Review Article

Diet and Gut Microbiota in Human Health

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There is growing concern and appreciation of the role of our dietary factors in modulating the composition and metabolic activity of the human gut microbiota (microbiome or microflora), which, in turn can impact host health. Beyond prevention and treatment of infections, microbes in the intestine are now known to influence cholesterol metabolism, blood glucose homeostasis, insulin sensitivity, obesity and demonstrate anti-hypertensive and anti-carcinogenic properties, by way of regulating host metabolic pathways and gene expression. Furthermore, recent research advances have expanded our understanding on the importance of the gut microbiome, and its potential involvement in neurological disorders impacting behaviour and mood. Mechanistic insights provide both neurotransmission and immune modulation inflammation routes, through which these effects can be mediated. The expression and function of the key players in adiposity and insulin resistance, such as glucagon-like peptide 1 (GLP-1), free fatty acid receptor 2 (FFAR2), also called G-protein-coupled receptor (GPR41) and peptide YY (PYY) are regulated by microbes and their products. Diet may potentially induce gut bacteria de-regulation and disrupt the basic functions of gut microbiota resulting in low grade inflammation, consequently paving the way for chronic non-communicable diseases (NCDs). In the present review, role of gut microbiome in obesity and diet induced inflammation and their health effects are discussed. Some insight into maternal microbiome in relation to reproductive health and pregnancy outcome, which is an emerging area, is also attempted.

Keywords: Gut microbiota; Obesity; Inflammation; Insulin Sensitivity; Maternal Microbiome; Vaginal Microbiome; Probiotics and Prebiotics

Introduction

The microbiota of the gastrointestinal tract, which is 150 times that of the host genome and 10 times that of host cells, (3.3 million non-redundant bacterial genes) is known to play a crucial role in the physiological and immunological development of the host (Qin *et al.*, 2010). Our gut microbiota contains trillions of microorganisms, belonging to 1000 different species of known bacteria, apart from several unknown microbes. Majorly, the predominant ones are; obligate anaerobes from genera *Bacteroides*, *Eubacterium*, *Clostridium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, *Bifidobacterium*, and *Fusobacterium*, and the less predominant facultative anaerobes are *Lactobacillus*, *Escherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, and *Proteus* (Savage, 1977; Manson *et*

al., 2008; Eckburg *et al.*, 2005; Martin *et al.*, 2007). *Firmicutes* is known as the largest bacterial phylum, consisting 200 genera, which includes *Lactobacillus*, *Mycoplasma*, *Bacillus*, and *Clostridium* (Caricilli and Saad, 2013; Caporaso *et al.*, 2011). *Enterococcus* and *Escherichia coli* constitute less than 1% of all intestinal microbiota. Anaerobes dominate upon facultative aerobes at the ratio of 1000:1. One third of the gut microbiota is said to be common to most people, while two thirds are unique to each one of us. The 16S rRNA gene sequencing has demonstrated the existence of a large number of yet-unidentified bacteria that inhabit the mammalian intestine (Caricilli and Saad, 2013; Caporaso *et al.*, 2011).

Microbes in the intestine are now known to influence cholesterol metabolism, blood glucose homeostasis, energy harvest, insulin sensitivity and

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blood pressure, apart from controlling gene expression and a number of host metabolic pathways (Claus *et al.*, 2008; Martin *et al.*, 2007; Velagapudi *et al.*, 2010; Wikoff *et al.*, 2009; Li M *et al.*, 2008; Zhao., 2007; Zhao and Shen, 2010). Microbial products such as the short chain fatty acids (SCFA), and conjugated and free fatty acids, are known for their health-promoting effects (Clemente *et al.*, 2012; Aziz *et al.*, 2013). The general functions carried out by gut microbiota (bacteria) are given in (Fig. 1). Given that 70% of the immune system is present in the GI tract, the cross-talk between the mucosal immunity and the microbes at the interphase in the intestinal lumen is the determining factor in keeping a delicate balance in the immune system by eliminating invading pathogens, while still maintaining self-tolerance and avoiding auto-immunity and inflammation (Blaise *et al.*, 2007).

