CHAPTER 2
REVIEW OF LITERATURE

Obesity typically results from over-eating (especially an unhealthy diet) and lack of enough exercise. In our modern world with increasingly cheap, high calorie food (fast food or junk food), ready to eat foods that have high amount of salt, sugars or fat, combined with our increasingly sedentary lifestyles, increasing urbanization and changing modes of transportation, it is no wonder that obesity has rapidly increased in the last few decades, around the world.

In perspective to this the present study was designed under the title, **"Sensory evaluation of fructooligosaccharide (FOS) added popular recipes of India and its role in modulating anthropometric indices, gut flora and lipopolysaccharide (LPS) in obese young adults of urban Vadodara".**

This chapter brings together the relevant review of literature under following heads:

Section 2.1: Prevalence of obesity across the globe and in India

Section 2.2 Obesity – causes and complications

Section 2.3 Obesity, GI health and its significance

Section 2.4 Obesity and gut microbiota

Section 2.5 Factors affecting intestinal microbiota and health

Section 2.6 Mechanism of action of colonic microbiota in the gut

Section 2.7 Obesity and endotoxemia (LPS)

Section 2.8 Obesity and atherogenic profile

Section 2.9 Probiotics and prebiotics

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2.1: Prevalence of obesity across the globe and in India

- ***Prevalence of obesity across the globe***

Non-communicable diseases or NCD's are those conditions that are usually not passed on from one affected person to another, but are caused as a direct result of inheritance, lifestyle and environmental factors. An alarming fashion has been seen in the last decade in the unfailing rise in NCD's on a global level.

NCD's unaccountably affect low and middle income countries where nearly three quarters of NCD's deaths (approx. 28 million deaths) occur. The amendable behavioral risk factors include tobacco consumption, physical inactivity, unhealthy diet and excessive intake of alcohol increase the risk of NCD's (World Health Organization, 2016). Approximately 3.2 million deaths can be attributed to insufficient physical activity per year, followed by elevated blood pressure (18% of global deaths) and by overweight and obesity and raised blood glucose (Lim et al., 2012).

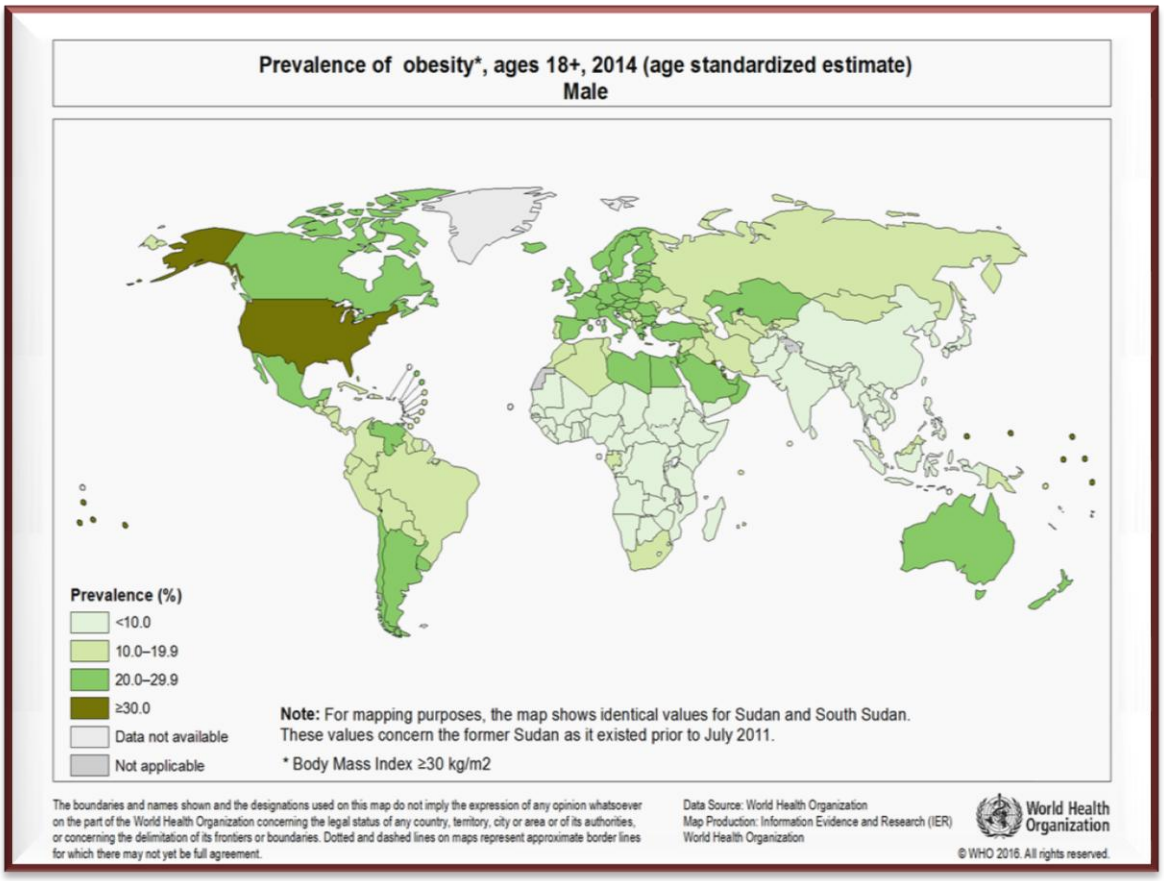
Obesity is one of the most pervasive and a major contributor to the global load of chronic disease and disability. Often coexisting with under-nutrition, it is a multifaceted condition, with serious social and psychological magnitude, affecting virtually all ages and socio-economic group.

Visscher and Seildell in 2010 estimated 1.5 billion adults, 20 years and older aged, are overweight. Out 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).

Obesity advances the odds of diabetes, hypertension, coronary heart disease and stroke, certain cancers, obstructive sleep apnea and osteoarthritis. (WHO, 2014). Overweight and obesity were anticipated for 3.4 million deaths per year and 93.6 million DALY's (Disability-adjusted life years) in 2010 (Lim et al., 2012).

NCD's are more common among older populations of India; Obesity, which is associated with hypertension, CVD, diabetes, and some cancers is projected to affect 52.1 million by 2030 (World Bank, 2011). In the South-East Asia Region 3,00,000/year died due to overweight/obesity (NIH, 2014).

Obesity outbreak has reached to an extent globally, with more than 1.9 billion adults overweight and at least 600 million of them clinically obese in 2014. 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight and about 13% of the world's adult population (11% of men and 15% of women) were obese in the year 2014. The worldwide prevalence of obesity has doubled between 1980 and 2014. Previously overweight and obesity was the disease of high-income group country, but it is now getting higher in low and middle-income group countries, particularly in urban settings (World Health Organisation, 2016). (Figure 2.1)



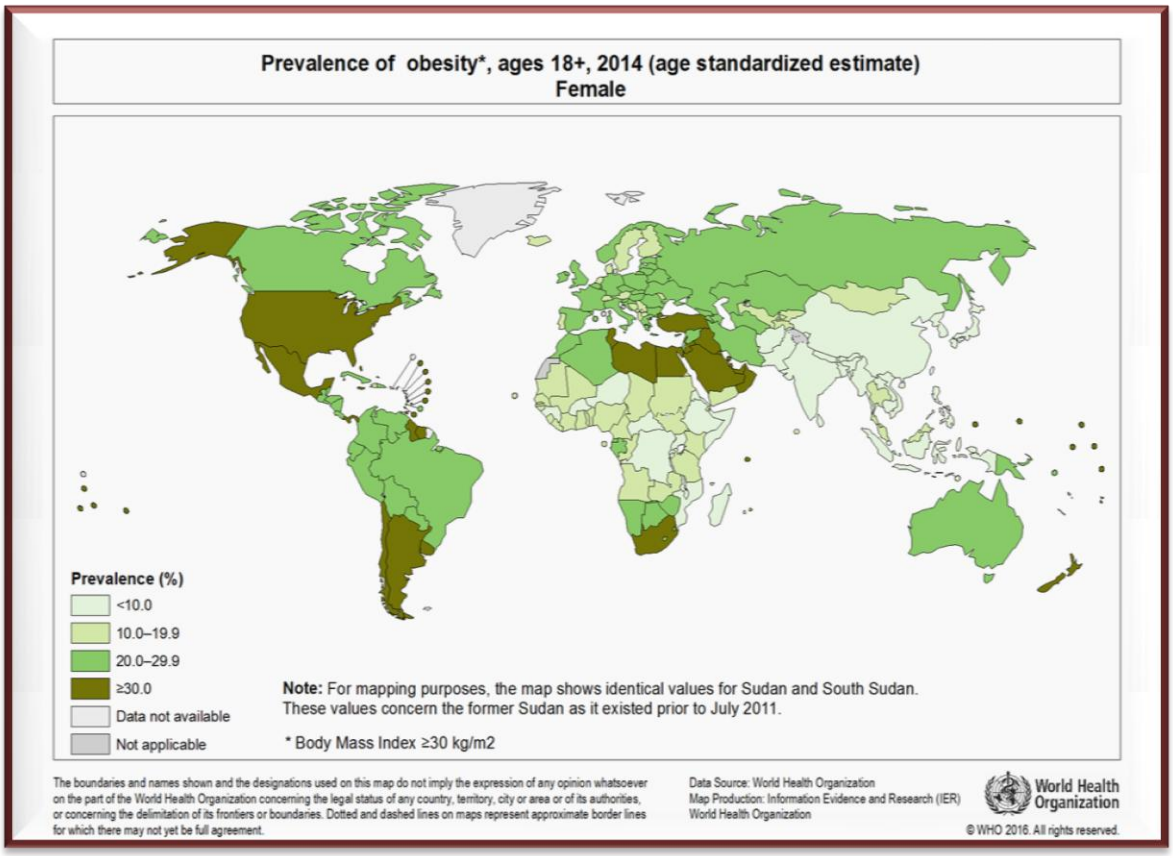


Figure 2.1: Prevalence of obesity across the globe in men and women

- ***Prevalence of obesity in India***

Chronic diseases are the foremost cause of death and disability worldwide (WHO, 2005). In India, chronic diseases are projected to account for 53% of all deaths (WHO, 2005). India, with 1.2 billion people is the second most populous country in the world and is currently experiencing rapid epidemiological transition. Under nutrition due to poverty which dominated in the past is being rapidly replaced by obesity associated with prosperity (Mohan and Deepa, 2006). Industrialization and urbanization plays a major role in high prevalence of obesity. Studies from different parts of India have provided evidence of the growing incidence of obesity (Bhardwaj et al., 2011, Deepa, Farooq, Deepa, Manjula, and Mohan, 2009, Misra and Khurana, 2008, Mohan and Deepa, 2006). However, most reports have been region specific (mostly from urban areas). Obesity is escalating swiftly in India. According to the National Family Health Survey 4 (NFHS-4), the percentage of women aged 15-49 years who are overweight or obese increased from 12.6% in NFHS-3 to 20.7% in NFHS-4 and the percentage of men aged 15-49 years who are overweight or obese increased from 9.3% in NFHS- 3 to 18.6% in NFHS-4. In urban settings 26.3 % men and 31.3% of women were obese stated NHFS-4 (MOHFW, 2016). This may be due to lesser physical activity in the urban areas. Furthermore, overweight and obesity are both higher for women than men. Earlier India was known for malnutrition but now Indians report more frequently with overweight, obesity and their consequences. Prevalence deviates within the country because of divergence in the lifestyle, mainly in prosperity, dietary patterns, and physical activity. In addition to this urbanization and industrialization are the main culprits for the increase in the prevalence of obesity.

The ICMR-INDIAB conducted a study in India to estimate the prevalence of generalized obesity (GO) and abdominal obesity (AO) among urban and rural residents of States of India. The results revealed that highest prevalence of both types of obesity (GO and AO) was found in Chandigarh followed by

Tamil Nadu, Maharashtra and Jharkhand. This is not surprising as Chandigarh has the highest per capita income among all the four regions studied and is highly urbanized. The prevalence of GO, AO and CO (combined obesity) was significantly higher among urban residents compared to rural residents in all the four regions studied (Pradeepa et al., 2015). The NFHS-IV also stated the prevalence of overweight or obese in women is highest in Chandigarh (41.5%), followed by Delhi (34.9%), Kerala (32.4%) and Punjab (31.3%), all of which are relatively richer states (MOHFW, 2016). The prevalence of underweight and overweight among men shows similar variations by age, education, and prosperity index.

The prevalence of overweight and obesity in Gujarat in women aged 15-49 years was 34.5% in urban area and 15.4% in rural area. For men prevalence was 25.9% in urban area and 14.4% for rural area. Overall prevalence of overweight and obesity was 34.5% for women and 25.9% for men in Gujarat in the year 2015-2016 (MOHFW, 2016).

