CHAPTER 2
REVIEW OF LITERATURE

Great people have great thoughts and there are few famous quotes that keeps inspiring and creating the basic structure of a scientific research. As once enunciated by Professor Earaldo Banovac, President of the Board of Commissioners' Office:

"People with a scientific mindset are analytical, open-minded and flexible. They are basically focused on what they do not know, and only exceptionally on what they do know"

Earaldo Banovac

Also, according to Charles F. Kettering, head of research at General Motors and holding 186 patents once said:

"Research is an organized method for keeping you reasonably dissatisfied with what you have"

Charles F. Kettering (1876-1958)

Looking into the exploding prevalence of obesity even after what we have known so far, being reasonably dissatisfied there is a strong need to reevaluate strategies and focus on underlying mechanisms that control body weight, specifically hormones that resist weight-loss and mechanisms that we do not know.

The present chapter focuses on unraveling the potential mechanisms that can form new line of treatment and help understand the newer aspects of our investigation entitled

"Acceptability Trials of Fructooligosaccharide (FOS) Added Popular Indian Recipes and Impact Evaluation of FOS Intervention in Modulating Gut Microflora, Gut Satietogenic Hormones and Anthropometric Indices of Young Obese Bank Employees of Urban Vadodara: A FAT – FIT Study"

Available research literature for the present study will flow under following heads:

2.1 Obesity – A Global Epidemic / A Global Public Health Issue

2.1.1 - Global Scenario

Today, obesity is one of the crucial public health concerns of the 21st century. Once, known as a condition of high-income countries, obesity rates are exploding worldwide. Despite of established causes and defined strategies to treat obesity, it seems as if there are many more unresolved underlying mysteries that are causing an unstoppable rise in obesity prevalence. Looking at the range of scientific literature on the underlying mechanism it makes us realize that we are yet on the tip of an iceberg.

Unfortunately, over the past 3 decades prevalence of obesity has doubled and is the 5th leading cause of death worldwide (WHO, 2018; EASO, 2017) (Figure 2.1). According to WHO (2018), today all regions across the globe are facing the dual burden of malnutrition (Figure 2.2). Till date where the government was struggling to eradicate under-nutrition, now it also has to deal with Obesity and dual burden of malnutrition along with an upsurge in risk factors for NCD's (Figure 2.1). Under-nutrition and Obesity now co-exist across the globe specifically in low and middle-income countries and their coexistence can commonly be found within countries, community and even at household level (WHO, 2018). Data on global estimates for prevalence of overweight and obesity as given by WHO (2018), depicts that up to 2016, 1.9 billion adults (>18 years or older) were overweight (39%) and 650 million adults were obese (11%). Obesity is not just limited to adults but has also affected 41 million children under age of five (WHO, 2018). Globally, in 2015 increased BMI has contributed to 4 million deaths (The GBD, 2015).

According to data from a trend analysis of 188 countries, performed by Christopher JL. Murray, Professor, IHME Director, University of Washington, stated that "No country has successfully reduced obesity rates in 33 years" (Murray, 2014). Study on Global Burden of Disease (GBD), results that 2.1 million people that account for 30% of the world's population are either overweight or obese (Ng et.al., 2014). Overweight and obesity rates have increased for both men (from 29% to 37%) and women (from 30% to 38%). According to National Health and Nutrition Examination Surveys, results revealed that, one of the strongest data providers on obesity prevalence rates described United States having the highest proportion of obese population (38%) in 2014 comprising 35% men and 40% women, followed by China and India together accounted for 15% of obese population (Hales et al., 2018; Flegal et.al., 2016).

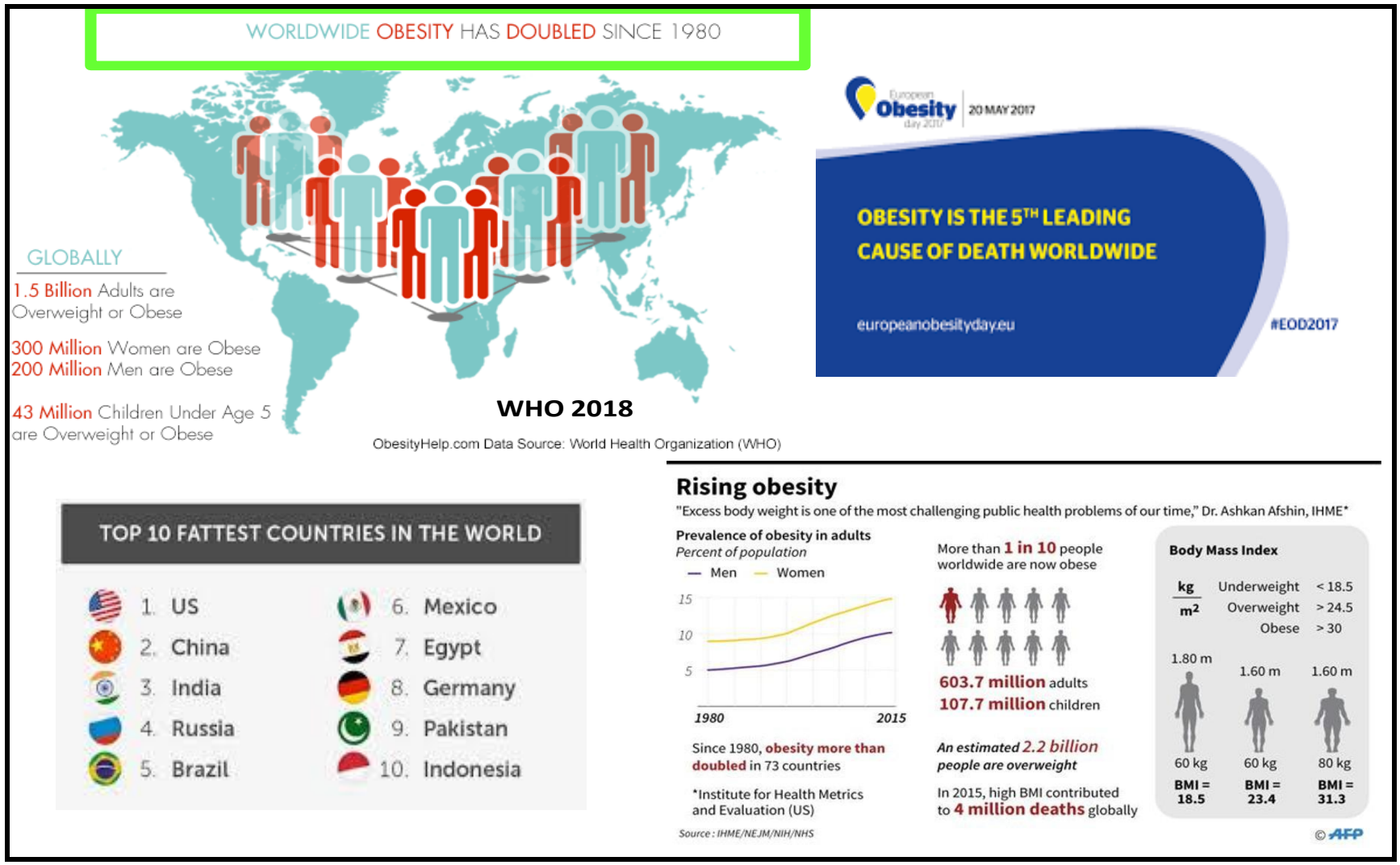


Figure 2.1: Prevalence of Obesity (WHO 2018; EASO 2017; IHME 2016)

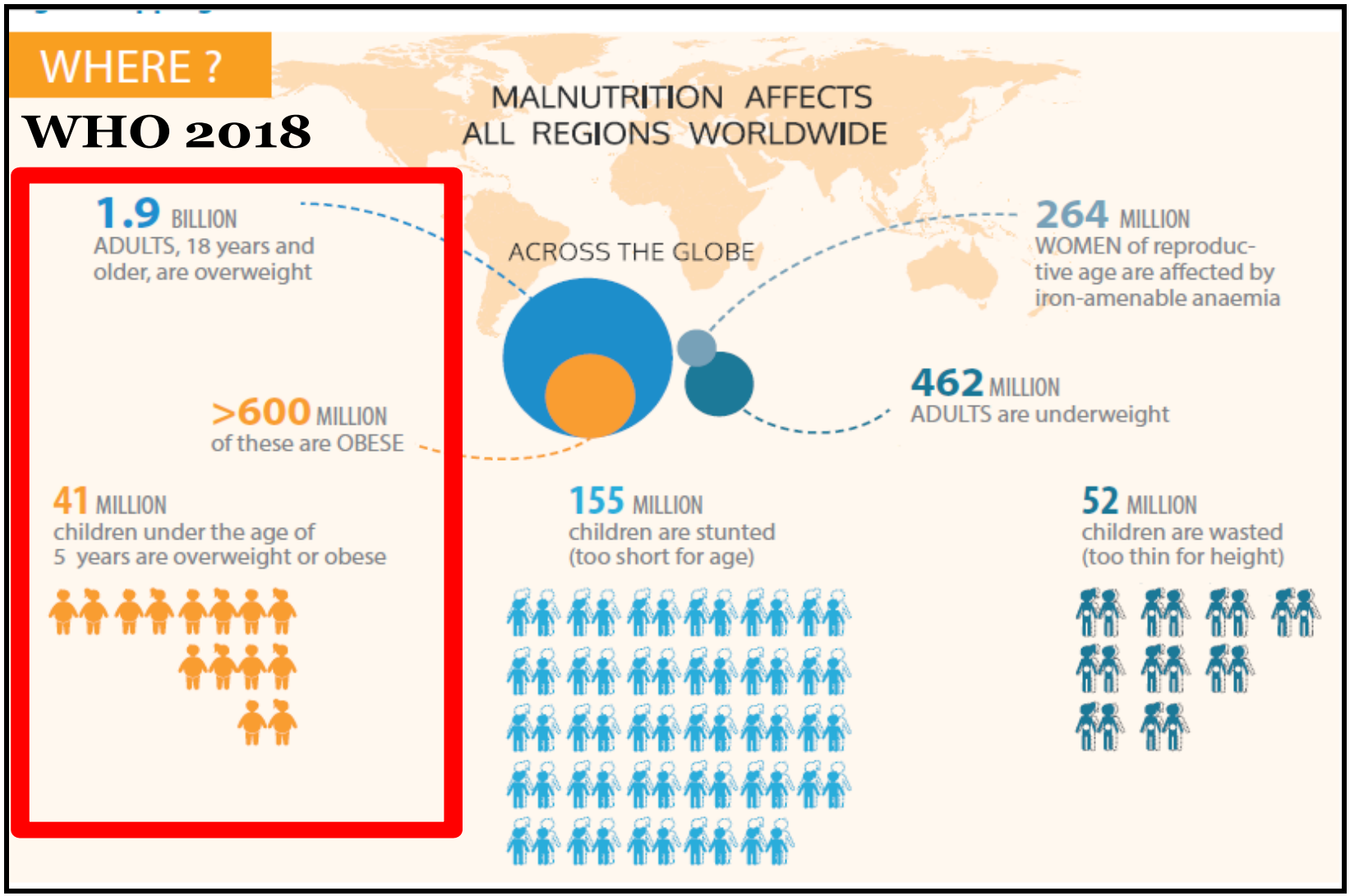


Figure 2.2: Dual burden of malnutrition (WHO 2018)

One of the interesting study conducted by Saab and Salvatore (2015), evaluated the cause of death in obese individuals through 849 retrospectively reviewed medical autopsies of Obese and Non-obese adults. As obesity increased the likelihood of various disease and conditions, the result of this study revealed that out of 849 autopsies 32.2% were of obese individuals and the leading cause of death in obese population was pulmonary embolism and liver disease. Obese people were less likely to die from neurologic and non-ischemic heart disease (Saab & Salvatore, 2015).

The major findings of the meta-analysis of 239 cohort studies carried out in Asia, Australia, New Zealand, Europe and North America by global BMI mortality collaboration 2016, depicted 39% increased the risk of death with an increase in every 5 additional points of BMI. In addition, men had a higher risk of death as compared to women and the higher risk was stronger for younger ages. There was also a higher risk of death due to heart disease, stroke and respiratory disease in people with BMI over 25 (Di Angelantonio et al., 2016).

In one of the review articles on overview of an obesity epidemic by Mitchell (2011) mentioned that increasing rates of obesity are now found in all socioeconomic classes and the gap within socioeconomic strata is diminishing. Also, the influence of high biological and economic preferences for high fat, high sugar and calories dense cheap food in less money is the prime reason where absolute rates are higher for obesity in people belonging to low income and low education level (Mitchell, 2011).

One of the studies comparing the risk assessment of disability-adjusted life-years (DALY's) and trends in exposure revealed top ten contributors in 2015 to global DALY's. Amongst that high blood pressure, smoking, high fasting plasma glucose, high BMI, high total cholesterol, alcohol use and diets high in sodium were largest contributors of DALY's. The odds for global death evaluating all risk factors together was 57·8% (95% CI 56·6–58·8) and for DALYs was 41·2% (39·8–42·8) (GBD Risk factors collaborators, 2015).

Obesity is not just aggrandizing alone but is also augmenting the prevalence of NCD's. Most of the literature had mentioned obesity as the gateway to all co-morbidities like cardiovascular diseases, diabetes, hypertension, stroke etc and is the single prime key modifiable risk factor for curtailing and /or ameliorating NCD's (WHO, 2018; EASO, 2017; Webber et al., 2012).

2.1.2 - Indian Perspective

Obesity is not just a problem of developed countries but now it is equally a matter of concern for developing countries like India. In India obesity paradoxically co-exist with under-nutrition. The main culprits for rapid rise in obesity rates in India are urbanization, industrialization, increase in purchasing power, accompanying lifestyle and India's integration in global food market (Gulati &Misra, 2017).

According to obesity update given by organization for economic cooperation and development (OECD, 2017), The self reported data for survey year 2015 India depicts 5% of total population aged 15 years and over as obese i.e. 65 million out of 1320 million total Indians were obese (Figure 2.3). Obesity rates in India have doubled in last 10 years. According to survey conducted by ministry of health and family welfare (MOHFW, 2016) and data recorded by national family and health survey (NFHS-4, 2015-2016) for BMI >25 for 15 – 49 years population, 19% – 21% of population was found to be obese. Obesity was more prevalent in urban India (26% -31%) as compared to rural India (Figure 2.4). Highest percentage of approximately 33 - 38% obese population was found in states like Andhra Pradesh, Andaman and Nicobar, Puducherry and Sikkim. In Gujarat, 21% - 24% of population was obese and 26% - 35% was found in urban Gujarat as compared to rural (NFHS-4, 2015-2016). Comparing the current data with previous report, the percentage of men and women increased from 9.3% and 12.6% in NFHS-3 to 18.9% and 20.7% in NFHS-4 respectively. Rates of overweight and obesity were almost double in urban areas (male 26.6% and female 31.3%) as compared to rural areas (male 14.3% and female 15%). Similarly, in Gujarat state also the overweight and obesity rates were more prevalent in urban area (24.9 - 34.5%) as compared to rural (14.4 – 15.4%) (Figure 2.4).

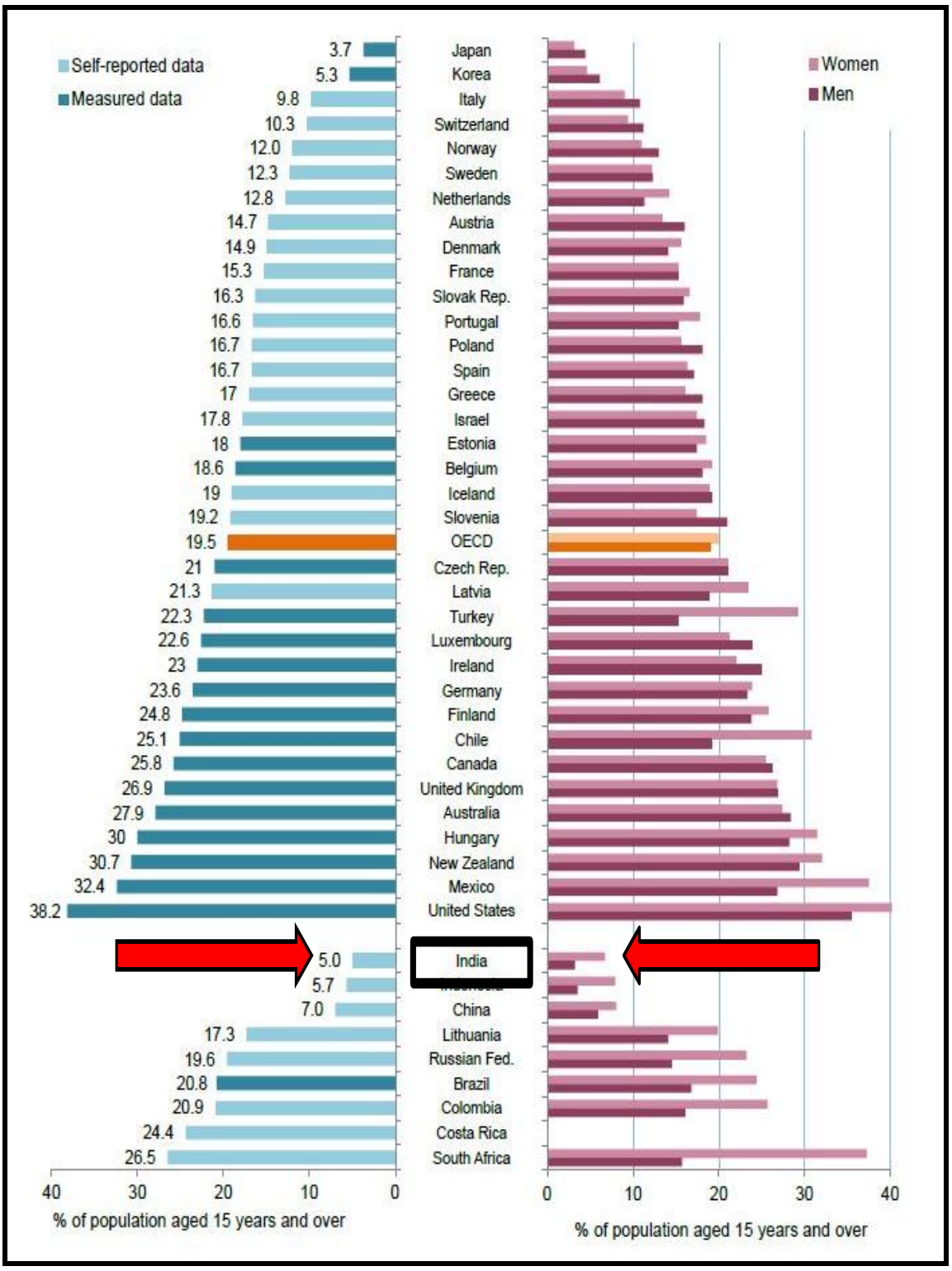


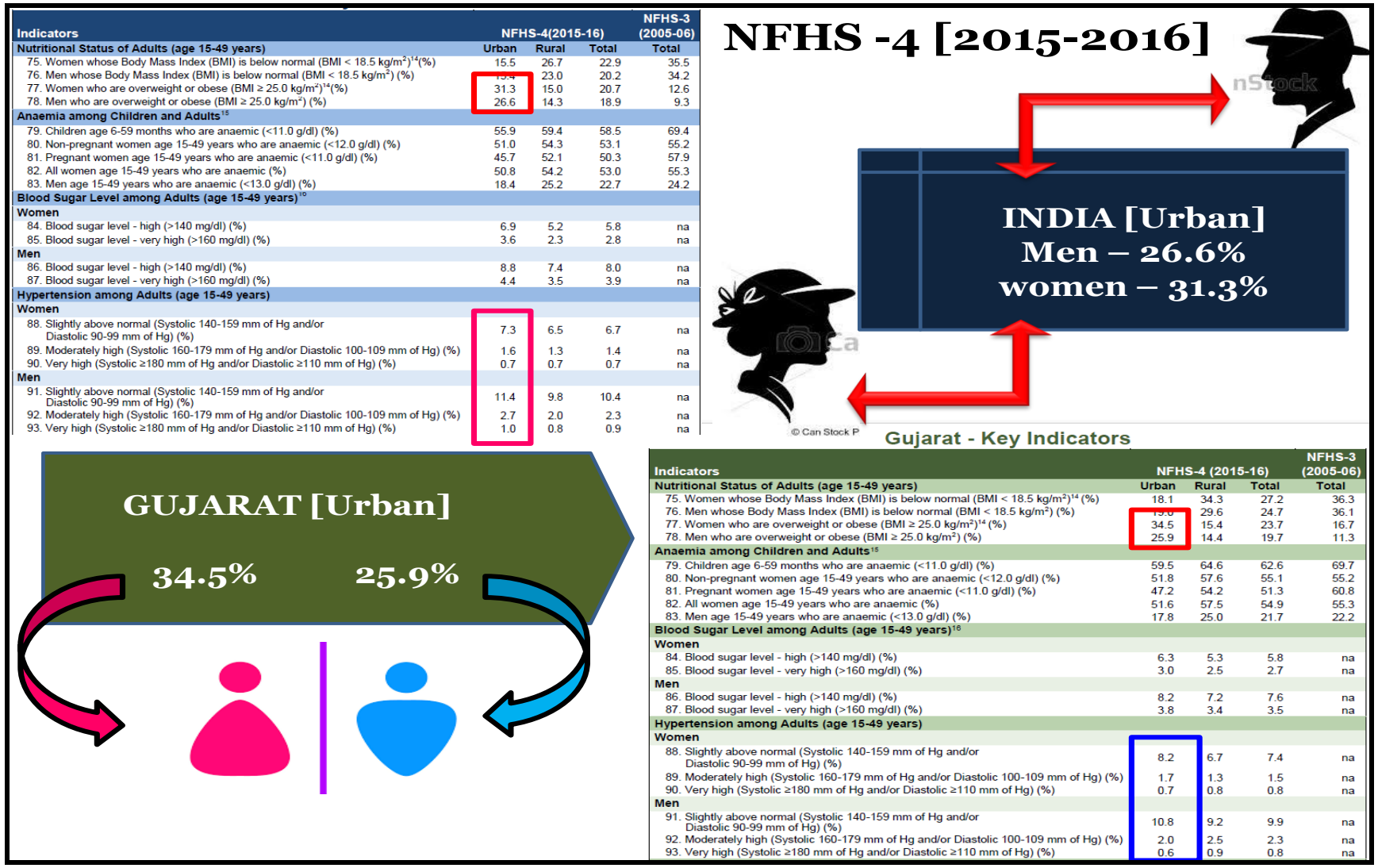
Figure 2.3: Obesity Amongst Adults, 2015 or Nearest Year (Source: OECD, 2017)

This may be due to lesser physical activity in the urban areas. Furthermore, overweight and obesity are both higher for women than men. This dual disease pattern in women may have an endocrine basis, but more probably has its roots in societal and cultural roles, which prevent women from leading a healthy lifestyle. The prevalence of overweight and obesity is three times higher among women with 12 or more years of schooling than those with no education (WHO, 2013, Ramachandran et al., 2010).

