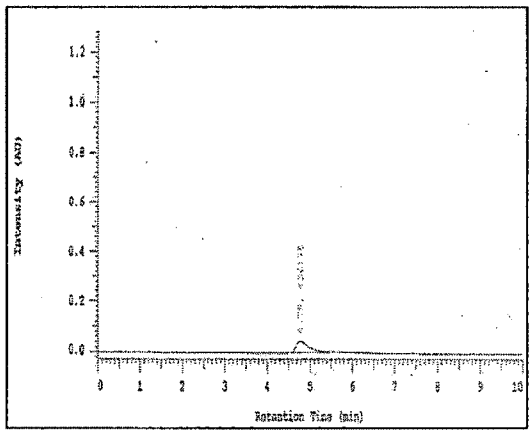
The standards of FOS (Brenntag Ingredients Pvt. Ltd.), fructose (D-fructose extra pure Qualigens Ltd.), glucose (D-glucose pure Qualigens Ltd.) and sucrose (Sucorse pure-merck chemicals Ltd.) were used as calibration samples (Fig 5.1.1.3 (a-c)). After standardizing and quantifying the standard FOS by HPLC, fructans (FOS) extracted from the foods namely *chapati, thepla, dhokla* and *patra* were determined. For determining the recovery of food products retention time (RT) and peak of standard FOS was compared with the RT peak of sample food products with variation of 1-2 minutes of RT.



(a) Sucrose



(b) Glucose



(c) Fructose

Figure 5.1.1.3: Chromatograms depicting standard for (a) Sucrose (b) Glucose (c) Fructose

Section 5.1.2 Processing changes of FOS in food products (*chapati, thepla, dhokla* and *patra*)

Table 5.1.2.1 and Figure 5.1.2.1 describes the values of raw mix of all the four food products and percent recovery of FOS after processing. The FOS content of wheat flour used for preparation of *chapati* was 5.2 g%. *Chapatis* prepared with 6% and 20% FOS addition revealed a percent recovery of 117 g% and 109 g% respectively indicating that roasting resulted in an overall percent increase in

FOS content. FOS content of raw *thepla* flour without addition of FOS was 6.6 g%. *Theplas* prepared with 6% and 20% FOS addition revealed a percent recovery of 97.7 g% and 91.8 g% respectively.

Whereas the percent recovery of FOS from *dhoklas* after addition of 6% and 20% FOS was 83.2 g% and 80.9 g% respectively. With respect to percent recovery of FOS in *patra*, 6% and 20% FOS added *patras* resulted in 81.1 g% and 86 g% FOS recovery respectively. Chromatograms for all the four products are depicted in Appendix III.

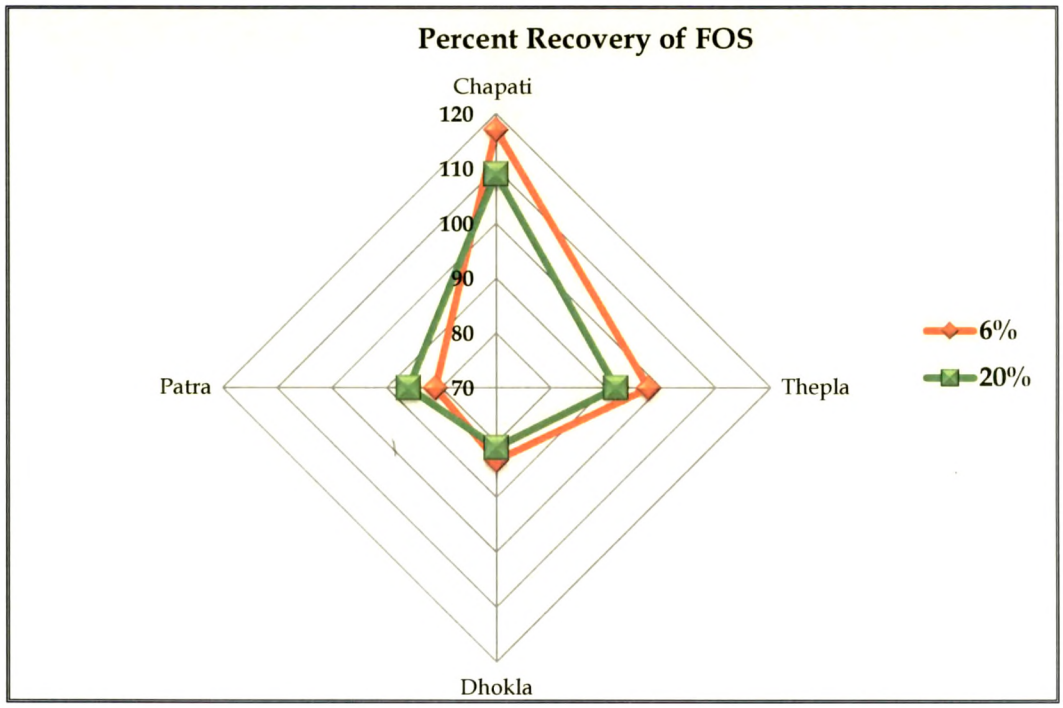


Figure 5.1.2.1: Webgraph showing percent recovery of FOS after using various processing techniques for *chapati, thepla, dhokla* and *patra*

Table 5.1.2.1: Recovery of FOS in the food products

Food Item	FOS content before processing (g%)	FOS content after processing (g%)	Percent Recovery of FOS (g%)
Chapati			
1. *Unprocessed*	5.2	-	-
2. *Roasted with 6% FOS*	11.2	13.2	117.1
3. *Roasted with 20% FOS*	25.2	27.5	109.0
Thepla			
1. *Unprocessed*	6.6	-	-
2. *Shallow fried with 6% FOS*	12.6	12.3	97.7
3. *Shallow fried with 20% FOS*	26.6	23.8	91.8
Dhokla			
1. *Unprocessed*	1.4	-	-
2. *Steamed with 6% FOS*	7.4	6.9	83.2
3. *Steamed with 20% FOS*	21.4	17.3	80.9
Patra			
1. *Unprocessed*	1.4	-	-
2. *Steamed with 6% FOS*	7.4	6.0	81.1
3. *Steamed with 20% FOS*	21.4	18.4	86

Roasting revealed maximum percent recovery of FOS followed by shallow frying and steaming. The results also revealed that both the type of product as well as the degree of substitution in the base material of FOS can affect the final recovery of FOS in the food products.

DISCUSSION

The main objective of the present phase of the study was to observe the effect of varying level of FOS concerning physical and organoleptic characteristics of various food products *viz. Chapati, Thepla, Dhokla* and *Patra* and to study the recovery of FOS in the processed products.

FOS incorporated *chapati* and *thepla* revealed a reduction in almost in all the physical attributes like total dough weight, cooked weight and percent water absorption capacity (WAP%). A similar study reported that inulin addition in the *chapatis* at 5%, 10%, 15% and 20% gradually reduced total dough weight, cooked weight and percent WAP by 14%, 5% and 13.3% respectively (Parnami and Sheth 2010). This may be because of reduction in water absorption power of the dough. FOS is highly hygroscopic in nature, and at low degree of polymerization, the water holding capacity of FOS decreases (Prapulla et al 2000). An experiment using 9 point hedonic scale by 20 semi-trained panel judges revealed that addition of combination of two prebiotic (FOS and resistance starch) and beta glucan to *chapatis* at 10%, 20% and 30% resulted in decreased water holding capacity of chapatis (Lorraine N et al 2011).

As the level of FOS substitution increased, the dough became sticky and was difficult to sheet. Additionally, it resulted in partial puffing of *chapatis* and reduced the dough distension. Development of poor dough might have resulted in denser texture. There is gradual decrease in dough weight of the *chapatis* and thepla. The dough became harder with increase in concentration of FOS in the base flour of *chapati* and *thepla*. In a study carried out by Renuka et al (2010), the hardness values of FOS and sucrose sweetened gulabjamun were higher compared to the control. Time required for cooking of *chapatis* decreased, as the level of FOS substitution was increased. This could be because of longer duration of roasting resulting in development of the burn spots. Also the reduction in the

time required for roasting of chapatis could be due to decreased water absorption capacity of dough. Similar results were obtained in a study where *chapati* containing lower level of water, roasted faster (Murthy, Manohar and Rao 1998).

Organoleptic assessment of *chapati* and *thepla* affirmed reduction in color and appearance scores, as increased number of blackish and brownish spots appeared at higher levels of FOS substitution. With the increase in level of FOS substitution, all samples exhibited an increase in whiteness. The whiteness of *chapati* and *thepla* depends upon many factors such as temperature, amount of water and the extent of non- enzymatic maillard reaction. Increase in level of FOS was attributed with increased burn spots on the surface of *chapati* and *thepla* which could be related to maillard reaction and caramelization (Lorraine N et al 2011).

Texture is widely recognized as an important quality attribute for product acceptability affecting consumer perception. The texture of *chapati* and *thepla* was judged by the hard/soft feel of *chapatis* and *thepla*, when touched or folded. In the present study a reducing trend in the texture scores were observed with the increase in level of FOS. In a research, soy granola bar made up from addition of 2% FOS to 100g of base material culminated in decreased softness and increase hardness values by 9 point hedonic scale (Chae-Jin Lee et al 2011).

Breakability is another attribute that was used for studying the texture of *chapatis* and *thepla*. Normally the product should have soft texture which can be folded and which doesn't turn brittle upon folding. In the present study *chapatis* and *thepla* made from wheat flour substituted with FOS became brittle when folded and the brittleness increased significantly ($p<0.01$) upon and beyond addition of 6% of FOS. Reduction in the taste and mouthfeel scores decreased significantly ($p<0.01$) upon substitution of wheat flour with 6% FOS, after which the taste

scores kept decreasing. This could be because of increased sweetness of *chapatis*, normally not preferred in an ideal *chapati*. Sweetness in *chapati* and *thepla* could be because FOS comprised of long chain polymers of fructose (Orafti 1996).

Chewiness is the length of time required to masticate the sample at constant rate of force application to reduce it to a consistency suitable for swallowing. *Chapatis* were fairly chewable upto 6% FOS substitution after which chewability scores decreased significantly. In case of *thepla*, it kept fluctuating from 6% to 20% level of FOS enrichment. This may be because increase in hardness with increase in FOS in *chapati* and *thepla*. The overall scores of *chapatis*, with increase in FOS were greatly affected because of the reducing scores of organoleptic attributes such as texture, taste and mouthfeel. A study also reported, addition of 20% of inulin in spaghetti reduced its overall quality. This result is due to the fact that the score of attributes such as firmness, color and taste decreased with the increase of the inulin amount influencing negatively the overall quality of the spaghetti samples (Mastromatteo M et al 2012).

Though, there are number of studies conducted on sensory attributes of FOS added food products by processing method of baking, freezing, frying etc. But there is scarcity of studies on effect of FOS added steamed products. Therefore, in the present study focused on steamed products like FOS incorporated *dhokla* and *patra* and were studied for their physical and organoleptic characteristics. Physical properties of *dhokla* and *patra* revealed that after incorporation of FOS there was a gradual decrease in batter weight, cooked weight and WAP%. Due to FOS addition thinning of batter was also witnessed. This may be because of synergestic effect of FOS. Volume of the *dhokla* increased by 5% after addition of FOS. A study reported that gluten development affects the height of the FOS added cookie, but not significantly as amount and type of sugar in the formulae can affect the height of cookie (Handa C et al 2011). Bulk density of the *dhokla* reduced gradually with increase in the level of FOS.

According to the organoleptic attributes, at higher levels of FOS incorporation, *dhoklas* were slightly harder than the standard. Mean values for cell size of the *dhokla* was also reduced thought the values remained non-significant. A research carried out by Renuka et al (2010) used Scanning Electron Microscope ultrastructure technology to study the microstructure of *gulabjamun* for its sponginess and revealed that a well-defined compact network was found in microstructure of the *gulabjamun* sweetened with FOS. This indicates slight hardness of *gulabjamun* sweetened with FOS. The overall FOS incorporated *dhokla* were very well accepted even at higher level as high as 20% level of substitution.

All the organoleptic attributes for *patra* increased as the level of FOS incorporation increased. This might be because of improved texture, after taste, taste and mouthfeel and overall acceptability. Slight sweet taste of FOS added *patra* was accepted by the panel of judges as it made the product more palatable and tasty. Many researches show the similar results for increased organoleptic attributes of cookies, ice-creams, meat sausages after FOS incorporation (Handa C et al 2011; Ting-ning lin and Gruen I 2012; Freitas Folly GA 2013) .

Analysis of the four food products for FOS content and its recovery using HPLC technique, showed variations due to the processing technique used. In the present study, FOS content of unprocessed *chapati* wheat flour was 5.2 g%. Several investigators have shown variation in FOS content of wheat flour between 1-6 g% (Van Loo J 1995; Campbell et al 1997; Coussement P 2000; Hogarth AJ et al 2000). Raw material of *thepla* which were prepared with wheat flour, bengal gram flour, garlic-ginger paste and fenugreek leaves had 6.6 g% FOS which was slightly higher than raw wheat flour alone. This slight increase in the product could be attributes to addition of 2 g garlic and 50 g fenugreek leaves. Studies have reported that FOS content of garlic varied between 0.2-13.5 g% (Campbell et al 1997; Hogarth AJ et al 2000; Espinos and Rico 2006; Muir G J

et al 2009). Whereas the FOS coming from fenugreek leaves would have been negligible (0.02 g%) as reported by Parnami S and Sheth M (2010).

The raw mix used for preparation of *dhokla* and *patra* with the major ingredient as bengal gram flour revealed 1.4 g% FOS indicating, lower FOS content in bengal gram , semolina and colocasia leaves used for preparation of these products. Few studies have reported small amounts of β-fructan in processed and raw legumes and pulses when analyzed using HPLC method (Pilar R 1998; Parnami S and Sheth M 2010).

Variations were observed amongst the four food products studied for recovery of FOS using various processing techniques such as roasting, shallow frying and steaming. In the present study roasting of *chapatis* showed more than 100% recovery of FOS. Study reported by Rumula P and Udayaskhara RP (1997) revealed no effect of roasting wheat flour *chapati* on the total dietary fiber content. Parnami S and Sheth M (2010) revealed a loss of 4% inulin in roasted *chapati* when analyzed using HPLC technique. Studies on recovery of resistant starch as a prebiotic in *chapati* have also revealed a in 1.5 fold increase (Vaidya R and Sheth M 2012). Similarly dietary fiber in *chapati* increased on roasting (Roopa S and Premvallalika KS 2006). Caramelization occurs when sugars are dry heated. Upon thermal heating of sugars dehydration and self-condensation reaction occurs giving rise to oligosaccharides and other dietary fibers with different degree of polymerization. Formation of these oligosaccharides depends upon d-fructose concentration, temperature and particle size (Brennan CS and Samyue E; Pereira ES et al 2010).

Shallow frying of *thepla* in the present study showed a minimal reduction in its FOS content. Other studies on shallow fried products have however revealed a slight increase in the prebiotic content of the product (Fillion L and Henry CJK 1998; Vaidya R and Sheth M 2012). The mechanism related to changes or

reduction in FOS content can be attributed to free fatty acid content of the oil used for shallow frying, temperature of heating and interactions between complex sugars when compared to roasted *chapati*.

Steaming as a technology used for preparation of *dhokla* and *patra* resulted in relatively lower percent recovery of FOS. Studies investigated on similar lines shows that steaming leads to decrease in total fiber content and xylooligosaccharides by 7-22% (Chang SNG and Lili H 2007; Sobota A and Zarzycki P 2013). This might be due to higher temperature of steaming more than 100^0 C and longer duration of heat treatment for 20 minutes. Processes involving heat-treatment like steaming may affect the dietary fibre in different ways. An increased temperature leads to a breakage of weak bonds between polysaccharide chains. Also glycosidic linkages in the dietary fibre polysaccharides may be broken. During heat treatment there may be decreased association between fibre molecules and / or depolymerization of the fibre, resulting in solubilization. If the depolymerization is extensive, alcohol soluble fragments may be formed, resulting in a decreased content of dietary fibre (Nyman M et al 1987).

To conclude, the processing technology does interfere the FOS retention in the foods. Therefore, this may be borne in mind while recommending foods rich in FOS for therapeutic uses.

CONCLUDING REMARKS

FOS can be incorporated in all the four food products studied. However, dhokla and patra remained the most acceptable products even at the higher (20%) level of FOS substitution. Recovery of FOS was maximum during roasting followed by shallow frying and steaming. Therefore depending on the use of foods, an appropriate processing method could be recommended. Looking at the prospects of use of FOS in the management of type 2 diabetes and other NCDs, all the four products studied may be recommended for consumption by this population group.

PHASE II Collection of baseline data of type 2 diabetic subjects attending health clinic of M.S. University of Baroda in terms of anthropometry, dietary, biophysical, glycemic, lipemic, GLP-1 and gut microbiota (*LAB, bifidobacteria* and enteric pathogen) and understanding the correlations between various parameters

Diabetes Mellitus is a group of chronic metabolic conditions, all of which are characterized by elevated blood glucose levels resulting from the body's inability to produce insulin or resistance to insulin action, or both. Indians have a number of characteristic features that make them highly susceptible to diabetes. The major contributory factors include genetic susceptibility, environmental factors, abdominal obesity, body fate percentage, urbanization, stress factor and cardio-metabolic risk factors. The economic burden of diabetes involves the chronic care of diabetes, which escalates many folds when vascular complications develop.

The intestinal microbiota may be identified as an important target for improving health through reduced disease risk. Recent studies have highlighted the associations of gut microbiota in reducing the glycemic and lipemic responses and enhanced gut incretins like GLP-1 which may have an indirect effect in the improvement of these metabolic conditions.

Therefore in the view of this, the present phase was planned to study the various risks which can impact diabetes and to further validate the existing information and determining the association between gut flora (*bifidobacteria, LAB* and enteric pathogen) glycemic, lipemic, and gut incretins of type 2 diabetic subjects. In this phase of the study an attempt were made to determine the general characteristics, medical history, activity pattern, anthropometric measurements, biochemical parameters (glycemic, lipemic and gut incretins), biophysical parameters and nutrient intake of type 2 diabetic subjects and studying the associations amongst these factors.

For achieving the desired objectives, a total of 120 diabetic adults were enrolled from M.S University health clinic, Vadodara. The methodology to collect the above mentioned information is elaborated in Material and Methods chapter and results are presented in sections 5.2.1 to 5.2.11.

The results of this section are divided into following sections

5.2.1 General characteristics of the subjects
5.2.2 Medical history of the subjects
5.2.3 Family history of the subjects
5.2.4 Activity pattern of the diabetic subjects
5.2.5 Anthropometric profile of the subjects
5.2.6 Biophysical and Biochemical parameters of the subjects
5.2.7 Food habits and Nutrient intake of the subjects
5.2.8 Frequency of consumption of foods and probiotics and prebiotic foods
5.2.9 Microbiological parameters of the subjects
5.2.10 Association of life style factors of subjects with biochemical parameters and microbial counts
5.2.11 Relationship of glycemic parameters with baseline, anthropometric, biochemical, microbial and dietary parameters

5.2.1 General characteristics of the type 2 diabetic subjects

Socio economic data of type 2 diabetic subjects revealed that majority of them were Hindus (95%) with 43% male and 57% female with a 100% literacy level. More than 45% subjects lived in nuclear family and 87 % of them showed per capita income more than Rs 3000 (Table 5.2.1).

Table 5.2.1: General characteristics of type 2 diabetic subjects

Parameters	Total Subjects N=120	Male N=52	Female N=68
Age(Mean age-55.2±7.58)			
40-50 y	42(35)	13(25)	29(42.6)
51-60 y	44(36.6)	18(34.2)	27(39.7)
61-70 y	34(28.4)	21(40.6)	12(17.7)
Sex	120(100)	52(43.4)	68(56.6)
Religion			
Hindu	114(95)	51(98)	63(92.6)
Muslim	6(5)	1(2)	5(7.4)
Education level			
Elementary	45(37.5)	4(7.7)	41(60.2)
High school/Diploma	49(41)	35(67.3)	14(20.5)
Graduation/higher studies	26(21.5)	13(25)	13(19.3)
Occupation			
Housewives	47(39.1)	0(0)	47(89.1)
Retired	27(22.5)	24(46.1)	6(8.8)
Working	26(38.4)	28(53.9)	15(22)
Type of family			
Nuclear	55(45.8)	26(50)	29(42.6)
Extended nuclear	51(42.5)	20(38.4)	31(45.5)
Joint	14 (11.7)	6(11.6)	8(11.9)
Per capita income			
<3000	33(27.5)	18(35)	17(25)
>3000	87(72.5)	34(65)	51(75)

Note: numbers in parenthesis indicate percentage

5.2.2 Medical history of the subjects

With regards to secondary complications of diabetes 18% subjects were dyslipidemic, 5% were having CVD, 2% had stroke. Retinopathy, Neuropathy and nephropathy were prevalent in 2.7, 3.5 and 5% subjects respectively. Almost 71% subjects were hypertensive and 61.5% subjects were obese (Figure 5.2.2).

Regarding the duration of diabetes and associated diseases, 22% subjects were recently diagnosed as diabetic for 5 years. About 21% subjects had diabetes for 5-15 years and 56% had diabetes for more than 15 years. About 71% subjects were hypertensive and 6% of them were hypertensive for more than 15 years. Coronary heart disease was prevalent in 42% of male diabetic subjects over the duration of 1-15 years and almost 12% in female diabetic subjects over the duration of 1-10 years. Almost 52% subjects were obese for 1-15 years and 9.1% subjects were obese for more than 15 years (Table 5.2.2).

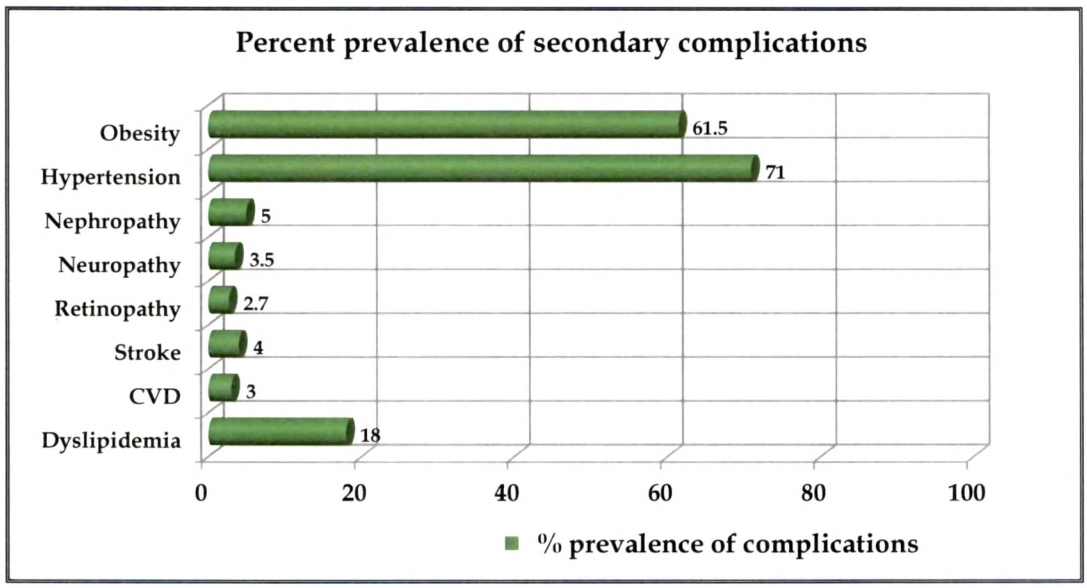


Fig. 5.2.2: Percent prevalence of secondary complications among the subjects

Table 5.2.2: Duration of type 2 diabetes and associated complications

Category	Percent Subjects N=120	Male (n=52)	Female (n=68)
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 CHD	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)

Note: numbers in parenthesis indicate percentage

5.2.3 Family history of Diabetes Mellitus, Hypertension and CHD among the subjects

About 88% subjects showed family history of diabetes with 56& subjects having single parent as history of diabetes mellitus. About 76% subjects revealed family history of hypertension with majority (60%) having single parent as hypertensive. Regarding CHD, 44.1% subjects had family history of CHD with either one, both parents and siblings (Table 5.2.3, Figure 5.2.3).