2.2: Obesity – causes and complications

- ***Defining and determining over weight and obesity***

Overweight and obesity are defined as 'abnormal or excessive fat accumulation that may impair health'. Measurement of body mass index (BMI) is the most frequently used method to define degree of obesity and quantify health risk (World Health Organisation, 2016). BMI can be calculated based on the height and weight of a person according to the formula:

BMI = Body weight (kg)/Height (m^2)

BMI calculators are available on numerous web sites and printed charts. BMI has shown a good correlation with total body fat and is relatively unaffected by height. Previously overweight is defined as BMI of at least 25 kg/m^2, obesity as BMI of at least 30 kg/m^2, and extreme obesity as BMI of at least 40

kg/m^2 (National Heart Lung and Blood Institute and National Institutes of Health (NIH) National Heart, Lung, and Blood Institute, 1998).

Mortality rates increase with increasing degrees of overweight, as measured by BMI. To achieve optimal health, the median BMI for adult populations should be in the range of 21 to 23 kg/m^2, while the goal for individuals should be to maintain a BMI in the range 18.5 to 24.9 kg/m^2. There is increased risk of co-morbidities for BMIs in the range of 25.0 to 29.9 kg/m^2, and moderate to severe risk of co-morbidities for a BMI greater than 30 kg/m^2 (WHO, 2015).

- ***Factors predisposing to obesity***

1. ***Physical inactivity***

Obesity results from energy imbalance: too many calories in and a small amount of calories burned. A number of factors influence how many calories people burn each day but the most erratic factor and the most easily modified factors is the amount of physical activity people do each day. Decline in physical activity is a key contributor to the global obesity epidemic and in turn to rising rates of chronic disease (WHO, 2016). Although, the etiology of obesity is likely to be multi factorial, the energy balance equation suggests that the obesity results from an imbalance between energy intake and expenditure (Hall et al., 2011; Blackburn 2002, Gortmaker and Cheung 1990).

The components of energy expenditure are the basal metabolic rate (BMR, about 60%), thermogenesis (about, 10%) and physical activity (about 30%) (Rising and Harper, 1994). The BMR varies by a small amount amongst individual. Similarly, adaptations in thermogenesis are also small. However, large variations occur in physical activity (PA) as it forms a major and modifiable component of energy expenditure. The low and decreasing levels of PA are primarily responsible for obesity (Du and Bennett, 2013; Andreasen 2008; Esparza et al., 2000).

Globally, around 31% of adults aged 15 and over were insufficiently active in 2008 in which 28% was men and 34% were women. Approximately 3.2 million deaths each year are owed by insufficient physical activity (WHO, 2014).

Several studies have suggested an inverse relationship between the BMI and the physical activity both among adults and children (Duvivier et al., 2013; Ross and Janssen 2001). A beneficial effect of PA on obesity has been demonstrated in many studies. A study showed that the prevalence of obesity was lower among individuals who were in the habit of performing exercise (Johansson et al., 2014; Collings et al 2013; Hiraoka 1998). Similar findings were observed in other studies also (Ekelund et al., 2015; Miller et al., 2013; Moayeri 2006).

Overall, strong evidence demonstrates that compared to less active adult men and women, individuals who are more active have lower rates of all-cause mortality, coronary heart disease, high blood pressure, stroke, type 2 diabetes, metabolic syndrome, colon and breast cancer, and depression and are more likely to achieve weight maintenance, have a healthier body mass and composition (World Health Organisation., 2015).

2. *Poor dietary practices*

In many developing countries, the progression of nutritional transition has been detected, characterized by a reduction in the prevalence of nutritional deficiencies and the more expressive occurrence of overweight and obesity in the adult population (Amin, Al-Sultan, and Ali, 2008). These characteristics are fundamentally associated with changes in lifestyle and eating habits (PT & I, 2004). During the last 2 decades overweight and obesity have increased globally among children, adolescents, and adults (Lissau, 2004). Consumption of large quantities of beverages rich in sugar, breakfast skipping, high-energy,

high fat and low-fiber food were shown to be related with overweight and obesity (Pérez-Escamilla et al., 2012).

Eating 3 or fewer meals per day or inadequate intake of carbohydrates (Filla Rosaneli et al., 2012) has been shown to be associated with a greater likelihood of being overweight or obese (Smetanina et al., 2010).

According to Popkin, an urban population has a distinctly different diet from a rural population. The urban diets include superior grains, more milled and polished grains, higher fat content, more animal products, more sugar, and more prepared and processed food (Mayén, et al 2014; Popkin 1996). The growing popularity of fast food is just one of many cultural changes that have been brought about by globalization and is a part of nutrition transition (Jeffery 2006; Popkin and Barry 2004; Drewnowski and Popkin 1997, Popkin 1994). The fat intake plays a major role in the energy imbalance and thus results in body weight changes Drewnowski and Specter 2004; Morton, 2006; Poti and Duffey 2014). The dietary fat has a higher energy density and is responsible for overeating and passive over consumption of high fat diets. The results revealed that infrequent intake of breakfast, frequent consumption of fast foods, low serving of fruits, vegetables and milk and milk products per day along with frequent consumption of sweets/candy and carbonated beverages all were proctors of the obesity and overweight (Amin et al.,2008; Poti and Duffey 2014).

Various studies have shown an association of consumption of these beverages and increased prevalence of adolescent obesity (Pereira, 2006; Sharma 2006). The increasing portion sizes especially of processed food items in developed countries are contributing to obesity (Morland, 2006; Canella et al., 2014).

Several investigators have shown that food portion size positively related to energy intake. The increase in portion sizes of food items like processed food, bakery, beverages, fast food etc. have led to increased caloric intake and obesity (Nestle, 2003; Muñoz-Pareja et al., 2013).The dietary intake patterns

have a role to play in causing obesity (Nozue et al., 2007). The dietary pattern varies widely across population and cultures. Regular snacking has been associated with the increased overall dietary intake in the affluent societies (Drummond, 1996; Muñoz-Pareja, et al., 2013).

3. Genetic factors

The obesity results from the phenotypic expression of a genetic susceptibility under the pressure of an obesogenic environment and characterized by a high phenotype heterogeneity linked most notably to differences in the stages of weight evolutions (Cuestas, 2008; Wajchenberg and Cohen, 2014) . Family studies have shown that heritability rates of total body fat mass are 50% (Henkin et al., 2003). In the last updates of this authoritative review, as many as 135 candidate genes had been identified as being linked and/or associated with obesity-related phenotypes, in addition to 253 quantitative traits (Perusse et al., 2005). A recent study on obesity-related genetic variants was performed in close to 250,000 individuals in whom 2.8 million single-nucleotide polymorphisms were genotyped. However, the combined effect of these genetic variants on obesity was modest, accounting for 6–11% of the genetic variation in BMI. Hence, factors other than DNA sequence variants alone are likely to explain the high heritability rates of obesity. These possibly include gene-gene interactions, gene-environment interactions, as well as epigenetics (Speliotes and Willer 2010). Genome-wide scans for measures of body fat distribution including waist circumference, or CT-measured visceral adipose tissue area, have led to the identification of several loci and candidate genes that could be of potential interest (Fox and Costa 2007; Norris et al., 2009; Perusse et al., 2001; Rice et al., 2002). Similarly, several studies have identified genetic variants that may be related to preferential accumulation of visceral adipose tissue accumulation in various populations (Katsuda et al., 2007, Berthier et al., 2004; Bouchard et al., 2004; Mussig 2009; Pausova 2010; Peeters and Beckers 2007). Several studies have also identified genetic variants that are associated with an increased susceptibility to the metabolic

complications of visceral obesity (Couillard et al., 2003). Most of these genetic variants need to be further validated and functionally characterized. Moreover, the relative impact of isolated variants, much like with overall adiposity measures, is quite low (Perusse et al., 2001). A study on twin adoption and family strongly suggests that the biological family members exhibit similarities in the maintenance of body weight (Wu and Suzuki 2006; Maffeis and Schutz 1997; Alison et al., 1998). (Table 2.1)

Table 2.1: Selected genes with variants that have been associated with obesity

Gene symbol	Gene name	Gene product's role in energy balance
ADIPOQ	Adipocyte-, C1q-, and collagen domain-containing	Produced by fat cells, adiponectin promotes energy expenditure
FTO	Fat mass- and obesity-associated gene	Promotes food intake
LEP	Leptin	Produced by fat cells
LEPR	Leptin receptor	When bound by leptin, inhibits appetite
INSIG2	Insulin-induced gene 2	Regulation of cholesterol and fatty acid synthesis
MC4R	Melanocortin 4 receptor	When bound by alpha-melanocyte stimulating hormone, stimulates appetite
PCSK1	Proprotein convertase subtilisin/kexin type 1	Regulates insulin biosynthesis
PPARG	Peroxisome proliferator-activated receptor gamma	Stimulates lipid uptake and development of fat tissue

Source: https://www.cdc.gov/genomics/resources/diseases/obesity/obesedit.html

4. Socio demographic factors

A number of aspects have been linked to obesity including age, gender and socio-economic status. The dominance of overweight and obesity become known in different socio-economic groups. Obesity has no longer remained a problem of only upper socio economic class but Indian middle class has also dragged into the so called "problem of affluent (Kujur and Kashyap, 2016). In

developed countries, levels of obesity are comparatively higher in the lower socio-economic groups (Braunschweig et al., 2005). A person's education is closely linked to his income and wellbeing (Battle and lewis 2002). Researchers have shown that obesity rose with a nation's economic development. In lower-income countries, people with higher SES were more likely to be obese. Conversely, in high-income countries, those with higher SES were less likely to be obese (Pampel and Denney 2012; Caballero 2007; Astrup, 2008). It may be that in lower-income countries, higher SES leads to consuming high-calorie food and avoiding physically tough tasks. But in higher-income countries, individuals with higher SES may have responded with healthy eating and regular exercise. The implication is that while economic development improves health, "problems of malnutrition are replaced by problems of overconsumption that differentially affect SES groups," noted the authors (Mayén et al., 2014; Pampel and Denney 2012). But some developing countries, such as India, are facing continued high levels of malnutrition along with a rise in obesity (Ravishankar, 2012).

With long standing history of infectious diseases, developing countries are now facing a rising tide of non-communicable diseases which is popularly known as the double burden of malnutrition (Coexistence of over and under nutrition) (Bishwajit, 2015).

Difference in the prevalence of obesity among different age groups was found and prevalence of obesity showed an increasing trend over the successive age period (Kujur and Kashyap, 2016). Age is also a major key factor in determining the role of childhood obesity. Studies have demonstrated that there are specific periods in the growth and development of a child which may lead to obesity. The childhood obesity has been identified as a risk factor for obesity in adulthood and is associated to an increased adult morbidity and mortality (Oner et al., 2004).

5. *Ethnicity*

The pronounced differences in regional adipose tissue distribution among various populations worldwide are well known. For a given amount of weight gain, some populations may be prone to accumulate adipose tissue in the subcutaneous adipose depots, whereas other populations may be more likely to accumulate adipose tissue in the visceral cavity. Ethnicity, therefore, critically needs to be considered in the identification of high-risk cases of obesity, especially in the definition of cut-off values of anthropometric measurements (Lear and James 2010; Lear and Humphries 2007; Katzmarzyk and Bray 2011). A national United States study of 9,179 individuals with over sampling of minority ethnic groups showed significant ethnic differences in body weight, with African- American and Hispanic populations at highest risk of obesity compared with Caucasian populations (McTigue and Garrett 2002). In Asian populations, a higher body fat content was reported at lower BMI values compared with Caucasians (Deurenberg, 2002). The significant ethnic differences in mean BMI may possibly be explained by intrinsic differences in body composition (Lear, 2009). Asians and Indian Asians seem to be especially prone to visceral fat accumulation despite lower total adiposity values compared with individuals from other ethnic backgrounds (Lear et al., 2007; Misra and Khurana 2009). Several hypotheses have been put forward regarding the physiological explanation of ethnicity-related differences in body fat distribution, the most plausible being related to genetic and epigenetic programming of the propensity of each fat compartment to store lipids (Misra and Khurana 2009; Sniderman, 2007). Overall, the greater propensity of some populations to accumulate visceral adipose tissue could contribute to their higher rates of type-2-diabetes and CVD.

6. *Birth Weight*

Studies have documented that adult obesity has an association with birth weight (McDonald and Han 2010). It has been reported that in intrauterine growth retardation (IUGR) newborns, a fetal programming is caused due to a

limited nutrient supply during pregnancy, which makes these children to alter their physiology of handling nutrients later in life. These affected children have persistent elevated insulin secretion that results in developing cardiac disease (Barker 1999). Recent studies suggest that the metabolic syndrome originates at fetal stage (Ozanne and Hales 2002, Levitt NS, Lambert, 2002). A birth cohort study undertaken in Finland on 5,210 subjects revealed that the incidence of obesity increased with increase in birth weight and ponderal index (Cnattingius et al., 2012; Eriksson, 2001). Another study done on 1,750 men and women has shown a positive association between birth weight and adult BMI (Tene, 2003). Birth weight and attained physical size during childhood were positively correlated with increased prevalence of obesity and overweight in adulthood (Monteiro and Monteiro, 2003). Evidence from the animal and human studies suggests that malnutrition in utero or in early life, endocrine development may be affected, which results in hormonal alteration and a predisposition to metabolic disorders and obesity (Barker, 1999).

