EDITORIAL

New-found link between microbiota and obesity

Chandra Kanti Chakraborti

Chandra Kanti Chakraborti, Department of Pharmacology, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela 769015, Orissa, India

Correspondence to: Dr. Chandra Kanti Chakraborti, Professor, Department of Pharmacology, Kanak Manjari Institute of Pharmaceutical Sciences, Chhend, Rourkela 769015, Orissa, India. chandrakanti_12@rediffmail.com
Telephone: +91-661-2480752
Fax: +91-661-2480752

Abstract

Due to the grave pathological role of obesity, worldwide research is being continued to find out the causative factors involved in it. Recent advances in this field reveal a possible relationship between the compositional pattern of gut microbiota and genesis of obesity. Several study results have shown that short-chain fatty acids (SCFAs, microbiota-induced fermentation products) and lipopolysaccharides (LPS, an integral component of Gram negative microorganisms) play the key role in linking the two. Though several SCFAs are produced as microbiota-fermentation products, three of them, *i.e.*, butyrate, propionate and acetate have been found to be definitely involved in obesity; though individually they are neither purely obesogenic nor antiobesogenic. Out of these, butyrate and propionate are predominantly antiobesogenic. Butyrate, though a major energy source for colonocytes, has been found to increase mitochondrial activity, prevent metabolic endotoxemia, improve insulin sensitivity, possess anti-inflammatory potential, increase intestinal barrier function and protect against diet-induced obesity without causing hypophagia. Propionate has been found to inhibit cholesterol synthesis, thereby antagonizing the cholesterol increasing action of acetate, and to inhibit the expression of resistin in adipocytes. Moreover, both these SCFAs have been found to cause weight regulation through their stimulatory effect on anorexigenic gut hormones and to increase the synthesis of leptin. Unlike butyrate and propionate, acetate, which is substantially absorbed, shows more obesogenic potential, as it acts as a substrate for hepatic and adipocyte lipogenesis. High fat diet increases the absorption of LPS, which, in turn, has been found to be associated with metabolic endotoxemia and to induce inflammation resulting in obesity. Multiple independent and interrelated mechanisms have been found to be involved in such linking processes which are discussed in this review work along with some possible remedial measures for prevention of weight gain and obesity.

Key words: Microbiota; Obesity; Butyrate; Propionate; Acetate

Core tip: The objective of this article is to relate gastrointestinal microbiota with obesity positively. This idea itself is most innovative. In this article, probable mechanisms involved in relating microbiota with obesity

have been discussed. Its key findings are: (1) The gut microbiota play a definite role both in genesis and retardation of obesity; (2) Microbiota-derived lipopoly-saccharides and short-chain fatty acids mediate the obesogenic action; (3) Fatty diet not only adds calories but also shifts microbiota compositional pattern in favour of obesity; and (4) The obesogenic actions are mediated through receptor activation, modification of cytokine and endocrine function and gene expression.

INTRODUCTION

Obesity, in both males and females, was considered simply as a negative criterion while assessing beauty. But recently, in addition to its previous role, it is considered to be an important marker for several diseases; particularly, hypertension, type 2 diabetes mellitus (T2DM) and metabolic syndrome where it plays a definite and significant pathological role[1,2]. Multiple etiological factors have been attributed to the genesis of obesity, of which hereditary predisposition, wrong dietary habits (fatty food) and life-style (lack of exercise) are important[3]. Besides these, certain hormonal imbalances[4] and side-effects of some drugs[5] also contribute towards its development. But unfortunately there are many obese individuals, in whom, these causative factors fail to explain the cause of their obesity. Therefore, because of its grave pathological role, research is still going on to find out the factors other than the above-mentioned ones, so that a remedial measure can be taken to prevent the development as well as progression of this worldwide epidemic[6].

Recently, it has been observed that the composition of gut microbiota of healthy persons is different from that of obese T2DM patients. Such observations suggested a possible relationship between the compositional pattern of gut microbiota and pathology of metabolic disorders. Human colon harbours a vast number of microorganisms which are extremely diverse. Out of these, three phyla, *Bacteroidetes* (Gram negative), *Firmicutes* (Gram positive) and *Actinobacteria* (Gram positive), are most abundant and have been found to play a dominant role in the pathophysiology of metabolic disorders - specifically, obesity. Other phyla also contribute, but to a lesser degree[6]. All these colonic microbiota cause fermentation of nondigestible carbohydrates resulting in the formation of short-chain fatty acids (SCFAs) along with gases like CO_2 and H_2[7]. It has been shown that acetate and propionate are mainly produced by the phylum *Bacteroidetes*, whereas butyrate is the predominant product of the phylum *Firmicutes*[6]. Of these SCFAs, butyrate mainly serves as an energy source for colonic epithelium[8,9], whereas propionate, getting absorbed through portal circulation, takes part in gluconeogenesis[8]. Acetate, on the other hand, reaches peripheral tissues after absorption through systemic circulation where it acts as a substrate for synthesis of cholesterol[8,10,11]. Butyrate, besides being an energy source for colonocytes, has been found to increase insulin sensitivity (in mice)[9], possesses obesity-related antiinflammatory action (in humans)[12], can give protection against diet-induced obesity without causing hypophagia[13], may protect against colon carcinoma[8,10], and increase the leptin gene expression[11]. Propionate, in addition to contributing towards gluconeogenesis, also reduces the intake of food[13] and cholesterol synthesis[11] along with a favorable effect on leptin gene expression[11]. Acetate, in addition to serving as a substrate for synthesis of cholesterol, also takes part in the *de novo* synthesis of lipids in liver[14]. Because of the above-mentioned functions of the microbiota-derived SCFAs, which appear to be closely related to obesity, both adversely as well as beneficially, an attempt has been made to review the work-results of several prominent investigators in this field, which may shed a light on the justification of "linking microbiota to obesity".

MICROBIOTA IN NORMAL GUT AND OBESITY

Microbiota in normal gut
The gut harbours the greatest density of microorganisms in the body (*e.g.*, about up to 1.5 kg of bacteria in the human gut) with *Firmicutes*, *Bacteriodetes* and *Actinobacteria* constituting the dominant phyla[7,15,16]. Generally, *Firmicutes* and *Bacteroidetes* are most abundant, followed by *Proteobacteria* and *Actinobacteria* with minor contributors like *Verrucomicrobia* and *Fusobacteria*[16].