Table 5.2.3: Family history of type 2 diabetic subjects

Category	Number of subjects N=120
Diabetes Mellitus	
Both parents	12(10)
Single parent	68(56.6)
Sibling	25(20.9)
No family history	15(12.5)
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)

Note: numbers in parenthesis indicate percentage

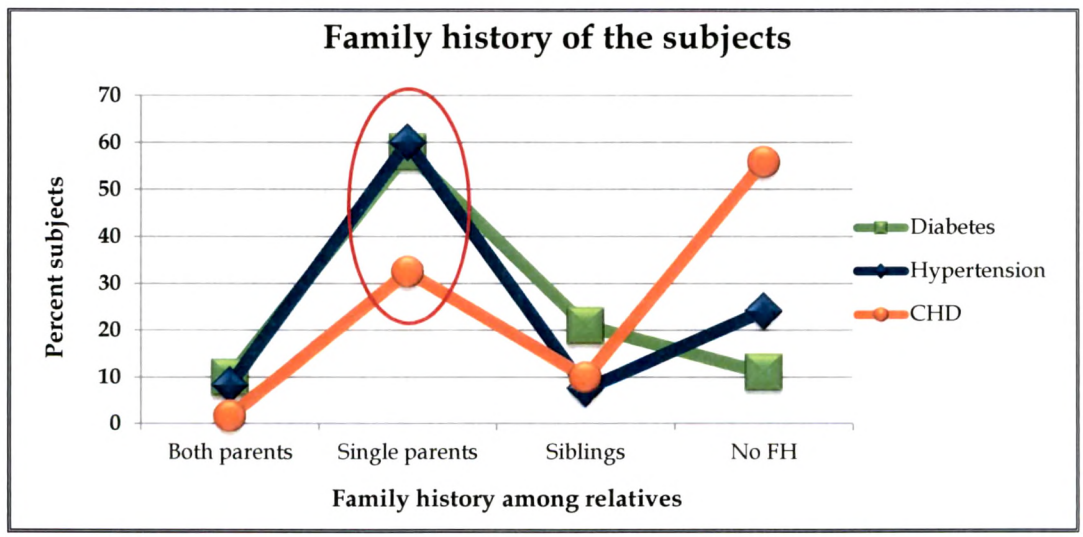


Figure. 5.2.3: Percent prevalence of family history among the subjects

5.2.4 Activity pattern of the diabetic subjects

The participants were divided into categories according to their physical activity level using the GPAQ questionnaire for physical activity developed by WHO (2008). Almost half (58.3%) of the subjects had low physical activity level (MET minutes <60). Around 32.5% had moderate physical activity level whereas only 9.2% of the total subjects had high physical activity level. Overall, the female subjects had lower physical activity level than male participants ($p<0.001$) (Table 5.2.4, Figure 5.2.4).

Table 5.2.4: Distribution of type 2 diabetic subjects according to physical activity pattern

Physical activity level (MET minutes)	Total N (120)	Male N (52)	Female N (68)	χ^2 value
Low (<60 mins)	70 (58.3)	46 (65.7)	24 (34.3)	6.26***
Moderate 139 (32.5) (≥60mins for ≥3 days)	20 (16.8)	13 (65)	7 (35)	
Moderate 2 (≥150 mins for ≥5days)	11 (9.1)	8 (72.7)	3 (27.3)	8.96***
Moderate 3 (≥600 mins for ≥5days)	8 (6.6)	6 (75)	2 (25)	
High 1 (≥1500mins for ≥3days) 11(9.2)	8 (6.6)	7 (87.5)	1 (12.5)	2.67*
High 2 (≥3000mins for ≥7 days)	3 (2.5)	3 (100)	0(0)	

***Statistically significant at $p<0.001$, ** $p<0.01$, * $p<0.05$
Note: numbers in parenthesis indicate percentage

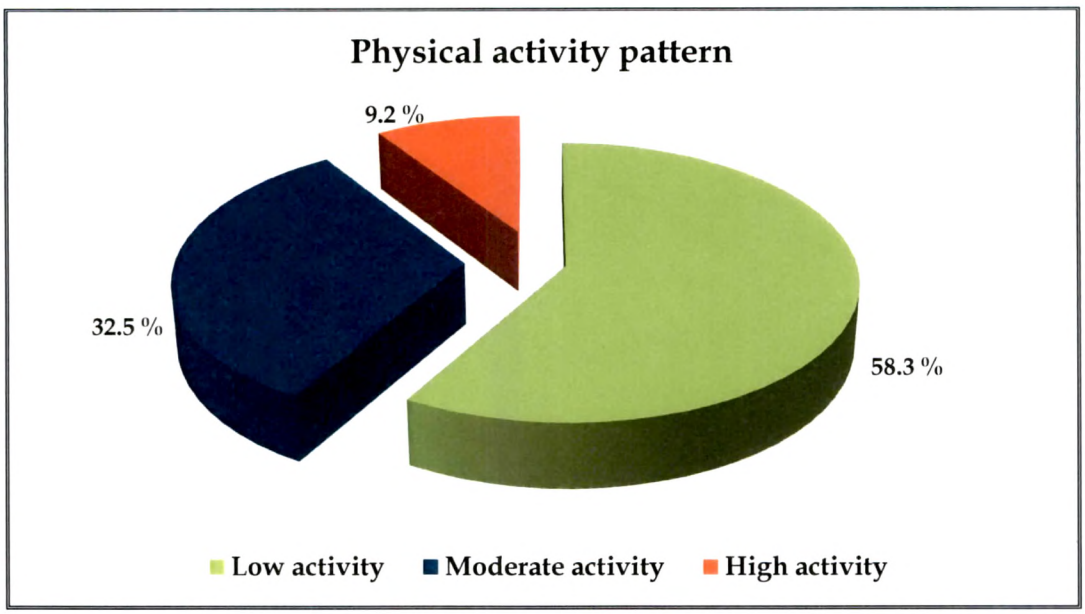


Figure 5.2.4: Physical activity pattern of type 2 diabetic subjects

5.2.5 Anthropometric profile of the subjects

The mean BMI of the subjects was 26.4 (Table 5.2.5.1). Mean waist circumference was 94.3 cm and mean hip circumference was 101.5 cm. About 20.8% and 61% subjects fell under the category of overweight and obese respectively according to Asia pacific BMI cut offs (Figure 5.2.5). On comparing the BMI cut off between males and females, results revealed that around 30% females were overweight as compare to 10% of males. Regarding the grade of obesity, more females (47.2%) were under obesity grade I where as more number of males (30.7%) were under obesity grade II (Table 5.2.5.2).

Table 5.2.5.1: Mean values of anthropometric measurements of the subjects

Variable	Percent subjects (N=120) Mean ±SD	Male (n=52) Mean ±SD	Female (n=68) Mean ±SD	't' test value
Height (cm)	160±8.4	166.9±7.1	155.3±5.2	9.8***
Weight (kg)	68±14.2	76.6±14.8	61.5±9.5	6.4***
Waist Circumference (cm)	94.3±3.5	98.4±3.8	90.2±3.2	6.5***
Hip circumference (cm)	101.5±3.8	99.5±4	103.5±3.4	0.68NS
Waist to Hip ratio (cm)	0.92±0.06	0.98±0.06	0.87±0.05	4.8***
Body mass index (BMI)	26.48±3.8	27.6±4.4	25.6±3.04	2.7**

Significance level **p<0.01, ***p<0.001, NS-Non significant

Table 5.2.5.2: Classification of type 2 Diabetic subjects according to Asia pacific classification

Category	BMI cut offs (Asia Pacific)	Total Subjects N=120	Male N=52	Female N=68
Underweight	<18.5	2(1.6)	1(1.9)	1(1.4)
Normal	18.5-22.9	20(16.6)	10(19.4)	10(14.7)
Overweight	23-24.9	25(20.8)	5(9.6)	20(29.4)
Obese I	25-29.9	52(43.3)	20(38.4)	32(47.2)
Obese II	>30	21(17.7)	16(30.7)	5(7.3)

Note: numbers in parenthesis indicate percentage

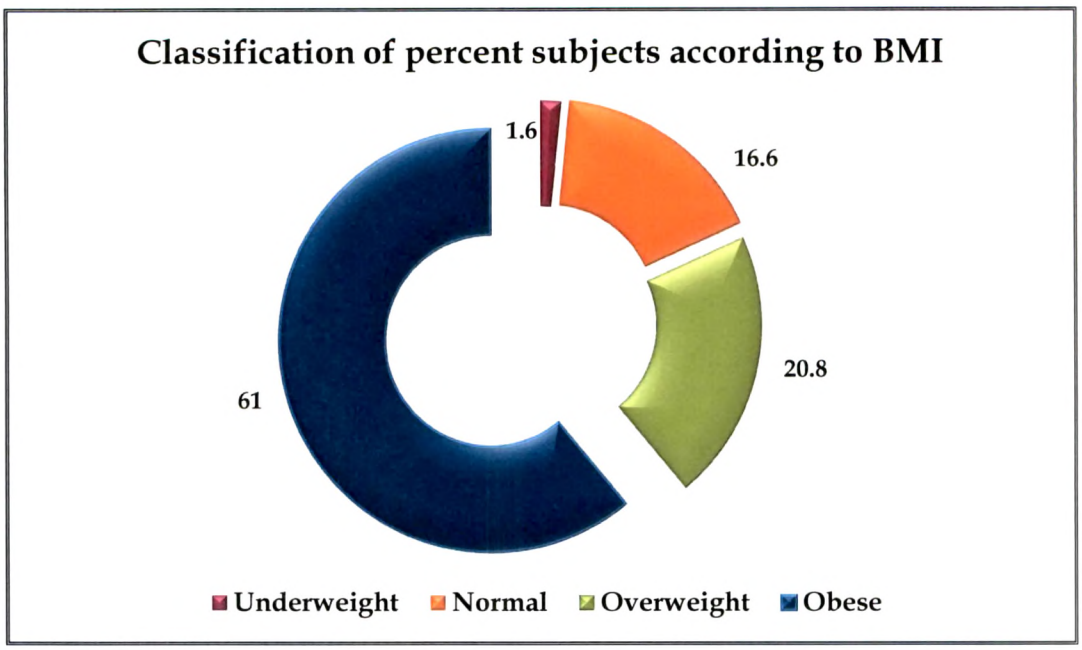


Figure 5.2.5: Percent subjects according to BMI as per Asia Pacific classification

5.2.6 Biophysical and Biochemical parameters of the subjects

The average systolic/diastolic blood pressure of the subjects was 136/86 mg/dl (Table 5.2.6.1). According to JNC VII 2003 classification, 67% subjects were borderline hypertensive and 22% subjects were having poor control of hypertension (Table 5.2.6.2, Figure 5.2.6.a).

Table 5.2.6.1 revealed that the mean fasting blood glucose levels and postprandial blood glucose levels were 143 mg/dl and 219 mg/dl respectively and the mean glycated hemoglobin levels of the subjects was 9.0. The mean gut incretin glucagon like peptide-1 (GLP-1) was 0.36 pmol/L. According to American diabetic classification, 2007, most of the subjects (86%) had poor control of diabetes (Table 5.2.6.2, Figure 5.2.6.b).

Table 5.2.6.1: Mean values for biophysical and biochemical profile of type 2 diabetic subjects

Parameters	Total subjects (N=120)	Male (N=52)	Female (=68)	't' test value
BP systolic (mmHg)	136.6±14.5	138±13.72	135.5±14.8	0.8^{NS}
BP diastolic (mmHg)	86±7.32	85.3±5.74	86.5±7.9	0.8^{NS}
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^{**}
Total cholesterol (mg/dl)	200.3±33.3	195.1±30.73	204.3±32.37	0.8^{NS}
Triglycerides (mg/dl)	142.3±37.07	135.4±32.72	147.6±36.9	1.5^{NS}
LDL-C (mg/dl)	132.5±27.3	131±26.39	137.2±24.3	1.4^{NS}
VLDL-C (mg/dl)	27±7.6	25±9.4	26.1±6.0	0.1^{NS}
HDL-C (mg/dl)	40.8±3.68	39.6±3.23	41.1±4.7	1.9^{*}
TC/HDL ratio	4.9±1.5	4.9±0.65	4.9±0.62	0.3^{NS}
GLP-1 (p mol/l)	0.368±0.32	0.433±0.38	0.288±0.18	1.6^{NS}

***Statistically significant at $p<0.001$, ** $p<0.01$, * $p<0.05$

Table 5.2.6.2: Glycemic, Lipemic and Biophysical status of the subjects categorized as good, borderline and poor

Parameters	Good N (%)	Borderline N (%)	Poor N (%)
Fasting Blood glucose	80-110	111-126	>126
N (%)	16(13.3)	18(15)	86(71.7)
Post prandial blood glucose	<140	140-200	>200
N (%)	0(0)	52(43.3)	68(56.7)
H_bA_{1c}	≤6	7-8	≥9
N (%)	0(0)	41(34.1)	79(65.9)
HDL (males)	>55	45-55	<35
N (%)	3(5.7)	3(5.7)	46(88.6)
HDL (females)	>55	45-55	<45
N (%)	0(0)	11(16.1)	57(83.9)
LDL	<100	100-130	>130
N (%)	40(33.4)	42(35)	30(31.6)
TC/HDL (males)	<6.4	-	>6.4
N (%)	45(86.5)	-	7(13.5)
TC/HDL (females)	<5.6	-	>5.6
N (%)	41(60.0)	-	27(40)
TC	<200	200-240	>240
N (%)	70(58.4)	34(28.3)	16(13.4)
TG	<150	150-200	>200
N (%)	77(64.1)	24(20)	19(15.9)
Hypertension	<130/80	130-150/80-90	>150/90
N (%)	31(25.8)	67(55.8)	22(18.4)

ADA, AHA 2009
Note: numbers in parenthesis indicate percentage

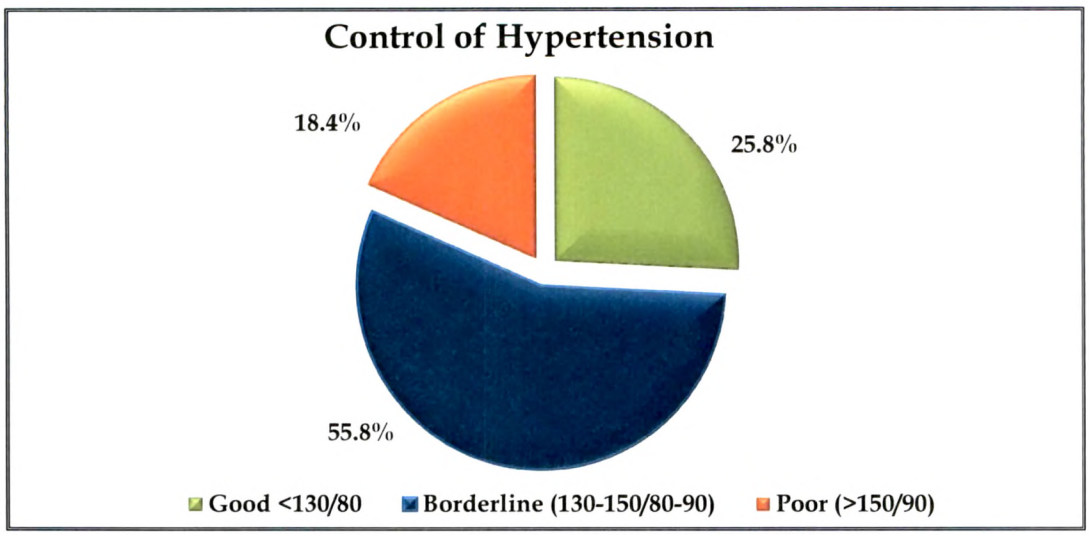


Figure 5.2.6.a: Percent subjects with varying control of hypertension

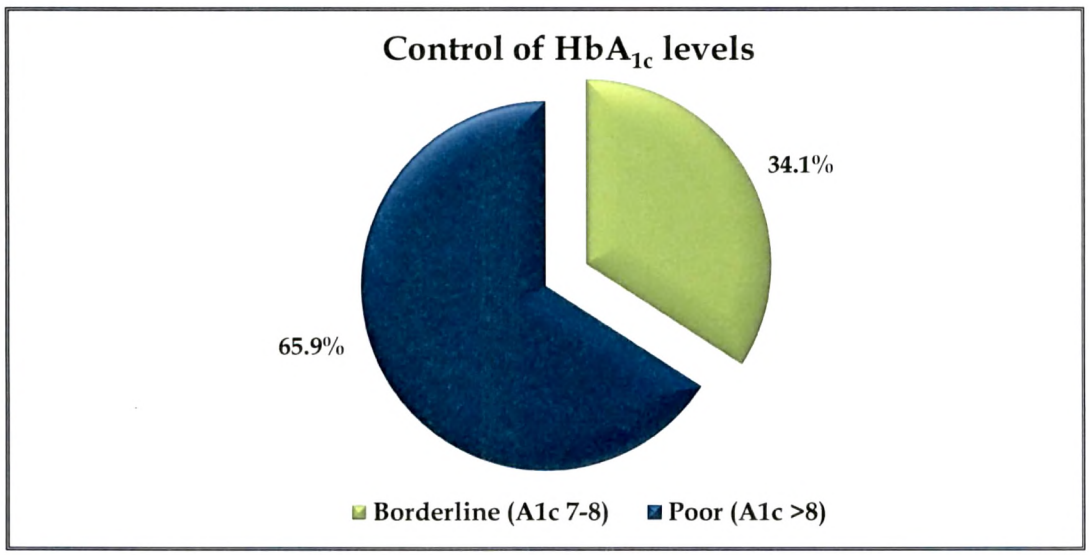


Figure 5.2.6.b: Percent subjects with varying control of HbA_{1c}

The outcomes of the lipid profile elicited that the mean cholesterol levels of the subjects was 200.3 mg. About 58.3% subjects had good control for blood cholesterol levels where as 28.3% and 13.4% had borderline and poor control on TC levels (Table 5.2.6.1, 5.2.6.2, Figure 5.2.6.c). According to WHO, IDA 2003,

64.1% of the subjects had good control on triglyceride levels with their TG levels <150 mg/dl where as 15.9% had TG levels more than 200 mg/dl (Figure 5.2.6.d). In the present study, 86.5% males and 60% females showed good control TC/HDL ratio i.e. <6.4 and < 5.6 respectively (Table 5.2.6.2).

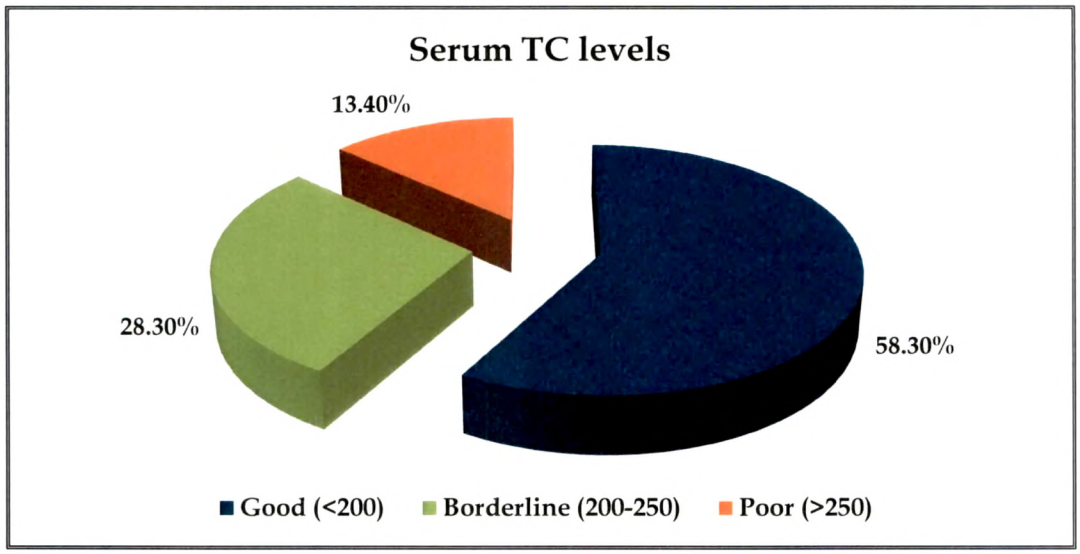


Figure 5.2.6.c: Percent subjects with varying levels of total serum cholesterol

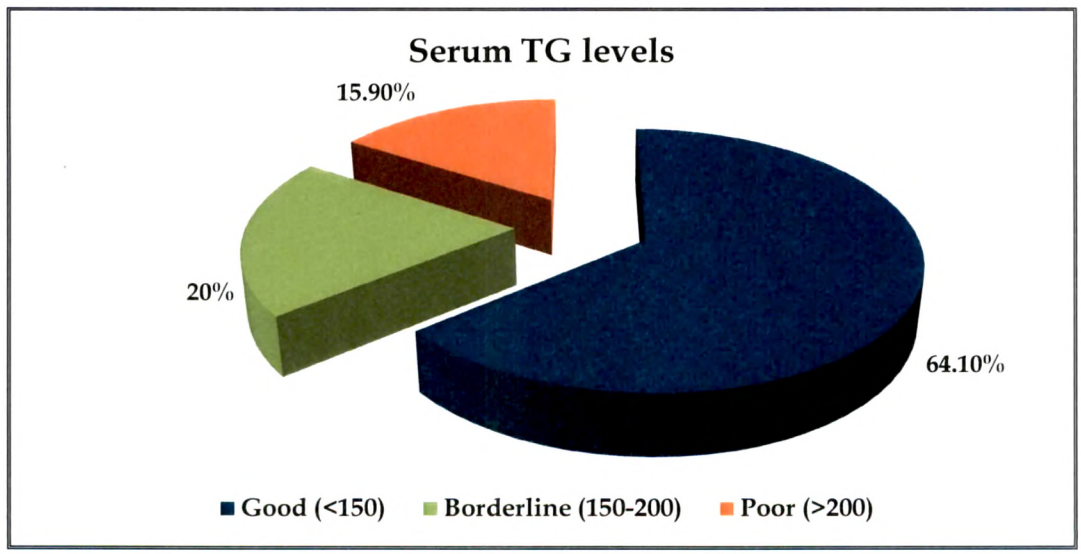


Figure 5.2.6.d: Percent subjects with varying levels of total serum triglyceride

Anthropometric, biophysical, biochemical profile of subjects based on age groups

To assess the difference in anthropometric, biophysical and biochemical profile of the subjects based on age groups, subjects were divided in three groups (Age 40-50y-A, 51-60y-B and 61-70y-C). Results on sub-categorization of age revealed that there was a significant difference amongst all the three groups with respect to systolic blood pressure ($p<0.05$). A significant difference was observed between group A and B and group A and C with regards to FBS, PP2BS, TC and TG values. However no significant difference was seen between group B and C for any of the mentioned parameters (Table 5.2.6.3).

Anthropometric, biophysical, biochemical profile of subjects based on family history of diabetes

Sub-categorization based on family history of diabetes revealed that, subjects who had family history of diabetes had significantly higher FBS, PP2BS and HbA_{1c} as compared to the subjects without family history of diabetes ($p<0.05$) (Table 5.2.6.4).

Anthropometric, biophysical, biochemical profile of subjects based on duration of diabetes

As can be seen in Table 5.2.2, almost 37% of the subjects were diabetes since 1-10 years and 62% subjects were diabetic for more than 10 years. Based on this information subjects were divided into two groups and the results revealed that no significant difference was observed for any of the parameters with respect to duration of diabetes. Although almost all the values for all the parameters were slightly on higher side in group A as compare to group B (Table 5.2.6.5).

Anthropometric, biophysical, biochemical profile of subjects based on BMI

The subjects were segregated based on BMI (Asia Pacific classification) into two groups. Subjects with lower BMI (≤23) were found to have significantly better

control on FBS, PP2BS, HbA_{1c}, TC and TG values as compared to their counterparts with higher BMI (>23) (Table 5.2.6.6).

Anthropometric, biophysical, biochemical profile of subjects based on HbA_{1c}

Table 5.2.6.7 reveals the distribution of subjects based on HbA_{1c} (a marker used to determine long term glycemic control) values that subjects with ≤8 HbA_{1c} values showed good control of FBS, PP2BS, TC and TG as compared to subjects with poor control of HbA_{1c} levels (>8).