7. *Environmental factor*

Changing societal structures due to economic transition have given rise to various problems like unemployment, overcrowding and family breakdown. These negative consequences play direct or indirect role which determine the nutritional and physical activity patterns which contribute to the development of obesity. Culture affects both food intake and physical activity pattern (Cruwys and Bevelander 2015; Agne and Daubert 2012; Richer, 2002). Cultural behaviours and beliefs are learned in childhood, are often deeply held. The cultural factors are among the strongestdeterminants of food choice (Verstraeten et al., 2014; WHO, 1997). There isincreasing evidence that children and adolescents of affluent families are overweightpossibly because of decreased physical activities, sedentary lifestyles, altered eating patterns

and increased fat content of the diet (Du and Bennett, 2013; Kapil and Bhasin, 2002).

8. Hypothalamo-Pituitary-Adrenal Axis, Stress, and Glucocorticoids

Excessive circulating glucocorticoid concentrations, as observed in Cushing's syndrome, create a pathological phenotype of abdominal obesity, dyslipidemia, insulin resistance, and hypertension (Peeke and Chrousos 1995). In most cases, cortisol hypersecretion origins from the pituitary gland (Cushing's disease) and results from excessive adrenocorticotropic hormone secretion. While individuals with idiopathic abdominal obesity share several of the morphological and metabolic alterations observed in Cushing's syndrome, alterations in the sensitivity and drive of the hypothalamo-pituitary-adrenal (HPA) axis have been shown to be much more subtle (Pasquali and Vicennati, 2000; Duclos et al., 2001). Early studies by the group of Björntorp and collaborators (Björntorp, 1993) had demonstrated that the cortisol response to stress induced by cognitive tests or cold exposure was positively related to abdominal sagittal diameter in premenopausal women. More recently, primate studies have suggested that social stress in primate colonies may be related to increased visceral obesity and coronary artery disease (Shively and Register 2009). A number of studies and review articles seem to point toward such an effect in humans (Dallman et al., 2004; De Vriendt and Moreno 2009; Donoho, 2011; Kyrou and Tsigos, 2008; Kyrou and Tsigos, 2007; Weigensberg and Corral, 2008; Vines et al., 2007). These studies suggest that chronic stress or poor coping in stressful situations is associated with mild hypercortisolemia and prolonged sympathetic nervous system activation, which in turn could favor accumulation of visceral fat (Kyrou and Tsigos, 2009).

9. Hypothyroidism

In this condition there is not enough thyroid hormone (thyroxine) to control normal rate of metabolism (Knudsen et al., 2005). Hence, the metabolism slows down. It can lead to fatigue and weakness of muscles, reducing the activity of the person. Thus underactivity of the thyroid gland can lead to increase in body weight (Moon et al., 2013).

10. Central mechanism in body weight regulation

In the CNS, the hypothalamus is the key region involved in the regulation of appetite (Murphy and Bloom, 2004). It had previously been hypothesized that satiety was controlled by the ventromedial hypothalamic nucleus, and that feeding was controlled by the lateral region (Vettor and Fabris, 2002).

i. Leptin: Expressed and secreted exclusively by white adipose tissue adipocytes, the circulating levels of leptin are proportional to fat mass (Ferrannini and Rosenbaum, 2014; Murphy and Bloom, 2004). Either peripheral or central administration of leptin has been demonstrated to reduce food intake and body weight and increase energy expenditure in rodents (Rosenbaum and Leibel, 2014; Friedman and Halaas 1998), and activation of hypothalamic neurons expressing the leptin receptor has suggested the mediation of its effects via this central region (Murphy and Bloom 2004). Leptin represents one of the core components of the physiological system that controls body weight in mammals. Humans with leptin deficiency are obese (Katarina 2014; Montague et al., 1997) and decreased leptin production from white adipose tissue has been demonstrated to contribute to a plethora of metabolic abnormalities associated with visceral obesity (Paz-Filho 2009). A study in obese men demonstrated that circulating leptin levels adjusted for body fat were inversely correlated with body weight, suggestive of a leptin deficient state associated with obesity (Paz-Filho 2009).

ii. Insulin: As an adiposity signal, insulin is believed to have a similar lipostatic role to that of leptin, although its central effects on food intake and energy homeostasis are less efficient (Katarina 2014). Similar to leptin, circulating insulin levels are proportional to the degree of adiposity (Rocha et al., 2011)Central administration of insulin in rodents has been shown to reduce food intake and body weight (Air and Benoit 2002), as with leptin, increased adiposity can lead to a decrease in insulin sensitivity and a state of insulin resistance (Adam et al., 2009). Adiposity might in fact be a consequence of insulin resistance itself (Morrison and Glueck 2008).

iii. Gut Hormones: Feeding is ultimately controlled by the central nervous system but is strongly influence by numerous physiological signal arising from the periphery that either promote or limit energy intake (Hameed and Dhillo 2009). Broadly speaking these gut hormone act via neuroendocrine mechanism to communicate information on changing energy status from the periphery of brain (Ritter 2004; Grill and Hayes 2012; Moran 2006). Some of these peptides are produced by gastro intestinal tract itself. Most of these gastro intestinal derived signal, including cholecystokinin, glucagon-like peptide-1, and peptide YY, promote meal termination; in contrast the hunger hormone ghrelin promotes the ingestion of food when readily available energy is low (Mietlicki-Baase and Hayes 2015; Cho 2011; le Roux et al., 2006). (Table 2.2)

Table 2.2: Peripheral effects of selected food intake-regulating gut hormones

Gut hormone	Site of synthesis	Food intake regulating receptor	Peripheral effect on food intake
CCK	Intestinal L-cells	CCKA	Decrease
Ghrelin	Stomach	GHS	Increase
PP	Pancreas/colon	Y4R	Decrease
PYY	Intestinal L-cells	Y2R	Decrease
GLP-1	Intestinal L-cells	GLP1R	Decrease
OXM	Intestinal L-cells	GLP1R?	Decrease

Abbreviations: CCK, cholecystokinin; CCK_A, cholecystokinin receptor subtype A; GHS, growth hormone secretagogue receptor; GLP-1, glucagon-like peptide-1; GLP1R, GLP-1 receptor; OXM, oxyntomodulin; PP, pancreatic polypeptide; PYY, peptide YY; Y2R, PYY Y2 receptor; Y4R, PP Y4 receptor

a. Cholecystokinin

CCK, the first gut hormone reported to affect appetite (Gibbs and Young 1973), has been shown to dose-dependently reduce food intake in both rats (Gibbs and Young 1973) and humans (Lieverse and Jansen 1995) and in response to meal initiation, plasma levels have been reported to rise within 15 min (Liddle 1985). Within the GI tract, CCK is predominantly synthesized and released from the duodenum and jejunum (Buffa and Solcia 1976), where its local regulatory effects include stimulation of gallbladder contraction and inhibition of gastric emptying (Hon et al., 2013; Dufresne and Seva 2006). Centrally-administered CCK has been shown to reduce food intake in rodents (Matson CA, Reid 2000), whereas peripheral administration has been shown to reduce food intake in both rodents and humans, through a reduction in meal size and duration (Ronveaux and Tome 2015; Kissileff 1981). As a result,

CCK has been investigated as a potential therapeutic target for the management of obesity (Hameed and Dhillo 2009).

b. Ghrelin

The 28–amino acid peptide hormone ghrelin, produced predominantly in the stomach (Kojima et al., 1999) represents the only known orexigenic gut hormone identified to date (Schubert and Sabapathy 2014; Hameed and Dhillo 2009). Ghrelin binds to the growth hormone secretagogue receptor which is highly expressed in the hypothalamus and brain stem (Kojima 1999). Ghrelin has also been shown to stimulate appetite in both lean and obese humans (Wren et al., 2001; Druce 2005), and infusion (intravenous) in healthy volunteers, at a concentration similar to that observed after a 24 h fast, has been shown to increase appetite and food intake at a buffet-style meal by almost 30% (Murphy and Bloom 2004). Subcutaneous injection has also been shown to significantly induce appetite and increase food intake (Druce et al., 2006) however, numerous other studies have reported no changes and increases (Ybarra et al., 2009) in circulating fasting and post- prandial ghrelin levels following GI surgery, thus highlighting the incomplete understanding of the effect of the surgery on circulating levels of this orexigenic gut hormone.

c. Pancreatic polypeptide

The 36-amino acid anorexigenic peptide PP, is primarily synthesized and released from the endocrine pancreas, and to a lesser extent, from the colon and rectum (Hameed and Dhillo 2009). Levels are low during the fasting state and rise in proportion to caloric intake (Schubert and Sabapathy 2014; Track and McLeod 1980). Peripherally-administered PP reportedly leads to a reduction in food intake, in both rodents and humans (Malaisse-Lagae 1977; Batterham et al., 2003). Peripheral PP administration has also been demonstrated to lead to an increase in energy expenditure and a reduction in

body weight in rodents, and a demonstrated reduction in appetite and food intake in both lean and obese humans has shed further light on its potential anti-obesity utility (Batterham et al., 2003).

d. Peptide YY

PYY, a member of the PP-fold family of proteins to which PP also belongs, is so named because of the tyrosine residues at both its N- and C-termini (Tatemoto and Mutt 1980). The full-length 36-amino acid peptide is synthesized and released from the L-cells of the GI tract. Circulating levels of PYY3−36 are influenced by meal composition and calorie content, and become elevated within 1 h post-feeding (Adrian et al., 1985) Similar to PP, peripherally-administered PYY3−36exerts its food intake-inhibiting effects (Schubert and Sabapathy 2014; Lumb et al., 2007) As circulating PYY3−36 levels are often lower in the obese state (Cahill et al., 2014), it has been suggested that this characteristic may in fact have a causative role in the development of obesity (Cahill et al., 2014; le Roux et al., 2006).

e. Glucagon-like peptide (GLP-1)

In the gut, GLP-1 is released from small intestinal and colonic L-cells in proportion to ingested calories (Herrmann and Goke 1995) In both lean and obese humans, peripherally-administered GLP-1 has been shown to exert anorexigenic effects (Schubert and Sabapathy 2014; Gutzwiller et al., 1999), with other possible influences on food intake being linked to a reduction in gastric emptying and a suppression of gastric acid secretion (Verdich et al., 2001). Both centrally- and peripherally-administered GLP-1 or GLP-1 receptor agonists have been shown to enhance satiety, reduce food intake, and promote weight loss in rodents and humans (Baggio and Drucker 2014; Vilsboll et al., 2007). GLP-1 are now in trial, and determination of their efficacy as anti-obesity agents is ongoing (Baggio and Drucker 2014; Steinert

et al., 2014; Neary and Batterham 2009). Further to the currently investigated approaches, future research aimed at better understanding the mechanisms involved in endogenous GLP-1 production would be beneficial. With the known additive satiating effects of GLP-1 and PYY, exploiting endogenous GLP-1 production may also yield a novel combinatorial anti-obesity approach.

f. Oxyntomodulin

Early work in rats on a peptide with inhibitory action on stomach oxyntic glands lead to the advent of the name OXM for the now well established gut hormone (Dubrasquet and Bataille 2006) OXM shares the same precursor molecule as GLP-1, is co-secreted with GLP-1 following feeding, and its release is also proportional to meal calorie content (Schubert and Sabapathy 2014; Druce and Bloom 2006). Centrally- and peripherally-administered OXM reduces food intake and increases energy expenditure in rodents, and reductions in body weight have been reported in response to chronic injections (Coll and Faruqui, 2007) Peripheral administration in humans increases satiation and reduces food intake, with repeated injections leading to decreases in body weight (Wynne et al., 2005). There has also been data in support of OXM promoting increased energy expenditure in humans (Murphy and Dhillo 2006).

11. Gut microflora

Formation of the gut is one of the first outcomes of multi cellularity. It appears on the 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 a highly dynamic ecosystem. The total area of the mucosal surface of the human gastrointestinal tract is 300 m^2 which makes it the largest surface area in the body that interacts with the external

environment (Bjorksten B, 2006). It comprises a series of complex and dynamic organs ranging from the stomach to the distal colon, which harbor immense microbial assemblages that are known to be vital for human health. Many species of bacteria have evolved and adapted to live and grow in the human gut. The number of bacteria in the human gut has been estimated to exceed the number of somatic cells in the body by an order of magnitude and that the biomass of the gut microflora may reach up to 1.5 kg (Karlsson, 2013). Due to the cost of weight reducing drugs and its side effects and inability to maintain healthy weight by means of life style modifications, recent studies have enlighten the importance of human gut microflora in various essential metabolic pathways. The various activities performed in the gut can play an outstanding role in treatment and prevention of metabolic disorders using prebiotics which have little side effects (Vieira, Teixeira, and Martins, 2013).

12. Access alcohol consumption

Miscellaneous factors linked to development of obesity include excessive alcohol consumption (Bhatt and Mehan 2014; Croezen, et al., 2009; Grochow 1985), The experimental metabolic evidence suggests that the consumption of moderate amounts of alcohol has to be accounted for in the energy- balance equation and may represent a risk factor for the development of a positive energy balance and thus weight gain. There seems to be a large individual variability according to the absolute amount of alcohol consumed, the drinking frequency as well as genetic factors. Presently it can be said that alcohol calories count more in moderate nondaily consumers than in daily (heavy) consumers. Further, they count more in combination with a high-fat diet and in overweight and obese subjects (Suter and Tremblay 2005).

- ***Co morbidities associated with obesity***

Obesity is associated with an increased incidence of hypertension, diabetes, coronary artery disease, osteoarthritis and overall increase in morbidity during adult life (Wormser et al., 2011; Winter and MacInnis, 2014).

a. Obesity, metabolic syndrome and other co morbidities.

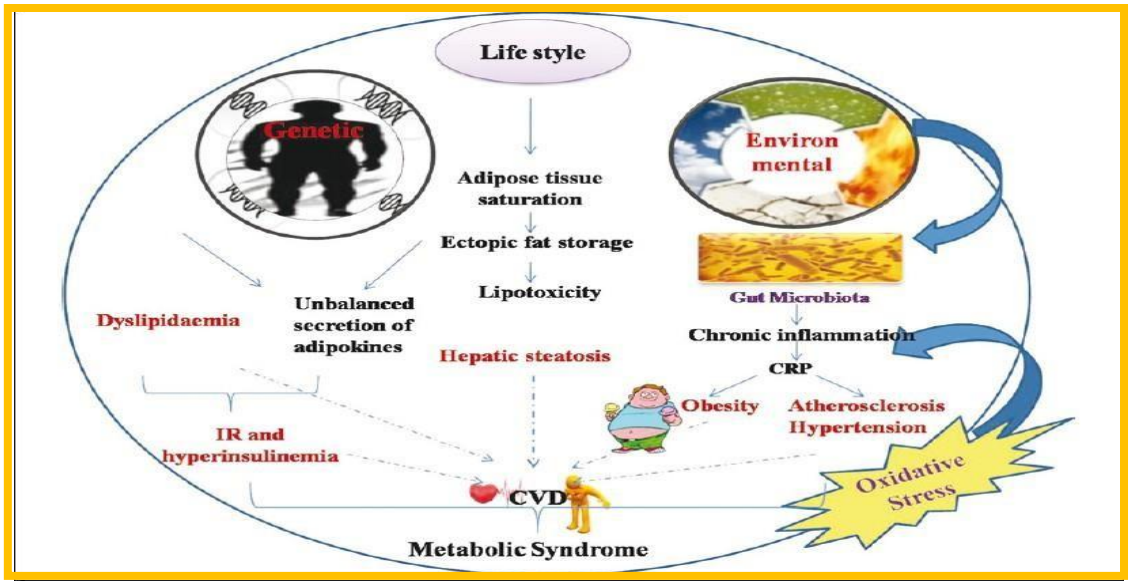
The transition from the traditional diet to calorie dense food and a sedentary lifestyle has led to a rapid increase in the prevalence of obesity both in children and adults. The constellation of abnormalities associated with obesity or abdominal obesity has been termed "metabolic syndrome," Although visceral adiposity and features of the metabolic syndrome are associated with an increased relative risk of CVD (Mottillo et al., 2010). This has been followed by the appearance of other 'diseases of affluence' such as diabetes mellitus and cardiovascular diseases (Belenchia and Tosh 2013; Fall 2001; Calle 2003). Obesity is a risk factor for a number of diseases including type-2-diabetes, cardiovascular disease. Other diseases related to obesity are hypertension, gallstones, sleep apnea, dyslipidemia, insulin resistance, psychological disorders and certain type cancers (Kotronen, 2008; Wormser et al., 2011; WHO, 1998 a).

The physiological risk factors like adipose tissue saturation, dyslipidemia, lipotoxicity, insulin resistance, chronic inflammation, oxidative stress are inter-related and associated with pathogenesis and progression of metabolic abnormalities like co- Morbidities obesity, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), hypertension, etc. These conditions often lead to pathophysiology of metabolic syndrome, which increases the risk of cardiovascular diseases (Savini, Catani, Evangelista, Gasperi, & Avigliano, 2013). (Figure 2.2)

b. Obesity and Cardiovascular Disease

Cardiovascular disease encompasses Cardiovascular Heart Disease (CHD), stroke and peripheral vascular disease. Obesity predisposes an individual to a number of cardiovascular risk factors including hypertension, raised

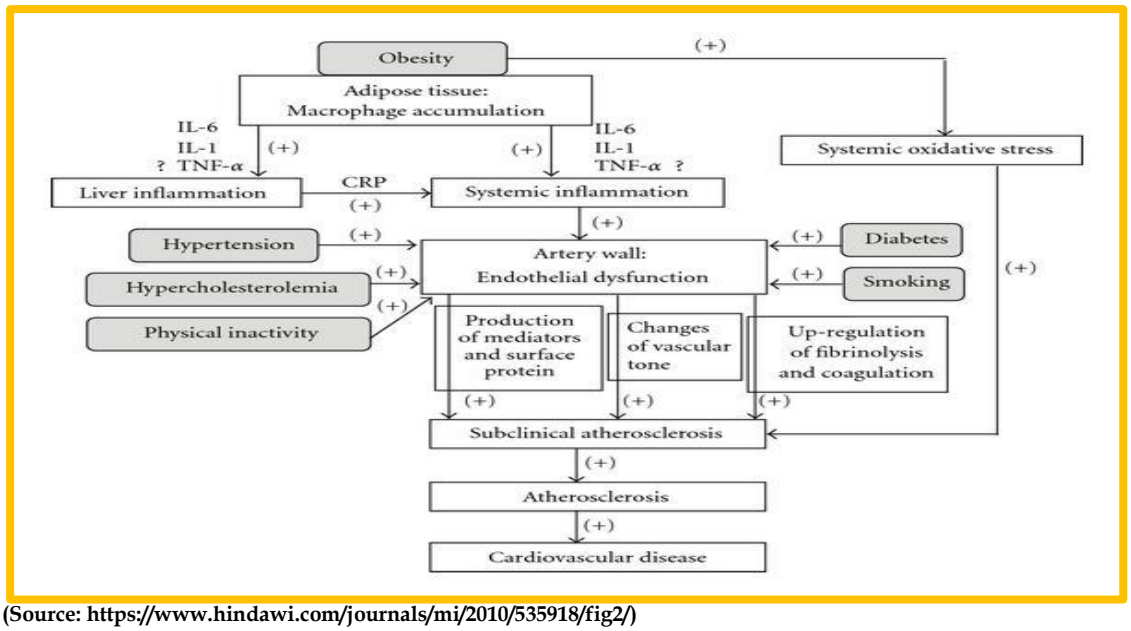
cholesterol and impaired glucose tolerance. Evidence suggests that the obesity is associated with increased risk for cardiac diseases (Wu, et al., 2014; Lavie and Milani 2009; Golley et al., 2006). On the basis of the robust evidences abdominal obesity and excess visceral adiposity is linked to an atherogenic dyslipidemic state (Dagenais and Mann 2005; Yusuf et al., 2005; Wormser et



al., 2011; Després2011). (Figure 2.3)

(Source: Mallappa et al., 2012)

Figure 2.2: The pathophysiology of obesity leading to metabolic syndrome



(Source: https://www.hindawi.com/journals/mi/2010/535918/fig2/)

Figure 2.3: The pathophysiology of obesity leading to cardiovascular disease

c. Obesity and Diabetes Mellitus

For more than two decades, abdominal obesity has been repeatedly associated with insulin resistance, and several seminal review papers have been published on this topic (Lovegrove 2006; Misra et al., 2008; Mahendra 2013). A study conducted on Asian Indians documented that the fasting insulin correlated significantly with the body mass index and waist circumference (Ley and Hamdy 2014). The odds ratio for hyperinsulinemia was 4.7 in overweight subjects and 6.4 with high waist circumference (Mishra et al., 2004), a finding fully concordant with the published evidence that patients with type 2 diabetes have more abdominal visceral fat and more ectopic fat than nondiabetic individuals matched for BMI (Ley and Hamdy 2014; Karter et al., 2005; Wang and Hu 2005; Balkau et al., 2007; Kotronen 2008). (Figure 2.4)

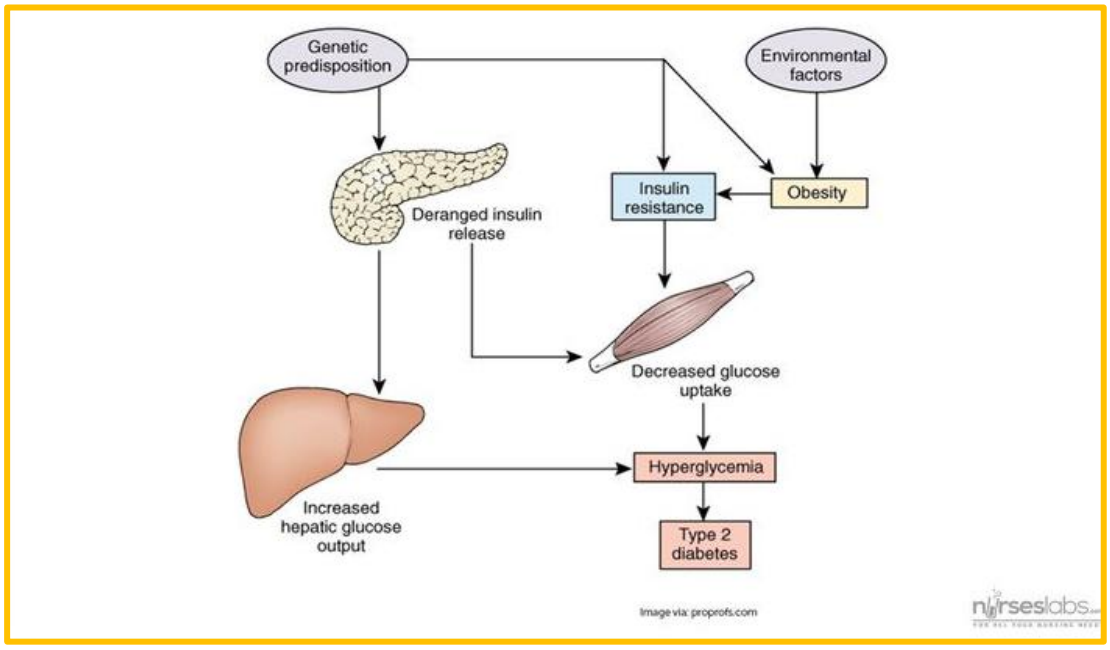
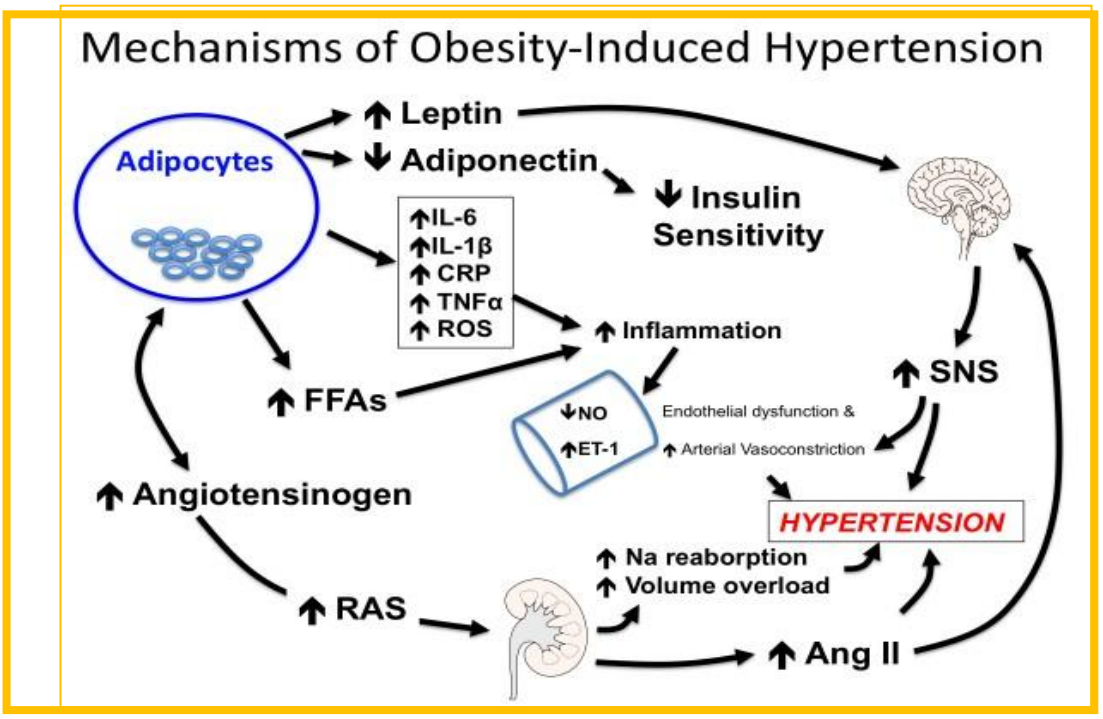