Faecalibacterium prausnitzii (*F. prausnitzii*) is the most abundant bacterium in the human intestinal microbiota of healthy adults (Table 1). It represents more than 5% of the total bacterial population. *F. prausnitzii* species is a major representative of *Firmicutes* phylum, *Clostridium* class, *Ruminococcaceae* family[17]. While the *Bacteroidetes* phylum mainly produces acetate and propionate, the *Firmicutes* phylum has butyrate as its primary metabolic end product[7].

Microbiota in obesity
Gut microbiota have been found to be significantly changed in humans and animal models of obesity, comprising a decrease in bacterial diversity[15,18] as well as composition, such as a reduced abundance of *Bacteroidetes* with a proportional increase in *Firmicutes* phylum[6,9,18-21].

In obese animals, Ley *et al*[22] found a difference in the ratio of *Bacteroidetes* and *Firmicutes*, where the obese mice displayed a decrease in *Bacteroidetes* with a corresponding increase in *Firmicutes* in comparison to their counterparts. In agreement with the results from

Table 1 Prevalence of gut microbiota in health and disease	
Microbiota in normal gut	**Microbiota in obesity**
Firmicutes phylum	Increase in *Firmicutes* phylum
Bacteriodetes phylum	Reduced abundance of *Bacteroidetes*
Actinobacteria phylum	A higher level of *Actinobacteria* phylum
Verrucomicrobia phylum	Lower proportion of *Verrucomicrobia*
Faecalibacterium prausnitzii species	Reduced abundance of *Faecalibacterium prausnitzii* species

animal studies, it seems that human obesity is linked with a reduced abundance of intestinal *Bacteroidetes* associated with a high abundance of *Firmicutes*. However, these results have been contradicted by other studies[11]. Studies of Duncan *et al*[23] did not show any difference in the proportions of *Bacteroidetes* and *Firmicutes* in the feces of lean and obese subjects. In another investigation, overweight and obese subjects had a ratio of *Bacteroidetes* to *Firmicutes* in favour of *Bacteroidetes*. Moreover, many authors have shown no change or even an increase of *Bacteroidetes* in overweight[6]. Besides these two phyla, a higher level of *Actinobacteria* has been demonstrated in obese persons[24]. On the other hand, Clarke *et al*[25] reported that the gut microbiota of obese individuals contained a lower proportion *Verrucomicrobia*, *i.e.,* abundance of this phylum in the gut is reduced in obese persons (Table 1). From these observations, it appears that the phylum level difference of the gut microbiota between obese and lean individuals may not be universally true[11]. But overall analysis of results point towards an increase in *Firmicutes*[6].

Methane-producing *Archaea*, a domain of single-celled microorganism, have been found to be present in greater abundance in obese mice and humans compared with lean subjects. Recently, in an investigation, germ-free mice were colonized with *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) (an adaptive bacterial forager of dietary polysaccharides) alone or either with *Methanobrevibacter smithii* (*M. smithii*) or the sulfate-reducing bacterium *Desulfovibrio piger* (*D. piger*). The results showed that cocolonization with *M. smithii* but not *D. piger,* induced *B. thetaiotaomicron* to ferment dietary fructans to acetate, resulting in a significant increase in host adiposity compared with monocolonized or *B. thetaiotaomicron*/*D. piger* cocolonized mice[20].

In an investigation, the numbers of hydrogen-producing *Prevotellaceae,* a family in the phylum *Bacteroidetes*, and *Archaea*, represented primarily by members of the order *Methanobacteriales* (hydrogen-oxidizing methanogens), were at a higher level in obese individuals compared with lean subjects and with those after gastric bypass. The investigators hypothesized that hydrogen transfer between bacterial and archaeal species may raise energy uptake by the large intestine in obese individuals *via* methanogens removing fermentation intermediates, such as H_2 or formate, thus relieving thermodynamic limitations and allowing greater production of SCFAs that are then available to be absorbed across the intestinal

epithelium[20]. On the contrary, Schwiertz *et al*[26] found no difference in the abundance of *Archaea* in overweight or obese humans, which brings into question the usefulness of *Archaea* as a potential biomarker of obesity.

The intestines of obese humans and mice have been found to be enriched with *Erysipelotrichi*, a class of bacteria within the phylum *Firmicutes*, and *Clostridium ramosum* (*C. ramosum*), a member of the *Erysipelotrichi*, is found to be linked with symptoms of the metabolic syndrome in humans. Thus, Woting *et al*[27] speculated that *C. ramosum* promotes obesity and related pathologies.

Obese children were found to display an elevated *Firmicutes*-to-*Bacteroidetes* ratio compared with their lean counterparts. Furthermore, low relative proportions of *Bacteroides vulgatus* and high concentrations of *Lactobacillus* spp. were found in the obese children and were positively correlated with plasma high-sensitivity C-reactive protein[21]. Million *et al*[28] have shown that *Lactobacillus reuteri* was linked with obesity in adults. These results thus indicate a possible role of *Lactobacillus* species in body weight and obesity. Moreover, *Staphylococcus* spp. were found to be positively linked with energy intake in all children[21].

Obese-prone (OP) donor and germ-free recipient animals have been found to harbour specific species from *Oscillibacter* and *Clostridium* clusters XIVa and IV, which were totally absent from their obese-resistant counterparts. Indeed, Duca *et al*[18] have reported high levels of bacteria from the *Ruminococcus* genus in OP rats, similar to that found in obese humans and high fat-fed mice. It is known that *Ruminococcus* is phylogenetically heterogenous, and most of its species fall under several *Clostridium* clusters, including *Clostridium* clusters IV and XIVa. But peculiarly, *Clostridium leptum* (cluster IV) has been found to be associated with both obesity and weight loss (Table 2). From the above discussion, it may be mentioned that unfavourable microbiome seems to be a predisposing factor for development of obesity.

While some gut bacteria groups correlated with energy intake, obesity, and metabolic changes, others, such as *F. prausnitzii*, linked with alteration in the inflammatory state and diabetes[29]. The presence of *F. prausnitzii* species is directly associated with the reduction in low-grade inflammation state in obesity and diabetes (independently of calorie intake)[17,29] (Table 1).

SCFAs

It is well established that the human intestine harbours a vast number of microorganisms, known as gut microbiota, whose metabolic end products (mainly SCFAs) interfere with the absorption of digestion end products as well as energy homeostasis of the host[19].