A community based cross sectional study conducted by Brahmbhatt and Oza (2012) assessing prevalence of obesity in adolescents of Ahmedabad city in 2012, depicted significant association of higher socio economic status, inadequate sleep at night, lack of physical activity and consumption of junk foods with prevalence rates of overweight in 13.3% and obesity in 5.4% of adolescents of Ahmedabad city (Brahmbhatt & Oza, 2012).

Results of yet another study conducted in 2012 on similar lines in Surat city demonstrated that 14.6% of adolescents were obese in urban area as compared to 12.8% in rural area. However, urban males showed higher prevalence ratio as compared to females. Higher socio economic status and male gender were significantly associated with higher risk of obesity (Parekh, Parekh & Vadasmiya, 2012).

Moving further, an effort was made by ICMR – INDIAB collaborative study group in 2015 to determine prevalence of generalized and abdominal obesity in India. The major results that surfaced from the study depicted presence of general obesity (GO) in 11.8% - 31.3% (Average 21.1%) of residents, abdominal obesity (AO) in 16.9% - 36.1% (Average 24.6%) and central obesity in 9.8% - 26.6% (Average 17.17%). Regression analysis revealed that hypertension, diabetes, higher socio-economic status, physical inactivity and urban residence were significantly associated with GO, AO and CO (ICMR – INDIAB, 2015).



Indicators	NFHS-4(2015-16)			NFHS-3 (2005-06)
Nutritional Status of Adults (age 15-49 years)	**Urban**	**Rural**	**Total**	**Total**
75. Women whose Body Mass Index (BMI) is below normal (BMI < 18.5 kg/m²)[14](%)	15.5	26.7	22.9	35.5
76. Men whose Body Mass Index (BMI) is below normal (BMI < 18.5 kg/m²) (%)	[illegible]	23.0	20.2	34.2
77. Women who are overweight or obese (BMI ≥ 25.0 kg/m²)[14](%)	31.3	15.0	20.7	12.6
78. Men who are overweight or obese (BMI ≥ 25.0 kg/m²) (%)	26.6	14.3	18.9	9.3
Anaemia among Children and Adults[15]				
79. Children age 6-59 months who are anaemic (<11.0 g/dl) (%)	55.9	59.4	58.5	69.4
80. Non-pregnant women age 15-49 years who are anaemic (<12.0 g/dl) (%)	51.0	54.3	53.1	55.2
81. Pregnant women age 15-49 years who are anaemic (<11.0 g/dl) (%)	45.7	52.1	50.3	57.9
82. All women age 15-49 years who are anaemic (%)	50.8	54.2	53.0	55.3
83. Men age 15-49 years who are anaemic (<13.0 g/dl) (%)	18.4	25.2	22.7	24.2
Blood Sugar Level among Adults (age 15-49 years)[16]				
Women				
84. Blood sugar level - high (>140 mg/dl) (%)	6.9	5.2	5.8	na
85. Blood sugar level - very high (>160 mg/dl) (%)	3.6	2.3	2.8	na
Men				
86. Blood sugar level - high (>140 mg/dl) (%)	8.8	7.4	8.0	na
87. Blood sugar level - very high (>160 mg/dl) (%)	4.4	3.5	3.9	na
Hypertension among Adults (age 15-49 years)				
Women				
88. Slightly above normal (Systolic 140-159 mm of Hg and/or Diastolic 90-99 mm of Hg) (%)	7.3	6.5	6.7	na
89. Moderately high (Systolic 160-179 mm of Hg and/or Diastolic 100-109 mm of Hg) (%)	1.6	1.3	1.4	na
90. Very high (Systolic ≥180 mm of Hg and/or Diastolic ≥110 mm of Hg) (%)	0.7	0.7	0.7	na
Men				
91. Slightly above normal (Systolic 140-159 mm of Hg and/or Diastolic 90-99 mm of Hg) (%)	11.4	9.8	10.4	na
92. Moderately high (Systolic 160-179 mm of Hg and/or Diastolic 100-109 mm of Hg) (%)	2.7	2.0	2.3	na
93. Very high (Systolic ≥180 mm of Hg and/or Diastolic ≥110 mm of Hg) (%)	1.0	0.8	0.9	na

Indicators	NFHS-4 (2015-16)			NFHS-3 (2005-06)
Nutritional Status of Adults (age 15-49 years)	**Urban**	**Rural**	**Total**	**Total**
75. Women whose Body Mass Index (BMI) is below normal (BMI < 18.5 kg/m²)[14] (%)	18.1	34.3	27.2	36.3
76. Men whose Body Mass Index (BMI) is below normal (BMI < 18.5 kg/m²) (%)	[illegible]	29.6	24.7	36.1
77. Women who are overweight or obese (BMI ≥ 25.0 kg/m²)[14] (%)	34.5	15.4	23.7	16.7
78. Men who are overweight or obese (BMI ≥ 25.0 kg/m²) (%)	25.9	14.4	19.7	11.3
Anaemia among Children and Adults[15]				
79. Children age 6-59 months who are anaemic (<11.0 g/dl) (%)	59.5	64.6	62.6	69.7
80. Non-pregnant women age 15-49 years who are anaemic (<12.0 g/dl) (%)	51.8	57.6	55.1	55.2
81. Pregnant women age 15-49 years who are anaemic (<11.0 g/dl) (%)	47.2	54.2	51.3	60.8
82. All women age 15-49 years who are anaemic (%)	51.6	57.5	54.9	55.3
83. Men age 15-49 years who are anaemic (<13.0 g/dl) (%)	17.8	25.0	21.7	22.2
Blood Sugar Level among Adults (age 15-49 years)[16]				
Women				
84. Blood sugar level - high (>140 mg/dl) (%)	6.3	5.3	5.8	na
85. Blood sugar level - very high (>160 mg/dl) (%)	3.0	2.5	2.7	na
Men				
86. Blood sugar level - high (>140 mg/dl) (%)	8.2	7.2	7.6	na
87. Blood sugar level - very high (>160 mg/dl) (%)	3.8	3.4	3.5	na
Hypertension among Adults (age 15-49 years)				
Women				
88. Slightly above normal (Systolic 140-159 mm of Hg and/or Diastolic 90-99 mm of Hg) (%)	8.2	6.7	7.4	na
89. Moderately high (Systolic 160-179 mm of Hg and/or Diastolic 100-109 mm of Hg) (%)	1.7	1.3	1.5	na
90. Very high (Systolic ≥180 mm of Hg and/or Diastolic ≥110 mm of Hg) (%)	0.7	0.8	0.8	na
Men				
91. Slightly above normal (Systolic 140-159 mm of Hg and/or Diastolic 90-99 mm of Hg) (%)	10.8	9.2	9.9	na
92. Moderately high (Systolic 160-179 mm of Hg and/or Diastolic 100-109 mm of Hg) (%)	2.0	2.5	2.3	na
93. Very high (Systolic ≥180 mm of Hg and/or Diastolic ≥110 mm of Hg) (%)	0.6	0.9	0.8	na

Figure 2.4: National Health and Family Survey -4 (2016); Ministry of Health and Family Welfare (MOHFW, 2016)

Looking into the prevalence rates of obesity between urban and rural population Yadav and Krishnan (2008), conducted study in north India assessing changing patterns of diet and physical activity in urban, rural and slum area. The results of this study revealed that intake of fruits and vegetable was proportionally inadequate in all three groups. However, physical activity was five times and seven times higher in rural men and women as compared to urban and urban slum men and women. Urbanization depicted increased prevalence of NCD's risk factors higher in women as compared to men (Yadav & Krishnan, 2008).

From above literature it is very much clear that prevalence of overweight and obesity is multiplying exponentially and rates are much higher in urban population as compared to rural areas of India.

2.2 Obesity is now recognized as "Disease": Adiposity Based Chronic Disease (ABCD) - A new diagnostic term

Obesity issue is now pandemic (WHO, 2018) and is now officially recognized as a "Disease" (CMA, 2015; AMA, 2013). Portugal was the first country in the entire Europe which officially recognized obesity as a "Disease" in 2004 (The Lancet Editorial 2017). Later it was followed by Scottish Inter-collegiate guidelines network in 2010. Moving forward, after lot of controversies American Medical Association in 2013 (AMA) and Canadian Medical Association in 2015 (CMA) official recognized Obesity as a Disease (AMA, 2013; CMA, 2015) (Figure 2.5).

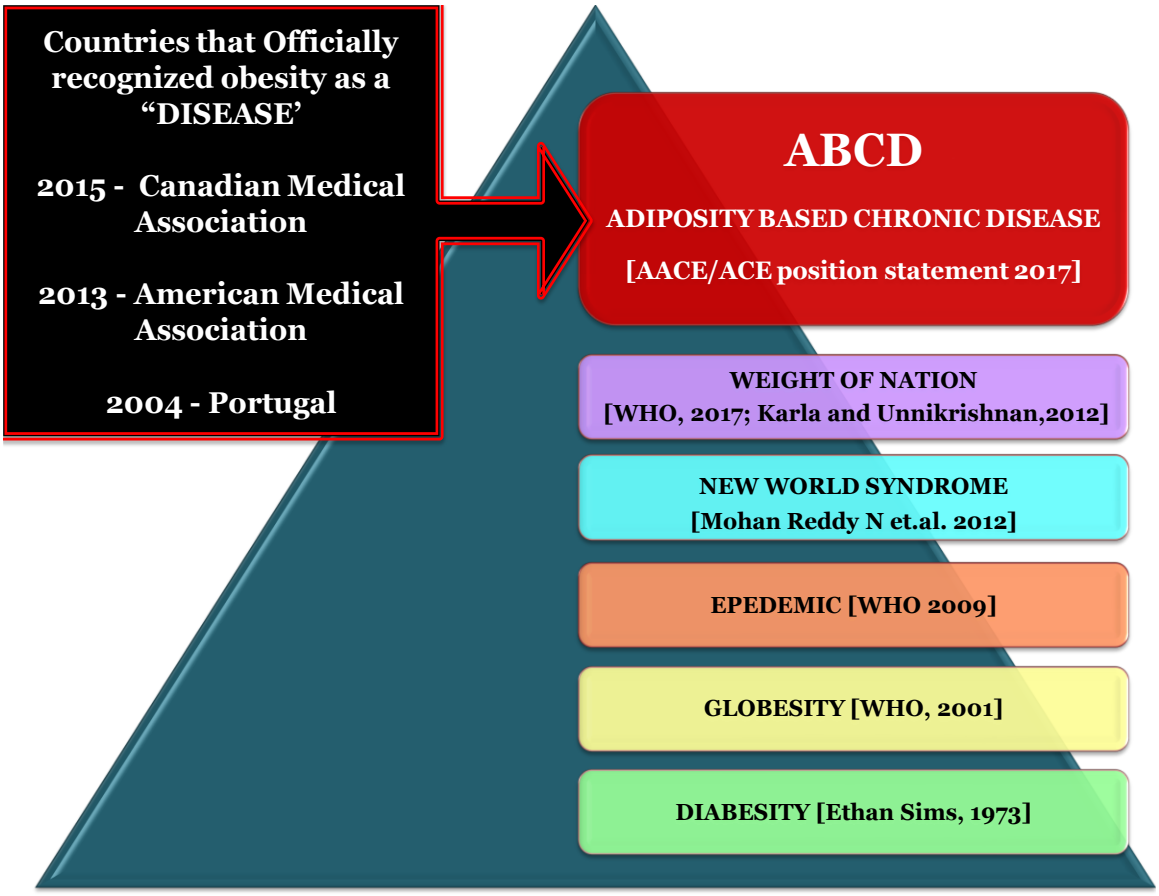


Figure 2.5: Hierarchy of Different Terminologies Coined For Obesity

Since obesity is affecting all age groups and even childhood obesity is on rise, leading to consequences of higher obesity rates in younger adolescents. World Obesity Federation (WOF) concerned about this gravely worrying trend argued in one of their position statement paper published in May 2017, that "early diagnosis and treatment of childhood obesity could be considered similar to vaccination —preventing a disease through proactive policy (Bray et al., 2017). But for effective prevention strategies such as these to be realized, obesity must be recognized as a disease". The argument contained the base of definition of disease which was defined as "A disease is caused by an agent that adversely affects the host". Obesity clearly fits into this definition where it can be defined as "chronic, relapsing and progressive disease" (Bray et al., 2017, WOF, 2017).

Several literatures had mentioned high calorie dense foods (high fat and sugar) as primary agent along with several other environmental factors like low physical activity. Similarly, obesity could also be considered as disease as its development and severity is directly proportion to the virulence of the agent and frequency of exposure of the host. When the host is frequently exposed to these agents in abundance they interact with genetic factors to produce a state of low grade inflammation that eventually affects and slowly causes organ damage, if left uncontrolled (The Lancet Editorial, 2017).

2.2.1 Adiposity Based Chronic Disease (ABCD) - A New Diagnostic Term

Recently, in 2017 according to a new position statement released by the American Association of Clinical Endocrinologists (AACE, 2017) and the American College of Endocrinology (ACE, 2017) had replaced term "Obesity" with a diagnostic term named "ABCD – Adiposity – Based Chronic Disease"**.** The authors insist on looking obesity as a "Complication Centric" Since BMI being a one dimensional approach and unable to differentiate between fat and muscles, authors find BMI a bit vague as a sole diagnostic tool and have emphasized more on "complications-centric" three pronged approach that considers assessment of excess body fat in terms of amount, distribution and its physiologic impact on health (Kedist, 2017).

To define overweight and obesity, BMI is applied universally as an assessment tool. However, several literatures have shown that even at lower points of BMI, significantly higher body fat, metabolic perturbation and risk factors for cardiovascular conditions were observed in Asian's compared to white population (Misra, 2015). A new question that arises out of these findings is that:

Will it be appropriate to classify people just based on BMI alone ?
Is something wrong with BMI ?

Since obesity is adiposity (accumulation of excess fat mass) and BMI is just a ratio of weight and height, it does not identify different compartments of body like fat, muscle, bone and water mass. BMI could be used as a sole parameter to classify status of

overweight and obesity only when other compartments of body remain constant and fat is only variable. Based on BMI there are possibilities and also mention in literatures that people may be classified as non-obese according to BMI but they may have higher fat mass and may enhance metabolic, cardiovascular risks and increase mortality (Bhardwaj & Misra, 2015; Hsu et al., 2015; Bodicoat et al., 2014)

Based on this AACE and ACE (2017), denied using one dimension and vague approach based on BMI and proposed using three dimensional approach and assessment of body fat using

- The amount of body fat
- The distribution of body fat
- The physiologic (health) impact of body fat

Another question that comes to mind is ***Why to Emphasize on Fat Distribution ?***

Several studies had demonstrated that proportion of body fat is very useful in identifying predisposition of disease. Accumulation of fat in abdominal region predisposes a person to higher risk of developing type 2 diabetes leading to Insulin resistance, high cholesterol leading to cardiovascular diseases, Leptin resistance and increased level of inflammatory cytokines. On the contrary when fat accumulates in buttocks or thighs (gluteo-femoral region) could prove to be protective as levels of hormone leptin will be high being directly proportional to fat and exerts anorexigenic effect (Manolopoulos, Karpe, & Frayn, 2010).

Hence, preventive measures can be initiated at as soon as adiposity and its pattern can be detected at an early stage and prevent metabolic derangement rather than just relying on BMI assessment.

2.3 Obesity – A State of Low Grade Inflammation

From previous literature review it is very clear that obesity is a state of adiposity and fat induced prolong inflammation (Mraz & Haluzik, 2014). Nevertheless, adipose tissue performs several functions apart from just storing fat as triglycerides (Mraz & Haluzik, 2014). Adipose tissue performs important functions like an endocrine organ and produces variety of molecules like adipocytokines such as IL-1, IL-6, IL-8, IFN, TNF, hormone Leptin and Resistin (Lowe et al., 2014). When adipocytes produce these molecules and are simultaneously destructed, this induces state of inflammation and influences other systems by altering their functions (Lowe et al., 2014). Obesity in itself is a state of low grade inflammation and unhealthy lifestyle is clearly associated with obesity. It can be postulated that unhealthy lifestyle modifies body's physiological response to adipocytokines and proinflammatory factors which are primarily related to chronic degenerative diseases (Lowe et al., 2014).

A person's lifestyle is of prime importance with regards to his health. Lifestyle has also been recognized by different areas of knowledge such as sociology, anthropology, medicine and psychology. To summarize, lifestyle is a result of formed behavior in relation to food, physical activity, alcohol consumption, caffeine, smoking and sleep pattern (Castro, Macedo-de la Concha, & Pantoja-Meléndez, 2017). Since obesity is clearly linked to unhealthy lifestyle that is distinguished by poor diet full of high fat and sugar intake, sedentary lifestyle and alcohol consumption (ENSANUT 2012), these factors bring change in metabolic processes and leads to state of low grade inflammation causing various disorders (Figure 2.6).

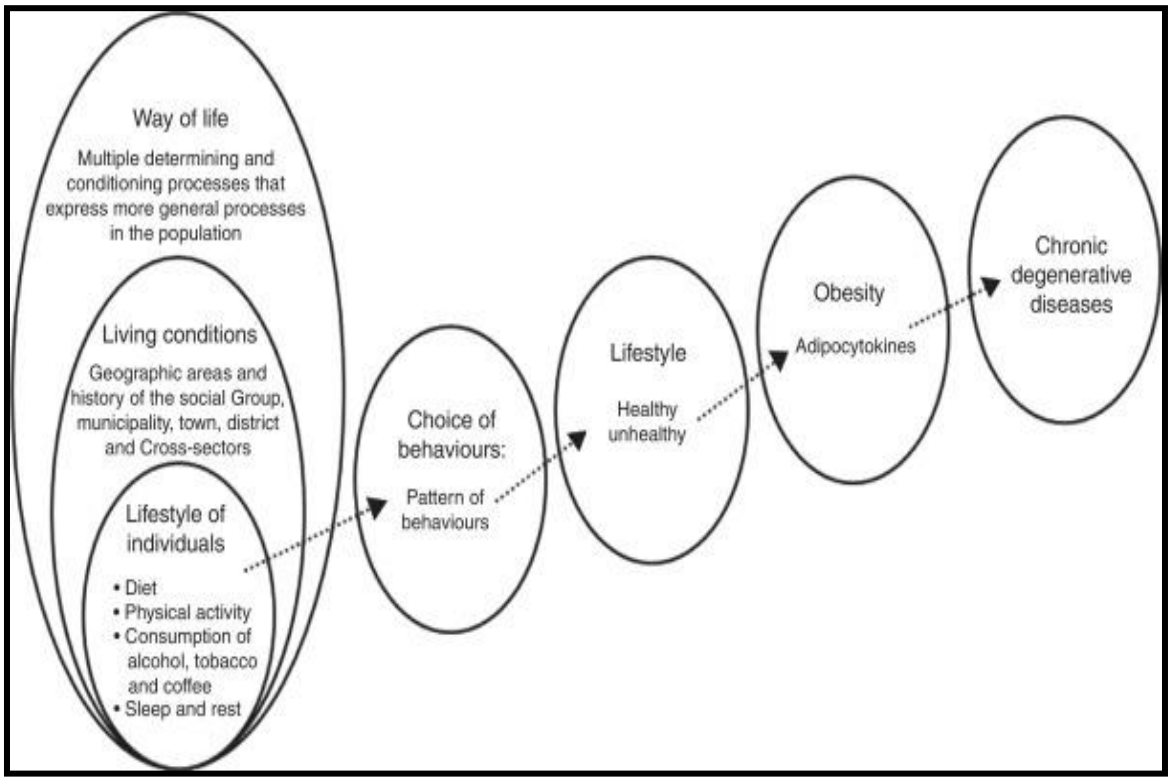


Figure 2.6: Sequence of variables for modifying risk factors for chronic degenerative diseases

(Source: Castro, Macedo-de la Concha, & Pantoja-Meléndez, 2017)

The common mediator between immune system and neuroendocrine system is hormone Leptin. Adipocytokines containing Leptin activates endothelial cells that in turn promote accumulation of macrophages in adipose tissue, which releases proinflammatory molecues that perpetuate the inflammatory process. The major risk factor in patients with type 2 diabetes is chronic low –grade inflammation (parainflammation) and high levels of adipocytokines present in adipose tissue (Lowe et al., 2014; O'Rourke et al., 2011).

Central nervous system and immune system communicate with each other and this communication plays a key role in inflammatory response to disease. This

communication initiates inflammatory reactions that are controlled by brain, including fever and behavioral changes [Guarner & Rubio-Ruiz, 2015; Gregor & Hotamisligil, 2011). Neurotransmitter produced during an inflammatory response act upon brain circuits and can easily influence mood and behavior (Castanon et al., 2014). In last two decades, strong association between chronic metabolic disorders and neuro-endocrine alterations with mood and behavioural changes has been demonstrated (Nguyen, Killcross, & Jenkins, 2014). Recent study by Slavich and Irwin (2014) depicted those patients with metabolic syndrome, diabetes and obesity positively correlated with the occurrence of depressive symptoms in response to increase in inflammation. Similarly, when these obese patients attempted for weight loss, reduction in inflammation along with reduction in depressive symptoms was observed (Slavich & Irwin, 2014).

Recent literature also suggests role of gut microbiota in obesity induced inflammation with regards to LPS related endotoxemia (Pereira & Alvarez-Leite, 2014). The first point of contact between body and diet is small intestine and is the first one to get exposed to ingested detrimental nutrients that leads to adiposity. This is the key reason why gastrointestinal tract is emerging as event initiator of systemic inflammation. The main contributor to these underlying mechanisms is westernized diet induced alteration in gut microflora, increased gut permeability and increased endotoxin release (Claire Barbier de La Serre et al., 2010).