Figure: 2.4: The pathophysiology of obesity leading to type-2-diabetes

d. Obesity and Hypertension

The link between obesity and hypertension has long been recognized, with obese patients having higher rates of hypertension than normal-weight

individuals (Chiang and Perlman 1969; Stamler and Riedlinger 1978). Waist circumference has been reported as the strongest independent predictor of systolic blood pressure and diastolic blood pressure (Hall et al., 2014; Lavie and Milani 2009; Hayashi et al., 2003). Furthermore, excess visceral fat has been found to be associated with hypertension (Hall et al., 2014). (Figure 2.5)



IL-6: interleukin-6; IL-1β: interleukin-1β; CRP: C-reactive protein; ROS: reactive oxygen species; FFAs: free fatty acids; NO: nitric oxide; ET-1: endothelin-1; RAS: renin-angiotensin system; SNS: sympathetic nervous system (increased tone). (Source: Kotsis et al., 2010)

Figure 2.5: Proposed mechanisms involved in the pathogenesis of obesity-induced hypertension.

e. Obesity and respiratory diseases

Obesity carry a risk of (i) restrictive airway disease caused by the difficulty in respiration from the mass of adipose tissue (ii) obstructive airway disease caused by fatty deposition along the airway, added to the tonsillar and adenoidal hypertrophy. Obstructive sleep apnea with carbon dioxide retention, hypoxia and right ventricular hypertrophy is a potential cause of severe morbidity and mortality in obese subjects (Rutten et al., 2010; Mottin

and Canani 2007; Malhotra and White 2002; Spiegel and Tasali 2004). Sleep disorder breathing is highly prevalent in childhood obesity. A cross-sectional studies has demonstrated an association between breathing disorders related to sleep and metabolic syndrome (Verhulst 2009).

f. Other related disorders

Cancer: Increasing body weight is associated with increased risk for specific cancers (Balentine et al., 2010; Bansen and Chang 2011; Pischon and Nothlings2008; Renehan and Tyson 2008). A number of studies have found a positive association between overweight and the prevalence of cancer, particularly hormone dependent and gastrointestinal cancers (Kim and Lee 2009). Greater risks of endometrium, ovarian, cervical and postmenopausal breast cancer have been documented for obese women, while there is small evidence for an increased risk of prostate cancer among obese men. The increased incidence of cancers in the obese subjects is greater in those with excess abdominal fat (Tiggemann and Pickering 1996). The incidence of gastrointestinal cancers, such as colorectal (Kang et al., 2010; Guiu et al., 2010; Nitori et al., 2009) and gallbladder cancer has also been reported to be positively associated with body weight. The renal cell cancer has consistently shown to be associated with overweight and obesity (Schapira 1994).

Sub-clinical inflammation: Obesity contributes to the development of vascular inflammation which raises markers of inflammation. High levels of C-reactive protein (CRP) levels denote future risk for development of type 2 diabetes mellitus and cardio vascular heart disease. In a recent study in Indian adolescents, high CRP levels were seen in 22 per cent in overweight subjects and in about 25 per cent in obese (Al-Hamodi 2014; Turer and Scherer 2012; Vikram et al., 2006). CRP levels show an association with per cent body fat, waist-hip ratio, waist circumference and triceps skin fold

thickness in Indian children (Vikram et al., 2003). Excess dietary intake of saturated fat has been found to be strongly correlated to high CRP levels in Indian adolescents (Nettleton et al., 2006; Arya et al., 2006).

Osteoarthritis: The extra weight an obese person carries, especially with fat distribution in the abdomen, puts increased stress on the weight-bearing joints. The common joints affected are knee join, hip joint and joints of the back bone. The individuals with BMI 30 Kg/m^2 or more had markedly higher risk for osteoarthritis as compared to the normal weight individuals. Obese children can suffer from orthopedic complications, including abnormal bone growth, degenerative disease and pain (Thijssen and Caam 2014; Gupta and Meuller 2002). Elevated risk of back pain has been observed in relation to obesity, particularly amongst adults who had childhood obesity (Han and Schoutel 1997). Recent studies have shown that adipocytokine leptin is a possible link between obesity and OA: Leptin levels in synovial fluid are increased in obese patients, leptin receptor (Ob-R) is expressed in cartilage, and leptin induces the production of matrix metalloproteinases (MMPs), pro-inflammatory mediators and nitric oxide (NO) in chondrocytes, not only leptin levels in the joint but also leptin sensitivity in the cartilage are enhanced in obese OA patients (Vuolteenaho and Koskinen 2014).

2.3: Obesity, GI health and its significance

Gastrointestinal symptoms occur commonly among the general population, however, the relationship between obesity and GI symptoms remains poorly understood. A recent meta-analysis evaluated a number of GI symptoms with obesity and increasing BMI (Eslick, 2012). Significant associations were identified between increasing BMI and upper abdominal pain, gastroesophageal reflux, vomiting, chest pain/heartburn, diarrhea, retching and incomplete evacuation (DiBaise and Foxx-Orenstein, 2013). In a

population-based study investigating associations among binge-eating behavior patterns, a disordered eating pattern that occurs commonly among obese individuals, and GI symptoms, after adjusting for BMI, age, gender, race, diabetes mellitus, socioeconomic status and physical activity level, binge-eating behavior was independently associated with the following upper and lower GI symptoms: acid regurgitation, heartburn, dysphagia, bloating, upper abdominal pain, diarrhea, urgency, constipation and feeling of anal blockage (Cremonini et al., 2009). This highlights the complex relationships among obesity, eating patterns and GI symptoms and the need for further study.

Obesity is also associated with a number of GI and hepato-biliary conditions. Many of the GI diseases that are commonly seen in normal weight individuals are seen up to 2–3 times more commonly in those who are obese (DiBaise and Foxx-Orenstein, 2013). An increase of obesity and gastroesophageal reflux disease (GERD) in Western populations in the past 50 years suggests an association between the two conditions (Friedenberg, Xanthopoulos, Foster, and Richter, 2008). Another recent report also demonstrated an association of visceral fat and fat near the gastroesophageal junction with Barrett's, and with increased esophageal inflammation and high-grade dysplasia in Barrett's patients, independent of BMI (Nelsen et al., 2012).

Obesity, the metabolic syndrome and rapid weight loss are modifiable risk factors of gallstone formation (Feneberg and Malfertheiner, 2012). A BMI >30 kg/m^2 is associated with about a threefold increase in risk of gallstone development, while a BMI > 45 $kg/m^2$is associated with a sevenfold increase in risk (Stinton and Shaffer, 2012). Obesity may disturb lipid and endogenous hormones metabolism and affect gallbladder motility, thereby increasing the risk of gallstones and gallbladder cancer (Wang, Wang, and Qiao, 2012).

In a recent report, overweight or obesity during early adulthood was associated with a greater risk of pancreatic cancer and a younger age of

disease onset, while obesity at an older age was associated with a lower overall survival in patients with pancreatic cancer (Li et al., 2009).

Obesity is associated with an increased risk of the spectrum of nonalcoholic fatty liver diseases (NAFLD) including simple steatosis, nonalcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma. Nonalcoholic fatty liver diseases has become one of the most common causes of liver disease worldwide and has been associated not only with obesity but also other features of the metabolic syndrome including insulin resistance, impaired glucose tolerance and dyslipidemia (Milic and Štimac, 2012). Importantly, there is evidence that obesity is associated with an increased risk of hepatocellular carcinoma independent of the presence of cirrhosis (Milic and Štimac, 2012; White, Kanwal, and El–Serag, 2012). Obese persons have a higher risk of colon adenomas including advanced lesions compared to normal weight persons (Okabayashi et al., 2012).

At its core, obesity results from an imbalance between energy consumed relative to energy expended. Energy intake and expenditure are tightly regulated by the brain, particularly the hypothalamus and brainstem, in a homeostatic mechanism that integrates neural, hormonal and metabolic afferent signals from both the periphery and centrally (Korner and Leibel, 2003; Huda, Wilding, and Pinkney, 2006; Klein, Wadden, and Sugerman, 2002).

Although the liver, muscle, adipocytes, pancreatic islets and CNS participate, the GI tract is the first organ affected by nutrient intake. There are a number of factors linking the gut to the pathogenesis of obesity including gut hormones, gut motility and the gut microbiota. Food ingestion results in the release of a number of gut hormones and activates gut motility, gastric and pancreaticobiliary secretion and the digestive and absorptive processes (Camilleri, 2006).

There has been an explosion of interest in recent years on the role of the microbes present within our GI tract in health and disease. It has been known for a much longer time that intestinal microbes play an important role in the metabolism of poorly absorbed nutrients. Recent experiments in mice suggest that differences in the composition of gut microbes between obese and lean animals may affect (i.e., enhance in obese mice) energy harvest and storage (Backhed et al., 2004; Turnbaugh et al., 2006; Ley et al., 2005). While intriguing, the studies to date in humans have been conflicting and the relevance of this to humans remains to be determined (DiBaise, Frank, and Mathur, 2012).

2.4: Obesity and gut microbiota

> ***"All disease begins in the gut"***
>
> ***"Health is determined by the microbiota in our gut"***
>
> ***-Hippocrates 460 BC – 370BC***

The etiology of obesity has been accredited to several factors like environmental, dietary, lifestyle, host, and genetic factors; nevertheless none of these entirely elucidate the increase in the incidence of obesity globally. Gut microbiota located at the line of host and surroundings in the gut are a new area of research being explored to explain the excess accumulation of energy in obese individuals and may be a potential target for therapeutic manipulation to reduce host energy storage. Evidence from animal and human studies clearly indicates controversies in determining the cause or effect relationship between the gut microbiota and obesity. Metagenomics based studies indicate that functionality rather than the composition of gut microbiota may be important (Khan, Gerasimidis, Edwards, and Shaikh, 2016).

Evidence linking the gut microbiota with obesity in humans is so far open to doubt and contentious. This may be partly due to marked inter-individual variations in the gut microbiota and metabolic activity in humans with age, diet, use of antibiotics, genetics, and other environmental factors (Delzenne, Neyrinck, Bäckhed, and Cani, 2011). Apart from the inter-individual variation in faecal microbiome and diversity, re-analysis of large datasets such as from the human microbiome project (HMP) and MetaHIT has shown inter-study variability which was far greater than the actual differences between the lean and obese phenotypes (Finucane, Sharpton, Laurent, Pollard, and Zafar, 2014). The first evidence showing higher Firmicutes and lower Bacteroidetes in obese versus lean adults before the onset of dietary intervention was presented by Ley et al. (2006) (Ley, Turnbaugh, Klein, and Gordon, 2006), followed by a number of studies reviewed in Table 2.3. The type of gut microbiota and their exact phylo-genetic level at which they exhibit differences are still under investigation. Evidence suggesting no phylum level differences between lean and obese gut microbiota (Schwiertz et al., 2010; Duncan et al., 2008) may indicate that functionality of bacteria may play a more important role than particular bacterial groups.

The energy harvesting capability of the gut microbiota in obese subjects is thought to be set at a higher threshold than in the lean with or without differences in the relative abundance of the gut microbiota. Obese adults had higher individual and total SCFA than lean adults in the absence of any difference in the relative abundance of major gut bacterial phyla (Fernandes, Su, Rahat-Rozenbloom, Wolever, and Comelli, 2014). Moreover, no significant correlation of the gut microbiota with dietary factors in early (Bergström et al., 2014) and later childhood (Bervoets et al., 2013) and a positive correlation with BMI SDS indicate that changes in the gut microbiota at these developmental stages may not depend on dietary factors.

On the other hand, evidence also suggests that diet plays an important role in altering the proportion of gut microbiota in individuals because the amount

and type of bacteria change significantly with diet (Santacruz et al., 2010; Walker et al., 2011). This varies between individuals and may be due to the distinct microbiota colonization during early life, altering the capacity for energy harvest from the diet. Composition and caloric content of the diet significantly alter the relative abundance of the gut microbiota (Walker et al., 2011). Similarly, SCFA production is affected by nutrient load and dietary carbohydrate available for fermentation (Duncan et al., 2007).

Long term changes in gut microbiota (such as lower counts of Bifidobacteria and higher Bacteroides) have been observed in children who were exposed to antibiotics in early childhood (Penders et al., 2006; Arboleya et al., 2012). Modulation of gut microbiota with antibiotics (e.g., norfloxacin and ampicillin) alters the expression of hepatic and intestinal genes involved in inflammation and metabolism thereby changing the hormonal, inflammatory, and metabolic milieu of the host (Membrez et al., 2008). The development of gut microbiota in infants and their tendency towards overweight and obesity in later childhood are linked to mother's pre-pregnancy, BMI and gut microbiota with significantly lower numbers of faecal Bifidobacteria and Bacteroides and significantly higher E. coli and Staph. aureus in overweight and obese compared to normal weight pregnant women (Santacruz et al., 2010).