Table 5.2.6.3: Anthropometric, biophysical and biochemical profile of the subjects based on age groups of diabetes

Parameters	Group A (n=42)	Group B (n=44)	Group C (n=34)
BMI (kg/m^2)	27.0±3.39	25.3±3.64	26.2±4.25
WHR	0.88±0.06	0.85±0.04	0.86±0.06
SBP (mmHg)	140.5±17.34[a]	133.0±13.53[b]	136.2±13.09[c]
DBP (mmHg)	88.4±9.25	85.3±7.12	87.8±6.57
FBS (mg/dl)	150.2±35.83[a]	140.8±34.10[b]	138.0±29.80[b]
PP2BS (mg/dl)	229.0±46.34[a]	207.7±43.23[b]	205±39.07[b]
HbA_{1c} (%)	9.1±1.76	8.9±1.33	8.7±1.47
Total cholesterol (mg/d)	216±43.76[a]	189.6±39.93[b]	193.9±45.67[b]
Triglycerides (mg/dl)	154.2±47.10[a]	136.8±36.79[b]	139.0±40.07[b]
LDL-C (mg/dl)	147.3±32.06[a]	122±25.99[b]	128.2±22.71[b]
VLDL-C (mg/dl)	27.7±10.73	25.8±6.28	25±3.56
HDL-C (mg/dl)	40.9±5.09	40.5±3.93	40.0±3.71
TC/HDL ratio	5.3±1.93	4.6±0.88	4.8±1.40
GLP-1 (p mol/l)	0.33±0.38	0.36±0.36	0.36±0.16

a,b,c- The non-identical letters in any two within the column denote a significant difference at a minimum of 5%

Group A- Age 40-50 y, Group B- age 51-60 y and Group C- 61-70 y

Table 5.2.6.4: Anthropometric, biophysical and biochemical profile of the subjects based on family history of diabetes

Parameters	FH (n=105)	WFH (n=15)
BMI (kg/m²)	26.7±3.72	24.9±4.90
WHR	0.87±0.06	0.85±0.05
SBP (mmHg)	140.2±14.64	136.6±17.99
DBP (mmHg)	86.0±7.83	85.7±9.03
FBS (mg/dl)	144.7±33.63	133.0±33.23*
PP2 BS(mg/dl)	218.6±37.57	199.4±36.46*
HbA_{1c} (%)	9.1±1.56	7.9±0.83*
Total cholesterol (mg/dl)	200±34.67	195.5±36.57
Triglycerides (mg/dl)	143.0±39.45	140.0±39.47
LDL-C (mg/dl)	132.5±28.33	130±20.94
VLDL-C (mg/dl)	27.3±8.09	25.6±4.08
HDL-C (mg/dl)	40.6±4.44	40.5±3.19
TC/HDL ratio	4.83±1.53	4.08±1.27
GLP-1 (p mol/l)	0.35±0.34	0.37±0.39

FH-Family history, WFH- without family history
Significantly different from the other group at *$p<0.05$

Table 5.2.6.5: Anthropometric, biophysical and biochemical profile of the subjects based on duration of diabetes

Parameters	Group A (n=45)	Group B (n=75)
BMI (kg/m²)	27.8±3.87	26.8±3.67
WHR	0.89±0.07	0.88±0.06
SBP (mmHg)	140.0±14.47	139.8±14.34
DBP (mmHg)	86.6±7.94	86.6±6.34
FBS (mg/dl)	147±33.78	145±33.23
PP2BS (mg/dl)	220±36.77	213±33.23
HbA_{1c} (%)	9.1±1.68	9.0±1.34
Total cholesterol (mg/dl)	202.7±34.00	189±33.23*
Triglycerides (mg/dl)	144.5±37.45	142.6±35.65
LDL-C (mg/dl)	138.1±27.85	126±26.12*
VLDL-C (mg/dl)	27±7.90	25.4±5.80
HDL-C (mg/dl)	40.5±3.99	40.4±4.09
TC/HDL ratio	5.0±1.65	4.9±1.46
GLP-1 (p mol/l)	0.37±0.36	0.37±0.32

Group A- Diabetes since between 1-10 years
Group B- Diabetes for >10 years
Significantly different from the other group at *$p<0.05$

Table 5.2.6.6: Anthropometric, biophysical and biochemical profile of the subjects based on BMI of diabetes

Parameters	BMI ≤ 23 (n=22)	BMI >23 (n=98)
WHR	0.84±0.08	0.86±0.06
SBP (mmHg)	134.0±14.22	137.2±14.01
DBP (mmHg)	82.6±7.42	86.8±7.89
FBS (mg/dl)	135.8±33.71	145.1±33.57*
PP2 BS(mg/dl)	205.5±41.41	220.2±36.63*
HbA_{1c} (%)	8.4±1.03	9.08±1.61*
Total cholesterol (mg/dl)	179.3±36.77	205.6±35.56**
Triglycerides (mg/dl)	123.8±37.63	146.9±32.45**
LDL-C (mg/dl)	115.1±27.54	135.7±26.33*
VLDL-C (mg/dl)	22.9±6.97	28.3±7.87
HDL-C (mg/dl)	41.5±3.46	40.30±4.58
TC/HDL ratio	4.2±0.80	4.9±1.59
GLP-1 (p mol/l)	0.37±0.35	0.35±0.37

Significantly different from the other group at *p<0.05, **p<0.001

Table 5.2.6.7: Anthropometric, biophysical and biochemical profile of the subjects based on HbA_{1c} of diabetes

Parameters	HbA_{1c} (7-8) (n=42)	HbA_{1c} (>8) (n=78)
BMI (kg/m^2)	27.1±3.44	27.6±3.79
WHR	0.84±0.06	0.86±0.06
SBP (mmHg)	136.8±16.16	140.6±14.97
DBP (mmHg)	88.7±8.04	89.6±8.47
FBS (mg/dl)	142.8±28.70	153.2±34. 91*
PP2 BS(mg/dl)	208.7±33.67	236.1±33.35*
Total cholesterol (mg/dl)	197.8±43.67	212.7±44.80*
Triglycerides (mg/dl)	148.7±38.48	168.8±40.67**
LDL-C (mg/dl)	129.8±26.04	142.5±26.65**
VLDL-C (mg/dl)	26.7±3.36	28.9±8.45
HDL-C (mg/dl)	40.6±4.13	40.5±4.06
TC/HDL ratio	4.9±1.76	5.2±1.64
GLP-1 (p mol/l)	0.37±0.34	0.35±0.32

Significantly different from the other group at *$p<0.05$, **$p<0.001$

5.2.7.1 Food habits of the subjects

In the present study, 77.4% subjects were vegetarian followed by 3.3% and 19.3% non-vegetarian and ovo-lacto vegetarian respectively. Almost 61% subjects had 2-3 cups of tea or coffee per day. Use of alternative therapies for diabetes was seen in 31% subjects whereas, rest of the subjects were on regular medications. Only 10% subjects consumed artificial sweetener on daily basis (Table 5.2.7.1).

Table 5.2.7.1: Food habits of type 2 diabetic subjects

Category	Total subjects (N=120)
Type of diet	
Vegetarian	93(77.4)
Non vegetarian	4(3.3)
Ovo Lacto vegetarian	23(19.3)
Number of tea/coffee/day	
1 cup	16(13.3)
2-3 cups	74(61.7)
>3 cups	30(25)
Amount of sugar added / coffee/tea	
>1 tsp.	38(31.6)
1-2 tsp.	68(56.6)
No sugar	14(11.8)
Alternative therapies to control diabetes	
Yes	38(31.6)
No	82(68.4)
Consumption of artificial sweetener	
Yes	12(10)
No	108(90)

Note: numbers in parenthesis indicate percentage

5.2.7.2 Nutrient intake of the subjects

Table 5.2.7.2 and Figure 5.2.7 elicits the mean nutrient intake for energy and other nutrients. Mean energy intake was 43.6% and 49.3% higher for females and males

respectively than the prescribed RDA. Protein intake was almost 14-20% lower in both the groups. Intake of fat was 98% and 119% higher in males and females respectively. Intake of iron was 60% and 56% lower in females and males respectively than the RDA. Almost all the micronutrients were below the normal range except vitamin C.

Mean intake of SFA, MUFA and PUFA was 7.3, 9.7 and 14.4 g respectively. As per ADA 2007 guidelines, calculated SFA, MUFA and PUFA of the subjects should be 2.6-3.8g, 5.7g and 3.8g respectively for females and 3.2-4.5g, 6.8g and 4.5g respectively for males. It indicates that subjects were consuming a diet which was high in SFA. An ideal ratio of 1:1.5:1 for SFA: MUFA: PUFA was also unbalanced in the diet of the subjects. An Ideal ratio of omega 6 and omega 3 fatty acids is 10:1 which was unbalanced (14.4:0.05) in the diet of the subjects. Total fiber intakes of male and females was 14.7 g and 12 g for males and females respectively which was almost 60% lower than the RDA (Table 5.2.7.3)

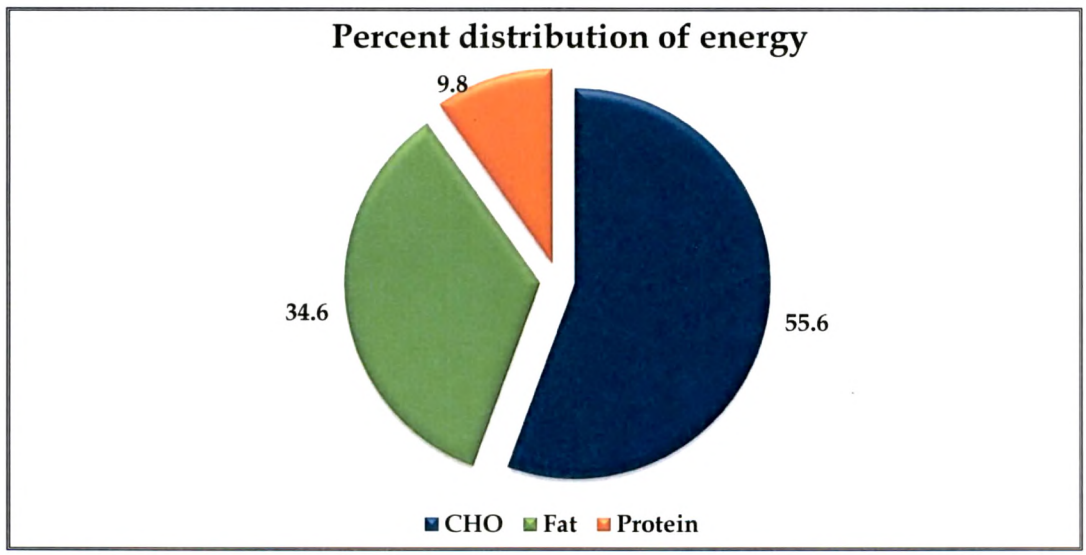


Figure 5.2.7.1: Unbalanced percent distribution of energy from macronutrients by type 2 diabetic subjects

Table 5.2.7.2: Mean intake of nutrients of type 2 diabetic subjects as per 24 hr dietary recall

Nutrients	Range	Total N=120	Female N=64	% RDA	RDA for Female	Male N=50	% RDA	RDA for Male	't' test
Energy (Kcal)	1234-2890	2205±405.5	1975±396	43.6↑	1375#	2465±415.1	49.3↑	1650##	3.08***
CHO (g)	133-329	307±45.8	259.8±40.7	17↑	223.4¥	350±49.4	30.5↑	268.1¥¥	2.87**
Protein (g)	30-72	52.5±11.6	43.8±12.4	14↓	50	62.7±10.9	19.2↓	60	2.70**
Fat (g)	37-100	85±15.1	83.8±15	119↑	38.1^	89.7±15.2	97.7↑	45.8^^	2.01*
Calcium (mg)	279-1398	618.4 ±294.7	593.1 ±218.9	1.16↓	600	810.7 ±325.8	35↑	600	3.26**
Iron (mg)	5-26	13.8±±4.8	12.0±4.3	60↓	30	15.8±4.5	56.4	28	2.95**
Sodium (mg)	73-726	285.9±143.9	267.0±117.0	-	-	306.1±167.7	-	-	0.83NS
Magnesium (mg)	158-859	326.9±146.6	315.0±164.9	-	-	339.7±125.7	-	-	0.50NS
Potassium (mg)	782-2457	1490.3±347	1416.8±315.5	-	-	1568.6±367.9	-	-	1.66NS
Zinc (mg)	2-9	4.6±1.4	4.2±1.2	-	-	5.0±1.5	-	-	2.57*
βCarotene (µg)	117-7260	2127±5146	2367.7±4495.8	11.3↓	2400	2144.6±5693	10.6↓	2400	1.60NS
Vitamin C (mg)	16-361	119.4±78.9	105.8±57.8	162↑	40	133.9±95.4	232↑	40	1.19NS
Vitamin B12 (µg)	0-1	0.3±0.2	0.3±0.1	70↓	1	0.4±0.2	60↓	1	0.92NS
Vitamin B6 (mg)	0-0.2	0.10±0.0	0.11±0.0	95↓	2	0.08±0.0	60↓	2	3.53**
Riboflavin (mg)	0.4-2.3	1.0±0.4	0.9±0.3	9↓	1.1	1.1±0.4	21.4↓	1.4	0.92NS

a) ^ Calculated as per 25% energy from fat for sedentary women (ADA, 2007)
b) ^^Calculated as per 25% energy from fat for sedentary men (ADA, 2007)
c) ¥Calculated as per 65% energy from CHO for sedentary women (ADA, 2007)
d) ¥¥Calculated as per 65% energy from CHO for sedentary men (ADA, 2007)
e) ***Statistically significant at p<0.001, ** p<0.01, * p<0.05
f) #Calculated by multiplying 25 with ideal mean body weight for sedentary >40 y old sedentary women (ADA, 2007)
g) ##Calculated by multiplying 25 with ideal mean body weight for sedentary >40 y old sedentary men (ADA, 2007)

Table 5.2.7.3: Mean intake of Fatty acids and Dietary fiber by type 2 Diabetic subjects as per mean 3 day 24 hr dietary recall

Nutrients		Reference Range	Total N=120	Female N=68	Male N=52	't' test
SFA (g)		F-2.6 -3.8g^ M-3.2-4.5g^	7.3±4.1 (6-15)	7.7±3.8	6.8±4.4	0.61^{NS}
MUFA (g)	1:1.5:1	5.7-6.8*	9.7±5.8 (6-22)	10.6±5.7	8.7±5.9	1.3^{NS}
PUFA (g)		3.8-4.5¥	14.4±8.3 (8-30	14.7±7.5	14.1±9.1	0.21^{NS}
n3 (g)		-	0.04±0.03 (0)	0.05±0.04	0.03±0.03	1.71^{NS}
n6 (g)		-	14.4±8.3 (0-30)	14.8±7.6	14.1±9.1	0.26^{NS}
n6:n3		10:1	14.4:0.04	14.8:0.05	14.1:0.03	
Insoluble dietary fiber (g)		-	9.0±4.0 (3-21)	9.0±4.0	10.6±4.4	1.42^{NS}
Soluble dietary fiber (g)		-	3.5±1.6 (1-7)	3.5±1.6	4.0±1.5	1.28^{NS}
Total dietary fiber (g)		25-35**	12.4±5.4 (4-27)	12.4±5.4	14.7±5.7	1.50^{NS}

^Calculated as per 7-10% energy from total fat (ADA, 2007)
* Calculated as per 15% energy from total fat (ADA, 2007)
¥ Calculated as per 10% energy from total fat (ADA, 2007)
** As per recommended by ADA, 2002

Ideally in diabetes, short and frequent meals are prescribed in which distribution of energy in a day is very important. In the present study subjects were consuming meals which were undistributed with 20% energy consumption in the breakfast, 35% energy in lunch, 20% energy at tea-time and 25% energy in the dinner (Figure 5.2.7.2).

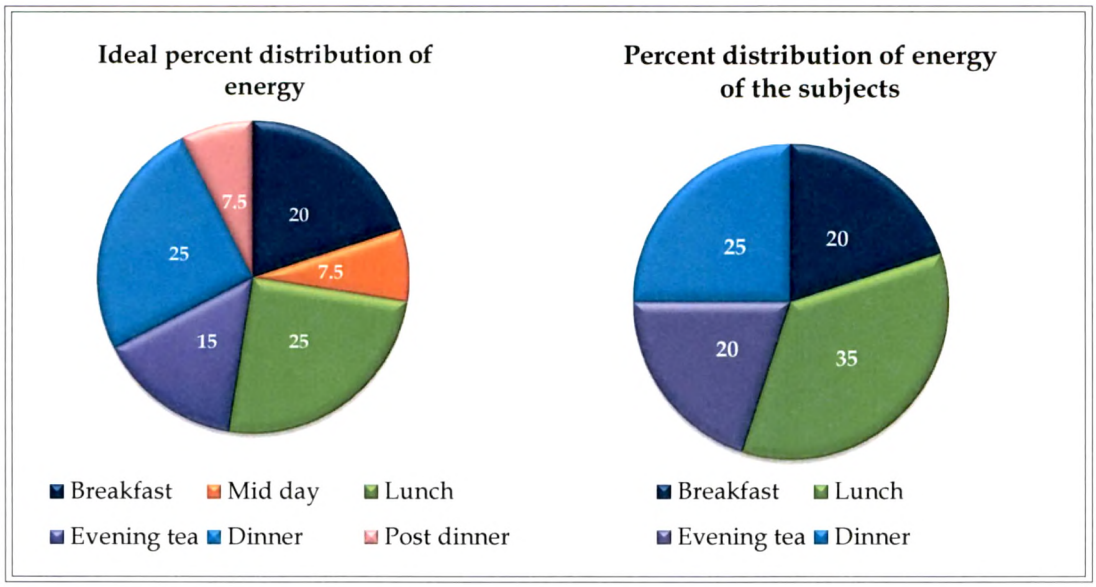


Figure 5.2.7.2: Ideal % distribution of energy v/s % distribution of energy by the subjects

5.2.8.1 Food intake of diabetic subjects as per Food frequency method

Table 5.2.8.1 demonstrates the frequency of consumption of various food groups by food frequency questionnaire (FFQ). Results shows that all the subjects consumed cereals on daily basis with maximum consumption of whole wheat flour followed by rice. Only 12.5% subjects consumed green leafy vegetables frequently. Fruits were consumed only by 20% subjects on frequent basis. Most subjects (90%) consumed milk and milk products on a daily basis. Almost 33% subjects consumed snacks like khakhara, bhajiya and fried papad on regular basis followed by 37% subjects who consumed snacks on less frequent basis. Sweets like kheer, icecream and peda was consumed by almost 30% subjects on less frequent basis, rest of the subjects consumed sweets rarely.

Table 5.2.8.1: Frequency of consumption of food groups by the subjects as per food frequency method

Food Groups	Frequent N (%)	Less frequent N (%)	Rarely N (%)
Cereals	120(100)	-	-
Whole wheat flour>Rice>Rice flakes>Bajra>Semolina			
Pulses/Legumes	115(95.8)	5(5.2)	-
Red gram dal>Green gram dal>Bengal gram dal>Lentil> Moth beans			
Green Leafy Vegetables	15(12.5)	102(85)	3(2.5)
Cabbage>Fenugreek leaves>Spinach>Colocasia leaves			
Roots & tubers	110(91.6)	10(8.4)	-
Onion>Potato>Carrot>Beet root>Colocasia			
Other vegetables	108(90.1)	12(9.9)	-
Brinjal>Ash gourd>Ladies finger>Cauliflower			
Fruits	24(20)	90(75)	6(5)
Tomato>Apple>Orange>Papaya>Banana			
Nuts & Oil seeds	10(8.3)	70(58.3)	40(33.4)
Groundnut>Sesame seeds>Almond>Coconut>Walnut			
Fats	120(100)	-	-
Groundnut oil>Cottonseed oil>Ghee>Gingelly oil>Butter			
Milk & milk products	108(90.1)	10(8.3)	2(1.6)
Milk (cow)>Buttermilk>Curd>Paneer>Srikhand			
Snacks	40(33.3)	45(37.5)	35(29.2)
Khakhra>Bhajiya>Papad>Dabeli>Samosa			
Sweets	-	35(29.2)	85(70.8)
Kheer>Icecream>Peda>Basundi>Gulabjamun			

Note: numbers in parenthesis indicate percentage

5.2.8.2 Frequency of consumption of probiotics and prebiotic foods

The probiotic rich foods studied were curd, buttermilk, yogurt, shrikhand and lassi and the prebiotic rich foods included were wheat, rice, bengal gram, black gram, green gram, apple, tomato, potato, onion, garlic, etc. Most of the subjects (71%) had frequent consumption whereas only 18% had most frequent consumption and 11% had less frequent consumption. Amongst the prebiotic rich foods consumed, wheat was consumed most followed by wheat, onion, tomato and garlic, on the other side amongst the probiotic rich foods, buttermilk was consumed most frequently followed by curd (Figure 5.2.8).

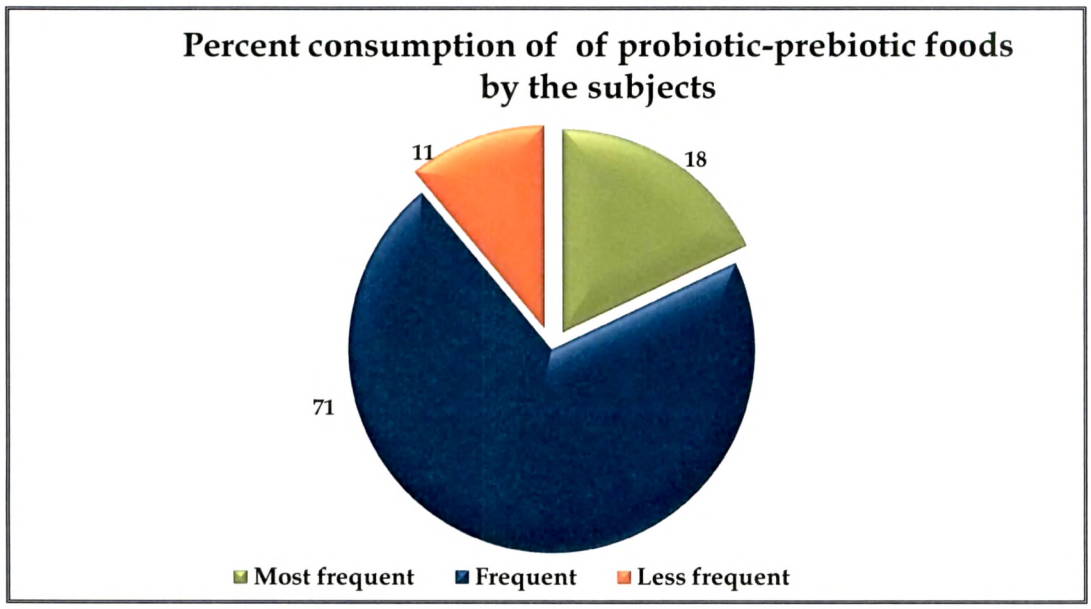


Figure 5.2.8: Frequency of consumption of probiotics and prebiotic foods by type 2 diabetic subjects

Most frequent:	Subjects consuming more than 15 foods daily, thrice a week and weekly from the list of prebiotic/ probiotic foods enlisted
Frequent:	Subjects consuming 9-15 foods daily, thrice a week and weekly from the list of prebiotic/ probiotic foods enlisted
Less frequent:	Subjects consuming less than 9 foods daily, thrice a week and weekly from the list of prebiotic/ probiotic foods enlisted

5.2.9 Fecal microbial counts of the subjects

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 5.2.9.1).

An attempt was made to analyze the difference between biophysical, biochemical, anthropometric and dietary fiber values based on *bifidobacterial* log counts and the results revealed that there was a significant difference in the FBS, PP2BS, HbA_{1c} and dietary fiber values in both the groups. However, no significant difference was observed in biophysical and lipemic parameters in both the groups (Table 5.2.9.2 and Figure 5.2.9).

