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Exploiting the Gut to Treat Obesity

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ABSTRACT

The current obesity pandemic is due to interplay of genetic, epigenetic and environmental factors. It is now increasingly realised that the gut plays an important role in energy homeostasis; and impairment in its functioning results in obesity. Three major players from the gut - gastrointestinal peptides, gut microbiota and bile acids are involved in energy homeostasis. Structural and functional changes of the above three have been observed in obesity. Thus, exploiting these pathophysiologic factors to develop the pharmacotherapeutic agents to correct the defect is the major cornerstone of obesity management.

INTRODUCTION

Obesity is global pandemic today, accelerating at an alarming speed. Between 1980 and 2014 the prevalence of obesity doubled. In 2014, according to the World Health Organization 1.9 billion adults were overweight, of which 600 million were obese[1]. In the same report, 41 million children under the age of 5 years were overweight or obese. According to the ICMR-INDIAB-3 study the predicted prevalence of generalised and abdominal obesity in India was 135 and 153 million respectively[2]. This pandemic threatens to nullify all the achievements mankind has achieved so far in the field of medical science in the past centuries. For this pandemic to be contained and managed effectively we need to understand why obesity occurs.

Obesity is a very complex disease. Our current 'obesogenic' lifestyle definitely has a big role in precipitating this pandemic. However obesity arises from interplay of various factors including, but not limited to, genetic, epigenetic and environmental factors. Environmental influences can have effects from as early as intrauterine life continuing throughout the life-span of an individual. Thus early life environment like mother's health and disease, mode of delivery, mode of feeding, exposure to antibiotics and later life environment like diet and physical inactivity interact in a very diverse way to result in obesity. Environment has a major role in influencing genetic expression or epigenetics.

ROLE OF THE GUT IN ENERGY HOMEOSTASIS

Our Gut is Programmed to Increase Efficiency of Nutrient Extraction

Our gastrointestinal tract (the gut) is the only 'natural and normal' route through which we get calories and nutrients for our survival. From an evolutionary perspective the gut is programmed and modified in such a way so that maximum nutrient is extracted from the food we consume. This 'effectiveness' of the gut was necessary for survival when food supply was scarce and intermittent. But in today's environment of excess and continuous food supply the adaptations made by the gut for survival becomes maladaptive. The gut does possess corrective mechanisms to prevent 'energy overload' by releasing peptide hormones that reduce appetite and decrease energy extraction from the food we consume. However, these corrective mechanisms are non-functional in obesity, both as cause and effect of obesity.

Therefore 'exploring' and 'exploiting' the gut would be an ideal, scientific and effective way to manage obesity. A lot of our understanding about the role of gut in the pathogenesis of obesity and thereby making it an ideal target for obesity management has emerged from the impressive weight loss seen with bariatric surgery. Bariatric surgery is associated with a number of operative, post-operative and lifelong complications and thus can never remain the permanent treatment of morbid obesity. But the understanding of the science of energy homeostasis from bariatric surgery remains the cornerstone for development of novel pharmacotherapeutic agents for the management of obesity.

Gut Peptides, Gut Microbiota and Bile Acids Play Major Role in Energy Homeostasis

The important players in the gut that have a role in energy homeostasis are i) gut hormones, ii) gut microbiota, and iii) bile acids. Their proper functioning results in good health and normal weight. But disturbance of these three important players singly or in combination can give rise to problems of either obesity or underweight. And thus, targeting these three becomes an important therapeutic tool for obesity.

GUT HORMONES: ROLE IN APPETITE AND WEIGHT HOMEOSTASIS

Hypothalamus integrates Energy Signals and Respond Accordingly

The body tries to maintain an intricate homeostasis of energy stores and thereby of weight. The hypothalamus plays the central role in this homeostasis by modifications of food intake, energy expenditure and energy allocation depending on signals it receives. The signals from the periphery especially from the gut and adipose tissues, tells the hypothalamus about the body's energy reserve and the hypothalamus respond accordingly. The hypothalamus also receives supply from the cortical and mesolimbic areas which are influenced by emotions, behaviour and other factors.

