Gut Microbiota and its Relation with Insulin Sensitivity and Obesity

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ABSTRACT

Gut microbes are an integral part of our body and they exhibit complex host-microbial relationship. Alterations in host conditions, including ingested food and use of antibiotics can alter the microbes and their metabolism and in turn would affect the host metabolism.

Short chain fatty acids, other metabolites and components of gut flora specially from gram negative bacteria influence host immune response and we have just started to understand its role in development of obesity, diabetes, CVD. Activation of immune response in turn affects various adipokines and cytokines which lead to decrease in insulin sensitivity and worsening of insulin resistance. Gut microbes also affect the secretion of gut peptides. This in addition to the LPS present in the gram negative bacteria of the gut flora increase insulin resistance and obesity. The ratio of negative to positive bacteria is increased in obesity.

Key-words : Obesity, Gut microbiota, Insulin resistance

Introduction :

Human Evolution is the process which took place over billions of years and is a result of constant interaction of host with the environment. Microbes are an integral part of our ecosystem and have colonized plants, soil and animals including humans establishing complex host - microbial relationship. Thus, as we have evolved, so are these microbes inhabiting human body[1]. This colony comprises of symbiotic, commensal and pathogenic microbes. They are present on the surface as well as inside the body in amazingly large numbers. The number estimated of these microbes only residing in gut is 10^3 - 10^4 microrganisms (dominated by anaerobic bacteria). The number which is many times more than the total number of cells in human body![2] The gut environment affects the genotype translating into phenotype of these organisms and in turn assist in many biological processes which human metabolism cannot perform[1,2]. The gut flora constantly educates and modifies host immune system. Host genetics play an important role in the establishment and shaping of the gut microbiota, as it has been demonstrated that composition of the bacterial community is influenced by specific host genomic loci (Benson et al., 2010; Spor et al., 2011) In this review we look at the role of gut microbiota in insulin sensitivity, insulin resistance and obesity[3,4].

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GUT MICROBIOTA COMPOSITION

Dominating bacterial phyla in the human gastrointestinal tract are the gram-positive Firmicutes and Actinobacteria, and the gram-negative Bacteroidetes. Firmicutes is known as the largest bacterial phylum, comprehending 200 genera, which includes *Lactobacillus*, *Mycoplasma*, *Bacillus*, and *Clostridium*[5,6]. Dietary changes have been shown to have significant effects on the microbiota. Shifting mice to a high-fat, high-sugar - Western diet, from a low-fat, plant polysaccharide-rich diet, changed the microbiota within 24 h[7]. Likewise, shifting from a high-fat / low-fiber diet caused notable changes in the gut microbiota within a day[8].

CROSSTALK BETWEEN GUT MICROBIOTA AND HUMAN PHYSIOLOGY

Gut microbes and Immunity

Innate immune system enables the recognition of general molecular patterns of the microbes through Toll like Receptor (TLR) present on immune cells. In case of mutations in TLR or its absence, normal mucosal and gut immunity fails to develop[9,10,11]. The

commensal bacteria enhance immunological tolerance and suppress inflammatory response through TLRs[12-14]. Gut microbiota thus plays an important role in the development of the immune system and helps maintain the intestinal homeostasis. For instance, they are essential for the emergence of T cell subsets and the differentiation of gut B cells into IgA-producing plasma cells[15-17]. This depends on the type of TLR expressed by the immune cells.

Gut Microbes and Metabolites

Gut microbes continuously produce short chain fatty acids (SCFA), branched short chain fatty acids, some bileacid derivatives and vitamins. This production depends upon the substrates available in the gut lumen like, amino acids, fats and carbohydrates. The microbes ferment carbohydrates in the gut to produce acetate, propionate, butyrate, and lactate, which are specific SCFAs. Their relative proportions and amount alter the host response. This metabolic collaboration between host and microbes largely depends upon bacterial genome[1]. Butyrate is known to be a primary energy source for colonocytes. Acetate is a cholesterol or fatty acid precursor, whereas propionate is gluconeogenic in the liver and the gut. Additionally propionate may also neutralize lipogenesis from acetate or glucose in the liver[18-21]. In addition to this, direct role in the de novo production of nutrients, it also has been demonstrated that these SCFAs can bind to specific receptors, such as G-protein coupled receptors FFAR 2 and FFAR 3, (also called GPR 43 and 41). These receptors are expressed in a wide variety of tissues and cells types immune cells, endocrine cells and adipocytes[22,23,24]. The SCFAs have been reported to have impact on inflammation, neutrophil chemotaxis, and proliferation of T regulatory cells. They also cause intestinal neoglucogenesis via gut-brain-neural circuitary with beneficial effects on insulin sensitivity and overall host physiology[22]. In addition to SAFAs, proteoglycans (PGNs) and lipopolysaccharides (LPS) from gut microbes enter host body influencing host metabolism[25-30].