Table 5.2.9.1: 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}

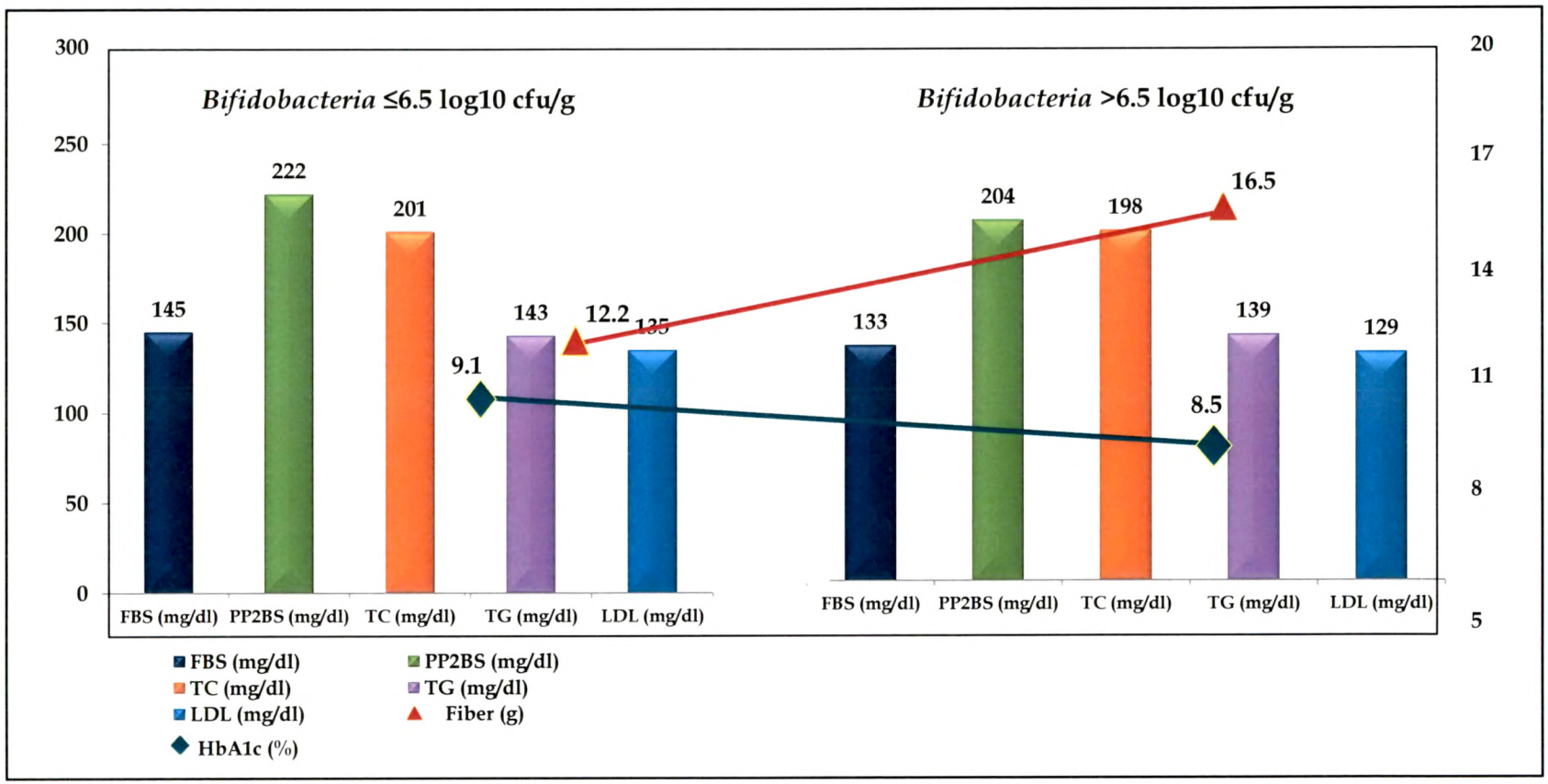
NS- Non significant

Table 5.2.9.2: Anthropometric, biophysical biochemical profile and total dietary fiber of the subjects based on *Bifidobacterial* counts

Parameters	*Bifidobacteria* ≤6.5 log10 CFU/g (n=90)	*Bifidobacteria* >6.5 log10 CFU/g (n=30)
BMI (kg/m^2)	26.8±3.64	25.5±4.09
WHR	0.85±0.06	0.85±0.07
SBP (mmHg)	138.9±13.78	136.8±18.70
DBP (mmHg)	86.8±7.52	86.2±9.25
FBS (mg/dl)	145.3±34.47	133.23±30.93*
PP2BS (mg/dl)	221.8±35.06	203.7±32.45*
HbA_{1c} (%)	9.1±1.60	8.5±1.25*
Total cholesterol (mg/dl)	201.67±42.65	198.5±47.45
Triglycerides (mg/dl)	152±44.19	149±35.97
LDL-C (mg/dl)	135±27.43	128±27.88
VLDL-C (mg/dl)	27.8±8.4	24.6±4.99
HDL-C (mg/dl)	40.3±4.28	41±4.37
TC/HDL ratio	5.2±1.55	4.9±1.32
GLP-1 (p mol/l)	0.334±0.32	0.382±0.33
Total dietary fiber	12.2±5.5	16.5±5.8*

Significantly different from the other group at *$p<0.05$

Figre 5.2.9: Difference in *bifidobacterial* counts on biochemical parameters and dietary fiber values of the subjects



5.2.10 Association of life style factors of subjects with biochemical parameters and microbial counts

As shown in Table 5.2.10.1(a,b), a positive correlation was observed between fasting blood sugar level and family history and CHO intake ($p<0.05$). Glycemic parameters like PP2BS and HbA_{1c} were not related with family history, however PP2BS revealed a significant positive correlation with physical activity and a negative correlation with fiber intake ($p<0.05$). GLP-1 was significant negatively correlated with WHR and fat intake ($p<0.05$). *Bifidobacteria* and *LAB* were significant positively correlated with total fiber whereas *Bifidobacteria* was negatively correlated with WHR and fat intake ($p<0.05$). Enteric pathogen revealed a non-significant positive correlation with BMI.

TC revealed an inverse significant positive correlation with physical activity and fiber and a significant positive correlation with WC, WHR, calorie and fat intake ($p<0.05$). A significant positive association was seen for TG with calorie and fat intake. However, TG showed a significant negative correlation with fiber intake ($p<0.05$). Intake of fiber was negatively correlated with LDL and TC/HDL ($p<0.05$). Moreover, LDL and VLDL was significant positively correlated with WC, WHR and calorie intake ($p<0.05$).

BMI revealed a negative correlation with physical activity and significant positive association with calorie, CHO and fat intake ($p<0.01$). Furthermore, WC was also observed to be significant positively correlated with calorie and fat intake ($p<0.05$). 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 HbA_{1c} 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 5.2.10.2).

Table 5.2.10.1(a): Correlation values determining the degree of association amongst lifestyle factors, glycemic parameters, dietary and microbial parameters of type 2 diabetic subjects

Life style factors	SBP	DBP	FBS	PP2	HbA1c	GLP1	*LAB*	*Bifido*	E. Pathogen
Age	0.33**	-0.22	-0.12	0.03	-0.06	0.03	-0.19	0.05	-0.18
Family history	0.08	-0.04	0.35**	-0.10	-0.09	-0.07	-0.01	0.19	0.06
Physical activity	-0.24	-0.08	-0.23	0.24*	-0.18	-0.14	-0.04	-0.05	0.17
BMI	0.18	0.10	0.08	-0.00	0.13	-0.05	0.03	0.07	0.24
WC	0.13	0.05	-0.05	-0.16	-0.03	-0.03	0.09	0.13	-0.14
WHR	0.14	0.06	0.05	-0.00	-0.06	-0.51*	0.21	-0.24*	-0.15
Calorie intake	-0.06	0.03	-0.07	-0.04	-0.12	-0.20	-0.13	-0.11	-0.01
CHO intake	-0.09	-0.01	0.24*	-0.13	-0.11	-0.01	-0.08	-0.12	0.03
Protein intake	-0.03	0.07	-0.22	-0.09	-0.24	-0.21	-0.14	-0.07	-0.03
Fat intake	0.00	0.10	-0.08	-0.06	-0.10	-0.28*	-0.15	-0.24*	-0.09
Fiber intake	-0.01	-0.01	0.12	-0.24*	-0.05	0.21	0.25*	0.26*	-0.21

* Correlation values are significant, $p<0.05$, ** Correlation values are significant, $p<0.01$

Table 5.2.10.1(b): Correlation values determining the degree of association amongst lifestyle factors, lipemic parameters, dietary and anthropometric parameters of type 2 diabetic subjects

Life style factors	TC	TG	LDL	HDL	VLDL	TC/ HDL	BMI	WC	WHR
Age	0.00	-0.18	0.06	-0.18	-0.20	0.05	-0.08	-0.03	-0.02
Family history	0.20	0.17	0.12	0.11	0.18	0.12	0.02	0.18	0.10
Physical activity	-0.27*	-0.08	-0.10	0.11	-0.06	-0.17	-0.24*	-0.15	-0.12
BMI	0.22	0.00	0.30*	-0.04	0.16	0.25*	-	-	-
WC	0.28*	-0.04	0.36**	-0.13	0.13	0.27*	-	-	-
WHR	0.26*	-0.17	0.29*	-0.10	-0.01	0.28*	-	-	-
Calorie intake	0.26*	0.32**	0.26*	0.10	0.26*	-0.20	0.28**	0.24*	0.19
CHO intake	0.21	0.20	0.20	0.05	0.26*	0.07	0.29**	0.20	0.10
Protein intake	-0.22	-0.20	-0.23	-0.13	-0.30*	0.05	0.13	0.03	0.02
Fat intake	0.31**	0.36**	0.20	0.22	0.11	-0.28*	0.48***	0.26*	0.22
Fiber intake	-0.28*	-0.24*	-0.32*	0.10	-0.20	-0.33**	-0.21	-0.12	-0.10

* Correlation values are significant, $p<0.05$, ** Correlation values are significant, $p<0.01$

Table 5.2.10.2: Correlation values determining the degree of association amongst glucose parameters and microbial parameters of type 2 diabetic subjects

Parameters	*Lactobacillus*	*Bifidobacteria*	*Enteric pathogen*
Mean A1c (%)	-0.4	-0.5	0.04
Mean FBS (mg/dl)	-0.5	-0.5	0.05
Mean PP2 (mg/dl)	-0.6	0.4	0.03
A1c 7-8%	0.3	-0.7*	-0.05
A1c >8%	0.4	0.5	0.04
FBS 80-110 (mg/dl)	-0.7*	-0.8*	0.08
FBS 110-126 (mg/dl)	-0.3	0.4	0.04
FBS>126 (mg/dl)	0.3	-0.4	0.03
PP2 140-200 (mg/dl)	-0.5	-0.9*	0.06
PP2>200 (mg/dl)	-0.5	-0.5	0.05

* Correlation values are significant, $p<0.05$

5.2.11 Relationship of glycemic parameters with baseline, anthropometric, biochemical, microbial and dietary parameters

To further assess the relationship between glycemic parameters with baseline, anthropometric, biochemical, microbial and dietary parameters linear multiple regression analysis was performed as it is a strong tool to predict the most affecting criterion variable on several other independent or predictor variables. In the present analysis shown in Table 5.2.11.2, glycemic parameters (FBS, PP2 and HbA_{1c}) are criterion or dependent variables and rest other factors are the predictors. Total twenty predictor determinants were identified to find out the most affecting predictor variables on criterion variables (Table 5.2.11.1).

Table 5.2.11.1: Predictor variables identified for most affecting variables

Parameters	Predictors
Baseline parameters	0. Family history 1. SBP 2. DBP
Anthropometric parameters	3. BMI 4. WHR 5. WC
Biochemical parameters	6. TC 7. TG 8. VLDL 9. LDL 10. HDL 11. TC/HDL ratio 12. GLP-1
Microbial parameters	*13. LAB* *14. Bifidobacteria* 15. Enteric pathogen
Dietary parameters	16. Energy 17. Fat 18. Protein 19. CHO 20. Dietary fiber

Table 5.2.11.2: Coefficient of relationships of glycemic parameters with baseline, anthropometric, biochemical and dietary parameters

Criterion variable	Predictor variable*	Standardized co-efficient Beta	Significance level	Criterion variable	Predictor variable*	Standardized co-efficient Beta	Significance level	Criterion variable	Predictor variable*	Standardized co-efficient Beta	Significance Level
FBS	0	**0.491**	0.05	**PP2BS**	0	0.072	0.66	**HbA_{1c}**	0	0.251	0.06
	1	0.166	0.94		1	0.042	0.93		1	0.266	0.06
	2	0.112	0.65		2	0.038	0.89		2	0.038	0.93
	3	-0.094	0.73		3	0.032	0.90		3	0.209	0.07
	4	-0.163	0.90		4	0.125	0.59		4	**0.313**	0.05
	5	-0.081	0.78		5	0.189	0.55		5	0.058	0.97
	6	0.076	0.67		6	0.212	0.06		6	-0.121	0.08
	7	0.071	0.65		7	0.231	0.05		7	0.167	0.07
	8	0.253	0.06		8	0.200	0.06		8	-0.05	0.89
	9	0.123	0.56		9	**-0.265**	0.06		9	**0.439**	0.02
	10	0.022	0.92		10	0.078	0.05		10	-0.045	0.87
	11	0.036	0.93		11	0.022	0.07		11	0.193	0.09
	12	-0.302	0.06		12	**-0.234**	0.05		12	-0.300	0.05
	13	0.298	0.06		13	0.192	0.06		13	0.198	0.08
	14	**0.352**	0.04		14	0.221	0.05		14	**0.337**	0.04
	15	-0.125	0.60		15	-0.178	0.07		15	0.219	0.06
	16	0.284	0.07		16	0.190	0.06		16	0.176	0.08
	17	0.227	0.07		17	0.003	0.97		17	0.286	0.06
	18	0.013	0.87		18	0.029	0.12		18	0.299	0.05
	19	0.219	0.92		19	0.201	0.06		19	0.265	0.06
	20	**0.366**	0.03		20	**0.279**	0.05		20	0.186	0.08

Predictor variables*- Refer Table 5.2.11.

RESULT HIGHLIGHTS

- *Majority of the diabetics surveyed in the study were Hindus (95.2%) belonging to the age group 40-70y with 52 males and 68 females.*
- *Almost 75% of the subjects suffered from diabetes for more than 15y.*
- *Around 11.2% and 25% subjects had microvascular and macrovascular complications of diabetes respectively.*
- *With reference to Asia pacific classification, 61% of the subjects were obese and 20.8% were overweight.*
- *According to JNC VII, 2003 classification, 55.8% subjects were pre-hypertensive and 25.8% were hypertensive.*
- *Almost 65.9% of the subjects had poor control of diabetes with HbA_{1c} >8. The mean FBS and PP2BS values of the subjects were 143mg/dl and 219 mg/dl respectively.*
- *The mean cholesterol and triglyceride levels of the subjects were 200 mg/dl and 142 mg/dl respectively. Almost 47% of the subjects had LDL levels less than 100 mg/dl. 46% of males and 57% of females had poor HDL levels.*
- *Regarding the dietary assessment, mean nutrient intake of energy, protein and fat was higher in both males and females. Fibre intake was 60% lower than the RDA. About 71% subjects had frequent consumption of prebiotic/probiotic rich foods.*
- *The mean log counts of lactic acid bacteria, bifidobacteria and enteric pathogen was 6.34, 6.34 and 4.47 respectively. The mean values of biochemical parameters were significantly higher in the subjects with ≤6.5 log_{10} counts of bifidobacteria.*
- *LAB and bifidobacteria were significant negatively associated with FBS, PP2BS, and HbA_{1c}.*
- *Fat intake of the subjects was significant negatively correlated with GLP-1 and Bifidobacteria. Fiber intake was inversely correlated with TC, TG, LDL, TC/HDL, PP2BS and positively correlated with LAB and bifidobacteria ($p<0.05$).*

DISCUSSION

In the present study 120 type 2 diabetic subjects were enrolled from the Health clinic of the M.S. University of Baroda to study their baseline profile and determining the associations amongst various factors. Baseline information of subjects was collected on socio economic status, anthropometric measurements, biophysical, biochemical, dietary, physical activity and microbial parameters. Study outcome indicated that 88% of diabetic subjects had family history of diabetes and 37% had diabetes for less than 10 years.

The role of heredity 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 (Hamman RF 1992). A similar study revealed that out of total 22 diabetic subjects almost 95% subjects had family history of diabetes with first degree relatives (Heideman et al 2012). Another study reported that type 2 diabetes has a strong familial component with family history of first relative having diabetes, and at least 50 genetic variants have been reported to influence susceptibility to type 2 diabetes (Pierce M et al 1995; Mc Carthy MI 2010; Paul WF 2012). Evidences also indicates that type 2 diabetes has a strong genetic component came from family-based studies, where the observation that diabetes clusters within groups of biologically related individuals led to the quantification of diabetes heritability and familial risk, through which having parents with diabetes was determined to double the risk of type 2 diabetes (Poulsen P et al 1999 and Wilson PWF et al 2007).

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 diabetes. According to an ICMR-INDIAB study, there are 199 million and 61.3 million people with

obesity and diabetes in India respectively (Mohan V 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). Studies have shown that obesity parameters like BMI and waist circumference are significantly higher among subjects with family history of diabetes (Mohan V et al 2005). 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 SR 2003; Snehlata et al 2003; Shetty 2012). It has been reported in several studies that visceral fat is associated with abdominal obesity and type 2 diabetes mellitus. Abdominal obesity causes unfavourable aberrations in the body metabolism of diabetics by the mechanism of desensitizing the insulin receptors which results in decrease of insulin levels (Parikh and Mani 2002). Blaha et al (2008) examined the relationship between WC and glucose deterioration in type 2 diabetes and it was found that WC alone may be a powerful tool for the glucose deterioration in type 2 diabetic subjects. 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 and 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 (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 (Zimmet P Alberti and Shaw 2001; Mohan V et al 2001, 2005).

Although type 2 diabetes is determined primarily by lifestyle and genes, dietary composition may affect both its development and complications. In the present study, subjects were consuming a diet which was 43-50% higher in calories and 97-119% higher in fat than the prescribed RDA. According to American diabetic association (ADA) 2007 guidelines, recommendations for SFA, MUFA and PUFA is 7-10%, 15% and 10% from the total fat intake respectively. Besides, in the present study subjects were consuming unbalanced ratios of fats with high SFA and unbalanced ratio of SFA, MUFA and PUFA. Animal studies 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 Walker and Frank Hu 2009). In a nurse's health study, a higher intake of n6 PUFA was associated with a significantly decrease risk of type 2 diabetes (Salmaron et al 2001).

Carbohydrate intake of the subjects was also 17-30% higher than the RDA (ADA 1987). Though subjects were consuming 55% of the total energy from CHO however, recently literature emphasized the importance of prescribed total fat and correspondingly the quantity of dietary carbohydrate (Penny M 2013). A randomized, crossover, multicenter study was conducted with 42 outpatients with NIDDM who were instructed to follow a high- MUFA diet that provided 45% of energy from fat and a high-carbohydrate diet that provided 55% of energy from carbohydrate and 30% of energy from total fat. Both diets were low in SFA (i.e. 10% of energy), and fiber content was comparable. The high-carbohydrate and MUFA diet increased the insulin values increased by 9% ($p<0.005$) (NCEP 1994). An anti-inflammatory mechanism is also related with omega 3 fatty acid. Presence of bacteria with

the capacity to synthesize conjugated linoleic acid (CLA), such as *Bifidobacterium and Lactobacillus*. CLA increases omega-3 polyunsaturated fatty acids in the host, exerting beneficial biological activities, including anti-diabetic and anti-inflammatory properties (Wall R et al 2009). In the present study, very low omega 3 intakes by the subjects might also be one of the possible reasons for poor glycemic control.

The intake of total fiber by the subjects under the study was 55%-60% lower than the RDA which is also evident from the low intake of fruits and GLV's in the diet of the subjects. According to ADA (2002) guidelines, minimum 500g/day intake of fruits and vegetables is recommended for therapeutic effects. Several studies have reported the positive effects of types of fiber in glycemic reduction. A study revealed that consuming a high-fiber diet (50 g fiber/day) reduces glycemia in subjects with type 1 diabetes and glycemia, hyperinsulinemia, and lipemia in subjects with type 2 diabetes (Franz MJ et al 2002). Braaten et al (1991) tested responses to glucose and glucose with oat gum and found reductions in glucose and insulin when the nine healthy subjects consumed solutions to which oat gum had been added. Yokoyama et al (1997) compared responses of five subjects to pastas containing wheat or wheat and 12 g beta glucans from barley. Consumption of barley-containing pasta resulted in lower glycemic and insulin indices. An *in-vitro* study also elicited the reduction in post prandial serum glucose reduction by consuming combination of soluble and insoluble dietary fiber (Shiyi Ou et al 2001).

Various mechanisms have been postulated for effect of fiber in glycemic reduction. First, dietary fibers increase the viscosity of small intestine juice and hinder diffusion of glucose; second, they bind glucose and decrease the concentration of available glucose in the small intestine; and, third, they retard α-amylase action through capsuling starch and the enzyme and might directly inhibit the enzyme. All of these decreased the absorption rate of glucose (Vinik and Jenkins 1988; Shiyi Ou et al 2001; Spiller GA 1993; ADA 2007). 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. Intake of type and amount of carbohydrate and fat in the diets may play an important role in improving the glycemic control in diabetic subjects.

Present study elicited that about 28.3% and 13.4% subjects had borderline and poor control over the TC levels. About 36% had borderline to poor serum TG levels. More than 50% of the subjects had borderline LDL and VLDL levels and low HDL levels.

Insulin resistance and hyperglycemia are known to affect each and every lipid and lipoprotein unit (Steven M Haffener 2006; Therese T et al 2012; Anand SS et al 2012). Chronically elevated glycemic levels determine the progression of the disease by exacerbating insulin resistance and causing β-cell exhaustion which in-turn decreases the responsiveness to glucose (Porte 2001). Insulin deficiency in diabetes causes reduced activity of the glycolytic enzymes in addition to five times increased hepatic gluconeogenic enzymes. It results in mediated uptake of glucose from the muscle and the unrestrained production of hepatic glucose. Impaired insulin action on adipose tissues impedes the suppression of free fatty acids (FFA) release. FFA increases in circulation and affects insulin induced uptake and utilization in muscle by interfering with glucose transport/ polyphosphorylation and inhibiting glycogen synthesis in muscle and stimulating gluconeogenesis in the liver (Stratton et al 2000 and Boden 1999). This leads to synthesis of TG while accumulating fatty acids (Anderson 1999).

A large body of epidemiological and pathological data documented a 50-100% contribution of type 2 diabetes to the elevations in plasma VLDL and total triglyceride (Wolffen B et al 2000; Jonker TJ 2013). Duration of exposure to diabetes also have an absolute effect of insulin on hepatic VLDL and triglyceride synthesis. Chronic hyperglycemia exaggerates VLDL triglyceride secretion from liver (Wiggins and Gibbons 1992; Eliasson B 2012; Kumawat M 2012).

The featuring lipid aberrations comprise of elevated TG due to abnormalities in VLDL, LDL and HDL. The increased body of FFA stimulates production of TG and secretion of VLDL and Apolipoprotein B. Decreased activity of insulin dependent enzymes lipoprotein lipase leads to decrease catabolism of chylomicrons, VLDL and IDL. This expanded pool of TG enriched VLDL and increased activity of cholesterol ester transfer protein produce directional change between TG of VLDL, and cholesterol esters of LDL and HDL (Kwiterovich 1998; Nirnajan G et al 2012; cited and adopted from Kumar SN and Mani UV 2010)

A study revealed that decreased blood glucose and disturbed mechanism of blood glucose control, increases glycosylation of apolipoprotein I and apolipoprotein II, which appears to aggravates HDL catabolism. Apolipoprotein are proteins which bind HDL lipids. Apo I and apo II are the major protein components of high-density lipoproteins; and is thought to act primarily in intestinal lipid absorption (Saito H et al 2004). The apolipoprotein promotes cholesterol efflux from tissues to the liver for excretion (Dastani Z et al 2006). There is a rapid clearance before they have circulated enough to acquire sufficient cholesterol to become HDL. Glycosylation of HDL incidentally also impairs its ability to boost cholesterol efflux from cells *in vitro*. The low HDL endures inspite achievement of glycemic control (Taskinen MR 1992; Yoshino G et al 1996; Garvey WT et al 2003). The disease-association data suggest that genetic variation at the apolipoprotein loci contributes to the development of non-insulin-dependent diabetes mellitus (NIDDM) (Kun-san X 1989 and Martins IJ 2006).

In the present study, 11.2% subjects had microvascular and 25% subjects had macrovascular complications of diabetes. There might be a correlation in early glycation products and degree of diabetic complications. Advanced glycated end products and glycoxidation products have been recognized as contributing factors of pathogenesis of secondary complication of diabetes (Kuichi et al 2001; Jakus 2000). At the molecular level, this leads to thickening

of basement membrane which hinders the normal functions of various organs. This altered glycosaminoglycan and lipid metabolism leads to development of secondary complications of diabetes which specifically damages the kidney, eyes, nerves, heart and blood vessels (Iyer U and Mani UV 1996; AHA 2009).