Fig. 1: General functions carried out by gut microbiota

At birth, the intestine is sterile, but intestinal colonisation starts right after birth and evolves as we grow. The newborn's digestive tract is quickly colonised by microorganisms from the mother (vaginal, faecal, skin, breast, etc.). From the third day onwards, the composition of the intestinal flora is dependent on how the infant is fed. While *Bifidobacteria* dominate the gut microbiome of the breast-fed neonate, babies fed with infant formulas have more gram-negative bacteria in the gut. By the age of three, microbiota becomes stable and similar to that of adults. Gut microbiota's balance can be affected by oral antibiotics, ageing, alcohol, smoking, environmental pollution and diets such as high calorie/high fat or low micronutrient diets (Michael

and Anthony, 2015; Claesson *et al.*, 2011; Makivuokko *et al.*, 2010). Both short- and long-term dietary changes, particularly macronutrients, have a major role in shaping the composition and activity of the intestinal microbiota. Knowledge regarding effects of carbohydrates is relatively better understood; but influence of fats and proteins on the gut microbiota is largely unknown (Michael and Anthony, 2015; Claesson *et al.*, 2011; Makivuokko *et al.*, 2010).

Probiotics and Prebiotics

With the advances in the knowledge on the important role of dietary components in influencing intestinal bacteria affecting health, the use of functional foods like probiotics and prebiotics have come into trend in the recent past. Probiotic was first conceptualised by a Russian Nobel prize winner and father of modern immunology, Elie Metchnikoff, a scientist at the Pasteur Institute. Elie Metchnikoff (1845-1916) identified an apparent link between consumption of fermented products containing Lactic acid bacteria with human health and longevity (Podolsky, 2012). Probiotic (Greek Language) "for life" was first used by Lilly and Stillwell in 1965 to describe "microorganism that secrete substances, which stimulate the growth of another" (Podolsky, 2012). The FAO/WHO defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO Report, 2001). They are selected from the strains most beneficial for the host such as *Lactobacillus*, *Bifidobacterium* and yeast. Yogurt and milk to which probiotic bacteria have been added, such as acidophilus milk and fermented milk products, such as *kefirs*, (fermented milk drink containing yeast and beneficial probiotic bacteria) are the primary food sources of probiotics. On the other hand, prebiotics are substances like inulin, oligofructosaccharides (OFS) that are not absorbed through the small intestine and are fermented in the large intestine (gut) by colonic bacteria. Pre-biotics promote the growth of beneficial bacteria in the host (Gibson and Roberfroid, 2004). Pre-biotics are present naturally in fruits, vegetables, onion, garlic, chicory, legumes, nuts, seeds, and most vegetables high in soluble fibre.

Gut Microbiota and Diet-Induced Obesity

Recent findings show that the gut microbiota has an important role in regulating weight and may be partly

responsible for the development of obesity (Shen *et al.*, 2013). Obesity has been linked with reduced bacterial diversity, altered expression of genes and impaired metabolic pathways. The first evidence linking obesity with gut microbiome came from studies on germ-free mice (Backhed *et al.*, 2004; Backhed *et al.*, 2007). Feeding high fat diet to normal mice lead to significantly more weight gain and fat mass and hyperglycemia, than the germ-free mice despite no differences in the diet intake between the two groups (Backhed *et al.*, 2004; Backhed *et al.*, 2007). Likewise, adult germ-free mice colonized with bacteria harvested from the colon of obese mice lead to an increase in body fat within two weeks, suggesting that the energy harvest phenotype could be transmitted by the gut microbiota (Turnbaugh *et al.*, 2006; Turnbaugh *et al.*, 2008). Colonization with obese flora and consequent increase in body fat was associated with decreased expression of fasting-induced adipocyte factor (Fiaf) gene in the ileum of germ-free mice (Backhed *et al.*, 2007). Colonization of germ-free mice with specific strains, such as *Methanobrevibacter smithii*, the methanogenic Archaea species (from the human gut), and *Bacteroides thetaiotaomicron* also led to body weight gain and increased fat mass (Samuel and Gordon, 2006). However, in experiments on resistin-like molecule- β (RELM- β) knockout mouse model, that gains less weight; high-fat diet induced decrease in *Bacteroidetes* and an increase in both *Firmicutes* and *Proteobacteria*. The change in microbiota occurred regardless of whether the mice were obese or not, suggesting that diet was the driving force behind this microbial change (DiBaise *et al.*, 2012; Hildebrandt *et al.*, 2009). In contrast, analysis of bacterial 16S rRNA, in genetically obese ob/ob mice and their lean counterparts fed the same polysaccharide-rich diet, showed 50% fewer *Bacteroidetes* and correspondingly more *Firmicutes* than their lean litter-mates. This difference was unrelated to differences in food consumption (Ley *et al.*, 2005). Fewer *Bacteroidetes* and more *Firmicutes* were also observed in obese humans compared with lean controls (Kotzampassi, 2014).

In humans, gut microbiota during early life has been suggested to predict the subsequent development of overweight and obesity (Kalliomaki *et al.*, 2014; Xiaolin Gao *et al.*, 2015). For instance, greater numbers of *Staphylococcus aureus* in infancy

predicted overweight in children at age seven (Kalliomaki *et al.*, 2014). In contrast, bifidobacterial numbers were higher in children with normal weight (Kalliomaki *et al.*, 2014; Xiaolin Gao *et al.*, 2015). Bifidobacteria produce conjugated linoleic acid, which modulates the fatty acid composition in the liver and adipose tissue in mice (Gorissen *et al.*, 2010). One recent study, found that higher prevalence of the phylum *Firmicutes* in early infancy was associated with greater weight gain later on (Kotzampassi, 2014). The KOALA cohort study also reported higher *Bacteroides fragilis* to be associated with greater childhood weight gain (Kalliomaki *et al.*, 2014). Similarly, in overweight pregnant women, *Bacteroides* and *Staphylococcus aureus* were relatively higher during pregnancy when compared to normal weight pregnant women (Collado *et al.*, 2008). Since mother's flora influences the microbial flora of the offspring it has been suggested to be one mechanism by which a tendency for obesity is conferred from the parent to the infant. Vaginal delivery seeds the infant's gut with *bifidobacteria* (Makino *et al.*, 2013). Colonization of *bifidobacteria* in the infant's gut is further supported by oligosaccharides that are present in the breast milk. Caesarean section delivery or use of antibiotics early in life and type of first feedings are factors that may influence the infants' gut microbiome. Early infant nutrition may thus have life-long consequences through microbial modulation (Makino *et al.*, 2013).

Mechanisms Linking Microbes in Diet Induced Obesity

The mechanisms implicated in intestinal microbiota and increased storage of calories as fat has been suggested to be due to alterations in the expression of several genes coding for enzymes involved in digestion, absorption and nutrient metabolism (Shen *et al.*, 2013). In addition, short chain fatty acids (SCFAs) are suggested to contribute to up to 10% of the basal energy requirements of people in developed countries. Paradoxically, this suggests that fibres, which are touted to be associated with weight maintenance, must be contributing to energy by way of SCFA's; especially, when methanogenic *Methanobrevibacter smithii* species and *Bacteroides thetaiotaomicron* are abundant in the gut (Samuel and Gordon, 2006). Methanogenic archaea are efficient in fermentation of fibres and

