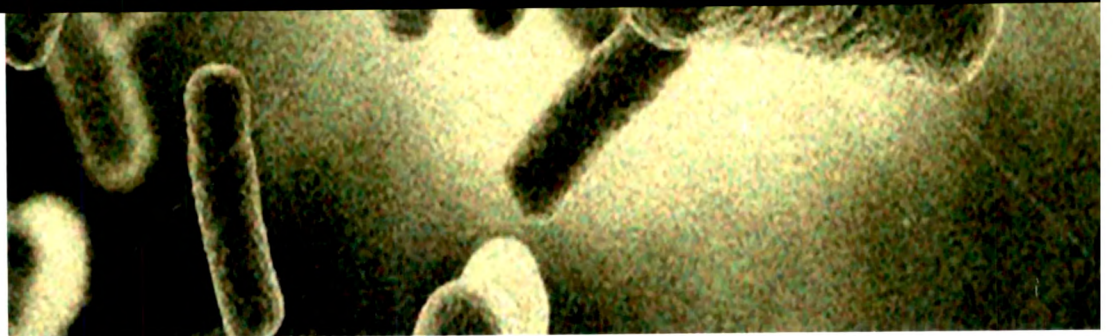




Review of Literature



CHAPTER 2

REVIEW OF LITERATURE

Chronic or non-communicable diseases (NCDs) are the largest cause of deaths in the world. Their health encounter on the society has compelled engrossment in the discovery of health favoring foods. Prebiotics and probiotics are among one of these functional foods, known for their plausible therapeutic effects in several diseased conditions. Amidst of the prebiotic foods, fructooligosaccharide (FOS) has been recognized for their great potential which is becoming apparent as a considerable food element in increasing the bacterial bionomics which might have a concrete role in reducing the burden of chronic diseases.

In perspective to this the present study was designed under the title, **“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”**. This chapter assembles the available review of literature for the study in to following sections.

- 2.1 Global and National prevalence of Non Communicable diseases
- 2.2 Global and National threat of Diabetes Mellitus
- 2.3 Diabetes – Types, Causes, Complications and Treatment
 - a. Pathophysiology of type 2 Diabetes
 - b. Portrayal of endocrine hormones in regulation of blood glucose
 - c. Long term complications of Diabetes mellitus
- 2.4 Preventive strategies in Diabetes mellitus
- 2.5 Overview of Gut environment: A complex ecosystem
- 2.6 Main functions of colonic microbiota in the gut
- 2.7 Probiotics, their types and mechanism of action
- 2.8 Prebiotics and their classification
- 2.9 Evolution of Fructooligosaccharide (FOS) as a prebiotic

- 2.10 Fructooligosaccharide (FOS) and its technological function
- 2.11 Fructooligosaccharide fermentation in the gut
- 2.12 Health implications of FOS
- 2.13 Role of Fructooligosaccharides in glucose homeostasis

2.1 Global and National prevalence of Non Communicable diseases

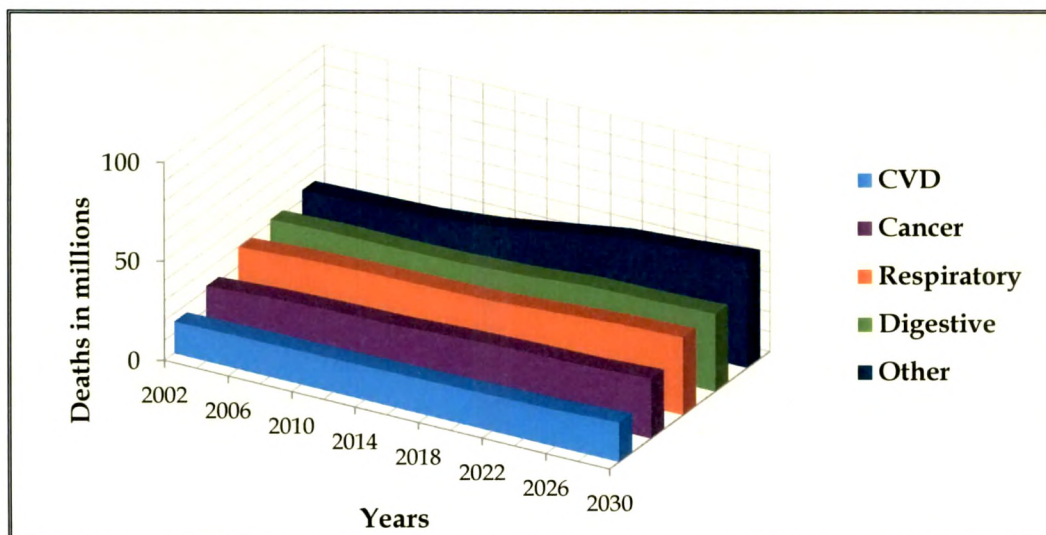
"Today, globalization has led to an unprecedented level of interdependence among countries and interwoven interests. Health factors and social determinants for NCDs exist in every country. Therefore, the prevention and treatment of NCDs is an inevitable option for our common interests and the healthy road for the common development of all mankind"

- WHO 2005

A non-communicable disease, or NCD, is a medical condition or disease which by definition is non-infectious and non-transmissible among people. NCDs may be chronic diseases of long duration and slow progression, or they may result in more rapid death such as some types of sudden stroke. They include autoimmune diseases, heart disease, stroke, type of cancers, asthma, diabetes, chronic kidney disease, osteoporosis, Alzheimer's disease, cataracts, and many more. The etiology of these diseases is usually multifarious (WHO 2005).

According to WHO fact sheet 2011, Non-communicable diseases (NCDs) kill more than 36 million people each year globally. Nearly 80% of NCD deaths (29 million) occur in low- and middle-income countries. More than nine million of all deaths attributed to non-communicable diseases (NCDs) occur before the age of 60; 90% of these "premature" deaths occurred in low- and middle-income countries. Cardiovascular diseases account for most NCD deaths, or 17 million people annually, followed by cancers (7.6 million), respiratory diseases (4.2 million), and diabetes (1.3 million). These four groups of diseases account for around 80% of all NCD deaths.

As shown in figure 2.1, in terms of the number of lives lost due to ill-health, disability and early death (DALYs) globally, NCDs accounted for 35 million of the total disease burden in the year 2002 which increased by 40 million in the year 2010 and predicted to increase by 55 million in 2030.



(Mather and Loncar 2006)

Figure 2.1: Projections of global death due to NCDs from year 2002 to 2030

Non-communicable disease continues to be an important public health problem in India, being responsible for a major proportion of mortality and morbidity. Demographic changes, changes in the lifestyle along with increased rates of urbanization are the major reasons responsible for the tilt towards the non-communicable diseases (Upadhyay PR 2012). In terms of the number of lives lost due to ill-health, disability and early death (DALYs) in India, NCDs (inclusive of injuries) accounts for 62% of the total disease burden while 38% is from communicable diseases, maternal and child health and nutrition (WHO 2004). Total deaths from NCDs are projected to increase by a further 17% over the next 10 years. In India, 53% of all deaths in 2005 were estimated to be due to non-communicable diseases (WHO 2007) (Figure 2.2).

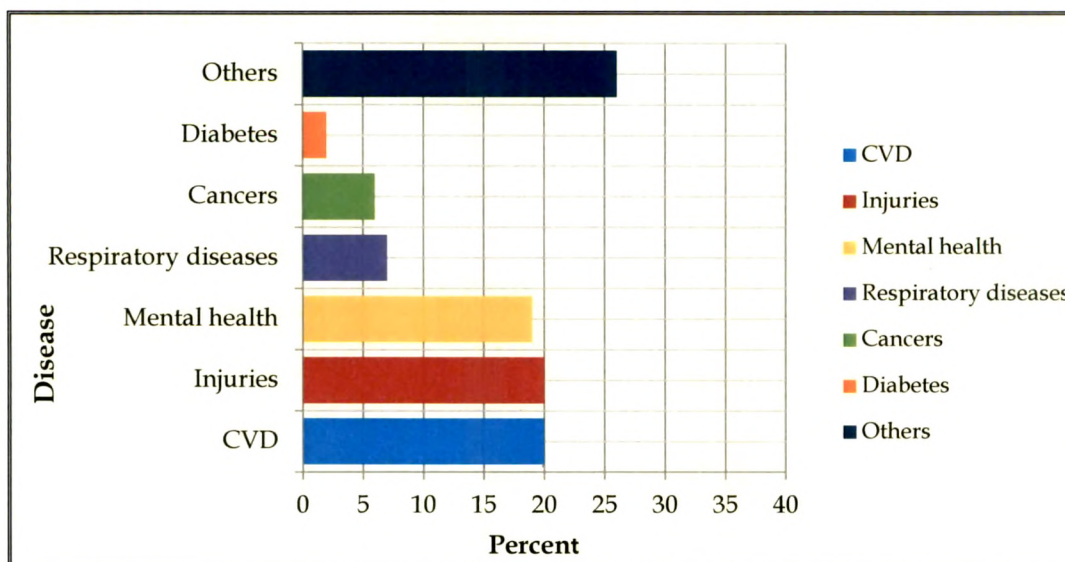


Figure 2.2: Pattern of NCDs related DALY's in India (WHO 2007)

The four leading chronic diseases in India, as measured by their prevalence, are in descending order: cardiovascular diseases (CVDs), diabetes mellitus, chronic obstructive pulmonary disease (COPD) and cancer. All four of these diseases are projected to continue to increase in prevalence in the near future (Taylor DW, 2010). The projected cumulative loss of national income for India due to non-communicable disease mortality for 2006-2015 is expected to be USD237 billion. By 2030, this productivity loss is expected to double to 17.9 million years (WHO 2005).

Cardiovascular diseases (CVD)

Cardiovascular diseases (CVD) have emerged out one of the leading cause of death and misery in India. It is predicted that by the year 2015, CVD would replace infectious diseases as the major killer in India. Indians have been reported to be at highest incidence of CVD amongst all ethnic groups in the world, which is almost 3.5 times higher than others. Moreover, the disease occurs much more prominently in India under 40 years; the risk is 4 times higher as compared to any other country. Triple vessel disease is 2-5 times more common and carries highest morbidity and mortality in India (Reddy

and Yusuf 1998; Sethi 2001). Every year CVD claims 5-6 million lives account for 64% of all deaths reported in India (WHO 2003).

According to WHO 2002, out of all NCDS, CVDs constitute 13%, maximum amongst the other contributors. There are about 1.9 million Rheumatic heart disease (RHD) in India. RHD constitutes 20-30% of hospital admission due to all CVD in India. It has been estimated that there are about 1 million cases of stroke occurring every year in the country. Of these, more than 1,00,000 dies. Based on the studies conducted, it has been estimated that, there are 2.5 million cases of ischemic heart diseases (IHD) in the country (WHO 2002). About 50% of the CVDs related deaths in India occur below the age of seventy as compared to 22% in the western countries. This trend is specifically alarming because its potential impact on one of the Asia's fastest growing economy.

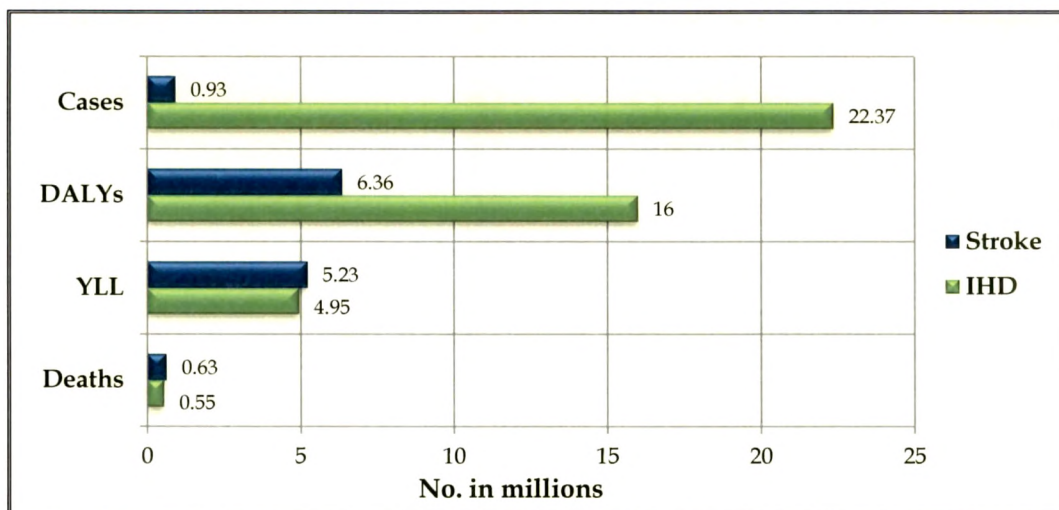


Figure 2.3: Burden of IHD and stroke in India (ICMR-WHO 2004)

Hypertension (HT)

Hypertension (HT) is also an important public health problem in India. Studies on Indian population has reported that in 1950's prevalence of HT was 1.4% to 3.5%, steadily increasing trend of HT prevalence from 5% in 1960's to 12-15% in 1990's. HT now accounts for 65.5 million cases in India

(Hazari MA 2012). The meta-analysis of eight studies carried out in urban areas gives a pooled prevalence rate of HT is 164.18 per thousand (ICMR-WHO 2004).

Cancer

Globally, the burden of new cases of Cancer in 2000 was estimated to be 10.1 million representing a 20% incidence over the previous decade with 53% occurring in the developing world. Similarly 56% of the estimated deaths from cancer occur in the developing world. This is projected by the WHO to dramatically increase to 20 million by 2020 with 70% in the developing world with access to only 5% of the global resources. The number of cases of cancer in 2004 were 8.2 lakh. The number of cancer cases among males is estimated as 3.9 lakh and among females as 4.3 lakh. The number of deaths and number of DALYs attributable to site specific cancers and all cancers in males and females are given in Figure 2.4(a), (b).

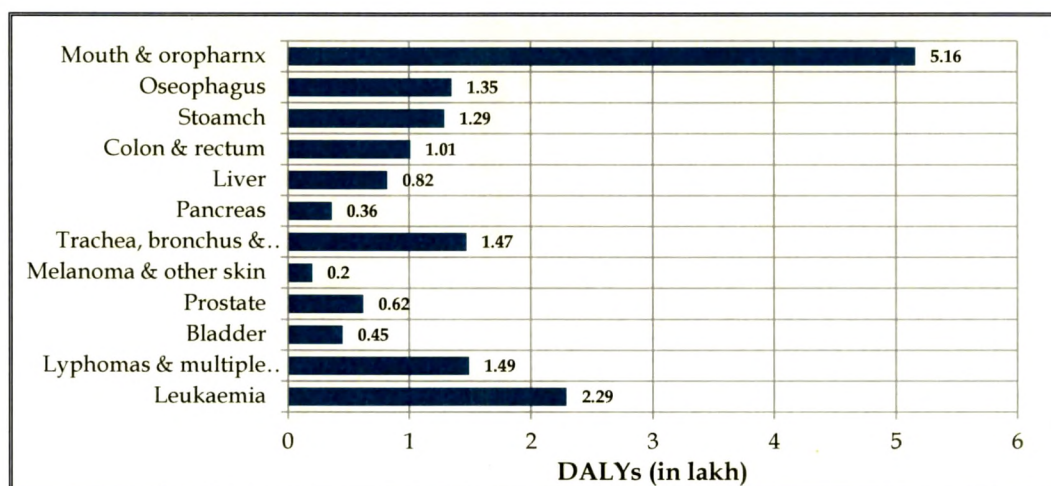


Figure 2.4(a): No. of DALYs (in lakhs) due to cancer in males in India (ICMR WHO 2004)

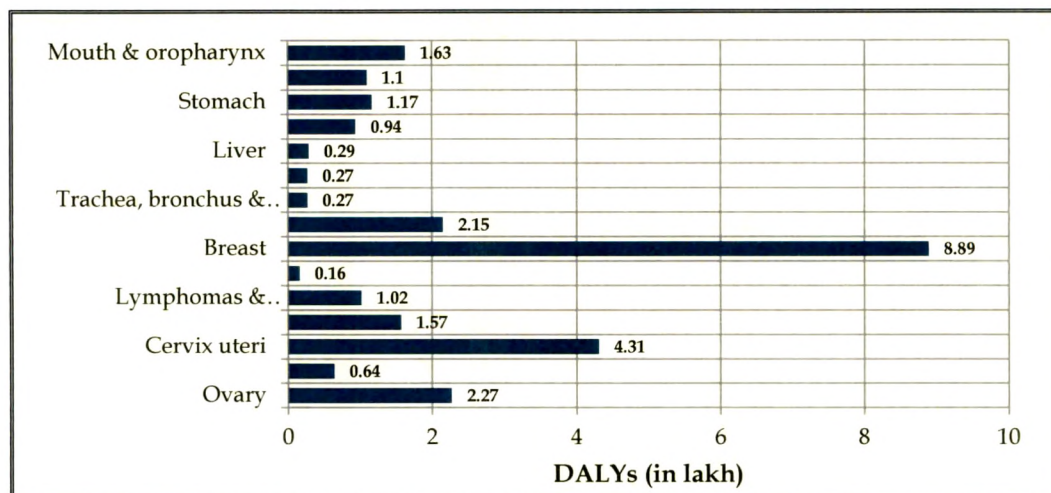


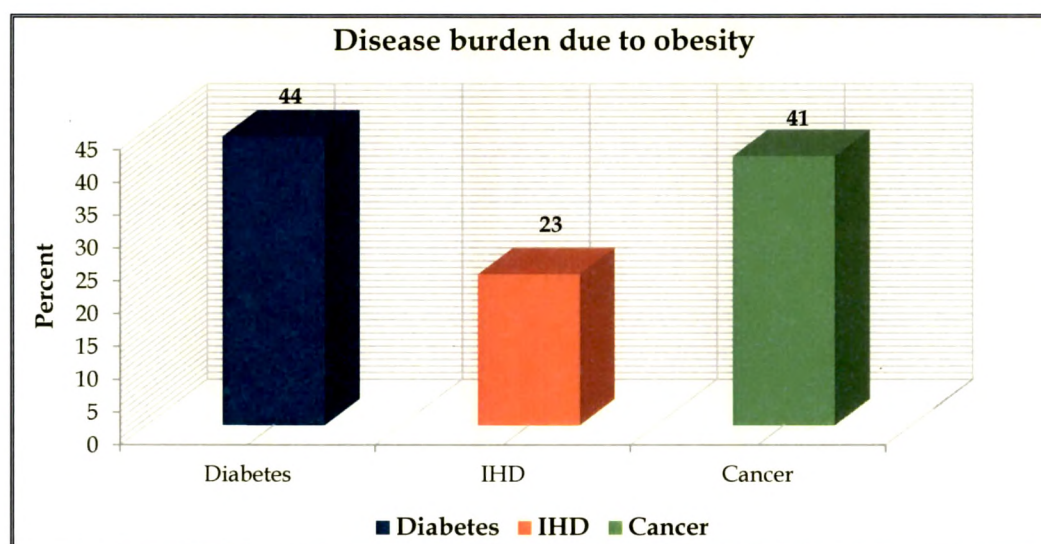
Figure 2.4(b): No. of DALYs (in lakhs) due to cancer in females in India (ICMR WHO 2004)

Obesity

As developing societies like India industrialize and urbanize, and as standards of living continue to rise, weight gain and obesity are beginning to pose a growing threat to the health of the citizens (Shetty P 2002). Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity. Approximate 1.5 billion adults, 20 years and older, are overweight. Of these 1.5 billion overweight adults, over 200 million men and nearly 300 million women are obese. Overall, more than one in ten of the world's adult population is obese (Visscher and Seildell 2010). There are several reports from various parts of India, mostly urban, which provide some insight into the problem. A study in Bombay revealed that the prevalence of obesity among young adult males varied from 10.7% to 53.1% (Dhurandhar NV and Kulkarni PR 1992), while another from urban Delhi, among a large representative sample of 13,414 adults (aged 25–64 years), showed an overall prevalence of 27.8% (Gopinath N et al 1994). The latter study indicated obesity was higher in females (33.4% vs. 21.3% among males) and that obesity

was associated with hypercholesterolaemia, hyperlipidaemia and lower levels of physical activity.

A report from the Kashmir of adults over 40 years old, studied by multistage sampling, showed the obesity prevalence to be 15.0%; females having a prevalence of 23.7% compared with 7.0% among males (Zargar AM et al 2000). A report from the Nutrition Foundation of India suggests that the prevalence of obesity varies with socio-economic status in urban India, with those in the upper strata having higher prevalence rates (32.2% among males, 50% among females) than the middle classes (16.2% males, 30.3% females), followed by the lower socio-economic groups (7.0% males, 27.8% females) and the poor in urban slums with the lowest (1.0% males, 4.0% females) (Gopalan C 1998). The National Family Health Survey showed a prevalence rate 30% female and 22% male to be obese (NFHS III 2005-06).



(Visscher and Seidell 2010)

Figure 2.5: Percent burden of disease caused by Obesity

2.2 Global and National threat of Diabetes Mellitus

"It is now recognized that it is the low- and middle-income countries that face the greatest burden of diabetes. However, many governments and public health planners still remain largely unaware of the current magnitude or, more importantly, the future potential for increases in diabetes and its serious complications in their own countries"

-IDF 2010

Diabetes- Global Scenario

Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century (Zimmet P 2000). The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide (Amos A et al 1997; King H et al 1998). Pronounced changes in the human environment, and in human behavior and lifestyle, have accompanied globalization, and these have resulted in escalating rates of diabetes. Globally, 366 million people had diabetes in 2011, by 2030 this will rise to 552 million. The greatest number of people with diabetes is between 40 to 59 years of age. Approximately 183 million people (50%) with diabetes are undiagnosed. Diabetes caused 4.6 million deaths in the year 2011. Diabetes caused at least USD 465 billion dollars in healthcare expenditures in 2011; 11% of total healthcare expenditures in adults (20-79 years) (IDF 2011). Table 2.1 shows 10 countries with the largest numbers of people with diabetes. The overall total predicted increase in numbers with diabetes from 2010 to 2030 is 54%, at an annual growth of 2.2%, which is nearly twice the annual growth of the total world adult population. Thirty-six percent of the anticipated absolute global increase of 154 million people with diabetes is projected to occur in India and China alone. These estimates suggest that in 2010 there were 285 million people worldwide with diabetes, with considerable disparity between populations and regions (Wild et al 2009).

Table 2.1: Top ten countries for number of persons with Diabetes

Rank	Country	Year 2000 People with T2DM (million)	Rank	Country	Year 2030 People with T2DM (million)
1	India	31.7	1	India	79.4
2	China	20.8	2	China	42.3
3	USA	17.7	3	USA	30.3
4	Indonesia	8.4	4	Indonesia	21.3
5	Japan	6.8	5	Pakistan	13.9
6	Pakistan	5.2	6	Brazil	11.3
7	Russia Fed	4.6	7	Bangladesh	11.1
8	Brazil	4.6	8	Japan	8.9
9	Italy	4.3	9	Philippines	7.8
10	Ukraine	3.2	10	Egypt	6.7

(Wild et al 2009)

Out of total pandemic of diabetes in world, India, China and USA ranks at the top three positions respectively.

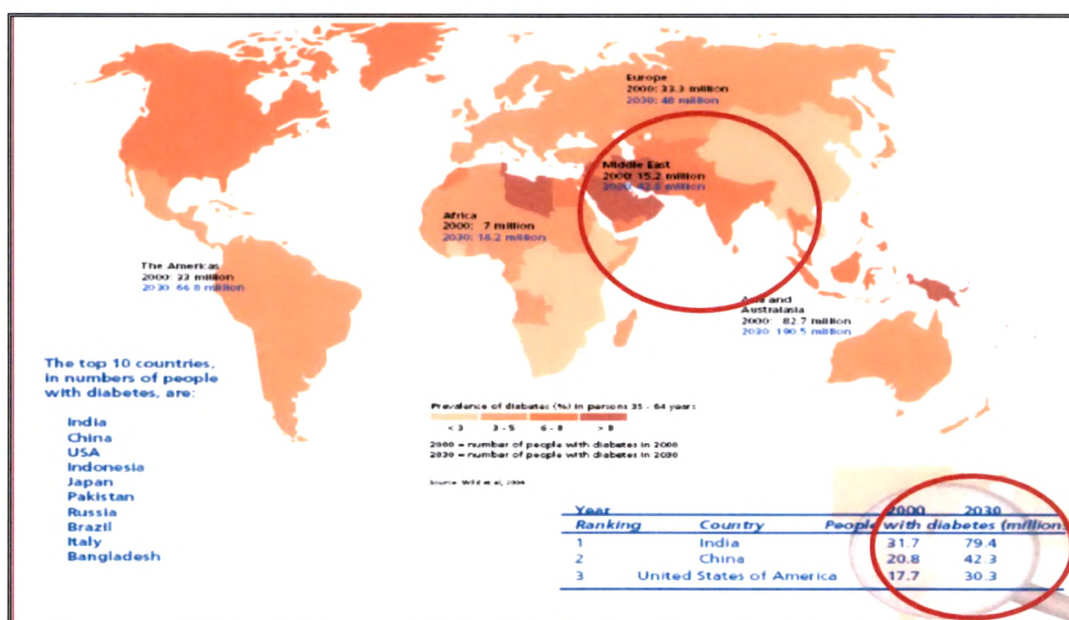


Figure 2.6: Global prevalence of Diabetes (WHO 2008)

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) collectively called as pre-diabetic states, have a high risk of conversion to diabetes. Several studies have shown that these pre-diabetic states are also high risk stages for cardiovascular disease (Novoa FJ et al 2005; NCD risk

surveillance 2006). Hence data on IGT and IFG are also urgently needed as they are indicators of future diabetes prevalence and burden on the nation.

Table 2.2 (a) summarizes the prevalence of diabetes and impaired glucose tolerance (IGT) in the year 2011 and projections for the year 2030.

Table 2.2 (a): Prevalence of diabetes and IGT at a glance

Diabetes at a glance	2011	2030
Total world population (billions)	7.0	8.3
Adult population (20-79 years, billions)	4.4	5.6
DIABETES AND IGT (20-79 YEARS)		
<i>Diabetes</i>		
Global prevalence (%)	8.3	9.9
Comparative prevalence (%)	8.5	8.9
Number of people with diabetes (millions)	366	552
<i>IGT</i>		
Global prevalence (%)	6.4	7.1
Comparative prevalence (%)	6.5	6.7
Number of people with IGT (millions)	280	398

IDF, 2011

Recent reports of International Diabetic Federation (IDF, 2012) on Diabetes and its prevalence (5th edition)

More than 371 million people have diabetes worldwide with highest number of diabetics in China (92.3 million) followed by India (63 million). Almost half of people worldwide are undiagnosed for diabetes (187 million cases). Four out of five people with diabetes live in low and middle income countries. Half of the people who die from diabetes are under the age of 60. In the year 2012, 4.8 million people died from diabetes and 471 billion USD were spent due to diabetes [Figure 2.7(a)-(d)].