The gut microbiota in the human body's single most important source of microbial stimulation and the newer researches have also reported the relationship between type and number of gut microflora and diabetes. The two most abundant beneficial micro-organisms in the gut are *lactobacillus* and *bifidobacteria* with typical bacterial counts of 10^8-10^9 and 10^9-10^{11} per gram of stool respectively in a healthy human gut (Andrew L et al 2008). In the present study the mean bacterial counts was 10^6 per gram of stool for *LAB* and *bifidobacteria* which are much lower than the healthy individuals.

Studies have also reported lower establishment of beneficial bacteria and higher establishment of harmful bacteria in diabetic and hyperlipidemic subjects (Vaidya R and Sheth M 2011; Parnami S and Sheth M 2010). An investigation provides molecular analysis of the faecal microbiota in type 2 diabetic patients. In order to characterise the gut microbiota in diabetic patients and to assess whether there are changes in the diversity and similarity of gut microbiota in diabetic patients when compared with healthy individuals, bacterial DNAs from 16 type 2 diabetic patients and 12 healthy individuals were extracted from faecal samples and characterized by PCR-denaturing gradient gel electrophoresis (DGGE). By comparing species diversity profiles of two groups, it was observed that there were no significant differences between diabetic and healthy group, although a few diabetic individuals exhibited a remarkable decrease in species profiles. Sequencing results also revealed that bacterial composition of diabetic group was different from that of the healthy group *B. vulgatus and Bifidobacterium* genus were low represented in the microbiota of diabetic group, and the significant decrease was observed for *Bifidobacterium* by real-time PCR. This suggests that

the gut microbiota of diabetes patients has some changes associated with occurrence and development of diabetes (Xiaokang et al 2010).

A study included 36 male adults with 18 diabetic and 18 non-diabetic individuals. Their fecal bacterial composition was investigated by real-time quantitative PCR (qPCR) and the results revealed that the proportions of phylum *Firmicutes* and class *Clostridia* were significantly reduced in the diabetic group compared to the control group ($p<0.05$). Furthermore, the ratios of *Bacteroidetes* to *Firmicutes* as well as the ratios of *Bacteroides-Prevotella* group to *C. coccoides-E. rectale* group correlated positively and significantly with plasma glucose concentration ($p<0.05$). Similarly, class *Beta proteobacteria* was highly enriched in diabetic compared to non-diabetic persons ($p<0.02$) and positively correlated with plasma glucose (Larsen et al 2010). A decrease in *Bifidobacterium* and *Faecalibacterium prausnitzii* has been recently described in humans with obesity and type 2 diabetes (Esteve E 2011). Together these results indicates that type 2 diabetes in humans is associated with compositional changes in intestinal microbiota.

In the present study diabetic subjects with good control of diabetes (A_{1c}, 7-8%) had better establishment of *Bifidobacteria* and *Lactobacillus* ($p<0.05$), when compared with subjects with poor control of diabetes. 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; Louis P, Flint HJ 2009; 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; Dewulf EM 2011).

CONCLUDING REMARKS

Unintermittedly, findings of the present study affirms that intertwine effects of heredity, sedentary lifestyle, dietary components and gut microbiota played a compelling role in the poor glycemic control and high prevalence of type 2 diabetes. It can also be interpreted that glycemic factors and microbial factors co-evolve with each other. In this context, the modification of microbiota induced by prebiotics will constitute new strategies in the treatment or modulation of metabolic disease like diabetes.

PHASE III Effect of Fructooligosaccharide (FOS) supplementation on Glycemic, Lipemic parameters, Gut incretin (GLP-1) and Gut Microflora in diabetic adults

Diabetes Mellitus is a complex metabolic disorder that involves numerous biochemical and dietary abnormalities, a heterogeneous clinical picture and a polygenic heredity component. Poorly controlled blood glucose levels can lead to numerous pathological conditions that ultimately result in long term microvascular and macrovascular complications. Hence it can be concluded that improved glycemic control retards the progression of secondary complications. In today's world many neutraceutical foods are coming up with improved health of diabetics.

Fructooligosaccharide (FOS) is one of the emerging neutraceutical prebiotic which has drawn its attention towards lowering blood glucose ability. This prebiotic is may be responsible for physiological effect on host in term of lowering glycemic and lipemic levels. With these considerations, the present study was designed to observe the effect of FOS supplementation on glycemic control, GLP-1 and gut microbiota in type 2 diabetic subjects.

The results of this phase of the study are presented in following sub sections:

5.3.1 Anthropometric and Biophysical profile of the subjects before and after supplementation trial

5.3.2 Nutrient intake of the diabetic subjects

5.3.3 Glycemic response of the subjects before and after supplementation

5.4.1 Lipemic response of the subjects before and after FOS supplementation

5.4.2 Atherogenic indices of the subjects before and after FOS supplementation

5.5 Gut microflora counts of the subjects before and after FOS supplementation

5.6 Association between gut microflora counts, anthropometric, biophysical and biochemical parameters of the diabetic subjects

5.7 Relationship of Glycemic parameters and GLP-1 with biophysical, biochemical, anthropometric and microbial parameters

5.3.1 Anthropometric and Biophysical profile of the subjects before and after supplementation trial

As shown in Table 5.3.1.1, no changes in the anthropometric measurements were observed in both control and experimental group. 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.

When comparison was made between male and female subjects, the body composition of diabetic subjects showed 2.37% reduction in waist circumference in female experimental group and 1.42% reduction in males. The experimental group showed a shift towards a better health profile with a significant reduction in systolic blood pressure ($p<0.05$, $p<0.01$) in both male and female experimental group and a marginal non-significant reduction in diastolic blood pressure (Table 5.3.1.2).

Table 5.3.1.1: Anthropometric and Biophysical profile of type 2 diabetic subjects before and after FOS supplementation

Parameters		Control (n = 20)	Experimental (n = 40)	*t* test
BMI	Pre	25.7±3.77	25.71±3.80	0.77 NS
(kg/m²)	Post	25.7±3.82	25.62±3.7	0.75 NS
	Paired t test	0.04 NS	0.10 NS	
	% difference	0.00	0.35 ↓	
WC	Pre	95.4±3.94	94.47±3.94	0.80 NS
(cm)	Post	95.3±3.94	94.00±3.94	0.88 NS
	Paired t test	0.00 NS	0.00 NS	
	% difference	0.01	0.04	
WHR	Pre	0.92±0.06	0.91±0.06	0.61 NS
	Post	0.90±0.06	0.90±0.06	0.68 NS
	Paired t test	0.01 NS	0.01 NS	
	% difference	0.01 ↓	0.02 ↓	
Diastolic BP	Pre	82.2±5.41	83.9±7.66	0.20 NS
(mmHg)	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
(mmHg)	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

Table 5.3.1.2: Body composition and biophysical parameters of male and female subjects before and after supplementation

Parameters		Control		Experimental	
		Males (N=12)	Females (N=8)	Males (N=21)	Females (N=19)
BMI (kg/m²)	Pre	25.18±3.67	26.34±3.91	25.19±2.28	26.35±3.96
	Post	25.19±4.05	26.40±4.05	25.10±2.41	26.25±3.92
	Paired t test	0.41 NS	0.41 NS	0.07NS	0.07NS
	% difference	0.03 ↓	0.22 ↓	0.35 ↓	0.37 ↓
WC (cm)	Pre	98.95±4.15	90.88±3.69	98.55±2.75	90.74±8.66
	Post	98.99±4.10	91.02±3.26	98.05±3.62	90.31±8.37
	Paired t test	0.25 NS	2.00NS	0.23NS	1.978NS
	% difference	0.10 ↑	0.32 ↑	2.37 ↓	1.42 ↓
WHR	Pre	0.98±0.08	0.85±0.04	0.98±0.018	0.86±0.38
	Post	0.98±0.07	0.85±0.41	0.97±0.03	0.85±0.03
	Paired t test	0.01NS	0.03NS	0.03NS	0.03NS
	% difference	0.00	0.00	0.01	0.00
Diastolic BP (mmHg)	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	1.10NS	1.00NS	0.447NS	1.03 NS
	% difference	2.09 ↑	0.34 ↑	2.40 ↓	3.00 ↓
Systolic BP (mm/Hg)	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	0.56NS	0.778NS	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

5.3.2 Nutrient intake of type 2 diabetic subjects

Table 5.3.2.1 describes a composite picture of dietary analysis of the subjects. Results revealed that both energy and carbohydrate intake was increased in experimental group as well as in control group after supplementation. The fat intake remained similar in control and experimental group where as protein intake was slightly reduced after supplementation. Fiber intake of

experimental group was significantly higher after supplementation compared to the initial values at baseline ($p<0.001$).

Table 5.3.2.1: Nutrient intake of type 2 diabetic subjects before and after FOS supplementation

Parameters		Control (n = 36)	Experimental (n = 36)	*t* test
Energy (Kcal)	Pre	2107±376.4	2100±344.5	1.90^{NS}
	Post	2165±368.4	2137±352.5	1.28^{NS}
	Paired t test	1.95^{NS}	1.62^{NS}	
	% difference	2.75 ↑	1.76 ↑	
CHO (g)	Pre	300±50.24	298±52.53	1.17^{NS}
	Post	305±50.32	306±49.36	1.59^{NS}
	Paired t test	1.89^{NS}	1.90^{NS}	
	% difference	1.66 ↑	2.68 ↑	
Protein (g)	Pre	43.0±12.4	43.5±11.4	0.17^{NS}
	Post	42.8±11.3	43.0±11.6	0.28^{NS}
	Paired t test	0.77^{NS}	1.67^{NS}	
	% difference	0.46 ↓	1.14 ↓	
Fat (g)	Pre	84.3±16.0	83.5±17.2	1.89^{NS}
	Post	86.3±17.9	85.2±17.4	1.90^{NS}
	Paired t test	1.97^{NS}	1.58^{NS}	
	% difference	2.38 ↑	2.03 ↑	
Fibre (g)	Pre	13.0±5.2	13.2±5.4	0.314^{NS}
	Post	12.6±5.0	24.5±4.6	13.78***
	Paired t test	0.80^{NS}	18.24***	
	% difference	3.0↓	84.6 ↑	

*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

5.3.3 Glycemic response and GLP-1 values of type 2 diabetic subjects before and after FOS supplementation

After supplementation, the glycemic response of the subjects reduced by 23.9%, 21.2% and 9.41% in terms of FBS ($p<0.001$), PP2BS ($p<0.001$) and HbA_{1c} ($p<0.001$). About 52% increase in GLP-1 values were also observed post

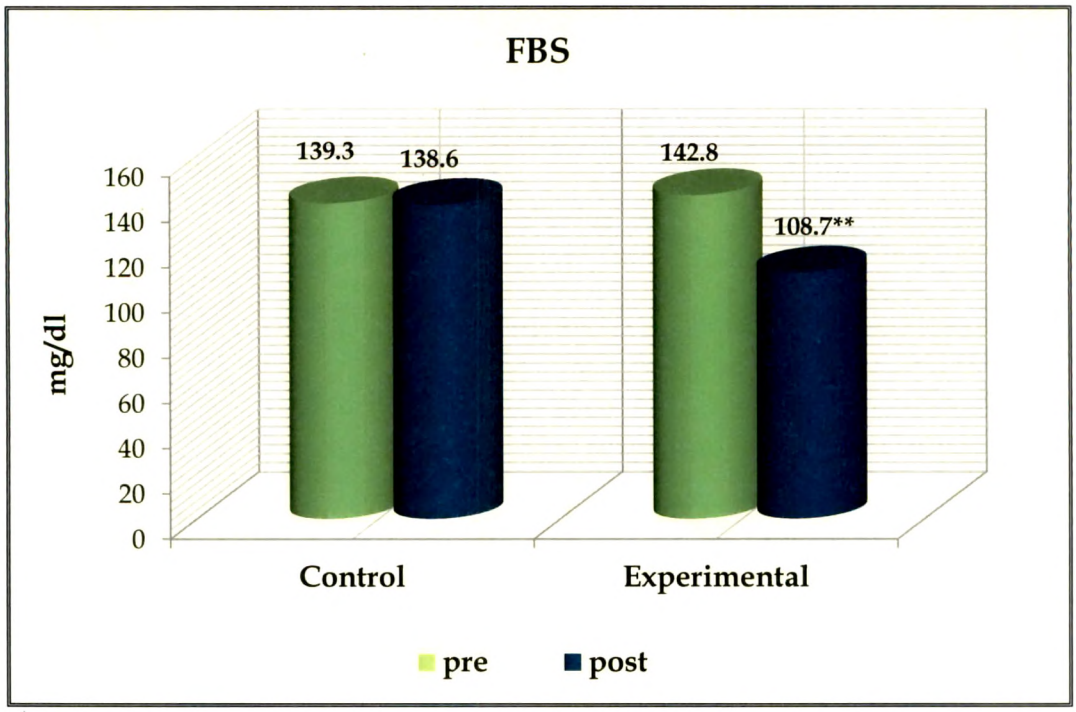
supplementation ($p<0.05$). As shown in Table 5.3.3.1, this reduction was statistically significant (Figure 5.3.3(a),(b)).

Table 5.3.3.1: Glycemic response and GLP-1 values of type 2 diabetic subjects before and after FOS supplementation

Parameters		Control (n = 20)	Experimental (n = 40)	*t* test
FBS	**Pre**	139.3±17.06	142.8±11.54	**0.22** NS
(mg/dl)	**Post**	138.6±11.12	108.7±11.22	**6.07*****
	Paired t test	**0.09** NS	**5.44*****	
	% difference	0.50 ↓	23.9 ↓	
PP2BS	**Pre**	201.5±31.32	207±27.65	**0.30** NS
(mg/dl)	**Post**	194.1±28.20	162.7±24.56	**7.05*****
	Paired t test	**0.61** NS	**5.81*****	
	% difference	3.41 ↓	21.2 ↓	
HbA_{1c}	**Pre**	8.16±0.98	8.5±1.08	**0.30** NS
(%)	**Post**	8.03±0.88	7.7±0.9	**2.17****
	Paired t test	**0.60** NS	**3.88*****	
	% difference	1.59 ↓	9.41 ↓	
GLP-1	**Pre**	0.50±0.74	0.42±0.43	**0.49** NS
(pmol/L)	**Post**	0.52±0.78	0.64±0.22	**2.90***
	Paired t test	**0.20** NS	**2.02***	
	% difference	4.00 ↑	52.38 ↑	

*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

As seen in Table 5.3.3.2, both male and female subjects shows a significant reduction in FBS, PP2BS and HbA_{1c} ($p<0.001$) and increment in GLP-1 values ($p<0.05$).



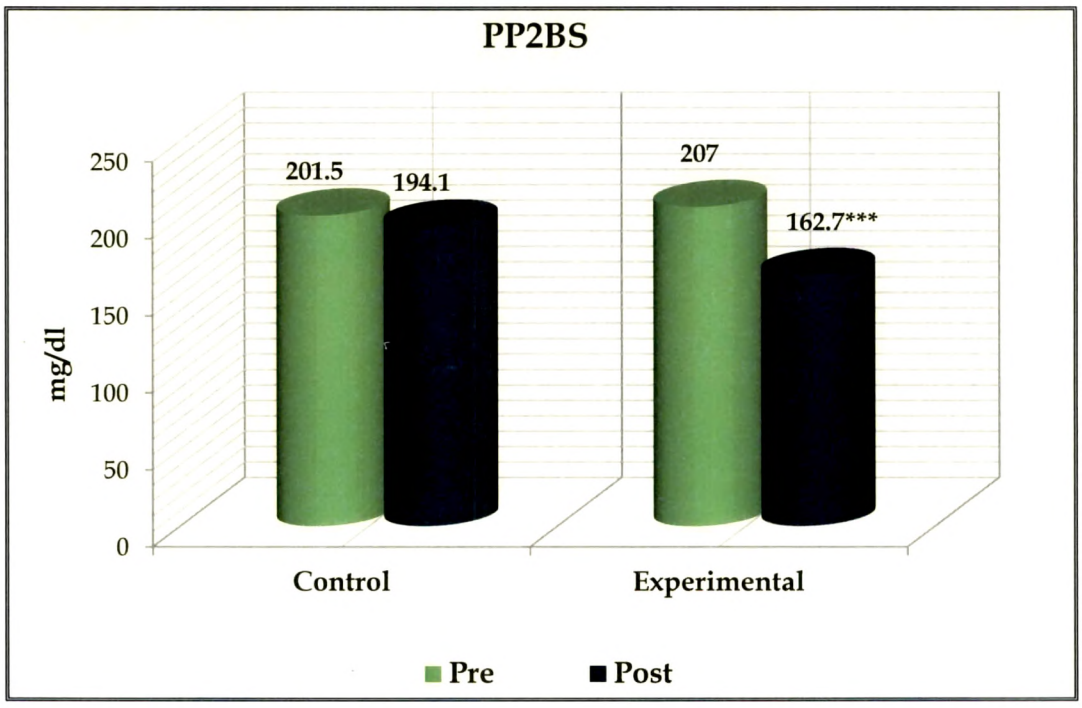
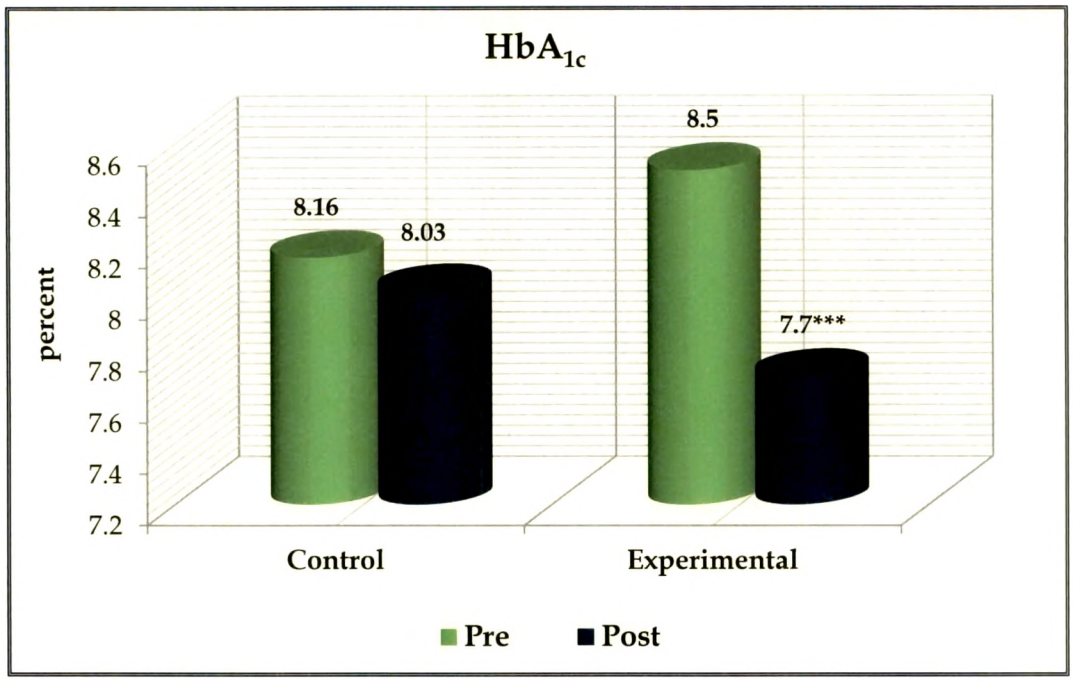


Figure 5.3.3.1(a): Glycemic response of the subjects before and after FOS supplementation



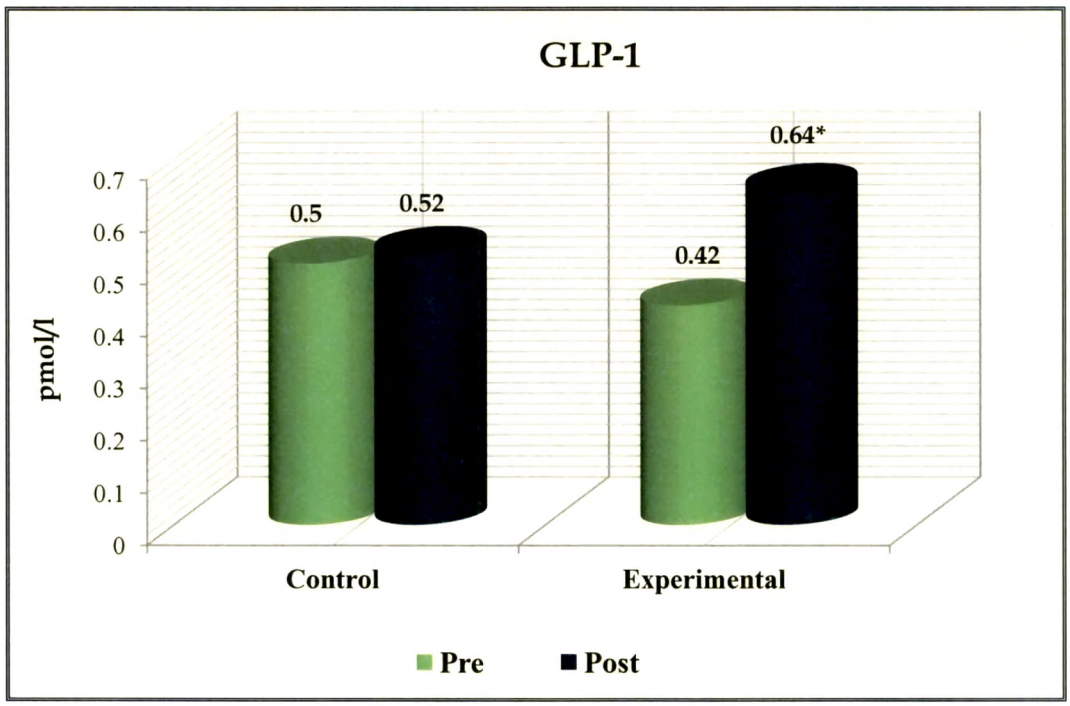


Figure 5.3.3.1(b): Glycemic response and GLP-1 levels of the subjects before and after FOS supplementation

Table 5.3.3.2: Glycemic response and GLP-1 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)
FBS	Pre	137.04±18.20	142.05±27.66	148.45±13.33	135.88±24.24
(mg/dl)	Post	134.86±19.22	143.16±28.41	104.45±15.06	113.88±25.34
	Paired t test	0.21^{NS}	0.11^{NS}	4.90***	2.7**
	% difference	1.59 ↓	0.70 ↑	29.71 ↓	17.03↓
PP2BS	Pre	190.77±25.60	214.77±23.41	210.72±26.94	202.44±29.96
(mg/dl)	Post	186.54±22.99	203.33±30.23	159.63±31.37	166.55±36.40
	Paired t test	0.27^{NS}	0.71^{NS}	4.96***	3.22**
	% difference	2.21 ↓	5.60 ↑	24.28 ↓	17.82↓
HbA_{1c}	Pre	7.94±0.76	8.44±1.17	8.42±0.72	8.79±1.40
(%)	Post	7.80±0.49	8.31±1.15	7.52±0.63	7.95±1.14
	Paired t test	0.68^{NS}	0.33^{NS}	4.35***	2.03*
	% difference	1.76 ↓	1.54 ↓	10.68 ↓	9.55 ↓
GLP-1	Pre	0.81±0.99	0.31±0.17	0.57±0.55	0.28±0.19
(pmol/l)	Post	0.90±1.03	0.34±0.20	0.94±1.03	0.35±0.20
	Paired t test	0.19 ^{NS}	0.27^{NS}	2.45*	2.40*
	% difference	11.1 ↑	9.67 ↑	64.9 ↑	25.00↑

*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 5.3.3.3 reveals that both controlled diabetic ($HbA_{1c}<8$) and uncontrolled diabetic ($HbA_{1c}>8$) had a significant reduction in glycemic response after supplementation. However the percent reduction in PP2BS, HbA_{1c} was higher

in uncontrolled diabetics (HbA_{1c}>8) and percent increase in GLP-1 was also higher in uncontrolled diabetes.

