The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease

Patrice D. Cani* and Nathalie M. Delzenne*

Université catholique de Louvain, Louvain Drug Research Institute, Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, Brussels, Belgium

Abstract: Obesity is now classically characterized by a cluster of several metabolic disorders, and by a low grade inflammation. The evidence that the gut microbiota composition can be different between healthy and or obese and type 2 diabetic patients has led to the study of this environmental factor as a key link between the pathophysiology of metabolic diseases and the gut microbiota. Several mechanisms are proposed linking events occurring in the colon and the regulation of energy metabolism, such as i.e. the energy harvest from the diet, the synthesis of gut peptides involved in energy homeostasis (GLP-1, PYY…), and the regulation of fat storage. Moreover, the development of obesity and metabolic disorders following a high-fat diet may be associated to the innate immune system. Indeed, high-fat diet feeding triggers the development of obesity, inflammation, insulin resistance, type 2 diabetes and atherosclerosis by mechanisms dependent of the LPS and/or the fatty acids activation of the CD14/TLR4 receptor complex. Importantly, fat feeding is also associated with the development of metabolic endotoxemia in human subjects and participates in the low-grade inflammation, a mechanism associated with the development of atherogenic markers. Finally, data obtained in experimental models and human subjects are in favour of the fact that changing the gut microbiota (with prebiotics and/or probiotics) may participate in the control of the development of metabolic diseases associated with obesity. Thus, it would be useful to find specific strategies for modifying gut microbiota to impact on the occurrence of metabolic diseases.

Key Words: high fat diet, metabolic endotoxemia- obesity, prebiotics, gut peptides, bifidobacteria, gut bacteria, cardiovascular diseases.

INTRODUCTION

Obesity is now classically characterized by a cluster of several metabolic disorders. Most of them are related to the glucose homeostasis and to the development of cardiovascular diseases (Fig. **1**) [1,2]. During the past decade, it became clear that a low-grade inflammation contributes to the development of the pathologies associated with obesity [3]. Unequivocal experimental, clinical or epidemiological evidence have causally linked inflammation, or the inflammatory signalling responses to the development of theses metabolic disorders associated with obesity. The analysis of the nutritional disorders associated with obesity reveals that the adverse health consequences of weight gain and obesity are especially prominent following prolonged periods of positive energy balance and is mostly associated with a high-fat diet ingestion in our Western countries. However, it is more difficult to understand the mechanisms by which high-fat diet feeding promotes low grade inflammation (Fig. **2**). What is the molecular link between high-fat or high-energy feeding and the development of this particular context? Why and by which mechanisms such metabolic diseases are so commonly linked to inflammatory processes? Those questions will constitute the core of this review paper (Fig. **2**).

New evidence supports the idea that the increased prevalence of obesity and type 2 diabetes cannot be attributed solely to changes in the human genome, nutritional habits, or the reduction of physical activity in our daily lives [4]. Over the past five years, studies have highlighted some key aspects of the mammalian host-gut microbial relationship. Gut microbiota could now be considered as a "microbial organ" placed within a host organism. In addition to the obvious role of the intestine in the digestion and absorption of nutrients, the human gastrointestinal tract contains a diverse collection of microorganisms, residing mostly in the colon. So far, the human gut microbiota has not been fully described, but it is clear that the human gut is home for a complex consortium of around 10^{13} to 10^{14} bacterial cells. As a whole, the microorganisms that live inside humans are estimated to outnumber human cells by a factor of ten. The microbiome represents overall more than 100 times the human genome [5,6]. Therefore, the gut microbiota and its microbiome provide us with genetic and metabolic attributes, sparing us from the need to evolve solely by our own. Accumulating evidence indicates that the gut microbiota is instrumental in the control of host energy metabolism. These findings open the way to better understand how the gut microbiota and the factors that influence its distribution and constituent microorganisms, are controlled and how they interact with the host organism.

The present review will discuss the recent data in order to propose how the gut microbiota may play an even more important role in the development of metabolic disorders associated with obesity.

*Address correspondence to these authors at the Unit PMNT-7369, Av. E. Mounier, 73/69, B-1200 Brussels, Belgium; