CHAPTER 1

INTRODUCTION

Changes in diets and lifestyles of human beings that has occurred with industrialization, urbanization, economic development and market globalization, has accelerated over the past decade. As a result, non-communicable diseases (NCDs) are increasing at alarming rates globally. NCDs are estimated to kill around 38 million people per year, accounting for 68% of all deaths worldwide (WHO 2015).

Rising incomes and urbanization has led to reduced physical activity, increased use of electronic appliances for domestic help, while family breadwinners have taken to desk jobs instead of ploughing the fields. These factors also encourage more sedentary pursuits such as television viewing and computer use, and well-off city-dwellers travel by car instead of walking or cycling.

At the same time, globalization puts junk food and fast food within easy reach of a population which is often hard-pressed to find time to cook healthy meals, but with more than enough money to buy a greasy lunch at a nearby restaurant. In India, these factors have contributed to the rise of bad eating habits and lack of exercise amongst a growing urban middle class, and their effects are startlingly visible obesity.

Obesity is considered as cluster of non-communicable diseases called 'New World Syndrome' creating an enormous socio economic and public health burden in developing countries. Recent studies from western populations have shown a plateau in the prevalence of both adult and childhood obesity during the last decade (Flegal et al., 2010, Lissner et al., 2010, Ogden et al., 2010). According to the World Health Organization (WHO), obesity is one of the most common, yet among the most neglected, public health problems in both developed and developing countries (WHO 2000). According to the WHO World Health statistics report 2015, In 2014, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 600 million were obese.

However, the problem is of a larger magnitude in developing countries like India where a significant proportion of the population belongs to younger age group (Adlakha A, 2011). A study in Bombay revealed that the prevalence of obesity among young adult males varied from 10.7% to 53.1% (Dhurandhar NV and Kulkarni PR 2009), while another study from urban Delhi, among a large representative sample of 13,414 adults (aged 25–64 years), showed an overall prevalence of 27.8% (Tandon et al., 2011). A report from Kashmir of adults over 40 years old, studied by multistage sampling, showed the obesity prevalence to be 15%; females having a prevalence of 23.7% compared with 7.0% among males (Zargar AM et al., 2009). The National Family Health Survey showed a prevalence rate 30% female and 22% male to be obese in India (NFHS III 2005-06).

Various health risks are associated with obesity. Epidemiological studies have shown that obesity poses a serious risk factor for coronary heart diseases (Eckel and Krauss, 1998). It is the underlying risk factor for cardiovascular disease, as it raises the risk for Atherosclerotic Cardio Vascular diseases (ASCVD) in combination with other risk factors. The latter include the major risk factors such as hypercholesterolemia, hypertension, hyperglycemia and emerging risk factors such as atherogenic dyslipidemia, insulin resistance, pro-inflammatory state and pro-thrombotic state. The relationship of obesity to major and emerging risk factors varies, depending on the genetic and acquired characteristics of individuals. The majority of obese persons who develop ASCVD typically have a clustering of major and emerging risk factors (metabolic syndrome) (Grundy, 2004).

In order to control the growing epidemic of obesity, the scientific community is prepared to develop or identify new therapeutic agents that can effectively control obesity and prevent the development and progression of its complications without compromising on safety. To date, many of the mechanistic studies have mainly focused in the biology of relationships between various human organs and cell systems. Nutraceutical avenues both at research level as well as at market level are marking their foot prints behind in dealing with obesity and its related complications. Nevertheless, there is an increasing body of literature focusing on the microbiota of the gut as means to combat obesity drawing our attention to Probiotics and Prebiotics as a means to fight against obesity.

Probiotics are the "live microorganisms which when administered in adequate amounts confer health benefit on the host"(FAO/WHO 2002). Amongst many bacteria of the intestinal microbiota considered as beneficial, *Lactic acid bacteria* and *Bifidobacteria*, normal inhabitants of human gastrointestinal tract, have raised great interest for their potential health benefits. The health promoting effects prompted by *Bifidobacteria* and *Lactic acid bacteria* is due to the growth inhibition of harmful bacteria, stimulation of immune functions, lowering of gas distention problems and improved digestion/absorption of essential nutrients and synthesis of vitamins (Gibson and Roberfroid 1995; Delzenne, 2001).

The human intestinal tract harbours a diverse and complex microbial community which plays a central role in human health. It has been estimated that our gut contains in the range of 1000 bacterial species and 100-fold more genes than are found in the human genome (Ley et al., 2006a; Qin et al., 2010).