production of SCFAs that may contribute to more than 10% of basal energy requirement (Samuel and Gordon, 2006). The mechanistic pathways associating weight maintenance and obesity with microbiome is still at exploratory stage and needs well designed studies to gain more insight into mechanisms. Nevertheless, the suggested mechanisms for microbiota-induced obesity include: (1) fermentation of indigestible polysaccharides and intestinal absorption of monosaccharides and short-chain fatty acids (SCFAs); (2) lipogenesis and glucogeneogenesis in the liver induced by high concentration of SCFAs such as acetate and propionate; (3) upregulation of sterol-response-element- binding proteins and increased hepatic lipogenesis; (4) suppression of lipoprotein lipase inhibitor, the Fiaf gene; (5) down regulation of AMP-activated protein kinase, that is triggered in response to metabolic stresses such as hypoglycaemia, exercise and hypoxia and (6) suppression of entero-endocrine expression of G-protein-coupled receptor 41 (Gpr41) and peptide PYY (anorexigenic) that inhibit gut motility and increase intestinal transit rate (Velagapudi *et al.*, 2010; Backhed *et al.*, 2004; Shen *et al.*, 2013):

Gut Microbiota, Probiotics and Prebiotics in the Prevention of Obesity

Microbial population that prevents obesity and that which promotes a healthy state may represent a possible therapeutic strategy in the prevention and management of overweight and obesity (William *et al.*, 2014). Recently, a butyrate-producing species, *Faecalibacterium prausnitzii*, was negatively associated with obesity and biomarkers of inflammation (Soko *et al.*, 2009). Lin *et al.* (2012) demonstrated protection from high-fat diet -induced obesity and insulin resistance in mice that were supplemented with acetate, butyrate or propionate. SCFA's modulate gene expression in colonic epithelial cells. For example, butyrate is the energy substrate for colonic epithelium, while acetate and propionate are substrates for lipogenesis and glucogeneogenesis in the liver. SCFA's such as butyrate, may function as microbe-derived signalling molecule that influences carbohydrate and fat metabolism by affecting gene regulation. For instance, butyrate-produced by intestinal bacteria can stimulate glucagon-like peptide 1 (GLP-1) secretion through the free fatty acid receptor 2 (FFAR2), also called G-protein-coupled

receptor, in the colonic mucosa. GLP-1 suppresses glucagon secretion, induces glucose-dependent insulin secretion and promotes glucose homeostasis (Lin *et al.*, 2012). The SCFA propionate also modulates energy homeostasis by promoting GPR41 (G protein-coupled receptor 41)-mediated activation of sympathetic neurons (Brown *et al.*, 2003; Kimura *et al.*, 2013). The ability to modulate sympathetic outflow provides another mechanism linking the gut microbiome to the energy expenditure, and metabolic homeostasis. SCFA's are also known to stimulate secretion of peptide YY (PYY), an appetite suppressing peptide (Psichas *et al.*, 2015).

These studies suggest that quality and concentration of SCFA's produced by resident bacteria might prevent obesity. Probiotics such as *bifidobacteria* regulate gut microbiota, increase portal plasma levels of two gut peptides, GLP-1 and PYY (anorexigenic) and decrease Ghrelin (orexigenic) (Li *et al.*, 2011; Parnell *et al.*, 2009). Prebiotics such as fructo-oligosaccharide have been shown to prevent many of the high-fat induced adverse effects including obesity. Apart from promoting the growth of bifidobacteria, bacteria in the gut, prebiotic feeding promotes GLP-1 synthesis in the proximal colon by a mechanism linked to the differentiation of precursor cells into entero-endocrine L-cells (Samuel *et al.*, 2008). All these features are associated with a reduced food intake, decreased body weight gain and fat mass development, and restoration of beta cell mass and glucose-induced insulin secretion (Kobyliak *et al.*, 2016). Prebiotics provide polysaccharides, which are not digestible in our small intestine and therefore make their way to our colon, where they are fermented by a diverse array of microbiota. Fibre-rich foods cultivate the various colonic microbiota so that no individual species is dominant. Thus, a dietary pattern rich in prebiotic is one way to promote robust microbial ecosystem that promotes health and prevents obesity and associated complications (Hemalatha *et al.*, 2015).