More than 371 million people have diabetes

TOP 10 COUNTRIES/ TERRITORIES FROM PEOPLE WITH DIABETES (20-79 YEARS)

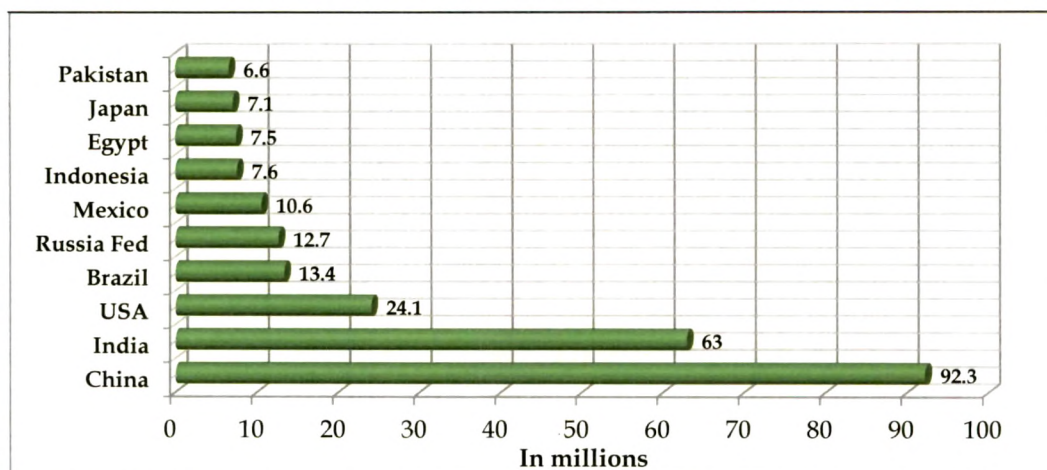
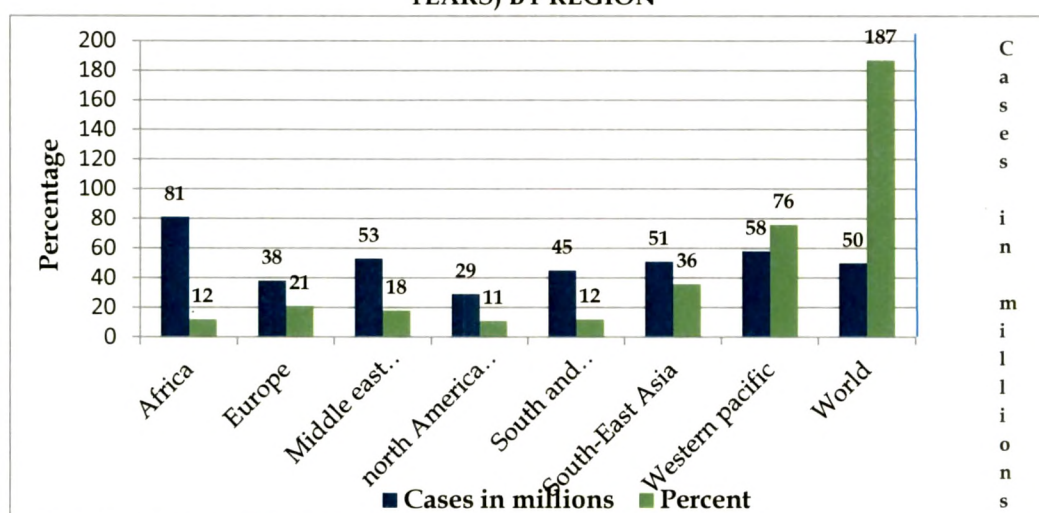


Figure 2.7(a): Top ten countries with diabetes globally

Half of people with diabetes don't know they have it

UNDIAGNOSED PERCENTAGE AND UNDIABNOSED CASES OF DIABETES (20-79 YEARS) BY REGION



4 out of 5 people with diabetes live in low and middle income countries

Figure 2.7(b): Undiagnosed percentage and cases of diabetes

PREVALENCE (%) ESTIMATES OF DIABETES (20-79 YEARS) BY INCOME GROUP AND AGE

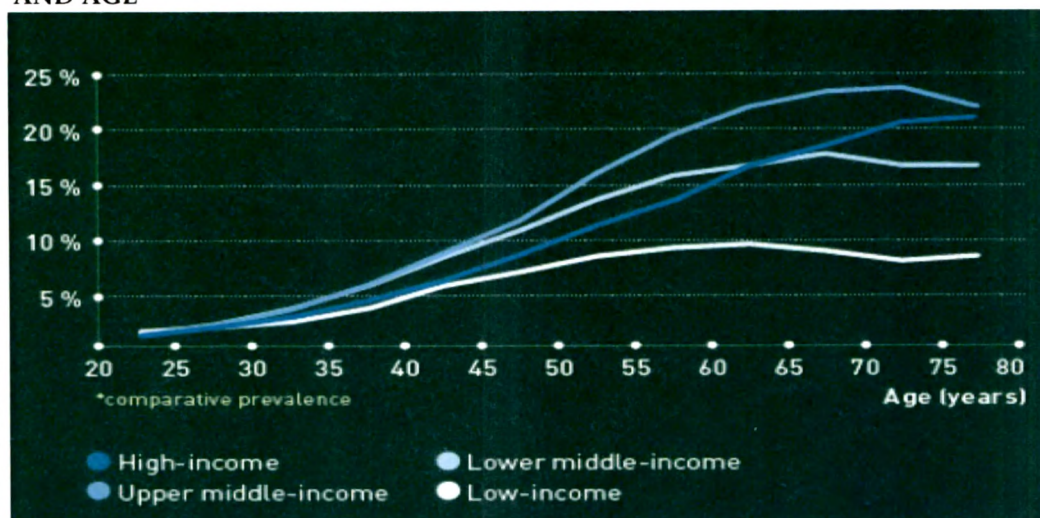
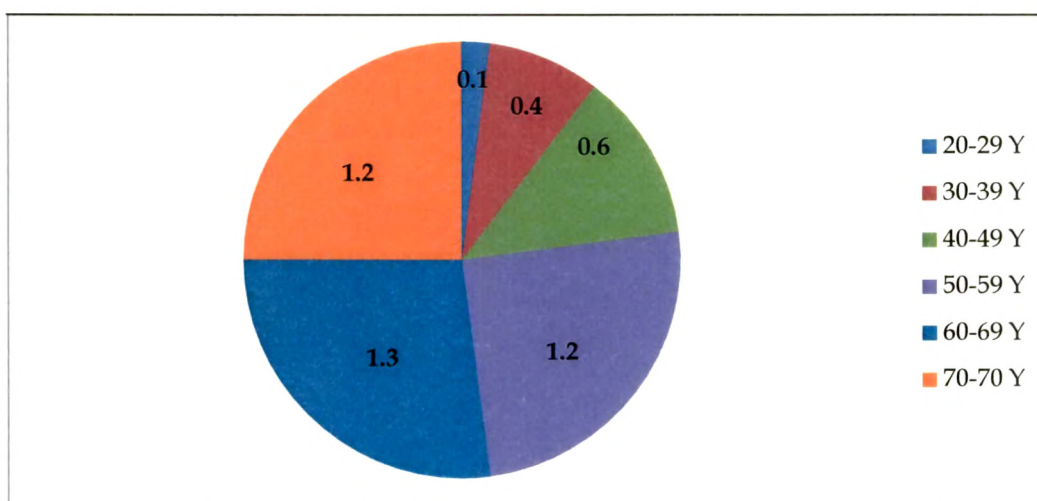


Figure 2.7(c): Prevalence of diabetes based on income groups

Half people who die from diabetes are under the age of 60

DEATHS ATTRIBUTABLE TO DIABETES (IN MILLIONS) BY AGE (20-79 YEARS)



Diabetes- South East Asian Scenario

Figure 2.7(d): Deaths attributable to diabetes (in millions) by age

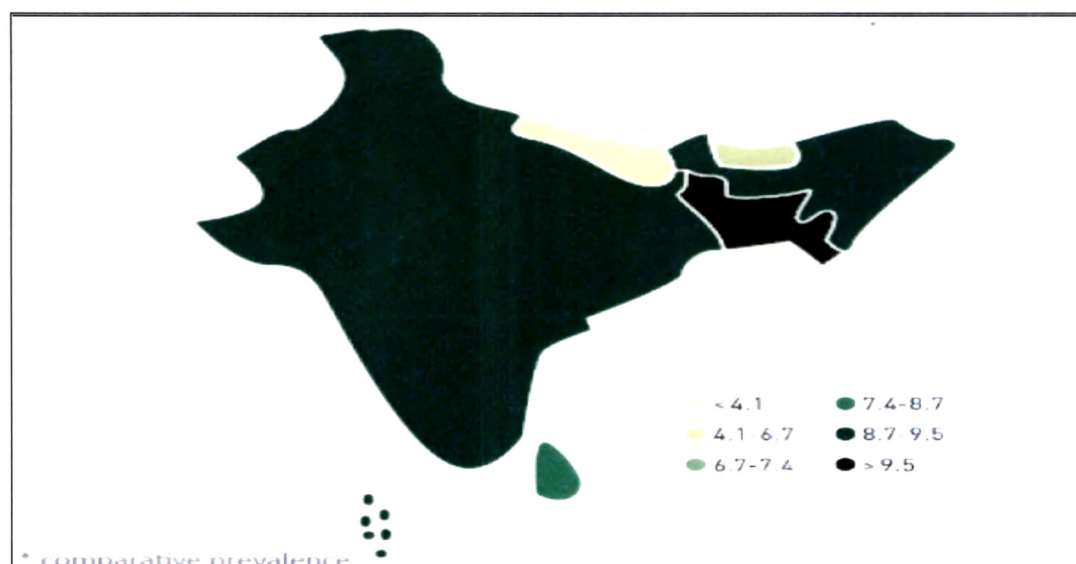
According to recent report of IDF 2012, more than 70.3 million people in the South East Asia (SEA) region have diabetes; by 2030 this will rise to 120.9 million. Almost 8.7% of adults have diabetes in SEA region. Diabetes can lead to serious and costly complication as diabetes caused 1.1 million deaths in the

SEA region last year. USD 4.6 billion was spent on treating diabetes in this region. In SEA region one fifth of all adults are living with diabetes. India has highest number of diabetics in SEA followed by Bangladesh and Sri-lanka (Figure 2.8, Table 2.2 (b)).

Table 2.2 (b): Highest numbers of diabetes in SEA region

Rank	Country	Cases
1	India	63 million
2	Bangladesh	5.5 million
3	Sri lanka	1.1 million
4	Nepal	506,727
5	Mauritius	141,644
6	Bhutan	22,362
7	Maldives	15,908

IDF, 2012



Prevalence* estimates of diabetes (20-79 years), SEA Region, IDF Diabetes Atlas, 5th ed. © International Diabetes Federation, 2012

Figure 2.8: Comparative prevalence of diabetes in South East Asia

Diabetes- An Indian Scenario

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world” (Mohan V et al 2007). The increased number of diabetics in India is likely to be due to a significant increase in the incidence of type 2 diabetes, caused by

unprecedented rates of urbanization, which results in environmental and lifestyle changes. According to World Health Organization (WHO 2004) estimates, the urban population in developing regions will increase from 1.9 billion in 2000 to 3.9 billion in 2030. It is estimated that, by 2030, nearly 46% of India's population will be living in urban areas (WHO 2002).

The first national study on the prevalence of type 2 diabetes in India was conducted between 1972 and 1975 by the Indian Council Medical Research (ICMR New Delhi) and found that the prevalence of diabetes was 2.1 per cent in urban population and 1.5 per cent in the rural population while in those above 40 yr of age, the prevalence was 5 per cent in urban and 2.8 per cent in rural areas (Ahuja MMS 1979). Subsequent studies revealed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in south India reported a prevalence of 5 per cent (Ramachandran A et al 1989). The prevalence of impaired glucose tolerance in the same study was 2 per cent. A national rural diabetes survey was done between 1989 and 1991 in different parts of the country in selected rural populations reported a crude prevalence of 2.8 percent (Sridhar GR et al 2002). A study carried out in 1988 in Chennai, reported a prevalence of 8.2 per cent in the urban and 2.4 per cent in the rural areas (Ramachandran A et al 1992). A subsequent study in the same urban area done after five years showed an age standardized prevalence of 11.6 per cent indicating a rising trend in prevalence of diabetes (Ramachandran A et al 1997).

Studies over the period 1990–2000, using standardized WHO (WHO 1985; Gupta R and Misra A 2007) or American Diabetes Association (ADA) criteria (ADA 1997) have demonstrated that the prevalence of diabetes in India has increased from 5% to 15% among urban populations, from 4.2% to 6.2% in semi-urban populations, and from 2% to 5% in rural populations, with wide regional disparities related to urban and rural settings (WHO 1999). There were 32 million diabetics in India in year 2000 which raised to 40.9 million in

just six years and is predicted to rise to 80 million in year 2030 (Wild S et al 2004; Sicree R et al 2006) (Figure 2.9).

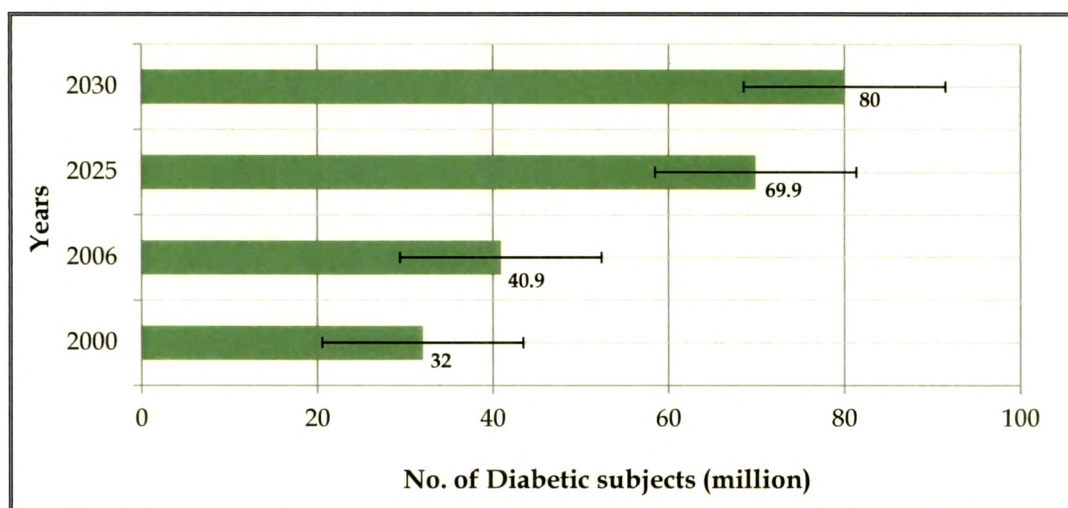


Figure 2.9: Estimated number of diabetic subjects in India

The National Urban Diabetes Survey (NUDS), a population based study was conducted in six metropolitan cities across India and recruited 11,216 subjects aged 20 yr and above representative of all socio-economic strata (Ramachandran A et al 2001). An oral glucose tolerance test was done using capillary glucose and diabetes was defined using the WHO criteria (Alberti KG and Zimmet PZ 1998). The study reported that the age standardized prevalence of type 2 diabetes was 12.1 per cent. This study also revealed that the prevalence in the southern part of India to be higher-13.5% in Chennai, 12.4%, in Bangalore, and 16.6% Hyderabad; compared to eastern India (Kolkata), 11.7%; northern India (New Delhi), 11.6%; and western India (Mumbai), 9.3%. The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14% with a high risk of conversion to diabetes.

A study carried out in western India showed age standardized prevalence of 8.6 per cent in urban population (Gupta A et al 2003). Another study reported a high prevalence (9.3%) in rural Maharashtra (Deo SS et al 2006). The Amrita Diabetes and Endocrine Population Survey (ADEPS), a community based

cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5 percent (Menon VU et al 2006). Figure 2.10 shows map of India showing the states where population-based studies have been done and it also shows the prevalence of type 2 diabetes reported in different regions of India.

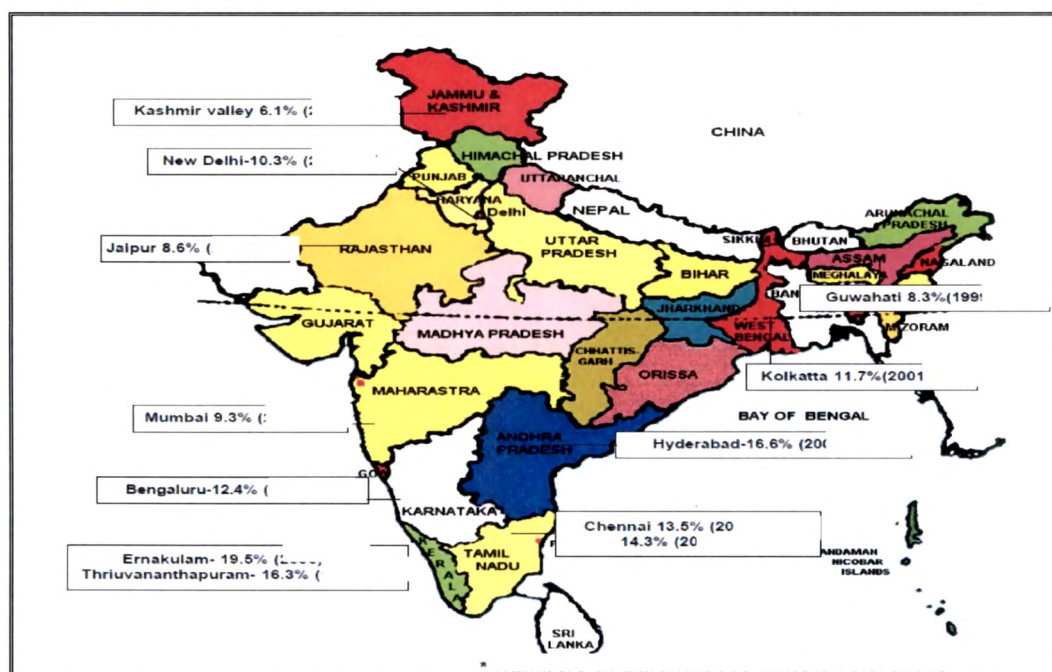


Figure 2.10: Population based study on prevalence of diabetes in various states of India

Recently documented by IDF, 2012 states total adult population in India is 752,631.15 thousands, out of which number of adults having diabetes is 63,013.87 thousand (prevalence rate 8.37%). Total number of deaths took place due to diabetes is 1,013,057 in the year 2012 (Table 2.3). Figure 2.11 shows the prevalence of diabetes in different regions of India.

Table 2.3: Statistics of diabetes in India, 2012

Diabetes in India (At a glance), 2012	Number
Total adult population (thousands)	752,631.15
Prevalence (%)	8.37
Number of adults with diabetes (thousands)	63,013.87
Number of adults with undiagnosed diabetes (thousands)	4,437.52
Number of deaths due to diabetes	1,013,057.00
Mean expenditure per person with diabetes (USD)	67.98

IDF, 2012

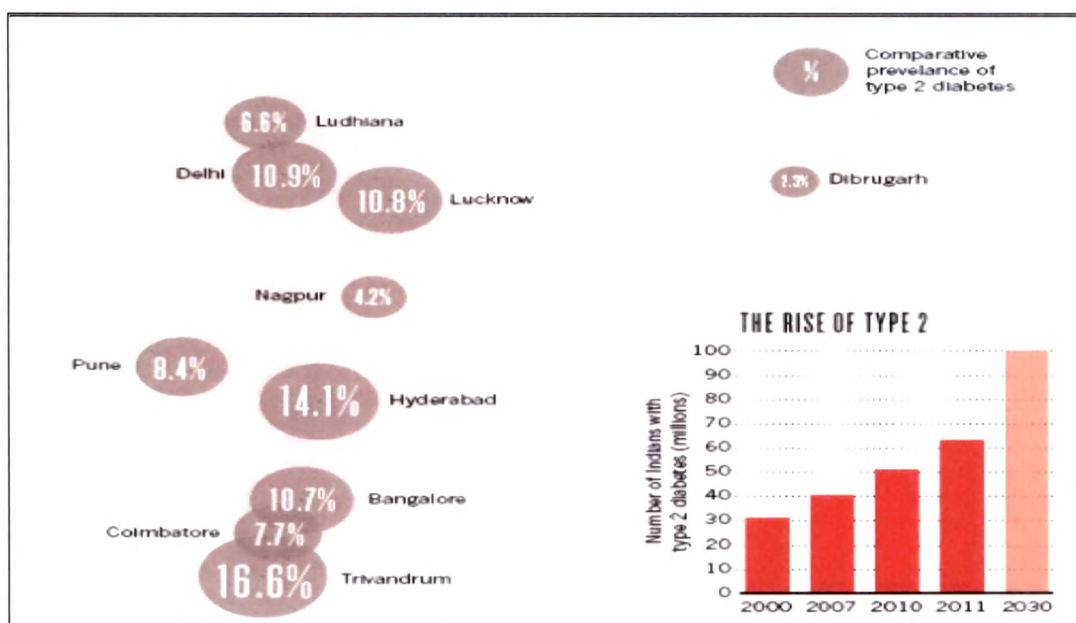
IDF atlas, 4th ed. 2009

Figure 2.11: Comparative prevalence of diabetes across various cities of India

Diabetes- Gujarat Scenario

According to Express India 2008 and Times of India 2010, Gujarat has second highest number of diabetics in the country after Tamil Nadu. There is scarcity of data for diabetes prevalence in Gujarat, India. A multicentric ICMR funded study reported 1.73% overall prevalence (3.7% of urban and 1.9% of rural) in

Ahmedabad (Gupta OP et al 1970; Ramaiya KL et al 1991). No such large community based research was conducted in Gujarat after 1970. People of Gujarat are known to be prone to develop diabetes because of genetic susceptibility, sedentary lifestyle and dietary preference for sweet and oily foods. A most recent study conducted on 904 diabetic subjects in Ahmedabad revealed that the overall prevalence rate of diabetes among the study population at Ahmedabad is 13.8%, 16.9% in males and 11.1% in females. The ratio of diagnosed to undiagnosed diabetes is 4:1 and the prevalence of IFG is 6%. Few studies carried out in the Gujarat region is summarized in Table 2.4.

Table 2.4: Summary of percent prevalence of diabetes in Gujarat region

Researcher	Year	Place	Type of population	Percent Prevalence
Iyer et al	2007	Vadodara	Industrial	6.4
Mehan et al	2005	Vadodara	Industrial	15.3
Mehan et al	2004	Vadodara	Industrial	10.4
Iyer et al	2002	Vadodara	Urban	9.5
Mani et al	2002	Vidhyanagar	Urban	5.3
Mani et al	2002	Vadodara	Urban	8.8
Desai et al	2000	Vadodara	Industrial	7.4

2.3 Diabetes – Types, Causes, Complications and Treatment

“INDIA’S DIABETES TIME BOMB: Epigenetics and lifestyle are conspiring to inflict a massive epidemic of type 2 diabetes in the subcontinent”

-Shetty P, Nature 2012

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; a hormone

produced by the β -cells of the pancreas that is necessary for the use or storage of body fuels (Sierra GN 2009). The existence of the disorder has been known since 600 BC when 'Susrutha' an Indian physician described it as an illness characterized by excessive thirst and excessive urination which was also sweet to taste. Abnormalities in the metabolism of carbohydrate, protein and fat are also present (Marion JF 2008). The following classification given by WHO is used to categories the type of diabetes-

1. Pre-diabetes

A stage of impaired glucose homeostasis that includes impaired fasting glucose (IFG) and glucose tolerance (GT) is called pre-diabetes. People with pre-diabetes have IFG, impaired glucose tolerance (IGT) or both. Individuals with pre-diabetes are at higher risk for future diabetes.

2. Type 1 Diabetes

The primary defect is pancreatic β -cell destruction, usually leading to absolute insulin deficiency and resulting in hyperglycemia, polyuria (excessive urination), polydipsia (excessive thirst), weight loss, dehydration, electrolyte imbalance and ketoacidosis. The rate of β -cell destruction is quite variable, proceeding rapidly in some persons and slowly in others. Persons with type 1 diabetes are dependent on exogenous insulin to prevent ketoacidosis and death. Type 1 diabetes has two forms, immune-mediated diabetes mellitus and idiopathic diabetes mellitus. Immune mediated diabetes mellitus results from an autoimmune destruction of the β -cell of the pancreas, the only cells in the body that produces hormone insulin that regulates blood glucose. Idiopathic type 1 diabetes mellitus refers to forms of disease that have no known etiology (ADA 2006). Risk factors for type 1 diabetes may be genetic, autoimmune or environmental. The genetic predisposition to HLA-DQ coded genes for disease susceptibility offset by genes that are related to disease resistance (ADA 2006). However the genetic factors that confer susceptibility remain unclear. Figure 2.12 shows the overview of type-1 diabetes.

3. *Type 2 Diabetes*

Type 2 diabetes is the most common form of diabetes and accounts for over 90% of all diabetes cases worldwide (Gonzalez EL et al 2009). Type 2 diabetes is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest. Type 2 diabetes usually occurs after the age of 35-40 years but may be diagnosed earlier, especially in populations with high diabetes prevalence. Type 2 diabetes can remain undetected (asymptomatic), for many years and the diagnosis is often made from associated complications or incidentally through an abnormal blood or urine glucose test. Type 2 diabetes is often, but not always, associated with metabolic abnormalities such as obesity, which itself can cause insulin resistance and lead to elevated blood glucose levels. In contrast to type 1 diabetes, people with type 2 diabetes are not absolutely dependent on exogenous insulin, but may require insulin for control of hyperglycemia if this is not achieved with diet alone or with oral hypoglycemic agents. Risk factors for type 2 diabetes include genetic and environmental factors, including family history of diabetes, older age, obesity, particularly intra-abdominal obesity, a prior history of gestational diabetes, pre-diabetes and race or ethnicity. Type 2 diabetes has a strong familial component, and at least 50 genetic variants have been reported to influence susceptibility to type 2 diabetes (McCarthy MI 2010).

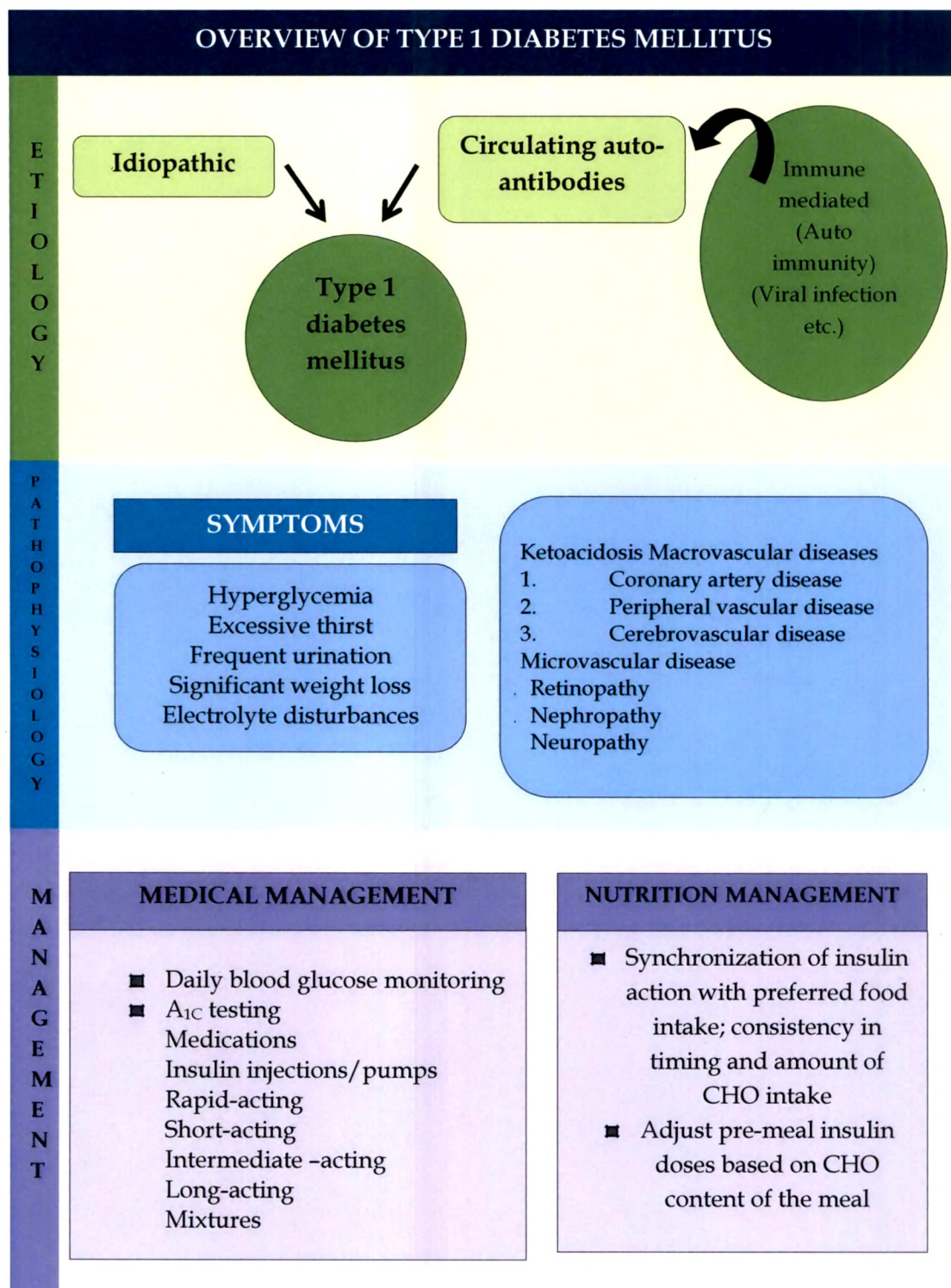
In most cases type 2 diabetes results from a combination of insulin resistance and β -cell failure. But the extent to which each of these factors contributes to the development of the disease is unclear. Endogenous insulin levels may be normal, depressed, or elevated; but they are inadequate to overcome concomitant insulin resistance (decreases tissue sensitivity or responsiveness to insulin); as a result, hyperglycemia occurs (Figure 2.13).