In intestine, the sites of production of SCFAs are distal small intestine and colon where nondigestible carbohydrates like resistant starch, dietary fiber, and other low-digestible polysaccharides are fermented

Table 2 Microbiota having doubtful role in obesity

Microbiota
Archaea (a domain of microorganisms)
Phylum *Firmicutes*: *Erysipelotrichi* (a class of bacteria)
Methanobacteriales (an order of bacteria)
Prevotellaceae (a family of bacteria)
Ruminococcus (a genus of bacteria)
Bacteroides thetaiotaomicron and *Methanobrevibacter smithii*
Clostridium ramosum (a member of the *Erysipelotrichi*)
Clustridium leptum (cluster IV) (associated with both obesity and weight loss)
Specific species from *Oscillibacter* and *Clostridium* clusters XIVa and IV
Lactobacillus spp. - *Lactobacillus reuteri*

by the saccharolytic bacteria which include the phyla *Bacteroidetes*, *Firmicutes* and *Actinobacteria*. Acetate and propionate are the main products of *Bacteroidetes* phylum and butyrate is mainly produced by *Firmicutes* phylum. Most bacterial activity is found in the proximal colon where substrate availability is the highest. But towards the distal colon, the availability of substrate decreases, and the extraction of free water lowers the diffusion of substrates and microbial products. This makes the proximal colon to be the principal site of fermentation, where, mainly nondigestible carbohydrates are fermented by saccharolytic bacteria, primary fermenters being *Bacteroidetes* and the main fermentation products are SCFAs together with gases like CO_2 and H_2[7]. Of the three SCFAs, butyrate is practically considered as a favourable marker (antiobesity) of obesity and its amount of production is determined by the composition of microbiota, population of the microorganisms producing it and the pH of the large intestine. Change in substrate bioavailability can alter the composition of butyrate-producing bacterial population and thus affect butyrate production[8]. It has been demonstrated that when the human fecal pH is 5.5; butyrate producing bacterial population (*Firmicutes* phylum) comprises 20% of the total bacterial population. But in the distal parts of large intestine, where fermentable dietary fiber availability is limited, the luminal pH is raised to 6.5. At this site, not only the bacteria producing butyrate, practically disappear completely, but also there occurs a significant increase in the population of acetate- and propionate-producing bacteria, whose products are mainly obesogenic[7].

An analysis of the population data regarding the production of SCFAs in proximal and distal colon shows that the production is in the order of acetate > propionate > butyrate. When calculated in a molar ratio, it was found to be 60:20:20 or 3:1:1, respectively[10]. It has been observed that out of the total SCFAs present in the colon, 90%-95% are constituted by acetate, propionate and butyrate together and their intraluminal individual concentrations have been found to be acetate 60%, propionate 25% and butyrate 15%[30].

After being produced in the colon, the above-mentioned three SCFAs are absorbed through gut epithelial cells but follow different patterns of absorption, distribution, metabolism and function. A substantial part of acetate is readily absorbed, reaches liver *via* portal circulation and subsequently, distributed throughout the whole body where it serves as a substrate for synthesis of cholesterol[11,13]. Because of the substantial absorption, plasma concentration of acetate is much more than the other two[30] and a small amount is available in the colon to be metabolized[10].

Propionate, like acetate, also reaches liver *via* portal circulation after absorption; but because of its primary utilization in gluconeogenesis (in the liver), its plasma concentration is less than that of acetate[10,11,30]. Butyrate, on the other hand, undergoes limited reabsorption, because it is primarily oxidized by the colonocytes and serves as a major source of energy for them[8,9,30].

It seems essential to mention here that absorption of these SCFAs through colonic epithelial cells alters the pH of colon, which in turn has an important influence on the composition and population of gut microbiota. It is so, because most of the SCFAs are absorbed in the colon being exchanged with bicarbonate and hence, the resultant luminal pH is determined by the rate of SCFA production by microbiota and the neutralizing capability of the bicarbonate. Due to its continuous absorption, decline in SCFA concentration from proximal to distal colon leads to a corresponding increase in pH from cecum to rectum. It has been demonstrated in animal and human fecal studies that gut pH has an important effect on the growth and composition of gut microbiota. Low luminal pH from ileum to cecum due to higher SCFA concentration, prevents the overgrowth of pH-sensitive pathogenic bacteria (like *Enterobacteriaceae* and *Clostridia*) and at pH 5.5, butyrate producing bacteria (*Firmicutes* phylum) comprise 20% of the total population (mentioned earlier). But as the luminal pH increases to 6.5 in more distal colonic sites due to less production of SCFAs (as fermentable dietary fibers are less available here) and their absorption in exchange with bicarbonate, the butyrate producing bacteria practically disappear along with a concomitant rise in acetate and propionate-producing bacteria (*Bacteroidetes* phylum)[7].

A detailed discussion has been made above about the multiple bacterial phyla producing several metabolites, of which three SCFAs play a dominant role in the development, progression as well as retardation of obesity. These three SCFAs are butyrate, propionate and acetate, produced during the fermentation of complex dietary carbohydrates (polysaccharides and oligosaccharides), proteins, peptides, and glycoprotein precursors by the microbiota in the colon and distal small intestine[10,11,13]. Chemically, SCFAs are saturated aliphatic organic acids containing one to six carbons (Acetate C2, propionate C3 and butyrate C4)[7].

FACTORS CONTRIBUTING TOWARDS GENESIS OF OBESITY

Besides the well known and established causes of

obesity like genetic predisposition, excessive intake of high calorigenic diet (fatty food) and lack of exercise[3] which favours storage of calorie in the form of fat in adipocytes, recently researchers in the field have shown the contribution and involvement of several other factors, like hormonal imbalance[4]; inflammatory cytokines of adipocyte and nonadipocyte origin; adipocytokines like adiponectin[31], leptin[32], and resistin[33], etc., toll-like receptors (TLR)[34] and many others in the genesis of obesity[33].

In addition to these, multiple study results have shown a close link between the compositional patterns of "intestinal microbiota" and "obesity"- the microbiota affecting the above - mentioned obesogenic factors through several mechanisms. A detailed account of the microbiota with their composition and population ratio and their metabolic end products (particularly SCFAs), have already been discussed. Here, an attempt has been made to discuss the various mechanisms involved in their obesogenic as well as antiobesity activity, although some of the observations appear to be controversial and inconclusive.