In addition to compositional differences between lean versus obese subjects (Ley et al., 2006), functional differences in the metabolome of the obese and lean phenotype may be more important. Calvani et al. (2010) in their preliminary study of 15 morbidly obese and 10 age matched controls found distinct gut microbial co-metabolites in urine of obese versus lean participants, including lower levels of hippuric acid (benzoic acid derivative), trigonelline (niacin metabolite), and xanthine (purine metabolism) and higher levels of 2-hydroxybutyrate (metabolite of dietary protein) (Calvani et al., 2010). Disturbance of this equilibrium is a hallmark of the obese phenotype as suggested by Ferrer et al. (2013) in a comparative metagenomic and meta-

transcriptomic analysis of faecal samples from obese and lean adolescents (Ferrer et al., 2013). Despite low compositional representation (up to 18%), up to 81% of the expressed proteins were contributed by Bacteroidetes (Ferrer et al., 2013). Moreover, the obese metagenome had higher aerobic and anaerobic vitamin B12 and 1,2-propanediol metabolism genes than the lean which expressed genes related to vitamin B6 metabolism (Ferrer et al., 2013). A recent study by Cottilard et al. (2013) has shown a reduced bacterial richness, reduced diversity, and higher dysmetabolism and low-grade inflammation in obese versus lean humans (Cotillard et al., 2013). Although dietary intervention partially improved gene richness, reduced measures of adiposity such as waist circumference and fat mass, and reduced plasma cholesterol, it was less efficient in improving low-grade inflammation (Cotillard et al., 2013). Controversies exist as to whether or not obese and nonobese individuals host a particular type of bacterial phyla or enterotype and whether the response of the gut microbiota to diet differs. Correlation of BMI with Bacteroides in obese and non-obese subjects on different dietary regimens (Duncan et al., 2008) is unclear as an inverse relationship has also been observed (Schwiertz et al., 2010), adding to the complexity of the relationship of diet, gut microbiota, and obesity. The population of gut microorganisms in the human intestine is affected by a variety of factors from birth till adulthood, of which some are known and others are largely unknown. Additionally, large inter-individual variations have been observed in all human studies suggesting host diet interaction at individual level.

Table 2.3 : Association of gut microbial species/genera with obesity or leanness in human studies.

Bacteria	Association* with obesity	Group	Level	Other associations	Reference
Lactobacillus reuteri	+ve	Firmicutes	Species	–	(Million et al., 2013) (Kong et al., 2013)
Clostridium cluster XIVa	+ve	Firmicutes	Group	Anti-inflammatory	(Kim, Hwang, Park, and Bae, 2013)
E. coli	+ve	Proteobacteria	Species	Non-alcoholic steatohepatitis (NASH)	(Kim, Hwang, Park, and Bae, 2013)
Staphylococcus spp.	+ve	Firmicutes	Genus	Energy intake	(Bervoets et al., 2013)
Bacteroides	−ve/+ve	Bacteroidetes	Genus	Controversial	(Ley et al., 2006)
Akkermansia muciniphila	−ve	Verrucomicrobia	Species	Mucus degradation	(Everard et al., 2013)
Methanobrevibacter smithii	−ve	Archaea	Species	Increase in anorexia	(Armougom, Henry, Vialettes, Raccah, and Raoult, 2009)
Clostridium cluster IV; F. prausnitzii	−ve	Firmicutes	Species	Anti-inflammatory	(Calvani et al., 2010)
Bifidobacteria	−ve	Actinobacteria	Genus	−ve association with allergy	(Million et al., 2013)

Source: (Khan et al., 2016)

Note: *Associations based on correlation or regression analysis or statistically significant differences between the lean and obese; +ve: positive association, −ve: negative association, and +ve/−ve: controversial.

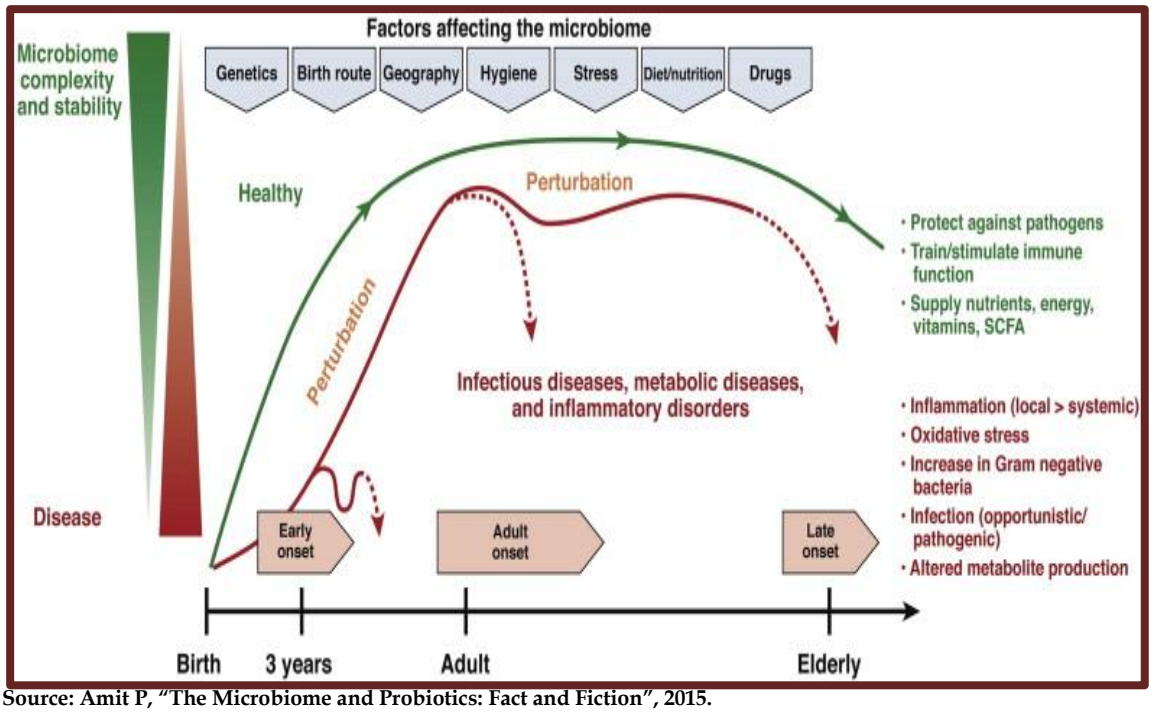
2.5 Factors affecting intestinal microbiota and health

Host genetic variation drives phenotype variation and our microbial phenotype is also influenced by our genetic state. The host genetic effect varies across taxa and includes members of different phyla (Goodrich et al., 2014; Org et al., 2015). The gut microbiome is environmentally acquired from birth (Costello, Stagaman, Dethlefsen, Bohannan, and Relman, 2012; Walter and Ley, 2011), therefore it may function as an environmental factor that interacts with host genetics to shape phenotype, as well as a genetically determined attribute that is shaped by, and interacts with, the host (Spor, Koren, and Ley, 2011; Nelson, Peterson, and Garges, 2011). Although gut microbiomes can differ markedly in diversity across adults (Costello et al., 2012; Qin et al., 2010), family members are often observed to have more similar microbiotas than unrelated individuals (Lee, Sung, Lee, and Ko, 2011; Tims et al., 2013; Turnbaugh et al., 2009; Yatsunenko et al., 2012). Familial similarities are usually attributed to shared environmental influences, such as dietary preference, a powerful shaper of microbiome composition (Cotillard et al., 2013; David et al., 2013; Wu et al., 2011). Yet related individuals share a larger degree of genetic identity, raising the possibility that shared genetic composition underlies familial microbiome similarities (Goodrich et al., 2014).

As has been reported in humans (Biasucci et al., 2010; Dominguez-Bello et al., 2010), mode of delivery likely has an influence on the composition of the murine GM. Vaginal delivery typically seeds offspring with microbes normally found in the maternal vaginal or intestinal microbiota (e.g., *Lactobacillus* spp., *Bifidobacterium* spp.) while Cesarian delivery tends to result in greater proportions of microbes normally associated with the external body surfaces (e.g., *Staphylococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp.) (Rodríguez et al., 2015).

The composition of the GM can shift rapidly and globally in response to abrupt changes in the macromolecular content of diet (Sonnenburg et al.,

2016). Due to insolubility or a lack of the necessary hydrolases, complex plant polysaccharides are often refractory to digestion in the small intestines. As such, undigested polysaccharides reach the colon and serve as a major energy source for the GM (Sonnenburg et al., 2010). Modulation of gut microbiota with antibiotics (e.g., norfloxacin and ampicillin) alters the expression of hepatic and intestinal genes involved in inflammation and metabolism thereby changing the hormonal, inflammatory, and metabolic milieu of the host (Membrez et al., 2008). (Figure 2.8)



Source: Amit P, "The Microbiome and Probiotics: Fact and Fiction", 2015.

Figure 2.6: Factors affecting gut microbiome

2.6 Mechanism of action of colonic microbiota in the gut

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 actions

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 microoganisms 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).

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 JI 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).

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 (Krinos 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.

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 1994). 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 localized niches (Guarner et al., 2005).

2.7: Obesity and endotoxemia (LPS)-a ferocious cycle

Gut microbiota contribute to the development of the insulin resistance and the low grade inflammation characterizing obesity. The concept of metabolic endotoxemia (increase in plasma LPS levels) as triggering factor in the development of the metabolic alterations associated with obesity (Beckhed, 2004; Cani, 2007). Endotoxemia is the presence of endotoxins in the blood. The term endotoxin was coined by Richard F. The term 'endotoxin' is used synonymously with the term lipopolysaccharide. Lipopolysaccharides (LPS), also known as lipoglycans, are found in the outer membrane of Gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals (Rietschel ET, et. al., 1994). It comprises three parts: (Figure 2.7)

- O antigen
- Core oligosaccharide
- Lipid A

The lipid A domain is responsible for toxicity. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock).

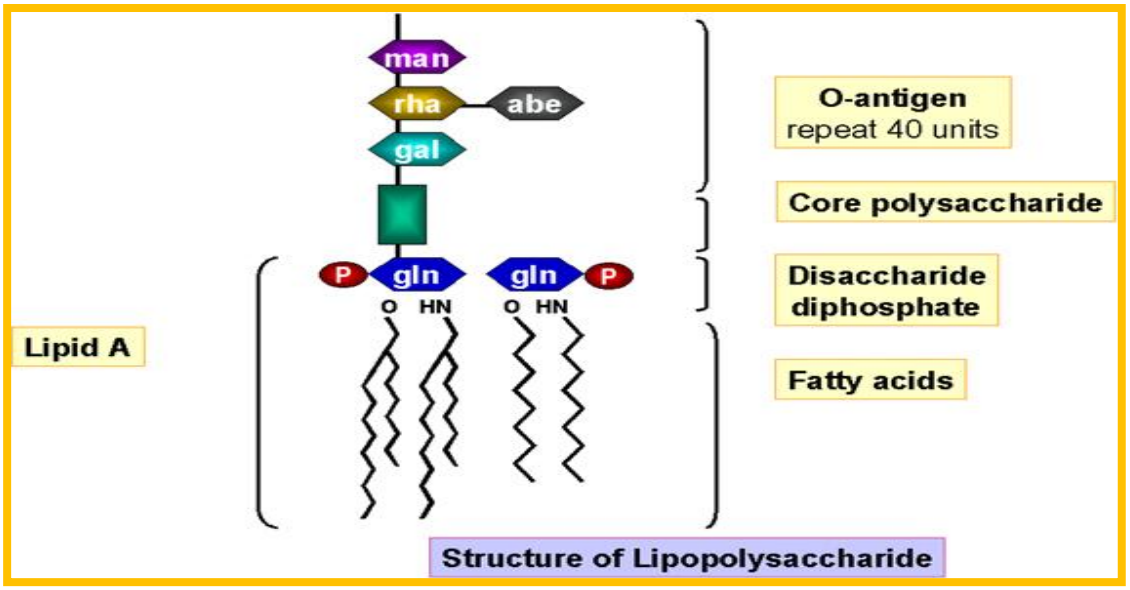


Figure 2.7: Structure of LPS molecule

Bacterial lipopolysaccharide (LPS, also termed endotoxin) released from dead Gram-negative bacteria in the gut and under conditions of gut damage can translocate into the circulation (as can whole bacteria) where it triggers an inflammatory response (Ulevitch et al., 1999). Using mouse models, Cani and colleagues have demonstrated that exposure to a high-fat diet increases systemic endotoxin concentration, a phenomenon termed 'ME' Metabolic endotoxemia, is characterized by chronic, but only moderately increased levels of plasma lipopolysaccharide (LPS) and is associated with a low-grade inflammatory status. In a mammal suffering from ME the concentration of circulating LPS is typically only 2-3 times higher than normal. This increase is 10-50 times lower than values reached during septicemia or septic shock (Cani et. al., 2007). LPS circulates in the plasma of healthy human subjects at low concentrations ranging between 1 and 10pg/ml, with transient increases rarely exceeding 80 pg/ml. Plasma LPS is derived mainly from the gut, which is a reservoir of≥ 1 g of LPS (Berg RD, 1996). Metabolic endotoxemia can cause chronic low-grade inflammation and mild disturbances in energy metabolism implicated in the development of CVD and type-2-diabetes (KR Feingold et al., 1992; U Maitra et al., 2011). These Plasma LPS conc. in metabolic endotoxemia are 10-50 times lower than that obtained during septic shock (Mitaka C, 2005). LPS is contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps stabilize the overall membrane structure. LPS binds the CD14/TLR4/MD2 receptor complex, which powerfully elicit the pro-inflammatory response of the innate immune system resulting in target organ damage (Beutler et. al., 2004). The major factor involved in the development of metabolic endotoxemia is related to the gut barrier function. Both nutritional and genetic obesity are associated with an increased gut permeability leading to the leakage of LPS. Although the clear mechanisms involved in the bacteria-host interactions are still under investigation (Cani et. al, 2009-10). Metabolic endotoxemia correlated negatively with *Bifidobacterium*

spp. *Bifidobacterium spp.* has been shown to reduce intestinal endotoxin levels (LPS) in rodents and improve mucosal barrier function (Wang et. al., 2006).