4. *Gestational diabetes*

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It occurs in about 7% of all pregnancies, resulting in more than 200,000 cases annually (ADA 2001). Women with known diabetes mellitus before pregnancy are not classified as having GDM. GDM is usually diagnosed during the second and third trimester of pregnancy. At this stage insulin antagonist hormone levels increases, and insulin resistance normally occurs.

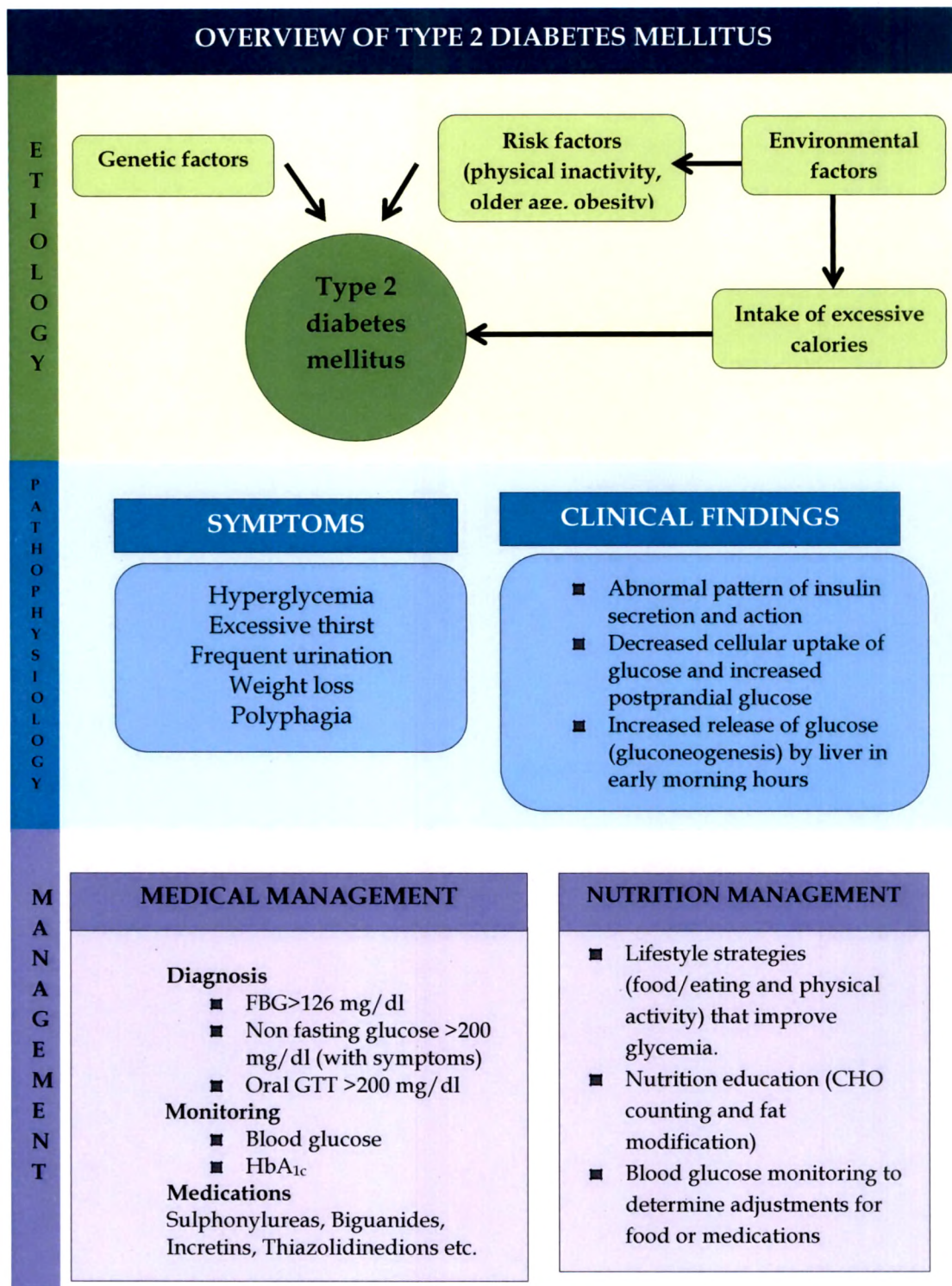
5. *Other types of diabetes*

This category includes diabetes associated with specific genetic syndrome (such as maturity-onset diabetes of youth), surgery, drugs, malnutrition, infections and other illnesses. Such type of diabetes account for 1% to 5% of all diabetes diagnosed cases.



Anderson JB and Garner CS, 2000 updated by Marion J Franz, 2006

Figure 2.12: Type 1 Diabetes: causes, symptoms, complications and management



Anderson JB and Garner CS, 2000 updated by Marion J Franz, 2006

Figure 2.13: Type 2 Diabetes: causes, symptoms, complications and management

Diagnosis criteria for diabetes

The diagnosis criteria for diabetes and pre-diabetes have been debated for several years and modified numerous times. In 1997 the fasting glucose cut-off level was lowered from 7.8 to 7.0 mmol/L (Alberti KG and Zimmet PZ 1998; Classification and diagnosis of diabetes 1997) and in 2003 the American Diabetes association (ADA) changed the threshold for IFG from 6.1 to 5.6 mmol/L (ADA 2003). Moreover, since 2010, ADA included the use of glycated hemoglobin (HbA_{1c}) to diagnose diabetes to identify individuals at increased risk for future diabetes (WHO report 2011)

Table 2.5: Present diagnostic criteria for diabetes, and non-diabetic hyperglycemia (IFG and IGT) according to serum/plasma levels

	WHO 2006	ADA 2011
<i>Diabetes Mellitus</i>		
Fasting glucose	≥7.0 mmol/L	≥7.0 mmol/L
2-hr glucose	≥11.1 mmol/L	≥11.1 mmol/L
HbA _{1c}	≥6.5%	≥6.5%
<i>Non-diabetic hyperglycemia</i>		
Fasting glucose	6.1-6.9 mmol/L	5.6-6.9 mmol/L
2-hr glucose	7.8-11.0 mmol/L	7.8-11.0 mmol/L
HbA _{1c}	-	5.7-6.4%

WHO report, 2011

a. Pathophysiology of Type 2 diabetes mellitus

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by abnormalities in carbohydrate and fat metabolism. The causes of type 2 diabetes are multi-factorial and include both genetic and environmental elements that affect beta-cell function and tissue (muscle, liver, adipose tissue, and pancreas) insulin sensitivity. Although there is considerable debate as to the relative contributions of beta-cell dysfunction and reduced insulin sensitivity to the pathogenesis of diabetes, it is generally agreed that both these factors play important roles (Scheen AJ 2003). Figure 2.14 gives a

composite picture of the role of insulin in the pathogenesis of the disorder and also the development of common symptoms.

Aberrations in insulin activity results in reduced activity of glycolytic enzymes like glucokinase (liver), hexokinase (muscle and adipose tissue), and phosphofructokinase and pyruvate kinase (Cavaghan et al 2000). There is a reduction in the insulin mediated uptake of glucose from muscles and other tissues and an increase in glucose production from the liver resulting in post prandial hyperglycemia followed by fasting hyperglycemia. There is a mean fold rise in activity of phosphoenol pyruvate carboxykinase (hepatic gluconeogenic enzyme) leading to increased gluconeogenesis. Muscle protein is broken down to release amino acids and FFA production from adipose tissues. There is an elevated influx of FFA to the liver, which continues gluconeogenesis, causing an increased synthesis causes an increased synthesis of triglycerides, while accumulating fatty acids (Anderson 1988). Thus lipid abnormalities are fairly common in diabetics (Mani and Mani 1988; Georg and Ludvik 2000). These basic defects in the early stages of the disorder coupled with insulin deficiency in the later stages together with the reciprocal changes in glucagon affects the carbohydrate, fat and protein metabolism (Weyer et al 2000).

Hyperinsulinemia and insulin resistance have a key role in the precipitation of peripheral vascular resistance and hypertension. The proposed mechanism involves increased renal sodium and water reabsorption, increased blood pressure sensitivity to dietary salt, augmentation of the pressure and aldosterone to angiotensin II, change in trans membrane electrolyte transport, stimulation of sympathetic nervous activity, reduced synthesis of vasodilatory prostaglandin and increased secretion of endothelin. Good glycemic control and tight control of blood pressure is vital in preventing and postponing micro and macroangiopathy in various target organs.

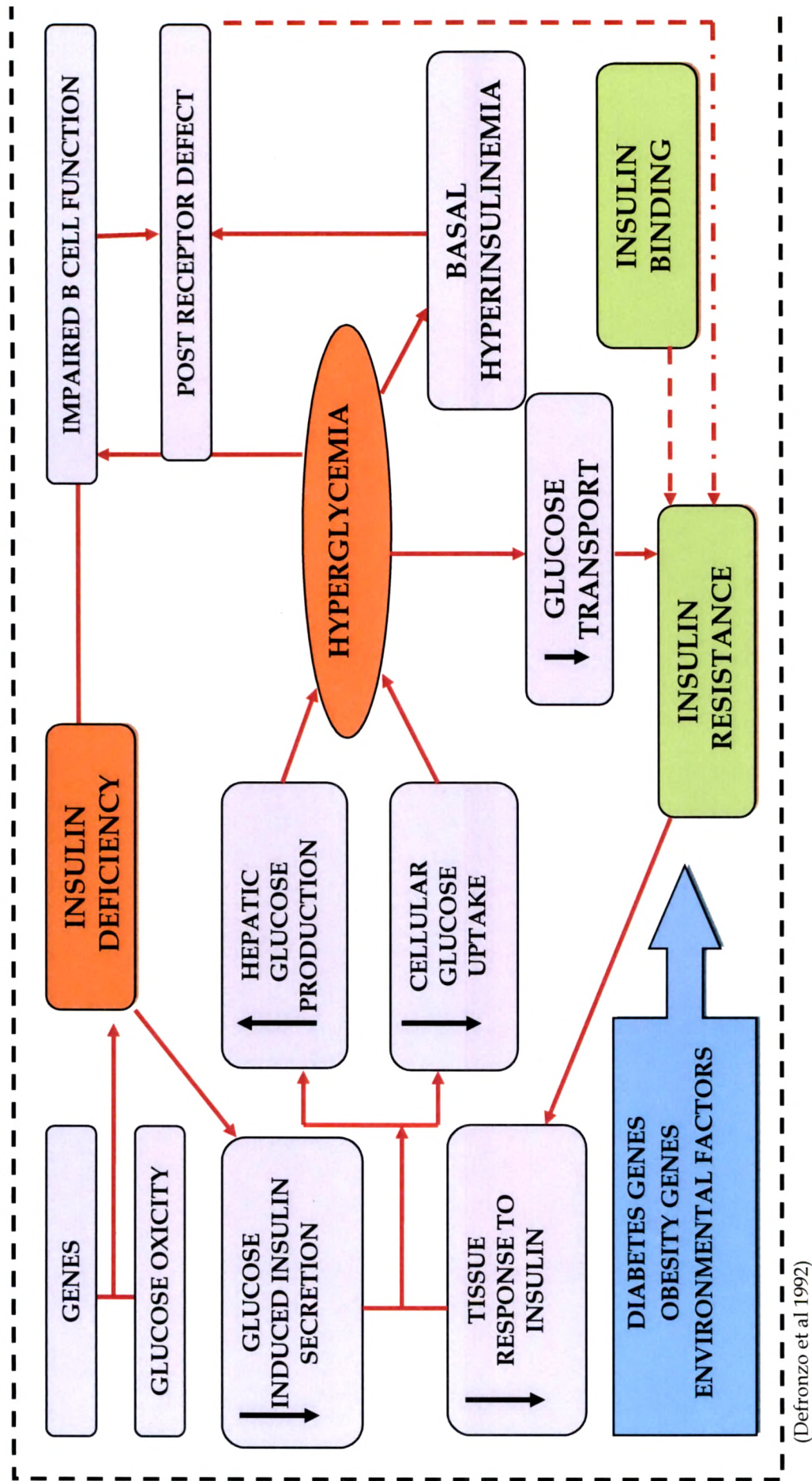


Figure 2.14: Pathophysiology of Type 2 Diabetes

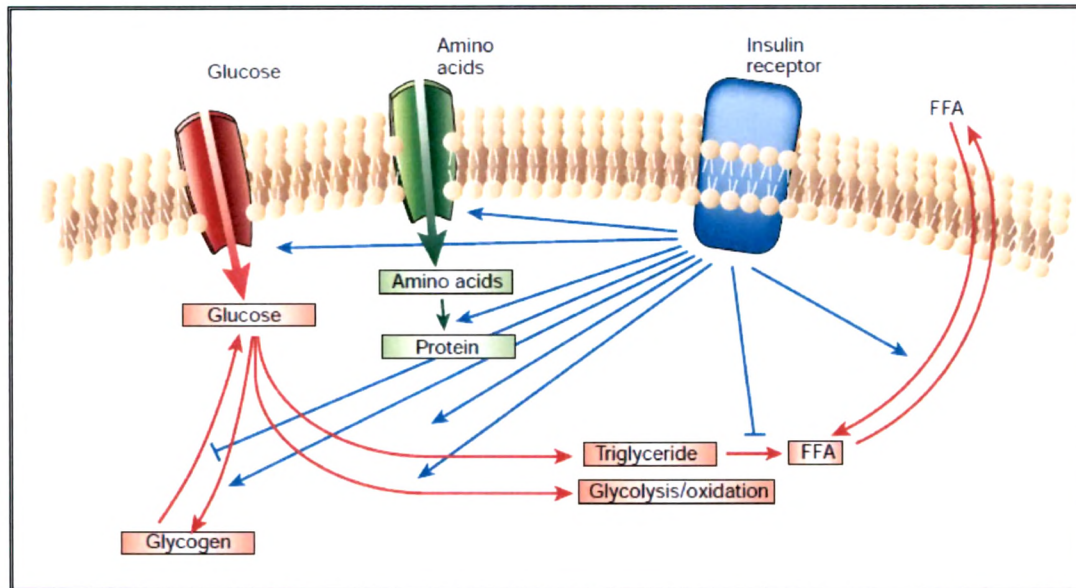
b. Portrayal of endocrine hormones/incretins in regulation of blood glucose

There are several hormones and incretins which have an important role in the regulation of blood glucose. Role of these hormone are described below

Insulin

Insulin is a hormone produced by the pancreatic β -cells and is the key hormone for the regulation of blood glucose. Insulin increases glucose uptake in muscle and fat, and inhibits hepatic glucose production, thus serving as the primary regulator of blood glucose concentration. Insulin also stimulates cell growth and differentiation, and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown. Insulin resistance or deficiency results in profound deregulation of these processes, and produces elevations in fasting and postprandial glucose and lipid levels (Saltiel AR and Kahn CR 2001). Insulin increases glucose transport in fat and muscle cells by stimulating the translocation of the transporter GLUT4 from intracellular sites to the plasma membrane. GLUT4 is found in vesicles that continuously cycle from intracellular stores to the plasma membrane. Insulin increases glucose transport by increasing the rate of GLUT4-vesicle exocytosis, and by slightly decreasing the rate of internalization (Pessin JE 1999). Although the exact mechanisms are unknown, it is likely that the insulin-responsive GLUT4 vesicle is tethered to intracellular sites, perhaps defined by a microtubule network (Guilherme A et al 2000). Up to 75% of insulin-dependent glucose disposal occurs in skeletal muscle, whereas adipose tissue accounts for only a small fraction (Klip A and Paquet MR 1990). Although insulin does not stimulate glucose uptake in liver, it blocks glycogenolysis and gluconeogenesis, and stimulates glycogen synthesis, thus regulating fasting glucose levels. Insulin action in tissues not normally considered insulin sensitive, including brain and pancreatic β -cell may also be important in glucose homeostasis (Bruning JC et al 1998; Kulkarni RN 1999).

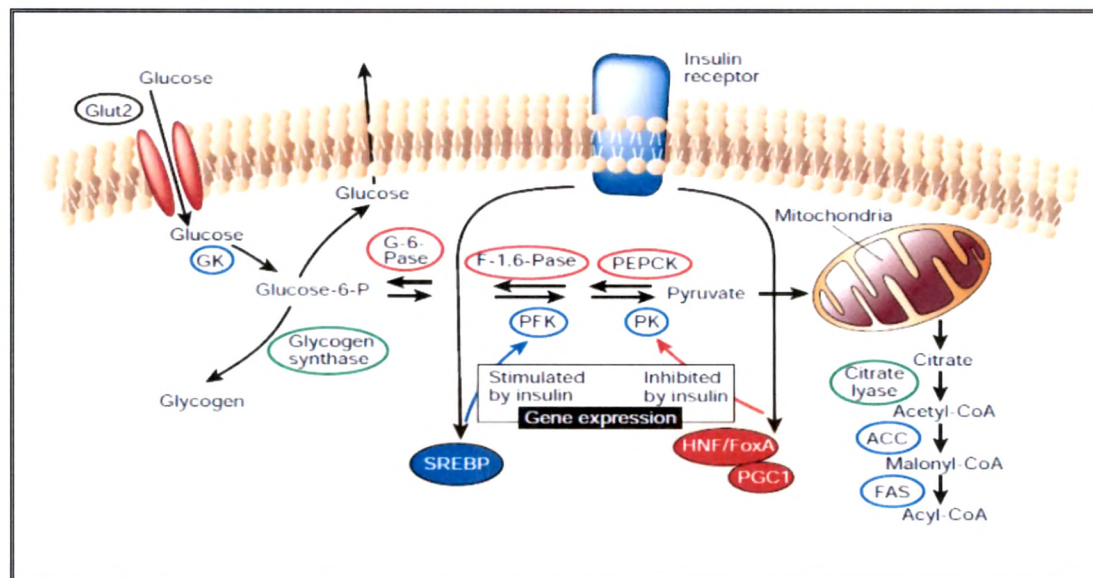
Regulation of glucose by insulin and regulation of insulin in the liver is diagrammatically presented in Figure 2.15 and 2.16.



Saltiel AR and Kahn CR, 2001

Insulin stimulates the uptake of glucose, amino acids and fatty acids into cells, and increases the expression or activity of enzymes that catalyse glycogen, lipid and protein synthesis, while inhibiting the activity or expression of those that catalyse degradation.

Figure 2.15: The regulation of metabolism by insulin



Saltiel AR and Kahn CR, 2001, GK, glucokinase; Glucose-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; F-1,6-Pase, fructose-1,6-bisphosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PK, pyruvate kinase; ACC, acetyl-CoA carboxylase; FAS, fatty-acid synthase

Figure 2.16: The regulation of glucose metabolism in the liver

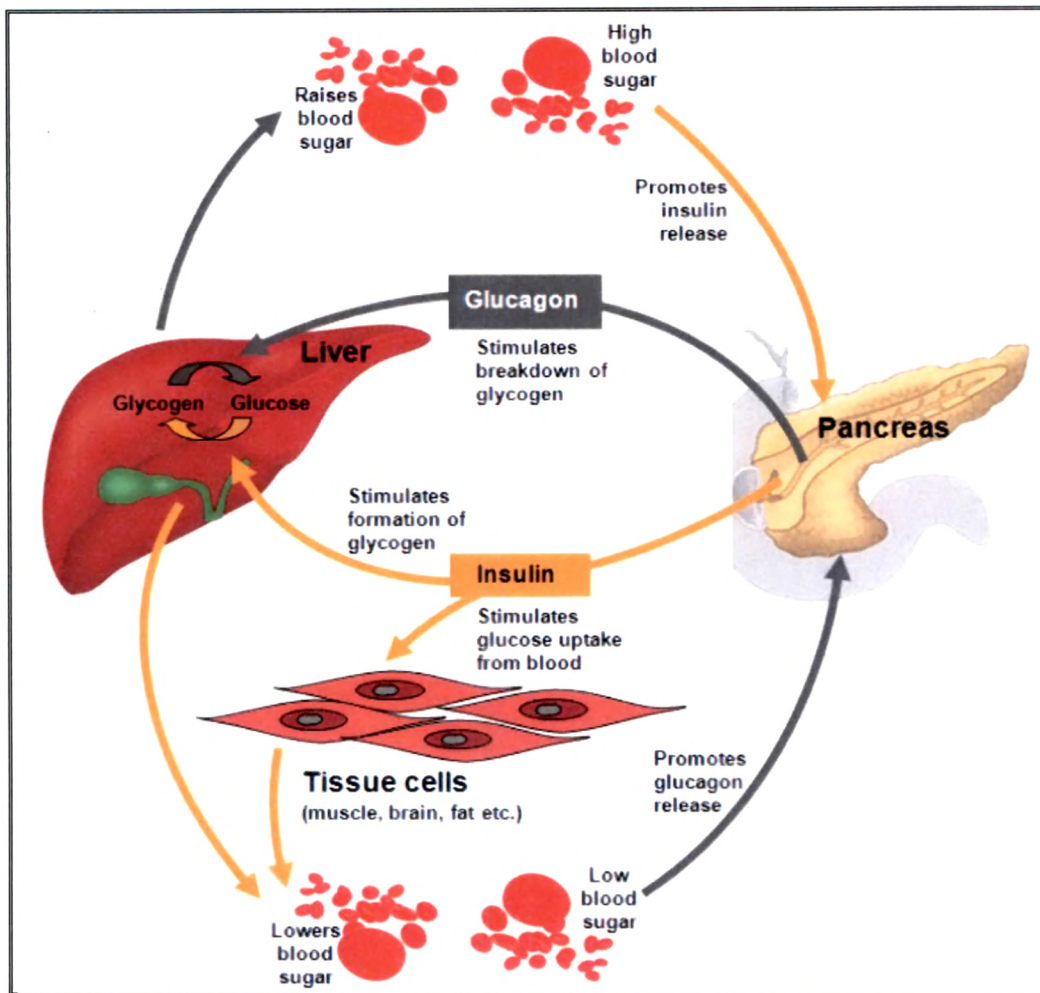
In the hepatocyte, insulin stimulates the utilization and storage of glucose as lipid and glycogen, while repressing glucose synthesis and release. This is accomplished through a coordinated regulation of enzyme synthesis and activity. Insulin stimulates the expression of genes encoding glycolytic and fatty-acid synthetic enzymes (in blue), while inhibiting the expression of those encoding gluconeogenic enzymes (in red). These effects are mediated by a series of transcription factors and co-factors, including sterol regulatory element binding protein (SREBP)-1, hepatic nuclear factor (HNF)-4, the forkhead protein family (Fox) and PPAR γ co-activator 1 (PGC1). The hormone also regulates the activities of some enzymes, such as glycogen synthase and citrate lyase (in green), through changes in phosphorylation state (Figure 2.16).

Glucagon

Glucagon is the key catabolic hormone secreted from the pancreatic α cells consisting of 29 amino acids. Roger Unger (1950) characterized glucagon as opposing the effect of insulin. Hepatic glucose production which is primarily regulated by glucagon maintains basal blood glucose concentrations within a normal range during the fasting state. When plasma glucose falls below the normal range, glucagon secretion increases resulting in hepatic glucose production and return to plasma glucose to normal range. Hence, glucagon and insulin are part of a feedback system that keeps blood glucose at the right level (Figure 2.17).

Amylin

Amylin is a neuro endocrine hormone co-expressed and co-secreted with insulin by pancreatic β cells in response to nutrient stimuli (Koda et al, 1992). Amylin slows the gastric emptying rate and suppresses post prandial glucagon secretion thereby decreasing glucagon stimulated hepatic glucose output.



IDF, Diabetes Atlas 2009

Figure 2.17: Synchronization of insulin and glucagon hormones

Gut Incretins

In 19th century, more than 40 gastrointestinal (GI) tract hormones have been discovered. These hormonal and paracrine signals are secreted from enteroendocrine cells located in the GI tract in response to the physicochemical properties of the ingested food passing along the lumen. However, many of the same hormones are also expressed in the central nervous system (CNS), acting to translate metabolic information between the GI tract and the CNS (Ranganath LR 2008; Badman MK and Flier JS 2005; Strader AD and Wood SC 2005; Riedy CA et al 1995; Williams DL et al 2006). To be labeled an "incretins", a hormone must fulfill two criteria: (i) it must be

released in response to oral ingestion of nutrients; and (ii) it must cause insulin release at physiological concentrations. The only known incretins in humans (Ranganath LR 2008; Strader AD and Wood SC 2005), GLP-1 and gastric inhibitory peptide (GIP) are peptide hormones with half-lives of only 3–5 min. These hormones are degraded mainly by the enzyme dipeptidyl peptidase (DPP)-IV shortly after secretion (Ranganath LR 2008). The incretin hormones are thought to constitute an important part of the enteroinsular axis, by which is meant the combined input to the endocrine pancreas of substrates, nerve impulses, and hormones generated by ingestion of mixed meals (Creutzfeldt W 1992).

Bloomgarden reported on TGR5, initially found by Kawamata et al (2003) a novel G-protein coupled receptor (GPCR) that is responsive to bile acids as a cell surface receptor. The immunosuppressive effects of bile acids in macrophages may be mediated by TGR5. Recently, it was also found that TGR5 is an important link between nutrition and metabolism (Nguyen A and Bouscarel B 2008; Thomas C et al (a), 2008; Thomas C et al (b), 2008). TGR5 stimulates the production of incretins, in particular glucagon-like peptide (GLP)-1 by enteroendocrine cells, leading to a reduction in appetite, enhanced insulin release, and a delay in gastric emptying.

A large number of peptides produced in the gastrointestinal tract, in particular those belonging to the glucagon-secretin superfamily of peptides, can be demonstrated to enhance glucose-induced insulin secretion. Among the peptides that actually are released in response to oral glucose, two additional members of the same peptide family, namely glucose-dependent insulinotropic polypeptide (GIP, formerly known as gastric inhibitory polypeptide) and glucagon-like peptide 1 (GLP-1) have attracted the most attention.

[illegible]

• • • • •

i) Glucose-dependent insulintropic polypeptide (GIP)

GIP is a peptide of 42 amino acids processed from a precursor of 153 amino acids (Takeda J et al 1987). The GIP receptor is a type II G protein-coupled receptor belonging to a superfamily of receptors the ligands of which (with a few exceptions) are constituted by the members of the secretin-glucagon family of peptides (Mayo KE et al 2003). It is expressed in the pancreatic islets and also in the gut, adipose tissue, heart, pituitary, adrenal cortex, and several regions of the brain.

GIP is secreted from specific endocrine cells, the so-called K cells, which exhibit the highest density in the duodenum, but GIP-producing cells may be found in the entire small intestinal mucosa (Mortensen K et al 2003). Secretion is stimulated by absorbable carbohydrates and by lipids. GIP secretion is therefore greatly increased in response to meal ingestion, resulting in 10- to 20-fold elevations of the plasma concentration. Interaction of GIP with its receptor on the pancreatic β -cells causes an elevation of cAMP levels, which, in turn, increases the intracellular calcium concentration and enhances the exocytosis of insulin-containing granules (Ding WG and Gromada J 1997; Wheeler MB et al 1995). Nauck et al (1989) demonstrated that the elevated GIP concentrations elicited by oral glucose can completely account for the accompanying augmented insulin release.

i) Glucagon like peptide-1 (GLP-1)

This hormone is secreted within a few minutes in response to glucose and lipids by the L-cells of the GI tract. L-cells are the second most abundant population of endocrine cells in the human intestine (Mojsov S 1986). GLP-1 secretion is stimulated by the presence of nutrients in the lumen of the gut (but additional neural or endocrine mechanisms may also operate), and the secretion of GLP-1 throughout the day is highly correlated to the release of insulin. GLP-1 is one of the most potent insulin-releasing substances known, with half-maximal effective concentrations for its effects on the β -cells 10 pmol/l (Fehmann HC et al 1995). It is strongly insulintropic in mimicry

experiments (Kreymann B et al 1987) and animal experiments involving an antagonist of the GLP-1 receptor have indicated that GLP-1 is responsible for a substantial part of the insulin response to oral glucose (Kolligs F et al 1995; Wang Z et al 1995). Recent clinical data also suggests a glycemia and lipid lowering effect of GLP-1 antagonist (ADA 2010; Holst JJ 2012; Buse JB et al 2011).

In a 13-week, randomized, double blind, placebo-controlled, parallel-group, phase II dose-ranging study, lixisenatide (GLP-1) administered at doses of 5, 10, 20, or 30 mg once daily or twice daily in patients with type 2 diabetes inadequately controlled with metformin significantly improved HbA_{1c} compared with placebo. Dose-dependent improvements were also observed for fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) associated with the morning meal and average self-monitored seven-point blood glucose levels. The dose of 20 mg administered once daily was found to provide the optimal balance of efficacy and tolerability (Ratner RE et al 2010; Fonseca VA et al 2012).