Genetically Modified Probiotic in the Prevention of Obesity

Genetic manipulation of known probiotic was investigated recently for prevention of diet induced obesity, with encouraging results. *Escherichia coli* (strain, Nissle 1917), a probiotic, originally discovered

by the German military surgeon, Alfred Nissle, during World War I, from the stools of a soldier who did not develop diarrhoea despite a cholera epidemic, is commonly used for treating diarrhoea. This strain (Nissle 1917) has been genetically modified to carry N-acyl-phosphatidylethanolamine (NAPE), a lipid produced in the small intestine. It is converted to N-acylethanolamide (NAE) that can reduce food intake and obesity (Chen *et al.*, 2014). The genetically modified NAPE-producing bacteria could lower food intake and weight gain in mice, in spite of feeding a high-fat diet for 8 weeks (Chen *et al.*, 2014). From all the above listed experimental evidences, it is clear that the high fat diet alters the gut flora, which is linked with metabolic endotoxaemia and associated insulin resistance. Probiotics or prebiotics might help prevent these metabolic changes.

Gut Microbiota Imbalance Implicated in Diet Induced Chronic Low Grade Inflammation

Low grade chronic inflammatory state and the resultant insulin resistance are known to be the underlying cause of many chronic diseases such as obesity, diabetes, atherosclerosis, cancer and arthritis (Hotamisligil, 2006; Arkan *et al.*, 2005; Wellen and Hotamisligil, 2005). Many age-related diseases such as Parkinson's and Alzheimer's disease are also attributed at least partially to inflammation. Our diet may induce inflammation depending on energy density and micronutrient content (Galland, 2010; Yassour *et al.*, 2016). High calorie diet is followed by high post-prandial spiking in glucose and lipids (FFA) that generates excess free radicals and stimulates inflammation (Galland, 2010). In addition to the direct effect of free fatty acid (FFA) on inflammatory cascade, a high calorie diet may induce gut bacteria de-regulation, endotoxemia and chronic low grade inflammation (Cani, 2007; Cani, 2008).

At the luminal content and host tissues, the intestinal bacteria are in cross-talk with the intestinal epithelium, which acts as the centre-stage integrating pro- and anti-inflammatory signals to regulate host immune responses. Any change in either the quality or quantity of the gut microbes can affect the inflammatory cascade and influence the development of conditions characterized by low-level chronic systemic inflammation, such as obesity and type-2 diabetes (Cani, 2007). A high calorie diet favours

reduction in gram-positive bifidobacteria and increase in gram-negative bacteria. Reduction in gram positive bacteria (*Bifidobacterium*, *Lactobacillus*, *Bacteroides-Prevotella*) is associated with down regulation of tight junction (TJ) protein and impairment of intestinal membrane integrity, which becomes more permeable to endotoxin (lipopolysaccharide-LPS) resulting in increasing endotoxin in the blood (Cani, 2007; Cani, 2008). High-fat diet increases gram-negative bacterial (GNB) load in the intestine and excess of LPS released from lysis of GNB results in translocation of LPS into the systemic circulation, wherein it stimulates TLR-4 that triggers chronic low grade inflammation (Cani, 2007). LPS stimulated release of pro-inflammatory cytokines is the key to induce insulin resistance. This was confirmed by continuous sub-cutaneous infusion of LPS that mimics high fat induced changes such as hyperglycemia, hyperinsulinemia, increased fat mass and inflammatory markers and liver triglyceride content (Cani, 2007). Figure 2 depicts impact of high fat/high calorie diet on intestinal bacteria and translocation of LPS into systemic circulation and the genesis of insulin resistance and metabolic syndrome.