Though intestinal microflora comprises several phyla of microorganisms, focus has been made on three phyla, namely Bacteroidetes, Firmicutes and Actinobacteria. These three phyla generate multiple metabolites out of which three SCFAs - butyrate, acetate and propionate have been shown to be definitely related with obesity. It may be mentioned in the beginning that none of these bacterial phyla is purely obesogenic or antiobesogenic. This is so, because individually they produce more than one SCFA, each of which possessing opposite actions as metabolites, which in turn possesses both the actions[7].

For this reason, while evaluating their obesogenic or the antiobesogenic potency, instead of taking the population of a single bacterial phylum, the population ratio of more than one phylum has been taken into consideration[6,7,19,21]. Several metabolic studies have suggested that imbalances in the intestinal bacterial population may result in obesity, systemic inflammation and metabolic dysfunction[14,35].

Gut microflora are involved in obesity through some of their constitutive structural materials and through some of their metabolic end products (SCFAs). Therefore, the mechanisms by which they contribute towards the development of obesity may be discussed under two headings: (1) The role of lipopolysaccharide (LPS) which is a structural component of bacteria; and (2) the role of SCFAs which are produced as bacterial metabolites of dietary compounds[11,14].

Role of LPS

Recently, it has been shown that obesity is associated with a chronic and systemic low-grade inflammation which is due to an innate immune response to LPS. It is an intrinsic constituent of Gram negative bacterial cell wall. It is considered as an endotoxin and found at low concentrations in the blood of healthy persons. But substantially high concentrations of LPS have been

demonstrated in obese individuals, where the obesity is diet-induced and has a genetic predisposition. High fat diet, both in animals and humans, has been found to alter the gut microbiota composition (more in favour of Gram negative phylum), which in turn increases the production and intestinal permeability of LPS, resulting in its high plasma concentration and development of "metabolic endotoxemia"[20]. Cani et al[36] have found that compositional pattern of microbiota, induced by a high-fat diet, could increase gut permeability which is an important hallmark of endotoxemia. Such microbiota were found to reduce the expression of host genes which code for the intestinal tight junction proteins like ZO-1 and occludin - necessary for normal gastrointestinal permeability character. Such microbiota-induced altered gastrointestinal epithelial integrity could result in intestinal absorption of the whole bacteria along with their products. It has been observed that in mice, taking a high-fat, such bacterial absorption is higher than those taking a standard chow and was found to be reversed by administering an appropriate probiotic bacterium[37].

LPS has been found to induce inflammation resulting in development of obesity. In a comparative study, it has been shown that when low doses of LPS were administered to mice for 4 wk, they developed obesity similar to 4 wk of a high-fat diet. LPS-induced inflammatory reactions are mediated through an immunoprotein called cluster of differentiation (CD) 14. When LPS was administered through $CD14^{-/-}$ rats, there was no weight gain. It is interesting to note that high fat diet is not only directly responsible for obesity but also indirectly aggravates it by increasing the absorption of endotoxin LPS via lymph by integrating it to chylomicrons. As high fat diet in humans increases the formation of chylomicron, more chylomicron is available to be integrated with LPS and hence, more absorption of this endotoxin in comparison to low fat diet. Mice develop endotoxemia when they consume high fat diet. Studies have shown that when such mice were treated with ampicillin and neomycin, endotoxemia was found to be reduced because of the antimicrobial-induced altered gastrointestinal microbiota. High plasma concentration of LPS has been found to be associated with increased levels of CD14 and interleukin-6 (IL-6) - the markers of inflammation. Because of these observations it may be inferred that regular intake of high fat diet, increases LPS absorption into systemic circulation, resulting in LPS-induced inflammation and obesity[37].

Chronic low-grade inflammation found in endotoxemia has been demonstrated to be due to activation of TLR-4 by LPS and dietary saturated fatty acids. TLR-4 activation induces upregulation of common intracellular inflammatory pathways like c-Jun N-terminal kinase and nuclear factor-kappa B in adipocytes and macrophages resulting in development of insulin resistance and increased adiposity[6]. Mice, lacking TLR-4, have been found to be resistant to diet-induced obesity and insulin resistant[37].

de La Serre et al[38] have demonstrated that high-fat

diet not only alters the composition of gut microbiota, but also causes increased activation of intestinal TLR-4. Such receptor activation results in gastrointestinal inflammation which in turn induces hyperphagia and thus, makes the animal an obese phenotype.

A neural mechanism has been suggested to explain LPS-induced obesity, in which the vagal afferents of diet-induced obese rats are found to be leptin resistant, and thus, develop hyperphagia and weight gain, which in turn, lead to increased food (fat) intake and LPS production, thereby increasing obesity and aggravating the inflammation further[37].

As mentioned earlier, LPS, which induces inflammation and increases adiposity resulting in obesity, is known to be a Gram negative bacterial product. But, there are confusing observations, where obese persons have more *Firmicutes* (Gram positive) and less *Bacteroidetes* (Gram negative) than lean individuals. Inspite of such confusions, recent observations show that obese person's microbiota are rich in *Prevotellaceae* (a subgroup of *Bacteroidetes*), which is a good source of LPS[37].

Though microbiota-constituent LPS is proinflammatory, some microbiota metabolite SCFAs possess immunoregulatory property and reduce inflammation. Studies have shown butyrate to have antiinflammatory action through inhibition of lymphocyte proliferation, and IL-2 and interferon-γ production. On the other hand, acetate and propionate increase interferon-γ level. The resultant effect of these three SCFAs is immunoregulatory[37].

Role of SCFAs

It has already been mentioned about the production of three SCFAs by different phyla of gastrointestinal microflora[7] and the obesogenic as well as antiobesogenic property of individual SCFAs which make it difficult to categories each of them as purely obesogenic or antiobesogenic. Of course, a broad characterization can be made where acetate appears to be predominantly obesogenic, whereas butyrate and propionate are mainly antiobesogenic[8-13,39].