To summarize, strong association between alteration in gut microflora caused due to high fat diet, increase in pathogenic bacteria, gut inflammation and obesity-induced inflammation was observed. Lipopolysaccharide (LPS) an endotoxin, which is mainly found in the outer membrane of Gram negative bacteria plays fundamental role in development of chronic low grade inflammation via immune system activation (Magnuson et al., 2015; Progatzky et al., 2014; Fei & Zhao, 2013; Teixeira et.al., 2012).

2.4 Determinants of Obesity – Direct and Indirect

2.4.1 – Direct Determinants of Obesity

Theoretically defining these terms, "Overweight is a state in which weight exceeds a standard based on height; and Obesity is a condition of excessive adiposity, either generalized or localized" (Mahan & Escott-Stump, 2017). The terms overweight and obesity are used parallel to each other. However, there are certain possibilities where both conditions can exist independently. For example, it is possible that a person is obese though his weight is in normal range according to standard tables and similarly it is very much possible that a person is overweight without being obese at the same time. There are several recent literatures that have validated the same (Bhardwaj & Misra, 2015; Hsu et al., 2015; Bodicoat et al., 2014).

2.4.1.1 - Body Mass Index (BMI) Cut-offs for Asian Indians

Universally, BMI classification is used to determine the prevalence of overweight and obesity. However, inspite of this standardized and accurate method that is used commonly for all there had been lot of controversies and recommendations to revise these universal BMI classification and make it ethnic specific especially for Asian Indians. Also, for World Health Organization to congregate a specific panel of experts on BMI classification there were three strong and specific factors.

First, previous literature clearly indicates that the higher prevalence rates of diabetes (NIDDM), and risk factors for CVD's in many parts of Asia even below the BMI cut-off points of < 25 kg/m^2 for overweight and BMI < 30 kg/m^2 for obesity (S. Behl & Misra, 2017).

Secondly, there was a lot of variation observed between BMI, body fat percentage and distribution of body fat across the population specifically in Asian population where

percentage of body fat was higher for specific BMI as compared to white Caucasians and European population.

Thirdly, there were previous two attempts to interpret the BMI cut-offs for Asians and that contributed to growing debates and possible need for ethnic specific BMI classification (WHO, 2011; James, Chunming, & Inoue, 2002; WHO/IASO/IOTF, 2000). Since there was lot of evidence that depicted increased risk of co-morbidities with obesity that occurs at lower BMI for Asians, proposal was formed to redefine the classification of obesity using BMI for Asian population (Table 2.1). However, the recommendations were based on two studies conducted by Ko et al. (1999) in Hong Kong and Deurenberg-Yap et al. (2000) in Singapore.

Table 2.1: Classification of weight status according to BMI in Asian adults (Source: Misra et al., 2009; WHO/IASO/IOTF, 2000)

BMI (kg/m^2)	Classification	Risk of Co-morbidities
< 18.5	Underweight	Low [but risk of other clinical complications]
18.5 – 22.9	Normal	Acceptable Risk
> 23	Overweight	
23.0 – 24.9	At Risk	Increased
25.0 – 29.9	Obesity Grade I	Moderate
≥ 30.0	Obesity Grade II	Severe

As compared to white Caucasians, Asian Indians have excess of body fat, increased subcutaneous, intra-abdominal fat, visceral fat, abdominal adiposity and deposition of fat in ectopic sites like liver and muscle. As Asian Indians exhibit these unique features and based on body fat percentage and morbidity data there was need to lower down the range of BMI for Asian Indians (Behl & Misra, 2017; NICE guidelines, 2014; Misra et al., 2009).

In view with the need of the hour, the Association of Physicians of India (Misra et.al., 2009) consulted with experts from various regions and medical disciplines, government

funded research institutions and policy making bodies of India before forming and recommended revised guidelines for diagnosis of obesity, abdominal obesity, metabolic syndrome, physical activity, drug therapy and bariatric surgery in Asian Indians.

Also before forming the guidelines the research questions that were addressed by Association of Physicians of India in 2009 aimed at:

a. Finding the best way to measure obesity
b. Determining BMI and WC cut-offs that are specific for Asian Indians
c. Determining appropriate definition for adults and children to assess metabolic syndrome for Asian Indians
d. To form specific guidelines for physical activity for Asian Indians
e. To form specific dietary guidelines for Asian Indians
f. To form treatment protocol for pharmacological treatment of obesity
g. To form treatment protocol for bariatric surgery

The recommendation based on the above questions as mentioned in the consensus statement for diagnosis of obesity by Misra et al., 2009 is given below

Consensus Statement for BMI Criteria:

Normal BMI: 18.0-22.9 kg/m2
Overweight: 23.0-24.9 kg/ m2
Obesity: >25 kg/m2

American Dietetic Association Position Statement 2015

Not only in India but in United Kingdom also Asian Indians and South Asians show differences in BMI cut-offs as compared to British white population and their situation differs with respect to diet, physical activity and exposure to vastly different condition than their homeland and reluctant stress. In United States some hospital and diabetes centers have been using lower BMI cut-points unofficially. Results of a recent study conducted by Hsu et al (2015), revealed occurrence of T2DM at a relatively lower BMI

cut-points. Investigators of this study clearly stated that "For screening of T2DM in Asian population residing in United States, a BMI cut-point of 23 kg/m^2 was appropriate. Similar information is also stated in position statement given by American Diabetes Association (2015). This is clearly a huge and prime approach for much needed health initiative for Asian population across the continent.

2.4.1.2 - Waist Circumference (WC) Cutoffs for Asian Indians

Deposition of fat around abdomen leading to increased waist circumference is one of the strongest and independent predictor of risk factors and morbidity associated to obesity, diabetes type 2, hypertension, dyslipidemia and cardiovascular risk factor as compared to the generalized adiposity. Waist Circumference is easy, simple and accurate way to measure in outpatient setting. (Singh, Sikri, & Garg, 2008; Mohan et al., 2007; de Koning et al., 2007; Joshi et al., 2007).

According to Behl and Misra (2017) in their recent review article on management of obesity in adult Asian Indians identified that "WC cut-offs of ≥ 90 cm in men and ≥ 80 cm in women identified high odds ratio of 4.2 and 2.2 respectively for cardiovascular risk factors and those with BMI ≥ 25 $kg/m^{2"}$. Data from several Asian countries like China, Singapore and Hong Kong provide evidence for high prevalence of NCD's below these cut-off points exist (China Obesity Task Force, 2002; Zhou, 2002; Jih et al., 2002; Deurenberg, 2001; Ko et al.,1999).

According to consensus statement given by Association of Physicians of India 2009 recommended two action levels with WC cut-offs for Asian Indian as mentioned below:

WC Cut-offs for Asian Indians,

A. Action level 1: Men: 78 cm, women: 72 cm. Any person with WC above these levels should avoid gaining weight and maintain physical activity to avoid acquiring any of the cardiovascular risk factor. These action level 1 cut-offs need to be researched further.

B. Action level 2: Men: 90 cm, women: 80 cm. Subject with WC above this should seek medical help so that obesity-related risk factors could be investigated and managed.

Thus based on recent evidence, WHO/IASO/IOTF 2000 proposed waist circumference cut-off points as mentioned below:

Table 2.2: Waist circumference cutoff points for increased risk to metabolic diseases (Source : Misra et al., 2009; MASO, 2005)

	MEN (WC)	WOMEN (WC)
WHO (1998)	**94 cm (37 inches)**	**80 cm (32 inches)**
WHO/IASO/IOTF (2000)	**90 cm (35 inches)**	**80 cm (32 inches)**

2.4.1.3 - Waist Hip Ratio (WHR) Cutoffs for Asian Indians

Waist hip ratio is also one of the surrogate criteria for measuring abdominal obesity and associated risk factors for cardiovascular disease. Waist hip ratio (WHR) is calculated by dividing WC by the maximum hip circumference. Measurement of body fat percentage through bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DXA) can be done at advanced centers' for obesity management; but are not required for routine management of obesity (Bhel & Misra, 2017).

However, as compared to WC, WHR is not accurate due to difficulty in measurement of hip circumference as it requires disrobing of clothes and also difficult to perform due to practical concerns related to the opposite gender. Also, changes in WHR not necessarily may reflect extent of obesity or weight change. In a retrospective study, the results of meta-regression analysis of WHR as predictor of cardiovascular events revealed that WHR was associated with risk of CVD incident (de Koning et.al., 2008). Nevertheless, there are some evidences where, WHR does reflect graded and significant association

with risk of myocardial infarction and also shown for Asian Indians (Misra et al., 2009; Joshi et al., 2007; Yusuf et al., 2005).

Mohan et al. (2007), conducted a study on South Indians (n=2350) and depicted that the optimal cut-offs for identifying any two risk factors was 87cm for men and 82 cm for women. Similarly, results of Misra et al. (2006) revealed that the cut-offs of 0.88 in men for identifying those with at least one CVD risk factor was optimum with sensitivity of 71% and specificity of 71.3%. Also, for women the WHR cut-off of 0.80 was optimum with sensitivity of 66.2% and specificity of 65.7%. Hence, for Asian Indians the WHR cut-offs in males and females are 0.88 and 0.80, respectively (Misra et al., 2009).

However, The WHO 2011, states that abdominal obesity is defined as a waist–hip ratio above 0.90 for males and above 0.85 for females (WHO expert consultation, 2011)

2.4.1.4 - Waist- to-Height Ratio (WHtR) / Waist Stature Ratio (WSR) Universal Cutoffs

The waist-to height ratio (WHtR) / waist stature ratio (WSR), is calculated by dividing waist circumference (WC) by height. Today, WSR has tremendously gained attention over WC as an index for central adiposity. It is also an easy-to-use and less age-dependent index to identify individuals with increased cardiometabolic risk (Ashwell & Hsieh, 2005). Since, It is very evident from the above mentioned literature on BMI and WC that Central obesity carries more health risk as compared with total obesity assessed by BMI. Therefore, when WC is used as a proxy for central obesity, it has been suggested that BMI should be included along with it. BMI and WC should be used as a "Matrix" to assess health risk (Ashwell & Gibson , 2016).

A cut-off of 0.5 for WSR is used and accepted as a universal cut-off for central obesity in children aged ≥ 6 years and adults. Also, it can also be used in different sex and ethnic groups (Ashwell, Gunn, & Gibson, 2012).

WSR has been established as a slightly better than WC and superior to BMI in predicting higher cardiometabolic risk assessment in prospective studies and meta-analysis in adults (Ashwell & Gibson, 2014).

It is also recommended that, additional use of WSR with BMI and WC may be helpful as WSR considers both height and central obesity. WSR also gives a very simple message of "Keep your WC to less than half of your height" (Yoo, 2016; Khoury et al., 2016; Petroff et al., 2015).

The credibility of WSR is also mentioned in one of the diagnostic accuracy study conducted by Caminha et al.(2017) on Brazilian women considering WSR as the best anthropometric predictor of hypertension. This study was first of its kind to highlight the importance and statistical superiority of WSR as a hypertension as a screening method especially when cutoff of 0.54 was used (Caminha et al., 2017).

Very similar kind of results was also observed in females with noticeably different facial and physical characteristics in western region (Dong et al., 2016; Kang et al., 2015; Zeng et al., 2014; Park & Kim, 2012).

The association between abdominal obesity and hypertension indicates that this pattern of body fat distribution can maximize the hemodynamic changes caused by obesity. The adipocytes, concentrated in the visceral area, have a more biologically active metabolism, tending to release greater amounts of fatty acids and proinflammatory adipokines. Thus, the Insulin resistance aspect gets worse, as well as the endothelial dysfunction, dysregulation of hepatic metabolism and, in consequence, it favors the onset of cardiovascular disorders (Caminha et al., 2017).

2.4.1.5 - Body Fat Percentage

The body fat percentage is a way to define fitness level of a person. It depicts amount of essential fat and extra stored fat inside body of an individual. It helps identifying derange compartments of body composition like water, lean mass, fat proportion and bone mass. Unlike BMI, this is kind of body measurement does not require height or weight and directly calculates a person's relative body composition. Body fat can be measured using several methods. Few of the methods are listed below

1. Skin Calipers

This is one of the most accessible methods for measuring body composition. It is performed using a skin fold assessment using three to seven different sites (meaning parts of the body). While performing the test skin is first pinched and then the skin caliper device is used to measure the thickness of the skin fold for each site. Specific sites for testing commonly include the chest, arms, abs and thighs. After estimating the numbers they are put into a formula and results calculated and practitioners can estimate body fat percentage (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Pros

This method is relatively inexpensive. Though being easily accessible method as compared to rest all other methods, it does take considerable practice. If well trained, a proper skin fold assessment can be completed within few minutes, at anytime and at any place (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Cons

Margin of error varies as only few body parts are considered for measurements and body areas that hold a greater amount of fat could be missed resulting in lower reading. Also accuracy of measurement highly depends on skills and experience of a technician,

consistency of holding and handling of calipers (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

2. Bioelectrical Impedance

Bioelectrical impedance works on principle of "Impedance". Tiny electrical impulses are send through body and body fat is determined on the pace those impulses return back. In theoretical model circuit for passing electric current is completed by placing electrodes on wrist and ankle. As fat is a bad conductor of electricity, impulses will take more time to return back and then the response time is correlated and results extrapolated. Similarly, lean tissues will conduct faster electrical impulses resulting in faster response time. Scales available for measuring body fat varies from simple bathroom scale where electrodes are under each foot to the complex with handhold electrodes in addition to the foot (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Pros

It is easy to measure as now they come as a built-in feature in traditional form of bathroom scale and are affordable to keep one in house. Users need no practice and just have to feed in certain data as mentioned in user manual of the specific model purchased and measurements are reflected in fraction of seconds (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Cons

It is less accurate than more advanced methods available like DEXA scans. Accuracy of results depends on state of body like hydration level at time of assessment [sipping a glass of water before measurement] , food intake [meals before measurement can give skewed results] and exercise [readings taken immediately after workout will provide lower body fat values (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

3. Hydrostatic Weighing

In this method of analyzing body composition, individual's body has to be completely submerged under water. Then two measurements are taken, weight outside water and weight while submerged underwater and are compared. Subject's density and body composition is accurately calculated by operators using these two measurements along with the density of the water (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Pros

Hydrostatic weighing uses true tried and tested a variable that has least percentage of error. It is an absolutely accurate technique for measuring body composition. For this reason, many experts used to refer hydrostatic weighing as the gold standard for measuring body composition until DEXA scan was developed. It's also commonly used in research settings (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Cons

Hydrostatic weighing is an expensive and bit inconvenient technique as subjects have to forcibly exhale air out of lungs to reduce potential error and sit submerged completely underwater. It also needs specific setup in any kind of lab or performance centers. This might be uncomfortable for some individuals (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

4. DEXA (Dual-Energy X-Ray Absorptiometry)

A DEXA scan uses X-ray radiations of different intensities that pass through body to analyze body composition. It is also used to analyze bone mineral density. It is performed under the medical supervision by a radiologist. These days DEXA scan is considered as to be the new gold standard in body composition testing replacing hydrostatic weighing. Procedure looks very similar to CT scan as individual has to lie down still on a table while a machine arm passes over their entire body exposing low energy radiations. The radiations are absorbed by different body parts and the readings are displayed on screen and also can be printed for submission. Measurements are displayed for bone mineral

density, lean mass and fat mass. This test can also be done only affected body part and not necissarily whole body to be exposed (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Pros

DEXA scan is now a gold standard in measuring body composition and is simple as the individual has to lie down, is quick, dry and painless unlike hydrostatic weighing (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Cons

It is expensive and needs prior appointment with hospital or research institute (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

5. 3D - Body Scan

3D body scan takes measures body circumference of different parts and tracks body fat via a corresponding app. There are several brands that offer devices that can be used at-home. Very soon this option will become more easily available and accessible to people. Many devices can also measure muscle mass. Device includes full-length mirror that is embedded with sensors and uses infrared light to provide a full-body three dimensional image. It also comes with a turntable that can rotate to 360 degrees and read weight (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Pros

This method is also provides precise readings to the accuracy of 2% similar to DEXA method. As device comes with a corresponding app it becomes very easy to frequently track progress and can even notice small changes. Accuracy is better and even simpler with a machine this when performed naked in the comfort and privacy of home.

Nevertheless, accuracy of measurement improves when scan is done at the same time of the day and need not worry about hydration or meal times like bioelectrical impedance (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

The Cons

Only one point of concern is that accurate results are obtained when this test is performed without clothes and completely naked. However, one can wear a tight pant and top, same outfit every day for reading and try to stand still as much as possible (DuVall & Creveling, 2018; Wells & Fewtrells 2006).

2.4.1.6 - Physical Activity (PA)

In 2011, World Health Organization stated that people are doing less of physical activity worldwide at their work place, while traveling and even at home despite of knowing the health benefits of physical activity. Also, globally, about one in three people is not getting enough physical activity to do (WHO, 2011).

Also, team of obesity prevention source from Harvard T.H. Chan School of Public Health, 2018 stated that, Physical activity levels are declining globally including wealthy countries like U.S. and also low- and middle-income countries like China. And it's clear evident that the key contributor to the pandemic of obesity is decline in physical activity levels and contributing to rising rates of chronic disease everywhere (Havard - obesity prevention source team, 2018).

Since obesity is a public health issue and has far reaching negative effects on health, the course of public health actions is mostly focused on encouraging individuals to eat healthier and to be physically active. Certainly these established strategies have not been successful and reasons of failure to restrict calorie intake or to maintain high levels of physical activity are of great controversy. Since voluntary exercise is the most

independent and discretionary component of energy expenditure and it still holds potential for curtailing the ongoing obesity epidemic (Wiklund, 2016).

Westerterp and Speakman (2008) conducted doubly-labeled water studies [optimal method to measure energy expenditure in free-living individuals] and depicted that daily energy expenditure had not declined between 1980 and 2005 in Europe and North America. Similar results were also observed in nearly 100 studies on doubly-labeled water in industrialized countries, and found that they did not had lower rate of daily energy expenditure as compared to populations in developing countries (Dugas et al., 2011). This indicates clearly that obese individuals have higher habitual energy expenditure due to their larger body size and resting metabolic rates as compared to normal weight individuals (Ravussin et al., 1982). Moving further, Leibel, Rosenbaum, and Hirsch (1995) demonstrated that increase in 10% of weight increases daily energy expenditure from 370 to 530 kcal, depending on their initial baseline weight. The very obvious implication of this condition results in increase in rate of energy intake accordingly, otherwise resultant weight loss will ensure (Leibel, Rosenbaum, & Hirsch, 1995).

Simultaneously, there were several findings that have been reported from US and European countries that depicted increase in estimated daily energy intake in adults by 500 kcal between 1970s and 2000s (Archer, Hand, & Blair, 2013; Swinburn, Sacks, & Ravussin, 2009 ; Balanza et al., 2007; Silventoinen et al., 2004).

Data from study conducted by Levitsky and Pacanowski (2012) showed that daily energy intake in the US increased slowly until the early 1980s, and then started to increase rapidly. Moreover, a recent global analysis concluded that in high-income countries increases in food-energy supplies are sufficient to explain increase in average population body weight (Vandevijvere et al., 2015). Thus, it seems unlikely that decrements in daily energy expenditure are driving the ongoing obesity epidemic.

Another study conducted by Prentice AM et al., 1996 on 319 adults from the UK indicated that obese individuals exhibit higher total energy expenditure (TEE, assessed by the doubly-labelled water method), basal metabolic rate (BMR), and AEE (activity-

induced energy expenditure) due to their larger body size (Prentice & Jebb 1995). According to these results, physical activity is not changed in obesity. Other studies conducted by Rising et al. (1994) and Ferraro et al. (1991) suggest contrary results stating that obese individuals are less active and expend less energy in physical activity than do lighter subjects.

Nevertheless, as reduced physical activity and obesity represents a vicious circle Pietiläinen et al. (2008) hypothesized that a vicious circle may arise between decreasing physical activity and weight gain from adolescence (16 – 18 yrs) to early adulthood in monozygotic (MZ) twin individuals. Results from this study revealed higher odds for physical inactivity and development of obesity (3.9%) and for abdominal obesity was 4.8% at age 25. Poor physical fitness in adolescence also increased the risk of overall (5.1, 2.0-12.7) and abdominal obesity (3.2, 1.5-6.7) in adulthood. Physical inactivity was both causative and secondary to the development of obesity discordance in the MZ pairs. TEE did not differ between the MZ co twins. PA levels were lower whereas BMR was higher in the obese co-twins. Physical inactivity in adolescence strongly and independently predicts total and especially abdominal obesity in young adulthood, favoring the development of a self-perpetuating vicious circle of obesity and physical inactivity. Physical (in) activity should be a major target of obesity prevention in the young (Pietiläinen et al., 2008).

Clearly, obesity results from excessive energy intake that has sustained over a long period of time. Currently, we do not understand why people consume more energy than they expend. It may be that PA has the ability to regulate food intake, but in the contemporary environment that is conducive for sedentary behavior, this regulatory mechanism has gone astray. Increasing PA most certainly can create energy deficit through increased energy expenditure. For this reason PA and exercise hold potential as part of the solution for the ongoing obesity epidemic.

2.4.1.7 - Dietary Intakes

Diet plays a very crucial role in the commencement of obesity. There are various factors that predetermine the amount of food a person consumes like personal choices related to food, advertising, social customs and cultural influences, food availability and pricing. Economic development of any country brings about improvements in a food supply and elimination of dietary deficiencies and hence improving overall nutritional status of the country's population. Along with it also brings about qualitative changes in production, processing, distribution and marketing of food. Increasing urbanization affects dietary patterns and lifestyle and not all of them have positive impact. Nutrition transition and changes in pattern of work and leisure contribute to the underlying factors of non-communicable diseases even in poorest countries. Nutrition transition include dietary shifts in structure of diet towards higher energy density foods, higher fat, sugar, saturated fats especially from animal sources, reduced intakes of complex carbohydrate and dietary fiber, reduced fruit and vegetable intake. In addition, these dietary changes are compounded by reduced physical activity. Moreover, these changes are also accelerating in low-income and middle-income countries (WHO, 2018).

Various eating patterns, such as a high consumption of low-quality food, sugar-sweetened soft drinks, snacks and sweets, and unfavorable meal patterns, such as a low number of daily meals and skipping breakfast, have been related to overweight (Eloranta, 2012; Sandercock, Voss & Dye, 2010; Toschke, Thorsteinsdottir, vonKries, 2009; Nicklas, et al., 2003). Moreover, some adverse eating behaviors', such as emotional overeating and food responsiveness, have been associated with childhood overweight in recent studies (Eloranta, 2012; Spence et al., 2011; Webber et al., 2009; Viana, Sinde, & Saxton., 2008). A high energy dense diet (Yasutake et al., 2014) and large meal volumes (Ledikwe et al., 2005) increase the intake of energy and such a pattern followed for a prolonged period of time results in obesity (Yasutake et al., 2014; Ledikwe et al., 2005).