Table 5.3.3.3: Glycemic response and GLP-1 values of the subjects before and after FOS supplementation with initial glycated hemoglobin levels >8 and <8

Parameters		Control		Experimental	
		HbA_{1c}<8 (n=6)	HbA_{1c}≥8 (n = 14)	HbA_{1c}<8 (n=9)	HbA_{1c}≥8 (n=31)
FBS	Pre	123.9±27.02	134.0±27.88	140.23±14.56	144.03±17.08
(mg/dl)	Post	126.1±27.52	130.70±23.07	108.07±12.24	113.81±15.34
	Paired t test	0.27^{NS}	1.52^{NS}	3.59**	4.16***
	% difference	2.43↑	2.98↓	23.09↓	20.83↓
PP2BS	Pre	191.00±29.18	198.64±25.60	194.92±34.47	212.81±14.56
(mg/dl)	Post	187.01±25.52	189.94±24.89	160.84±21.27	163.66±21.56
	Paired t test	0.60^{NS}	0.59^{NS}	2.97**	5.12***
	% difference	2.09↑	4.54 ↓	17.43↓	23.11↓
HbA1c	Pre	7.52±0.33	9.03±0.91	7.66±0.25	9.03±1.05
(%)	Post	7.56±0.34	8.95±0.90	7.05±0.50	8.03±0.89
	Paired t test	0.44^{NS}	0.26^{NS}	3.89***	3.75***
	% difference	0.53↑	0.88↓	7.96 ↓	11.07↓
GLP-1	Pre	0.57±0.36	0.56±1.01	0.76±0.57	0.24±0.15
(pmol/l)	Post	0.65±0.51	0.59±1.01	0.85±0.55	0.43±0.88
	Paired t test	0.41 ^{NS}	0.05^{NS}	2.80*	2.16*
	% difference	14.03↑	5.35 ↑	11.84↑	79.16↑

*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

As can be seen in Table 5.3.3.4, BMI status did not affect values of glycemic parameters. However, subjects with higher BMI (≥23) had higher percent increase in GLP-1 values.

Table 5.3.3.4: Glycemic response of the subjects before and after FOS supplementation with initial BMI levels <23 and ≥23

Parameters		Control		Experimental	
		BMI<23 (n=6)	BMI≥23 (n = 14)	BMI<23 (n=16)	BMI≥23 (n=24)
FBS (mg/dl)	**Pre**	133.8±31.90	138.0±31.08	148.1±32.43	140.2±28.94
	Post	136.0±25.65	139.8±26.84	112.2±26.64	107.00±25.48
	Paired t test	**0.35**^{NS}	**1.04**^{NS}	**4.37*****	**4.47*****
	% difference	2.25 ↑	1.30 ↓	24.32↓	23.57 ↓
PP2BS (mg/dl)	**Pre**	205.3±24.53	202.7±25.6	213.76±38.01	203.7±31.61
	Post	209.1±43.42	205.0±34.5	166.69±36.72	160.8±32.33
	Paired t test	**0.44**^{NS}	**0.34**^{NS}	**3.21****	**4.92*****
	% difference	1.95↑	1.48 ↑	22.06 ↓	21.18 ↓
HbA_{1c} (%)	**Pre**	8.21±0.94	8.25±1.01	8.56±1.02	**8.60**±1.13
	Post	8.24±0.81	8.17±0.99	7.63±0.68	7.75±1.01
	Paired t test	**0.37**^{NS}	**0.26**^{NS}	**2.78***	**2.87****
	% difference	0.36↑	0.96 ↓	10.94↓	9.88 ↓
GLP-1 (pmol/dl)	**Pre**	0.52±0.47	0.58±0.82	0.47±0.52	**0.4**±0.40
	Post	0.56±0.53	0.62±0.86	0.54±0.55	0.69±0.88
	Paired t test	**0.11**^{NS}	0.19^{NS}	**2.03***	2.89*
	% difference	**7.69** ↑	6.89 ↑	14.89 ↑	68.29 ↑

*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 5.3.3.5 shows that subjects with lower (<0.3) and higher (≥0.3) GLP-1 values had almost similar percent reduction in glycemic values.

Table 5.3.3.5: Glycemic response of the subjects before and after FOS supplementation with initial GLP-1 levels <0.3 and ≥0.3

Parameters		Control		Experimental	
		GLP-1 <0.3 (n=6)	GLP-1 ≥0.3 (n = 14)	GLP-1 <0.3 (n=16)	GLP-1 ≥0.3 (n=24)
FBS	Pre	148.8±31.90	142.0±31.08	148.1±32.43	140.2±28.94
(mg/dl)	Post	146.0±25.65	138.8±26.84	112.2±26.64	107.00±25.48
	Paired t test	0.35^{NS}	1.04^{NS}	4.37***	4.47***
	% difference	1.36↓	2.81↓	24.32↓	23.57↓
PP2BS	Pre	215.3±24.53	202.7±25.6	213.76±38.01	203.7±31.61
(mg/dl)	Post	208.1±43.42	201.1±34.5	166.69±36.72	160.8±32.33
	Paired t test	0.44^{NS}	0.34^{NS}	3.21**	4.92***
	% difference	3.25↓	0.49↓	22.06↓	21.18↓
HbA_{1c}	Pre	8.34±0.94	8.25±1.01	8.56±1.02	8.60±1.13
(%)	Post	8.31±0.81	8.17±0.99	7.63±0.68	7.75±1.01
	Paired t test	0.37^{NS}	0.26^{NS}	2.78*	2.87**
	% difference	0.35↓	0.96	10.94↓	9.88↓

*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

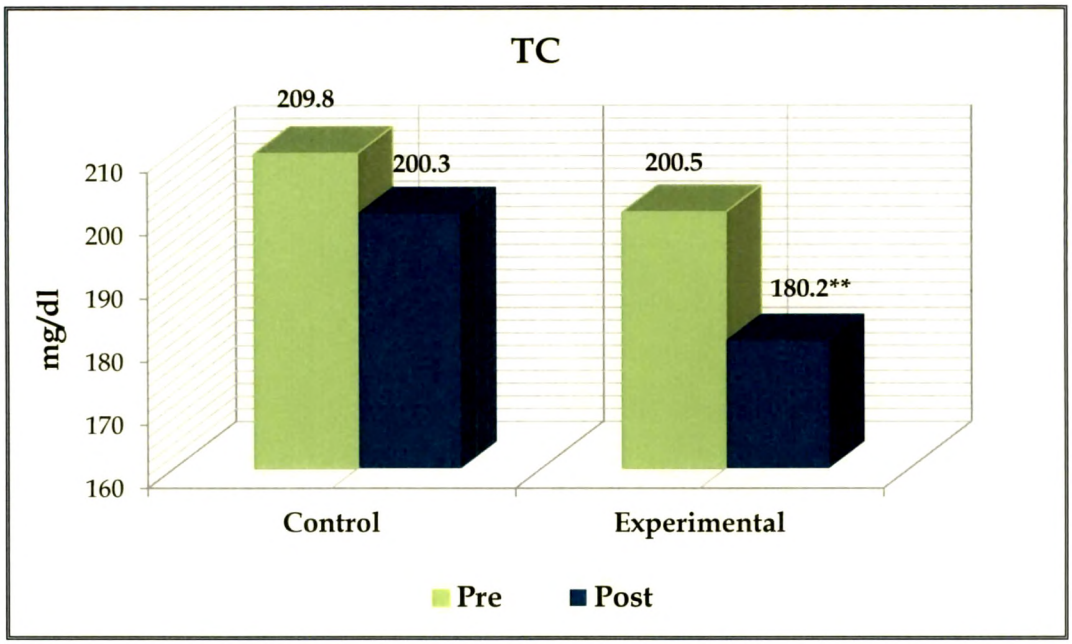
5.3.4 Lipemic response of the subjects before and after FOS supplementation

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 5.3.4.1).

Table 5.3.4.1: Lipemic response 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
(mg/dl)	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
(mg/dl)	Post	139.7±43.03	137.7±30.59	1.69 NS
	Paired t test	0.51 NS	2.70*	
	% difference	4.53↓	5.46 ↓	
LDL	Pre	138.0±28.46	131.6±36.87	0.00 NS
(mg/dl)	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
(mg/dl)	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
(mg/dl)	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	

*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



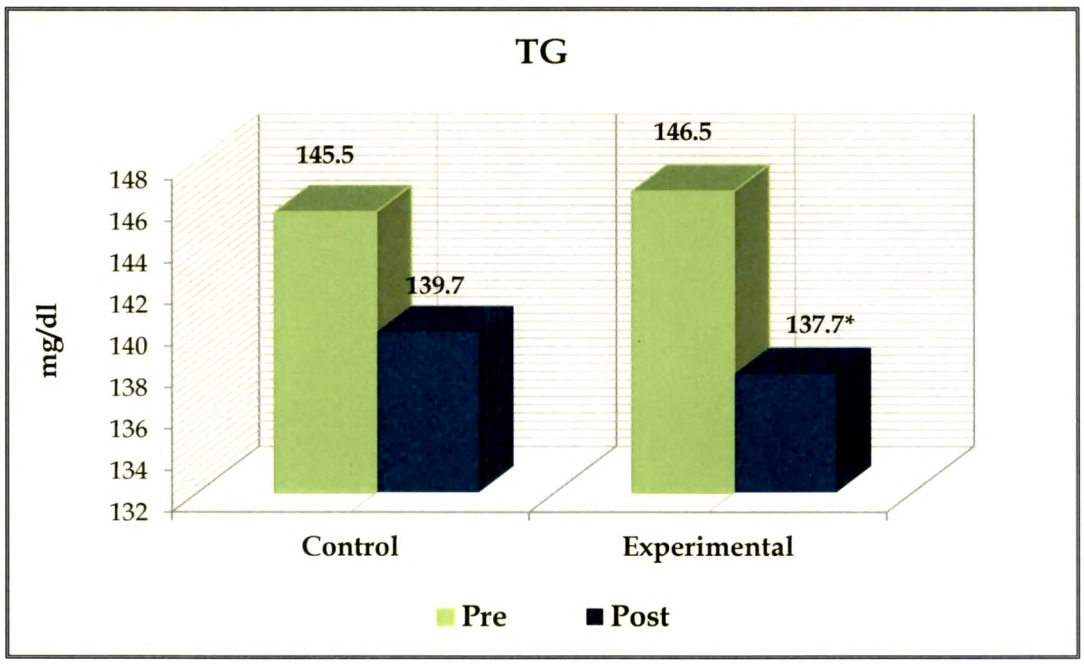


Figure 5.3.4.1: Lipemic response of the subjects before and after FOS supplementation

As can be seen in Table 5.3.4.2, FOS supplementation resulted in 11.9% and 8.13% reduction in TC in males and females respectively. About 10.9% and 11% significant 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 5.3.4.2: Lipemic response 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 (mg/dl)	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 (mg/dl)	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 (mg/dl)	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 (mg/dl)	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 ↑

*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

Effect of FOS supplementation on lipid profile of the subjects in relation to their initial glycated hemoglobin levels

Considering the initial HbA_{1c} levels of the subjects, glycemic status of the subjects did not reveal any significant difference in TG and HDL. However, uncontrolled diabetics (HbA_{1c}>8) had higher percent reduction in TC, LDL and VLDL values by 10.8%, 8.6% and 12.1% respectively (Table 5.3.4.3).

Whereas controlled diabetic subjects showed 10.4% higher percent reduction in TC values by 12.6% (Table 5.3.4.3).

Effect of FOS supplementation on lipid profile of the subjects in relation to their initial BMI values

Table 5.3.4.4 shows a composite picture of lipid profile of the diabetic subjects in relation their BMI status. Subjects with higher BMI (≥23) had higher significant percent reduction in TC and LDL levels by 9.2% and 11.6% respectively. However no changes were observed for TG, HDL and VLDL as per BMI status.

Table 5.3.4.3: Lipemic response of the subjects before and after FOS supplementation with initial glycated hemoglobin levels <8 and ≥8

Parameters		Control		Experimental	
		HbA1c<8 (n=6)	HbA1c≥8 (n = 14)	HbA1c<8 (n = 9)	HbA1c≥8 (n = 31)
TC	Pre	207.47±24.09	204.76±19.26	201.46±34.53	202.11±34.54
(mg/dl)	Post	205.95±34.85	203.29±22.99	180.15±33.33	180.22±29.91
	Paired t test	0.14 NS	0.06 NS	2.48*	2.55*
	% difference	0.96↓	0.49 ↓	10.44↓	10.89 ↓
TG	Pre	138.76±36.84	145.28±32.43	144.25±36.33	146.58±29.32
(mg/dl)	Post	141.08±29.83	144.64±36.35	143.23±34.30	143.21±38.10
	Paired t test	0.13 NS	0.66NS	0.06 NS	0.88 NS
	% difference	2.66↑	0.61 ↓	0.69 ↓	2.28 ↓
LDL	Pre	136.30±33.98	137±18.73	132.76±35.44	131.11±38.10
(mg/dl)	Post	135.95±33.23	136.20±22.42	128.12±25.90	119.82±24.88
	Paired t test	0.13NS	0.22NS	0.47NS	2.08*
	% difference	2.66 ↓	3.23 ↓	3.03 ↓	8.61 ↓
HDL	Pre	42.30±3.22	42.47±2.80	42.23±4.03	41.74±3.25
(mg/dl)	Post	41.95±3.45	42.41±2.98	42.53±3.32	42.25±2.83
	Paired t test	0.35 NS	0.05NS	1.78NS	0.62NS
	% difference	0.83 ↑	0.14 ↑	0.82↑	1.22 ↑
VLDL	Pre	29.86±7.06	28.23±6.44	27.92±7.47	28.97±13.17
(mg/dl)	Post	29.50±5.87	28.76±6.50	26.62±7.54	25.42±5.58
	Paired t test	0.13NS	0.23NS	0.07NS	2.05 *
	% difference	1.18↓	2.09↑	1.15↓	12.11↓

*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 5.3.4.4: Lipemic response of the subjects before and after FOS supplementation with initial BMI levels <23 and ≥23

Parameters		Control		Experimental	
		BMI <23 (n=6)	BMI ≥23 (n = 14)	BMI <23 (n = 9)	BMI ≥23 (n = 31)
TC	Pre	204.20±26.75	208.10±31.75	202.8±35.76	206.40±34.29
(mg/dl)	Post	205.70±30.61	205.60±31.39	185.8±29.44	187.40±31.39
	Paired t test	**0.29** NS	**0.29** NS	**2.01***	**2.45***
	% difference	2.03↑	1.44 ↓	8.86↓	9.22 ↓
TG	Pre	145.20±22.40	146.6±38.40	137.0±39.83	145.2±39.11
(mg/dl)	Post	146.25±24.63	142.3±35.89	131.0±19.89	139.1±34.63
	Paired t test	**0.25** NS	**0.43**NS	**0.47** NS	**0.60** NS
	% difference	0.61 ↑	2.73↓	4.37↓	4.26↓
LDL	Pre	138.1±30.60	140.2±28.08	138.8±27.92	137.0±40.97
(mg/dl)	Post	139.4±31.69	138.7±28.68	130.8±20.31	121.7±27.78
	Paired t test	**0.25**NS	**0.20**NS	**0.81**NS	**2.27***
	% difference	1.01↑	1.42↓	5.79 ↓	11.6↓
HDL	Pre	40.9±2.53	42.9±3.13	41.7±2.71	42.1±3.86
(mg/dl)	Post	41.0±3.17	42.6±3.10	41.6±2.71	42.5±3.08
	Paired t test	0.14 NS	**0.38**NS	**0.07**NS	**0.42**NS
	% difference	0.24↑	0.69↑	0.23↑	0.95↑
VLDL	Pre	25.0±4.1	28.07±7.48	23.0±7.39	27.2±13.22
(mg/dl)	Post	26.4±3.94	27.60±6.95	21.3±3.25	24.5±7.03
	Paired t test	**0.80**NS	**0.24**NS	**0.78**NS	**1.92**
	% difference	5.66↑	1.60↑	7.39↓	9.92↓

*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

5.4.1 Atherogenic indices of the subjects before and after FOS supplementation

As shown in table 5.4.1.1, 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. Comparison between male and female diabetic subjects also

revealed significant changes in TC/HDL, LDL/HDL and non-HDL (Table 5.4.1.2)

Table 5.4.1.1: 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

Table 5.4.1.2: Atherogenic indices of male and female subjects before and after supplementation

Parameters		Control		Experimental	
		Males (N=12)	Females (N=8)	Males (N=21)	Females (N=19)
TC/HDL	Pre	3.85±0.58	4.45±0.59	4.33±1.33	4.78±1.01
	Post	3.87±0.61	4.39±0.58	3.53±0.67	3.80±0.90
	Paired t test	0.12^{NS}	0.31^{NS}	2.52*	3.06**
	% difference	1.29 ↑	1.34 ↓	18.47 ↓	20.5 ↓
LDL/HDL	Pre	2.34±0.67	2.77±0.49	2.47±0.70	3.02±0.79
	Post	2.37±0.72	2.76±0.51	2.35±0.64	2.35±0.41
	Paired t test	0.13^{NS}	0.07^{NS}	2.58*	2.74*
	% difference	1.28 ↑	0.36 ↓	4.85 ↓	12.25 ↓
TG/HDL	Pre	2.75±0.89	3.39±1.36	3.16±0.70	3.28±0.87
	Post	2.76±0.89	3.07±1.01	3.01±0.48	3.15±0.75
	Paired t test	0.03^{NS}	0.79^{NS}	0.92^{NS}	0.89^{NS}
	% difference	0.36 ↑	9.43 ↓	3.22 ↓	3.96 ↓
Non HDL	Pre	156.72±52.6	180.61±39.57	150.45±32.2	166.55±29.4
	Post	137.86±40.4	183.00±40.54	123.59±32.7	147.05±24.4
	Paired t test	1.33 ^{NS}	0.17^{NS}	2.74*	2.15 ^{NS}
	% difference	12.17 ↓	1.60 ↑	18.0 ↓	11.4 ↓

*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

5.5 Gut microflora counts of the subjects before and after FOS supplementation

Table 5.5.1 shows the gut microbial counts of the subjects before and after FOS supplementation. The fecal log counts of *Lactic acid bacteria* and *bifidobacteria* showed a significant increase by 9.3% and 10.9% respectively ($p<0.001$). There was a significant reduction by 4.8% ($p<0.001$) of fecal log counts of Enteric pathogen in diabetic subjects after FOS supplementation (Figure 5.5.1) (Plate 5.5.2, 5.5.3, 5.5.4)

Table 5.5.1: 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 ↓	12.69 ↑	
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

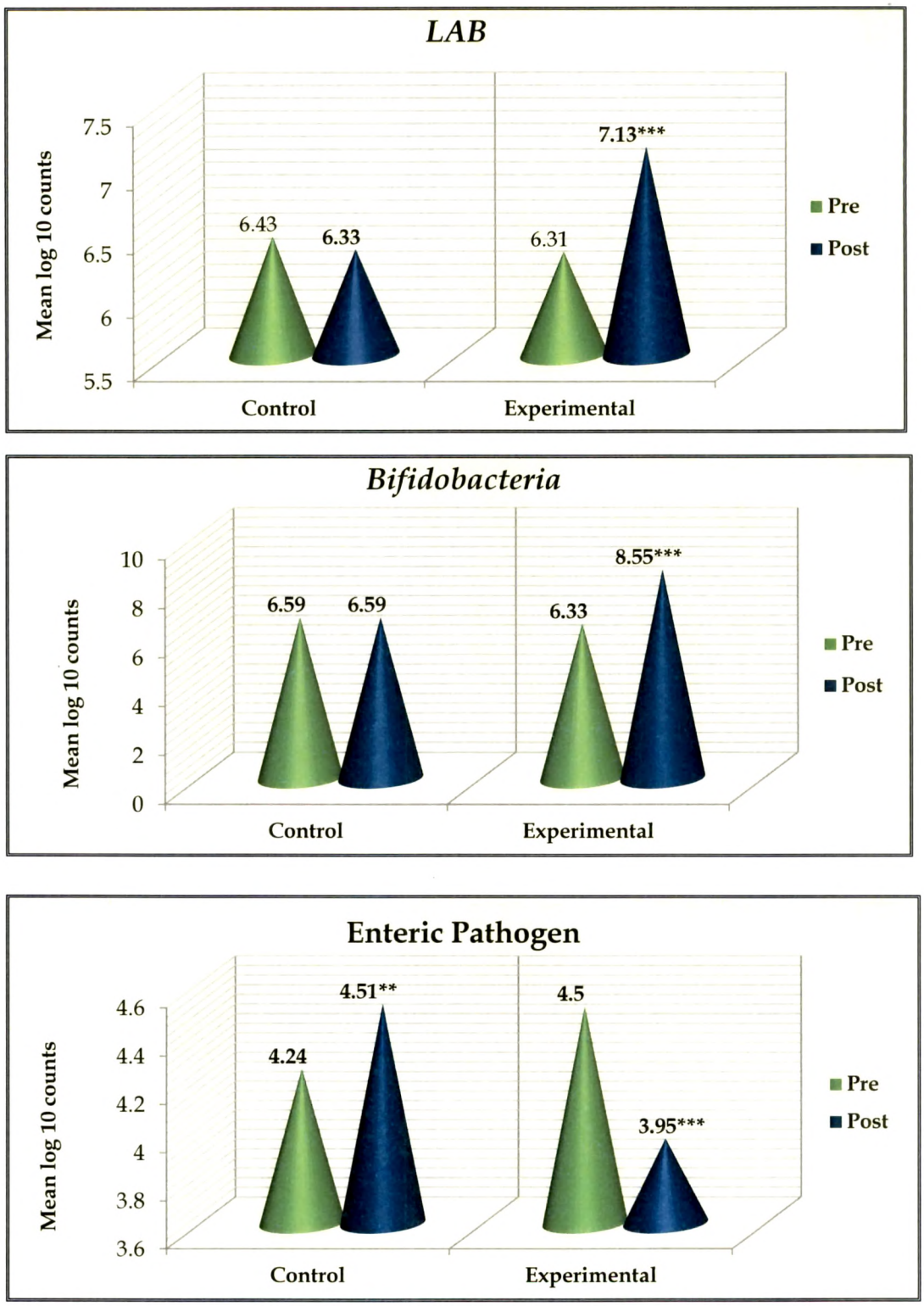


Figure 5.5.1: Microbial colonization of the subjects before and after intervention