Long Term and Short Energy Signals Arise From Adipose Tissue and Gut Respectively

Signals from the periphery to the hypothalamus are of 2 major types: long term and short term energy reserve signals[3,4]. The long term adiposity signals arise mainly from the white adipose tissue in the form of leptin. Insulin is also a long-term adiposity signal. When body energy stores are increased leptin and insulin relay to the hypothalamus to decrease energy intake and increase energy expenditure[3]. Obesity is associated with both leptin and insulin resistance[5].

Short term peripheral signals regarding meal initiation or termination arise from the gut in the form of hunger or satiety signals. These signals reach the hypothalamus, mainly at the arcuate nucleus (ARC) which is partially permeable to the blood brain barrier[6]. In the ARC there are two groups of neurons: appetite suppressing pro-opiomelanocortin (POMC) and appetite stimulating agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons[7]. These neurons project into the ventro-medial and lateral areas of the hypothalamus involved respectively in satiety and hunger[8].

Appetite is Modulated by Two Major Mechanisms: Homeostatic and Hedonic

Appetite is a complex issue and controlled by at least two mechanisms: homeostatic and hedonic mechanisms[9]. The homeostatic mechanism described above depends on the body energy store and responds to correct any imbalances in it. Thus ghrelin is released from the stomach when energy stores are depleted, stimulating hunger and promoting consumption of food[10]. Contact with nutrients and distension of gut following eating, results in the release of a number of gastrointestinal peptides like glucagon-like peptide 1 (GLP-1), peptide YY (PYY), pancreatic polypeptide (PP), cholecystokinin (CCK) and oxyntomodulin (OXM) which delays gastric emptying and suppresses appetite by central and peripheral mechanisms[11]. The hedonic control of appetite is more complex and is under the control of the mesolimbic (reward) areas and others. This pathway is controlled by emotion, taste, texture, colour and smell of food. This pathway can continue to be active even when homeostatic mechanism has replenished energy stores. However the two mechanisms of appetite regulation are not exclusive of each other and frequently interact. Thus ghrelin, a hormone mainly acting via the homeostatic pathway also stimulates the hedonic pathway[12].

Gut Signals are Impaired in Obesity: Aim of Treatment is to Replace the Defective Signals

Gut signals are impaired in obesity and bariatric surgery acts by correcting this defect to a certain extent. Modulation of gut hormone levels by pharmacotherapy forms an important cornerstone of obesity treatment, without the risks of bariatric surgery.

As discussed above, important gut hormones involved in satiety are GLP-1, PYY, PP, CCK and OXM. GLP-1 is released from the L cells of the small intestine and colon in response to nutrients like glucose, amino acids and fatty acids in the gut[13]. It promotes weight loss by central mechanisms and peripheral mechanisms. Centrally it acts directly on the hypothalamus causing an anorexigenic effect and peripherally it slows gastric emptying resulting in satiety. It also inhibits gastric acid secretion. It stimulates glucose dependent insulin secretion and inhibits glucagon secretion and therefore is a good anti-diabetic agent. Obesity is associated with decreases in post-prandial GLP-1 levels. However obese individuals respond well to the anorexigenic effects of externally administered GLP-1 agonists[14]. Bariatric surgery has been shown to enhance post-prandial GLP-1 response[15]. Endogenous GLP-1 has a short half-life of around 5 minutes due to the effects of dipeptidyl peptidase-IV (DPP-IV) enzymes. Long acting analogues are already available for the treatment of diabetes. Liraglutide, a long acting GLP-1 analogue at a dose of 3mg subcutaneously daily has been approved by the USFDA for the treatment of obesity.