Average Calorie Consumption

Whatever we eat is finally broken down into calories. Traditionally, calorie is a calorie regardless of its source. When intake of total calories exceeds the expenditure, weight gain is observed. Hence, for weight watchers and obese individuals the best advice is to keep check on total calories consumed and exercise more. However, there are emerging researches that suggest eating patterns and macronutrient composition of the meal influences achieving early satiety, avoid overeating and achieving optimum health benefits.

Studies conducted by National Health and Nutrition Examination Survey in United States from 1971 – 2000 depicted increase in average consumption of 335 calories per day by women from 1542 calories to 1877 calories and for men, the average increase was 168 calories per day from 2450 calories to 2618 calories (Wright et al., 2004). Based on the previous data and trends as mentioned in WHO report on global and regional food consumption pattern and trend in year 2018, it was predicted that by the year 2030 there would be increase in calorie intake of 130 kcal/day from 2850 kcal/day in year 2015 to 2980 kcal/day in year 2030 (WHO, 2018). Majority of excess calories are contributed by carbohydrate consumption along with increase consumption of fat over same period of time (Wright et al., 2004).With changing economy and traditions, majority of food is consumed away from home that includes consumption of salty and fried snack, soft drinks and fast foods (junk food) that accompanies with large portion sizes (Vadera et al., 2010; Wright et al., 2004).

Does Type of Macronutrient Intake Matter in Obesity ??

Carbohydrate Intake - Sugar Sweetened Beverages (SSB)

These days majority of carbohydrate is contributed by the simple sugar present visibly and invisibly in our food apart from the major food group of cereals'. If we analyze closely and check out all junk food, breads, bakery products and sugar sweetened

beverages from our diet, the average carbohydrate content of our diet will never ever exceed RDA and will also provide sense of satiety.

Consumption of sweetened beverages and soft drinks accounts for almost 25% or more of daily calories of young adults in America. These intakes may underestimate the amount of calories consumed as they are dependent on person's accurate dietary recall (CDC, 2016; Caballero, 2007).

Sugar-sweetened beverages are mostly referred to soft drinks / soda pops. However, they are not just limited to regular soda but also include fruit juices, sports - energy drinks, sweetened water, tea and coffee. Substance used to sweeten these beverages can range from simple sugar, brown sugar, corn sweetener, corn syrup, dextrose, fructose, glucose, high-fructose corn syrup, honey, lactose, malt syrup, maltose, molasses, and sucrose (U.S. Department of Agriculture, 2015).

According to Centers for Disease Control and Prevention 2017 data collected during 2011 – 2014 depicted that, 63% of young adults and 49% of adults consumed one SSB per day. Approximately, 143 calories / day was consumed by U.S. youth and 145 calories /day by U.S. adult from SSB intake (Rosinger et al., 2017). On an Average 65% of Americans from different geographical locations consumed minimum one SSB per day (Park, McGuire, Galuska, 2015). Also, 52% of Americans drink SSB at home and 48% of people drink away from home (Kit et al, 2013).

Consumption of Sucrose and Fructose are the main culprits for weight gain. They are also referred as "Empty Calories". Sweetened beverages contain either sucrose or in addition fructose which gets metabolized into fat when consumed in excess. Large scale cross sectional and prospective cohort studies supports consumption of sweetened beverages as prime reason for adult and childhood obesity pandemic (WHO, 2018; Obesity Prevention Source, Havard, 2018; Lyssiotis & Cantley, 2013; Tappy & Lê, 2010; Malik, Schulze, & Hu 2006).

Several studies have reported that SSB contains approximately 15 – 18 teaspoons of sugar, providing 240 calories. Soda drinks available in large size packaging of 64 ounce provides around 700 calories. Though these SSB are loaded with sugar and calories and are calorie dense, after its consumption it fails to provide sense of satiety. Resultant people continue eating their solid food in addition to the intake of SSB and end up putting on weight due to excess of total calorie intake. Studies have very well established a strong correlation between development of obesity and consumption of SSB (CDC, 2016; Ervin 2013; US Department of Agriculture, 2015; Pan & Hu, 2011; Hu & Malik, 2010; Woodward-Lopez, Kao & Ritchie 2010; Johnson et al., 2009; Malik et al., 2006).

High glycemic load of sugar sweetened beverages could probably be the major contributor towards development of NCD's (CDC, 2016; de Koning et al, 2012; Malik et al., 2010). The American Heart Association estimated increase in 50% of total calorie intake over past 3 decades that is attributable to sweetened beverages (Johnson, 2009). Furthermore, sugar may also be an addictive substance potentiating to the existing problem of excessive consumption (CDC, 2016; Avena, Rada, & Hoebel, 2008)

The bloom in soda industry could be attributed to the tremendous turnover of the product at lower selling price, advertising and marketing of the product in a tempting way along with allotting $700 million in year 2000 as advertising budget .Furthermore, at lower cost of sweetened beverages the standard serving size increased from 8-ounce to 12-ounce bottles between 1977 – 1996 [Siegel et.al., 2016; Lyssiotis & Cantley, 2013; Ervin, 2013; Woodward-Lopez, Kao, & Ritchie, 2009)

Protein intake

Protein is one of the most important macronutrient in our diet as it has vast array of biological functions to execute. Unlike carbohydrate and fat, role protein in weight gain and obesity has been uncontroversial forever. Protein has been very well accepted as a weight loss macronutrient. The mechanism through which protein exerts or aids in weight loss could be attributed to

1. Satiation Effect – Mellinkoff's aminostatic theory that involves intestinal gluconeogenesis. Protein intake generally leads to early satiety and may facilitate reduced calorie intake (Westerterp-Plantenga, Lemmens, Westerterp, 2012; Keller U, 2011; Douglas Paddon-Jones et al., 2008; Astrup et al., 2005; Yancy et al., 2004; Vandewater, 1996; Mellinkoff, 1956).
2. Thermogenesis Effect – Protein intake elevates thermogenesis in body augmenting energy expenditure and influencing satiety (Raben et al., 2003; Parker et al., 2002; Eisenstein, 2002).
3. Maintenance of Fat Free Mass (FFM) – helps stimulate muscle protein anabolism and retaining lean muscle mass along with improvement in metabolic profile (Lejeune et al., 2005; Westerterp-Plantenga et al., 2004; Layman et al., 2003).
4. Enhanced glycemic control – protein intake exerts a delayed postprandial increase in blood glucose levels due to protein-induced stimulation of pancreatic glucagon secretion (Layman et al., 2003; Farnsworth et al., 2003).
5. Stimulation of gut hormone release – (Nakamura et al, 2011).

Protein Ingestion and Satiety

Protein intake has long been considered to maintain normal wear and tear of cells, for cosmetic health benefits in females and as over the counter supplement for adolescents and adult males to increase muscle mass. However, the more we explore this macronutrient, more benefits surface up at molecular level. Growing evidence from various researches indicates that it plays a very important role in promoting satiety and achieving weight-loss (Mathews, 2018).

The mechanism by which protein helps in promoting satiety is through peptides formed during protein digestion. It involves intestinal gluconeogenesis in the control of food intake. These peptides lead to the suppression of μ-opioid (Mu-opioid) receptors (MORs) lined up on walls of portal vein that acts as communication channel between gut and brain. MORs sends appetite stimulating signals to brain and these signals are inhibited by the protein peptides and communicate satiety signals to brain by suppressing MORs and curbing appetite (Carreiro et al., 2016; Duraffourd et al., 2012).

There are several studies that support the central role of proteins in appetite regulation. Results of study conducted by Griffioen-Roose et al. (2012) on healthy humans indicated that when protein intake is low it promotes desire to eat protein containing savoury foods and higher protein intake results in lower hunger pangs (Carreiro et al., 2016; Griffioen-Roose et al., 2012). Ogden et al. (2012) observed that over the last three decades of exploding weight incidence in United States at population level cross-culturally protein intake was surprisingly consistent unlike carbohydrate and fat (FAOSTAT database, 2002). Based on this observation Simpson (2005) suggested a biological base and proposed a protein leveraging hypothesis. According to this postulate it can be interpreted that when body protein needs are not met, it will increase appetite to increase food intake until an appropriate amount of protein is ingested. Antithetically, diets rich in protein are ostensibly ingested in lesser quantity as they suffice the requisite amount of protein with proportionately lesser calories (Simpson, 2005).

It is also evident from various studies that high protein diet induces weight –loss and also helps in weight maintenance due to its satiating, low-caloric value properties and positive effects on lean mass (Westerterp-Plantenga et al., 2012; Douglas Paddon-Jones, 2008; Lejeune et al., 2005; Westerterp-Plantenga 2005; Layman et al., 2005; Westerterp-Plantenga, 2004; Layman, 2004), improves glycemic control and also improves lipid profile of T2DM patients over 6 – 12 months period (Westman et al., 2007; Yancy, 2004).

However, underlying mechanisms related high protein intake inducing enhanced satiety and weight loss are unclear. To understand this mechanism Jefferson et al. (2009) conducted study on pubertal children and effect of different types of macronutrients (CHO, Fat and Protein) on Ghrelin and PYY secretion. Results revealed that in response 30 min after consuming high-carbodydrate meal, PYY levels peaked and significantly declined thereafter in normal weight and obese children. However, after high-protein meal steady increase in morning concentration of PYY levels was observed in both the groups. Additionally, lower AUC hunger and higher AUC satiety was observed in obese group children in response to high protein meal (Jefferson et al., 2009).

In addition, Gerald et al. (2015), formed hypothesis predicting possible release of gut hormones either by proteins or amino acids (AA). Results revealed no difference in either fasting or postprandial increments for Insulin, Ghrelin and PYY. However, plasma essential AA concentrations remained elevated up to only 60 minutes for normal protein diet and for high protein diet they remained elevated for 3-5 hours were greatest (Gerald et al., 2015).

2.4.1.7 – Alcohol Intake

Globally, alcohol is one of the most commonly consumed drink when people are on vacation, partying or on recreational trip. Use of alcohol is kind of a status symbol of the people belonging to high income group and offering drinks on the house reflects lavish lifestyle. Alcohol provides 7kcal/g consumed and contributes to extra calorie intake, which is never compensated for (Traversy & Chaput, 2015; Yeomans, 2010). There are several health issues associated with alcohol intake, but relationship between alcohol intake and weight gain has been extensively explored in cross-sectional, longitudinal and experimental studies (Bendsen et al., 2013; Sayon-Orea et al., 2011; Yeomans, 2010). Apart from obesity, excessive consumption of alcohol is also one of the major concerns of public health issue as it is the 3rd leading cause of death in United states smoking and obesity (Traversy & Chaput, 2015; O'Keefe et al., 2014).

According to global status report on alcohol and health by WHO (2014), stated that 13.5g of pure alcohol per day is consumed by each individual aged 15 years and older worldwide (WHO, 2014).

In a study conducted by Rosalind et al. (2013) on diets of drinkers in US and data collected from NHANES 2003-2008 survey data reported that intake of calories due to alcohol went exorbitantly high on drinking days as compared to non-drinking days. Also, men consumed higher calories (433 extra calories) as compared to women (299 extra calories) (Rosalind et al., 2013). Figure 2.7 depicts serving size and calorie content of a standard drink.

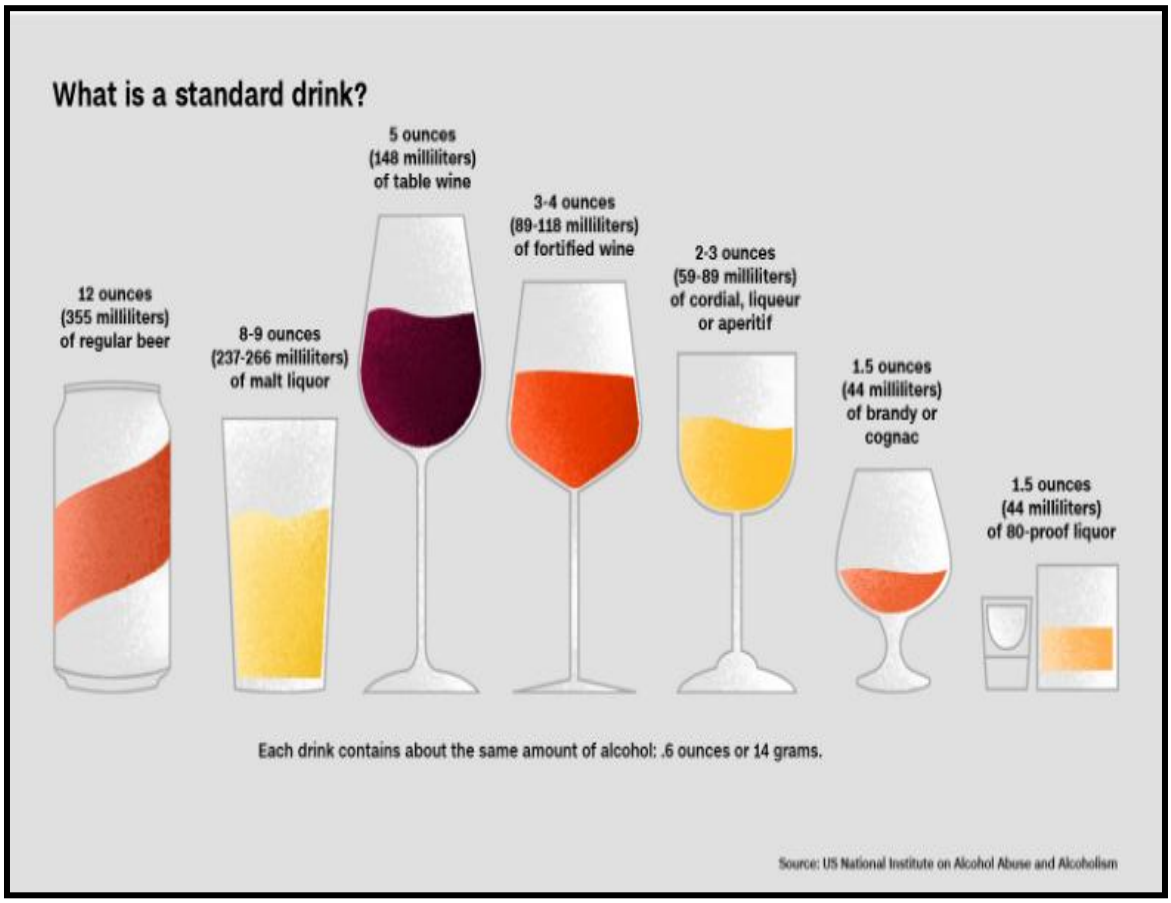


Figure 2.7: Serving Size and Calorie Content of a Standard Drink

(Source: US National Institute on Alcohol Abuse and Alcoholism)

This brought up the concept of "Binge drinking" in UK by National Health Service (NHS) 2018 defined as,

> **"Drinking lots of alcohol in a short span of time or drinking to get drunk"**
>
> **(NHS, Binge Drinking, 2018)**

Definition of binge drinking as given by The National Institute on Alcohol Abuse and Alcoholism, 2004 is as follows:

"Pattern of drinking that brings a person's blood alcohol concentration (BCA) to 0.08 gm percent or above. This typically happens when men consumes 5 or more drinks or women consumes 4 or more drinks in about 2 hours"

(The National Institute on Alcohol Abuse and Alcoholism, 2004)

In yet another national survey by Office for National Statistics on adults drinking habits in Great Britain 2005 – 2016 revealed that 2.5 million people admitted to habit of binge drinking in one weeks time (Dr Ben Windsor-Shellard, 2017). Also, according to Centers for Disease Control and Prevention stated that one in six adults dump about eight drinks at a time and four times a month when they binge drink (Kanny et al, 2013).

There are recent prospective studies that have demonstrated heavy drinking being strongly associated as a major risk factor for weight gain as compared to light-moderate drinking (Macinnis et al., 2014; Sayon-Orea et al., 2011; Schütze et al., 2009; Rissanen et al., 1991)

A study was conducted by Shelton and Knott (2014), determining association between incidence of overweight and obesity and calories contributed by alcohol intake in English adults. Calories consumed from alcohol intake were recorded for only those days when individuals had highest intake of alcohol. Results revealed that the heaviest drinking group had 70% higher risk of obesity as compared to lightest drinking group (Shelton & Knott, 2014).

Heaviest drinking group on their heaviest drinking day consumed additional calories of 75% of their total daily energy intake only from alcohol as compared to the lightest group whose intake was <24% of daily energy intake (Shelton & Knott, 2014). In few studies risk of obesity and large WC was associated with binge drinking (Lee, 2012; Arif &

Rohrer, 2005). In another study conducted by Fan et al. (2008), reported association of excess alcohol intake with heavy drinking episode and abdominal obesity (Fan et al., 2008).

Hence, cross-sectional studies demonstrated that excess body weight, higher body fat percentage are closely associated with heavy drinking and binge drinking unlike light – moderate drinking (Shelton & Knott, 2014; Lee, 2012; Wakabayashi, 2010; Croezen et al., 2009; Alcácera, 2008; Vågstrand, 2007; Tolstrup et al., 2005; Arif & Rohrer, 2005; Lukasiewicz et al., 2005). However, cause and effect relationship was not clearly demonstrated by these cross-sectional studies (Traversy & Chaput,, 2015).

There are certain potential mechanism related to alcohol intake and obesity that have shown to influence satiety hormones. Several studies have proposed that effects of Leptin and glucagon-like-peptide-1 (GLP-1) are inhibited influencing energy intake by alcohol intake (Röjdmark & Calissendorff, 2008; Raben & Agerholm-Larsen, 2003). However, evidences till date does not support role of Peptide YY (PYY), Ghrelin, Gastric Inhibitor Peptide (GIP) or Cholecystokinin (CCK) (Traversy & Chaput,, 2015; Calissendorff, 2006; Raben & Agerholm-Larsen, 2003; Manabe & Sawai, 2003). Alcohol intake also influences immediate appetite and effects energy storage. Oxidation of fat is inhibited by alcohol intake suggesting fat sparing action leading to higher body fat in long term (Traversy & Chaput, 2015; Yeomans, 2003).

2.4.2 – Indirect Determinants of Obesity

2.4.2.1 – Depression

Depression and obesity share a bidirectional relationship. In simple words obesity and depression feed each other in a vicious self destructive cycle (Dr. Michael Craig Miller 2013). So the question that comes in first place is, Which of the two conditions comes first ?? Depression or Obesity ??

Defining depression is like resolving a puzzle. There are many people who are not aware that they are suffering from depression and vice a versa many people around us look depressed but are not. To understand depression we need to look into two primary components that is cognitive component (mood) and somatic component (sleep and appetite) (Engstrom, 2018).

How to measure Depression ?

One of the most respected and commonly used instruments for measuring depression is Becks Depression Inventory (BDI). BDI consists of 8 cognitive and 13 somatic sub scales for assessment of depression (Engstrom, 2018; Obesity Action Coalition, 2018).

Cognitive sub-scale consists of: (Engstrom, 2018; Obesity Action Coalition, 2018).

- Pessimism
- Punishment feelings
- Self-criticalness
- Past failures
- Self-dislike
- Worthlessness
- Guilty feelings
- Suicidal thoughts or wishes

Somatic sub-scale consists of: (Engstrom, 2018; Obesity Action Coalition, 2018).

- Sadness
- Agitation
- Loss of energy
- Change in appetite
- Loss of interest in sex.
- Loss of pleasure
- Loss of interest
- Change in sleep patterns
- Concentration difficulties
- Crying
- Indecisiveness
- Irritability
- Tiredness and/or fatigue

According to the Data from NHAHES 2014, 43% of adults in US with depression were found to be obese and adults with depression had higher odds for becoming obese as compared to adults without depression. This effect was more prominent in women i.e women with depression were more likely to be obese than women without depression. With the increase in severity of depressive symptoms, increase in proportion of adult obesity was observed (CDC 2015, NHANES 2014).

During the survey year of 2005 – 2010, out of 34.6% of obese adults aged 20 and over in U.S., 7.2% of them had depression (CDC 2015, NHANES 2014). Obesity and Depression

both impose a major health risk of NCD's including functional limitations. Also, 11% of population are on antidepressant medication and were positively related to obesity (NCHS, 2014; Pratt, Brody, & Gu, 2011; Luppino et al., 2010; McElroy, 2004).

Till date it was assumed that obesity and depression coincidentally existed together. However, in a meta-analysis of longitudinal studies conducted by Luppino and colleague (2010), they found that in American studies the development of depression was strongly influence by obesity. They also accentuated the possibility that overweight, obesity and depression had a biological link between them (Gwyn Cready-Obesity Action Coalition, 2018; IARD, 2017; Faith et al., 2011; Luppino & colleague, 2010; Stunkard et al., 2003).

As obesity and depression both are associated with inflammation, brain responds in a manner leading to elevated pro-inflammatory cytokines. Since in obese individuals fat tissues are loaded with macrophages, they release inflammatory hormones such as TNF-alpha and interleukin-6 that constantly activate the immune system at a low level, leading to chronic inflammatory state (Gwyn Cready-Obesity Action Coalition, 2018; Bastard et al., 2006).

Hence, from above studies we couldn't find substantial evidence for direct casual pathway that leads to depression form obesity. However, to recognize these pathways and factors is of utmost importance to know when to intervene to prevent depression in obese people (Deina et al., 2012).

Dr Miller also explains his views stating that in obesity parts of brain that regulate mood gets affected leading to low energy and motivation that gets translated into less activity and exercise resulting into weight gain. Also, if both problems have a hold on an individual then he enters into a vicious cycle.

2.4.2.1 – Hunger and Satiety

To understand why people gain weight seems like resolving a mystery. It would be much more luminous and would make perfect sense, the day we understand the reason of gaining weight or cause of it (Tony Bednarowski, 2018). Hypothalamic responses control energy balance however, they can be confounded by hedonic and brain reward system. This combination in addition to availability of cheap and palatable junk foods rich in energy density in form of sugar and fat may probably explain increase in overweight and obesity epidemic. As shown in Figure 2.8, Full4Health is an EU-funded project conceived to advance our understanding of hunger and satiety mechanisms (Tehmina & Julian, 2016).