Interesting and novel mechanisms have been found to be involved in the causation and prevention of obesity by the above-mentioned three SCFAs. It may be convenient to mention the contribution of individual SCFAs towards the genesis as well as prevention of obesity and subsequently, discuss the underlying mechanisms involved in such actions: (1) Butyrate has been found to be a major energy source for colonocytes[8-11,13,30]. In the colonocyte-mitochondria 70% to 90% of the SCFA (butyrate)[10] is oxidized into acetyl-CoA, which is subsequently processed through tricarboxylic acid cycle to generate large quantity of ATP[8]. It has been shown that in addition to producing butyrate, the butyrate-producing microbes also increase the expression of the enzymes taking part in the colonocyte-mitochondrial SCFA-oxidative reactions[9]; (2)

Besides supplying energy, butyrate also has a controlling role over the mechanisms involved in cellular apoptosis, proliferation and differentiation[10]; (3) Butyrate has been shown to possess some mixed metabolic effects which include an increase in mitochondrial activity, prevention of metabolic endotoxemia and activation of intestinal gluconeogenesis. These actions are mediated through gene expression and regulation of hormonal activity[9]; (4) Butyrate, when given orally to mice, has been found to improve insulin sensitivity and increase energy expenditure by improving mitochondrial function which may result in reduction of obesity[9]; (5) Some studies have indicated the antiinflammatory potential of butyrate which may contribute towards a decrease in obesity-associated metabolic complication, because of its capability to increase intestinal barrier function[12]. These effects of butyrate support the observation that decreased population of intestinal butyrate producing bacteria is associated with metabolic risk in humans; (6) Butyrate has been found to be protective against diet-induced obesity without causing hypophagia. Acetate which is considered as obesogenic, also possesses this beneficial function like butyrate[13]; (7) Butyrate and propionate (beneficial SCFAs) cause weight regulation at least partially by controlling food intake; the action appears to be mediated through their stimulatory effect on the anorexigenic gut hormones. It may be mentioned here that acetate also inhibits weight gain, but through mechanisms which are independent of suppression of food intake and acute gut hormone effect[13]; (8) Xiong *et al*[40] had demonstrated the potential of butyrate and propionate to increase the expression of the gene coding for synthesis of leptin (Table 3); and (9) Besides these antiobesogenic properties, both butyrate and propionate have been shown to possess a definite protective role against colon carcinogenesis[8,10].

Like butyrate, propionate also possesses favourable some effects in obesity. They are as follows: (1) The SCFA has been found to reduce food intake and regulate body weight, similar to butyrate[13]; (2) It decreases cholesterol synthesis by inhibiting the activity of the enzyme acetyl-CoA synthetase (the enzyme converts acetate to acetyl-CoA), thereby antagonizing the cholesterol increasing action of acetate[10,11]; (3) Moreover, propionate has been found to be a precursor for gluconeogenesis in the liver[10,14]. This may decrease the hepatic synthesis of cholesterol because fatty acids necessary for cholesterol synthesis are diverted towards synthesis of glucose (gluconeogenesis)[14]; (4) It has been shown that like butyrate, propionate also stimulates the formation of the anorexigenic hormone leptin[40] (Table 3); and (5) However, propionate inhibits the expression of resistin in human adipose tissue[39].

Of all the three SCFAs, acetate seems to be more obesogenic than butyrate and propionate because: (1) It is a substrate for lipogenesis[8,14] and cholesterol synthesis in liver and other tissues[8,11]. This SCFA is readily and substantially absorbed by the colonocytes and though,

Table 3 Gross mechanisms involved in short-chain fatty acids-induced obesity
Butyrate
A major energy source for colonocytes
Involved in cellular apoptosis, proliferation and differentiation
Possesses metabolic effects like increase in mitochondrial activity, prevention of metabolic endotoxemia and activation of intestinal gluconeogenesis
Improves insulin sensitivity and increases energy expenditure by improving mitochondrial function resulting in reduction of obesity
Increases intestinal barrier function - an antiinflammatory potential
Protects against diet-induced obesity without causing hypophagia - the action being mediated through stimulation of anorexigenic gut hormones
Increases the expression of the gene coding for synthesis of leptin
Propionate
Increases the expression of the gene coding for synthesis of leptin
Protects against diet-induced obesity without causing hypophagia - the mechanism being similar to butyrate
Decreases cholesterol synthesis by inhibiting the activity of the enzyme acetyl-CoA synthetase
Acts as a precursor for hepatic gluconeogenesis thereby decreasing the hepatic synthesis of cholesterol
Inhibits the expression of resistin in human adipose tissue
Acetate
Acts as a substrate for lipogenesis and cholesterol synthesis in liver and other tissues
Gives protection against diet-induced obesity without causing hypophagia

some part of it is utilized in the liver for lipogenesis, a significant amount reaches systemic circulation and is delivered to the peripheral tissues[13] for synthesis of cholesterol (specifically in adipose tissues and mammary glands, whose cytosol contains acetyl-CoA synthetase, the enzyme essential for utilization of acetate for lipogenesis)[10]. Human studies have shown that when lactulose (synthetic nonabsorbable sugar, metabolized by microbiota to produce high amounts of acetate) was administered to the diets of six volunteers for two weeks, there was a significant increase in both total and low-density lipoprotein cholesterol, apolipoprotein B and plasma concentration of acetate in comparison to the control group[11]; and (2) Though predominantly obesogenic, some workers have demonstrated the obesity-protecting role of acetate, which is less than that of butyrate and propionate. Like butyrate, it gives protection against diet-induced obesity without causing hypophagia and thus, the action is independent of suppression of food intake and does not have any acute effect on gut hormones[13] (Table 3). It has been demonstrated that acetate increases cholesterol synthesis and propionate, though regulates it, does not affect serum cholesterol levels[8,11] and is primarily utilized for gluconeogenesis[8,10,14]. But when the two SCFAs are administered simultaneously, serum cholesterol level does not rise[11]. This may be due to increased gluconeogenesis by propionate consuming more fatty acids and thus diverting them from getting utilized by acetate for synthesis of cholesterol[14]. Therefore, though acetate increases fatty acid synthesis, they take part in gluconeogenesis rather than being used for synthesis of cholesterol and thus, plasma level of cholesterol does not rise[10,11].

It has been demonstrated that fecal concentration of SCFAs are 20% higher in obese individuals than their lean counterparts. But such higher SCFAs concentration in feces may reflect a compensatory protective mechanism against obesity, in which a greater amount is eliminated from the increased amount of SCFAs produced, thereby preventing increased accumulation

of SCFA in the intestinal lumen for obesogenic action[37].