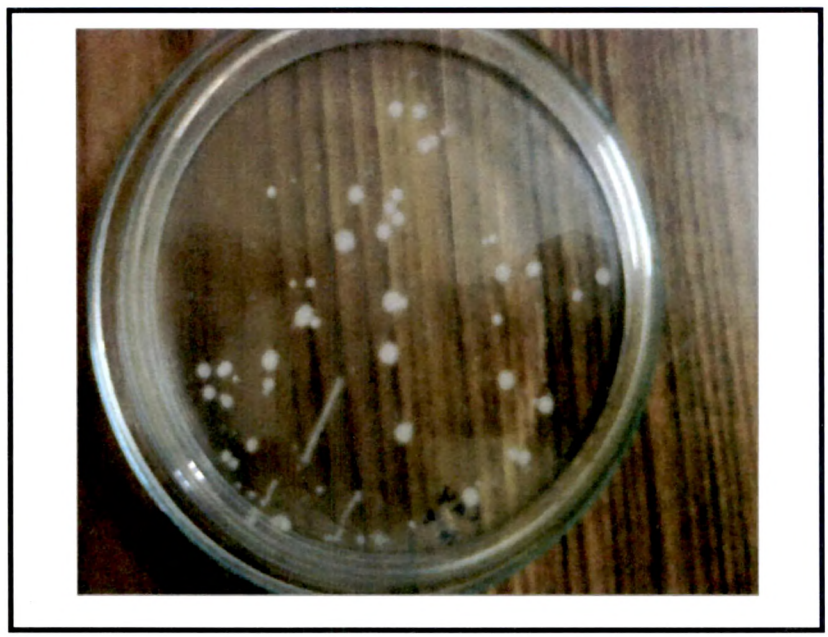


Plate 5.5.2.a: *Bifidobacteria* counts before FOS supplementation

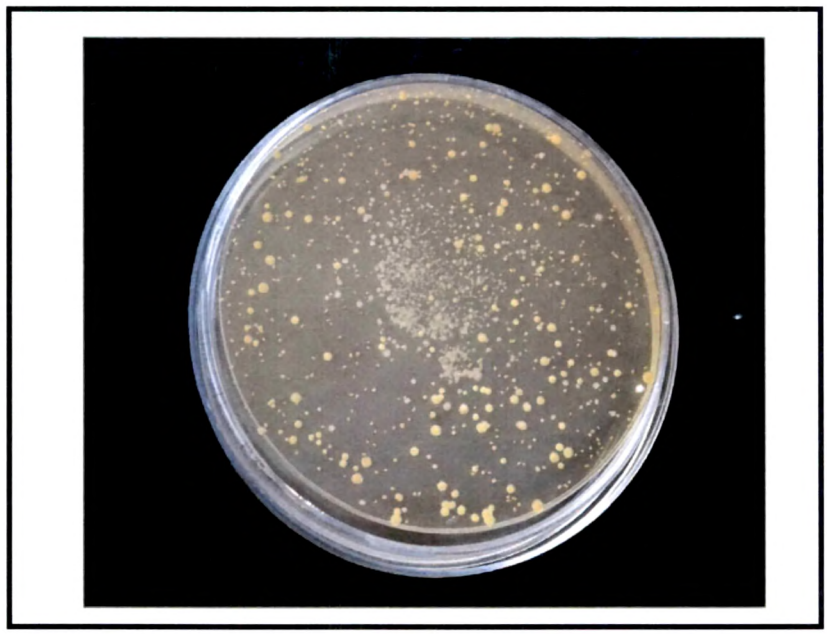


Plate 5.5.2.b: *Bifidobacteria* counts after FOS supplementation

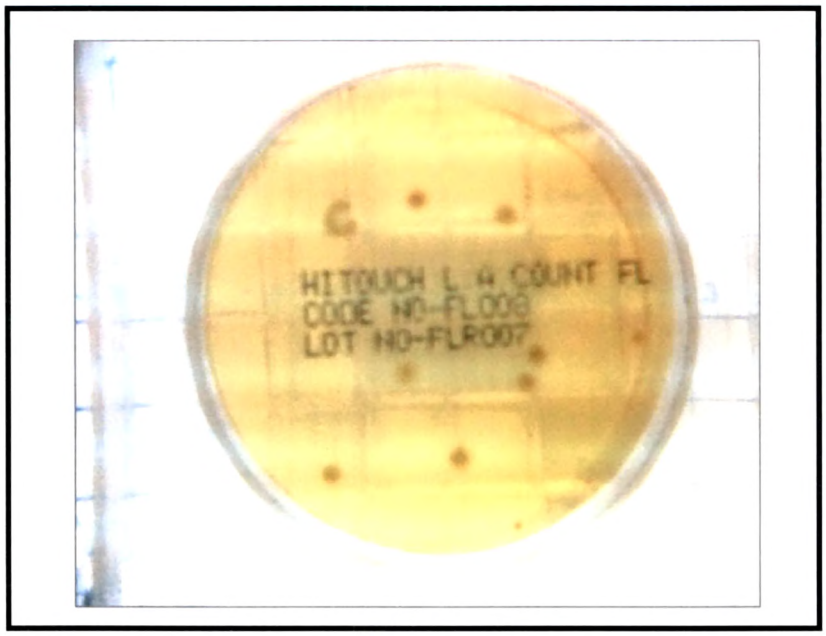


Plate 5.5.3.a: Lactic acid bacteria counts before FOS supplementation

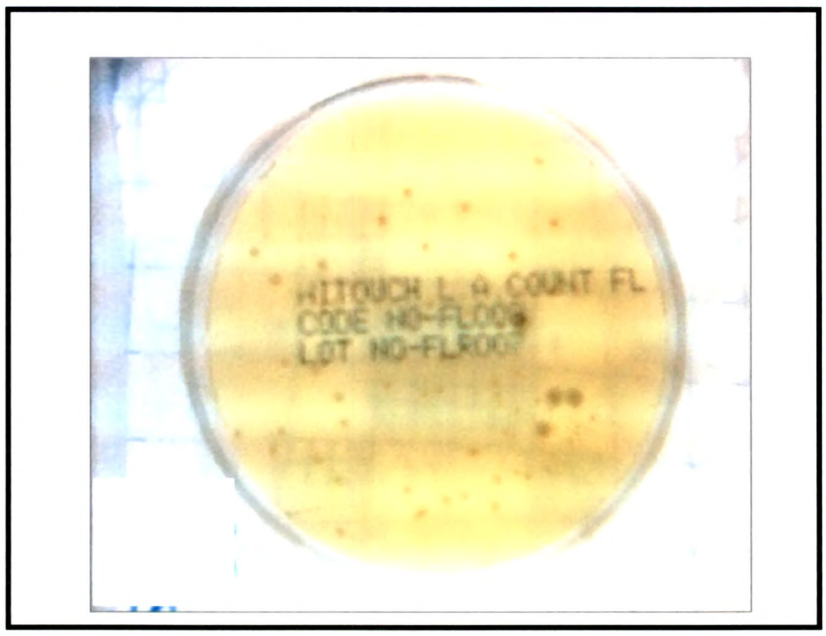


Plate 5.5.3.b: Lactic acid bacteria counts afterFOS supplementation

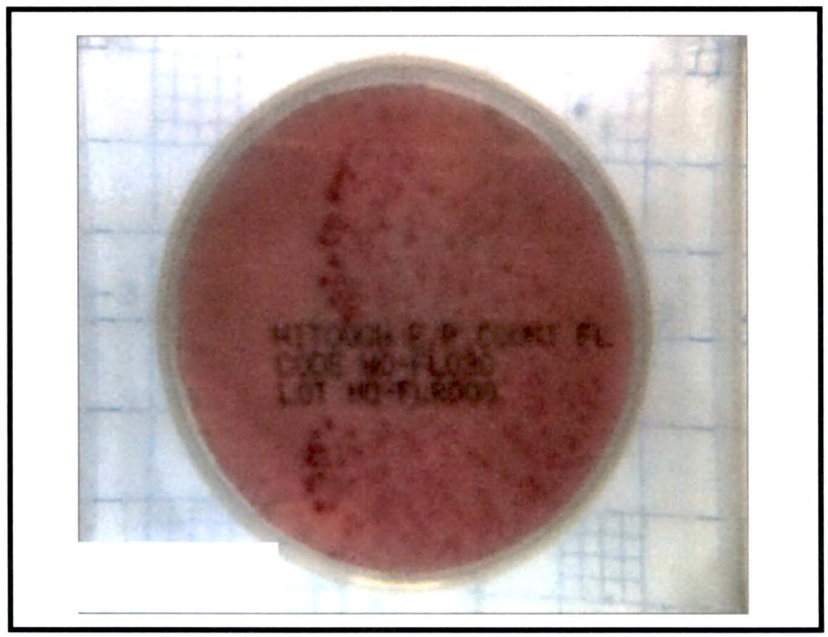


Plate 5.5.4.a: Enteric pathogen counts before FOS supplementation

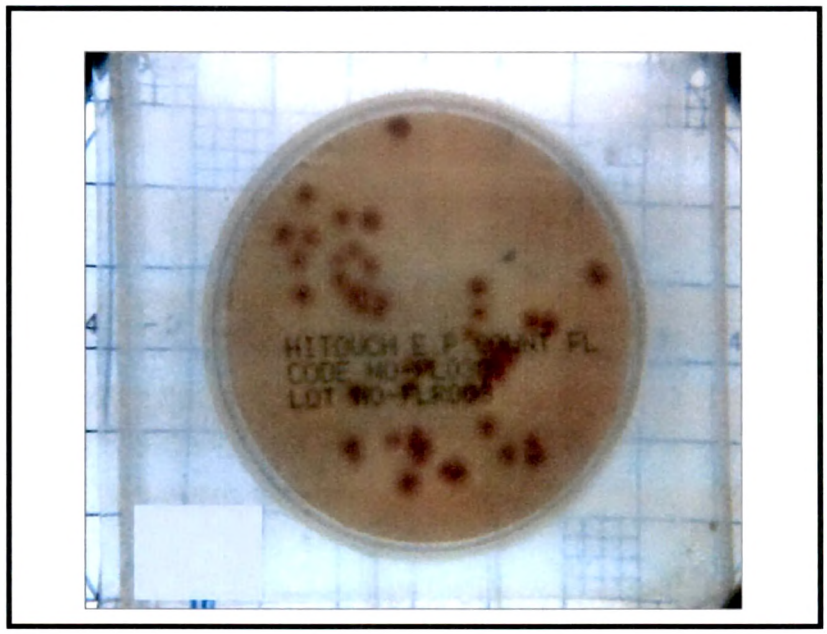


Plate 5.5.4.b: Enteric pathogen counts after FOS supplementation

Table 5.5.2 reveals that, FOS supplementation resulted in significant increase in fecal *lactic acid bacteria* and *bifidobacteria* counts in both males and females after. Enteric pathogen showed higher reduction in female subjects as compared to males. However, there was no significant difference was seen between males and females fecal log counts after supplementation trial.

Table 5.5.2: Gut microbial counts of male and female subjects before and after supplementation

Parameters	Control		Experimental	
	Males (N=12)	Females (N=8)	Males (N=21)	Females (N=19)
Lactic acid bacteria				
Pre	6.56±0.21	6.54±0.23	6.28±0.23	6.39±0.21
Post	6.40±0.22	6.48±0.21	7.15±0.44	7.10±0.55
Paired t test	2.50*	0.76^{NS}	8.11***	5.11***
% difference	2.43 ↓	0.91 ↓	13.85 ↑	11.11 ↑
Bifidobacteria				
Pre	6.40±0.22	6.53±0.62	6.29±0.23	6.39±0.16
Post	6.63±0.88	6.66±0.55	8.40±1.70	8.74±0.12
Paired t test	0.16^{NS}	0.67^{NS}	5.73***	5.84***
% difference	3.59 ↑	1.99 ↑	33.54 ↑	36.77 ↑
Enteric Pathogen				
Pre	4.26±0.27	4.22±0.37	4.52±0.26	4.48±0.26
Post	4.53±0.21	4.48±0.22	3.95±0.80	3.96±0.45
Paired t test	2.53*	2.50*	3.16**	4.11***
% difference	6.33 ↑	6.16 ↑	13.27 ↓	11.60 ↓

*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

Effect of FOS supplementation on gut microflora of the subjects in relation to their initial glycated hemoglobin levels

As shown in Table 5.5.3, both controlled diabetics ($HbA_{1c}<8$) and uncontrolled diabetics ($HbA_{1c}\geq8$) had a significant increment in *Lactic acid bacteria* counts and *bifidobacteria* counts after supplementation ($p<0.01$, $p<0.001$). On the other hand, percent decrease in enteric pathogen counts was significantly higher in

subjects with their initial glycated hemoglobin levels more than 8 (uncontrolled diabetics).

Table 5.5.3: Gut microflora counts (log_{10} cfu/ml) of the subjects before and after FOS supplementation with initial glycated hemoglobin levels < 8 or ≥ 8

Parameters	Control		Experimental	
	HbA_{1c}<8 (n=6)	HbA_{1c}≥8 (n = 14)	HbA_{1c}<8 (n=9)	HbA_{1c}≥8 (n=31)
Lactic acid bacteria				
Pre	6.61±0.18	6.47±0.24	6.38±0.20	6.30±0.23
Post	6.47±0.20	6.39±0.23	7.06±0.40	7.16±0.53
Paired t test	**2.43***	**1.01^{NS}**	**5.44*****	**7.64*****
% difference	2.11↓	1.23↓	10.65↑	13.65↑
Bifidobacteria				
Pre	7.91±1.16	7.02±1.20	6.39±0.22	6.31±0.19
Post	6.42±0.21	6.36±0.17	8.77±0.11	8.45±1.53
Paired t test	**2.01***	**2.00^{NS}**	**7.44*****	**7.16*****
% difference	18.8↓	9.40↓	27.1↑	25.44↑
Enteric Pathogen				
Pre	4.17±0.32	4.34±0.30	4.42±0.31	4.54±0.22
Post	4.48±0.22	4.55±0.20	3.96±0.44	3.95±0.75
Paired t test	**0.89^{NS}**	**0.99^{NS}**	**3.0****	**3.93*****
% difference	7.4↑	4.83↑	10.4↓	12.99↓

*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

5.6 Association amongst anthropometric, biophysical, glycemic, GLP-1 and lipemic parameters of type 2 diabetic subjects

Table 5.6.1 summarizes the association amongst anthropometric, biophysical glycemic, GLP-1 and lipemic parameters. FBS was significant positively correlated with PP2BS (p<0.001), HbA_{1c}, TC, TG and VLDL (p<0.05). PP2BS was found to be positively correlated with SBP, DBP, HbA_{1c} and TC (p<0.05). HbA_{1c} was significant positively correlated with FBS and PP2BS (p<0.05),

GLP-1 showed significant negative correlation with WC ($p<0.01$) and WHR ($p<0.05$). Among glycemic and lipemic parameters GLP-1 was significant negatively correlated with PP2BS ($p<0.01$) and with TC and HDL ($p<0.05$).

Table 5.6.1: Correlation amongst anthropometric, biophysical parameters and glycemic parameters of type 2 diabetic subjects (r value)

Variables	FBS	PP2BS	HbA1c	GLP-1
WC	0.071	0.016	0.026	-0.405**
WHR	0.219	0.178	-0.064	-0.349*
BMI	0.038	0.120	0.187	0.177
SBP	0.195	0.431**	0.168	-0.351*
DBP	0.220	0.390*	0.156	-0.048
FBS	-	0.695**	0.335*	-0.110
PP2BS	0.695**	-	0.406*	-0.606**
HbA1c	0.335*	0.406*	-	-0.112
GLP-1	-0.110	-0.606**	-0.112	-
TC	0.296*	0.280*	0.090	0.339*
TG	0.281*	0.080	0.087	-0.168
LDL-C	-0.010	0.081	0.097	0.180
VLDL-C	0.366*	0.132	0.048	0.165
HDL-C	-0.086	-0.003	-0.186	0.369*

*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

5.6.2 Association between anthropometric and lipemic parameters of type 2 diabetic subjects

As shown in Table 5.6.2, TC revealed a significant positive correlation with DBP, HDL, VLDL-C ($p<0.05$), TG and LDL-C ($p<0.001$). TG was significant positively correlated with TC, VLDL-C, HDL-C ($p<0.01$) and LDL-C ($p<0.05$). LDL-C was significant positively correlated with WC, SBP, TG, VLDL-C ($p<0.05$) and TC ($p<0.01$). However, non-significant negatively correlated with BMI and non-significant positive correlated with WHR and HDL-C.

Table 5.6.2: Correlation amongst anthropometric, biophysical parameters and lipemic parameters of type 2 diabetic subjects (r value)

Variables	TC	TG	LDL-C	HDL-C	VLDL-C
WC	0.190	0.032	0.294*	-0.074	0.020
WHR	0.169	0.062	0.241	-0.108	-0.029
BMI	0.169	0.010	-0.208	0.034	0.086
SBP	0.216	0.084	0.251*	-0.102	-0.049
DBP	0.267*	-0.100	0.092	-0.162	0.046
TC	-	0.621**	0.612**	0.402*	0.580**
TG	0.621**	-	0.443*	0.501**	0.866**
LDL-C	0.612**	0.443*	-	0.295	0.456*
HDL-C	0.402*	0.501**	0.295	-	0.466*
VLDL-C	0.580*	0.866**	0.456*	0.466*	-

*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

5.6.3 Association amongst anthropometric, biophysical, glycemic, GLP-1, lipemic parameters and gut microflora of type 2 diabetic subjects

An association was determined amongst anthropometric, biophysical, glycemic, GLP-1, lipemic parameters and gut microflora as shown in Table 5.6.3. *Lactobacillus* was significant negatively associated with FBS, HbA_{1c} ($p<0.001$) and 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 DBP, PP2, HbA_{1c} ($p<0.01$). *Enteric pathogen* was significant positively associated with DBP, PP2 ($p<0.001$), SBP, FBS and HbA_{1c} ($p<0.05$) and inversely associated with GLP-1.

Table 5.6.3: Correlation amongst anthropometric, biophysical, glycemic, GLP-1, lipemic parameters and gut microflora of type 2 diabetic subjects (r value)

Variables	*Lactobacillus*	*Bifidobacteria*	*Enteric pathogen*
WC	-0.058	-0.059	0.081
WHR	0.153	-0.070	0.083
BMI	-0.076	0.142	0.031
SBP	-0.215	-0.371*	0.328*
DBP	0.098	-0.499**	0.595**
FBS	-0.429**	-0.395*	0.364*
PP2	-0.342*	-0.447**	0.436**
$HbA1_c$	-0.556**	-0.520**	0.342*
GLP-1	0.065	0.258*	-0.355*
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

5.7 Relationship of Glycemic parameters and GLP-1 with biophysical, biochemical, anthropometric and microbial parameters

To further assess the relationship between glycemic parameters and GLP-1 with biophysical, biochemical and 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. In the present analysis as shown in Table 5.7.1 (a, b, c, d) glycemic parameters (FBS, PP2BS) are criterion or dependent variable and biophysical, biochemical, anthropometric and microbial parameters are the predictors or independent variables. In Table 5.7.2 (a, b, c, d) criterion variables are $HbA1_c$ and GLP-1 and independent variables will be same.

Table 5.7.1.a: Model summary of relationship of FBS and PP2BS with biophysical, biochemical, anthropometric and microbial parameters

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.733[a]	.537	.824	9.57835
2	.640[a]	.410	.756	10.6734

a. Predictors: (Constant), DBP, VLDL, WHR, Lacto, BMI, GLP1, Bifidobacteria, LDLC, HDL, SBP, TC, WC, Enteric, TG

FBS (Model 1), PP2BS (Model 2)

Table 5.7.1.a revealed that, adjusted R square value for the above model 1 is .824 which represents that the present model accounts for 82% of variance in the independent variables. Adjusted R value for the model 2 is .756 which represents that the model 2 accounts for 75% variance in the predictor variables.

Table 5.7.1.b represents the ANOVA value, which assesses the overall significance of the model 1 and model 2 as the significant level is <0.05. It implies that both the models are significant.

Table 5.7.1.b: ANOVA of relationship of FBS and PP2BS with biophysical, biochemical, anthropometric and microbial parameters

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	13779.392	14	984.242		
	Residual	11859.008	25	474.360	2.075	.044[a]
	Total	25638.400	39			
2	Regression	17910.623	14	1279.330	1.241	.049[a]
	Residual	25768.877	25	1030.755		
	Total	43679.500	39			

a. Predictors: (Constant), DBP, VLDL, WHR, Lacto, BMI, GLP1, Bifido, LDLC, HDL, SBP, TC, WC, Enteric, TG. b. Dependent Variable: FBS (Model 1), PP2BS (Model 2)

Table 5.7.1.c: Coefficient of relationship of FBS with biophysical, biochemical, anthropometric and microbial parameters

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	5.022	146.061		.034	.973
	TC	.184	.195	.219	.941	.356
	TG	-.274	.287	.326	.954	.349
	LDLC	-.358	.236	-.354	-1.518	.142
	HDL	-.237	1.569	-.027	-.151	.881
	VLDL	2.893	1.327	.702	2.180	.039
	Lacto	8.718	8.981	.167	.971	.341
	Bifido	-10.738	6.101	-.531	-1.760	.031
	Enteric	11.099	12.756	.287	.870	.393
	GLP1	4.730	7.518	.122	.629	.535
	WC	-3.994	2.005	-.414	-1.993	.049
	WHR	4.981	2.912	.358	2.017	.050
	BMI	2.029	1.625	.299	1.248	.224
	SBP	.306	.414	.149	.739	.467
	DBP	.046	1.101	.011	.042	.967

Dependent Variable FBS

Table 5.7.1.d: Coefficient of relationship of PP2BS with biophysical, biochemical, anthropometric and microbial parameters

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-20.022	215.307		-.093	.927
	TC	.201	.288	.184	.700	.490
	TG	-.326	.423	.298	-.771	.048
	LDLC	-.035	.348	-.027	-.102	.920
	HDL	.159	2.312	.014	.069	.946
	VLDL	1.609	1.956	.289	.822	.419
	Lacto	-11.038	13.239	.162	.834	.412
	Bifido	-13.687	8.994	-.640	.410	.005
	Enteric	-8.649	18.803	-.171	-.460	.650
	GLP1	-1.737	11.082	-.034	-.157	.877
	WC	-.190	2.955	-.022	-.064	.949
	WHR	99.534	98.635	.202	1.009	.323
	BMI	-.878	2.396	-.099	-.367	.717
	SBP	.836	.611	.311	1.368	.033
	DBP	-.460	1.623	-.084	-.283	.779

Dependent Variable PP2BS

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. Table 5.7.1.c revealed that reduction in FBS (criterion variable) is affected most by *Bifidobacteria* followed by WC and WHR (predictor variables). Table 5.7.1.d elicited that reduction in PP2BS (criterion variable) is effected maximum by *Bifidobacteria* followed by SBP and TG (predictor variables) (Figure 5.7.1).

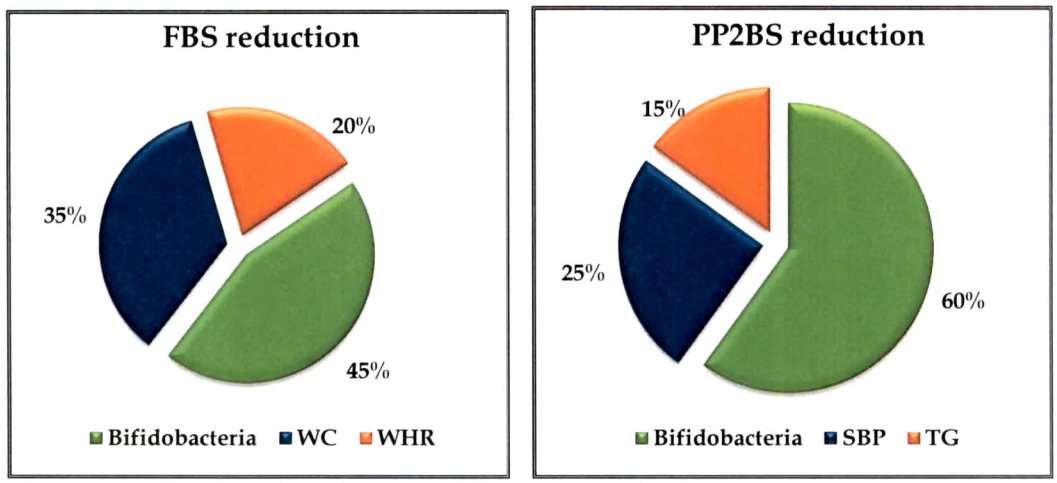


Figure 5.7.1: Percent contribution of the predictor variables in reduction of FBS and PP2BS as per regression analysis

Table 5.7.2.a: Model summary of relationship of HbA1c and GLP-1 with biophysical, biochemical, anthropometric and microbial parameters

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
3	.598[a]	.357	.714	.912
4	.724[b]	.525	.892	.592

$HbA1_c$ (Model 3) a. Predictors: (Constant), DBP, VLDL, WHR, Lacto, BMI, GLP1, Bifido, LDLC, HDL, SBP, TC, WC, Enteric, TG
GLP-1 (Model 4) b. Predictors: (Constant), PP2, HDL, WC, HbA1c, Lacto, SBP, Enteric, VLDL, WHR, TC, DBP, BMI, LDLC, FBS, Bifido, TG

Table 5.7.2.a revealed that, adjusted R square value for model 3 is .714 which represents that the present model accounts for 71% of variance in the independent variables. Adjusted R value for the model 4 is .892 which represents that the model 4 accounts for 89% variance in the predictor variables.

Table 5.7.2.b: ANOVA of relationship of $HbA1_c$ and GLP-1 with biophysical, biochemical, anthropometric and microbial parameters

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.577	14	.827		
	Residual	20.836	25	.833	.992	.048[a]
	Total	32.413	39			
2	Regression	8.884	16	.555		
	Residual	8.049	23	.350	1.587	0.031[b]
	Total	16.933	39			

a. Predictors: (Constant), DBP, VLDL, WHR, Lacto, BMI, GLP1, Bifido, LDLC, HDL, SBP, TC, WC, Enteric, TG. Dependent Variable: $HbA1_c$ (Model 3)
b. Predictors: (Constant), PP2, HDL, WC, HbA1c, Lacto, SBP, Enteric, VLDL, WHR, TC, DBP, BMI, LDLC, FBS, Bifido, TG. Dependent Variables: GLP-1 (Model 4)

Table 5.7.2.b reported the ANOVA value, which assesses the overall significance of the model 3 and model 4 as the significant level is <0.05. It implies that both the models are significant.