Hunger and Satiety are sensations, where hunger represents physiologic need to eat food and satiety is absence of hunger and feeling of fullness (Oxford University Press, 2017). Unpleasant hunger sensation manifests within few hours without eating and after eating satiety is achieved between 5 – 20 minutes (Steen, Juliette, 2016).

Hunger pangs are contractions that occur in stomach. One single hunger contraction lasts for about 30 seconds and pangs for around 30 – 45 minutes and then subsides for around 30 - 150 minutes until next contraction. Hunger contractions are inhibited in emotional states and are activated by hypoglycemia (Carlson, 1931)

The biological mechanisms that regulate hunger and satiety involve fluctuation of hormone Leptin and Ghrelin motivating an individual to eat. Ghrelin initiates the feeling of hunger and after consuming meal, adipocytes in response to meal triggers the release of Leptin resulting inhibition of Ghrelin and reducing motivation to eat (signal's brain to stop eating). Again, after few hours of non-consumption Leptin levels drop and Ghrelin reinitiates feeling of hunger and the cycle continues (Malik, McGlone, Bedrossian, & Daghe, 2007).

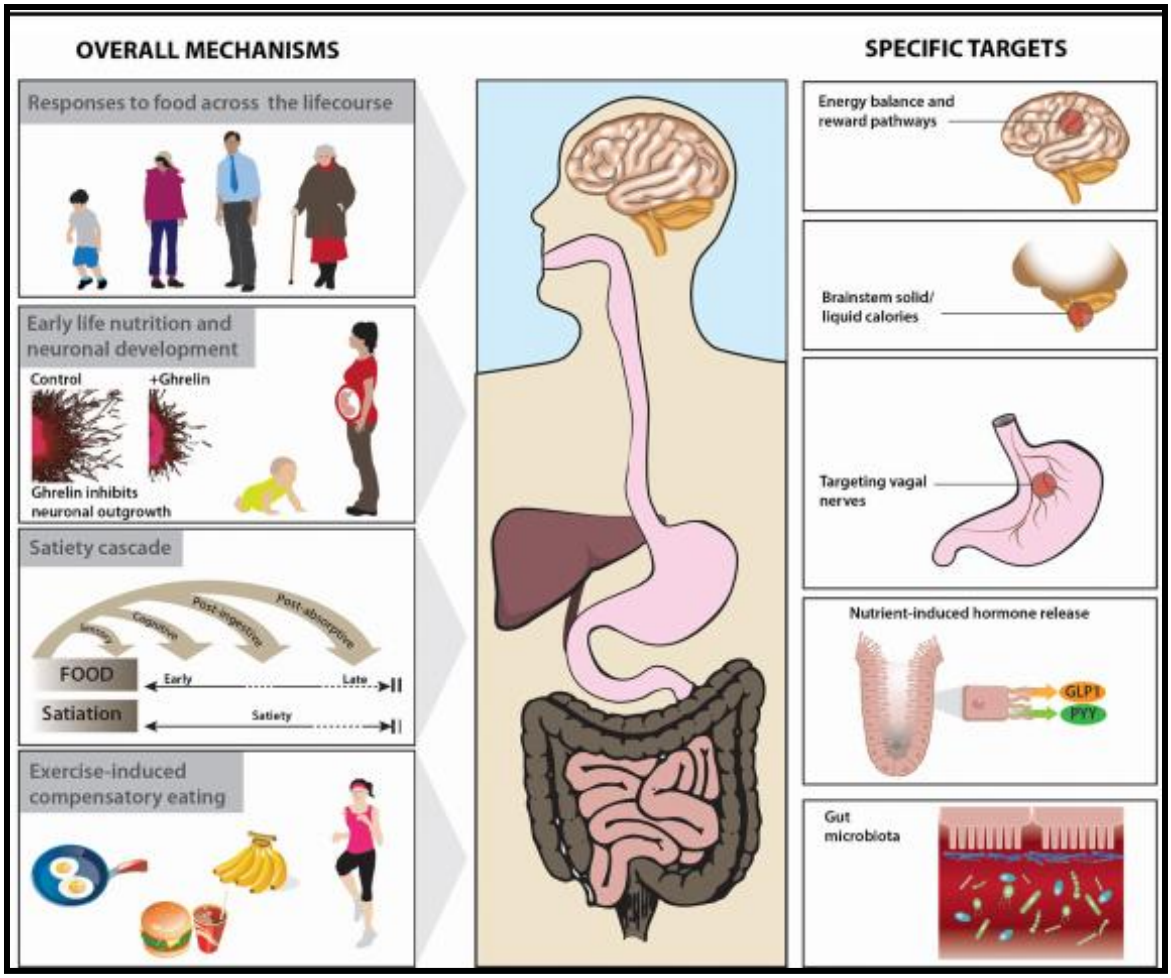


Figure 2.8: Hunger and satiety: overview of mechanisms and specific targets.

Source: Tehmina & Julian 2016.

Selected aspects of the Full4Health project include responses to food intake across the life course, the impact of early life nutrition on neuronal development and the effect of exercise on feeding behaviour. An integrated physiological system—the 'food-gut-brain axis'—controls what we choose to eat, when we eat it and the impact on our subsequent behaviour and thus on body weight. Different foci in this axis, as discussed in the text, may provide targets for adapting and exploiting responses to food and could deliver alternative solutions to the problem of overweight and obesity.

Hunger and Satiety signals are also regulated by neural signals from gastrointestinal tract (GI tract). Vagal nerve fibers carry signals between brain and GI tract. Studies have demonstrated that difference in macronutrients can be sensed by brain. Also, appetite is

inhibited by sending signals along vagus afferent pathway upon distension of GI tract (Marieb & Marieb, 2010).

There are several psychological factors that play role in short term regulation of food intake (Morewedge, 2010). Huh, et al. (2016) conducted research on "Selective Sensitization: Consuming a Food Activates a Goal to Consume its Complements" and its results depicted that when an individual introduced one kind of food it induced craving for another complement food that was desirous to add pleasure to the current one. The authors Huh et al. (2016) explained this theory as study participants who consumed a sip of cola were also willing to pay more for a cheeseburger voucher that could be redeemed later as compared to participants of control group (Huh et al,2016).

Hence, wide range of comprehensive researches over last 3 decades have depicted evidences involvement of GI tract, gut peptides, peripheral nerves, neuroendocrine and reward systems in brain modulating molecular and neuroanatomical mechanisms controlling energy balance. Presumably it is now high time to utilize natural properties of food inducing satiety and individuals able to control hunger pangs which is one of the biggest reason for dietary failure. It's now time for 'Tailor made foods / designer foods" (Tehmina & Julian, 2016).

2.5 Gut Brain Axis – Underlying Molecular Mechanisms

The biochemical signaling between two organs namely GI tract and central nervous system (CNS) is termed as gut –brain axis (GBA). In broader terms GBA includes central nervous system, neuroendocrine and neuroimmune systems, including hypothalamic – pituitary – adrenal axis (HPA axis), sympathetic and parasympathetic arms of the autonomic nervous system, including vagus nerve and the gut microbiota Occasionally , the role of gutflora is in interplay when term GBA is used. However, when term "microbiome-gut-brain axis" is used it clearly indicates the biochemical signaling events that takes place between the GI tract and CNS (Dinan et al., 2014; Sudo, Chida, & Aiba, 2004) (Figure 2.9).

Gut-brain-axis also includes role of gut hormones that seem to communicate signals from the GI tract to the regulatory appetite centers within the CNS. This information is probably transferred to the CNS either via vagal or non-vagal afferent nerve signaling or directly via blood circulation (Buhmann et al., 2014; Bueter & le Roux, 2009). Forebrain and brainstem that control feeding behavior and energy homeostasis consist of complex neural networks and homeostatic feeding behavior is integrated within the hypothalamus. A number of nuclei including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN) and the lateral hypothalamic area (LHA) are all contained in the hypothalamus and are interconnected by energy homeostasis-regulation circuits (Buhmann et al., 2014; Schwartz et al., 2000; Cone et al., 2001) (Figure 2.9).

One of the most important nuclei in hunger and appetite regulation is ARC that receives peripheral appetite signals and responds in two distinct neuronal populations namely neuropeptide Y (NPY) and agouti-related peptide (AgRP) with modulated neuropeptide release. This in turn increase stimulates eating and body weight gain via increase in hunger and appetite sensations (Suzuki & Jayasena, 2012; Cone & Cowley, 2001; Schwartz, 2000) (Figure 2.9).

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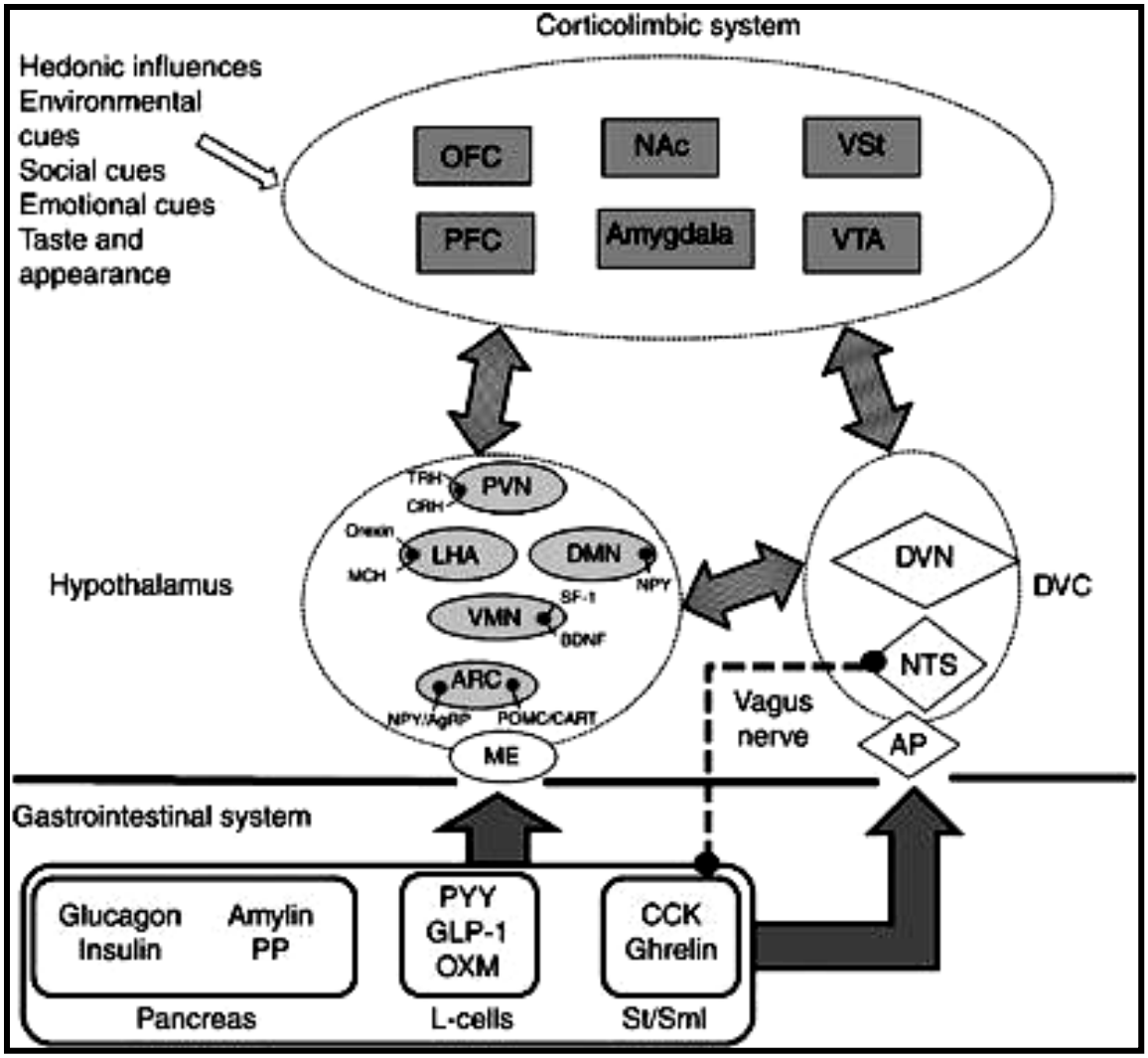

Figure 2.9: Regulation of Food Intake via Gut Brain Axis

On other side, in the lateral ARC, the neurons co-express pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART) that is responsible for inhibiting hunger signals and inducing satiety signals leading to reduced eating and subsequent weight loss. Hence, it is very important that the balance is maintained between the activities of these neuronal signals to maintain body weight (Buhmann et al., 2014; Suzuki, Jayasena, & Bloom, 2012; Flier, 2004). After meal consumption, sensory information is forwarded from the GI tract to the CNS through afferent fibers or via gut hormones via blood stream (Buhmann et al., 2014; Murphy & Bloom, 2006) (Figure 2.10)

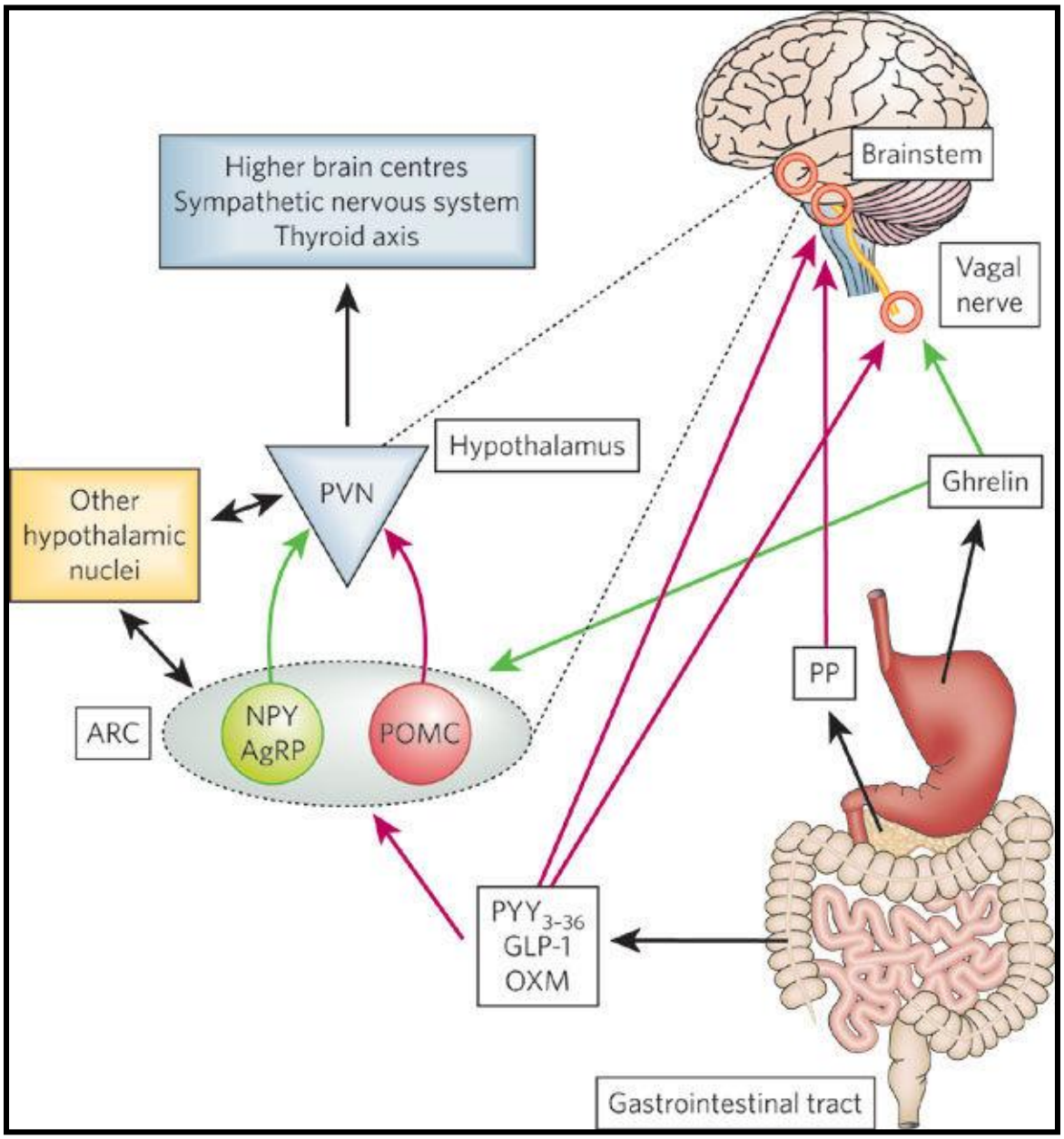

Figure 2.10: Peripheral Signals Regulating Hunger and Appetite Signals via ARC

What really matters is that, information is communicated in bidirectional way regarding energy stores and food intake between brainstem and hypothalamus influencing the perception of hunger and satiety. Also, whenever there is change in body adiposity,

mechanisms that resist weight change are triggered in brain through physiological homeostatic mechanisms by modulating neurotransmitters, metabolic rate and bringing change in appetite (Buhmann et al., 2014; Ellacott, Halatchev, & Cone, 2006; Schwartz & Woods, 2003; Leibel & Rosenbaum, 1995; Brady et al., 1990; Grill & Smith, 1988).

2.6 Role of Gut Satietogenic Hormones in regulation of body weight

Over the last few decades, gut hormones have been extensively studied and their intricate interplay between regulation of food intake through appetite modulation and with central nervous system (Perry & Wang, 2012; Hameed, Dhillo, Bloom, 2009; Woods & D'Alessio, 2008). From the vast ocean of several gut hormones only few of these circulating hormones have been known to influence appetite in humans (Batterham & Le Roux, 2003) namely Ghrelin, the only known hunger hormone (orexigenic hormone) (Tschop & Weyer, 2001) and group of anorexigenic gut hormones that includes cholecystokinin (CCK), pancreatic polypeptide (PP), peptide YY (PYY), oxyntomodulin (OXM) and glucagon-like peptide (GLP-1) (Batterham, 2003).

Hunger and satiety regulation and body weight is a homeostatic process. This homeostatis is maintained by Long-term humoral sginals and short-term neural signals. Long–term humoral signals communicate information regarding general health and short-term signals regulate meal initiation and termination by neural signals from brain and humoral signals from the gut (Perry & Wang 2012; Wilding, 2002).

Gut hormones like ghrelin, glucagon –like peptide (GLP-1), peptide YY (PYY), pancreatic polypeptide (PP) and cholecystokinin (CCK) are known to induce satiety and meal termination leading to dramatic impact on energy balance homeostasis (Mishra, 2016).

2.6.1 Peptide YY (PYY)

Peptide YY is 36 amino acid short peptide and is released from cells in the ileum and colon in response to feeding. Peptide YY also known as peptide tyrosine

tyrosine is a satiety hormone belonging to family of neuropeptide Y (NPY) along with pancreatic polypeptide (PP) and induces anorexigenic effects attributing to delayed gastric emptying (ileal brake), is dose related and dependent on the amount of fat in the meal (Lean & Malkova, 2016; De Silva & Bloom, 2012). The name PYY is derived due to the presence of tyrosine residue and is co-secreted by L cells along with GLP-1 in the lower intestine (Lean & Malkova, 2016; le Roux et al., 2006). PYY $_{(3\text{ -}36)}$ is the major circulating form in both fed and fasted state (Batterham, et al., 2006). PYY is secreted in response to meal and peak within 2 hour of eating and is proportional to the size and type of meal consumed (Yang et al., 2009; le Roux, 2006; Batterham et al., 2006).

Studies have reported that the strongest stimulant of PYY secretion is fat followed by protein and carbohydrate has limited effect in obese or non-obese individuals (Lean & Malkova 2016; Yang & Liu, 2009; le Roux, 2006). However, some studies have also reported protein rich meals to cause peak in PYY levels as compared to other macronutrients (Bewick, 2012; Batterham, 2006). This could probably explain role of high protein diet and their high thermal effect in achieving weight-loss

Studies have also reported diminution of postprandial PYY in relation to obesity (Cahill et al., 2011; Brownley et al., 2010; Zwirska-Korczala. et al., 2007; Essah et al., 2007; Feinle-Bisset et al., 2005). In obese adult, children and infants, fasting PYY negatively correlated with obesity markers and have shown blunted postprandial response (Mishra, 2016; Batterham et al., 2003.)

Studies have also demonstrated that administration of PYY reduces food intake with a significant reduction in cumulative 24 hour caloric intake in obese and lean humans (Bewick, 2012). However, obese subjects reflected lower endogenous PYY response at each meal compared to normal weight volunteers indicating that this relative deficiency could have reinforced obesity (Drucker, 2006).

2.6.2 Gut Incretin - Glucagon-Like Peptide (Active GLP-1$_{(7-36)}$)

GLP-1belongs to class of molecules referred as "Incretins" and is a long peptide hormone made up of 30 amino acids and is derived from post translational processing of tissue – specific proglucagon gene. It is secreted in response to meal by intestinal enteroendocrine L-cells and certain neurons located within the nucleus of solitary tract in brainstem upon food consumption. GLP-1 $_{(1-37)}$ is an initial product that undergoes amidation and proteolytic cleavage which gives rise to the two truncated and equipotent biologically active forms, GLP-1$_{(7-36)}$ amide and GLP-1 $_{(7-37)}$ amide. GLP-1$_{(7-36)}$ is the active form and possesses incretin properties enhancing secretion of Insulin and helps reducing blood sugar levels in a glucose- dependent manner (Lean & Malkova, 2016; de Graaf et al., 2016; Ma, Guan, Hua, 2014). Hence, the prime focus of pharmaceutical research has diverted towards the development of GLP-1 based treatment.

GLP-1$_{(7-36)}$ is secreted in response to food intake and is biphasic. After food intake the early phase occurs within 10 -1 5 min and the second peak is achieved at 30 – 60 min (Lean & Malkova, 2016; Madsbad, 2014).