A reversal of endotoxaemia with concomitant reduction in systemic inflammation was shown in both high-energy fed and ob/ob mice treated with antibiotic, thereby showing that dietary lipids are not sufficient to induce endotoxemia and systemic inflammation on a background of low number of gram-negative bacteria following antibiotic treatment (Membrez, 2008). Treatment with a prebiotic also had a similar outcome. By enhancing beneficial gram-positive bacteria, prebiotic supplementation prevented body weight gain and fat mass development, reduced endotoxemia, inflammation, oxidative stress, and visceral adiposity. The use of a prebiotic, oligo-fructosaccharide, was shown to lower the adverse effects of high fat diet with selective increase of the gut *Bifidobacterium* spp. which is reported to reduce intestinal endotoxin levels and improve mucosal barrier function (Roberfroid, 2010). It was demonstrated that the prebiotic modulation of gut microbiota lowers intestinal permeability by a mechanism involving endogenous intestinotrophicproglucagon-derived peptide (GLP-2) production, thereby taming inflammation and metabolic disorders in obesity and diabetes (Cani, 2007; Cani, 2009). Further, it was shown that the GLP-2 antagonist abolished most of the prebiotic effects.

Pharmacological GLP-2 treatment mimicked prebiotic-induced changes in gut microbiota and decreased gut permeability and inflammation (Cani, 2007; Cani, 2008; Cani, 2009). Put together, these results confirm that the intestinal gram negative bacteria cell wall component- LPS/endotoxin is a pre-requisite for chronic low grade systemic inflammation and metabolic disorders and these effects are perhaps mediated through GLP-2.

Bacterial LPS Induced Inflammation May Affect Insulin Signalling and Induce Type 2 Diabetes (T2D)

In a study, that tested the hypothesis that bacteraemia is an early marker in the development of T2D, phylum proteobacteria was found to be predominant in a cohort of 3280 subjects. Total 16S rRNA concentration was higher in those who developed insulin resistance and diabetes 9 years later (Amar *et al.*, 2011). 16S rRNA concentration may be an early biomarker for predicting T2D. Saturated fatty acids, after a high fat/high calorie diet, can directly stimulate TLR molecule. Alternatively, LPS, the outer membrane component of Gram-negative bacteria, may stimulate TLR4 and induce expression of several cytokines, such as TNF- α and IL-6, subsequently resulting in impaired insulin signalling (Fig. 2). Mutation in TLR4 or TLR4 knockout prevents insulin resistance induced by obesity or free fatty acids, suggesting an important

role of TLR4 in the genesis of insulin resistance (Cani, 2007; Cani, 2008). Furthermore, even in preadipocytes, LPS activates TLR4 and increases the expression of several cytokines, TNF- α and IL-6, and impairs insulin signalling in adipocytes. Studies have suggested that bacteria derived LPS can promote the expression of iNOS, NF- κ B and activate Mitogen-activated protein kinases (MAPK), all of which interfere with insulin signalling (Rafi *et al.*, 2007). The cumulative effect of LPS-induced release of cytokines (TNF- α and IL-6) and nitric oxide may worsen the insulin sensitivity. Added to this, increasing levels of circulating fatty acids may further increase production of nitric oxide and deteriorate insulin sensitivity due to impaired lipoprotein lipase (LPL) activity and increased lipolysis. Dietary supplementation with SCFA (acetate, butyrate or propionate), prebiotic-fructo-oligosaccharide, or probiotic-*Bifidobacteria* protected against high fat diet-induced obesity and insulin resistance in mice (Lin *et al.*, 2012).