PYY is a 36 amino acid polypeptide secreted from the L cells of the intestine and belongs to the same family as PP. PYY_{3-36} is the more active form of the peptide and acts via the Y family of G-protein coupled receptors especially Y2[16]. Similar to GLP-1 it decreases appetite and induces weight loss by peripheral and central mechanisms. Post-prandial levels are low in obesity and levels of PYY_{3-36} rises following bariatric surgery[17]. Nasal sprays of PYY_{3-36} have shown modest weight loss in humans[18]. CCK, PP and OXM are other gastrointestinal peptides released in response to a meal and associated with weight loss by peripheral and central mechanisms. CCK released from the jejunum and duodenum was the first gut peptide known to affect appetite in humans[19]. CCK receptor antagonists have been studied in obese humans but the resultant weight loss was unsatisfactory[20]. PP is secreted mainly from the pancreas but also from L cells of the intestine and acts via the Y4 receptor in the hypothalamus[21]. OXM is released from the oxyntic cells of the stomach[22]. Like other gut peptides they cause weight loss by central and peripheral mechanisms and have also been found to increase energy expenditure[23,24]. All the gut peptides described above are low in obesity and rise to varying levels after metabolic surgery.

Ghrelin is the only gut peptide that stimulates appetite[25]. It acts via its growth hormone secretagogue receptor in the hypothalamus and the brain. The expected fall in post-prandial ghrelin levels does not occur in obesity and hence hunger persists. In earlier studies bariatric surgery was shown to decrease ghrelin levels. However over the ensuing months after surgery, the ghrelin levels continue to rise reaching pre-operative levels months after surgery[26]. Ghrelin antagonists have been used in experimentally induced obesity in mice resulting in weight loss.

Following Roux-en-Y gastric bypass (RYGB), GLP-1 levels have been shown to rise by 10-fold a few days after surgery[27]. Levels of other gut peptides especially PYY and OXM increase after bariatric surgery. We have partially

exploited the gut peptides in the management of obesity, but more needs to be done. Liraglutide is already being used for the management of obesity. But a cocktail of gut peptides especially GLP-1, PYY and OXM could mimic many of the effects of RYGB on appetite, and could result in weight loss similar to bariatric surgery without the risks[28].

THE GUT MICROBIOTA: ROLE IN OBESITY PATHOGENESIS

The Size and Products of the Gut Microbiota is Impressive

The gut microbiota is composed of organisms that reside in our gut. The microbiota composition is variable between individuals but remains relatively stable in a single individual. It is composed of organisms that permanently reside in our gut and the organisms that temporarily pass through it. Initial colonization at birth may influence the permanent residents of the gut. The stomach and duodenum harbour 10^3/ml with the numbers increasing to 10^{11}-10^{12}/ml at more distal tracts[29]. There are at least 1000 different species of organisms, mainly anaerobes. The microbiota contains at least 100 times more genes than our own genome[30]. Bacteroidetes and Firmicutes account for more than 90% of all[31]. The volume of the gut microbiota is so impressive that it has an approximate weight of 1-2kg in an average size adult[32] and an estimated 90% of all the cells in the human body is derived from microorganisms that reside in our body[33,34].

Our understanding of the gut microbiota and microbiome has increased exponentially due to greater reliance on culture-independent techniques, especially genomic methods. This is possible because of large projects like the NIH sponsored Human Microbiome Project (http://commonfund.nih.gov/hmp), the European-funded Metagenomics of the Human Intestinal Tract (http://www.metahit.eu) consortium, and the International Human Microbiome Consortium (http://www.human-microbiome.org).

Gut Microbiota is a Virtual Endocrine Organ

The gut microbiota is a virtual complex endocrine organ. It satisfies all the definition of an organ because it collectively influences the functioning of the body and is responsive to secretions and signals from other organs of the host. It secretes a vast array of hormones and other neurotransmitters with the amount exceeding those secreted even by the brain[35].