MECHANISM OF ACTION OF SCFAS AT THE MOLECULAR LEVEL

Some important actions of these three SCFAs have been found to be mediated through activation of endogenous free fatty acid receptor (FFAR) like FFAR2 and FFAR3 which are otherwise designated as Gpr43 and Gpr41, respectively, because they belong to G-protein coupled receptor family of receptors[13,37]. Presence of both these receptors has been demonstrated in adipocytes, epithelial cells and enteroendocrine cells. Activation of these two receptors leads to an increase in expression of satiety hormone polypeptide YY (PYY) and increase in intestinal motility. In addition to the above effect, Gpr41 activation also increases the expression of leptin in adipocytes. It has been observed that when SCFA-producing bacteria were administered to germ-free mice, the mice gained weight along with an increase in body fat. But, mice (both germ-free and conventional), deficient in Gpr41 did not show such effects. Such observation indicates that weight gain occurs through activation of Gpr41[37]. Moreover, Samuel et al[41] have shown that the expression of PYY in the above-mentioned mice was lower in the mice with intact Gpr41. Reduced production of PYY leads to decreased gut motility and hence, decreased dietary energy harvest[19]. Besides increasing leptin expression in adipocytes, Gpr41 activation also increases hepatic lipogenesis. Hence, this receptor is considered as a probable regulator of energy balance of the host[37].

SCFAs, like butyrate and propionate, increase the formation of the gut hormone glucagon-like peptide-1 (GLP-1). It reduces food intake by decreasing appetite. Maximal induction of GLP-1 requires activation of Gpr41, but is not essential[13].

Nondigestible carbohydrates (NDC) are known to be antiobesogenic because they are not digested in the intestine but are fermented in the large bowel resulting in the formation of SCFAs. Ultimately, they (SCFAs) mediate some of the antiobesogenic actions of NDC.

Propionate stimulates Gpr43 in caloric enteroendocrine cells leading to increased release of PYY and GLP-1 (anorexigenic gut hormones). It also activates Gpr43 in adipocytes, which reduces output of FFAs into circulation and thus, it results in increased insulin sensitivity. Hence, the formation of more propionate in the colon, by consuming NDC, may be beneficial in obesity[42].

However, food rich in fermentable fibers are seemed to stimulate obesity through harvested energy by their SCFAs (metabolites). But epidemiological study results suggest that they prevent it rather than promoting. It may be explained by the fact that these SCFAs, by stimulating FFARs, cause satiety *via* increased production of GLP-1 and PYY[18]. Thus, they are not obesogenic[14,18].

Certain study results have shown that mirobiota-derived SCFAs modulate (increase) the secretion and gene expression of *GLP-1* and *PYY* which are known to be satiety hormones[18,37]. Fasting-induced adipocyte factor (Fiaf) has been found to suppress the production of adipocyte-LPL (hormone sensitive lipase) which leads to an increase in lipolysis of triglycerides in adipocytes and modulation of fatty acid oxidation in adipocytes and skeletal muscles. It has been shown that physiological appetite regulators regulate the expression of Fiaf in the hypothalamus and exert their anorexigenic effect through inhibition of hypothalamic AMP-activated protein kinase (AMPK) activity. This suggests a central regulatory role of Fiaf in energy metabolism[43].

Investigations on germ-free and conventionalized mice have shown that one of the mechanisms of energy harvest and adipocyte hypertrophy by microbiota is through inhibition of enterocyte Fiaf, leading to suppression of the actions of intestinal LPL and increased activity of PYY[19,44].

Metabolic degradation of a given source of energy is more with *Firmicutes* than with *Bacteroidetes*, resulting in increased absorption of calories and hence more weight gain[45]. Increased population of *Firmicutes* has been found to raise the number of lipid droplets, thereby proportionately intensifying fatty acid absorption[46]. Such a finding seems to involve several mechanisms. Microbiota may increase the metabolism of the host along with modification and increase in bile salt production. It favours more fatty acid (FA) absorption and hence, increased bioavailability[47]. In addition, intestinal microbes may directly prevent the lipolytic activities of the host[48]. They may indirectly change the physiological responses in the gut of the host, resulting in increased absorption. Finally, microbes may lower the rate of FA oxidation, which increases FA absorption[46]. In addition to these, *Firmicutes*-induced increased FA absorption may involve other specific mechanisms[45].

Methanogen, like *M. smithii* is found in 70% of human beings. It generates methane through anaerobic fermentation. It has been found to enhance the fermentation of polysaccharides and other carbohydrates by removing hydrogen atoms, leading to greater production of SCFAs and hence, their increased absorption. These SCFAs function as an extra source of energy which contributes towards weight gain and subsequent

obesity[49].

Some gastrointestinal microbiota-components have been found to suppress the expression of the host genes which code for the synthesis of intestinal epithelial tight junction proteins and Fiaf, leading to increased adipocyte lipoprotein lipase (LPL) activity and hence, increased storage of liver-derived triglyceride in host fat cells and weight gain[22,37,43]. Interesting experiments on mice has been conducted to demonstrate the combined effect of microbiota and diet resulting in development of obesity. When mice reared in germ-free environment (hence absence of gastro-intestinal microbiota) were fed with a western-style diet (high fat, high sugar), they did not gain weight as compared with colonized mice with similar diet. This may be due to suppression of microbiota-induced gene expression and hence, inhibition of Fiaf formation resulting in increased fat metabolism, lower fat storage and decreased sugar absorption. Such altered lipid metabolism and storage is supported by the fact that germ-free mice were having higher levels of Fiaf and hence, lower LPL activity, higher muscle and hepatic levels of the key enzyme (phosphorylated AMPK) necessary for β-oxidation and lesser monosaccharide absorption from the intestine in comparison with colonised mice[37]. Thus, gut microbiota may be considered as an important environmental factor increasing dietary energy harvest and energy storage in the host[19]. But such observations may not be taken conclusive, because another study has demonstrated that germ-free mice significantly gained weight with western-style diet[37].

It has been shown that in the mucosa of small intestine of gnotobiotic mice, who harbour intestinal *C. ramosum,* there is upregulation of Glut2 and CD36 transcription. It suggests that this organism is responsible for more gain in body fat by an increase in intestinal absorption of glucose and lipid[27].

It may be mentioned here that though bacterial product LPS disrupts normal gastrointestinal integrity, bacterial SCFA metabolites acetate and butyrate strengthen it by increasing the secretion of mucin-2 (MUC-2) - the mucus secreted by goblet cells, which plays an important role to maintain healthy intestinal epithelial barrier. It has been shown that butyrate, when added to goblet cell lines, increased the secretion of MUC-2 23-fold and, thus, considered as a protective SCFA against intestinal translocation of bacteria and their products[37].