However, Dipeptidyl peptidase-4 (DPP-4) rapidly degrades GLP-1$_{(7-36)}$ endogenously resulting in half-life of approximately 2 minutes. As a result amount of GLP-1$_{(7-36)}$ that reaches intact into circulation is only 10 – 15%, leading to fasting plasma levels of only 0 – 15 pmol/L (0 - 49.5pg/ml). In order to overcome this limitation, therapeutic treatments have developed GLP-1$_{(7-36)}$ receptor agonists and DPP-4 inhibitors to increase GLP-1activity. Also, GLP-1$_{(7-36)}$ based treatment suffices two important considerations for T2DM patients of inducing weight loss and reducing risk of hypoglycemia (Lean & Malkova, 2016; Marathe et al., 2013; Calanna et al., 2013; Seino et al., 2010)

GLP-1$_{(7-36)}$ also reduces appetite by collectively inhibiting gastric emptying, acid secretion and motility. Since it decelerates gastric emptying, postprandial excursion of glucose is reduced which attracts researches, medical practioners and phrama industries

to consider it for diabetes treatment (DellaValle Brian et al., 2016; Sharma et al., 2014; Seino et al., 2010) (Figure 2.11).

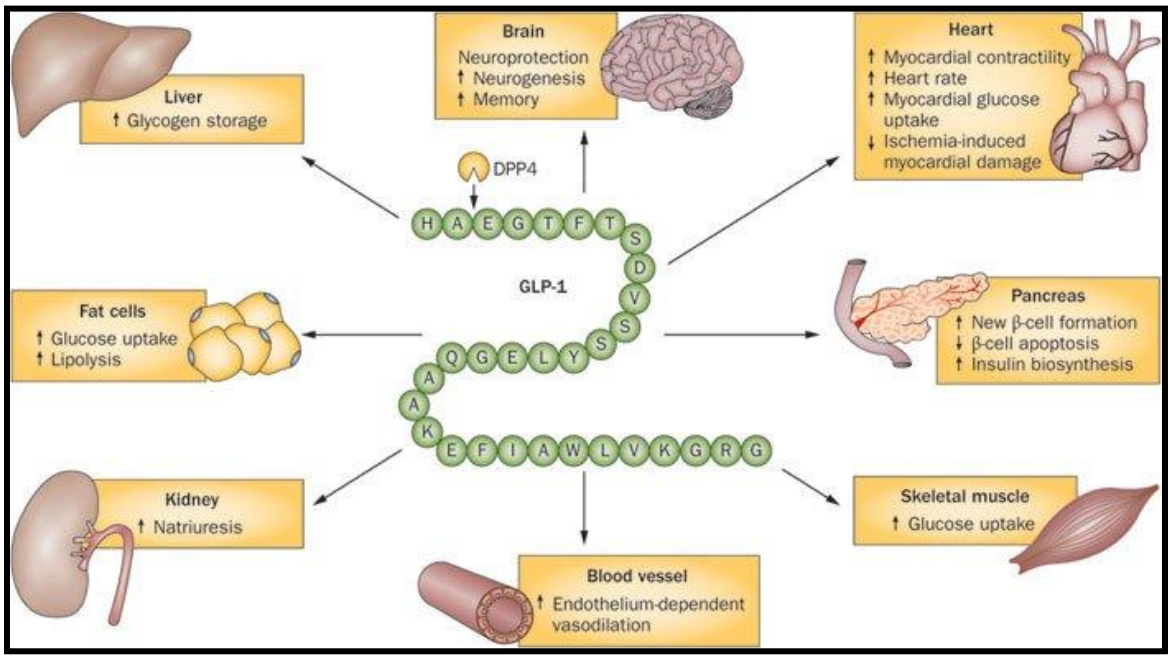

Figure 2.11 : Functions of GLP-1

(Source: https://www.selfhacked.com/blog/glp-1-a-hormone-that-causes-weight-loss-and-good-cognitive-effects/)

In a study conducted by Adam & Westerterp-Plantenga (2005) revealed that preprandial GLP-$1_{(7-36)}$ levels were similar for obese adults as compared to normal weight individual. However, the postprandial GLP-$1_{(7-36)}$ was significantly attenuated in obese adults after 30 min as compared to controls (Adam & Westerterp-Plantenga, 2005). In yet another study conducted by Carroll et al (2007) revealed that in obese subjects GLP-$1_{(7-36)}$ levels declined markedly in the first 20 min as compared to normal weight subjects where GLP-$1_{(7-36)}$ levels increased 10 min after a standard liquid meal (Lean & Malkova, 2016; Carroll et al., 2007).

There are several studies that have examined the effects of dietary interventions for weight loss on GLP-$1_{(7-36)}$ levels. Results of these studies revealed that reduction of 8% of body weight in obese subjects on 8 – week low calorie diet (1000 kcal/day), had lower

levels of fasting GLP-$1_{(7-36)}$ and greater appetite as compared with same patients after 6 month of high monounsaturated fat diet leading to a modest weight regain of 4.2% (Lean & Malkova, 2016; Sumithran, 2011). The above studies postulated to favor increased appetite and weight regain that explains why inspite of achieving weight loss through calorie restriction it is so difficult to achieve and/or maintain for obese individuals.

Few more studies evaluated weight loss with exercise and surprisingly the response induced was opposite to low calorie induced weight loss. One study conducted by Martins et al., 2010 who were first to examine the effect of aerobic exercise training on fasting and postprandial levels of GLP-$1_{(7-36)}$ in obese individuals. Study results of 12 – week supervised exercised training found no impact on fasting GLP-$1_{(7-36)}$ concentration. However, postprandial release increased tremendously explaining positive effects of long-term exercise training in achieving weight loss and maintenance [Schubert et al., 2014; Martins et al., 2010).

2.6.3 Gut Incretin –Gastric Inhibitory Polypeptide / Glucose–dependent Insulinotropic Peptide (GIP)

Gastric inhibitory polypeptide (GIP) is now also known as glucose-dependent Insulinotropic peptide (GIP) due to its characteristic property to stimulate Insulin secretion and consist of 42-amino acid polypeptide produced in small intestine by K cells. Previously, it was known to protect small intestine from acid damage by reducing stomach acid secretion and slowing down food transition time from stomach (gastric emptying) (Kim & Egan 2008). However, later it was found that these similar effects are seen with secretin hormone and were achieved only with higher physiological level and. GIP also belongs to class of molecules referred as “Incretins” along with GLP-1 (Pederson & McIntosh, 2016).

GIP is synthesized and postprandial released from the duodenum and proximal jejunum depending on several factors including meal content and pre-existing health status like obesity, diabetes, age etc. The potent stimulator of GIP was found to be fat that was augmented by bile and mediated through fatty acid-binding protein 5 and G protein –

coupled receptor 120 through direct actions on K-cells, followed by glucose (Satoko et al., 2017; Cho et al., 2014; Yip & Wolfe, 2000). With prior exposure to high-fat diet, the response of GIP to oral glucose was enhanced. It was also depicted that in obese individual's reduction in postprandial plasma triglyceride was associated with elevated plasma GIP levels, suggesting its role in triglyceride uptake (Satoko et al., 2017; Shibue, et al., 2015; Iwasaki et al., 2015).

The expression of endogenous GIP appeared to coordinate with nutritional status and was linked to over-nutrition leading to development of obesity soon after its discovery. In this era of modern society, there is tendency to accumulate fat due to sedentary lifestyle and availability of plentiful food all year round. In a study conducted by Miyawaki et al.(1999) have shown that may be a potential target for antiobesity drugs and had demonstrated direct links of overnutrition to obesity (Satoko et al., 2017; Miyawaki et al., 1999).

According to study data by Ballinger (2003), fasting blood glucose and plasma Insulin concentrations were found to be same in GIP receptor knockout and wild type mice depicting that the physiological role of GIP in fasting state seems to be less important (Ballinger, 2003; Miyawaki, 2002). Furthermore, in same study conducted by Ballinger (2003), high fat diet was fed to mice for 43 weeks and results demonstrated 35% increase in body weight as compared to control diet fed mice. Fat accumulation was observed in visceral area, subcutaneous and in liver. Mice lacking GIP receptor were prevented and did not show weight gain and triglyceride synthesis on high fat diet. Hence, high fat diet induced obesity was prevented by interruption of GIP signaling pathway. It was also found that resistance to obesity due to inability to store fat in adipocytes and higher energy expenditure were the prime reason in GIP receptor knockout mice. Investigators also revealed that Insulin resistance and obesity induced by high fat diet was prevented due to inhibition of GIP signaling (Satoko et al., 2017; Daniela 2014; Ballinger, 2003).

To conclude, hyper secretion of GIP was induced due to overnutrition caused due to excessive fat intake, increased nutrient uptake into fat cells leading to obesity and

resultant Insulin resistance and hyperinsulinemia (Satoko et al., 2017; Daniela 2014; Ballinger, 2003).

2.6.4 Leptin – The Energy Expenditure Hormone

In greek Leptin / Leptos means "thin". Leptin is also known as the hormone of "Energy expenditure" and is predominantly made by cells of adipose tissue, comprising of 167 amino acids. The amount of fat in an individual's body is directly proportional to amount of circulating leptin (Muhammad 2015; Clément et al., 1998). Leptin opposes the action of hormone Ghrelin that is "hunger hormone". It induces satiety signals by inhibiting Ghrelin. Leptin and Ghrelin play a vital role in appetite regulation and in order to achieve energy homeostasis, both hormones act on receptors present in the arcurate nucleus (ARC) of hypothalamus. In obesity, similar to Insulin resistance, despite of high energy stores and high levels of Leptin, decreased sensitivity to Leptin occurs resulting in an inability to detect satiety (Figure 2.12) (Pan, Guo, Su, 2017).

Leptin and Ghrelin hormones regulating energy homeostasis through neuroendocrine control develop resistance. The development of resistance of both hormones is a hallmark of obesity (Figure 2.13). However, strategies that were developed till date to slow down the current epidemic of obesity have been hampered. This is attributed largely to lacking knowledge owing to the underlying mechanisms and their resistance to the action of hormones (Cui, López, & Rahmouni, 2017).

The primary function of leptin is regulation of fat stores, however; it also plays a role in many other physiological processes (Muhammad, 2015). There are many conditions in which Leptin no longer correlates with body fat levels and deviates from their strict role of communicating nutritional status between brain and body.

- Critical role is played in adaptive response to starvation (Friedman, 2009)
- Short-term fasting of 24-72 hours depicts decrease in Leptin levels with no change in fat mass (Chan & Heist, 2003)

- Sleep deprivation reduces serum levels of Leptin (Copinschi et al., 2014; Knutson et al., 2007)
- Paradoxically, higher levels of Leptin are observed in obesity and emotional stress (Otsuka, 2006)
- Reduced by physical exercise training (de Salles et al., 2010)
- Leptin levels are increased by Insulin (Kolaczynski, 1996)

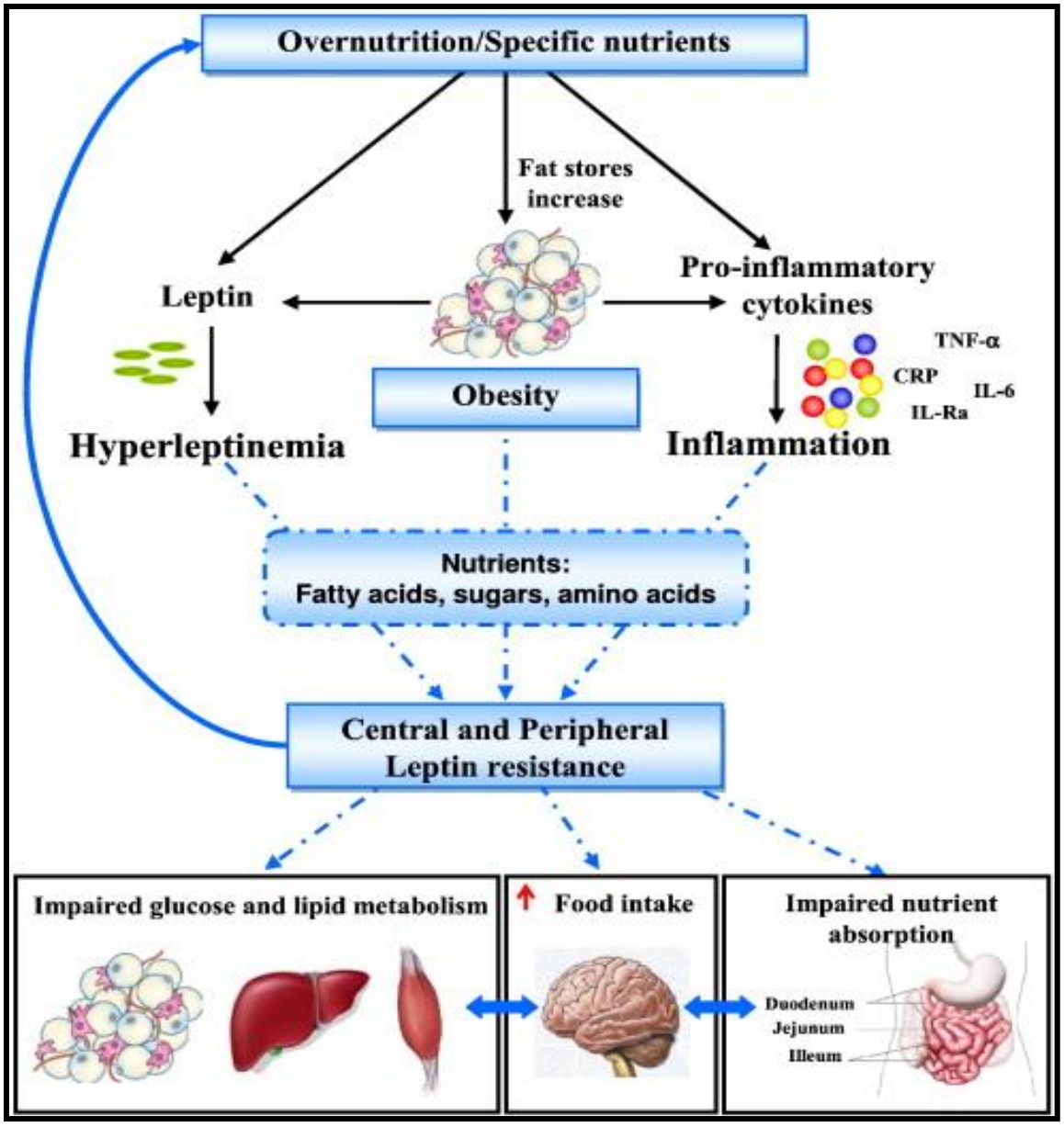

Figure 2.12: Obesity and Leptin resistance

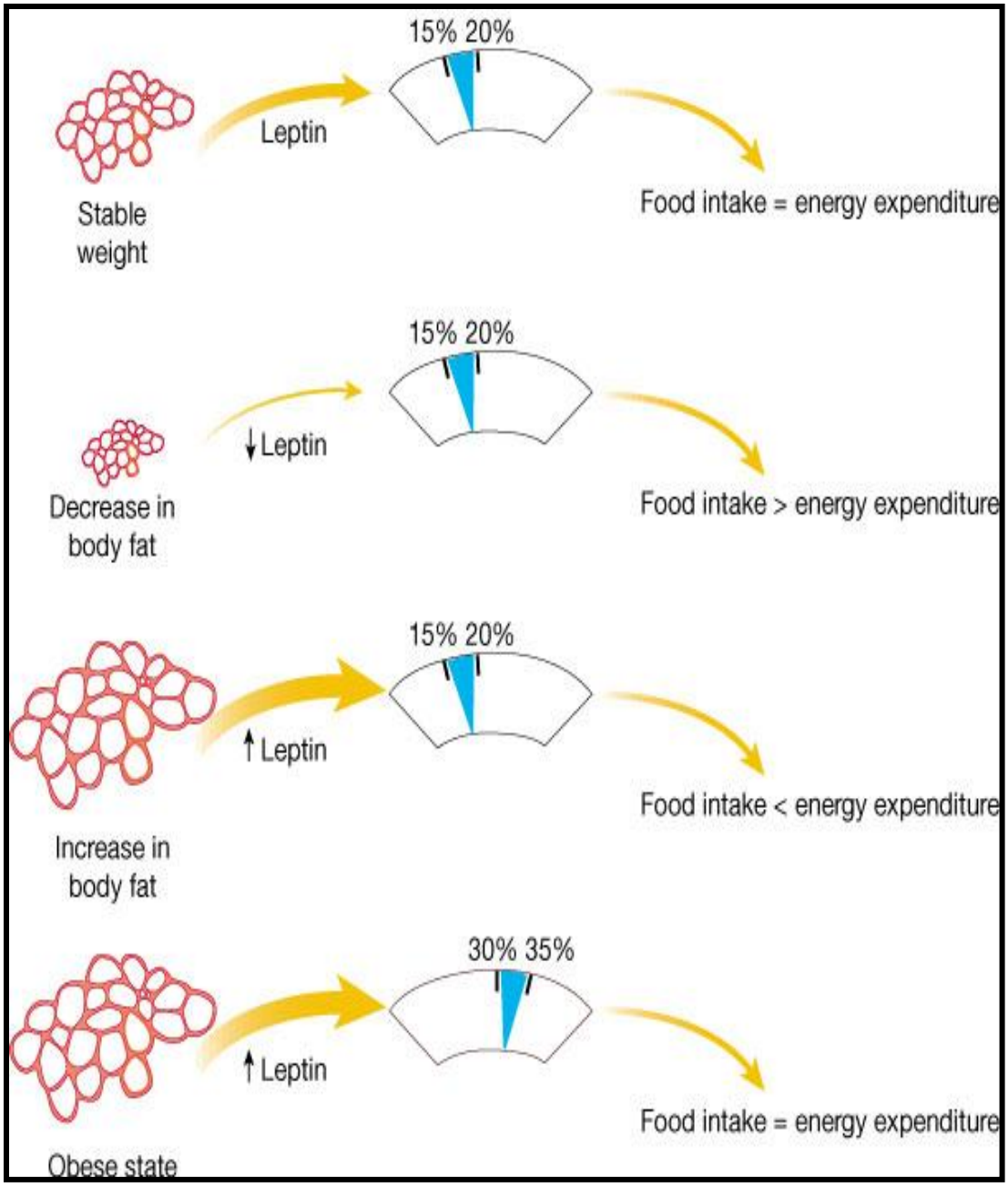

Figure 2.13: Leptin and Body fat

2.6.5 Ghrelin – The Hunger Hormone

Ghrelin is also known as "Hunger hormone" and is 28-aa Ser3 acylated peptide. Growth hormone secretagogue receptor (GHS-R) is its functionally relevant endogenous receptor.

Ghrelin being an orexigenic peptide is a key regulator for meal initiation, appetite and nutrient sensing (Sato et al., 2012; Kirchner, 2012). Researches in the last decade has shown that apart from its orexigenic effect, Ghrelin has regulatory roles in many organs and systems like obesity, Insulin resistance and diabetes (Choi et al., 2013; Varela et al., 2011; Perez-Tilve, 2011; Andrews et al., 2010; Sun, 2006; Lin, et al., 2011). However, the most recent findings about the new regulatory functions of Ghrelin are in glucose homeostasis, energy – homeostasis (Zakhari et al., 2012) heart disease (Cao. 2013), muscular atrophy (Sugiyama, 2012), bone metabolism (van der Velde, 2012) and cancer progression and development (Majchrzak, 2012).

In obesity, body adopts a compensatory mechanism towards prolonged positive energy balance by lowering down Ghrelin levels as it acts on indication of energy insufficiency. Emilia Korek et al. (2013) demonstrated that obesity was associated with lowered basal plasma Ghrelin level. Findings of previously published studies which demonstrated that fasting plasma Ghrelin levels are negatively correlated with body fat percentage and body weight supports the results of Emilia Korek et al. (2013) (Tschop, 2001).

2.6.6 Insulin

Insulin levels are lowest in anorexic individuals and highest in obese individuals, reflecting the nutritional status of people. In study conducted by Emilia Korek et al. (2013), results depicted that Insulin levels rose after a meal in all three groups [obese, normal-weight and anorexic] and highest rise (4-fold increase in Insulin levels) was observed in obese subjects leading to hyperinsulinaemia. Though statistically correlations between basal Insulin and Ghrelin did not differ in study group, the author noted lowest level of Ghrelin in subjects with hyperinsulinaemia and on the contrary highest Ghrelin was found in anorexic patients (Emilia Korek et al., 2013; Flanagan et al., 2003). Insulin and Ghrelin share a two-way street of regulation mechanism. Ghrelin being a potent regulator of Insulin level can reduce Insulin secretion that is glucose induced; on the contrary, ghrelin release is inhibited by high Insulin levels (Emilia Korek et al., 2013; Tong et al., 2010).

In addition, strong positive correlation was also observed between fasting Leptin and fasting Insulin concentration. It is clearly evident that hyperinsulinaemia and Insulin resistance as observed in obese subjects are directly associated with high fasting Leptin levels (Emilia Korek et al., 2013; Friedman, 2004). Hence, in state of obesity hyperinsulinaemia and Insulin resistance are positively correlated with fasting Leptin and Insulin levels (Emilia Korek et al., 2013).

2.7 Gut microflora and obesity – An Inner rain forest

There are several tenable factors that play role in development of obesity but role of gut microflora plays a very important role. People these days can now be identified based on the structure of individuals gut microbiota using microbiota "fingerprints" with help of metagenomics approach (Dahiya. & Puniya, 2017; Franzosa et al., 2015).

Disequilibrium in energy homeostasis being the prime reason of obesity could be induced by dysbiosis in gut microbiota. In experimental model there are numerous mechanisms by which gut microflora induces obesity. However, specific microbial community that is directly linked to obesity is unknown due to its complex nature (Dahiya & Puniya, 2017). Recent studies provide evidence for the paramount role of gut microflora present in the human GI tract in the onset and establishment of obesity. The nutrient acquisition and energy homeostasis gets affected by the adhered gut microflora and influences the number of effectors molecules that decide fat storage in adipocytes (Dahiya & Puniya, 2017; Rosenbaum et al., 2015).

The gut harbors a complex microbial community comprising of trillion microbes and approximately 1000-1100 different bacterial species representing altogether 10^{14} – 10^{15} microbes and resembles a "world within a world" (Qin et al., 2010). Previously, there are studies in past that have established that gut of an adult human being is mainly inhabited by bacteria from three major divisions like, Gram-positive *Firmicutes*, Gram-negative *Bacteroidetes* and Gram-positive *Actinobacteria* and they all together mark up more than 90% of total bacteria present in gut.