Gut Microbiota Increase Energy Extraction, Neurotransmitter Release and Modulate Bile Acids

The symbiotic role of the gut microbiota with the human host has been known for a long time. The microbiota is known to protect the host from other pathogens, synthesize vitamins, and digest indigestible polysaccharides. However, the gut microbiota is far more complex. In the context of obesity, the functions of the gut microbiota may be considered as follows i) efficiency of energy extraction, ii) release of neurotrasmitters that act on the enteric nervous system (ENS) and central nervous system (CNS), and iii) impact on bile acid metabolism.

i. Efficiency of Energy Extraction: Experimentally produced germ free mice are protected from diet induced obesity. Introduction of gut microbiota from conventionally raised mice to the germ free mice increased body weight by 60% in 2 weeks despite decrease in food consumption by 29% and increased activity by 27%[36]. The microbiota composition of the obese gut is different from the lean gut in both humans and mice. Firmicutes are higher and Bacteroidetes are lower in the obese and there is lesser bacterial diversity[37,38]. Successful and sustained weight loss in obese humans improved the Bacteroidetes/Firmicutes ratio[38]. Metagenomic studies have shown that the microbiota in the obese gut is enriched with genes coding for enzymes involved in digestion of indigestible polysaccharides[39].

   The gut microbiota increases the efficiency of energy extraction by the following mechanisms i) intestinal absorption of monosaccharides and short chain fatty acids (SCFA) after digestion of non-digestible polysaccharides by gut microbiota. Among the SCFA propionate serves as a precursor for gluconeogenesis in the hepatocytes and acetate serves as a precursor of de novo lipogenesis in hepatocytes and adipocytes[40]; ii) suppression of fasting induced adipocyte factor (FIAF), which inhibits lipoprotein lipase (LPL). Inhibition of FIAF increases LPL activity. This promotes lipolysis of triglyceride in lipoproteins and facilitate greater fat storage in adipocytes[41]; iii) decrease activity of the AMPK which acts as a 'fuel gauge'. AMPK plays an important role in maintaining body weight (by fuel utilisation) and preventing lipotoxicity[42,43]; iv) SCFA inhibits the release of PYY[44] by acting on the G-protein coupled receptor 41 (Gpr41) in the enteroendocrine cell.

ii. Release of Neurotransmitters: A number of neurotransmitters are produced by gut microbiota. Important among them are SCFA, serotonin, dopamine, noradrenaline, acetylcholine and GABA[45-49]. SCFA can modulate secretion of serotonin and PYY[50]. SCFA like butyrate and propionate can cross the blood brain barrier and are major energy source for cellular metabolism, especially during brain development. They can also affect neurotransmitter synthesis in the CNS via regulation of tyrosine kinase gene activation[51]. In mice models SCFA butyrate has been found to have anti-depressant effects[52].

iii. Impact on Bile Acid Metabolism: the microbiota can transform the primary bile acids to secondary bile acids. The effects of bile acids on metabolism will be discussed later.

Mode of Delivery, Mode of Feeding, Antibiotics and Mother's Health Determine Initial Gut Colonization

The gut is sterile in the intrauterine life. The microbiota

becomes established at the time of birth and therefore the mode of delivery determines the character of the microbiota. At 1-3 years of age adult-like microbiome is present[53]. Immediately after vaginal delivery infants have bacterial composition in the gut similar to those in mother's faeces[54]. Infants delivered by caesarean section have microbiota derived from mother's skin, air and nursing personnel[55]. Children born by vaginal delivery have greater diversity of the microbiota, higher number of Bacteroides fragilis, Bifidobacteria and lower Clostridium difficile compared to those born by caesarean section[56,57]. Breast fed children have higher Bifidobacteria, probably due to bifidobacterial growth factors in breast milk and have lower rates of colonization by E. Coli and Clostridium difficile[56,58]. Other factors that can adversely affect microbial composition in the infant are antibiotic use[56] and maternal gut dysbiosis due to high fat diet, obesity and antibiotics. The composition of gut microbiota in early life determines the development of overweight and obesity in future. Bifidobacteria were higher in fecal samples of infants who remained normal weight at 7 years, whereas staphylococcus aureus was higher in fecal samples of infants who subsequently became overweight or obese at 7 years[57].