Obesity and related metabolic disorders are associated with low-grade inflammation, which contributes to the onset of some degenerative diseases (Olefsky and Glass 2010; Cani et al., 2007a, 2009). The gut microflora has been linked with chronic diseases such as obesity in humans Cani et al., 2007; Cani et al., 2008; Everard and Cani 2013). It has been shown that obese and lean subjects present different gut microflora composition profile. Obese subjects present lower proportion of Bacteroidetes in comparison to lean subjects (Angelakis et al., 2012). These and impaired metabolism. The evidences from animal models suggest that it is possible that the microbiota of obese subjects has higher capacity to harvest energy from the diet providing substrates that can activate lipogenic pathways. In addition, microorganisms can also influence the activity of lipoprotein lipase interfering in the accumulation of triglycerides in the adipose tissue. The interaction of gut microflora with the endocannabinoid system provides a route through which intestinal permeability can be altered. Increased intestinal permeability allows the entrance of endotoxins to the circulation, which are related to the induction of inflammation and insulin resistance in mice (Zao, 2013). Lipopolysaccharides (LPS) are unique glycolipids in the cell wall of gram- negative/pathogenic bacteria. LPS molecules, also known as bacterial endotoxins, may trigger acute and chronic inflammation, leading to immune cell activation and cytokine release (Laetitia et al., 2013). HDL cholesterol is one of the most important factors involved in the elimination of LPS molecules from circulation. In healthy subjects, LPS is mainly bound to HDL, whereas in patients with sepsis, LPS is redistributed toward LDL and VLDL lipoproteins (Tran-Dinh et al., 2013; Wendel and Paul 2007). High LPS activity combined with low HDL levels increases the risk for type II diabetes mellitus and cardiovascular disease (Jayashree et al., 2014; Pussinen et al., 2007). LPS infusion in mammals leads to the appearance of factors known to be associated with the (Metabolic Syndrome) MetS: elevated levels of

proinflammatory markers, dyslipidemia, fasting hyperglycemia, insulin resistance, and obesity (Jayashree et al., 2014; Parekh et al., 2014). (Figure 2.8)

(Source: Moreno-Indias, 2014)

(1) chronic bacterial translocation due to increased intestinal permeability that drive a systematic inflammation leading to macrophage influx into visceral adipose tissue, activation of hepatic Kupffer cells and insulin resistance. (2) Short chain fatty acids normalize intestinal permeability and alter de novo lipogenesis and gluconeogenesis via reduction of free fatty acid production by visceral adipose tissue.

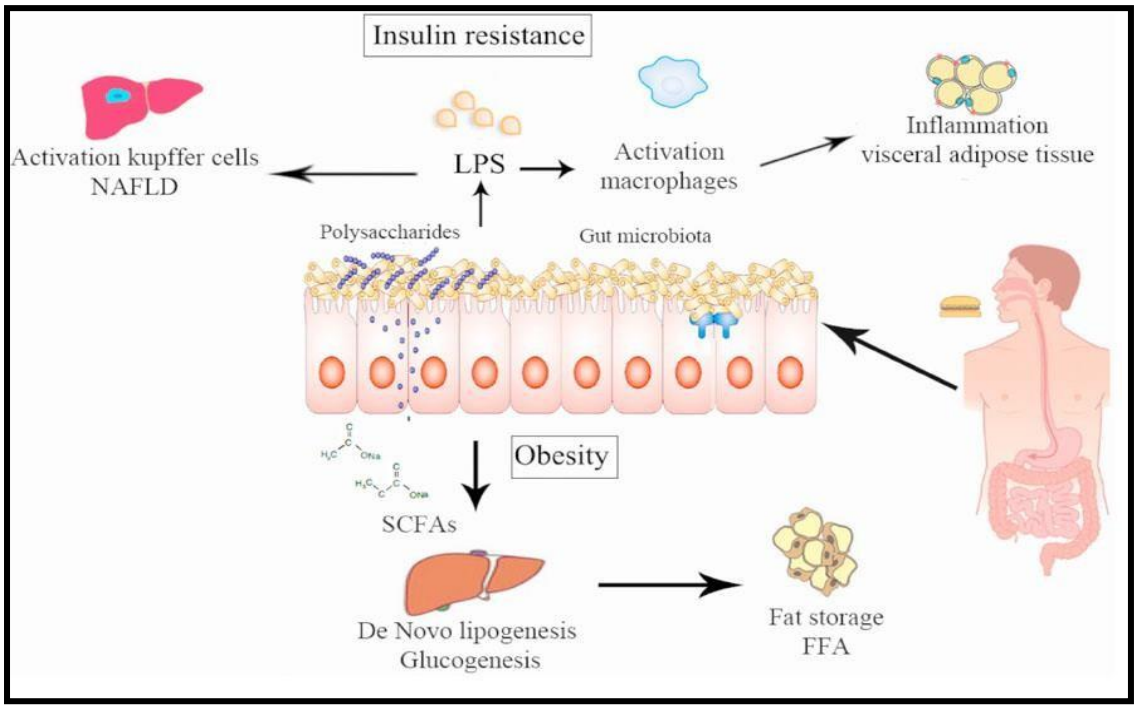


Figure 2.8: Pathway via which intestinal microbiota can alter human metabolism producing obesity and insulin resistance

2.8 Obesity and atherogenic profile

Obesity is associated with increased basal lipolysis in adipose tissue, and elevated circulating FFAs (Van Hall et al., 2003). Acute-phase serum amyloid A (SAA), a lipolytic adipokine in humans, stimulates basal lipolysis. The lipolysis has been postulated to be an autocrine feedback mechanism by which increased SAA production from enlarged adipocytes A into the circulation may contribute to insulin resistance. The SAA act through the CLA-1 and the extra-cellular signal regulated kinase signaling pathway to stimulate lipolysis directly (Souza et al., 2003). Alternatively, increased

lipolysis by SAA may be indirect, i.e. through the stimulation of the lipolytic cytokines viz IL-6 and TNF-α (Singla, Bardoloi, & Parkash, 2010).

Plasma triglyceride (TG) concentration is another metabolic variable, most affected in obesity. It has been suggested that there is tissue resistance to insulin mediated glucose uptake, which in turn accelerates the very low density lipoprotein (VLDL), TG production rate and leads to endogenous hypertriglyceridemia (Barter and Nestel, 1973; Kissebah, Alfarsi, Adams, and Wynn, 1976; Durrington, Newton, Weinstein, and Steinberg, 1982). In obesity there is decreased Lipoprotein lipase-mediated lipolysis of chylomicron-TG and ineffective inhibition of hormone-sensitive lipase-mediated lipolysis in adipose tissue (Lewis, Uffelman, Szeto, and Steiner, 1993). Postprandial lipemia and elevated plasma FA levels are well-recognized abnormalities in obesity. Excess fatty acid availability early in the postprandial period (when it is normally suppressed by insulin) is estimated to influence glucose uptake by as much as 50% (Yu and Cooper, 2001). SAA has also a direct effect on cholesterol metabolism. Being an apolipoprotein by nature, it is the apoprotein of high-density lipoprotein (HDL) (Van Lenten et al., 1995). The inter-action of SAA with HDL may impair the function of HDL as an anti-atherogenic molecule (Benditt, Hoffman, Eriksen, Parmelee, and Walsh, 1982) and facilitate its degradation (A. Wu, Hinds, and Thiemermann, 2004). The increase of adipose tissue derived SAA in obesity may be a link between obesity, low HDL and increased coronary artery disease risk (Jung & Choi, 2014).

2.9 Probiotics and prebiotics

- **Probiotics and its types and benefits**

Probiotics—a word derived from Latin and Greek meaning literally ''for life''—has been defined in many ways since it was first coined 50 years ago. FAO/WHO joint report (2001) defines Probiotics are "living micro-organisms which when administered in adequate amount confer health benefits of the host". This definition fits well in with that of functional foods. Probiotics are usually bacterial components of the normal human intestinal flora, for example *lactobacilli* and *Bifidobacteria*, that produce as end products of metabolism lactate and short chain fatty acids such as acetate and butyrate (Hill et al., 2014).

'Probiotic' is a useful and accepted term. The FAO/WHO definition has been widely adopted and has proven valuable to researchers, regulators and consumers. Organizations and agencies such as Codex (which comes under the FAO/WHO, 2002), Health Canada, the World Gastroenterology Organization, the European Food Safety Authority (EFSA) and the Institute of Food Technologists use the FAO/WHO definition when referring to probiotics. The panel noted, however, that a more grammatically correct definition would be worded as, "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" and supports use of this wording going forward. This definition is inclusive of a broad range of microbes and applications, whilst capturing the essence of probiotics (microbial, viable and beneficial to health). The definition differentiates live microbes used as processing aids or sources of useful compounds from those that are administered primarily for their health benefits. The distinction between commensal microorganisms and probiotics is also inferred from this definition. Although commensals in the gut are often the source of probiotic strains, until these strains are isolated, characterized and a credible case presented for their health effects, they cannot be called 'probiotics'. So to claim any microflora as probiotic it

should be member of a safe species which is supported by a sufficient evidence of general beneficial effect in humans or a safe microbe with a property (e.g. structure , activity or end product) for which there is sufficient evidence for beneficial effects in humans (Hill et al., 2014). (Table 2.4)

Table 2.4: Microorganisms used as probiotics in humans and animals

Lactobacillus species	***Bifidobacteria species***	***Other lactic acid bacteria***	***Non lactic acid bacteria***
L. acidophilus	*B. adolecentis*	*Enterococcus faecalis*	*Saccharmycescereisiae*
L. brevis	*B. animalis*	*Enterococcus faecium*	*Saccharomyces boulardii*
L. casei	*B. bifidum*	*Lactococcus lactis*	
L. fermentum	B. breve	*Leuconostoc mesenteroides*	
L. gallinarum	*B. infantis*	*Pediococcus acidilactici inulinus*	
L. gasseri	*B. longum*	*Sporolactobacillus*	
L. johnsonii		*Streptococcus salivarus*	
L. plantarum			
L. reuteri			
L. rhamnosus			

Source: Balcazar JL, 2007.

2.10 Prebiotics and its types and mechanism of action

Prebiotic is a nondigestible substance of food origin when administered in adequate amounts, is beneficial to the consumer due to the selective promotion of growth and/or activity of one or more bacteria already present in the gastrointestinal tract or taken together with the prebiotic (Hill et al., 2014).

Any food that contains oligosaccharides is potentially a prebiotic but in order to be classified as a prebiotic it must fulfill the following criteria: it

should neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract and it should be selectively fermented by one or limited number of potentially beneficial bacteria commensal to the colon for example *Bifidobacteria* and *lactobacilli* which are stimulated to grow and become metabolically activated. These requirements have been classified as three prebiotic criteria (Hill et al., 2014; Bai 2014). Prebiotics are found naturally in some plants or are produced enzymatically from sucrose, and often are used in dietary supplements.

Criteria for classification of a food ingredient as a prebiotic

- The must not be hydrolyzed nor absorbed in the upper digestive tract.
- The must represent selective substrate for one or more beneficial bacteria species in the colon; stimulating their growth or activity.
- The must be able to modify the intestinal microflora of the colon promoting a healthy composition.