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 in the fasting state after glucose loading or meal ingestion (Orskov C et al 1993; Qualmann C et al 1995). In contrast to GIP, the insulinotropic and glucose-lowering actions of GLP-1 are preserved in human subjects with type 2 diabetes (Elahi D et al 1994; Nauck MA et al 1993) in both the fasting and the postprandial states (Nauck MA et al 1993). Similarly, GLP-1 inhibits gastric acid secretion (O'Halloran DJ et al 1990) and gastric emptying in humans (Wettergren A et al 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; Nauck MA 1999). GLP-1 may also enhance glucose clearance in humans (D'Alessio DA et al 1995); however, the majority of these actions are likely mediated indirectly

through effects on insulin and glucagon (Toft-Nielson M et al 1996; Ryan AS et al 1998; Vella A et al 2000).

Action of DPP-IV on GLP-1

The dipeptidyl peptidase IV (DPP-IV), also known as CD26, is a trans-membrane and circulating protease responsible for cleaving polypeptides containing a proline or alanine residue in the N terminal position. DPP-IV is constitutively expressed on epithelial cells of kidney, intestine, liver (bile duct) and pancreas. GLP-1 (7-36) amide entering the portal venous circulation is rapidly inactivated by the DPP-IV dependent cleavage into GLP-1 (9-36 amide), accounting for the short half-life (2-5 minutes) of this peptide (Hansen L et al 1999).

Mechanism of action of GLP-1

There are several mechanisms and sites of GLP-1 in regulating the glucose homeostasis. Firstly, evidence revealed that GLP-1 acts as a glucose sensitizer. Thus GLP-1 may facilitate glucose-dependent mitochondrial ATP production (Tsuboi T et al 2003). Secondly, reports also suggest activation on cAMP by action of GLP-1. It has been shown that cAMP generated by activation of the GLP-1 receptor may also influence the exocytotic process directly, and this process has been estimated to account for up to 70% of the entire secretory response. ATP may directly influence the exocytotic process, which may therefore represent another site of convergence for the glucose and GLP-1-mediated signals (Gromada J et al 1998).

Thirdly, GLP-1 also stimulates all steps of insulin biosynthesis as well as insulin gene transcription (Fehmann HC and Habener JF, 1992), thereby providing continued and augmented supplies of insulin for secretion. Activation of pancreatic duodenal homeobox-1, a key regulator of islet growth and insulin gene transcription, may be involved (Buteau J et al 1999). In addition, GLP-1 upregulates the genes for the cellular machinery involved in insulin secretion, such as the glucokinase and GLUT2 genes (Buteau J et al 1999). Finally and most importantly, GLP-1 has been shown to have trophic

effects on β -cells (Egan JM et al 2003). Not only does it stimulate β -cell proliferation (Xu G et al 1999), it also enhances the differentiation of new β -cells from progenitor cells in the pancreatic duct epithelium (Zhou J et al 1990). A proliferation was also induced in aging glucose-intolerant rats, been shown to be capable of inhibiting apoptosis of β -cells (Farilla L et al 2003; Li Y et al 2003). Because the normal number of β -cells is maintained in a balance between apoptosis and proliferation (Bonner-Weir S 2003) (Figure 2.18).

ii) Cholecystokinin (CCK)

Cholecystokinin (CCK) is another GI hormonal satiety signal, secreted primarily from I cells within the duodenal and jejunal mucosa. Two receptors mediate CCK action: CCK-1 in the GI tract and CCK-2 in the CNS (Badman MK and Flier JS 2005; Strader AD and Wood SC 2005). Following its release, CCK has multiple effects on the GI system, including regulation of gut motility, pancreatic enzyme secretion, gallbladder contraction, gastric emptying, and gastric acid secretion. CCK is a short-term signal that causes a dose-dependent reduction of the size of the meal consumed following its administration to normal humans. It also appears to interact with long-term signals of energy balance, such as leptin and insulin (Strader AD and Wood SC 2005; Riedy CA et al 1995).

iii) Ghrelin

In contrast with all other known GI hormones, ghrelin increases food intake in humans (Badman MK and Flier JS 2005; Cummings DE et al 2001; Wren AM et al 2001). Ghrelin is secreted from gastric X/A-like cells and has been confirmed as the endogenous ligand for the growth hormone secretagogue receptor.

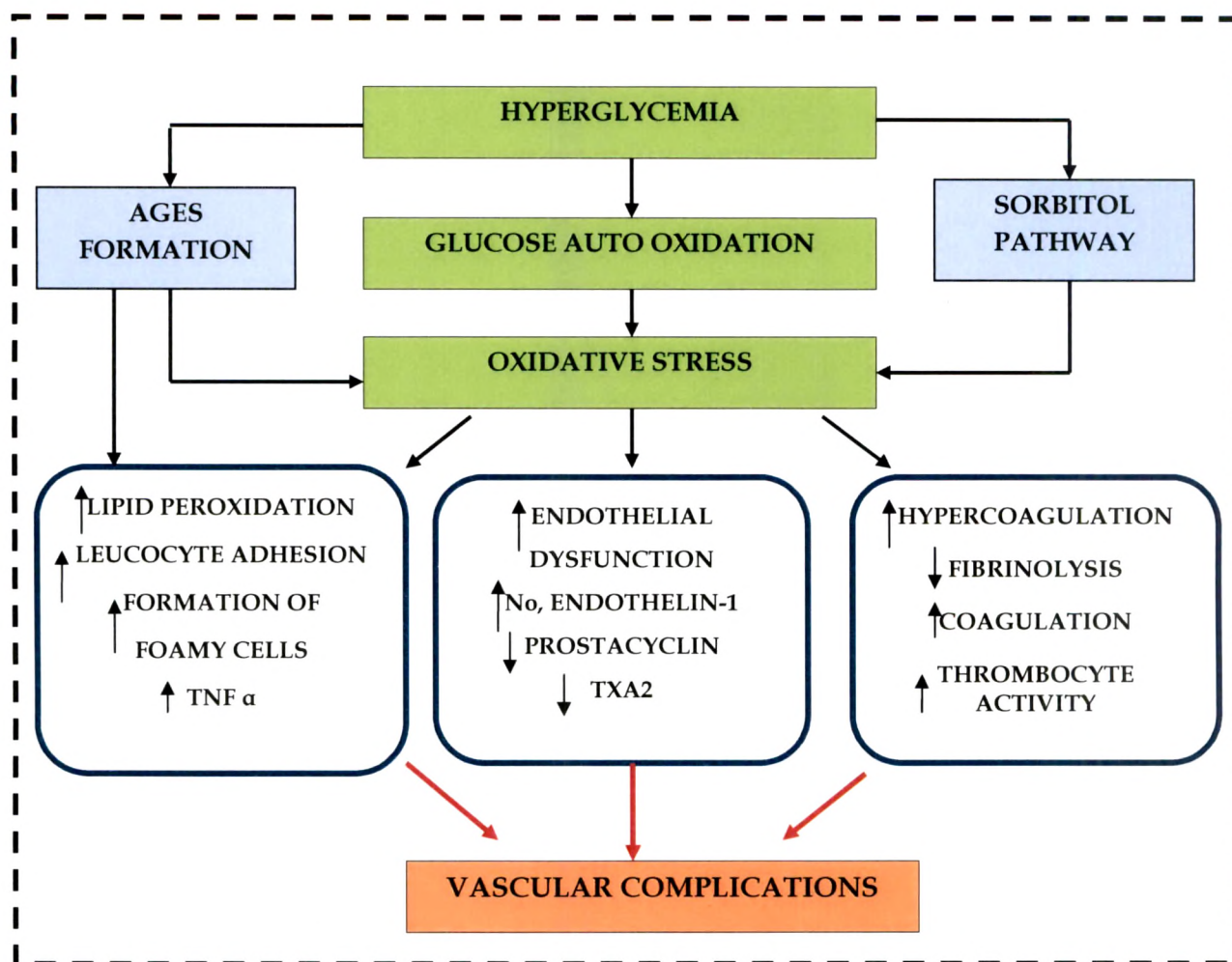
iv) Other gut hormones

Many other GI hormones participate in the regulation of food intake, gastric motility, and gastric acid secretion, including the bombesin family of peptides, glicentin, GLP-2, oxyntomodulin (OXM), peptide tyrosine-tyrosine

(PYY), and apolipoprotein A-IV (Korner J and Leibel RL 2003; Strader AD and Wood SC 2005; Riedy CA et al 1995). These hormones are secreted from various cells in the GI tract and all reduce food intake, forming a coordinated set of satiety signals that prevent over consumption of calories, acting to reduce the rate of increase of postprandial blood glucose and lipid concentrations.

c. Long term complications of Diabetes Mellitus

Long term complications of diabetes include macrovascular disease, microvascular disease, and neuropathy. Macrovascular diseases involve diseases of large blood vessels; microvascular diseases associated with diabetes involve the small blood vessels and include nephropathy and retinopathy. In contrast, diabetic neuropathy is a condition characterized by damage to the nerves. In microvascular and macrovascular diseases persistent hyperglycemia plays a dominating role. Complications of diabetes are not solely caused by hyperglycemia. It is preferably the deleterious effect of excessive glucose which arises because of long term hyperglycemia which is intermediated and clouded as a consequence of oxidative stress (Figure 2.19).



AGES – Advanced Glycosylation End Products, TXA₂ – Thromboxane A₂, TNF α – Tumour necrotizing factor, NO – Nitric oxide, (Goycheva 2006)

Figure 2.19: Schematic representation of oxidative stress and vascular complications

Role of endothelium dysfunction in diabetes

The endothelium is a complex organ with a multitude of properties essential for control of vascular functions. Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic micro and macro vascular diseases.

Endothelium Functions

The endothelium is the biological active inner layer of the blood vessels, which serves as an important locus of control of vascular and thus organ functions (Cines DB 1998). The endothelium actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the sub-endothelial matrix, the adhesion and extravasation of leucocytes, and inflammatory activity in the vessel wall. It also affects the functions of other cell types, such as vascular smooth muscle cells, platelets, leucocytes, retinal pericytes, renal mesangial cells and large artery macrophages. On the other hand, these cells can affect endothelial cells. To carry out its above-mentioned functions, the endothelium produces components of the extracellular matrix such as collagen and a variety of regulatory mediators, including NO (nitric oxide), prostanoids, ET-1 (endothelin-1), Ang II (angiotensin II), t-PA (tissue-type plasminogen activator), PAI-1 (plasminogen activator inhibitor-1), vWF (von Wille brand factor), adhesion molecules and cytokines. The production of these moieties is responsive to various stimuli (Quyyumi AA, 1998).

Endothelium dysfunctions

Endothelial dysfunction in diabetes originates from three main sources (Hammes HP et al 2003; Singh R 2001). First, hyperglycemia and its immediate biochemical sequel directly alter endothelial function (Brownlee M 2001). Glucose transport into endothelial and vascular smooth muscle cells occurs by facilitated diffusion and is thus insulin-independent. Glucose transport is auto regulated by glucose in smooth muscle cells, but not in endothelial cells, in which an increase in blood glucose concentration will thus increase the intracellular accumulation of glucose and its metabolites. Thus endothelial cells exposed to high glucose *in vitro* increase the production of extracellular matrix components, such as collagen and fibronectin, and of procoagulant proteins, such as vWF and tissue factor, and

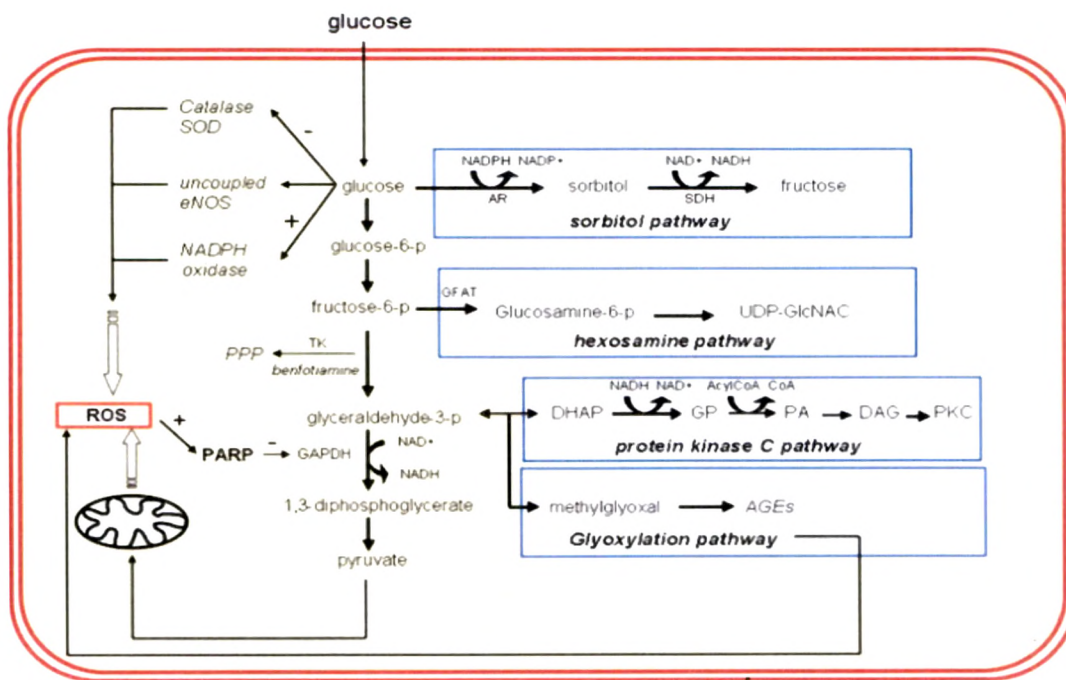
show decreased proliferation, migration and fibrinolytic potential, and increased apoptosis (Mc Ginn 2003; Graier WF 1995). Secondly, high glucose influences endothelial cell functioning indirectly by the synthesis of growth factor and vasoactive agents in other cells (Kofler S 2005). Thirdly, the components of the metabolic syndrome can affect endothelial function (Cacicedo JM 2004).

Various mechanisms have been proposed to explain how hyperglycemia directly causes diabetic vascular complications. An increase in intracellular glucose will lead to an increase in the flux of glucose to sorbitol via the polyol pathway, an increase in glucosamine-6-phosphate via the hexosamine pathway, and the activation of PKC (protein kinase C) via *de novo* synthesis of DAG (diacylglycerol). In addition, glucose and glucose derived dicarbonyl compounds react non-enzymatically with the basic amino acids lysine and arginine in proteins to form AGEs (advanced glycosylation end-products) both extra- and intra-cellularly. These different pathways are interrelated and potentiate each other. Figure 2.20 shows how, intracellularly, these four biochemical mechanisms may all be the consequence of hyperglycaemia-induced overproduction of ROS in mitochondria (Schalkwijk CG and Stehouwer DA 2005).

Microvascular complication of diabetes

a) Diabetic retinopathy

Diabetic retinopathy may be the most common microvascular complication of diabetes. Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes. There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy (Fong DS et al 2004). Aldose reductase may participate in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol).



(Brownlee M 2001) p (phosphate), PPP (pentose phosphate pathway), TK (enzyme transketolase), PA (phosphatidic acid), SOD (superoxide dismutase), AR (aldose reductase), SDH (sorbitol dehydrogenase), GFAT (enzyme glutamine:fructose-6-phosphate amidotransferase), ROS (reactive oxygen species), AGEs (advanced glycosylation end-products), PK (Protein kinase)

Figure 2.20: Potential mechanisms by which hyperglycemia and its immediate biochemical sequel induce hyperglycemic damage

High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes (Gabbay KH 2004). Cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the non-enzymatic formation of advanced glycosylated end products (AGEs). In animal models, these substances have also been associated with formation of micro aneurysms

and pericyte loss. Evaluations of AGE inhibitors are underway. Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation (Fong DS et al 2004). Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β , have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy (Keenan HA et al 2007; Aiello LP et al 2005).

b) Diabetic nephropathy

Diabetic nephropathy is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or "microalbuminuria". Microalbuminuria is defined as albumin excretion of 30–299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes (Gross JL et al 2005). The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy.

c) Diabetic neuropathy

Diabetic neuropathy is recognized by the American Diabetes Association (2007) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes." As with other microvascular complications, risk of developing diabetic neuropathy is

proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications. The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Boulton AJ 2005).

Macrovascular complication of diabetes-Cardiology

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles. Monocytes then infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes, in turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction (Boyle PJ 2007).

In addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability in type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in patients

with diabetes. The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in type 2 diabetes (Beckman JA et al 2002). Figure 2.21 shows the flowchart of progression of CHD and related diseases.

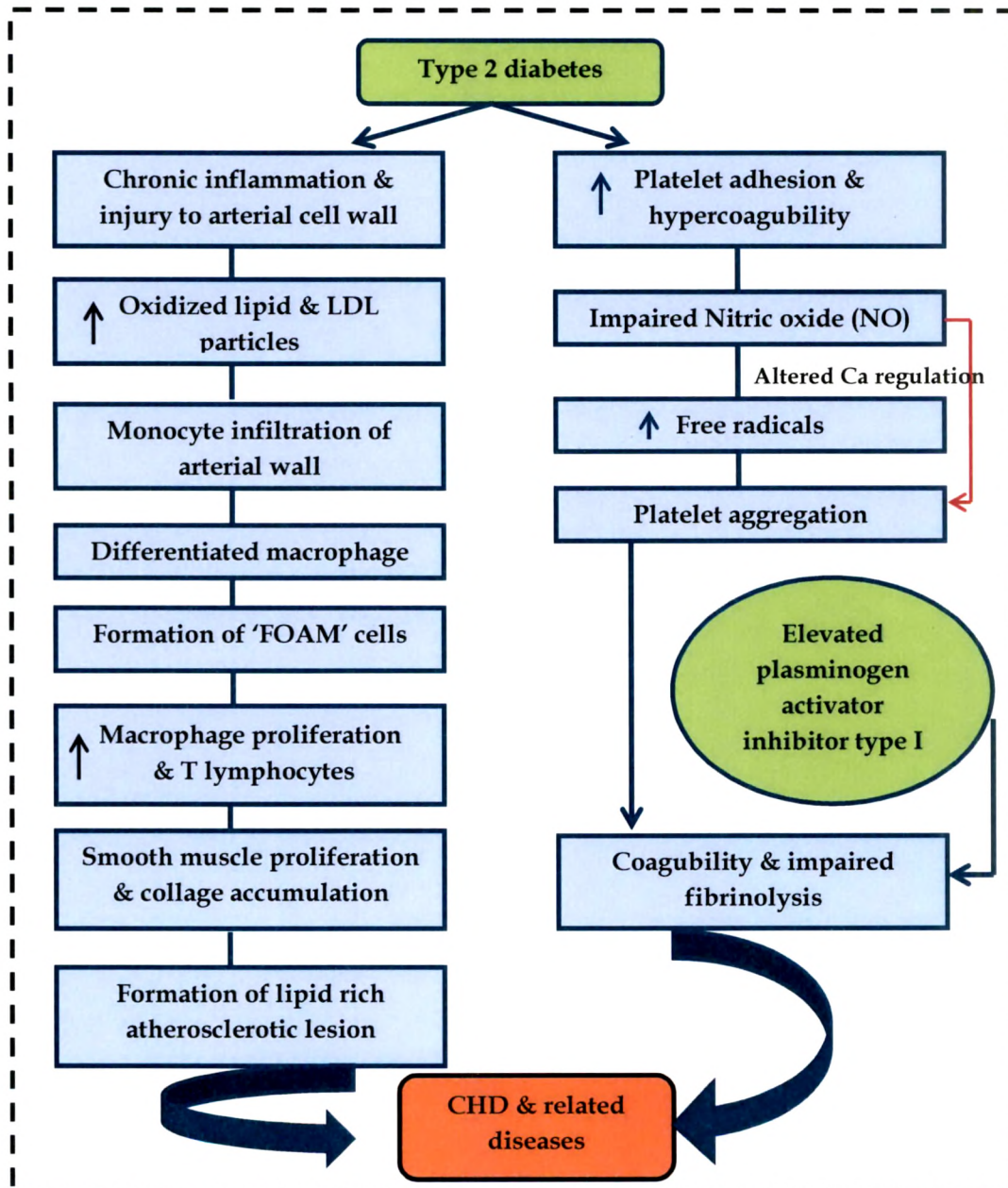


Figure 2.21: Flowchart depicting the progression of CHD due to type 2 diabetes mellitus

2.4 Preventive strategies in Diabetes mellitus

"By promoting the importance of lifestyle and diet changes and creating the social and environmental supports, burden of diabetes can be reduced and one can live a health life"

-Ministry Reports, 2012

Diabetes has long been viewed as a disorder of carbohydrate metabolism due to its hallmark feature of hyperglycemia. Thus, a primary goal in the management of diabetes is the regulation of blood glucose. Current strategies for optimal control on diabetes include diet, physical exercise and medications.

A cohort study of middle aged women showed a combination of several life style factors including maintaining a BMI < 25, eating a diet high in cereal fibre and PUFA and low in SFA and trans fats and glycemic load, exercising regularly, abstaining from smoking and consuming alcohol moderately was associated with approximately 90% lower incidences of diabetes mellitus than that found in women without these factors (Frank et al 2001). These result outcomes indicate that diet and lifestyle has a major role to play in the control of diabetes.

For decades, exercise has been considered a corner stone of diabetes management, along with diet and medication. Many clinical trials, and a number of large cohort studies, provide strong evidence for the value of physical activity in reducing the incidence of type 2 diabetes. The Da Qing IGT and Diabetes Study was the first randomized trial evaluating lifestyle interventions for the prevention of type 2 diabetes. In this study, 577 people with IGT from 33 clinics were randomized, by clinic, to diet only, exercise only, diet plus exercise, or control. After 6 years of follow-up, cumulative incidence of type 2 diabetes was 68% in control, 44% in diet only, 41% in exercise only, and 46% in diet plus exercise groups. This study provides evidence that both diet and exercise can be

effective diabetes prevention modalities (Pan XR et al 1997). Physical inactivity is estimated to cause around 27% of diabetes burden (WHO 2009). Exercise reduces insulin resistance, improves glycemic control and insulin sensitivity thus helps prevent type 2 diabetes (Warburton et al 2006).

Review of dietary patterns of south Asians revealed that these diets result in increased adiposity especially abdominal adiposity, insulin resistance and dyslipidemia, predisposing South Asians to obesity, type 2 diabetes and CVD (Lovegrove 2007; Misra et al 2008). Excess energy intake either in the form of fats, carbohydrates results in accumulation of fat and increase in risk of type 2 diabetes (Krauss et al 2000; Mani and Tiwari 2002; Desai and Mani 2002). A diet high in fiber is associated with improved ability to lower blood glucose, insulin resistance and visceral adiposity (Davis et al 2009; Weickert and Pfeiffer 2008). A cohort study on 1141 diabetic women revealed that whole grain, cereal fiber and dietary magnesium has a protective role against diabetes (Meyer KA et al 2000).

Findings of a review study indicated that a higher intake of polyunsaturated fat and possible long chain n-3 fatty acids could be beneficial, whereas a higher intake of saturated fat and trans fat could adversely affect glucose metabolism and insulin resistance (Hu FB et al 2001).

In view of pharmacotherapy, the choice of specific anti-hyperglycemic agents is predicted on their effectiveness in lowering glucose, extra glycemic effects that may reduce long term complications, safety profiles, tolerability, ease of use and expense. The UKPDS compared 3 classes of glucose lowering medications (sulfonylurea, metformin or insulin) but was unable to demonstrate clear superiority of one drug over the others with regard to diabetic complications. However, different classes do have variable effectiveness in decreasing glycemic levels and the principle in selecting a particular intervention will be its ability to achieve and maintain glycemic goals.

A cross sectional national survey of 875 diabetics by Piette et al, 2004 showed that out-of-pocket medication costs pose a significant burden to many adults with diabetes and contribute to decreased treatment adherence. A total of 19% of respondents reported cutting back on medication use in the prior year due to cost, 11% reported cutting back on their diabetes medications, and 7% reported cutting back on their diabetes medications at least once per month. Moreover, 28% reported forgoing food or other essentials to pay medication costs, 14% increased their credit card debt, and 10% borrowed money from family or friends to pay for their prescriptions.

Lately, apart from conventional strategies like dietary modifications, physical activity and pharmacological interventions alternative therapies are being sought to tackle the diabetic pandemic. Several Nutraceutical/Functional foods have been found to improve blood glucose and many more are still being explored. Many clinical trials have shown that these functional foods act similarly as anti-diabetic agents. The use of herbs has more than tripled over the last 10 years (Eisenberg 1998). One of threat areas among these nutraceutical is probiotics and prebiotics, which have enlighten the importance of human gut microbiota in various essential metabolomics pathways. Several unrevealed mechanisms by these gut flora can play an exceptional role in treatment and prevention of metabolic diseases like type 2 diabetes mellitus.

2.5 Overview of Gut environment: A complex ecosystem

"A complex community of microorganisms inhabits the mammalian gastrointestinal tract from mouth to anus, but the colon is, far by, the main site of this microbial colonization. Over the last 20-25 years, the knowledge on the complexity of this microbiota has increased considerably"

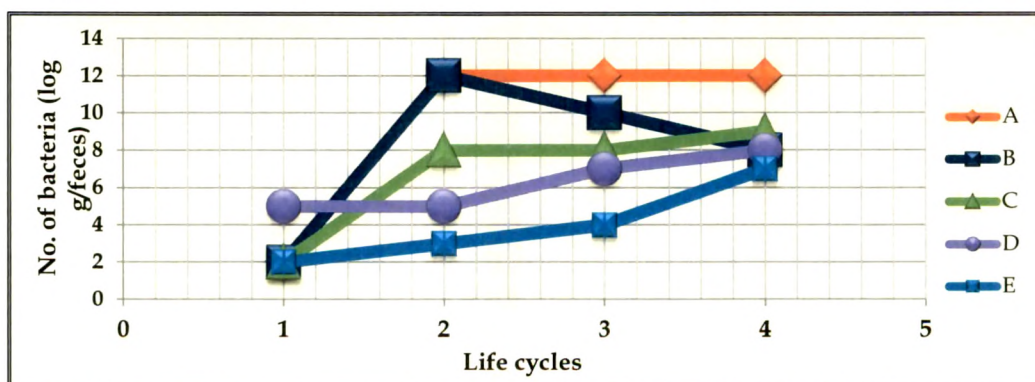
-Roberfroid MB, 2008

The human gastrointestinal (GI) tract is highly specialized ecosystem that has evolved over time, both physiologically and microbiologically. At least in part, this is consequence of the host and environment pressures it must counteract in order to maintain eubiosis. It appears on first impression to be quite a simple organ as it is an epithelial tube comprising different cells surrounded by a layer of muscle. However, the human gastrointestinal tract is highly dynamic ecosystem. The total area of the mucosal surface of the human gastrointestinal tract is 300 m² which makes it the largest surface area in the body that interacts with external environment (Bjorksten B 2006).

The human GI tract is composed of highly adapted regions for mediation of its diverse functions, many of which impact markedly upon host health and welfare. Major functions of the gut microflora include metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on intestinal epithelia and on immune structure and function, and protection of the colonised host against invasion by alien microbes. Gut flora might also be an essential factor in certain pathological disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases. Nevertheless, bacteria are also useful in promotion of human health. Probiotics and prebiotics are known to have a role in prevention or treatment of some diseases like CVD's, hypertension, obesity and diabetes (Guarner F and Malagelada JR 2003).

a) Composition of gut microflora during life cycles

Colonization of the gastrointestinal tract of newborn infants starts immediately after birth and occurs within a few days. Initially, the type of delivery (the birth canal versus caesarean section) and the type of diet (breast versus formula feeding) might affect the colonization pattern (Harmsen HJ et al 2000; Hooper LV 2001). The initial colonization is therefore very relevant to the final composition of the permanent flora in adults (Guarner F and Malagelada JR 2003). The choice of diet for the newborn is also of great importance as the microbiota of breast-fed infants is predominated by *bifidobacteria*, whereas formula fed infants have a more complex flora which resembles the adult gut in that *bacteroids*, *clostridia*, *bifidobacteria*, *lactobacilli*, *gram positive cocci*, *coliforms* and other groups are all represented in fairly equal proportions (Benno Y et al 1984). During the weaning stage, the microbiota becomes more developed and the ecosystem is thought to be fairly stable around 2 years of age. During the first few years of life and upon weaning, the infant microbiota normalizes. This composition will remain stable throughout most of adult life. Recent studies have shown that the gut microbiota changes in old age, with an increased number of bacterial groups represented in the predominant elderly gut microbiota (Kimura K 1997; Mitsuoka 1982). (Figure 2.22)



A-bacterioids, eubacteria, peptococci; B-bifidobacteria; C-E.coli, streptococci; D-lactobacillus; E-Clostridium. Horizontal axis, 1-at birth, 2-young children, 3-adult, 4-old age (Mitsuoka 1982)

Figure 2.22: Changes with Age in Number of Bacteria in Faeces

b) The human gastrointestinal tract and its microbiota: a state of art

The GI tract begins with the oral cavity which is comprised of the mouth, nose and throat. In the oral cavity a particularly complex microbiota exists. These include members of the *prevotella*, *porphyromonas*, *peptostreptococcus*, *bacteriodes*, *fusobacterium*, *eubacterium* and *desulfovibrio* genera. Bacterial number falls dramatically to $<10^3$ colony forming units (CFU) as they encounter stomach, as it provides a highly effective barrier against invading microorganisms. Few microorganisms with the exception of acid-tolerant *lactobacilli*, yeasts and *Helicobacter pylori* can survive the strongly acidic and peristaltic nature of the stomach (Willis CL 1999).