2.7.1 Evidence of dysbiosis in obesity

Since, microbiota has a crucial role in obesity then probably the phenotype of obese individuals should have a distinct microbial composition than lean individuals (Ley et al., 2005). During the study conducted on ob/ob mice, lean ob/C and wild-type counterparts analyzing differences in their gut microflora Ley et al. (2006) found that genetically obese mice had less of *Bacteroidetes* and more of *Firmicutes* as compared to lean mice. Turnbaugh et al. (2006) when transplanted obese mictobiota to germ free mice, the obese phenotype was transferred and was observed that *Firmicutes* helped to draw more calories form the ingested diet leading to obesity (Dahiya & Puniya, 2017; Turnbaugh et al., 2006). Ley et al. (2006) also observed similar findings in his study where *Bacteroidetes* were less and *Firmicutes* were more. On initiation of a low calorie diet the proportions reversed and increase in short chain fatty acids (SCFAs) was observed (Riva et al., 2017). Overall, the microbial diversity is reduced in obese phenotype in comparison to lean ones and to improve microbial richness dietary interventions are recommended (Dahiya & Puniya, 2017; Le Chatelier et al., 2013; Cotillard et al., 2013).

In a study conducted by Armougom et al. (2009) and Million et al. (2012) revealed that alterations in microbial population of lactobacilli also occurred at genus and species level, but these results were not consistent. In some studies higher lactobacilli counts correlated positively with pro-obesity effects in obese subjects (Armougom et al., 2009; Million et al., 2012). On the contrary, there are several studies that have documented anti-obesity effects of lactobacilli discussed in a review article by Arora et al. (2013). This conflict was resolved in a meta-analysis study which depicted that activity of lactobacilli and their anti-obesity properties are species-specific and cannot be attributed to whole genera (Million et al., 2012). Similarly, Yin et al. (2010) and An et al. (2011) also depicted that *Bifidobacteria* is negatively correlated with obesity (Yin et al., 2010; An et al., 2011).

The role of gut microbiota in obesity was further proved by antibiotic experiments in mice. Interesting evidence from these antibiotic experiments on body weight depicted

that early exposure to antibiotics results in dysbiosis leading to increased fat mass. Altered gut microbiota also predisposed mice towards adiposity by negatively modulating hepatic metabolism and its associated hormones (Dahiya & Puniya, 2017; Cox et al., 2014; Cho et al., 2012).

Recent studies have also proved progression of several diseases in human beings such as obesity, diabetes, NAFLAD, certain form of cancers and even anxiety and depression due to dysbiosis of gut microflora (Leung et al., 2016; Perez-Chanona & Trinchieri, 2016; Luna & Foster, 2015)

2.7.2 Gut Microbiota and Energy Harvesting

Energy in our body is generated by process of glycolysis in form of ATP (adenosine triphosphate) from influx of simple sugars fermented from complex oligosaccahride. More ATPs are generated by further hydrolysis of these biological molecules. Acetate, propionate and butyrate are the most important end product SCFAs of gut-situated microbial species (Koh et al., 2016). The energy utilized by the host colonic epithelial cells (10%) and for cellular respiration (70%) is obtained from SCFAs. Colonic epithelial cells preferably utilize butyrate as source of energy (Kasubuchi et al., 2015). Extra fat starts getting deposited in body when energy for SFCAs is persistently acquired. Studies on animal models and also human subjects have demonstrated reduced butyrate level in the female matter. However, as fiber composition greatly varies in a human diet, it significantly alters the SCFA production (Dahiya & Puniya, 2017; Koh et al., 2016; Kasubuchi et al., 2015; Louis & Flint., 2009).

Since, the end product of bacterial fermentation of nutrients produces SCFA, in addition to their role as a substrate in energy metabolism; SCFAs also reduces the pH of the gut. The production of butyrate is reduced when pH increased from 5.5 to 6.5 and leading to rise in the population of propionate producers and simultaneously flourishing the predominance of *Firmicutes*. From these findings it can be suggested that one microbial group outclasses another group/species at a specific luminal pH. However, exact nature

and mechanisms are yet to be established (Dahiya & Puniya, 2017; Duncan et al., 2009) (Figure 2.14).

2.7.3 Gut Microbiota and Satiety

SCFAs also function as ligands for some receptors. These receptors known as Free Fatty Acid Receptor -FFAR3 (earlier they were known as G-protein coupled receptors - GPR41) and FFAR2 (earlier known as GPR43) are important targets for SCFA ligands. Presence of these receptors indicates that SCFAs influence satiety and host metabolism via modulation of intestinal enteroendocrine L cells derived peptides, prominently GLP-1 and PYY (De Silva & Bloom 2012; Brown et al., 2003). From these findings it could be postulated that SCFAs produced from microbial fermentation of dietary oligosaccharide have direct influence on L cells resulting in rise of intestinal and plasma GLP-1 levels. There are several studies in animal and human studies that demonstrate the upregulation of GLP-1 and PYY through microbial fermentation of indigestible oligosaccharide and production of SCFAs (Tolhurst et al., 2012; Tarini & Wolever, 2010; Zhou et al, 2008).

In addition, one more way in which gut flora modulates energy balance is through SCFAs and its effect on Leptin secretion from adipocytes through GPR41/43 dependent process and acting as a significant messenger amidst gut microbiota and host metabolism (Zaibi et al., 2010) (Figure 2.14).

2.7.4 Gut Microbiota and Bile acids

The primary function of bile acids is to facilitate digestion and absorption of fats in small intestine and helps in removal of lipids and toxic metabolites. However, the feedback inhibition process done through underlying molecular mechanism of bile acid is unclear. Still, important role of a nuclear receptor farnesoid X receprot (FXR) is mentioned in regulation of bile acid. There are two key genes namely, cholesterol 7a-hydroxylase (CYP7A1) and CYP27A1 and their expression is negatively regulated by FXR where CYP7A1 initiates classic pathways of bile synthesis and CYP27A1 manages alternative pathways. Yet there is one more mechanism of activation of G-protein coupled bile acid receptor 1 (GPBAR1 / TGR5) by which energy metabolism is regulated. GPBAR1 is activated by interaction with secondary bile acids which in turn activates secretion of GLP-1. Thus FXR and GPBAR1/TGR5 signaling is influenced by modulation in bile acid metabolism via gut microbiota and indirectly contributes to development of obesity (Aron-Wisnewsky et al., 2013) (Figure 2.14).

Hence, it can be postulated that the population of gram-negative bacteria including important pathogens are favored when levels of bile acids in gut decrease and conversely, gram-positive *Firmicutes* are promoted when bile acid increases especially those bacteria that convert the primary bile acids to toxic secondary bile acids by 7a-dehydroxylation (Ridlon et al., 2014).

2.7.5 Gut Microbiota and Lipogenesis

When dietary oligosaccharide are fermented by microbes in gut there is increased influx of monosaccharide's to the liver, leading to activation of lipogenic enzymes by ChREBP and perhaps SREBP-1. In this process liver tackles this flooding influx in two ways: Firstly, by initiating the inefficient futile cycles of metabolism secondly, storing surplus calories as fat in peripheral tissues (Backhed et al., 2004) and thirdly, initiating de novo lipogenesis and triglyceride (TG) synthesis in HepG2 cells by production of t10, c12 and conjugated linoleic acid isomer (CLA) induced by gut microbiota (Dahiya & Puniya, 2017; Go et al., 2013) (Figure 2.14).

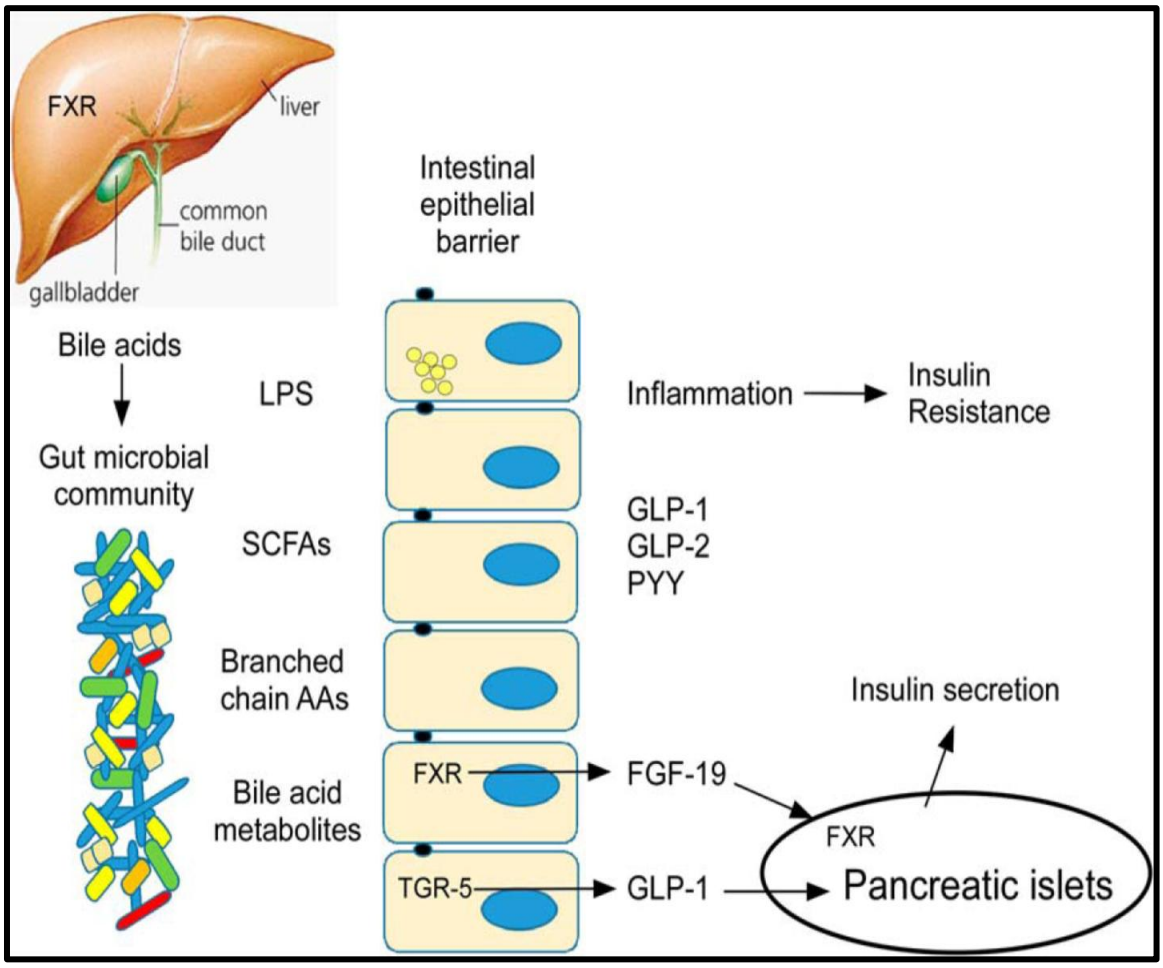

Figure 2.14: Mechanisms Linking the Gut Microbiome, Bile acids, Glucose Metabolism and Satiety hormones (Source Kristina M. Utzschneider et al., 2016)

Schematic of potential mechanisms linking the gut microbial community with glucose metabolism. Potential mechanisms include 1) systemic absorption of LPS, either through "leaky" tight junctions or via chylomicron uptake, with subsequent inflammation; 2) bacterial production of SCFAs with signaling effects to stimulate secretion of gut hormones GLP-1 and GLP-2 and PYY as well as nutrient effects; 3) bacterial synthesis and absorption of branched chain amino acids (AAs) that may result in insulin resistance; 4) bacterial metabolism of bile acids with local and organ-specific signaling effects, including stimulation of fibroblast growth factor-19 (FGF-19) and GLP-1. FGF-19 has metabolic effects on the FXR in the pancreatic β-cell and in the liver.

2.7.6 Gut Microbiota and Metabolic Endotoxemia

As it is evident from previous literature that obesity is associated with the activation of TNF-a, IL-1, IL-6 which are low grade inflammatory signaling molecules form adipose tissue that disrupt normal metabolic processes and mediate Insulin resistance (Ouchi et al., 2011). Cani (2007) was first to propose that lipopolysaccharide (LPS) was responsible or early onset of inflammation, Insulin resistance obesity and diabetes. They also demonstrated that high fat diet group had elevated LPS levels and were associated with a decrease abundance of *Bacteroides* and *Bifidobacterium* species and increased *Clostridium* species (Cani et al., 2007). Also, dramatic reduction in population of *Lactobacillus* spp., *Bifidobacterium* spp., and *Bacteroides* spp., was observed in high fat diet group (Cani et al., 2013; Wang et al., 2006) (Figure 2.15)

2.7.7 Gut Microbiota and Innate Immunity

A group of proteins known as Tol-like receptors (TLRs) play an important role in the innate immunity. They generally recognize structurally conserved motifs of microbes called pathogen-associated molecular patterns (PAMSPS) (Medzhitov, 2001). It is clearly evident that interaction between gut microbiota that we harbor and epithelium TLRs is very important to maintain the immune homeostasis (Peterson et al., 2015). TLR-5 is predominantly expressed in the intestinal mucosa and prevents bacterial flagellin from invading. TLR-5 is also postulated to play a role in adiposity progression and its associated metabolic syndrome as increased adiposity is strongly associated with secretion of proinflammatory cytokines IL-1b and INF-g. In a study on TLR-5 deficient mice, the authors found that these TLR5KO mice exhibited hyperphagia, hyperlipidemia, hypertension, hypercholesterolemia, Insulin resistance and enhanced fat deposition as compared to normal mice (Kumar et al., 2010) (Figure 2.15).

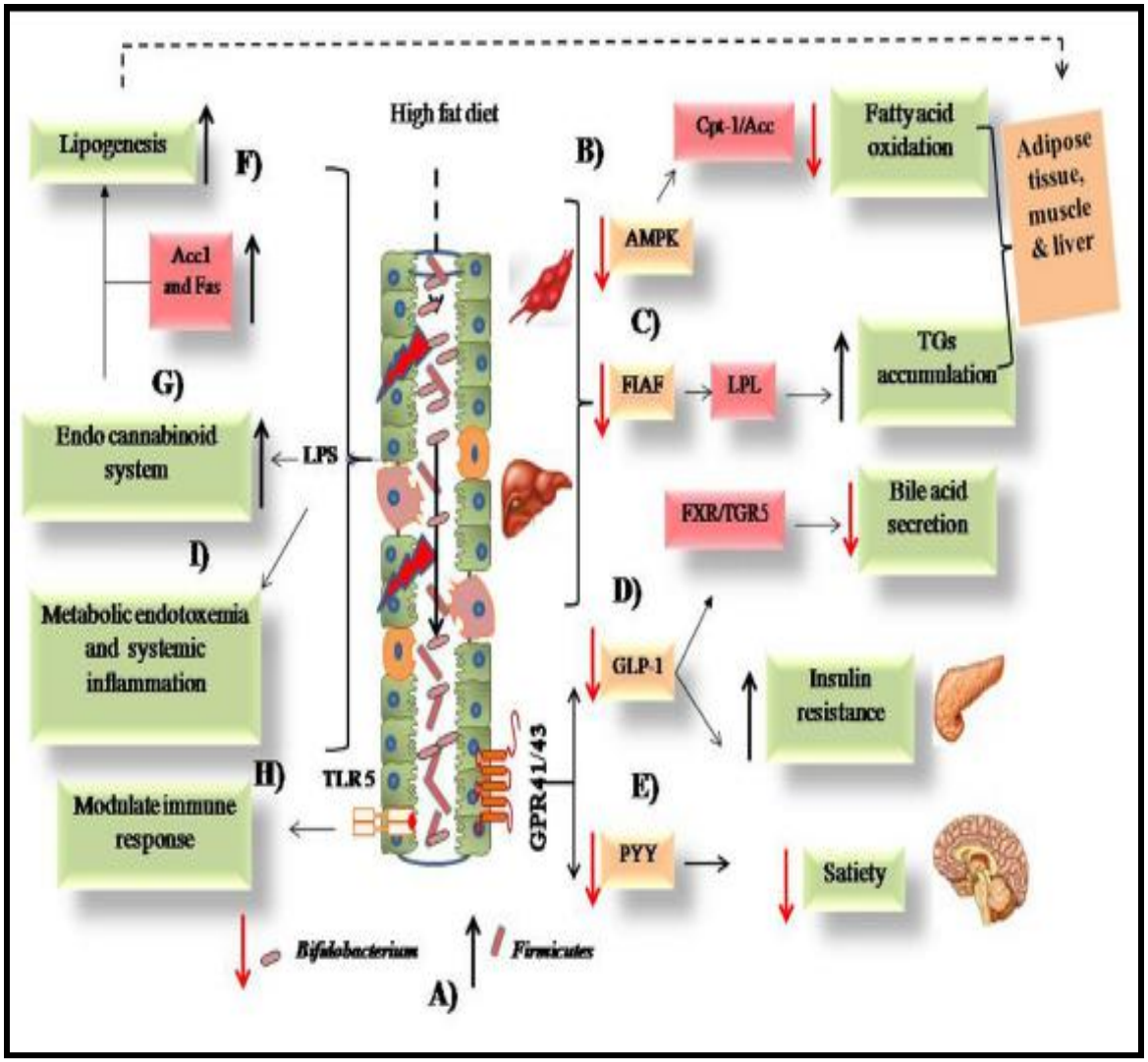

Figure 2.15: All Possible Mechanisms Regulating Gut Microbiota and Obesity Considering Central Role of Dysbiosis

(Source: Dahiya et al., 2017)

(A) High fat diet causes alteration in intestinal microbiota from low to high Firmicutes and high to low Bifidobacterium. (B) Low expression of AMPK leads to decreased fatty acid oxidation. (C) FIAF expression causes activation of LPL that leads to TGs accumulation. (D) Low GLP-1 leads to increased insulin resistance and decreased bile acid secretion from liver. (E) Decreased PYY causes low satiety in obese host. (F) Increased lipogenesis via upregulated Acc1 and Fas enzymes. (G) Activation of endo cannabinoid loop via release of LPS due to damages intestinal epithelium. (H) Modulation of intestinal immune response via TLR-5 downstream signaling. (I) Systemic inflammation caused by inflammatory cytokines and bacterial LPS

2.8 Factors influencing Gut Microbiota and Health

There are several factors that alter the composition of gut microbiota and studies in animal models have demonstrated including genetics; the mode of delivery at birth; the method of infant feeding; the use of medications, especially antibiotics; and the diet. (Li Wen & Andrew Duffy, 2017).

During childhood the composition of gut varies widely during the first few years of life depending on gut physiology, introduction of solid foods, therapeutic drugs and host genotype (Koenig et al., 2011).

Since decades, there has been use of antibiotics in promoting growth and body weight of animals in livestock sector. This surely indicates that weight is modulated indirectly by role of gut microflora and in addition also leads to increased fat mass and is negatively associated with hepatic metabolism and associated hormones (Cox et al., 2014; Cho et al., 2012). Recently in last few decades there is drastic increase in use of antibiotics in animal feeds. In an experiment with mice conducted by Cox et al. (2014) depicted that when antibiotic was administered early in mice, it altered their gut microbiota, increased fat deposition and negatively modulated hepatic and hormonal metabolism. Alterations in gut microbiota, due to intake of certain antibiotics can increase the risk of infections with opportunistic pathogens such as *Clostridium difficile* (Suhrawardy, 2014; Backhed et al., 2012). Researchers have now started seriously reviewing early administration of antibiotics on human adiposity (Podolsky, 2017; Turta & Rautava, 2016; Mueller et al., 2014).

2.9 Role of Prebiotics and its Interventional Studies in Obesity

The concept of prebiotic came into existence before 2 decades. The original definition of prebiotic was given by Glenn Gibson & Marcel Roberfroid in 1995. They defined a prebiotic as :

"A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health"

(Glenn Gibson & Marcel Roberfroid, 1995)

Since it's well known that all prebiotics are dietary fibers, but not all fibers can be classified as prebiotics. For any food ingredient to qualify as a prebiotic, the scientific prerequisites for that ingredient as specified by Gibson and Roberfroid, 1995 are:

- Food ingredient should resist gastric acidity, hydrolysis by mammalian enzymes and absorption in the upper gastrointestinal tract;
- Is fermented by the intestinal microflora;
- Selectively stimulates the growth and or activity of intestinal bacteria potentially associated with health and well-being.

(Slavin, 2013; Gibson & Roberfroid, 1995)

Recently, with the aim to clarify the prebiotic concept, Bindels et al., 2015, proposed to define prebiotic as:

"A non-digestible compound that, through its metabolism by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host"

(Bindels et al, 2015)

1] Selective stimulation of the growth and/or activity of intestinal bacteria associated with health, mainly *Lactobacilli* and *Bifidobacteria* (Carlos Gomez Fallego & Seppo Salminen, 2015)

2] Production of short chain fatty acids (SCFAs), particularly butyrate, which have antimicrobial activity by reduction of intestinal pH and other immunological and physiological activities (Bindels et.al. 2015).

The usual target genera for prebiotics are *Lactobacilli* and *Bifidobacteria*. As *Bifidobacteria* usually resides in the human colon than *Lactobacilli* exhibiting a preference for oligosaccharides, changes in their proliferation is more likely to be seen as compared to *Lactobacilli* (Slavin, 2013). Prebiotic fibers improve gut barrier function and immunity of host by potentially reducing subpopulations of pathogenic bacteria like clostridia (Slavin, 2013).

Fermentation of dietary components lead to an increase in bacterial mass and consequently fecal mass, as a result produces stool bulking effect. Approximately 30g of bacteria are produced for every 100g of fermented carbohydrate (Slavin, 2013).

Natural sources of prebiotics include chicory, leeks, asparagus, Jerusalem artichokes, garlic, onions, wheat, oats and soybeans. Non-digestible carbohydrates and oligosaccharides have estimated caloric value of 1-2 kcal/g. Prebiotic Inulin possesses low digestible properties and is associated with impaired gastrointestinal tolerance, especially when consumed in large quantities. However, other prebiotic fibers like wheat dextrin, polydextrose exhibit high gastrointestinal tolerability up to 30 – 45g/day (Gibson et al., 2010; Pasman et al., 2006)

Fructooligosaccharides, galactooligosaccharides, lactulose, and non-digestible carbohydrates Inulin, cellulose, resistant starches, hemicelluloses, gums, and pectins are the most commonly used prebiotics in practice because they fulfill the criterion as suggested by Gibson et al. (2004).