These includes 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 2.5. Major prebiotics include fructans and galactooligosaccharides (GOS). Fructans include inulin derived from chicory, whole grains, fruits (e.g., bananas), vegetables (e.g., onions, artichokes) or fructo-oligosaccharides (FOS) hydrolyzed from chicory or enzymatically from sucrose. GOS are made from lactose as a by-product of dairy-food processing (Bai 2014).

Table 2.5: Classification of Prebiotics

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

Although probiotic and prebiotic approaches are likely to shares 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.

2.11 Development of FOS as a prebiotic

a) 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-(2)1) fructosyl- fructose linkages (Waterhouse AL and Chatterton NJ 1993). A starting a-D-glucose moiety can be present but is not necessary. GpyFn [glucopyranosyl- (fructofuransoyl)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 Composite family, i.e., chicory. Native chicory inulin is a non-fractionated 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 a-glycosidic bonds. They have thus been classified as ''nondigestible'' oligosaccharides (Sabater-Molina et al., 2009; Femia et al., 2010; Xu et al., 2009; Delzenne N and Roberfroid MB 1994; Roberfroid MB et al 2000).

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 macro-ingredients, 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 (Coussement 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) (Lied et al., 2011; Grabitske and Slavin 2009; Sabater-Molina et al., 2009; Femia et al., 2010; Xu et al., 2009; Pasman 2006).

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, Roberfoid (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, Orafti 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 Orafti declared both inulin and oligofructose as generally Recognized as Safe in 1992 (Slavin2013; Kolbye et al., 1992).

2.12 Fructooligosaccharide (FOS) - its formation, types and technological Functions

Nowadays one of the tendencies in food segment is the healthiness and wellness, associated to the growth of food industry in answering the consumer exigencies who is more conscious that an adequate feeding with healthy ingredients are indispensable to a better life wellness to children and adults.

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'Sullivian 2001; Bruno and Shah 2002). Many researches about of developing food products, involving milk and derivatives, bread, cake, etc., adding value due contain prebiotic ingredients. . It is valid to highlight that these products must positively answer to the nutritional and sensory characteristics, and remain in appropriate conditions during the processing and storage (Barreto et al., 2003).

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.

Sensory analysis is a decisive phase during the food product development (Morais et al., 2014) developed chocolate dairy dessert with addition of prebiotics and replacement of sucrose with different high-intensity sweeteners. The relative sweetness analysis showed that sweeteners had the highest sweetening power compared with the prebiotic chocolate dairy dessert containing 8% sucrose. The study of sweetness in this product is important because consumers desire healthier functional products with no added sugar.

Cruz et al., 2013 aimed to evaluate the effect of increasing concentrations of oligofructose addition on physicochemical, rheological and microbiological characteristics of non-flavored yogurt. The addition of oligofructose showed no influence on the pH, proteolysis or the viability of *Streptococcus thermophilus* or *Lactobacillus bulgaricus* during 28 days of refrigerated storage ($p > 0.05$). In another study, inulin was supplemented in bread and results are smaller loaves, harder crumb and darker colour. Sensory studies reflected acceptability decreases with inulin content, yeast invertase and dry heat degrade inulin, and fructo- oligosaccharide/inulin fortification in bread at 5% seems achievable (Morris and Morris 2012).

Study to determine the effect of a prebiotic (fructooligosaccharide) on the sensory properties and consumer acceptability of peach-flavored drinkable yogurts was carry out. The yogurts containing the prebiotic were not significantly different from their comparable controls indicating that a prebiotic can be added without impacting acceptance (Gonzalez et al., 2011).

2.14 Prebiotic effect of Fructooligosaccharide on weight, gut microbiota, atherogenic profile and LPS

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 and coworkers in 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 37^0C for 48 h. Growth and activities of the cultures were determined. Viability of each strain was assessed after 4 weeks of refrigerated storage at 4^0C. 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 β(2→1) fructosyl-linkages, and that its

specificity decreases as the degree of polymerization (DP) of the fructan increases (Janer C et al., 2004).

A bibliographic survey on 61 original articles from PubMed, ScienceDirect, Lilacs and SciElo databases carried out to understand the relationship between gut microbiota, obesity and possible impact of prebiotic and prebiotic concluded that after dietary manipulation with prebiotics and probiotics, the growth of *bifidobacteria* was obtained in 10 studies, involvement with weight reduction, adipogenic effects of diet, intestinal permeability and inflammatory markers (Da Silva, dos Santos, & Bressan, 2013).

Very few data are available on effect of FOS on weight reduction and other anthropometric indices till date. Findings of a study conducted by Sheth and Gupta (2014) observed significant weight loss and BMI reduction (1.06%) in Sixty five obese subjects working in an industrial setting (BMI 25-31kg/m2, aged 25-55 yrs) supplemented with 12 g FOS for 12 weeks.

A study by Parnell JA et al where in, oligofructose supplementation, independently from any lifestyle changes, were able to decrease body weight, primarily by losing fat mass, and could help manage caloric intake in overweight and obese adults (Parnell JA, et al., 2009). A study conducted by Nakamura Y and team in 2011 assessed efficacy of FOS supplementation on suppression of high fat induced body fat accumulation revealed that body weight and percent body fat were lower in mice fed FOS than in controls. Furthermore, the weight of the visceral adipose tissue, and the weight and triglyceride content of the liver were significantly lower in the high-fat plus FOS group. These results indicate that dietary FOS suppresses high-fat diet-induced body fat accumulation, and inhibit intestinal absorption of dietary fat (Nakamura Y et al., in 2011).

A study conducted by causey et al (Jennifer LC et al., 2000), who observed a significant reduction in serum TG in subjects with moderate hyperlipidemia given 18 g/d inulin for 3 weeks. In a study conducted on fifty-eight middle aged subjects with moderately raised blood lipid concentrations, subjects consumed 10 g/d of inulin in a powdered form found no significant changes

in total LDL or HDL cholesterol either of the groups over the 8 weeks intervention with reduced serum TG levels by 19% after intervention in the inulin treated group (Kim G et.al., 1999), indicating that a higher dose of FOS supplementation for at least 3 months period is required to bring about desirable changes in the LDL cholesterol whereas, lower levels of supplementation for a shorter duration may bring about improvements in serum TG levels. Although evidence suggests that TG lowering effect of prebiotic occurs via a reduction in VLDL and TG secretion from the liver due to reduction in the activity of all lipogenic enzymes and in the fatty acid synthase, via modification of lipogenic gene expression (Delzenne NM and Kok N, 1998 and Rebecca Wall et.al., 2012), one of the proposed mechanisms is also through the type of beneficial gut microbiota which gets colonized in the gut.

In 2007 Cani PD and team suggested that specific modulation of gut microbiota with prebiotics influences fat mass development and lipid metabolic disorders associated with obesity (Cani et. al., 2007). A study conducted on 40 institutionalized elderly subjects (>60 years) revealed significant 7.4% reduction in mean total cholesterol values and increased counts of Bifidobacteria and lactobacillus after supplementing probiotic curd for 6 weeks (Parnami S and Sheth M, 2011).

The impact of gut microbiota on the progression or slowing down of obesity is not yet fully known. It is believed that obesity is associated with elevated serum levels of lipopolysaccharide (LPS), which is a component of the cell wall of Gram-negative bacteria (Amar *et al.*, 2011a; Amar *et al.*, 2011b). It was shown that the growth of beneficial microbiota, consequently closing the intestinal barrier and changes in the metabolism of endotoxin in the blood can be modulated by the addition of prebiotics to the diet (Everard *et al.*, 2013). Prebiotics unchanged reached to the large intestine where they are food for the bacteria (Kowalska-Duplaga, 2003). Resistant dextrins derived from potatos had a bifidogenic effect and stimulate the growth of gut microbiota, thus limiting the growth of *Clostridium* strains (Barczynska *et al.*, 2010; Barczynska *et*

al., 2012). Lecerf et.al., in 2012 conducted a study where a mixture of inulin and xylooligosaccharides added to the diet effectively lowered the blood plasma LPS level (Lecerf *et al.*, 2012). A similar result was found where FOS and inulin (10g/d) added to the diet encouraged the growth of bifidobacteria, in particular *Bifidobacterium adolescentis* (Ramirez-Farias *et al.*, 2009).

Several studies have shown that *Bifidobacterium* spp. may be involved in the regulation of gut barrier function and in the diminution of gut lumen endotoxin levels in addition to improvement of mucosal barrier function (Griffiths EA et.al., 2004; Wang Z et.al., 2004 and 2006). Among the probable mechanisms clearing up the development of metabolic endotoxemia, obese and diabetic mice display enhanced intestinal permeability, that participate to the occurrence of LPS-induced inflammation and metabolic disorders (Brun P et.al., 2007; Cani PD et.al., 2008-2009).

FOS has been extensively studied as a prebiotic and there is ample evidence in human subjects, including infants, as well as in animal and in vitro studies that, prebiotics significantly increase the proportion of fecal *Bifidobacteria* and sometimes *Lactobacillus* even at fairly low levels of consumption (5–8 g per day) (ILSI Europe, 2011).

These findings are supported by a study (Cani PD and Delzenne NM, 2010), analyzing the effects of fermentable (oligofructose) and non-fermentable (cellulose) fibers on the intestinal microbiota of obese mice, revealing significant increased total content of, *Bifidobacteria* and *Lactobacillus* in the groups that received oligofructose, compared to control. Another study (Shinohara K et.al., 2010) that assessed the effects of oligofructose consumption in healthy volunteers and found increased content of *Bifidobacteria* and *Lactobacillus* in faeces while *Bacteroides* presented reduction. The authors contend that the increased amount of SCFA, resulting from the increase of certain groups of bacteria by prebiotic fermentation, inhibited the growth of *Bacteroides*. Several animal studies that analyzed microbiota modulation reported increased amount of *Bifidobacteria*, which was followed by reduced weight gain. These findings suggest that the reduced counts of *Bifidobacteria*

and *Lactobacillus* play a significant role in the development of obesity and its related comorbidities. Also, a 12-week FOS supplementation to obese humans resulted in weight loss and meal related suppression of the orexigenic hormone ghrelin (Parnell JA and Reimer RA 2009).

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 and coworkers in 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 and coworkers in 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 (Sheth and Assudani 2015; Scott et al., 2014; Mendlik K et al., 2012; Boler BV 2013).

2.15 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).

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 (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 (Guandalini and Cernat 2014). Both human and animal data have shown a powerful impact on improved gastrointestinal tract diseases by inulin type fructans (Vidella 2001).

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 FOS can offer an opportunity to prevent or mitigate gastrointestinal disease and their symptoms.

Gut microflora has recently been proposed as an environmental factor responsible for the weight gain and the altered energy metabolism that accompanies the obese state (Moran and Shanahan 2014; Harris et al., 2012). Several studies reported that the gut microflora differs at phylum level depending on weight status (Angelakis et al., 2012; Eckburg PB et al., 2005; Turnbaugh PJ 2006).

Prebiotics are defined as food ingredients that stimulate the growth of a limited number of microbial genus/species in the gut microbiota that are hypothesized to confer health benefits to the host (Guandalini and Cernat 2014; Slavin 2013). The administration of oligofructose to high-fat-fed mice increased the abundance of *Bifidobacterium* and *lactobacilli* (Everard et al., 2013; Slavin 2013) and normalized endotoxaemia (Zhao 2013; Cani et al., 2007; Neves et al., 2013) and the inflammatory tone associated with the high-fat diet (Martin et al., 2009; Million et al., 2013). The administration of oligofructose to genetically obese mice induced increases in the levels of *Lactobacillus*, *Bifidobacterium*, and *C. coccoides*, *E. rectale* , which led to a reduction in intestinal permeability and an improvement in tight junction integrity and inflammatory markers, such as lipopolysaccharides and cytokines (Cani et al., 2007; Million et al., 2013) (Figure 2.16) .

Parnell and Reimer 2009 evaluated the effects of oligofructose supplementation on body weight and concentrations of ghrelin and PYY as a measure of satiety in overweight and obese adults. FOS induced carbohydrate fermentation results in the production of SCFAs, (Xu J and Gordon JI 2003; Samuel et al., 2008; Tolhurst et al., 2012) which ultimately results in the regulation of gut hormones such as glucagon- like peptide (GLP-1) and peptide YY (PYY) (Bomhof et al., 2014; Neyrinck et al., 2012; Conterno 2011; Knauf C et al., 2008; Chaudhri OB et al., 2008) (Figure 2.17). These gut hormones are responsible for satiety through regulating the

production and release of digestive enzymes (Baggio and Drucker 2014). The randomized 48 healthy adults (BMI>25 kg/m2) to receive either oligofructose (21 g/daily) or placebo (maltodextrin) for 12 weeks. They found a reduction of 1.03 ±0.43 kg in patients with oligofructose as compared to an increase of 0.45± 0.31 kg (p= 0.01). This led to conclude that oligofructose supplementation has a potential benefit in promoting weight loss as well as improving glucose regulation in overweight and obese adults (Parekh et al., 2014).

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; Bomhof et al., 2014).