High Fat Diet Can Induce Rapid Changes in the Gut Microbiota

Another very important fact is that diet can rapidly affect the microbiota. Results obtained from mouse models with humanised gut microbiome showed that gut microbiota can shift rapidly in a single day when high-fat, high-sugar diet is consumed in contrast to low-fat, plant-based polysaccharide diet[59]. In experimental mice, high fat diet is associated with an increase in Firmicutes and Protobacteria, and a decrease in Bacteroidetes[59]. In humans, it has been shown that short term increases in nutrient load rapidly changes the gut microbiota in lean, but not in obese individuals, with increased Firmicutes and decreased Bacteroidetes, leading to increased energy harvest[60]. This suggests that the microbiota of the obese and lean respond differently to nutrient load.

Obese Gut Microbiota Promotes Greater Energy Extraction and Metabolic Endotoxinemia

As detailed above, the microbiota of the obese encode for genes that are efficient in energy extraction. A high fat diet contributes to 'metabolic endotoxinemia' by increasing lipopolysaccharides (LPS)[61]. LPS derived from the cell-wall of gram negative bacteria are increased in obesity and this result in a low grade chronic inflammation. High fat diet changes the quality of the gut microbiota and can promote increase capillary permeability favouring LPS absorption[62]. In *in vitro* model of human colorectal adenocarcinoma cells formation of chylomicrons favoured the absorption of LPS[63]. All plasma lipoprotein class can bind LPS[64]. Thus dietary fat increases LPS absorption by at least 3 mechanisms: changes in composition of gut microbiota, increased chylomicron formation and increase capillary permeability. Bifidobacteria which is the prominent species in the lean gut do not degrade intestinal mucus glycoproteins as other pathogenic bacteria, promoting a healthy gut that is less permeable, preventing LPS translocation[65]. The metabolic endotoxinemia results in low grade inflammation contributing to insulin resistance, adipocyte hypertrophy and β cell dysfunction[66].

After bariatric surgery, especially after RYBG changes in the gut microbiota have been observed. The possible mechanisms could be anatomical rearrangement of the gut with rapid delivery of undigested nutrients to distal small gut, alterations in entero-hepatic bile flow, increase in pH with bacterial overgrowth, use of antibiotics and changes in food preference[28].

Transfer of Microbiota from Obese to Lean or Vice Versa Can Induce Phenotypic Characteristics of the Donor

Are changes in the gut microbiota a cause or consequence of obesity? The probable answer is that it could be both. Germ-free mice on receiving microbiota transplant from obese mice develop the obese phenotype[67,39], whereas they lose weight on receiving microbiota from an animal who has lost weight following RYGB[68]. In genetically obese mice (ob/ob mice), there is a decrease in Bacteroidetes and increase in Firmicutes, suggesting that obesity by itself could determine the character of the gut microbiota[69].

Modulation of Gut Microbiota: One Promising Aspect of Obesity Pharmacotherapy

It is therefore clear that the gut microbiota is altered in obesity and changes in the composition of the gut microbiota similar to a lean gut can bring about metabolic improvements. The therapeutic options that can be exploited for bringing out changes in the gut microbiota include the use of prebiotics, probiotics or both, antibiotics and gut microbial transplantation (GMT).

Probiotics are living non-pathogenic microbes that when consumed in appropriate amounts promote health. Probiotics have been shown to decrease adipocyte size which is the strongest marker of insulin resistance. Possible mechanisms include fecal excretion of neutral sterols and bile salts, decreased absorption of triglyceride, cholesterol and phospholipids and increased lipolysis[70,71]. Studies using dahi supplemented with probiotic strains of Lactobacillus acidophilus and Lactobacillus casei have shown that this product can improve the stigmata of diabetes, i.e. hyperglycemia and hyperinsulinemia, in high-fructose induced rat models of diabetes[72,73]. In another study administration of fermented milk containing Lactobacillus gasseri SBT2055 for 12 weeks lead to a significant reduction of abdominal visceral and subcutaneous fat areas by 4.6 and 3.3 % respectively as measured by computed tomography[74]. The human studies on probiotics are small scale and have short-term follow up. Larger and longer studies with different strains of microbiota will give more convincing results.