Vaginal Microbiome, Reproductive Health and Pregnancy Outcome

The human vaginal microbiota, which is predominantly *Lactobacillus* community, plays an important role in the maintenance of a woman's health. When this predominant *Lactobacillus* community is disrupted and replaced by anaerobes and gram-negative bacteria, the condition is called bacterial vaginosis (BV) (Hemalatha *et al.*, 2012; 2012). Women with BV have impaired local immunity and increased risk of HIV and other sexually transmitted infections (Fig. 3). BV has been associated with ascending infections and obstetric complications, such as chorioamnionitis, preterm delivery and low birth weight (LBW). In BV the overgrowth of anaerobes produces substances like polyamines and other compounds that increase the vaginal pH and trigger the release of pro-inflammatory cytokines IL-1 β and IL-8 (Fig. 3). These pro-inflammatory cytokines, IL-1 β and IL-8 associated with BV can profoundly affect reproductive health, reproduction, fertilization, pregnancy and associated complications (Hemalatha *et al.*, 2012; 2012). Exogenous strains of lactobacilli have been suggested as a means of re-instituting a normal healthy vaginal flora (Fig. 3). Carefully selected probiotic strains can eliminate BV associated organisms and restore vaginal health (Mastromarino *et al.*, 2014). Probiotic lactobacilli used in the prevention and

Fig. 2: Impact of high fat/high calorie diet on intestinal microbiota and translocation of LPS into systemic circulation and the genesis of insulin resistance and metabolic syndrome

Fig. 3: Mechanism of action of *Lactobacilli* used in the prevention and treatment of BV

treatment of BV exert antibacterial and antiviral effects, restore vaginal pH, ameliorate local inflammation and prevent foetal and neonatal infections (Hemalatha *et al.*, 2012). The administration of beneficial microorganisms such as probiotics either orally or locally (vaginally) are used to restore and maintain a healthy vaginal ecosystem and to maintain reproductive health.

Apart from BV related organisms, disruption of normal vaginal ecosystem and increased vaginal inflammation may also occur with high fat diet. It is seemingly possible that maternal malnutrition and infections may share many of the same adverse consequences mediated through inflammation. In both cases, alterations in physiological functions can be induced and may lead to the development of offspring phenotype that increases the susceptibility to non-communicable conditions.

Maternal Microbiome and Pregnancy Outcome

Placenta once considered to be sterile, is actually composed of non-pathogenic commensal microbiota belonging to *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria*-phyla (Aagaard *et al.*, 2014). Recent research has found the placenta microbiome to be different in term and preterm delivery. A direct correlation of certain bacterial taxa such as *paenibacilli* was found in term deliveries, while placentas from preterm deliveries had *Burkholderia* (Aagaard *et al.*, 2014). Likewise, a recent study reported an increase in placenta *Fusobacteria* and a decrease in *Cyanobacteria* phyla

in low birth weight group, compared with normal birth weight; and a positive correlation between *Lactobacillus* with increasing birth weight (Zheng *et al.*, 2016). Interestingly, placental microbiome has been shown to be linked with oral microbiome, strengthening the proposed association between maternal dental health and foetal outcomes (Aagaard *et al.*, 2014). An increase in gram-negative bacteria implicated in periodontal disease results in adverse pregnancy outcomes such as the preterm birth and still births (Han *et al.*, 2006; Zi *et al.*, 2015). Even in the absence of periodontal disease, low levels of *Lactobacilli* in saliva were associated with preterm birth (Durand *et al.*, 2009). Furthermore, every unit increase in log (10) of *Actinomyces naeslundii* genospecies 2 (gsp-2), in the oral flora, there was a 60 gm decrease in birth weight and a 0.17 week decrease in gestational age (Dasanayake *et al.*, 2005). In contrast, per unit increase in log (10) of *Lactobacilli casei* levels, there was a 42 gm increase in birth weight and a 0.13 week increase in gestational age (Dasanayake *et al.*, 2005). These findings are interesting, in the sense that oral flora can potentially be a good marker for predicting birth outcome.

Other Health Effects of Colonic Bacteria

There are other less well studied health benefits of colonic bacteria that are not mediated through intestinal integrity, immunity inflammation or energy metabolism. Specific colonic bacteria or probiotic bacteria in fermented milk have been shown to regulate blood lipids, reduce colonic carcinogens and regulate blood pressure.