Consumption of dietary fibers alter high body weight and obesity by SCFA –mediated physiological effect influencing satiety in addition to their satiating abilities and

formation of fat-fibre complex (Slavin, 2013). Nevertheless, reduction in body weight, body fat and adipocyte size is brought about by microbiota modulation coupled with SCFA-mediated satiety effect (Gerard P, 2016)

In human interventional studies prebiotic fiber have been reported to be involved in appetite regulation by modulating gut peptides, induce satiety and increase breath-hydrogen excretion. It also prompted the growth of *Bifidobacteria* and *Lactobacilli.* However, it was unknown whether these prebiotic fibers stimulated growth of whole *Bifidobacteria* genus or a particular species (Dahiya & Puniya, 2017; Parnell & Reimer, 2009; Cani et al., 2009, 2006; Gibson et al., 2004).

In one of the study of 17 human volunteers supplemented with oligofructose led to significant increase in *B.longum* and *B.adolescentis* species (Joossens et al., 2011). In similar kind of study in obese women demonstrated increase in population of *Bifidobacterium, F.prausnitzii* and *Lactobacilli*. Correlation analysis speculated that serum LPS levels negatively correlated with *Bifidobacterium* and *F. prausnitzii*. Also positive correlation with changes in body composition and glucose homeostasis was demonstrated by *B. intestinalis* and *B. vulgates* (Dewulf et al, 2012).

Investigators also tried to establish correlation between metabolic markers and *Bifidobacterium* species and SCFAs. Results of Salazar et al. (2015) also depicted increase in colonies of *B. longum* and *B. adolescentis* and they negatively correlated with serum cholesterols. Surprisingly, *B. longum* negatively correlated with serum LPS (Salazar et al., 2015). Authors , concluded that prebiotics helps in reducing weight by inducing satiety via L cells in gut and reducing production of LPS by modulating gut microbiota (Dahiya et al., 2017).

Supplementation study conducted by Abrams et al. (2007) using Inulin –type fructans prebiotic (8g/day) for one year demonstrated significant benefit in the maintenance of BMI and fat mass in non-obese young adolescents (Abrams et al., 2007).

In a randomized clinical trial on FOS supplementation in healthy obese adults of 21g/d for 3 months in experimental group demonstrated decreased food intake, body weight gain (1.03 ±0.43 kg, $p<0.01$) and fat mass development in obese subjects of experimental group, making evident weight reduction properties of prebiotics. The investigators also found higher plasma PYY levels following a meal, along with drop in Ghrelin over a 6 – hour meal tolerance test (Parekh et al., 2014; Parnell & Reimer, 2009).

A study on healthy individuals with 16g/d of prebiotic supplementation for two weeks depicted modulation of gut peptides with increase in GLP-1, PYY and GIP resulting in an effect that correlated with a reduction in glycemic response and reduced energy intake (Cani, 2009). This study further postulated the possibilities of improvement in obesity outcomes by prebiotic supplementation through modulation of endocrine function of gut and proliferation of number of L cells in jejunum and colon (Cani &Delzenne, 2011).

Simultaneously, the over-secretion of GLP-2 (co-secreted with GLP-1) is impeached in the lower systemic inflammation i.e. decrease in circulating LPS and proinflammatory cytokines in obese mice. Prebiotic supplementation in animal model also revealed decrease in LPS absorption through an improvement of the e gut barrier function. The most valuable findings depicted that modulation of gut microbiota with prebiotic supplementation promotes the normalization of endocannabinoid system (eCB) responsiveness in gut, resulting in reduced gut permeability and metabolic endotoxemia and development of fat mass. However, these effects are yet to be unravelled in human trials (Delzenne et al., 2011; Muccioli et al., 2010; Cani & Delzenne, 2009; Cani & Lecourt, 2009; Cani & Neyrinck, 2007). Since, several studies demonstrate involvement of gut microbiota in development of a low-grade inflammation, the "anti-inflammatory" effect of prebiotics seems particularly interesting (de LG, de La.& Raybould, 2011; Delzenne et al., 2011).

Moving further, Everard and colleagues formulated study on obese and diabetic mice investigating the effect of prebiotic administration. Study results demonstrated decrease in the *Firmicutes* and propotional increase in the *Bacteroides* populations. Moreover,

prebiotic supplementation improved glucose tolerance, reduced adiposity and low-grade inflammation (Everard et al., 2011).

A meta-analyiss conducted da Silva and colleagues with 61 original articles described the relationship between the microbiota and obesity and the possible impact of prebiotic and prebiotic exploring the fact that the main effect of associated weight loss was related to an increase in *Bifidobacteria* (da silva et al., 2013).

Bomhof et al. (2014) designed a study to assess the efficacy of supplementing prebiotic oligofructose (OFS) and probiotic BB-12 and their independent and combined effects in obese rats. Study results revealed prebiotic significant weight loss via reducing fat mass and reduced energy intake, elevated secretion of portal GLP-1 and amplified colonization of *Bifidobacteria* and *Lactobaclli* in OFS group rats ($p<0.05$). However, secretion of GLP-2 was stimulated with probiotic BB-12 ($p<0.05$). BB-12 alone failed to demonstrate bifidogenic properties (Bomhof et al., 2014).

Since prebiotics are soluble dietary fibers, there are few studies that demonstrate their role in relieving constipation. In a recent study conducted by Yu et al. (2017) on determining the effects of prebiotics and synbiotics on functional constipation (FC) in adults. Study comprised of 13 RCTs with participation of 199 patients intervened with prebiotic and 825 patients with symbiotic administration. Results reveal that prebiotics increased weekly frequency of stool (1.01 bowel movements/week, 95% CI: 0.04-1.99) and improved stool consistency (-0.59, 95% CI: -1.16 to -0.02). Analysis of subgroup revealed specific effects for Galacto-oligosaccharides on stool frequency, consistency, ease of defecation and abdominal pain. Synbiotics also significantly improved stool frequency (1.15bowel movements/week, 95% CI: 0.58-1.71), consistency (0.63, 95% CI: 0.33-0.92) and reduced whole-gut transit time (13.52, 95% CI: -26.56 to -0.49) in patients with FC. Detailed analysis revealed effects of Fructooligosaccharide and prebiotic combinations on stool frequency, consistency, straining defecation and bloating. The authors from their findings concluded that Galactooligosaccharides and synbiotics made

up of Fructooligosaccharides with probiotic combinations may improve stool frequency, consistency and some other symptoms related to constipation.

Vandeputte et al. (2017), set up a randomised, doubleblind, placebo-controlled; cross-over trial in chicory-derived Orafti inulin was supplemented to healthy adults with mild constipation with dose of 12 g/day of for a 4-week treatment period. Study results established significant increase in stool frequency resulting from Inulin consumption (Vandeputte et al., 2017; Micka et al., 2017) leading to a first positive opinion by the European Food Safety Authority on chicory Inulin and 'maintenance of normal defecation' (EFSA Panel, 2015)

Moreover the analysis revealed association between Bilophila and Patient Assessment of Constipation Quality of Life (PACQoL) - assessed physical discomfort and treatment satisfaction scores ($r=0.20$, q-value <0.05 and $r=-0.30$, q-value <0.01). According to authors they claimed this as a first report demonstrating shift in abundance of a bacterial genus being associated with both softer stools and a favorable change in constipation specific quality-of-life measures following a dietary intervention with Inulin.

2.10 Fructooligosaccharide (FOS) as a potential prebiotic

A potential prebiotic candidate that can enhance satiety along with retaining positive organoleptice and bifidogenic properties which can foster incorporation into various recipes is Fructooligofructose (FOS) (Boulangé et al., 2016; Van Hoffen et al., 2008; Cani et.al., 2006; Daddaoua, 2006; Delzenne, et al., 2001). Obesity primarily being a state of energy imbalance, it becomes sort of mandatory to develop "designer novel foods" that can dilute the energy density of foods without compromising on the sensory and organoleptic attributes and in addition promotes satiety. Eventually, these "designer foods" would provide first line of defense in maintaining energy homeostasis (Valéria Maria Caselato de Sousa et al., 2011; Amar et al., 2008).

Modern consumers are progressively interested in their personal health and expect the foods to be safe and healthy - beyond tasty and attractive. Non-digestible carbohydrates such as dietary fibers, oligosaccharides, and resistant starch have various physiologic functions, and health implications on well-being, better health, and reduction of the risk of diseases. FOS presents important physicochemical and physiological properties beneficial to the health of consumers leading to their extensive use as food ingredients and presenting a significant growth on the functional food market all over the world. Europe nutraceuticals market is expected to have a share of over 20% until 2017. In view of the great demand for FOS as food ingredients, the opportunity exists for the screening and identification of novel strains capable of producing enzymes with transfructosylation activity and for developing improved and less expensive production methods (Dominguez et al., 2014).

Most of the studies on prebiotics have been focused on Fructans, such as Inulin-type fructans, Fructooligosaccharides (FOS) Galactooligosaccharides (GOS) (Gomez Gallego and Salminen, 2016) and Lactulose (Ait-Aissa & Aider, 2014). The location where they seems to exert their activity depend of their degree of polymerization (DP), acting in proximal areas of the colon those with a low DP, and in distal areas those with a high DP (Allsopp, 2013). Several new sources of prebiotics and plants with high Fructooligosaccharide content are under investigation (Gomez Gallego & Salminen, 2016).

Fructooligosaccharides (FOS) are isolated from plants belong to the group of oligosaccharides. They are joined by α-glycosidic bonds (1---2) between terminal fructose and glucose and consist of three to ten monosaccharide units (Valéria Maria Caselato de Sousa et al., 2011). FOS and Inulin molecules are defined using the degree of polymerization (DP), consisting of the number of monosaccharide units, with FOS having a DP<10 and Inulin a DP between 2 – 60 (Borges, 2001; Spiegel et al., 1994).

Flamm et al. (2001) have evaluated the caloric value of FOS and found that the energy yield for the host would be in the range of 1.5 kcal/g to 2.0 kcal/g. By using another

method based on lipogenesis balance, Roberfroid (1993) stated that the caloric value of FOS is around 1.0 kcal/g to1.5 kcal/g (Flamm et al., 2001; Roberfroid 1993). 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 diabetic subjects (Roberfroid, 1999).

FOS is considered as an ingredient and not additive as per law. FOS has a status of GRAS (Generally Recognized as Safe) in the United States. Ingestion of FOS may cause flatulence, but the severity of this symptom is associated with the amount of FOS consumed: the higher the quantity, the greater the symptom (Hauly & Moscatto, 2002). Consumption of FOS up to 20g is regarded as safe according to FAO-WHO, 2001 (FAO, WHO, 2001).

FOS is naturally present in some foods with onion being the food with the highest levels of FOS. Other natural sources include bananas, garlic, onion, tomato, wheat, asparagus, artichoke, leek, honey, rye, brown sugar, barley, triticale, beer, lettuce, chicory, burdock, beetroot, apples and oat (Valéria Maria Caselato de Sousa et al., 2011).

Prebiotics in food applications

According to Saxelin et al. (2003) for food application purpose prebiotics are classified as

> **"Food products that contain prebiotic ingredients in sufficient concentration, so that after their ingestion, the postulated benefit is obtained"**
>
> **(Saxelin et al., 2003)**

The potential interest for using prebiotic in daily foods is mainly due to its low calorie value, bifidogenic properties, dietary fibre effects and hypocariogenic effect (Roberfroid, 1993). Prebiotics can be easily added to liquid based dairy products such as yoghurts and

its drinks, spreads, fresh cheeses, and milk, and other emerging food products such as sport products, functional waters, nutrition bars, weight loss products, soymilk, and mineral supplements (Kaur & Gupta, 2002; Niness, 1999).

Properties of prebiotics like their selective stimulation of the growth of *Bifidobacteria* and the production of SCFA as end products of fermentation has been very well established in many in vitro and in vivo studies (Roberfroid, 2002; Gibson & Wang, 1994). They are increasingly used in functional foods, especially dairy products and breads at typical amounts of 3-8 g per serving to allow the bifidogenic claims (Franck, 2000; Coussement, 1999). Compared to Inulin, other types of non-digestive oligosaccharides such as oligofructose are either branched or composed of several types of glycosidic bonds, which makes them less readily accessible for bacterial hydrolysis (Roberfroid, 1998).

However, both Inulin and oligofructose have been demonstrated to be effective prebiotics (Menne et al., 2000). The other commercially available prebiotic (Murphy, 2001; Cummings et al., 2001) and currently marketed as bifidus factor for infant formula is lactulose. Its ingestion contributes to the growth of gut microflora in bottle-fed babies in the same way as breast-fed babies (Strohmaier, 1998; Salminen et al., 1998). Fibre enhancer, another remarkable functionality of prebiotics in food formulations is their roles as a dietary fibre. The dietary fibre is defined as "remnants of plant cells resistant to hydrolysis by the human digestive enzymes" (Trowel & Burkitt, 1986). As discussed previously, several prebiotic substances such as inulin fall under this definition (Flamm et al., 2001).

Moreover, from a physiologic point of view the effects of these prebiotics on intestinal function, blood lipid parameters, and caloric value meet the properties of dietary fibers (Gibson et al., 1995; Roberfroid, 1993). These effects are related to reduce the risk of coronary heart disease, colon cancer and other colonic disorders. Compared to insoluble fibre such as bran, soluble prebiotic ingredients are more palatable and have greater functional properties (Dreher, 1999). Resistant starch is related to increased fibre content

in baked goods and pasta products without any grainy appearance (Murphy, 2001). Supplementation with Inulin in baked goods allows not only fibre enrichment, but also better moisture retention properties and improved texture (Franck, 2000). Their solubility also allows fibre incorporation in drinks, dairy products, soup and table spread. Such additions are in the range of 3-6 g per serving and increase up to 10 g in extreme cases (Miremadi & Shah, 2012; Coussement, 1999).

FOS as a Sugar replacer

Functional properties of FOS are very much similar to glucose syrup though having minimal contribution to calories and delivering bulking properties. It can effortlessly replace sugar in variety of foods like dairy and bakery products such as chocolate filling biscuits, chewing gums, confectionary and ice-creams (Miremadi & Shah, 2012; Frank, 2000).

In addition, similar to sugar properties oligofructose oppresses the freezing point of frozen desserts and acts as a binder in nutrition bars. FOS is highly soluble with 30% of sweetness as of sucrose and along with addition to other sweeteners such as aspartame, oligofructose can provide a desired sweetness and better flavor profile (Miremadi & Shah, 2012; Kaur & Gupta, 2002). Sugar alcohols contribute fewer calories like sorbitol, mannitol, xylitol, lactitol, non-digestive oligosaccharides such as oligofructose and lactulose ranging 1-2 kcal /g as compared to sugar (4 kcal /g) (Murphy, 2001; Salminen et al., 1998) allowing the development of low-sugar-energy products. This is particularly useful in development of sugar-free confections such as hard candies, chewing gums and marshmallows, sugar-free added baked goods and ice-creams. More importantly, these low-calorie ingredients offer advantages over traditional digestible carbohydrates especially for obese, diabetic and cardiac patients (Miremadi & Shah, 2012; Schumann, 2002; Hidaka et al., 1986).

FOS as Fat replacer

The pre-requisites for any prebiotic to act as a fat replacer and texture modifier would be to (1) reduce total fat or partial fat content, (2) modify smoothness and creaminess, (3) increase perception of body and richness, (4) improve an overall eating quality and an acceptable appearance.

Inulin is well-recognized prebiotic for its ability to replace fat in the manufacturing of low-calorie foods (Miremadi & Shah, 2012). Inulin forms gel composed of tridimensional gel network of insoluble sub-micron crystalline inulin particles with large amounts of immobilized water when mixed with water. This inulin gel provides the same texture and mouth feel as fat (Miremadi & Shah, 2012; Franck, 2000; Silva, 1996). The chain length of Inulin plays a key role in gel quality. A high DP Inulin facilitates gel formation at lower concentrations and can be formulated to replace fat up to 100%.

Fat replacement by Inulin is successfully applied in most water-based foods such as dairy products, frozen desserts, dressings, table spreads, sauces, soups and even meat products, but not in dry foods such as snacks, bakery and confectionery products (Murphy, 2001). Typically, 1 g of fat can be replaced by a 0.35 g of Inulin in most foods (Miremadi & Shah, 2012; Coussement, 1999). Formulating foods with Inulin also helps to maximize freeze-thaw stability and minimize emulsion separation phased due to its ability to immobilize water and to work with most gelling agents such gelatin, gellan gum, and maltodextrin (Bishay, 1998).

Inulin also gives a richer texture to liquid products and spreads and provides crispiness and expansion to extruded snacks and cereals. In addition to Inulin, resistant starch is also used as a fat mimetic and a texture enhancer in low-moisture foods e.g. crackers and cookie. In extruded cereals, the use of resistant starch improves crispness and expansion (Miremadi & Shah, 2012; Murphy, 2001).

FOS as Fibre enhancer

Another remarkable functionality of prebiotics in food formulations is their roles as a dietary fibre. The dietary fibre is defined as "remnants of plant cells resistant to hydrolysis by the human digestive enzymes" (Miremadi & Shah, 2012; Trowel & Burkitt, 1986). As discussed previously, several prebiotic substances such as inulin fall under this definition (Flamm et al., 2001).

Moreover, from a physiologic point of view the effects of these prebiotics on intestinal function, blood lipid parameters, and caloric value meet the properties of dietary fibers (Roberfroid, 1993; Gibson et al., 1995). These effects are related to reduce the risk of coronary heart disease, colon cancer and other colonic disorders. Compared to insoluble fibre such as bran, soluble prebiotic ingredients are more palatable and have greater functional properties (Dreher, 1999).

Resistant starch is related to increased fibre content in baked goods and pasta products without any grainy appearance (Murphy, 2001). Supplementation with Inulin in baked goods allows not only fibre enrichment, but also better moisture retention properties and improved texture (Franck, 2000). Their solubility also allows fibre incorporation in drinks, dairy products, soup and table spread. Such additions are in the range of 3-6 g per serving and increase up to 10 g in extreme cases (Miremadi & Shah, 2012; Coussement, 1999).

FOS Acceptability Trials

Recently, the use of bioactive compound in the matrix of functional food is the global consumer trend (Verbeke, 2005; Mark-Herbert, 2004). Amongst various types of bioactive compounds, some of them are antioxidants, dietary fibre, omega-3 fatty acids, plant sterols, probiotic and prebiotic (Health Canada, 2010). Currently, the new area emerging in the management of obesity concentrates on use of Prebiotics as bioactive compounds. Fortification of foods and beverages with novel functional ingredients like Prebiotic Fructooligosaccharide is a recent development in this direction. The market for

functional foods in global market is thriving as consumers are more inclined towards consuming foods and beverages containing prebiotic (Stanton, 2005).

Various FOS based products like beverage concentrates, spreads and honey have been studied successfully and their processes have been patented (Renuka, 2009; Ramesh, 2004). FOS added soups and beverages namely butter milk, lemon juice, milk and tomato soup have also been studied and were highly acceptable at 7.5% level of addition (Gupta. & Sheth, 2011).

Feasibility of FOS substitution in Indian recipes commonly consumed in Gujarat region were attempted and successful substitution was achieved in *Dhokla and Patra* at highest level of 10 g, whereas *Thepla* was acceptable at 5 – 8 g and C*hapati* was least accepted (Mahendra & Sheth, 2013).

A number of studies have looked at the rheological properties of dough prepared with FOS or Inulin (Morris Cecile, 2012; Peressini, 2009; Mirsaeedghazi, 2008; Karolini-Skaradzinska, 2007). Studies on humectants properties of FOS revealed that it has solubility of 80% in water at room temperature, sweetness of about 35% in comparison with sucrose (Franck, 2008; Crittenden, 1996). FOS is very hygroscopic in nature and with the increase in the degree of polymerization, the water holding capacity of sugars also increase (Prapulla, 2000).

Reduction in the WAP (%) has been observed in several studies with prebiotic dietary fibers (Mahendra. & Sheth, 2013; Morris Cecil, 2012; Hager et al., 2011; Parnami & Sheth, 2010; Wang et al., 2002). In a recent study there was a reduction in WAP (%) by 32% in the dough of *Chapatti* and *Thepla* and for steamed products like *Dhokla* and *Patra* it reduced by 30 % and 18.8% respectively (Mahendra. & Sheth, 2013). Other studies also revealed reduction in WAP (%) with the increase in addition of up to 7.5 % of Inulin. Reduction in WAP (%) was more pronounced for the shorter chain Inulin which was explained by a lubricating effect of the sugars and oligosaccharides present in

Inulin (Mahendra. & Sheth, 2013; Morris Cecil, 2012; Hager et al., 2011; Parnami & Sheth, 2010; Wang et al., 2002).

Increase in bulk density of FOS added products have been observed in many studies. This could be attributed to FOS being a soluble fibre and having high solubility of 80 % at room temperature, which helps in retaining moisture and reducing moisture loss. Incorporation of FOS is an ideal ingredient for increasing bulk properties of the product and requires only minor adaptation of the production process, if any (Guggisberg, 2011; Wang et al., 2002; Crittenden, 1996)

Burn spots, which could be related to non-enzymatic maillard reaction and caramelization of sugars was observed for *chapatti* and *thepla* at the highest level of 10 g FOS addition in a recent study (Mahendra. & Sheth, 2013). Increase in burn spots and darker color of FOS / Inulin enriched products have been explained by a greater number of reducing ends involved in a maillard reaction. Shorter chain Inulin's result thus in even darker color as it possesses more low molecular weight fructans (Poinot, 2010; Mandala, 2009; Peressini, 2008).

Preparation and sale of FOS based spreads, chocolates, beverage concentrate and honey like product has been successful and are easily available in the market (Ramesh et al., 2004).

In a study on FOS addition in breads, an increase in crumb hardness of bread was observed along with smaller loaves and darker color, when Inulin was added at 3 percent to 5 percent levels (Cecile Morris & Gordon Morris, 2012; O'Brien et al., 2003). An enhancement of bread crust coloration was also reported for breads prepared with as little as 3 percent and up to 10 percent Inulin addition, the crust became darker as the level of addition increased (Hager et al., 2011). Similar observations have been found in a study where the investigators have reported that oligofructose contributes humectancy to soft baked goods. Authors concluded 5% level of addition in bread was feasible and achievable (Cecile Morris & Gordon Morris, 2012; Kaur & Gupta, 2002).

In a recent study on coconut cookies where FOS was added at 6 different levels of 0% (Standard), 10%, 12.5%, 15%, 17.5% and 20%. Coconut cookies showed good acceptability at all lower levels up to 15% showing no statistical difference amongst them. However, cookies were highly acceptable at specific 15% level of addition along with improvement in physic-chemical composition in response to higher fiber concentration and lower moisture ($p<0.05$) as compared to standard coconut cookies (Stadler & Franciélly et al., 2017).