There is a high degree of variability between the stomach, small intestine and colon in terms of type and number of bacterial populations. This is predominantly due to transit time, secretions and nutrient availability. The duodenum also has low microbial populations due to its short transit time and the secretion of intestinal fluids, which create a hostile environment (Fooks LJ and Gibson GR 2002). However; there is a progressive increase in both number and species along the jejunum and the ileum. The small intestine harbors *enterococci*, *enterobacteria*, *lactobacilli*, *bacteriodes* and *clostridia*. These rapidly increase in numbers from 10^4 - 10^6 CFU mL⁻¹ in the small intestine to 10^{11} - 10^{12} CFU mL⁻¹ in the large intestine (Sanford PA 2005).

Transit in the distal colon is slower and nutrient availability is minimized. The majority of bacteria are non-spore forming anaerobs, of which the most numerically predominant are *bacteriodes* and *bifidobacterium*, *eubacterium*, *clostridium*, *lactobacillus*, *fusobacterium* and various gram positive cocci. Bacteria present in lower numbers include *enterococcus*, *enterobacteriaceae*, *methanogens* and dissimilatory sulphate-reducing bacteria (Sanford PA 2005).

Yeasts, including *candida albicans*, are also present in the gut microbiota; although in healthy individuals counts do not exceed 10^4 CFU g⁻¹ faeces (Gibson GR and Roberfroid MB 1995). The vast majority (>90%) of the total cells in the body are present as bacteria in the colon. The intestinal microbiota has been estimated to consist of at least 400 different species although only 40 species predominate (Bernhardt H et al 1995; Freter R 1998). A composite picture of type of microbes present in the human body has been shown in Figure 2.23.

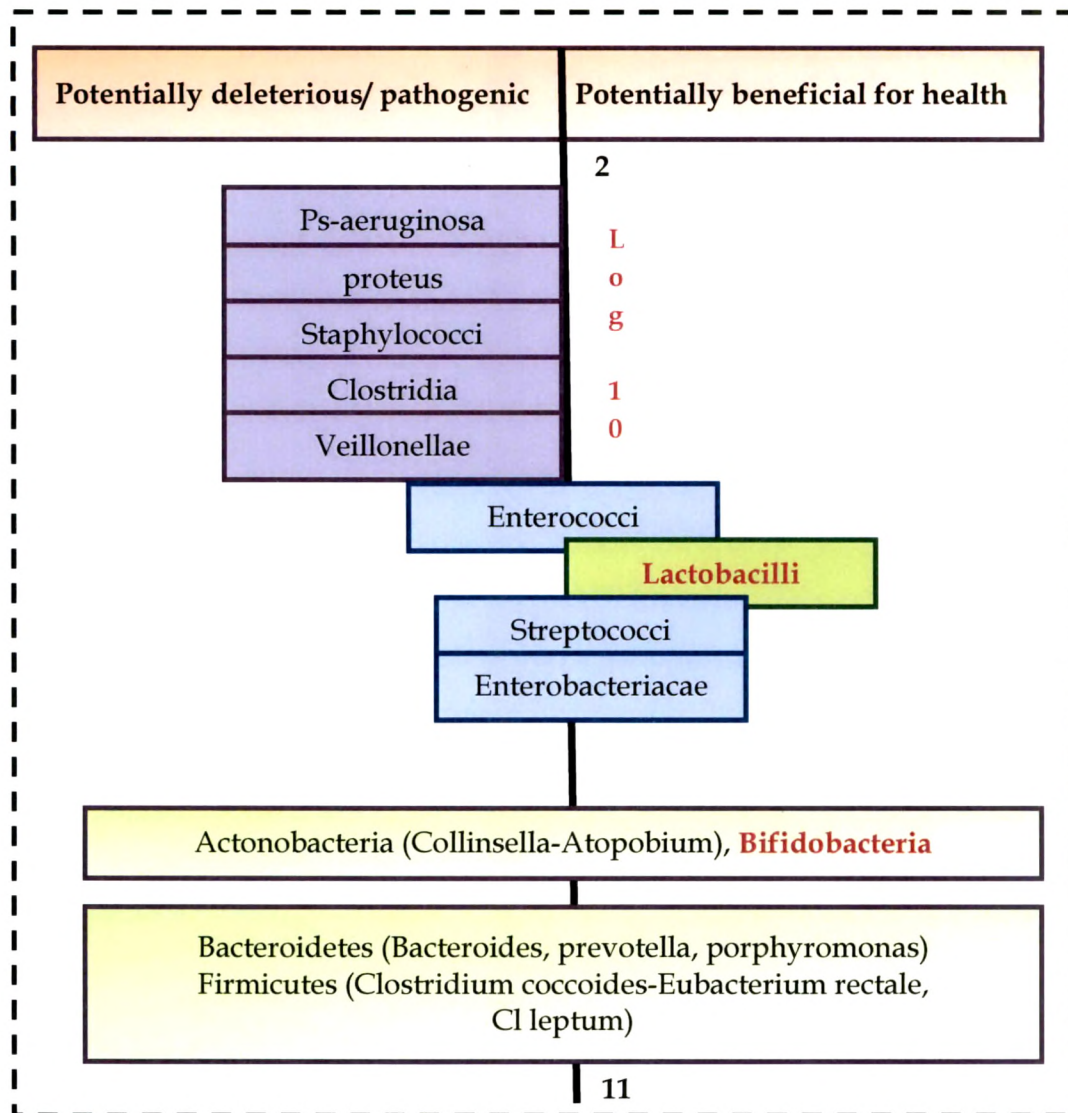
Log 10	Firmicute	Bacteroidites	Actinobacteria		Enterobacteria	Lactobacillus
			Bifidobacteria	Collinsella Atopobium		
10	3-5.3*10 ¹⁰ 30-53%					
		0.9-4.2*10 ¹⁰ 9-42%	0.7-1.0*10 ¹⁰ 1-14%			
9				0.3-0.4*10 ⁹ 0.7-10%		
					0.4-1.0*10 ⁹ 0.3-3.7%	
8						
						2*10 ⁸ 2%

Adopted and modified from Roberfroid MB 2008

Figure 2.23: Quantitative overview of the predominant human microbiota

c) Microbial diversity and importance of maintaining a balance in the gut

Humans can be considered as “super organisms” with an internal ecosystem of diverse microorganisms. Their homeostatic balance is dependent upon the interactions between the host and its microbial components (Rezzi S 2007). A balanced intestinal flora is a precondition for a fairly stable ecosystem in which both host-related factors and antagonistic interactions among intestinal bacteria play a role.



Adopted and modified from Roberfroid MB 2008

Potentially deleterious (left side) and potentially beneficial (right side)

Figure 2.24: Schematic average distribution of dominant, sub-dominant and minor components of human fecal microflora

When the main types of generally recognized beneficial bacteria, *Bifidobacteria* and *Lactobacilli* are at optimum levels they constitute approximately one-third of the bacterial population in the gastrointestinal tract. The numbers of *Bifidobacteria* are regarded as a marker of the stability of the human intestinal microflora

(Mutai M Tanaka R 1987; Turrone et al 2008; Van Der Waaij et al 2005; Harmsen et al 2002). These beneficial bacteria may act as wards regulating the activity of the other bacteria in the colon. The other bacteria, such as *Salmonella*, *Shigella*, *Clostridia*, *Staphylococcus aureus*, *Candida albicans*, *Campylobacter jejuni*, *Escherichia coli*, *Veillonella*, and *Klebsiella*, have varying potential to cause disease and are much less numerous (Ventura et al 2009). However, these pathogenic bacteria can produce harmful local and systemic effects if they overgrow as a consequence of a gut microflora imbalance. Research has shown beneficial bacteria, particularly *Bifidobacteria* and *Lactobacilli* keep these potential disease-causing organisms under control, preventing several disease-related dysfunctions related to an imbalances GI situation (Elmer GW 1996).

Reducing or eliminating more of the healthy gut microflora, like *Bifidobacteria*, has its consequences. When the human diet influences the species composition and metabolic characteristics of the intestinal microflora, toxic metabolite production is affected, such as the conversion of pro-carcinogens to active carcinogens (Perman JA 1989; Roland N et al 1995). In addition to producing toxic metabolites, several harmful bacteria, such as *Salmonella*, *Shigella*, *Listeria*, *Bacteroides*, *Proteus*, *E. coli*, *Clostridium perfringens* and *Vibrio cholerae* also have association with diarrhea, infections, liver damage, carcinogenesis and intestinal putrefaction.

Pathogenic effects associated with harmful intestinal microflora such as *E. coli* not only include colonic disorders but also have implication with possible vaginal infections and systemic disorders (Gibson GR and Roberfroid MB 1995). Major factors in the biology of these disorders are the overgrowth of pathogenic bacteria such as *clostridia*, *E. coli*. As well as parasites, viral infections, extensive burn injury, post-operative stress, and antibiotic therapy. These disorders are often associated with bacterial translocation due to intestinal barrier failure (Gibson GR and MacFarlane GT 1994). *Lactobacillus*, *Bifidobacteria* produce strong

acids, i.e. acetic and lactic acid the production of these acids reduces intestinal pH which results in lower blood ammonia levels and a reduced hepatic load (Levrat MA et al 1993).

Recent studies of the gut microbial ecosystem have identified more than 1000 species and possibly over 7000 strains, of which the largest part (80%) remains uncultured (Zoetendal et al 2008; Blaut and Clavel 2007; Rajilic-Stojanovic et al 2007; Backhed et al 2005). However, new approaches for culturing previously uncultured colonic microbes are being developed (Zoetendal et al 2008; Duncan et al 2007). In addition to this, new powerful tools for amplification and sequencing of genomic DNA from minute quantities of a sample and barcoded pyrosequencing can be expected to give new insights into the composition of the gut microbiota at high spatiotemporal resolution (Anderson et al 2008; Marcy et al 2007).

Functional aspects of microbial diversity in the human large intestine are now being discussed by many scientists (Dethlefsen et al 2008; Flint 2006; Ley et al 2006). Recent meta-genomics based studies have indicated that the gut microbiome has a coding capacity that vastly exceeds that of the human genome and encodes biochemical pathways that humans have not evolved (Kurokawa et al 2007; Turnbaugh et al 2007; Gill et al 2006; Ley et al 2006; Backhed et al 2005). Today, a great challenge is to expand and discover newer arenas of complex relationships and mechanisms related to responsiveness, diversity and resilience of the gut ecology, and its interactions with diet and related aspects of human host which affects each and every aspect of life.

2.6 Main functions of colonic microbiota in the gut

"Evidence based on many studies has suggests that gut microflora have specific functions which are very important and crucial for maintaining a balance and equilibrium for the gut ecology"

The presence of the gut microbiota has influenced human evolution in that the human host cannot perform certain vital intestinal functions without them. Germ free animal models have provided useful insights into the extensive roles of the microflora and the extent of interaction between the host and the gut microflora. The gut microbiota can be thought of as a microbial organ within a human organ as the processes performed by this diverse population are extensive; it can communicate with itself and with the host. It is also a site of energy consumption, transformation and distribution. Based on this knowledge, functions of gut microbiota are divided into three main categories; *Metabolic functions, Tropic functions and Protective functions.*

a) Metabolic functions

A major metabolic function of colonic microflora is the fermentation of non-digestible dietary residue and endogenous mucus produced by the epithelia (Roberfroid MB et al 1995). Gene diversity in the microbial community provides various enzymes and biochemical pathways that are distinct from the host's own constitutive resources. Overall outcomes of this complex metabolic activity are recovery of metabolic energy and absorbable substrates for the host, and supply of energy and nutritive products for bacterial growth and proliferation. Fermentation of carbohydrates is a major source of energy in the colon. Non-digestible carbohydrates include large polysaccharides (resistant starches, cellulose, hemicellulose, pectins, and gums), some oligosaccharides that escape

digestion, and unabsorbed sugars and alcohols (Cummings JH et al 1996; Preter et al 2011). The metabolic endpoint is generation of short-chain fatty acids.

Anaerobic metabolism of peptides and proteins (putrefaction) by the microflora also produces short-chain fatty acids but, at the same time, it generates a series of potentially toxic substances including ammonia, amines, phenols, thiols, and indols (Smith EA et al 1996; Cummings JH 1987). Available proteins include elastin and collagen from dietary sources, pancreatic enzymes, sloughed epithelial cells and lysed bacteria (Salminen S et al 1998). Substrate availability in the human adult colon is about 20–60 g carbohydrates and 5–20 g protein per day (Silvester KR et al 1995; Fallingborg J 1999). In the caecum and right colon, fermentation is very intense with high production of short-chain fatty acids, an acidic pH (5–6), and rapid bacterial growth (Macfarlane GT et al 1992). By contrast, the substrate in the left or distal colon is less available, the pH is close to neutral, putrefactive processes become quantitatively more important, and bacterial populations are close to static.

Colonic microorganisms also play a part in vitamin synthesis (Conly JM et al 1994; Hill MJ 1997) and in absorption of calcium, magnesium, and iron (Miyazawa E et al 1996; Roberfroid MB et al 1995; Younes H et al 2001). Absorption of ions in the caecum is improved by carbohydrate fermentation and production of short-chain fatty acids, especially acetate, propionate, and butyrate. All of these fatty acids have important functions in host physiology. Butyrate is almost completely consumed by the colonic epithelium, and it is a major source of energy for colonocytes. Acetate and propionate are found in portal blood and are eventually metabolised by the liver (propionate) or peripheral tissues, particularly muscle (acetate) (Cummings JH and Englyst HN 1987).

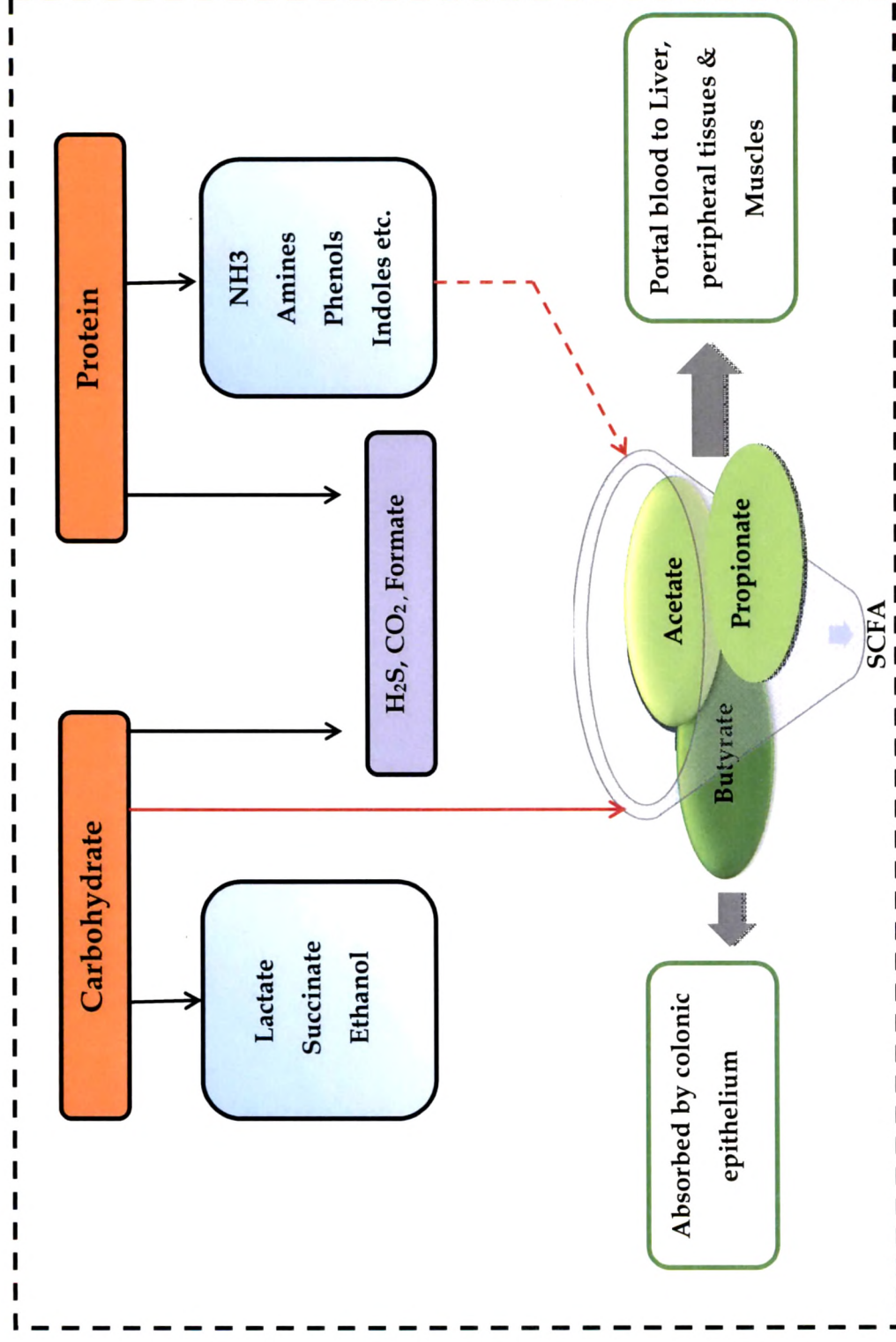


Figure 2.25: Metabolic pathways of intestinal microbiota

b) Tropic functions

Major two types of tropic functions are performed by the colonic microbiota, first is epithelial cell growth and differentiation and second is interactions between gut bacteria and host immunity.

Epithelial cell growth and differentiation: Differentiation of epithelial cells is greatly affected by interaction with resident microorganisms (Hooper LV et al 2001; Gordon JJ et al 1997). All three major short-chain fatty acids stimulate epithelial cell proliferation and differentiation in the large and small bowel *in vivo*. However, butyrate inhibits cell proliferation and stimulates cell differentiation in epithelial cell lines of neoplastic origin *in vitro* (Siavoshian S 2000). Moreover, butyrate promotes reversion of cells from neoplastic to non-neoplastic phenotypes (Gibson GR et al 1992). The role of short-chain fatty acids in prevention of some human pathological states such as chronic ulcerative colitis and colonic carcinogenesis has been long suspected.

Interactions between gut bacteria and host immunity: The intestinal mucosa is the main interface between the immune system and the external environment. The dialogue between host and bacteria at the mucosal interface seems to play a part in development of a competent immune system. Microbial colonisation of the gastrointestinal tract affects the composition of gut associated lymphoid tissue. Immediately after exposure to luminal microbes, the number of intraepithelial lymphocytes expands greatly (Umesaki Y et al 1993; Helgeland L 1996) germinal centres with immunoglobulin producing cells arise rapidly in follicles and in the lamina propria (Cebra JJ et al 1998) and concentrations of immunoglobulin increase substantially in serum (Butler JE et al 2001). In mice and rats, a non-pathogenic and non-culturable segmented filamentous bacterium that preferentially attaches to Peyer's patch epithelium stimulates development of mucosal immune architecture and function (Umesaki Y et al 1995; Jiang HQ et al 2001).

Many and diverse interactions between microbes, epithelium and gut-associated lymphoid tissue are involved in modeling the memory mechanisms of systemic immunity. For instance, flora has been implicated in oral tolerance. The systemic response to a specific antigen can be abrogated after ingesting the same antigen. This effect persists for several months in mice with conventional flora, whereas in germ free mice systemic unresponsiveness persists for only a few days. The interaction between gut-associated lymphoid tissue and flora early in life seems to be crucial for appropriate development of complex mucosal and systemic immune regulatory circuits (Moreau MC, Routhiau GV 1996).

In adults, immunity may be constantly reshaped by persistent interactions between the host and its bacteria that take place in the gut. Commensal organisms try to circumvent the immune response. For instance, *Bacteroides fragilis*, a predominant species in the human colon, can change its surface antigenicity by producing distinct capsular polysaccharides (Krinis CM et al 2001). Surface diversity seems to allow the organism to escape immune surveillance and maintain an ecological niche of predominance in the intestinal tract. However, host defences adapt and keep an active control of bacterial growth.

The immune response to microbes relies on innate and adaptive components, such as immunoglobulin secretion. Most bacteria in human faeces are coated with specific IgA units (Van der Waaij LA 1996). Innate responses are mediated not only by white blood cells such as neutrophils and macrophages that can phagocytose and kill pathogens, but also by intestinal epithelial cells, which coordinate host responses by synthesising a wide range of inflammatory mediators and transmitting signals to underlying cells in the mucosa (Kagnoff MF and Eckmann L 1997) The innate immune system has to discriminate

between potential pathogens from commensal bacteria with use of a restricted number of preformed receptors.

Mammalian cells express a series of toll-like receptors, which recognise conserved motifs on bacteria that are not found in higher eukaryotes (Aderem A and Ulevitch RJ 2000). The system allows immediate recognition of bacteria to rapidly respond to an eventual challenge. For example, incubation of nonpathogenic bacteria with inflamed human intestinal mucosa elicits different types of immediate cytokine responses, which are transduced to the underlying tissue and promote changes in the phenotype of lamina propria lymphocytes (Borruel N et al 2002).

c) Protective functions: the barrier effect

Resident bacteria are a crucial line of resistance to colonisation by exogenous microbes and, therefore, are highly relevant in prevention of invasion of tissues by pathogens. Germ-free animals are very susceptible to infection (Baba E et al 1991; Taguchi H 2002). Colonisation resistance also applies to opportunistic bacteria that are present in the gut but have restricted growth. The equilibrium between species of resident bacteria provides stability in the microbial population within the same individual under normal conditions. However, use of antibiotics can disrupt the ecological balance and allow overgrowth of species with potential pathogenicity such as toxigenic *Clostridium difficile*, associated with pseudomembranous colitis (Van der Waaij D 1999).

Several mechanisms have been implicated in the barrier effect. *In vitro*, bacteria compete for attachment sites in the brush border of intestinal epithelial cells. Adherent non-pathogenic bacteria can prevent attachment and subsequent entry of pathogen entero invasive bacteria into the epithelial cells (Bernet MF 1996). Furthermore, bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by administering and consuming all resources

eg, in the genotobiotic mouse mono colonised with *Bacteroides* (Hooper LV 1999). The host actively provides a nutrient that the bacterium needs, and the bacterium actively indicates how much it needs to the host. This symbiotic relationship prevents unwanted overproduction of the nutrient, which would favour intrusion of microbial competitors with potential pathogenicity for the host. Finally, bacteria can inhibit the growth of their competitors by producing antimicrobial substances called bacteriocins (Brook I 1999; Lievin V 2000). The ability to synthesise bacteriocins is widely distributed among microbial collectivities of the gastrointestinal tract. The host can control production of such substances since most of them are protein compounds degradable by digestive proteases. Thus, the role of bacteriocins is mainly restricted to localised niches.

2.7 Modulating the composition of microflora- Probiotics, their types and mechanism of action

"Many species of bacteria have evolved and adapted to live and grow in the human intestine. The intestinal habitat of an individual contains 300–500 different species of bacteria. Of which, some has potential pathogenic properties and some bacteria (probiotics) have positive health effects in terms of prevention of gut infection or a reinforcement of innate defenses"

-Andrew L et al 2008

Probiotics, which means, 'for life', have been used for centuries as natural components in health promoting foods. The original observation of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Eli Metchnikoff, who in the beginning of the 20th century suggested that it would be possible to modify the gut flora and to replace harmful microbes by useful microbes (Metchnikoff E 1907). The term 'probiotics' was first introduced in 1953 by Kollath, contrasting antibiotics; probiotics were

defined as microbally derived factors that stimulate the growth of other microorganisms. In 1989 Roy Fuller suggested a definition of probiotics which has been widely used "A live microbial fed supplement which beneficially affects the host animal by improving its intestinal microbial balance". However recent most definition proposed by FAO/WHO joint report (2001) defines Probiotics are **"Living micro-organisms which when administered in adequate amount conferring health benefits of the host"**.

Furthermore, to be considered as a probiotics, an important criteria should be satisfied which states that 'bacteria's have probiotic properties when they have phenotype and genotype stability which includes plasmid stability, carbohydrates and protein utilization patterns, acid and bile tolerance survival and growth, bile metabolism, intestinal epithelial adhesion properties, production of antimicrobial substances, antibiotic resistance patterns, ability to inhibit known gut pathogens, spoilage organisms or both and immunogenicity.

There are varieties of probiotics which impart several beneficial functions, *bifidobacteria* and *lactobacillus* still remains the most abundant, benefitting most common known probiotics used in altering the host mechanisms.

a) Type of probiotics

Lactic acid bacteria (LAB)

The *lactobacillus* genus comprises a diverse group of Gram-positive bacteria; their most typical features include non-sporulation and lack of cytochromes; they are nonaerobic but aerotolerant, and have fastidious and acid-tolerant cocci and rods that produce lactic acid as a major fermentation end product. The genus *Lactobacillus* heterogeneous and contains species with 32-53% G+C content of the chromosomal DNA content, which is classified into three groups based on differences in sugar metabolism due to the presence or absence of fructose-1, 6-diphosphate aldolase and phosphoketolase. The *lactobacillus* species has

classified into six subgroups based on DNA sequencing homology and cell wall compositions. The six groups include A1 (*L. acidophilus*), A2 (*L. crispatus*), A3 (*L. amylovorus*), A4 (*L. gallinarum*), B1 (*L. gasseri*), and B2 (*L. johnsonii*) (Fujisawa T et al 1992; Johnson JL et al 1980). The most common intestinal *Lactobacillus* isolates are from the acidophilus group and include *L. salivarius*, *L. casei*, *L. plantarum*, *L. fermentum*, *L. reuteri* and *L. brevis* (Mitsuoka 1992). A study conducted across Europe whereby, the composition of the microbiota was determined for healthy adults and the elderly in four countries. It was found that *Lactobacillus-Enterococcus-Lactococcus-Enterobacteria* and *Eubacteria cylindroides* each composed less than 1% of bacteria in the study sample (n=230) (Mueller S et al 2006). Therefore, LAB are important functionally but not predominant numerically.

Bifidobacteria

Bifidobacteria are another key group of probiotics found in gastrointestinal tract, with typical bacterial counts of 10^9 - 10^{11} per gram of stool. They have been found in six distinct ecological niches including human oral cavity and gut, food, sewage, and the gastrointestinal tract of insects and animals (Ventura M et al 2004). The common human group isolates include *B. bifidum*, *B. longum*, *B. infantis*, *B. breve*, *B. adolescentis*, *B. angulatum*, *B. catenulatum*, *B. pseudocatenulatum*, and *B. dentium*. They share some phenotypic features with lactic acid bacteria, although the genus *bifidobacterium* is actually related to the *Actinomycetes* branch and have a high G+C content. Sugar metabolism of *Bifidobacterium* spp. is unique as they lack aldolase and glucose-6-phosphate dehydrogenase. Hexose sugars are exclusively degraded within the fructose-6-phosphate pathway by the key enzyme fructose-6-phosphate phosphoketolase. This enzyme is also used as a taxonomic tool to identify *Bifidobacterium* species; however, it cannot be used to discriminate between species (Lauer E and Kandler O 1993). *Bifidobacterium* spp. can utilize a wide range of substrates for fermentation including various hexoses (lactose, galactose, raffinose, sucrose, mannitol and sorbitol) and polysaccharides

(amylopectin, amylose, xylan and mucin). They can also metabolize substrates such as fructooligosaccharides, which selectively encourage their proliferation, resulting in positive shift in microbial ecology as *bifidobacteria* have positive reported health benefits, as do LAB (Gibson GR and Roberfroid MB 1995).