Gut Microbes and Gut Peptides

Hypothetically the region of the GUT where maximum microbes colonize, is the same region of the colon which has maximum number of L cells that release gut peptides viz : GLP-1, and Peptide YY (PYY). Both these peptides are anorexigenic. Studies have revealed that alteration of gut microbiota with pre-biotic supplementation, increases the concentration of Peptide YY and GLP-1 in portal vein in rodents. Consequently, the expression of preproglucagon and prepro Peptide YYmRNA in the ileum and in the colon is also increased. Concomittantly the researchers found reduction in the expression of orexigenic hormone ghrelin thus linking gut microbiota with modulation of behavior of brain with respect to food intake and insulin sensitivity[31-34]. Many other studies have confirmed this finding. L cells express GPR 43 and GPR 41 which are receptors for SCFAs (metabolites produced by gut microbes). This expression is increased when there is supplementation of probiotics inulin-type fructans and with abundance of SCFAs propionates and butyrates.

Cani et al. have used two different approaches. They first used genetic and pharmacological manipulations and found that mice lacking the GLP-1 receptor (GLP-1R) were not sensitive to the impact of prebiotics[35]. In other words, in the absence of GLP-1R, mice remained obese, resistant to insulin, and did not reduce their food intake. In another approach researchers injected GLP-1 antagonist for 4 weeks which completely abolished the beneficial effects of prebiotics on food intake, hepatic insulin sensitivity and glucose tolerance[35]. GUT microbiota also influence the differentiation of stem cells to L cells. Thus the total number and density of L cells increase under the influence of prebiotics[36]. From this data there is a reason to believe that composition of gut microbiota influences release of GLP-1 and Peptide YY, which modulate feeding behavior of humans and also influence insulin release.

Supplementation with nondigestible carbohydrates like oligofructose caused increased satiety, significant reduction in hunger and increase in endogenous gut peptide production.

Insulin Resistance, Obesity and Gut microbiota

Many chemokines and cytokines are implicated in development of insulin resistance, impairment of insulin signaling and development of obesity. Many of them are expressed by adipose tissue. Diabetics who are physically lean and metabolically hyperactive also exhibit pathological profile of adipocytokines contributing to development of insulin resistance. Under pro-inflammtory state, T-cells produce Interferon Gamma (IFN-γ), Interleukin-17 (IL-17), TNF-alpha, IL-6, MCP-1. These are also released from inflammatory macrophages from adipose tissue[37-40]. Innate immune system activation can also contribute to impaired insulin signaling. This response is mediated via TLRs, mentioned above. The TLR-4 is stimulated by Lipopolysaccharides (LPS) which is a component of gram negative bacteria. The activation of TLR 4 signaling induces upregulation of inflammatory pathways related to the induction of insulin resistance. Activation of TLR-4 increases the expression and release of pro-inflammatory cytokines from adipose tissue and also increases expression of NF κB and MAPK pathway promoting insulin resistance. Studies have reported that LPS also increases expression of iNOS leading to increased levels of Nitric Oxide (NO) which worsens insulin resistance by increasing levels of circulating fatty acids, due to hampering Lipoprotein Lipase (LPL) activity and increased lipolysis[40-44]. Likewise activation of TLR-2 also is LPS dependent and leads to higher TNF-alpa secretion. There appears to be some co-ordination in TLR-2 and TLR-4 effects mediated via LPS in causing insulin resistance[45]. Both the mechanisms can be activated through LPS.

Animal studies have shown that mice fed with high fat diet have higher amount of LPS, a result of higher gram negative / gram positive ratio. This suggests that intestinal flora alters metabolic functioning of the host, increases adipose tissue inflammation, oxidative stress. After treating high fat fed diet mice with antibiotics reduced the levels of LPS, and all the parameters of inflammation, including inflammatory cytokine production[40,46].