It is now apparent that our gut microbiome coevolves with us (Ley *et al*. 2008) and that changes to this population can have major consequences, both beneficial and harmful, for human health. Indeed, it has been suggested that disruption of the gut microflora (or dysbiosis) can be significant with respect to pathological intestinal conditions such as obesity (Ley et al*.,* 2006b; Zhang et al., 2009) and malnutrition (Kau *et al*. 2011), systematic diseases such as diabetes (Qin et al., 2012) and chronic inflammatory diseases such as inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD) (Frank et al*.,* 2007).

As the composition and functional potential of the gut microflora increases, the number of diseases that have been linked with alterations in our gut microbial community has also expanded. Although lifestyle factors, diet and exercise contribute largely to the modern epidemic, it has also been indicated that the microbial communities within the human intestine play an important role in obesity (Ley, 2010; Ley et al., 2005; Tilg and Kaser, 2011; Turnbaugh et al*.,* 2006). Although a number of studies of the microbiota of lean and obese mice have indicated that genetically (*ob*/*ob*) and diet-induced obese mice contain higher proportions of *Firmicutes* and lower levels of *Bacteroidetes* than their lean counterparts (Ley et a*l*., 2005). Further studies have indicated a lower proportion of *Bacteroidetes* in obese individuals, an increased abundance of *Actinobacteria* while the levels of *Firmicutes* remained unaltered (Turnbaugh et al., 2009a). The importance of the *Firmicutes* to *Bacteroidetes* ratio in obesity, however, is still not clear with some conflicting studies published to date in this area (Duncan et al., 2007; Schwiertz et al*.,* 2009) and scanty data is available on the role of body weight on gut health. Therefore there is a need to study the gut microflora of the Indian population of both obese and normal weight subjects.

Glucagon-like peptide-1 (GLP-1) is a neuropeptide and an incretin derived from the transcription product of the proglucagon gene. The major source of GLP-1 in the periphery is the intestinal L cell that secretes GLP-1 as a gut hormone. Colonization of probiotic bacteria promotes GLP-1 synthesis in the proximal colon by a mechanism linked to the differentiation of precursor cells into entero-endocrine L-cells. GLP-1 which is known to affect satiety center and likely to affect obesity on long run thus resulting in reduced food intake, body weight and fat mass development, a restored beta cell mass and glucose-induced insulin secretion (Chaudhri et al., 2008, Druce and Small 1004, Cowley et al., 2003, Knauf et al., 2005).

Lipopolysaccharide (LPS) has role in metabolic endotoxemia, it is the major molecular component of the outer membrane of Gram-negative bacteria and serves as a physical barrier providing the bacteria protection from its surroundings. 1) LPS is a constituent of Gram negative bacteria present in the gut microflora, 2) LPS triggers the secretion of proinflammatory cytokines when it binds to the complex of CD14 and the toll like receptor 4 (TLR4) at the surface of innate immune cells (Wright et al., 1990), 3) LPS is continuously produced within the gut by the death of Gram negative bacteria and is physiologically carried into intestinal capillaries through a TLR4 dependent mechanism (Neal et al., 2006), 4) LPS is transported from the intestine towards target tissues by a mechanism facilitated by lipoproteins, notably chylomicrons freshly synthesized from epithelial intestinal cells in response to fat feeding (Tomita M and Ohkubo 2004, Moore et al., 1991, Vreugdenhil et al., 2003, Black DD and Tso P 1983).

In order to determine the role of metabolic endotoxemia as a triggering factor in the development of metabolic disorders associated with obesity, scientist had mimicked the metabolic endotoxemia by developing a mouse model chronically infused with a very low dose of LPS to reach the same plasma LPS levels as the one measured in the high-fat diet fed mice. The four weeks chronic low dose LPS infusion mimicked the high-fat diet fed mice phenotype namely, fasting hyperglycemia, obesity, steatosis, adipose tissue macrophages infiltration, hepatic insulin resistance and hyperinsulinemia (Cani et al., 2007). Hence, first phase of the study focuses on determining GLP-1, LPS and gut microflora of the normal weight and the obese adults.

A number of recent studies provide novel insight that might help establish a link between non digestible carbohydrates that changes the composition of gut microflora, and reduce obesity and insulin resistance. These compounds are called prebiotics. Prebiotics, promote the growth or activity of a limited number of bacterial species especially probiotic bacteria in the gut. They selectively nourish beneficial intestinal flora, stimulate their proliferation and reinforce their action and imparts beneficial health effects in humans (Ziemer and Gibson, 1998).

Oligofructose (prebiotic) is naturally found in significant amounts in edible fruits and vegetables like banana, onion, garlic, artichoke etc. Using this ingredient in food formulations allows the nutritional value of the end product to be improved by increasing the dietary fibre content, reducing the calorie content and increasing the *bifidus*-promoting capacities. Studies have shown fructooligosaccharides(FOS) supplementation resulted in reduced colonization of Clostridium and Bacteroides by along-with significant increase in the colonization of *Bifidobacteria* and *Lactobacilli* (Sheth M and Assudani A 2014, Sheth M and Gupta N 2014).