Depending on the type, substrate availability, host, environment and internal interactions these probiotics have been studied since long and have been found to impart several health benefits in arenas of gut and associated diseases. Recently the focus of potentially beneficial bacterias has been shifted to cure various metabolic conditions like diabetes, obesity, dyslipidemia, hypertension, cancer and many more. The highlights of benefitting in these diseases conditions have been summarized in Table 2.6.

Table 2.6: Health benefits of probiotics

S N	Name of the Researcher	Microbial species	Study population	Result highlights
1	Saavedra JM et al 1994	and	Chronically Hospitalized infants	Prevented diarrhoeal disease
2	Raza S et al 1994; Shornikova AV et al 1997; Guarino A et al, 1997; Shornikova AV et al 1997; Pedone CA 1999; Guandalini S et al 2000	L rhamnosus, Lactobacillus reuteri, or Lactobacillus casei	Children	Probiotic supplementation significantly decreased duration of diarrhoea.
3	Majamaa H et al 1995; Link-Amster H et al 1994	LAB	Rotavirus infected children and adults	Orally administered probiotics enhance d specific IgA responses Salmonella typhi in adults
S	Name of the	Microbial	Study population	Result highlights

N	Researcher	species		
4	Yadav H et al 2008	L. acidophilus, L. casei	Diabetic rats	Supplementation of probiotic dahi of STZ induced diabetic rats by inhibiting depletion of insulin
5	Elmer GW et al 1996	L. acidophilus, L. burgaricus	Hypercholesterolemic subjects (16)	Supplementation for 16 weeks reduced serum cholesterol by 7%
6	Aso Y et al 1992	L. casei	48 human subjects with tumor	Supplementation for 2 weeks reduced recurrence of tumor growth in bladder cancer
7	Matsuzaki T et al 2009	L. casei	AXN induced diabetic rats	Supplementation for 7 week reduced blood glucose levels
8	Cani PD et al 2007	Bifidobacterium	Diabetic rats	Supplementation significantly improved glucose tolerance, and decreased endotoxaemia
9	Geier MS et al 2007	L. fermentum BR11, B. lactis, S. thermophilus	DSS induced colitis rats	Probiotic feeding prevented distal colon crypt and reduced colitic symptom

b) Mechanism of action of probiotics

The mode of action probiotics is complex and not completely understood. There have been a large number of probiotic species identified, most of which have differing mechanisms of action. Further, complexity stems from the finding that the mode of action of a given probiotic can differ based on the presence of other probiotics or enteric bacteria in the surrounding environment, and also the disease setting in which the probiotic is used (Dieleman et al 2003; Shanahan et al 2004). There are however a number of common mechanisms that are evident in a wide variety of probiotic strains. One such mechanism is adherence to the intestinal mucosal surface which prevents colonisation of pathogenic bacteria (Guarner and Malagelada 2003). There is a form of competition between the two species. Evidence for this model systems for example, pre-incubation with *L. rhamnosus* GG has been shown to adherence of *B. vulgatus* to most epithelial cells (Dieleman et al 2003). A further common mode of action is via stimulation of the intestinal immune systems. Probiotics are believed to be involved in the modulation of cytokines and promoting production of anti-inflammatory cytokines (Dieleman et al 2003).

Probiotics are also believed to function via the modulation of cell proliferation and apoptosis (Ichikawa et al 1999; Yan and Polk 2002). The administration of 10^7 colony forming units/ml of LGG and *clostridium butyricum* in rats has been shown to increase epithelial cell proliferation rates in the small intestine, caecum and distal colon (Ichikawa et al 1999). This increase in epithelial cell proliferation is believed to be due to the capability of probiotic strains to produce short chain fatty acid (SCFAs) like acetate, propionate and butyrate via the fermentation of polysaccharides.

Lactobacill, *Bifidobacteria* produce strong acids, i.e. acetic and lactic acid the production of these acids reduces intestinal pH. One effect of lowering the

gastrointestinal pH might be the production of toxic ammonia (NH_3) to produce ammonium ion (NH_4^+), which is non-diffusible and could result in lower blood ammonia levels and a reduced hepatic load (Levrat et al 1993, Miller-Catchpole 1989).

Acetic acid has been observed to exert a greater antimicrobial effect than lactic acid, most likely due to a greater amount of undissociated acid at intestinal pH values (5.8) common to *Bifidobacteria* and *Lactobacilli* (Moller PL et al 2005). Because *Bifidobacteria* produce almost two-fold more acetate than lactate, the undissociated acetic acid would be approximately 11-fold greater than lactate. This is an important factor as the growth of many potential pathogenic bacteria is very sensitive to concentrations of undissociated acid (Moller PL et al 2005).

The anti-apoptotic effects of probiotics have been demonstrated in an *in vitro* model, in which LGG has shown to prevent apoptosis in human and mouse colon cells (Yan and Polk 2002). There are opposition views as to the potential for probiotics to alter the gut microbiota in normal and diseases gut. Tannock (2005) suggested that it is unlikely that probiotics could significantly alter the gut microbiota as even when administered in large numbers, the probiotic strain could account for approximately 1% of the total bacterial count. However, numerous studies have indicated the probiotic administration can indeed alter the composition of the intestinal microbiota in animals (Gaudier et al 2005) and in humans (Cui et al 2004; Kuechbacher et al 2006).

Accumulating evidence indicates that the gut microbiota has a crucial role in conditions including obesity, diabetes, CVD, Inflammatory bowel diseases, diarrhoea, colitis, immune conditions and even cancer. However, continued long term administration of probiotics may resolve the complex metabolomics of the gut friendly bacteria.

2.10 Prebiotics, and their types

"A wealth of information has been gathered over the past decade on prebiotics through experimental, animal and human studies and also on their organoleptic properties in food industries. However, information is still scanty. Therefore, novel researches need to be done with an aim to understand the mechanism of actions and elucidate their beneficial health effects to the human host"

In 1995, Gibson and Roberfroid defined prebiotic as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon, and thus improves host health". This definition only considered microbial changes in the human colonic ecosystem. Later, it was considered timely to extrapolate this into other areas that may benefit from a selective targeting of particular microorganisms and to propose a redefined definition of a prebiotic as "A selectively fermented ingredient that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits" (Gibson et al 2004). These definitions have attracted, and still continue to attract, a great deal of interest in the field of nutrition both in scientific and in food applications.

A food ingredient can be termed as 'prebiotic' when it can be classified under following criteria

- Resist gastric acidity
- Is not hydrolyze by mammalian enzymes
- Is not absorbed in the upper gastrointestinal tract
- Is fermented by the intestinal microflora

- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being (Gibson and Roberfroid 1995).

Although probiotic and prebiotic approaches are likely to share common mechanism of action, as their effect is impacted through increase in beneficial colonic bacteria, they differ in composition and metabolism. Prebiotics are found naturally in some plants or are produced enzymatically from sucrose, and often are used in dietary supplements. However, the prebiotic property has been demonstrated adequately for only a few food ingredients. These include non-digestible carbohydrates, which are often described as soluble fibers, include non-starch polysaccharides, resistant starches and soluble oligosaccharides. The classification of natural and also some synthetic prebiotic types is listed in Table 2.7.

Table 2.7: Classification of Prebiotics

Classification	Origin/ Manufacturing process
Disaccharides	from lactose, synthetic
Lactulose	from lactose, synthetic
Lacticol	
Oligosaccharides	Legumes, vegetables, cereals
Fructose Oligosaccharides (FOS)	Extraction/hydrolysis
Soyabean Oligosaccharides	Soyabean
(Trans) Galactooligosaccharides	Extraction/hydrolysis
Inulin	From lactose, Synthetic
	Legumes, vegetables, cereals
	Extraction
Polysaccharides	Legumes, vegetables, cereals
Resistant Starch	Extraction

2.11 Evolution of Fructooligosaccharide (FOS) as a prebiotic

"Inulin and oligofructose have the potential to elevate indigenous bifidobacteria and lactobacilli levels in the colon and thus influence the whole body's physiology and consequently health and well-being"

-Conway, 2001

a) Inulin type fructans: chemistry and nomenclature

Inulin-type fructans are natural components of several edible fruits and vegetables, and the average daily consumption has been estimated to be between 3 and 11 g in Europe (Van Loo J et al 1995) and between 1 and 4 g in the United States (Moshfegh AJ et al 1999). The most common dietary sources are wheat, onion, banana, garlic, and leek. Chemically, inulin-type fructans are a linear polydisperse carbohydrate material consisting mainly, if not exclusively, of β -(21) fructosyl-fructose linkages (Waterhouse AL and Chatterton NJ 1993). A starting α -D-glucose moiety can be present but is not necessary. GpyFn [glucopyranosyl- (fructofuranosyl)n-fructose] and FpyFn [fructopyranosyl- (fructofuranosyl)n-fructose] compounds are included under that same nomenclature; they are both a mixture of oligomers and polymers that are best characterized by the degree of polymerization (DP), either as the average (DPav) or the maximum (DPmax) value.

The plant that is most commonly used industrially for the extraction of inulin-type fructans belongs to the Compositae family, i.e., chicory. Native chicory inulin is a nonfractionated inulin extracted from fresh roots (De Leenheer L 1996). Because of the β -configuration of the anomeric C2 in its fructose monomers, inulin-type fructans resist hydrolysis by human small intestinal digestive enzymes, which are specific for α -glycosidic bonds. They have thus been classified as "nondigestible" oligosaccharides (Delzenne N and Roberfroid

MB 1994; Roberfroid MB et al 2000). Structure of inulin and fructooligosaccharide has been displayed in Figure 2.26.

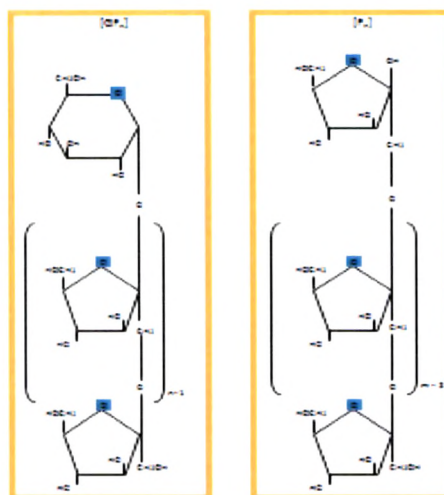


Figure 2.26: Structure of inulin (left) and Fructooligosaccharide (right)

Fructooligosaccharide formation from inulin

i) Partial enzymatic hydrolysis of inulin using an endoinulinase

The DP of chicory inulin varies from 2 to 60 units with a DP_{av} $\frac{1}{4}$ 12. About 10% of the fructan chains in native chicory inulin have a DP ranging between 2 (F2) and 5 (GF4). The partial enzymatic hydrolysis of inulin using an endoinulinase produces oligofructose that is a mixture of both GpyFn and FpyFn molecules, in which the DP varies from 2 to 7 with a DP_{av} $\frac{1}{4}$ 4 (Roberfroid M 2005). It is composed primarily of lower-DP oligosaccharides, namely, 1-kestotriose, 1,1-kestotetraose, and 1,1,1-kestopentaose, as well as inulobiose, inulotriose, and inulotetraose.

ii) Enzymatic synthesis (transfructosylation)

Oligofructose can otherwise be obtained by enzymatic synthesis (transfructosylation) using the fungal enzyme β -fructosidase from *Aspergillus niger*. In that reaction, in a process similar to the plant biosynthetic pathway,

sucrose serves as a substrate to which 1, 2, or 3 additional fructose units are added by forming new β -(2)1 linkages. In such a synthetic compound, DP varies from 2 to 4 with DP_{av} $\frac{1}{4}$ 3.6, and all oligomers are of GpyFn type. By applying physical separation techniques, it is also possible to eliminate all oligomers with DP ≤ 10 to produce a highmolecular- weight inulin-type fructan or inulin HP, a mixture of GpyFn molecules with a DP ranging from 10 to 60 and DP_{av} $\frac{1}{4}$ 25. A mixture of 2 distinct populations of the low-molecular weight oligofructose and the high-molecular-weight inulin is known as oligofructose-enriched inulin or Synergy. It is a unique type of oligosaccharide (Roberfroid M 2005).

Even though the inulin hydrolysate and the synthetic compound have slightly different DP_{av} (4 and 3.6, respectively), the term oligofructose shall be used to identify both. Indeed, oligofructose and (short-chain) fructooligosaccharides are considered to be synonyms to name the mixture of small inulin oligomers with DP_{max} ≤ 10 (Quemener B et al 1994; Coussement P 1999; Roberfroid MB and Delzenne N 1998; Perrin S et al 2001; Roberfroid M 2002). Moreover, as outlined by Farnworth (Farnworth ER 1993), "although the initial findings (on the effects of inulin) were based on Neosugar (the synthetic or so-called short-chain fructooligosaccharide), it has become evident that many of the conclusions extend to other sources of dietary fructans and especially inulin and inulin derivatives." Synergy will be used to identify the 30/70 mixture (wt:wt) of oligofructose and inulin HP.

Fructooligosaccharide (FOS) are plant carbohydrates that, because of the β -(2)1 configuration of the fructosyl-fructose glycosidic linkages, resist digestion in the upper gastrointestinal tract but are quantitatively fermented in the colon. They are thus undoubtedly part of the dietary fiber complex, and they must be labeled as dietary fiber on consumer food products. However, because of their specific fermentative properties, FOS does have characteristic features different from

those of other dietary fibers. Therefore, they may contribute in a significant way to deliver many health benefits.

b) Safety and tolerance of Fructooligosaccharide (FOS)

Fructooligosaccharide and inulin are a significant part of the daily diet of most of the world's population. Daily intakes for the U.S. and Europe have been estimated at up to 10 g, specifically 1–4 g for the 97th percentile in the U.S. Because both inulin and oligofructose are macroingredients, it is difficult to apply classical toxicology tests. Although some high dose animal tests have been performed, none have revealed any toxic effects. The safety of inulin and oligofructose for use in foods was evaluated by many legal authorities worldwide. As a result, both inulin and oligofructose are accepted in most countries as food ingredients that can be used without restrictions in food formulations. In the U.S., a panel of experts performed a generally accepted as safe (GRAS) Self-Affirmation Evaluation in 1992 and concluded similarly. At high doses, increased flatulence and osmotic pressure can cause intestinal discomfort. These doses vary widely from person to person and also depend on the type of food in which inulin or oligofructose is incorporated. With regard to labeling, both inulin and oligofructose are gradually being accepted as "dietary fibers" in most countries around the world. The mention of their "bifidogenic effect" on food labels has also been legally accepted in several countries (Paul AAC 1999). According to the U.S., FDA notice on GRAS of FOS with notice number GRAS Notice No. GRN 000118, based on the proposed uses FDA estimates that dietary intake of inulin at the 90th percentile level would be approximately 6 grams per day for infants less than one year of age, approximately 15 grams per day for infants one year of age, and approximately 20 grams per day for the general population (i.e., two years of age and older) (Kruger CL 2003).

c) Caloric value of fructooligosaccharide (FOS)

Longer chain native oligosaccharides (Inulin) and shorter chain synthetic fructooligosaccharides (Neosugar)-GF2,GF3,GF4 reach the large intestine virtually intact and, as such, were considered not to be a major source of energy (Oku et al 1984). Furthermore, in the rat model, there appear to be no hydrolytic enzymatic adjustments in the small intestine to long-term ingestion of these factors. Nilsson and others (1988 a,b) used oral intubation to give fructans with a DP of about 9 or DP 16 to rats and found that both proceeded as undigested material through the gastrointestinal tract to the colon. However, due to the bacterial fermentation that occurs in the colon, these oligosaccharides do contribute to the energy pool. The caloric value of a fructosyl unit of oligofructose is calculated at 30 to 40% of a digested fructose molecule or between 1-1.5 kcal/g (Roberfroid et al 1993). Ranhotra and coworkers (1993) reported a caloric value for oligofructose of 1.48 kcal/g. They determined usable energy value based on efficiency of conversion of gross food energy to net energy (carcass energy) using young rats as the test model. Molis et al 1996 further defined the energy value of fructooligosaccharides (44% GF2; 46% GF3; and 10% GF4) working with six healthy human subjects. Calculated mean energy value of the fructooligosaccharide was 9.5±0.6 kJ/g (range: 8.3-11.7 kJ/g) or about 2 kcal/gram. For nutrition labeling purposes, Roberfroid (1999) recommends that inulin and oligofructose, as well as all nondigestible oligosaccharides that are mostly fermented in the colon, be assigned a caloric value of 1.5 kcal/g (6.3 kJ/g).

d) Legal classification of fructooligosaccharide (FOS)

Fructooligosaccharide (FOS) and inulin are legally classified as food or food ingredients, and not as additives, in all countries in which they are used. Although this seems evident if one considers the nutritional properties and the

use of both substances, it has not been easy to obtain confirmation of this legal status from many of the legal authorities in the world. As a consequence, neither inulin nor oligofructose are listed as accepted food additives in the standard positive lists from the European Union or from Codex Alimentarius. EU Directive EC 95/2 explicitly lists inulin as a substance that is not an additive. The EU Standing Committee meeting of June 1995 confirmed that oligofructose is a food ingredient. In Europe, both inulin and oligofructose were brought to market long before the Novel Foods Regulation (EC 258/97) came into force. Since 1987, Orafit has applied for authorization as a food ingredient for both substances in all European countries separately. In most countries, the files were submitted to the Superior Health Council (or the corresponding government body) for advice. None of the European countries has ever expressed reservations with regard to the safety of inulin or oligofructose. In all countries, both substances are accepted for food use without limitations. No ADI were fixed. In the U.S., a committee of experts convened by Orafit declared both inulin and oligofructose as Generally Recognized As Safe in 1992 (Kolbye et al. 1992)

As a follow-up of International Life Science Institute (ILSI North America 1994), mini-workshop on complex carbohydrates, a workshop was held at the AOAC International meeting in Nashville in (1995) to determine if there was agreement among representative scientists as to a definition of complex carbohydrates and dietary fiber (AOAC International Workshop 1995). There was general agreement among the workshop participants that dietary fiber should be included in the definition of complex carbohydrates, but more importantly, they agreed that resistant oligosaccharides, namely, inulin and oligofructose, be included in the dietary fiber complex. The results of three AOAC International surveys also supported the expansion of the dietary fiber definition to include resistant oligosaccharides (Prosky L 1999). Further, representatives of the FDA in December of 1995 stated that they would consider inulin and oligofructose as

dietary fiber if the method for their measurement would pass the scrutiny of an AOAC International collaborative study.

2.12 Fructooligosaccharide (FOS) and its technological functions

"An increasing important determinant in food choice is the growing consumer concern about nutrition and health. Therefore, the food industry can contribute by reducing the number of energy dense products; by improving the nutrient profile and development of health focused designer and functional foods"

-Nehir S EI & Simsek S 2011

Over the last 20 years, there has been a significant interest, by both consumers and food manufacturers in the production and consumption of prebiotics in daily diet (O'Sullivan 2001; Bruno and Shah 2004) as consumers are becoming more aware of maintaining their 'internal health' by modifying gastro intestinal flora. The main objective of prebiotic is to improve the gut microflora through dietary means. Using prebiotic in food formulation process has some advantage over the probiotic strategy as they could reduce the problem of keeping the organisms alive during transit through upper gastrointestinal tract as well as during storage (Crittenden 1999). Thus, the prebiotic approach involves the interaction of a non-digestible food ingredient and beneficial micro-organism such as *bifidobacteria* and *lactobacillus* in the human colon.

Among the prebiotics researched so far fructooligosaccharide and inulin hold the key position in the food industry because of its interesting technological characteristics. Refined native inulin powder from chicory is white, amorphous, and slightly hygroscopic; has a specific gravity of about 1.35 and an average molecular weight of about 1,600. It is neutral in odor and taste. Commercial inulin contributes a marginally sweet taste due to a small amount of naturally

occurring mono-and disaccharides. OFS is soluble in water with the solubility dependent on the temperature of the water, degree of polymerization, distribution of the molecular chains, degree of molecular branching and how the molecule is processed. FOS exhibit minimal influence on the organoleptic characteristics of a product and possesses nutritional benefits and health claims.

a) FOS as a fat replacer

Specific kinds of prebiotics such as oligosaccharides and inulin have been developed as fat replacers and texture modifiers as they would be able to 1) reduce total fat or partial fat content, 2) modify smoothness and creaminess, 3) increase perception of body and richness, 4) improve an overall eating quality and an acceptable appearance. Oligofructose is well-recognized prebiotic for its ability to replace fat in the manufacturing of low-calorie foods (Silva, 1996; Franck, 2000). When oligofructose is mixed with water, it forms gels composed of a tri-dimensional gel network of insoluble sub-micron crystalline OFS particles with large amounts of immobilized water. This OFS gel provides the same texture and mouth feel as fat (Silva 1996; Franck 2000).

Fat replacement by oligofructose and inulin is successfully applied in most water-based foods such as dairy products, frozen desserts, dressings, table spreads, sauces, soups and even meat products, but not in dry foods such as snacks, bakery and confectionery products (Murphy, 2001). Typically, 1 g of fat can be replaced by a 0.35 g of FOS or inulin in most foods (Coussement 1999). Formulating foods with OFS and inulin also helps to maximize freeze-thaw stability and minimize emulsion separation phased due to its ability to immobilize water and to work with most gelling agents such gelatin, gellan gum, and maltodextrin (Bishay, 1998). OFS also gives a richer texture to liquid products and spreads and provides crispness and expansion to extruded snacks and cereals.

The increasing demand of low fat food has led the food industry to develop or modify traditional food products to contain less fat. In this regard, a number of studies have been conducted to investigate the effect of replacing fat or oil with oligofructose and inulin in foods. Schaller LA and Smith DE (1999) developed ice cream using inulin corn syrup as fat replacer at 50% and 100% levels. Results revealed that replacing inulin corn syrup with fat increased in chewiness, texture, and storage life of the ice-cream was also increased by 6 weeks. Another study aimed to develop biscuits containing reduced fat which exhibits functional properties. Raftilose (fructooligosaccharide) was used as a fat replacer and the results elicited a 25% reduction in fat in FOS incorporated biscuits (Gallagher E et al 2003).

Role of FOS as a fat replacer in meat products has also been studied by many researchers. In a study conducted by Caceres E et al (2004) on sensory properties of reduced-fat cooked meat sausages has been studied and the results depicted a 40% less fat reduction and a low caloric value of the product. A study on similar lines revealed a 6-22% reduction in fat content in German type mortadella sausage formula which was replaced with oligofructose at different concentrations (Nowak B et al 2007). A couple of studies revealed that FOS incorporated fermented and cooked sausages exhibited around 15%-50% reduction in fat and increased sensory properties of the final product (Salazar P et al 2009; Santos BAD 2012).

A study has also focused on sensory properties of chocolate with incorporation of FOS as a fat replacer. Farzanmehr H and Abbasi S (2009) carried out a study on inulin as a bulking agent and fat replacer in milk chocolate and results of the study elicited that chocolate made from inulin were accepted with better sensory and textural attributes and replaced fat by 5% in comparison to the control chocolate. A more recent study on handmade chocolate and nut bar replaced FOS with fat at different levels and revealed good acceptance and textural

properties (Folly de G et al 2013). Together all these findings indicate that FOS and inulin are good replacers of fat and can be used in variety of food products.

b) FOS as a sugar replacer

Several types of non-digestive oligosaccharides and polyols can be used as sugar replacers due to their physiological characteristics such as having minimal contribution to energy intake and performing bulking properties (Frank 2000).

Oligofructose delivers some functional properties similar to glucose syrup and is often used to replace sugar in various foods, mainly dairy and bakery products such as chocolate filling biscuits, chewing gums, confectionary, dairy desserts, and ice-cream (Franck 2000). In addition, oligofructose depresses the freezing point of frozen desserts and acts as a binder in nutrition bars, in much the same way as sugar. The solubility of oligofructose is higher than sucrose but its sweetness is about 30% of sucrose. In combination with other sweeteners such as aspartame, oligofructose can provide a desired sweetness and better flavour profile (Kaur and Gupta, 2002). More importantly, these low-calorie ingredients offer advantages over traditional digestible carbohydrates such as sucrose, glucose and fructose in terms of having low glycemic index (especially helpful for those patients with diabetes, heart problems and obesity) (Hidaka et al 1991; Schumann 2002).

FOS as a sugar substitute has been tried out in a variety of food products like peanut brittle, chocolate chips, chocolate coating, nougat, ginger snap cookies, cheese cake and all the products adjudged to be satisfactory (Barndt RL et al 2003). An Indian study on preparation of low calorie gulab jamun revealed that FOS is a potent low calorie healthier alternative over sucrose without altering any of the sensory characteristics (Renuka B et al 2004). Another study carried out by Renuka et al (2009) prepared fruit juice beverage with fortification of FOS

as a low calorie prebiotic sweetener and showed that FOS did not affected the sensory attributes of the beverages and even the shelf life increased with an addition of FOS. A recent study in conducted by Choonhahirun A and Akesowan A (2012) prepared *thai* pandanus custard replaced with cocnut milk and sugar with soy milk and inulin and were compared with quality characteristics of the regular formulation (control). Custard replaced 5% inulin as sugar replacement presented desirable characteristics in relation to regular formation. The product has about 20.7% reduction in energy and storage time was also increased by 3 weeks.

The above researches illustrate the ability of FOS as a highly effective sugar substitute in a huge range of food products.

c) *FOS as a texture modifier*

FOS owe a high solubility in water over 'classical fibres' it can be used to fortify dairy products such as milk drinks, yoghurt, cheeses and desserts, which have been traditionally difficult to fortify (Niness 1999). The functionality of oligofructose or inulin is based on its effect on water solutions at various solid levels. At lower concentrations it causes significant viscosity increase and can be used as a rheology modifier. At a concentration of 40-45% a gel or cream is formed which is firm but with a fatty creamy feel. This cream offers many advantages. Water is tightly bound in the gel, helping to maximize freeze-thaw stability and to inhibit syneresis. In this form oligofructose and inulin are stable in acidic conditions or at high temperatures owing to lack of available water (Silva 1996).

Production of yogurt using oligofructose and inulin has been used in the food industry since long. A study on fat free plain yogurt prepared by Aryana KJ et al (2007) revealed that inulin and *L. acidophilus* containing yogurt had significantly

lower pH than the remaining yogurts, high flavor scores, less synergesis, better body and texture. Preparation of gluten free bread with combination of inulin, oligosaccharide syrup and bitter free chicory flour with 3%, 5% and 8% additions exhibited best effects on sensory features with medium level of 5% incorporation (Korus J et al 2008). A most recent study on development of prebiotic yogurt revealed that addition of 2%, 4%, 6% and 8% FOS increased physiochemical, rheological and microbiological characteristics of low calorie yogurt (Cruza AG 2013).

Chocolate mousse prepared by addition of inulin and *L. paracasei* showed an advantageous, conferring potentially effective as it enhanced textural and sensory properties of the product. Inulin addition also increased the storage duration by 28 days (Cardarelli HR et al 2008).

Oligofructose is also used in bakery products for improved textural characteristics. Orange cakes with addition of inulin and fructooligosaccharide were investigated regarding sensory aspects and the findings depicted that cakes with addition of prebiotic (3g in 60 g serving of cake) presented greater crust brownness and dough beigeness. It implies that addition of prebiotic in orange cakes is feasible, based on the sensory result which may facilitate marketing of this functional food with sensorial qualities equivalent to conventional products (Larissa FVP et al 2012).

Using oligofructose in food formulations allows the nutritional value of the end product to be improved by increasing textural and organoleptic features, dietary fibre content, reducing the calorie and fat content and increasing the *bifidus*-promoting capacities. As more and more scientific data become available, the nutritional benefits of inulin became further apparent. This ingredients offer a unique combination of nutritional and technological advantages. Therefore it is

often taken as practical illustrations of active food ingredient for 'functional foods' (Van Loo et al 1999).