As mentioned earlier, acetate is known to be obesogenic because of its peripheral action. However, it has been shown that it can also control weight gain through its central action, where it produces an anorexigenic signal in the hypothalamic arcuate nucleus, through increased generation of gamma-aminobutyric acid (GABA), by augmenting the glutamate-glutamine (transcellular) cycle involved in GABA production[50].

CONCLUSION

The beneficial role of gastrointestinal microbiota for maintenance of proper health of the host is well

established. From the above discussion, it seems that out of the millions of species harbouring the gastrointestinal tract, only a few are linked with the genesis of obesity. Moreover, individual species of these is not harmful entirely; each of them possessing obesogenic as well as antiobesogenic property, for which, ratio of two species (like *Firmicutes* and *Bacteroidetes*) are taken into consideration when grouping them into harmful or beneficial group. Several researchers have observed that it is the dietary habit (fatty food) of the host which alters the population and composition of the microbiome, thereby shifting the ratio of the concerned pair in favour of obesity. Hence, by altering the nature of the diet (less fat and more NDC), an individual, in addition to reducing the total calorie intake, may also be able to shift the ratio in the opposite direction (antiobesity).

As one of the causes of obesity has been attributed due to the structural components (LPS) and metabolic end products (SCFAs) of certain gastrointestinal microorganisms, it is not wrong to consider obesity (at least partially, if not fully) as an infectious disease. Further research in this respect is needed to confirm this possibility and to find out selective chemotherapeutic agents, which will reduce or abolish the more harmful bacterial population. Another possible mechanism, which can cause weight loss or decrease obesity, is to implant the useful bacterial species in appropriate ratio.

Probiotics and prebiotics are known to alter the compositional pattern and population of gastrointestinal microflora and are used to prevent or ameliorate some of the antimicrobial chemotherapy-induced gastrointestinal side effects and some other gastrointestinal diseases. Because of the new found link between these microflora and obesity (both obesogenic and antiobesogenic), pharmaceutical industries may focus more on manufacturing the required pre- and probiotics which may be beneficial to counter this worldwide epidemic and its complications.

REFERENCES

1   **Steinberger J**, Daniels SR. Obesity, insulin resistance, diabetes, and cardiovascular risk in children: an American Heart Association scientific statement from the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). *Circulation* 2003; **107**: 1448-1453 [PMID: 12642369 DOI: 10.1161/01. CIR.0000060923.07573.F2]

2   **Kaur J**. A comprehensive review on metabolic syndrome. *Cardiol Res Pract* 2014; **2014**: 943162 [PMID: 24711954 DOI: 10.1155/2014/943162]

3   **Rang HP**, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. New Delhi: Churchill Livingstone, 2006: 394-403

4   Get Your Hormones Checked and Lose Weight. [Accessed 2013 Nov 20]. Available from: URL: http://www.dietdoctor.com/get-hormones-checked-lose-weight

5   **Ness-Abramof R**, Apovian CM. Drug-induced weight gain. *Drugs Today* (Barc) 2005; **41**: 547-555 [PMID: 16234878 DOI: 10.1358/dot.2005.41.8.893630]

6   **Abdallah Ismail N**, Ragab SH, Abd Elbaky A, Shoeib AR, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes

7   **den Besten G**, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**: 2325-2340 [PMID: 23821742 DOI: 10.1194/jlr.R036012]

8   **Shoaie S**, Karlsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J. Understanding the interactions between bacteria in the human gut through metabolic modeling. *Sci Rep* 2013; **3**: 2532 [PMID: 23982459 DOI: 10.1038/srep02532]

9   **Hartstra AV**, Bouter KE, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 2015; **38**: 159-165 [PMID: 25538312 DOI: 10.2337/dc14-0769]

10 **Hijova E**, Chmelarova A. Short chain fatty acids and colonic health. *Bratisl Lek Listy* 2007; **108**: 354-358 [PMID: 18203540]

11 **Harris K**, Kassis A, Major G, Chou CJ. Is the gut microbiota a new factor contributing to obesity and its metabolic disorders? *J Obes* 2012; **2012**: 879151 [PMID: 22315672 DOI: 10.1155/2012/879151]

12 **Brahe LK**, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? *Obes Rev* 2013; **14**: 950-959 [PMID: 23947604 DOI: 10.1111/obr.12068]

13 **Lin HV**, Frassetto A, Kowalik EJ, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* 2012; **7**: e35240 [PMID: 22506074 DOI: 10.1371/journal.pone.0035240]

14 **Sanz Y**, Santacruz A, Gauffin P. Gut microbiota in obesity and metabolic disorders. *Proc Nutr Soc* 2010; **69**: 434-441 [PMID: 20540826 DOI: 10.1017/S0029665110001813]

15 **Petriz BA**, Castro AP, Almeida JA, Gomes CP, Fernandes GR, Kruger RH, Pereira RW, Franco OL. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics* 2014; **15**: 511 [PMID: 24952588 DOI: 10.1186/1471-2164-15-511]

16 **Sun J**, Chang EB. Exploring gut microbes in human health and disease: Pushing the envelope. *Genes Dis* 2014; **1**: 132-139 [PMID: 25642449 DOI: 10.1016/j.gendis.2014.08.001]

17 **Miquel S**, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol* 2013; **16**: 255-261 [PMID: 23831042 DOI: 10.1016/j.mib.2013.06.003]

18 **Duca FA**, Sakar Y, Lepage P, Devime F, Langelier B, Doré J, Covasa M. Replication of obesity and associated signaling pathways through transfer of microbiota from obese-prone rats. *Diabetes* 2014; **63**: 1624-1636 [PMID: 24430437 DOI: 10.2337/db13-1526]

19 **Park JS**, Seo JH, Youn HS. Gut microbiota and clinical disease: obesity and nonalcoholic Fatty liver disease. *Pediatr Gastroenterol Hepatol Nutr* 2013; **16**: 22-27 [PMID: 24010102 DOI: 10.5223/pghn.2013.16.1.22]

20 **DiBaise JK**, Frank DN, Mathur R. Impact of the Gut Microbiota on the Development of Obesity: Current Concepts. *Am J Gastroenterol Suppl* 2012; **1**: 22-27 [DOI: 10.1038/ajgsup.2012.5]