Other factors that may contribute to IR : Nucleotide oligomerization domain (NOD) - 1 and - 2 proteins are intracellular pattern recognition receptors that sense bacterial cell wall peptidoglycan (PGN) moieties, which induce stress and inflammation pathways. NOD-1 detects PGN structures found in gram-negative bacteria, whereas NOD-2 detects PGN segments specifically found in gram-positive strains. Different researchers have established relationship between NOD-1 and different levels of insulin resistance in different tissues like adipose cells, hepatocytes and skeletal muscle[52].

Low grade endotoxemia, increased absorption of endotoxins through various mechanisms can lead to low grade inflammation in host body and could be another route through which intestinal flora influence insulin resistance. This also explains relative abundance of gram negative microbes compared with gram positive in diabetic patients[50].

Trimethylamine-N-Oxide (TMAO). Microbial metabolism of phosphatidylcholine may play a role in atheroprogression. Phosphatidylcholine, a phospholipid integral to cell membranes is present in higher-fat foods. Gut microbiota releases choline from dietary phosphatidylcholine which is then metabolized to trimethylamine (TMA). TMA is transported to the liver via the portal vein where it is oxidized by flavin monooxygenase-3 to trimethylamine - Noxide (TMAO). Wang et al. showed that increasing levels of plasma TMAO, choline, and betaine had dose-dependent relationships with the presence of CVD in a cohort of 1,876 men and women, after controlling for established risk factors and medication use[53].

Development of Obesity and Gut Microbiota

Obesity which is excess deposition of adipose tissue was so far believed to be a result of classical risk factors like sedentary lifestyle and excess intake of energy rich food. But recently one more environmental factor is being studied as a contributor to obesity. That is gut microflora. The types of microbes and their products both are shown to have potential to cause obesity though a few contradictory studies have also been reported.

Several studies have reported different composition of microbe phyla in obese when compared with lean individuals. They have shown that there is relative low abundance of intestinal Bacteroidetes and high abundance of Firmicutes in obese as compared with lean individuals. The same findings are also reflected in animal studies obese models[54,55]. Zhang et al. found that obesity was associated withan increase of family Prevotellaceae in morbidly obese persons posted for bariatric surgery. He further concluded that the level of prevotellaceae fell down to lean levels after bariatric surgery[56].

EVENTS ALTERING GUT MICROBIOTA

Use of antibiotics

After treatment with antibiotics, colonization with foreign microbes is observed, that leads to permanent changes in the structure of the microbiota. This alteration can even cause diseases[32]. The altered taxa are different among individuals, some of them are not able to recover months after treatment and there is decrease in the bacterial diversity in most of the cases studied[57].

Cho et al studied the effect of antibiotic use in infant mice and concluded that there is a possibility of modulation of infant gut microbiome after the use of antibiotics and these changes can have long term consequences affecting adiposity and bone development[58].

Interestingly, a recently published study of 64 000 children has reported a 1.1-fold increased risk of obesity at age 2-5 years among children receiving antibiotics four or more times during their first 2 years of life, but, no association was observed between narrow-spectrum antibiotics and obesity. Penicillin and vancomycin have shown to worsen insulin sensitivity.

Type of food

It remains a major modulator of gut microbiota since birth. Breast feeding vs formula fed infants, high/ low fiber diet, high fat diet all influence the composition of gut microbes. A low-fat, high-fibre diet has been linked to a more diverse gut microbiota compared with a diet rich in fat and low in fibre[41], and it has been demonstrated that the human gut microbiota is capable of adapting to a shift from a plant-based diet to an animal based diet and vice versa within only 2 to 4 days[59].

Use of Alcohol and Smoking

Alcohol seems to increase permeability of enterocytes for endotoxins and altered gut microbiota in composition as well as function. But studies on smoking and gut microbes are inconclusive[59].

SUMMARY

Gut microbes and human host have strong biological association. Alterations in host conditions, including ingested food and use of antibiotics can alter the microbes and their metabolism and in turn would affect the host metabolism.

Short chain fatty acids produced by gut microbes and their relative proportion affect the host response significantly. This in turn affects various adipokines and cytokines which lead to decrease in insulin sensitvity and worsening of insulin resistance. Gut microbes also affect the secretion of gut peptides. This in addition to the LPS present in the gram negative bacteria of the gut flora increase insulin resistance and obesity.

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