2.11 Role of Fructooligosaccharide fermentation in the gut

"Nutritional interest in inulin and oligosaccharide lies in the fact that the selectively stimulate the growth of lactobacillus and bifidobacteria in the human and well as in animals, which has long been regarded as beneficial to health"

-Gibson and Roberfroid 1997

a) Role of Fructooligosaccharides in enhancing the gut microbiota

The fermentability of FOS and inulin by fecal bacteria has been extensively investigated in several *in vitro* models (Langlands SJ et al 2004; Vuyst de Luk and Leroy F 2011). Wang and Gibson (1993) determined *in vitro* the prebiotic efficacy of FOS and inulin as compared to a range of reference carbohydrates (starch, polydextrose, fructose and pectin) in 12 h batch cultures with mixed populations of gut bacteria. Bacterial growth data showed preferential fermentation by *bifidobacteria* while populations of *Escherichia coli* and *Clostridium perfringens* remained at relatively low levels, which showed the Bifidogenic properties of this prebiotic. Shin HS et al (2000) cultured two commercial strains of *Bifidobacterium* spp (Bf-1 and Bf-6) in 12%(w/w) non-fat dry milk containing 0.5, 1.0, 3.0 and 5.0%(w/v) fructooligosaccharide (FOS), galactooligosaccharide (GOS) and inulin. Inoculated samples were incubated anaerobically at 37°C for 48 h. Growth and activities of the cultures were determined. Viability of each strain was assessed after 4 weeks of refrigerated storage at 4°C. Growth promotion, enhancement of activity and retention of viability were greatest when *Bifidobacteria* Bf-1 and Bf-6 were grown in the presence of FOS followed in a

descending order by GOS and inulin. The effects of oligofructose and inulin increased with increasing carbohydrate concentration and was maximal at 5%(w/v).

The degree of polymerisation of oligofructose is also important in affecting the level of growth and viability of bacteria. For instance, in a study β -fructofuranosidase gene from *Bifidobacterium lactis* was identified and characterised. This gene showed high identity with a similar gene in *Bifidobacterium longum*. The deduced enzyme showed maximum activity towards oligofructose, to a lesser extent to inulin and a minimum activity towards long chain inulin. From this data it appears that the characterized enzyme is highly selective for oligofructose and has a high affinity towards $\beta(2\rightarrow1)$ fructosyl-linkages, and that its specificity decreases as the degree of polymerization (DP) of the fructan increases (Janer C et al 2004).

Experiments with a three-stage continuous culture model of the human colon (*in vivo*) further confirmed the Bifidogenic effect of FOS (Gibson GR and Wang X 1994). Karpinen et al (2000) compared the fermentability of inulin by human fecal bacteria of the rye, wheat, oat bran in non pH controlled batch cultures. Inulin was the most rapidly fermented of the test substrates giving the highest butyrate production. In a 2 week study upon the effects of 4g/day FOS on 10 healthy individuals, Williams et al (1994) reported a significant increase in *bifidobacteria* levels and an increase in *lactobacilli* in six volunteers. In a similar study, Buddington et al (1996) investigated the influence of FOS supplementation on the fecal microflora composition of 12 healthy adult humans. Subjects were fed a controlled diet for 42 days, which was supplemented with 4g/day FOS. The controlled diet increased *bifidobacteria* levels but the highest increase was observed during FOS supplementation.

Tuohy et al (2001) further used fluorescent in situ hybridization (FISH) to investigate the prebiotic efficacy of biscuits delivering 6.6 g/day short chain FOS

(scFOS) in a double blind, placebo-control study of 31 healthy adults. A significant increase in *bifidobacteria* levels was observed at the end of supplementation. Bouhnik et al (1999) assessed the tolerance and threshold dose of scFOS which significantly increased fecal *bifidobacterial* counts in an 8-day study of 40 healthy human volunteers. Volunteers were divided into six treatment groups each given a treatment between 0 and 20g/day scFOS. They reported that the optimal dose for increased bifidogenesis without significant side effects, such as flatulence, was 10g/day. Most recent both *in vitro* and *in vivo* studies on Bifidogenic properties of FOS on humans also exhibit the similar results of increasing *bifidobacteria* (Mendlik K et al 2012; Boler BV 2013).

b) Role of Fructooligosaccharides in production of Short chain fatty acid

Upon reaching the large intestine FOS is preferentially utilized by a group of healthy bacteria, *bifidobacteria* and *lactobacilli*, that are present in the ceco-colon (Hartemink and Rombouts 1997). The gastrointestinal tract is an extremely complex ecosystem containing about 10^{11} CFU (colony forming units) of bacteria per gram of intestinal content. This large population of bacteria plays a key role in the nutrition and health of the host (Roberfroid M 2000). The colonic microbiota ferments organic material that cannot be digested otherwise by the host in the upper gut. These include resistant starch, non-digestible carbohydrates (fructans) as well as some proteins and amino acids (Topping D and Clifton P 2001). The main products of fructans metabolism in the colon are linear SCFAs, mostly acetate (C2:0), propionate (C3:0) and butyrate (C4:0) (Gibson G 1999; Topping D and Clifton P 2001; Cummings J and Macfarlane G 1991). However, other fermentation products may be lactate, succinate as well as ethanol (Roberfroid M et al 1998), which are sometimes only intermediates in the global process of carbohydrates fermentation by the microbiota, and are metabolized in varying degrees to SCFAs by interactions and/or collaboration of

present bacteria in the ecosystem, so that generally do not accumulate to any significant extent in the colon (Bernalier A et al 1998). Fructans fermentation also produces a few gases as CO₂, CH₄, H₂ and additionally heat (Flamm G et al 2001; Topping D and Clifton P 2001). The presence of both, non-digestible carbohydrates and SCFAs in the colon can positively alter the colonic physiology drastically (Wong J et al 2006).

Various studies on microbial population have shown that SCFAs production is in the order of C₂:0 > C₃:0 > C₄:0 in a molar ratio of approximately 60:20:20 mainly in the proximal and distal colon (Cummings J et al 1997). An increased in SCFAs synthesis also creates a more acidic environment in the gut, which is important *in vivo* in terms of colonization resistance against pathogens (Roberfroid M 2001; Gibson GR 1999). The production of SCFAs is affected by many factors, including the source of substrate (Cook S and Sellin J 1998), in particular, the chemical composition of the fermentable substrate, the amount of substrate available, its physical form (e.g. particle size, solubility, association with undigestible complexes such as lignin) (Macfarlane G and Macfarlane S 2007), the bacterial species composition of the microbiota (Roberfroid M 2005) ecological factors (competitive and cooperative interactions between different groups of bacteria) and intestinal transit time (Cummings J et al 1997).

The gut of human or mice comprises four sections: caecum, proximal, transverse (medial) and distal colon. The caecum and proximal colon are the main sites where fermentation is carried out, given the number of bacteria and the availability of substrate, because as it moves through the intestine toward the distal colon, there is a lower concentration of water as well as a depletion of carbohydrates and increased pH (Bernalier A et al 1999). SCFAs are rapidly absorbed in the caecum and colon being excreted in the faeces only from 5% to 10% of them (Wong J 2006). The major SCFAs (C₂:0, C₃:0 and C₄:0), are absorbed at comparable rates in different regions of the colon. Once absorbed, SCFAs are

metabolized at three major sites in the body: 1) cells of the caecum-colonic epithelium that use C4:0 as a major substrate for maintenance-energy; 2) liver cells that metabolize residual C4:0 and C3:0 used for gluconeogenesis and 50% to 70% of C2:0 is also taken up by the liver; and 3) muscle cells that generate energy from the oxidation of residual C2:0 (Van Loo J et al 1995).

C2:0 is the principal SCFA produced in the colon, this is readily absorbed and transported to the liver, and therefore is less metabolized in the colon (Cook S and Sellin J 1998). The presence of acetyl-CoA synthetase in the cytosol of adipose and mammary glands allows the use of C2:0 for lipid metabolism 166 lipogenesis once it enters the systemic circulation (Wong J et al 2006). C2:0 is the primary substrate for cholesterol synthesis. In the host, it may be absorbed and utilized by peripheral tissues also. On the other hand, C3:0 is produced via two main pathways: 1) by fixation of CO₂ to form succinate, which is subsequently decarboxylated (the "dicarboxylic acid pathway") and 2) forms lactate and acrylate (the "acrylate pathway") (Cummings J 1981). C3:0 is also a substrate for hepatic gluconeogenesis and it has been reported that this acid inhibits cholesterol synthesis in hepatic tissue (Venter C 1990; Cheng H and Lai M 2000). The ratio of C3:0 to C2:0 in the colon is relevant since it lowers cholesterol synthesis coming from the C2:0 pathways (Cheng H and Lai M 2000).

Finally, C4:0 is the preferred fuel by the colonic epithelial cells but also plays a major role in the regulation of cell proliferation and differentiation (Topping D and Clifton P 2001). It is the most important SCFA in colonocytes metabolism, where 70% to 90% of C4:0 is metabolized by the colonocytes. C4:0 is used preferentially over C3:0 and C2:0 in a ratio of 90:30:50 (Cook S and Sellin J 2001). Approximately 95% of the C4:0 produced by colonic bacteria is transported across the epithelium, but concentrations in portal blood are usually undetectable as a result of a rapid utilization (Pryde S et al 2002). C4:0 production might also occur through the use of other fermentation products such

as C2:0 or lactate that can act as precursors of C4:0 (García AH and Lopez G 2013) (Figure 2.27).

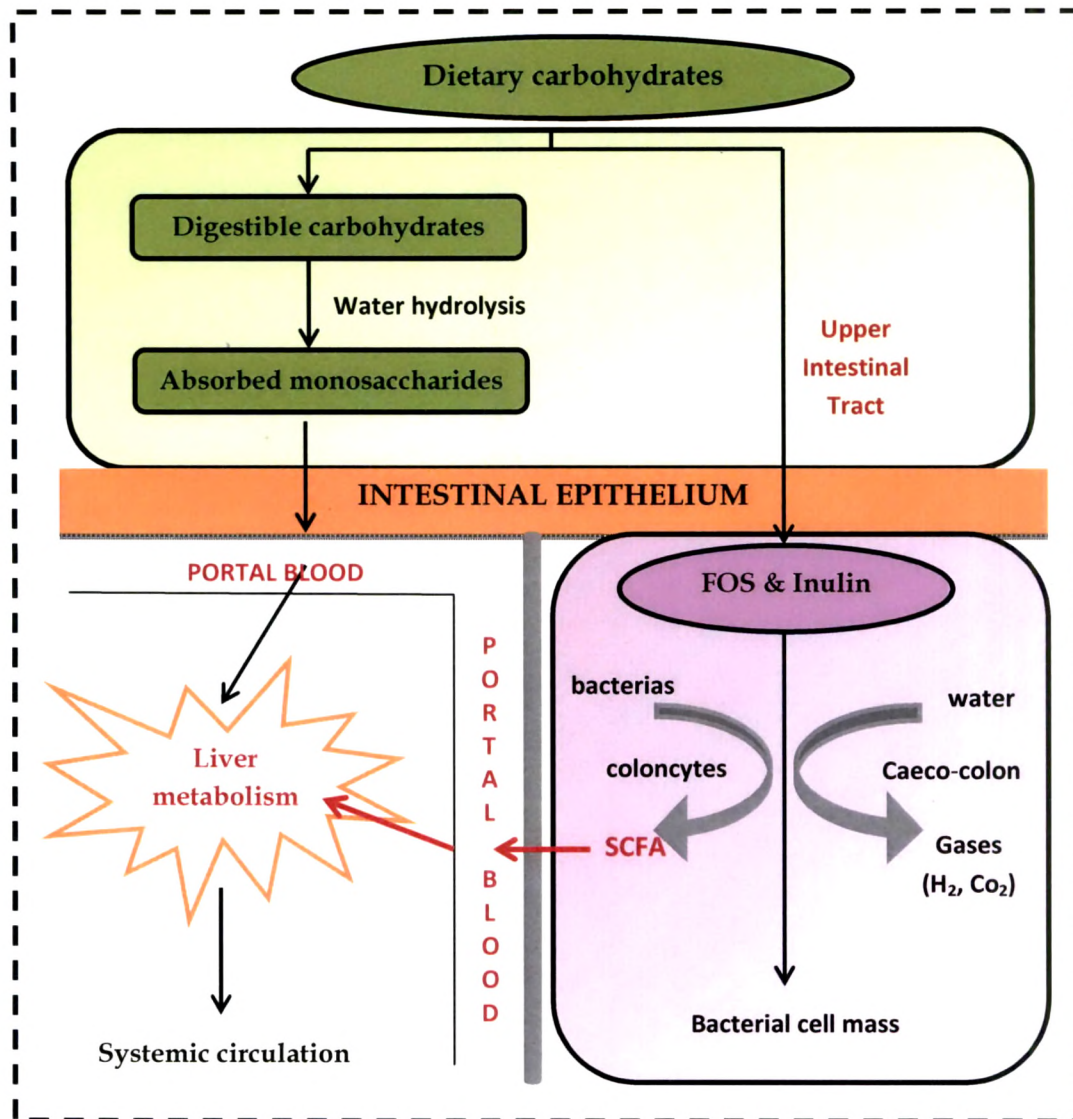


Figure 2.27: Schematic of production and bioavailability of SCFA from FOS/inulin

Most *in vitro* and *in vivo* studies show that oligofructose serve as an excellent substrate for the production of short chain fatty acids. Amongst them few studies revealed that the highest levels of C3:0 acid were found in caecum and proximal

and distal colon of rats fed with inulins, whereas the highest levels of C4:0 acid were found in caecum and proximal and distal colon of rats fed with FOS (Nilsson and Nyman 2005). Similar results were obtained by Licht et al (2006) who fed rats with different dietary carbohydrates. These authors concluded that C3:0 acid concentrations reached statistical significance in animals fed with inulin, whereas the concentration of C4:0 acid was significantly higher in animals receiving FOS.

Van de Wiele et al (2007) compared the fermentation of FOS and inulin *in vitro* with faecal inoculum, observing that FOS and inulin increased the production of SCFAs by about 30%. This increment in SCFAs production was attributed to an increase mainly in C3:0 and C4:0 acids. Where inulin showed a higher production of C3:0 acid (almost 2-fold) than FOS. The authors concluded that these differences correlated well with the structural differences, FOS has a short DP and inulin a long DP.

This tremendous increase in short chain fatty acids by the endogenous bacterias has several health implications in various metabolic conditions.

2.12 Health implications of FOS

"Recent use of FOS as a food ingredient has stimulated much research to know its functionality and its effects on human health. Thus, its potentially beneficial effects in preventing and controlling some diseases have been extensively discussed"

-Conterno L et al 2011

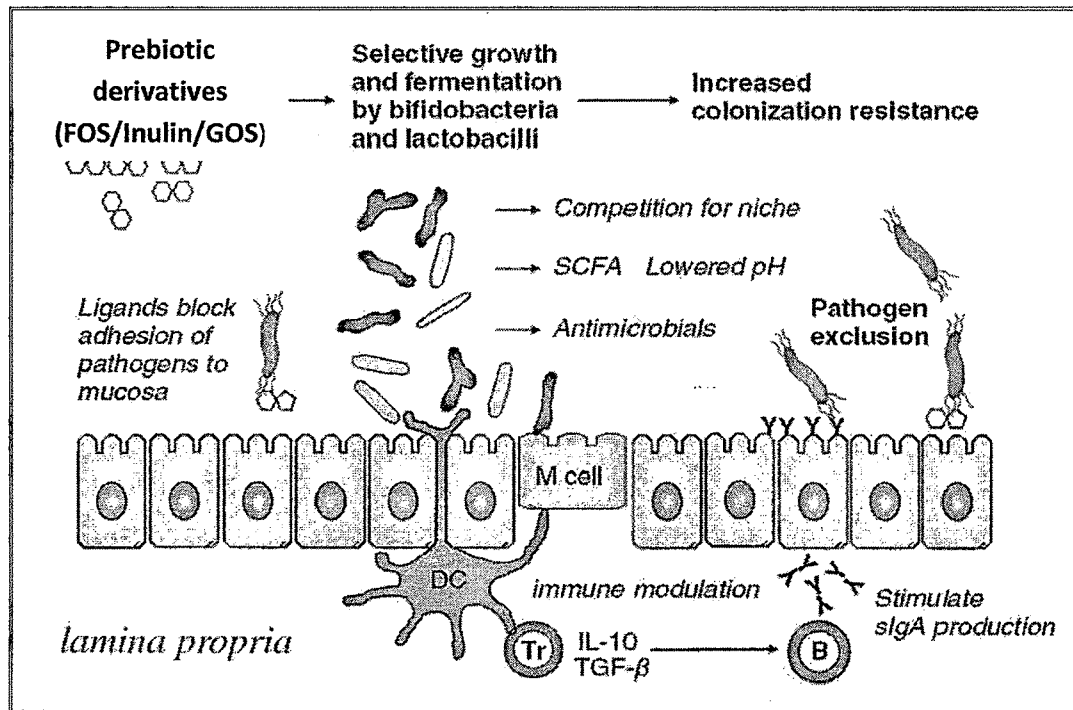
a) Effect of fructooligosaccharide in Immune function

As the gut matures during infancy, so does the immune system and this process is markedly affected by early diet. Human breast milk is a rich source of

prebiotics including 'bifidus factor'. As a result, the gut flora of breast fed infants is dominated by *Lactobacillus* and *Bifidobacteria*, whereas formula-fed infants have more complex composition of gut flora (Gibson GR Delzenne N 2008). This clearly indicates the immunity builds up from the very scratch of life. The immune system consists of two types, innate immune system and acquired immune system. A challenge to the innate system often leads to activation of the acquired immune system. In the past decade, FOS has been studied extensively in humans as well as in animal models for its modulating immune properties. In a small randomized, double blind controlled trial including human subjects with ulcerative colitis supplementation of *B. longum* and OFS resulted in an improvement of the chronic inflammation. Furthermore, intestinal mRNA levels of the proinflammatory cytokines (IL-1 β) and tumor necrosis factor (TNF- α) were significantly reduced (Furrie et al 2006).

In a randomized controlled trial with 259 infants at high risk of atopy, intake of formula providing 0.8g prebiotics/100 ml (FOS+GOS) resulted in a significantly reduced incidence of atopic dermatitis suggesting that these prebiotics altered postnatal immune development (Moro et al 2006). Another randomized controlled study investigated the effect of the same prebiotic mixture on fecal SIgA secretion in infants. Results revealed that an enhanced secretion of fecal SIgA which is considered to be associated with a significantly fast clearance of pathogenic bacteria in the intestine (Bakker Z AM et al 2006). Potential underlying mechanisms by which FOS might induce immune alterations are 1. Selective increase/decrease in specific bacteria that modulate cytokine and antibody production. 2. Increase in intestinal SCFA production and enhanced binding of SCFA to G-coupled protein receptors on leukocytes. 3. Partial absorption of prebiotics resulting in local and systemic contact with the immune system. 4. And lastly by interaction of FOS with carbohydrate receptors on leukocytes. Figure 2.28 shows a diagrammatic presentation of mechanism

involves in immune modulation (Akira et al 2001; Abreu 2003; Ichikawa et al 2002; Karkai et al 2006).



Playne and Crittenden 2009

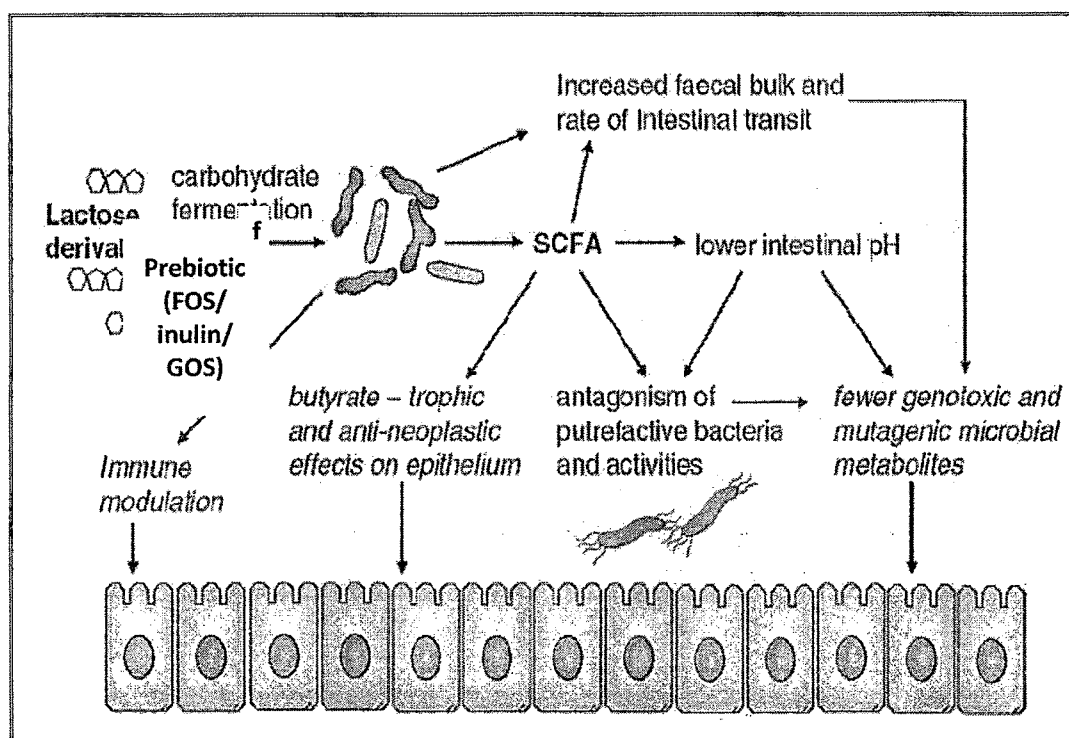
Figure 2.28: Immune modulating effect of FOS

b) Effect of fructooligosaccharide in colon cancer

It is believed that colon cancer develops because of interactions between diet, other environmental and disease factors, gut microflora and immune system. Oligosaccharides are being studied for affecting cancer risk by mediating the colonization of specific microbial populations, thus triggering a biological chain of events (Pool-Zobel BL 2005; Rafter J et al 2007). Although data from animal studies support a role of oligofructose and inulin in prevention of the disease, trials on human beings are still in the early stages (Roller M et al 2004). In a large multi-centric European study, biomarkers of colonic cancer risk, such as cell proliferation and toxicity of feces (fecal water) improved when participants were

given a synbiotic (FOS+*L. rhamnosus* and *B. lactis*) for 12 weeks (Rafter J et al 2007). Klessen B et al (2001) suggested that oligosaccharides may play an important role in reducing colon cancer risk through a decrease in colonic pH and by shifting the resident microflora and production of SCFA. In chemopreventive studies in colon cancer induced rat models, an increase in cecum contents weight and a reduction of cecal pH were reported for inulin like fructans (Jacobsen H et al 2006; Hsu CK et al 2004).

Evidence exists that FOS can reduce the formation of aberrant crypt foci, hallmarked by exaggerated cell size and a thickening of the colonic epithelial lining in animals, a putative colonic preneoplastic lesion modeling early carcinogenic events (Hsu CK et al 2004). As fermentation of FOS increases, intestinal permeability rises via the dose-dependent production of SCFA. In turn, these SCFA stimulates more mucin production and protect GI lumen against cellular damage (Ten B SG et al 2005). Studies also reported that bacteria of the gut microflora possess a variety of enzymes that can metabolize exogenous and endogenous compounds, different bacterial enzymes such as azoreductase, nitroreductase or β -glucuronidase have been associated with colon cancer. These enzymes are involved in the transformation of pro-carcinogens into carcinogens (Gorbach SI and Goldin BR 1990). Figure 2.29 depicts the pathway of colon cancer reduction.



Playne and Crittenden 2009

Figure 2.29: The pathways of colon cancer reduction by oligofructose

c) Effect of fructooligosaccharide in mineral absorption

A number of food constituents have attracted attention as potential enhancers of mineral absorption. There is extensive evidence in experimental animals that prebiotics, such as inulin type fructans, can increase the absorption of a variety of minerals including calcium, magnesium, iron and zinc (Scholz-Ahrens KE et al 2001 and 2002). A study on rat model has shown that ingestion of fructooligosaccharide resulted in increased calcium and magnesium absorption, reduced the occurrence of post gastrectomy osteoporosis, and improved bone mineralization. These effects are associated with an increase in the weight of cecal contents, an increase in the amount of cecal short chain fatty acids, and a decrease in cecal pH (Ohta A et al 1998; Delzenne et al 1999; Lopez HW 2000; Heijnen AM 1993; Coudray C 2000, 2003, 2005). A study investigated by Abrams

S (2005) reported an improvement in calcium absorption, bone mineral content and bone mineral density after 1 year supplementation with 8 g/d of oligofructose and inulin.

Animal models of menopause have been used to determine the likely impact of oligofructose and inulin on osteoporosis risk. Results of several studies have revealed improvement in calcium absorption, bone mineral content, and bone mineral density and reduction in ovariectomy-induced bone loss after supplementation with inulin type fructans (Coxam V 2005; Holloway L et al 2007; Tahiri M 2003). The results suggests that inulin type fructan not only target mineral absorption but also other aspects of bone health, especially bone mineralization, bone density, and bone accretion and resorption. Though human studies indicate no significant changes in mineral absorption by inulin type fructans (Yamada S 1999; Griffin IJ et al 2003; Griffin IJ et al 2005). Some animal studies have shown beneficial effects of prebiotics on absorption of other minerals, such as iron, zinc and copper (Yasuda K et al 2006; Coudray C et al 2006; Douwina B et al 2003), although human data are limited (Coudray C et al 1997).

A variety of mechanisms have been proposed to explain the effect of prebiotics on mineral absorption. First theory states that non absorbed prebiotics enter the large intestine undigested where they are fermented into short chain fatty acids. These acids lower the pH of the large intestinal contents, increases solubility of calcium, magnesium and other minerals in the luminal contents and so increase passive concentration-dependent calcium absorption in the colon (Scholz-Ahrens KE 2002) (Figure 2.30). The second theory hypothesize that effect of prebiotics on calcium absorption is through, tropic effect on the gut and increase absorptive surface area and so increases passive calcium absorption (Scholz-Ahrens KE 2002). The third theory reveals that increased calcium absorption could be attributed to its increased availability by transfer of calcium from the small

intestine into large intestine and the osmotic effect of inulin type fructans that transfers water into the large intestine, thus allowing it to become more soluble (Kaur and Gupta 2002).

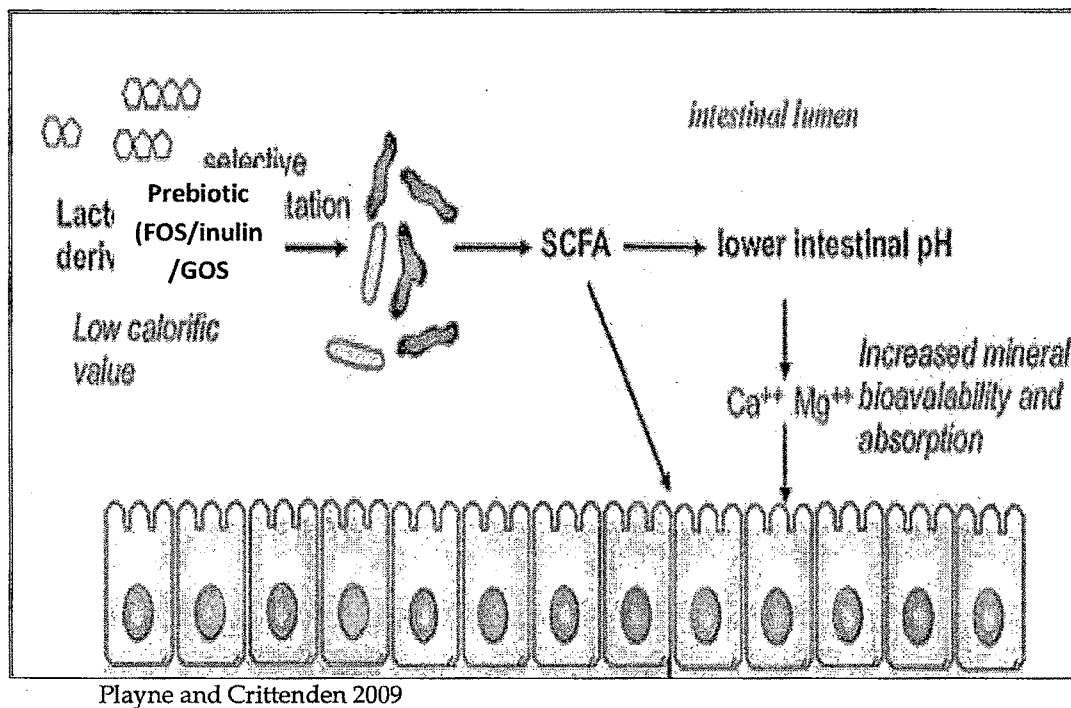


Figure 2.30: The pathway of mineral absorption by oligofructose

d) Effect of fructooligosaccharide in Gastrointestinal tract

The inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis and pouchitis are chronic conditions of unknown etiology characterized by persistent mucosal inflammation at different levels of the gastrointestinal tract (O'Hara Am and Shanahan F 2007). There is evidence showing that the microbiota of patients with IBD differs from healthy subjects. Differences include low biodiversity of dominant bacteria, temporal instability, and changes both in composition and spatial distribution: high numbers of adherent bacteria in the mucus layer and at the epithelial surface (Guarner F 2005; Manichanch C et al 2006; Ott SJ et al 2004). Numerous studies have shown that prebiotics like inulin and oligofructose

increases saccharolytic activity within the gut and promote the growth of *bifidobacteria*. By increasing the number of 'friendly' bacteria on the mucosal surface, inulin and oligofructose could improve the barrier function in IBD and prevent mucosal colonization. Both human and animal data have shown a powerful impact on improved gastrointestinal tract diseases by inulin type fructans.