Kok, et al., observed that feeding rats with a prebiotic fibre oligofructose (OFS) lead to an increase in total caecal GLP-1 and jejunum GIP concentrations. Several data show that prebiotics containing short chain oligosaccharides reduce food intake, body weight gain and fat mass development. Studies have shown 20g FOS supplementation for 90 days resulted in reduced body weight, BMI, WC, WHR and body fat (Sheth M and Assudani A 2014).

Inulin type fructans are well studied and clearly effective in humans and animal models to stimulate growth of health promoting species belonging to *Bifidobacteria* and *lactobacilli* and modulating gut hormone GLP-1 (Macfarlane et at., 2006 and Flamm et al., 2001). Many researchers have reported that prebiotic supplementation increases gut hormone signaling (GLP-1, PYY), in a study rats fed with prebiotic (resistant starch) had increased GLP-1 and PYY expression (Cani et al., 2009).

Studies confirmed that mice fed a high-fat diet exhibit a higher endotoxemia, a phenomenon completely abolished through dietary supplementation with the prebiotic dietary fibers. In prebiotic treated-mice, *Bifidobacteria* bacterium-spp. significantly and positively correlated with improved glucose-tolerance, glucose-induced insulin-secretion, and normalized low-grade inflammation (decreased endotoxemia, plasma and adipose tissue proinflammatory cytokines). Scientists also found that metabolic endotoxemia correlated negatively with *Bifidobacteria* bacterium spp. (Tuohy and Gibson 2005, Cani et al., 2007).

A need was felt to study the relevance of these studies in Indian context due to limited available literature in this area. Hence one of the objectives of the present study was to evaluate metabolic effect of FOS on gut microflora in fecal samples, gut incretin (GLP-1) and LPS of obese adults.

The current knowledge regarding advantages of probiotics and prebiotics has attracted many researchers to manufacture designer foods that will enhance the friendly bacteria in the gut, hence deliver many health benefits (Miremadi, F. and Shah, N. P 2012; Santos BAD 2012). The consumption of foods and beverages containing prebiotics and probiotics is the current consumer global trend (Mark-Herbert 2004: Verberke, 2009), fortification with novel functional ingredient such as prebiotic like FOS is a recent development in this direction (Luckow, Sheehan, Fitzgerald and Delahunty, 2006). Consequently the global, functional food market is thriving with recent estimates indicating up to a $ 50 billion annual share (Stanton, Ross, Fitzgerald and Van Sinderen, 2005). FOS has been studied for its organoleptic properties and it has been widely used as sugar and fat replacer. According to FAO-WHO (2001), FOS is regarded as safe when consumed up to 20 g. For nutrition Labeling purposes, Roberfroid (1999) recommends that inulin and oligofructose, as well as all non-digestible oligosaccharides that are mostly fermented in the colon, be assigned a caloric value of 1.5 kcal/g (6.3 kJ/g), which is very low in calorie and hence, provide an attractive agent for obese subjects.

FOS has attracted special attention because of its prebiotic properties and also due to its sweet taste being very similar to that of sucrose (Yun 1996). It acts as functional food ingredient that exhibit specific physiological effect such as growth stimulating beneficial *Bifidobacteria* in the digestive tract, decrease in total cholesterol and lipid in serum, relief of constipation and general improvement of human health (Tomomatsu, 1994).

In addition, FOS being slightly sweet in taste is likely to blend well with many products. In pure form, it has sweetness of about 30-35% in comparison to sucrose. Its sweetening profile closely approaches that of sugar. The taste is very clean without any lingering effect. It mingles very well with delicate aromas and even enhances fruit flavors (Franck, 2002; 2008). FOS was used for partial substitution of sucrose in fruit juices without significantly affecting the overall quality (Prapulla and Renuka, 2009).

However, there is scarcity of Indian food products of Gujarat region prepared from fructooligosaccharides in the market which are consumed on day to day lives. Therefore, first phase of the study was taken up to study the acceptability of FOS added food products such as *Buttermilk, Lemon juice, Milk, Soup, Potato curry, Dal, Kadi, Kheer* and *Khichdi*.

Thus present study entitled ***"Sensory evaluation of fructooligosaccharide (FOS) added foods and its impact on gut health and biochemical parameters in obese industrial employees of rural Vadodara"*** was undertaken in the following three phases:

PHASE I – Sensory evaluation of FOS added popular Indian food products.

PHASE II– Comparative analysis of obese and normal weight subjects of an industry for their anthropometric parameters, nutrient intake, fecal gut microflora, GLP-1, LPS, hunger and satiety.

PHASE III- Anthropometric and metabolic responses of obese subjects to supplementation of FOS.