Renin-Angiotensin-converting enzyme (ACE) system normally maintains blood pressure. ACE inhibitors are commonly used to control hypertension. Supplementation of milk fermented with *Lactibacillus helveticus*LBK-16h had demonstrated significant antihypertensive effects in humans. The mechanism behind this may be ACE inhibitor peptides-Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP) that are produced naturally when milk is fermented with *Lactibacillus helveticus* and *Lactibacillus debrueckii bulgaricus*. Large clinical trials are required to confirm this finding. 10-12 mmHg reduction in systolic blood pressure and 5 mmHg reduction in diastolic blood pressure may reduce the risk of stroke by 40%, coronary heart disease by 16% and all-cause mortality by 13% (Jakala P and Vapaatalo H, 2010).

Fermented milk using specific strains of some lactobacilli could lower total cholesterol and low-density lipoprotein (LDL) cholesterol and increase high density lipoprotein (HDL). Bile Salt Hydrolase (BHS) present in lactobacilli and *bifidobacteria*, deconjugate bile acids and increase their rates of excretion (Kumar R *et al.*, 2013). Cholesterol being a precursor of bile acids, is converted to bile acids replacing those lost during excretion. This facilitates reduction in serum cholesterol. Free bile salts are less efficient in the solubilisation and therefore absorption of lipids in the gut is impaired. Cholesterol absorption is reduced in the presence of specific probiotic strains in the intestinal lumen that de-conjugate bile salts (Kumar M, 2012).

Dimethylhydrazin (DMH) is a procarcinogen normally removed by colonic bacteria by adsorption and excretion in faeces. Oxidative radicals and inflammatory molecules such as COX2/PGE2 up-regulate colorectal carcinogens and promote formation of carcinogen, azoxymethanemethyldiazoniumion (colorectal cancer) from pro-carcinogens. Beneficial bacteria may reduce the risk of colorectal cancer by promoting excretion of carcinogens, reduction of oxygen radical formation and taming of inflammation. In addition, microbes generate a variety of short chain fatty acids (SCFA's-acetate, butyrate, propionate, valeric, formate) by fermenting dietary carbohydrates (fibre). SCFA's such as acetate, propionate and also butyrate regulate apoptosis and epithelial cell proliferation and reduce the colonic pH (Liong, 2008). Most importantly, SCFA's stimulate differentiation of colon mucosal cells and impede mucosal atrophy when delivered colorectally or intra-peritoneally in animal models. The low luminal pH caused by SCFA inhibits

formation of secondary bile acids, which are insoluble and are not taken up by colonocytes and thus lower the carcinogenic potential of secondary bile acids (Liong, 2008). Secondary bile acids are cryptotoxic (toxic to intestinal crypts present in intestinal epithelium) and favour selection of apoptotic resistant cells and promote development of cancer (Kumar *et al.*, 2012).

Conclusion

In India, a large proportion of population is insulin-resistant and the prevalence of diabetes and CHD is high. Accumulating evidence indicates that microbiota of the human intestinal tract plays an important role in health, in particular by mediating many of the effects of diet upon health. Recent research has shown increased proportion of *Actinobacteria*, *Bacteroidetes* and gram-negative bacteria and decreased proportion of genus *Bifidobacteria* with increased serum LPS levels, insulin resistance, increased body weight gain and associated complications such as metabolic syndrome. Elucidating the mechanisms and filling the gaps that exist in the area of diet and gut microbiota are important to develop preventive and therapeutic targeting of the microbiome as an effective strategy for reducing NCDs risk.

Low levels of *Lactobacilli* in saliva and placenta are associated with preterm and low birth weight babies (LBW). Increasing proportion of oral *Actinomyces naeslundii* species was also associated with LBW. Studies are needed to unravel the predictive ability of oral microbiota that can be potentially used as a first trimester biomarker to improve maternal health and pregnancy outcomes.

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