A study carried out by Vidella S (2001) in colitis, induced by chemical dextran sodium sulphate (DSS) in the rat models. Daily administration of inulin by oral route increased counts of indigenous *lactobacilli* in the cecal lumen and reduced intra-colonic pH; it also resulted in an extension of the saccharolytic areas of acidic environment. A subsequent study investigated the effect of oligofructose and inulin alone and in combination with probiotic *bifidobacteria* in the DSS model. The prebiotic alone or synbiotic both significantly improved the disease activity indexes and decreased colonic myeloperoxidase activity, as well as an expression of inflammatory mediators (Osman et al 2006). It was concluded that these beneficial bacteria might have resulted in production of short chain fatty acids and its likely to be the principle mechanism mediating anti-inflammatory effect.

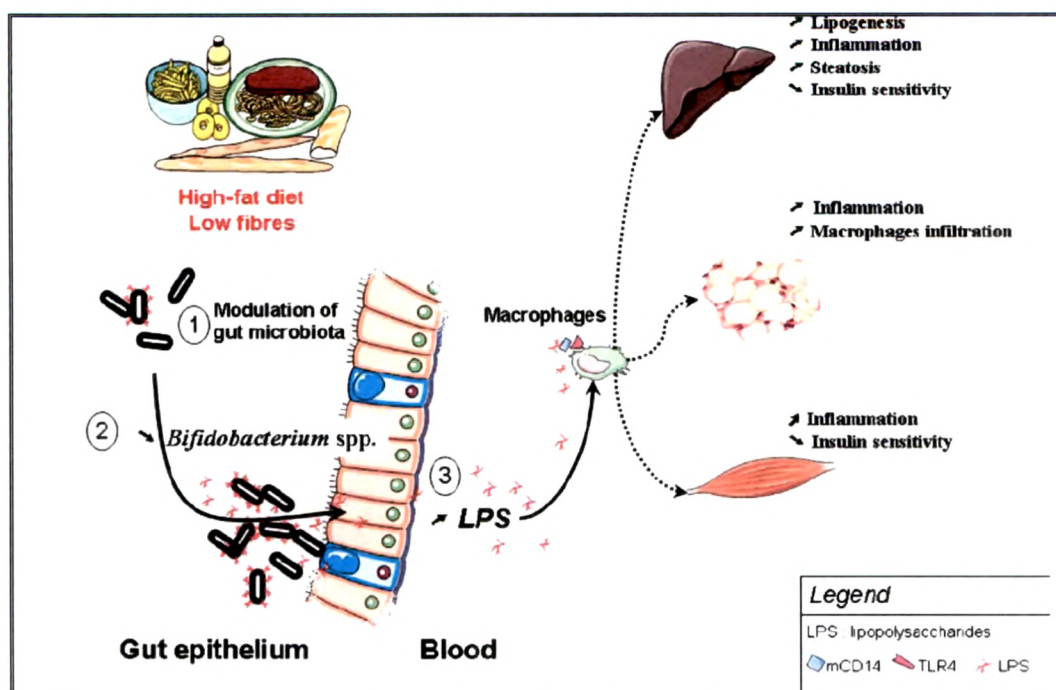
Almost similar results have been drawn from clinical human trials. In a double blind crossover design, twenty patients with mild gastrointestinal disease were supplemented 24g/d of inulin (dissolved in 200 ml of commercial available milk) for 3 weeks which resulted in increased fecal butyrate and decrease in the counts of bacterioids in feces. A significant reduction of endoscopic and histological parameters of inflammation of the mucosa of the ileal reservoir was also reported in this study (Welters CFM 2002). FOS has also been given to patients with ulcerative colitis and crohn's disease with different doses ranged from 12g/d to 15g/d for the duration of 3-4 weeks and reported overall improvement and

decrease mucosal inflammation in both the gastrointestinal disease conditions (Furrie E et al 2005; Lindsay JO et al 2006).

From these strong experimental and clinical studies it can be concluded that inulin and OFS can offer an opportunity to prevent or mitigate gastrointestinal disease and their symptoms.

e) Effect of fructooligosaccharide on Obesity

Gut microbiota has recently been proposed as an environmental factor responsible for the weight gain and the altered energy metabolism that accompanies the obese state (Harris K et al 2012). Several studies reported that the gut microbiota differs at phylum level depending on weight status (Eckburg PB et al 2005; Turnbaugh PJ 2006). Compositional changes of the human gut microbiota in response to weight change have been examined by many groups. Ley et al (2006) studied the fecal gut microbiota in 12 obese subjects participating in a weight-loss program by consuming restricted diets for a year. Following weight loss, the proportion of *Bacteroidetes* increased while the number of *Firmicutes* reciprocally decreased. Studies have also demonstrated in both animal models as well as in humans that high fat diet was a triggering factor for development of obesity and inflammation and the eligible candidate is an inflammatory compound of bacterial origin which is bacterial lipopolysaccharide (LPS). It is a constituent of gram negative bacteria. LPS triggers the secretion of pro-inflammatory cytokines and it binds to the complex of CD14 and the toll like receptor 4 (TLR 4) at the surface of innate immune cells (Wright SD et al 1990), LPS is continuously produced within the gut by the death of gram negative bacteria and is physiologically carried into intestine capillaries towards target tissues (Neal MD et al 2006; Tomita M et al 2004). High fat feeding and LPS promotes low-grade inflammation-induced metabolic disorders (Figure 2.31)



Cani PD and Delzenne NM 2009

Figure 2.31: High fat feeding diet changes the gut microflora, promote endotoxaemia and obesity

Inulin type fructans are well studied and clearly effective in humans and animal models to stimulate growth of health promoting species belonging to *bifidobacterium* and *lactobacillus* (Macfarlane S 2006; Flamm G 2001). A study reported that oligofructose feeding (20g/d) significantly increased plasma GLP-1 after mixed meal (Piche T et al 2003). Furthermore, a study demonstrated that in healthy humans, feeding of 16g/day FOS promoted satiety followed breakfast and dinner and reduced hunger after dinner. This was accompanied by a significant 10% lower total energy intake (Cani PD et al 2006). A similar research have also reported that fructans added as fat replacer in food were able to lower energy intake during a test day (Archer BJ et al 2004).

Several mechanisms have been postulated for the lower energy intake by fructans, first is the reduction in the LPS activity by intake of higher levels of

fructans which reduced endotoxaemia (Cani PD et al 2007) (Figure 2.32). Second mechanism is excessive production of fasting-induced adipocyte factor (FIAF), which suppresses hepatic *de novo* lipogenesis and inhibits triglyceride storage in white adipose tissues (Backhed F et al 2007). It also inhibits lipoprotein lipase (LPL) thereby blocking the disassociation of fatty acids from triglycerides for uptake into tissues (Mandard S et al 2004). Third is the production of SCFA (Xu J and Gordon JI 2003) and activation of gut peptides GLP-1, GIP, Ghrelin which have been proposed as important modulators of food intake and energy expenditure (Knauf C et al 2008; Chaudhri OB et al 2008)

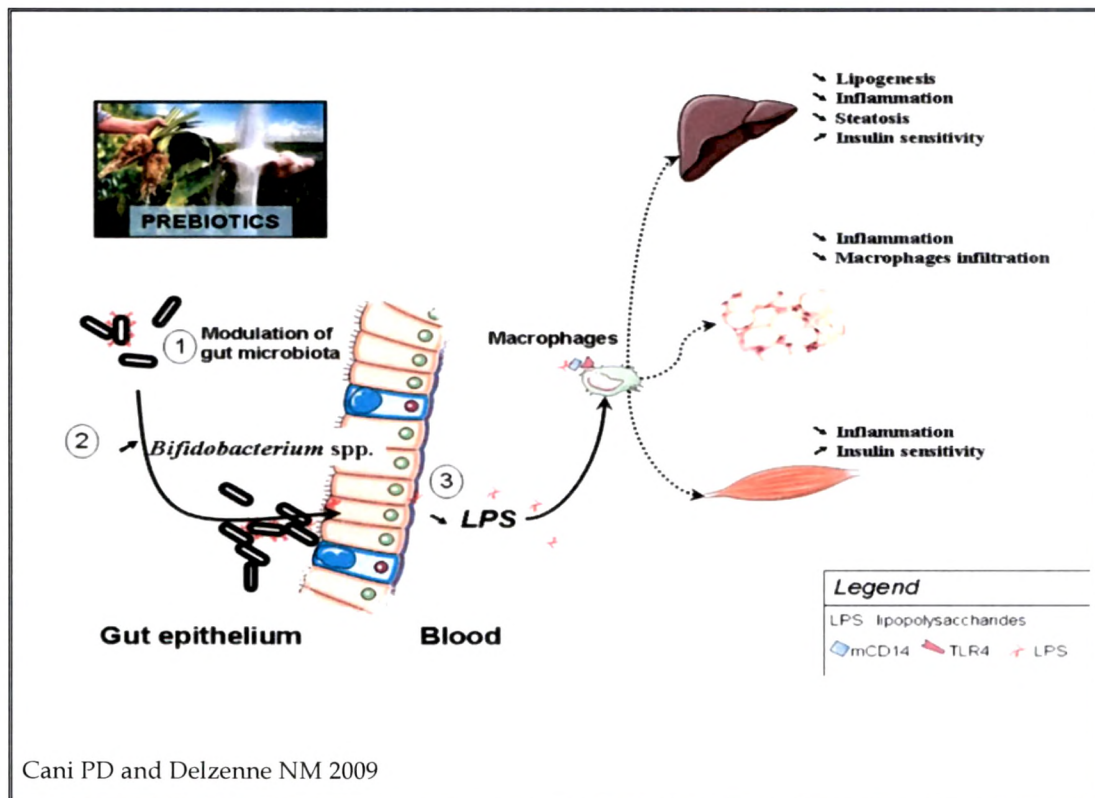


Figure 2.32: Changing in gut microbiota by prebiotic (FOS) and decrease in LPS activity

f) Effect of fructooligosaccharide on lipid metabolism

Modulation of either digestion/ absorption or the metabolism of lipid is another physiological effect of inulin type fructans that affect triglyceridemia and cholesterolemia as well as the distribution of the lipids among different lipoprotein in favor of a pattern more beneficial for health (Moshfegh AJ et al 1999). Studies using animal models have indicated that moderate amount of inulin and oligofructose may reduce blood cholesterol levels. Rat studies have repeatedly shown that an inulin and OFS consumption of 9-20 g/d induces significant reduction (40%) in serum and liver triglyceride, LDL and total cholesterol concentrations (Fiordaliso et al 1995; Delzenne et al 2002; Fava et al 2006; Rault N et al 2006).

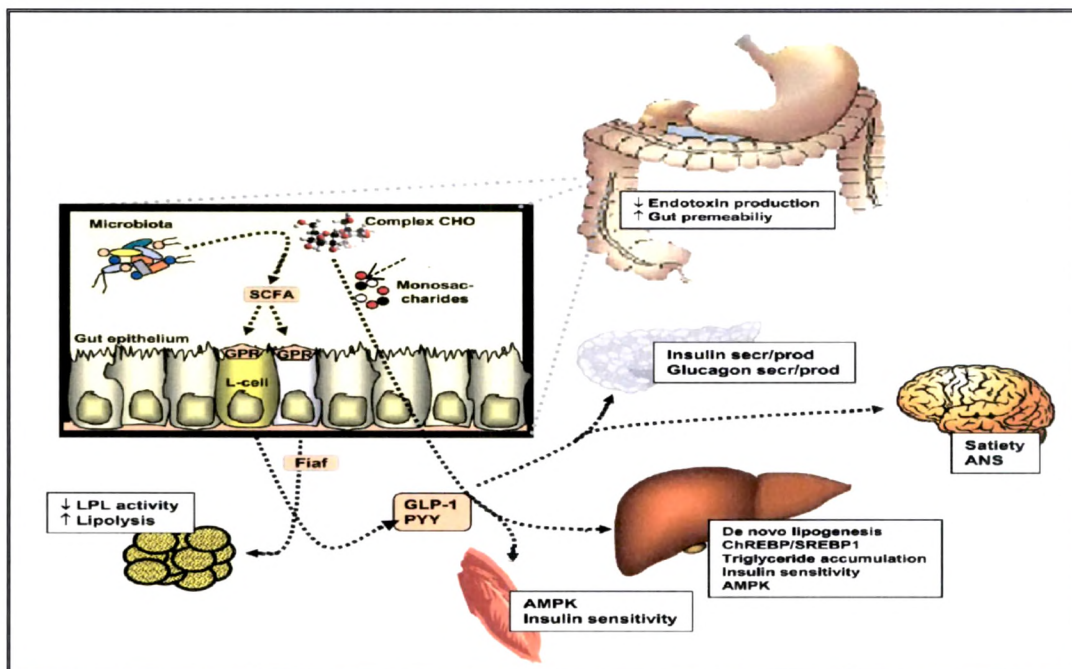
Bruserolls et al (2003) revealed that OFS is protective against pro-oxidative stress, lower heart lipid oxidation and thus contribute to be cardio protective. A study reported by Moroti C et al (2012) investigated the effect of synbiotic shake (*L. acidophilus*, *B. bifidum* and 2% FOS in 200 ml shake) supplementation in 20 hypercholesterolemic patients and found a reduction in total cholesterol and triglycerides and increment in HDL cholesterol. Studies have also shown significant serum TG and TC reduction in hypercholesterolemic and hyperlipidemic subjects, consuming 20 g inulin and OFS (Jackson KG et al 1999; Causey JL et al 2000). On the contrary, some studies show that the dietary supplementation with 15–20g/d FOS for 4 weeks had no effect on serum TC or TG levels in type 2 diabetic subjects (Alles MS et al 1999; Lou J et al 2000).

Various mechanisms have been suggested by the scientist regarding reduction in blood lipid levels via fructans. Ingestion of dietary fructans increases the beneficial microflora in the gut. Kiebling et al (2002) reported a significant increase in HDL levels with administration of FOS and LAB in humans. Intestinal breakdown of FOS leads to the production of substantial amounts of SCFA

which are almost completely absorbed along the digestive tract. Butyrate widely metabolize by enterocytes, propionate and acetate can reach the liver through the portal vein (Demigne C et al 1999). When acetate enters the hepatocyte, it is mainly activated by the cytosolic acetylS CoA synthetase 2, and then enters the cholesterologenesis and lipogenesis pathways. This effect has been proposed as a rationale behind the hypercholesterolemic effect of fructans. Conversely propionate is a competitive inhibitor of the protein devoted to the entrance of acetate in the liver cells (Delzenne NM et al 2002), a phenomenon which contributes to a decrease in lipogenesis and cholesterologenesis. Thus, production of high concentration of propionate has been proposed as an explanation of reduction in serum and hepatic cholesterol through fructans (Lopez HW et al 2001; Delzenne NM and Kok N 2001). Another mechanism related to SCFA is increase in acetate which activates AMP kinase in the liver which in turn inhibits *de novo* lipogenesis through a corresponding reduction of the activity of all lipogenic enzymes, is a key event in the reduction of VLDL-triglyceride levels (Sakakibara S et al 2006). The effect of inulin (10g/d for 3 weeks) supplementation on hepatic lipogenesis and cholesterologenesis has been analyzed in normal subjects in a double blind, placebo-controlled crossover study and reported a decrease in hepatic *de novo* lipogenesis (Letexier D et al 2003).

Another underlying mechanism related to lipid reduction is ability of probiotic bacterias in reducing the resorption of bile acids through enterohepatic circulation. This is due to the fact that conjugated bile acids are directly absorbed back in the liver. *Lactobacillus* and *bifidobacteria* cells can hydrolyze the conjugate bile acids, excrete them faster and reduce the extent to which they are absorbed (Ooi and Liong 2010). Figure 2.33 depicts diagrammatically the mechanisms by which gut microflora influence the host cardio-metabolic phenotype complex dietary carbohydrates (CHO) can be digested by certain intestinal microbiota species into monosaccharides and short-chain fatty acids (SCFAs). Increased

intestinal uptake of the digestions products may increase overall food-derived energy uptake. Nutrient-related selection of specific gut microbiota species may lower intestinal endotoxin levels and promote mucosal barrier function. Supply of monosaccharides and SCFA may enhance hepatic *de novo* lipogenesis and triglyceride (TG) accumulation. Altered supply of gut-derived nutrients may additionally affect transcription factors such as ChREBP and SREBP-1 and modify AMP-activated protein kinase (AMPK) activity in both liver and skeletal muscle. Finally, gut microbiota may suppress gut-derived fasting-induced adipose factor (Fiaf), a circulating lipoprotein lipase (LPL) inhibitor, leading to increased LPL-activity and lipolysis.



Diamant M et al 2011

Figure 2.33: Mechanisms proposed by which gut microbiota may influence the host cardio-metabolic phenotype

2.13 Role of Fructooligosaccharides on glucose homeostasis

"Research surrounding prebiotics has historically focused on digestive health. However, over recent years, scientist have been investigating the potential role of fructooligosaccharide in blood glucose homeostasis"

The first reported attempt to study the fate of inulin type fructans in man was by Kulz (1874) who investigated its metabolism in diabetic subjects. Later in 19th century, Root and Baker (1925) used oligofructose-containing Jerusalem artichokes in the diets for diabetic patients. When Jerusalem artichokes were substituted for other carbohydrate-rich foods, glycosuria was reduced. Feeding of Jerusalem artichokes resulted in decrease blood levels. These findings offered some promise for the practical use of inulin type fructans containing foods in diabetic diets.

In more recent years, others also have suggested that inulin-containing food products may be beneficial to persons with diabetic disease due to effects on reducing glucose uptake and thereby reducing postprandial hyperglycemia (Kim and Shin 1996). Yamashita and others (1984) fed 8 grams of fructooligosaccharide (Neosugar) for 14 days to 18 diabetic subjects. By the end of the study the diabetic subjects experienced 15 mg/dL decline in fasting blood glucose levels while control subjects showed no change. The implications from this study suggest that inulin-containing products may be useful carbohydrate substitute in diabetic diets. Contrary a study reported that feeding 15 g/d of FOS for 20 days to 20 men and women with non-insulin dependent diabetes (NIDDM) did not favorably affect either serum glucose or lipid concentrations (Alles et al 1999).

In eight healthy human subjects, Rumessen and others (1990) also examined the effects of sultans from Jerusalem artichoke on blood responses. After 20 gram

load, these subjects demonstrated a lower glycemic response and insulin peak than when lactose was fed but a glucose response was experienced, nonetheless. The short-term nature of this study hinted that further long-term studies would be needed to further assess the physiological and nutritional benefits of using inulin products in both healthy and diabetic persons. Koivisto and Yki-Jarvinen (1993) studied 10 patients with type 2 diabetes and reported improved glycemic control and less insulin resistance after 4 week of a high-fructose diet (20% of energy) compared with a control diet.

In 1996, Lou and others worked with 12 healthy male subjects in a double-blind crossover study. In separate four-week periods 20 grams of fructooligosaccharide was included in the diets. Results indicated that fasting levels of plasma glucose and insulin did not differ significantly. However, productions of short chain fatty acids were noted which could have an effect on liver glucose production. Further studies of the role of short chain fatty acids in regulating hepatic glucose and metabolism were encouraged. According to Kaur and Gupta (2002), inulin and oligofructose modulate the hormonal level of insulin and also the metabolism of carbohydrates by their ability to lower blood sugar levels. A study investigated on effect of FOS on post prandial insulin on veal calves revealed an increase in insulin levels after supplementation of 10g/d FOS for 3 weeks (Kaufhold J et al 2001).

Another study by Voss et al (2008) evaluated the postprandial glycemic, insulinemic, and GLP-1 responses of 48 individuals with type 2 diabetes, after the consumption of 10.7 g tube feed formula consisted of FOS a significant reduction in post prandial blood glucose and an increment in plasma insulin and GLP-1 levels was witnessed. In a clinical study carried out by Cicek B et al (2009), consumption of 20g/d of oligofructose to type 2 diabetic subjects exhibited a significant reduction in blood glucose levels after 6 weeks. A more recent study revealed that consumption of 200 ml symbiotic shake (2% FOS, LAB and

Bifidobacteria) resulted in a significant reduction in fasting glycemia in diabetic and hyperlipidemic subjects (Moroti C et al 2012).

Pioneering animal and human studies have unveiled potential mechanisms through which reduction in blood glucose levels could be explained. First mechanism suggests that the reduction in glucose levels may be attributed to transit time. Rats, fed with 10% and 20% FOS in their diet for 6 weeks, revealed reduced mouth to anus time by 25 and 50% respectively. This reduction in transit time confirms a dose dependent effect possibly similar to other dietary fibers: OFS and inulin influence the absorption of macronutrients, especially carbohydrates, by delaying gastric emptying and shortening small intestine transit time (Oku et al 1984; Bornet FRJ 1994).

Several studies provided evidence regarding difference exists in the microbial colonies of diabetics and healthy humans. Larsen et al (2011) characterised intestinal microbiota in type 2 diabetic patients and described that proportions of phylum *firmicutes* and class *clostridia* were reduced in diabetic group compared to controls. This suggested that type 2 diabetes in humans is associated with compositional changes in intestinal microbiota. Studies on similar lines also reported lower establishment of colonies of *bifidobacteria* in diabetic subjects, resulting in reduced intestinal permeability and increased endotoxaemia (Wu X et al 2010; Cani PD et al 2007; Cani PD 2008).

Another proposed mechanism is by lowering the lipopolysaccharide (LPS) which reduces the level of endotoxaemia and hence reduce the severity of diabetes. Animal studies showed that, bacterial LPS derived from gram negative bacteria, triggers pro-inflammatory cytokine production when it binds to the CD14/TLR4 complex at surface of immune and gut epithelium cells resulting in body weight, fat mass gain, liver steatosis and diabetes (Cani PD et al 2007 (a) (b); Creely SJ et al 2007; Wellen KE and Hotamisligil GS 2005; Hotamisligil GS 2006).

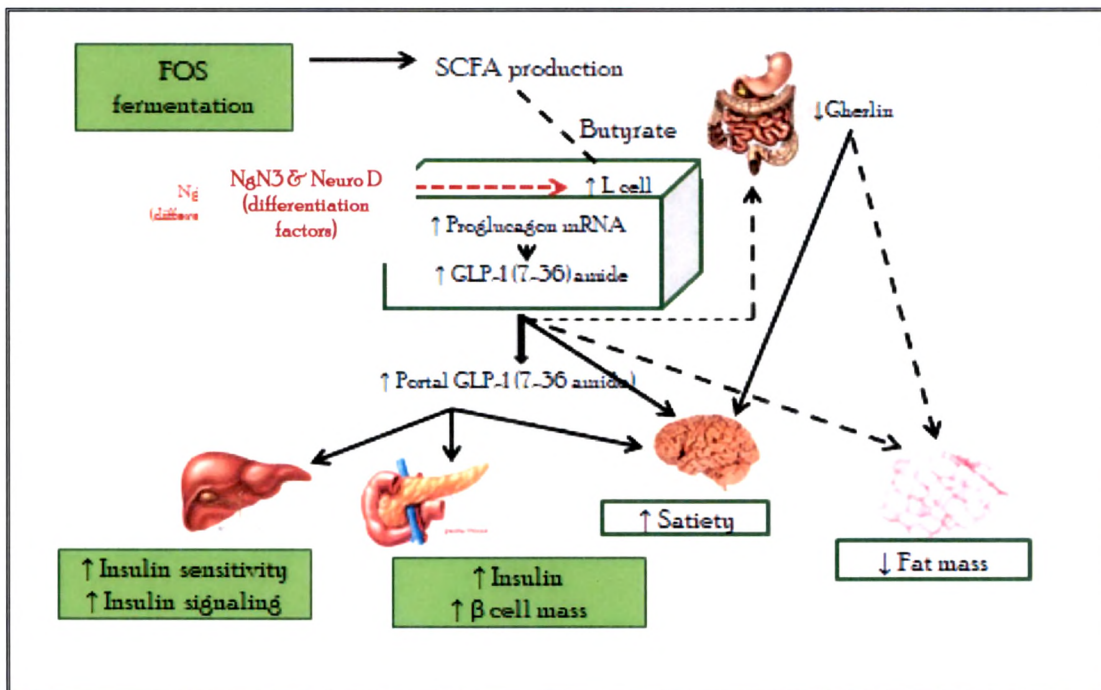
A significant increase in *bifidobacteria* and *lactobacilli*, slight increase in plasma insulin and GLP-1 levels and reduction in severe glycosuria has also been demonstrated in a study in poloxamer-407-induced diabetic rats when supplemented with fructooligosaccharide (Bharti SK et al 2013). A study reported that intake of 10g/d of short chain fructans increased *bifidobacteria* counts in 30 diabetic subjects (Giacco R et al 2004). Increase in fermentable bacterial counts gives rise to SCFA such as propionate, acetate and butyrate (Alles MS et al 1999). Le Blay G et al (1999) observed that when 9% fructan was present in the diet of rats, intestinal butyrate concentration was doubled with an increase in both acetate and propionate levels. Moreover, the profile of SCFA in terms of proportions of butyrate, acetate and propionate differs with varying degree of polymerization of fructans in rat models (Nayman M 2002).

Furthermore, a mechanism have been postulated which states that an increase in SCFA concentration (especially butyrate) increases the gut incretins which have a direct effect in increasing insulin levels thereby, affecting overall blood glucose homeostasis. Kok et al (1998) observed that OFS feeding leads to an increase in total cecal GLP-1 and jejunum GIP concentrations in rats. Therefore, it was postulated that modulation of gut peptides could be a key process mediating the effect of FOS on glucose metabolism. However, putative mechanism by which FOS exerts its effects on glucose metabolism was shown to be majorly is dependent on specifically GLP-1 (Cani PD et al 2005). It has also been investigated that modifications of SCFA could result in differential modulation of proglucagon mRNA content by fructans (Aanini Y and Brubaker PL 2003; Reimann F et al 2005). In mice, a high fiber diet (300g/kg) increased serum GLP-1, which increased proglucagon m RNA content in colon, jejunum and ileum (Nian M et al 2002).

It has been demonstrated that modulation of intestinal GLP-1 by FOS, observed in the proximal colon might be related to significant increase in GLP-1 positive

number of L-cells. Studies have also elicited the role of FOS in differentiation of L-cells in the proximal colon in animal models (PD Cani et al 2007; Nauck MA 2012). It has been hypothesized that terminal differentiation in L-cells is dependent on a cascade mechanisms of basic helix loop (bHL) transcription factors (Schonhoff HC et al 2004). Increase in L-cells number was found to be positively correlated with the two key differentiation factors NeuroD and NGN3. This suggests that fermentation occurring in the proximal colon could promote the specific differentiation of stem cells into GLP-1 producing L-cells (Cani PD et al 2007). Figure 2.34 presents a summary of effects of FOS on glucose homeostasis.

Collective information on animals and humans conclude that FOS feeding modulate GLP-1 incretin and thus appears to be a key hormone involved in regulation of glucose metabolism and homeostasis.



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Figure 2.34: Summary of effect of FOS in regulation of glucose homeostasis via GLP-1

By understanding the new ecological strategies based on prebiotics, it is evident that Fructooligosaccharide is unequivocally associated with glycemic, insulinemic and lipemic responses. In this context, FOS is a substantial nutraceutical approach in management of metabolic disorders like obesity, cardio-vascular diseases and type 2 diabetes. Multidisciplinary research based on animal studies firmly supports the above evidence of consumption of FOS in combating these diseased conditions. Nevertheless, tremendous lack of data limits the current knowledge of the complex mechanisms linking diet, gut microbiota and metabolic disorders in humans. Therefore, long term human studies will constitute new knowledge in determining the effects of FOS consumption on the metabolic syndrome and meta-dynamics behind it