Prebiotics promote the growth of beneficial bacteria. Prebiotics (oligosaccharides like galacto-oligosaccharides; the inulin derivatives like the fructo-oligosaccharides; and soluble fibers) are proposed to stimulate the growth of beneficial bacteria (i.e. Bifidobacteria and Lactobacilli) in

the gut, to generate fermentation products i.e. SCFAs with anti-inflammatory effects[75], to reduce the appetite, and to mimic the pathogen binding sites that coat the surface of gastrointestinal epithelial cells inhibiting enteric pathogen adhesion and infection[76].

Antibiotic treatment reduces metabolic endotoxinemia and LPS levels in ob/ob mice resulting in improved metabolic profile[66]. However, the impact of antibiotics on metabolic health is variable. As discussed earlier, antibiotic use can cause obesity by changing the composition of the gut microbiota and by destruction of intestinal mucosa causing increased LPS absorption. In fact, farmers have used antibiotics for a long time in farm animals to increase fat mass and body weight. The possible explanation for increase in body weight are changes in the gut microbiota with resultant obesity[77,78].

Fecal transplantation or gut microbiota transplantation (GMT) refers to transfer of feces from a healthy donor to a recipient. It is already in use for the treatment of Clostridium difficile pseudomembranous colitis since 1950's[79]. As discussed above transfer of gut microbiota from obese gut induces obese phenotype and lean microbiota induces lean phenotype in germ free mice. GMT can actually be considered a probiotic treatment because what is being transferred is the organisms from healthy gut. In one study, GMT was done via a naso-duodenal tube in nine men with metabolic syndrome, whereas another nine men with metabolic syndrome received placebo[80]. Six weeks after GMT, treated subjects had an impressive 75% increase in insulin sensitivity. Moreover, GMT was associated with favourable changes in the gut microbiota like greater bacterial diversity and a 2.5-fold increase in butyrate-producing bacteria[80]. However, at this moment challenges to GMT exist. Some of the challenges are identification of healthy donor, mode of delivery and the psychological stress that could be associated with fecal transfer.

BILE ACIDS: METABOLIC EFFECTS

Modulation of Bile Flow and Content after Bariatric Surgery Confer Metabolic Benefits

Bile flow alteration following bariatric surgery contributes significantly to improvements in weight and correction of dysglycemia. In fact, many of the beneficial effects after bariatric surgery can be reproduced in rats by simply diverting bile from the common bile duct to the mid-distal jejunum via a catheter[81]. After RYGB fasting and post-prandial concentrations of bile acids rise. Bariatric surgery could actually alter the expression of nuclear receptors of bile acids. Bile acids have multiple effects on metabolism. It increases GLP-1 secretion by TGR5 receptor present on the L-cells[82]. It also increases the levels of other satiety hormones like PYY and CCK[83,84]. Bile acids have been known to suppress genes associated with lipogenesis[85]. Acting via the FXR, bile acids have been shown to activate apo-CII and apoA-V expression which are co-activators of lipoprotein lipase. This inhibits serum triglyceride levels[86]. Bile acids may also contribute to changes in the gut microbiota following bariatric surgery. The results of modification of bile acids and bile acid dynamics should be explored in greater detail, as this will guide novel pharmacotherapy for obesity.

CONCLUSION

In the course of evolution complex mechanisms have been developed by our gut to improve the efficiency of nutrient extraction. Pathways exist to keep the system in 'check' so that overnutrition does not occur. In the modern day 'obesogenic' environment, an intricate interplay of factors starting from intrauterine life and continuing in later life interferes with the proper functioning of the gut and results in obesity. The three important players in energy homeostasis for which we have a fair understanding are gut peptides, gut microbiota and bile acids. Obesity is associated with functional and/or structural alterations in the above three. Correction of these alterations can result in significant and sustained weight loss as shown by bariatric surgery. Bariatric surgery with its complications can never be the final treatment for obesity. The science of weight loss following bariatric surgery which manipulates the gut structurally and functionally has been understood to a large extent. We need to urgently develop pharmacotherapy to exploit the gut based on this understanding, so that the biggest epidemic to threaten mankind can be dealt with effectively and on time.

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