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. Table 5.2.1.c revealed that reduction in $HbA1_c$ (criterion variable) is effected most by *Bifidobacteria* followed by WC and *Enteric pathogen* (predictor variables). Table 5.7.2.d elicited that reduction in GLP-1 (criterion variable) is effected maximum by WC. *Bifidobacteria* and *Enteric pathogen* accounted almost similar effect in increase in the plasma GLP-1 values (predictor variables) (Figure 5.7.2).

Table 5.7.2.c: Coefficient of relationship of HbA_{1c} with biophysical, biochemical, anthropometric and microbial parameters

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	13.474	6.122		2.201	.037
	TC	.000	.008	-.007	-.026	.979
	TG	.005	.012	.160	.397	.695
	LDLC	.000	.010	.007	.027	.979
	HDL	-.117	.066	-.379	-1.774	.088
	VLDL	.006	.056	.038	.099	.922
	Lacto	-.184	.376	-.099	-.490	.629
	Bifido	-.609	.256	-.847	-2.383	.005
	Enteric	.851	.535	.619	1.592	.024
	GLP1	.141	.315	.102	.448	.658
	WC	-.153	.084	-.660	-1.817	.081
	WHR	1.132	2.805	.085	.404	.690
	BMI	.176	.068	.228	2.581	.016
	SBP	-.001	.017	-.007	-.030	.976
	DBP	.023	.046	.155	.500	.621

a. Dependent Variable: HbA_{1c}

Table 5.7.2.d: Coefficient of relationship of GLP-1 with biophysical, biochemical, anthropometric and microbial parameters

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	4.201	4.254		.987	.334
	TC	-.005	.005	-.232	-.942	.356
	TG	.001	.008	.047	.126	.901
	LDLC	.008	.007	.308	1.205	.240
	HDL	-.058	.044	-.259	-1.322	.199
	VLDL	-.011	.040	-.108	-.290	.774
	Lacto	-.200	.247	-.149	-.807	.428
	Bifido	.286	.183	.746	1.561	.042
	Enteric	-.742	.346	-.722	-2.146	.043
	WC	-.142	.056	-.848	2.521	.019
	WHR	.008	1.965	.001	.004	.997
	BMI	-.081	.049	-.463	-1.633	.116
	SBP	.007	.012	.138	.628	.536
	DBP	-.047	.029	-.438	-1.651	.112
	HbA1c	.049	.130	.068	.380	.708
	FBS	.006	.007	.233	.867	.395
	PP2	-.003	.005	-.166	-.694	.494

a. Dependent Variable: GLP1

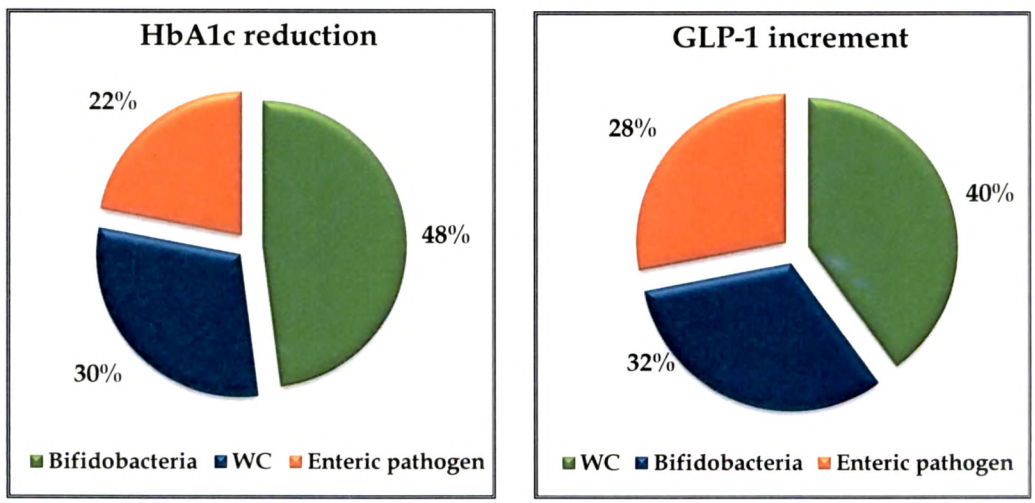


Figure 5.7.2: Percent contribution of the predictor variables in reduction of HbA_{1c} and GLP-1 as per regression analysis

RESULT HIGHLIGHTS

- *Biophysical parameters revealed that there was significant reduction in systolic blood pressure by 3.6% after fructooligosaccharide supplementation for 8 weeks.*
- *The dietary intake showed energy and CHO intake of the subjects was higher than baseline, post supplementation and fiber intake of the supplemented group significantly increased by 84% after supplementation.*
- *The glycemic response reduced by 23.9%, 21.2% and 9.4% in terms of FBS, PP2BS and HbA_{1c} levels after FOS supplementation. The percent reduction was higher in the subjects with their initial HbA_{1c} levels more than 8.*
- *GLP-1 values increased by 52.3% post FOS supplementation and this percent reduction was higher in the subjects with their initial HbA_{1c} more than 8.*
- *The lipid profile showed a significant reduction in TC (10%), TG (5.4%), and LDL (6.8%).*
- *A significant reduction in TC/HDL, LDL/HD and non-HDL-C was observed in diabetic subjects after FOS supplementation by 10.4%, 7.6%, and 13% respectively.*
- *The fecal log counts of lactic acid bacteria and Bifidobacteria showed a significant increase by 12.6% and 34.7% respectively and a significant reduction by 11.1% of fecal log counts of enteric pathogens in diabetic subjects after FOS supplementation.*
- *Lactobacillus was significantly inversely associated with FBS, HbA1c and VLDL and Bifidobacteria was significant negatively associated with DBP, PP2, HbA1c, SBP, FBS, and TC and positively correlated with GLP-1.*

DISCUSSION

In the present study supplementation of 10 g fructooligosaccharides (FOS) for a period of 8 weeks to type 2 diabetic adults brought about a significant reduction in glycemic and lipemic parameters. Consonant with these results there was a significant improvement in GLP-1 levels and significant increment in mean log counts of beneficial gut microbiota with a significant reduction in fecal enteric pathogen counts of the subjects.

Post supplementation fasting blood sugar, post prandial blood sugar and HbA_{1c} reduced by 24%, 21% and 9.4% respectively along with concentration of Glucagon like peptide-1 (GLP-1) increased by 52%.

A combined review of 13 studies conducted on similar lines revealed that serum glucose concentration decreased in the group containing inulin-fructan compared with the corresponding control in four (31%) of the comparisons. The fructan consumption in these four group trials varied in between 8-16 g/day. The trial that showed significant decreases in blood glucose levels used the least amount of FOS (8 g/day). The duration of the trials that showed lowering effects ranged between 14-56 days (Yamashita K et al 1984).

An animal study has shown attenuation of both insulin and glucose concentrations following long-term neosugar (Oligofructose) feeding in rats. The effects were attributed to the actions of oligofructose (OFS) on secretion of the gut hormones glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1(Hata Y et al 1996). Oku et al (1984) revealed 17% and 26% reductions in postprandial glycemia and insulinemia respectively among rats after feeding by a diet containing 10% short-chain fructooligosaccharides (FOS) for 30 days.

Results from a RCT trial found that after inulin consumption for 90 days a significant decrease in glucose concentration and increased insulin and glucagon was noted (Forcheron F and Beylot M 2007). Furthermore it was supported by a study where in trends towards decreased fasting blood

concentration was seen in humans who were consuming fructans (Luo J et al 2000).

Present study revealed 12% and 35% increment in *LAB* and *bifidobacteria* counts respectively and 11% reduction in enteric pathogen counts as a result of daily intake of 10 g FOS for 8 weeks. A similar study conducted in mice showed that FOS increased the counts of *bifidobacteria* (Kok NN et al 1998). A study reported by Handan K and Robert WH (2000) where *Lactic acid bacteria* and *bifidobacteria* were screened for their ability to ferment FOS on MRS agar, the results showed that of 28 strains of LAB and bifidobacteria examined, 12 of 16 *LAB* strains and 7 of 8 *bifidobacteria* strains fermented FOS. Present study is also supported by a study where, in the small intestine digesta, the viable counts of *bifidobacterium* and *lactobacillus* significantly increased in broilers, fed diet with 4g/kg FOS (ZR Xu et al 2003). Moreover, human trials also elicited that oligosaccharides that are fermented by colonic microflora enhanced that growth of beneficial commensal organisms like *bifidobacteria* and *lactobacillus* (Giovanni M et al 2010). A study conducted on 8 human subjects revealed that after eating a diet supplemented with 8 g/d chicory oligofructose for 2 week, the number of *bifidobacteria* in feces had increased significantly ($p<0.01$). At the end of the treatment period, after eating the usual home-cooked diet supplemented with 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) for an additional period of 3 week, the number of *bifidobacteria* in feces were still significantly ($p<0.01$) (Evelyne M and Nicolas G et al 2000).

Present study revealed an inverse association of glycemia i.e. FBS, PP2BS and HbA_{1c} with *LAB*, *bifidobacteria* and positive association with enteric pathogen ($p<0.05$, $p<0.01$). The results also showed a positive association between GLP-1 and *bifidobacteria* ($p<0.05$) as well as a negative association between *LAB* and GLP-1.

Many studies have shown that supplementation of FOS, which lead to increase in SCFA formation in the gut and related beneficial effects on the host metabolism like increased satiety, fat loss, and improvement in insulin

sensitivity and glucose tolerance (Cani PD et al 2006, 2007; Alles MS et al 1996). Gut microbiota synthesize a large array of glycoside hydrolases needed to digest complex dietary polysaccharides to monosaccharaides and SCFA, mainly acetate, propionate and butyrate (Flint HJ 2008). In a study butyrate supplementation to mice on high fat diet prevented diet induced obesity and insulin resistance, by promoting energy expenditure and inducing mitochondrial function (Gao Z 2009). SCFA, mainly butyrate, has been proposed as best nominee to resolve the effect of fermentable carbohydrates on intestinal pro-glucagon expression (Tappenden KA et al 1999). A study reported that when 9% fructan was present in the diet of rats, intestinal butyrate concentration was doubled along with an increase in propionate and acetate (Nynam M 2002). The reason for this butyrogenic effect is currently not very clear. The chemical structure of FOS may be partly involved, although other sugars composed of fructose and glucose do not induce a similar increase in butyrate (Campbell et al 1997). Other factors such as the pH of cecal contents and transit time are probably involved (Gibson and Wang 1994; El Oufir et al 1996).

In a study, the effect of ingestion of acute test meals containing FOS on blood glucose and insulin and C-peptide levels in healthy adults showed a trend for a lower glycemic response and peak insulin levels, following FOS enriched meals (Rumessen JJ et al 1990). These effects were attributed to the actions of FOS on the secretion of the gut hormones like GIP and GLP-1 (Kok NN et al 1998). These hormones are secreted from the small intestine (GIP) and the terminal ileum colon (GLP-1), and contribute towards the secretion of insulin following the meal (Morgan LM 1998). A study also reported that GLP-1 concentration in type-2 diabetic subjects was 4-10 times higher after ingestion of standard meal (Vilsboll T et al 2001). The degradation of GLP-1 incubated with human plasma *in vitro* shows a half-life of around 20 minutes (Deacon CF, Johnsen AH and Holst JJ 1995). These findings are consistent with the present study where a significant inverse correlation existed between PP2BS and GLP-1 values ($p<0.01$).

Another study showed that dietary fiber increased pro-glucagon expression in rat intestinal cells possibly by SCFA action on GPR43 receptors on L-cells (Reimer RA and McBurney MI 1996; Karaki S 2006). In a study, FOS feeding was associated with increased portal glucagon-like peptide (GLP)-1 levels and the number of enteroendocrine L-cells in the proximal colon. High-fat feeding supplemented by FOS in GLP-1 receptor mice showed that the beneficial effects of FOS were mediated by GLP-1 receptor activation (Cani PD et al 2006). Also, FOS in mice increased the number of intestinal *bifidobacteria* and reduced the impact of high-fat diet-induced endotoxaemia and inflammation (Cani PD et al 2006; Kok NN et al 1998). However, in the present study, analysis of SCFA would have been given a better picture of association between GLP-1 and insulin sensitivity.

In the present study almost 52% increase in GLP-1 values was observed following FOS supplementation. In a similar study where FOS feed to animals (mice) was associated with increased portal GLP-1 levels and amount and the number of enteroendocrine L-cells in the proximal colon. A study revealed that supplementation of fermentable dietary fiber for 14 days to diabetic adult dogs is associated with increased ileal proglucagon gene expression increased intestinal glucose transport capacity, increased secretion of GLP-1 and insulin and improved glucose homeostasis (Stefan PM et al 1998).

Study on animal model revealed that high fat feeding supplemented with FOS lead to activation of GLP-1 receptor in mice (Cani PD et al 2006). The physiological actions of GLP-1 reflect the involvement of organs in which GLP-1 receptors are expressed. GLP-1 is the most potent known peptide stimulus for insulin release (Nauck MA et al 1993). In the β cells, GLP-1 stimulates transcription of the pro-insulin gene and promotes insulin bio-synthesis. Furthermore, evidence indicates that GLP-1 stimulates the proliferation and neogenesis of β cells and inhibits their apoptosis (Xu G et al 1999; Farilla L et al 2003; Stoffers DA et al 2000; Brubaker PL and Drucker DJ

2004). Dardevet et al (2005) showed that physiological intra-portal levels of GLP-1 increases hepatic glucose utilization and hepatic glycogen synthesis.

The majority of GLP-1 actions delineated in preclinical experiments have also been demonstrated in human studies. Infusion of GLP-1(7-36) amide into normal human subjects stimulated insulin secretion, reduced glucagon secretion, and significantly reduced blood glucose (Kreymann B et al 1987; Qualmann C et al 1995 and Larsen PJ et al 2001). GLP-1 inhibits gastric acid secretion (O'Halloran et al 1990) and gastric emptying in humans (Wettergren A 1993) and the GLP-1- dependent attenuation of gastric emptying contributes to decreased glycemic excursion and, consequently, reduced glucose-stimulated insulin secretion (Nauck MA et al 1997). Consistent with the importance of gastric emptying and glucagon secretion for glycemic control, GLP-1 also lowers blood glucose in type 1 diabetic subjects (Dupre J et al 1995; Creutzfeld WO 1996). Analogous to studies demonstrating the induction of glucose competence in rodent cells, GLP-1 infusion enhances cell function and insulin secretory dynamics in human subjects with impaired glucose tolerance or type 2 diabetes (Ritzel R et al 2001; Kjems LL et al 2003). GLP-1 may also enhance glucose clearance in humans however; the majority of these actions are likely mediated indirectly through effects on insulin and glucagon (Vella A 2000). Together these results suggests role of fermentable fructooligosaccharides in explaining the reduced glycemic levels and the underlying mechanism behind it.

The present study also demonstrated the effect of FOS supplementation in reduction of lipemic indices such as TC, TG and LDL by 10% 4.9% and 7.8% respectively in the supplemented group.

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 (1999) 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 remained significantly lower 4

weeks after the end of intervention. Total cholesterol and LDL cholesterol levels were reduced by 5% and 7% respectively with inulin. A study conducted by Causey et al (2000) also observed a significant reduction in serum TG in subjects with moderate hyperlipidemia given 18 g/d inulin for 3 weeks. Yamashita et al (1984) 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. 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 et al 1998).

In contrast with the findings of the present study Luo and co-workers (1996), 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 was observed in either treatment or placebo groups, although there was a strong trend for free fatty acid (FFA) concentrations to be reduced on oligofructose. Pedersen et al (1997) 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.

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 or HDL cholesterol in either of the groups over the 8-week intervention. However, serum TG levels were 19% lower after intervention in the inulin treated group (Jackson et al 1999), which are consistent with the findings of the present study.

A number of possible mechanisms have been proposed. 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. 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. (Wolever et al 1989; Demigne et al 1995).

Oligofructose have 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 (Demingne 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 and Kok 1998; Rebecca Wall et al 2012).

One of the proposed mechanisms is also through the type of beneficial gut microbiota which gets colonized in the gut. 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 and Sheth 2010). 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.

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 1985). 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 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* (Gilliland et al 1984) and *Bifidobacterium bifidum* (Rasic et al 1992) have the ability to assimilate cholesterol during in vitro studies, but only in the presence of bile salts and under anaerobic conditions.

Cholesterol binding to bacterial cell walls has also been suggested by a possible mechanism for the hypocholesterolemic effect of probiotics. Honsono and Tono-oka (1995) reported that *Lactococcuslactis* had the highest bonding capacity for cholesterol for a range of bacteria tested. It was speculated that differences in binding of the bacteria were due to chemical and structural properties of their cell walls (Manoj Kumar 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 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 (Namakmura 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 et al 2002). In another study, Lairon et al (2005) suggested

that the reduction of obesity upon consumption of prebiotics such as fiber could prevent the elevation of blood pressure.

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 et al 2005). Past studies have shown promising evidence on the correlation of dietary calcium and hypertension. Allender et al (2004) conducted 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. Diets high in calcium have been found to reduce peripheral vascular resistance and blood pressure leading to a reduced risk of hypertension (Zemel et al 2005). Increased calcium intake in the present study 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 NP 2000; Donkor et al 2005; QaisarM 2012).

There is a significant inverse correlation seen between GLP-1 and systolic blood pressure ($p<0.05$), post supplementation in the present study. There are

two theories as to the mechanisms underlying the antihypertensive effects of incretins. The first theory proposes that the relaxant effect of GLP-1 is entirely mediated via the GLP-1R, which is a type of G-protein. Nystrom et al (2004) have reported that the relaxant effect of GLP-1 is totally inhibited by the specific GLP-1R antagonist exendin (9-39). It has also been shown in patients with T2DM and coronary heart disease that activation of the GLP-1R results in vasodilation of the brachial artery (Nystrom T 2005).

The second 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 JP 2005; Liu Q 2010). A recent study in db/db mice found that treatment of mice for 12 weeks with exendin-4 inhibited the development of hypertension, despite decreased urinary sodium excretion and elevated BP in response to a high-salt load (Hirata 2009).

There was a significant negative correlation observed between GLP-1 and waist circumference (WC) ($p<0.01$), GLP-1 and waist to hip ratio (WHR) ($p<0.05$). Recently, role of gut microflora in development of obesity received a tremendous attention among scientific community and has been considered one of the potential therapeutic targets against obesity. Experimental models connect an altered microbiota composition to the development of obesity in the host through various mechanisms: increased energy harvest from the diet, composition in adipose tissue and liver, modulation of gut peptide YY and GlP-1 secretion, activation of the lipopolysaccharide toll like receptor axis, and modulation of intestinal barrier integrity by GLP-2 (Defronzo RA 2009; Cani PD and Delzenne NM 2011).

In humans, it has been shown that microbial population in the gut are different in obese and lean subjects, and that when the obese subjects lost weight their microbiota reverted back to the observed in a lean person, suggesting that obesity may have a microbial component (Larsen N et al 2010). Inulin type fructans with prebiotic properties have been successfully

used in animal models because they promote *bifidobacteria* in the gut and exert effects that are beneficial to the host. The beneficial effect observed include: a decrease in food intake though modulation of the production of gastrointestinal peptides; a decrease hepatic lipogeneisis and steatosis; an improvement in hepatic insulin resistance. Certain strains of *Lactobacillus* reported to decrease body fat percentage in healthy volunteers were also shown to exert beneficial effect on the onset of diet-induced obesity by reducing the cell size of white adipose tissue (Cani PD et al 2005).

Several underlying mechanisms have been put forward to answer the mechanisms of energy homeostasis by modulation of various gut peptides like GLP-1, YY, oxyntomodulin and hormone ghrelin which are implicated in regulation of body weight and food intake (Druce MR et al 2004; Wynne K et al 2005; Brubaker Pl and Drucker DJ 2004).

First, conventionally raised mice had over 40% more total body fat compared with those raised under germ-free conditions (Bäckhed F et al 2004). Conventionalization of germ-free mice via colonization with cecum-derived distal microbial community resulted in a significant increase in total body fat. The mechanism proposed to underlie this body fat increase was the suppressed intestinal expression of fasting-induced adipose factor (FIAF, or angiopoietin-like protein 4), a circulating lipoprotein lipase (LPL) inhibitor that is also produced by liver and adipocytes. Increased LPL activity leads to triglyceride accumulation in adipocytes. Second, activation of AMP-activated protein kinase (AMPK), a fuel gauge that monitors cellular energy levels, in liver and skeletal muscle, resulting in increased fatty-acid oxidation and insulin sensitivity, and decreased glycogen levels, protected germ-free mice from diet-induced obesity (Bäckhed F et al 2007).

Third, gut microbiota synthesize a large array of glycoside hydrolases needed to digest complex dietary polysaccharides to monosaccharides and SCFAs, mainly acetate, propionate and butyrate (Flint HJ et al 2008). These SCFAs represent an important energy source for our body, and are ligands for G-

proteincoupled receptors (GPCRs): Gpr41 and Gpr43 (Brown AJ et al 2003; Le Poul E et al 2003). These GPCRs are expressed by gut epithelial and enteroendocrine cells, but also by adipocytes (Xiong Y et al 2004). SCFAs, by stimulating Gpr41, where shown to increase leptin expression in mouse-cultured adipocytes (Xiong Y et al 2004). Gpr41 -/- mice, whether conventionally raised with complete gut microbiota or whether reared germ-free, gained less weight than their wild-type (WT) (+/+) littermates, despite similar chow ingestion. Gpr41 deficiency was associated with decreased expression of PYY, increased intestinal transit rate, reduced harvest of energy from the diet and decreased hepatic lipogenesis (Samuel BS et al 2008).

Thus, manipulation of SCFA activation of Gpr41 in the distal gut could serve as a therapeutic target modulating efficiency of caloric extraction from polysaccharide-rich diet. Changes in the relative abundance of the two dominant bacterial phyla, the *Baceteroidetes* and *Firmicutes*, were found in obese insulin resistant ob/ob mice, with a greater *Firmicutes* over *Baceteroidetes* ratio (Ley RE et al 2005), and this 'obese gut microbiome' was associated with an increased capacity for energy harvest (Bäckhed F et al 2004). In the ceca of the obese vs. lean mice, higher contents of the SCFAs acetate and butyrate were found. Transplantation of the obesity-associated gut microbiota to germ-free mice resulted in a greater increase in total body fat gain than colonization with a 'lean microbiota' (Turnbaugh PJ et a, 2006).

Fourth, a microbiota-related signal may link high-fat diet to the development of obesity, insulin resistance and T2DM, by promoting a low-grade pro-inflammatory state (Cani PD and Delzenne NM 2007; Wellen KE and Hotamisligil GS 2005). Bacterial lipopolysaccharide (LPS) derived from Gram-negative intestinal bacteria, that triggers a pro-inflammatory cytokine production when it binds to the CD14/TLR4 complex at the surface of immune and gut epithelial cells, may be an eligible candidate (Wolowczuk I et al 2008; Wright SD et al 1990 and Neal MD et al 2006). High-fat diet feeding resulted in an increase in the Gram-negative/Gram-positive ratio in mice, in

association with elevated plasma LPS levels, body weight and fat mass gain, liver steatosis, diabetes and a pro-inflammatory state (Cani P et al 2007).

Supplementation with inulin-type fructooligosaccharides (FOS), stimulated growth of *Bifidobacterium* spp. and in some cases *Lactobacillus* spp. in humans (Bouhnik Y et al 2004; Kolida S et al 2007 and Macfarlane S et al 2006). These groups of bacteria, often administrated as probiotics, were associated with reduction of intestinal endotoxin levels and improvement of mucosal barrier function (Griffiths EA et al 2004; Wang Z et al 2006 and Cani PD 2007). In a pilot study, 2-week FOS supplementation in healthy subjects (n=10) was shown to promote satiety, reduce hunger and food ingestion (Cani PD 2006). Also, a 12-week FOS supplementation to obese humans resulted in weight loss and meal related suppression of the orexigenic hormone ghrelin (Parnell JA and Reimer RA 2009).

CONCLUDING REMARKS

From the above results and supporting studies it can be concluded that FOS is an attractive therapy for the management of type 2 diabetic subjects. As FOS supplementation resulted in reduction in the blood glucose, blood lipid and GLP-1 as well as colonization of beneficial microorganisms also improved. Indicating an overall metabolic control.