21 **Bervoets L**, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C, Goossens H, Desager KN, Vankerckhoven V. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathog* 2013; **5**: 10 [PMID: 23631345 DOI: 10.1186/1757-4749-5-10]

22 **Ley RE**, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**: 11070-11075 [PMID: 16033867 DOI: 10.1073/pnas.0504978102]

23 **Duncan SH**, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes* (Lond) 2008; **32**: 1720-1724 [PMID: 18779823 DOI: 10.1038/ijo.2008.155]

24 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm

in gut microbiota in obese and normal weight Egyptian children and adults. *Arch Med Sci* 2011; **7**: 501-507 [PMID: 22295035 DOI: 10.5114/aoms.2011.23418]

M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]

25   **Clarke SF**, Murphy EF, Nilaweera K, Ross PR, Shanahan F, O'Toole PW, Cotter PD. The gut microbiota and its relationship to diet and obesity: new insights. *Gut Microbes* 2012; **3**: 186-202 [PMID: 22572830 DOI: 10.4161/gmic.20168]

26   **Schwiertz A**, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* (Silver Spring) 2010; **18**: 190-195 [PMID: 19498350 DOI: 10.1038/oby.2009.167]

27   **Woting A**, Pfeiffer N, Loh G, Klaus S, Blaut M. Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. *MBio* 2014; **5**: e01530-e01514 [PMID: 25271283 DOI: 10.1128/mBio.01530-14]

28   **Million M**, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, Valero R, Raccah D, Vialettes B, Raoult D. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int J Obes* (Lond) 2012; **36**: 817-825 [PMID: 21829158 DOI: 10.1038/ijo.2011.153]

29   **Furet JP**, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, Mariat D, Corthier G, Doré J, Henegar C, Rizkalla S, Clément K. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 2010; **59**: 3049-3057 [PMID: 20876719 DOI: 10.2337/db10-0253]

30   **Puddu A**, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. *Mediators Inflamm* 2014; **2014**: 162021 [PMID: 25214711 DOI: 10.1155/2014/162021]

31   **Lau CH**, Muniandy S. Novel adiponectin-resistin (AR) and insulin resistance (IRAR) indexes are useful integrated diagnostic biomarkers for insulin resistance, type 2 diabetes and metabolic syndrome: a case control study. *Cardiovasc Diabetol* 2011; **10**: 8 [PMID: 21251282 DOI: 10.1186/1475-2840-10-8]

32   **Chakraborti CK**. Possible links between obesity and type 2 diabetes mellitus. *Int J Pharm Sci Res* 2012; **3**: 1935-1945

33   **Bastard JP**, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006; **17**: 4-12 [PMID: 16613757]

34   **vinh quốc Lu'o'ng K**, Nguyễn LT. The beneficial role of vitamin D in obesity: possible genetic and cell signaling mechanisms. *Nutr J* 2013; **12**: 89 [PMID: 23800102 DOI: 10.1186/1475-2891-12-89]

35   **Escobedo G**, López-Ortiz E, Torres-Castro I. Gut microbiota as a key player in triggering obesity, systemic inflammation and insulin resistance. *Rev Invest Clin* 2014; **66**: 450-459 [PMID: 25695388]

36   **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]

37   **Graham C**, Mullen A, Whelan K. Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. *Nutr Rev* 2015; **73**: 376-385 [PMID: 26011912 DOI: 10.1093/nutrit/nuv004]

38   **de La Serre CB**, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G440-G448 [PMID: 20508158 DOI: 10.1152/ajpgi.00098.2010]

39   **Al-Lahham SH**, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, Rezaee F, Venema K, Vonk RJ. Regulation of adipokine production in human adipose tissue by propionic acid. *Eur J Clin Invest* 2010; **40**: 401-407 [PMID: 20353437 DOI: 10.1111/j.1365-2362.2010.02278.x]

40   **Xiong Y**, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA* 2004; **101**: 1045-1050 [PMID: 14722361 DOI: 10.1073/pnas.2637002100]

41   **Samuel BS**, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA* 2008; **105**: 16767-16772 [PMID: 18931303 DOI: 10.1073/pnas.0808567105]

42   **Frost GS**, Dhillo WS, Ashby D, Bloom SR, Blundell JE, Murphy K. Increased propionate production in the colon is associated with reduced appetite body weight and improved insulin sensitivity. [Accessed 2015 Sep 23]. Available from: URL: http://gtr.rcuk.ac.uk/project/8FD29977-D6E2-4AFE-ADFA-D445CF5BB17C

43   **Tilg H**, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest* 2011; **121**: 2126-2132 [PMID: 21633181 DOI: 10.1172/JCI58109]

44   **Zak-Gołąb A**, Olszanecka-Glinianowicz M, Kocełak P, Chudek J. [The role of gut microbiota in the pathogenesis of obesity]. *Postepy Hig Med Dosw* (Online) 2014; **68**: 84-90 [PMID: 24491899 DOI: 10.5604/17322693.1086419]

45   **Kallus SJ**, Brandt LJ. The intestinal microbiota and obesity. *J Clin Gastroenterol* 2012; **46**: 16-24 [PMID: 22064556 DOI: 10.1097/MCG.0b013e31823711fd]

46   **Semova I**, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, Rawls JF. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host Microbe* 2012; **12**: 277-288 [PMID: 22980325 DOI: 10.1016/j.chom.2012.08.003]

47   **Swann JR**, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4523-4530 [PMID: 20837534 DOI: 10.1073/pnas.1006734107]

48   **Ringø E**, Strøm E, Tabachek JA. Intestinal microflora of salmonids: a review. *Aquacult Res* 1995; **26**: 773-789 [DOI: 10.1111/j.1365-2109.1995.tb00870.x]

49   **Basseri RJ**, Basseri B, Pimentel M, Chong K, Youdim A, Low K, Hwang L, Soffer E, Chang C, Mathur R. Intestinal methane production in obese individuals is associated with a higher body mass index. *Gastroenterol Hepatol* (N Y) 2012; **8**: 22-28 [PMID: 22347829]

50   **Frost G**, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S, Carling D, Swann JR, Gibson G, Viardot A, Morrison D, Louise Thomas E, Bell JD. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* 2014; **5**: 3611 [PMID: 24781306 DOI: 10.1038/ncomms4611]

Baishideng®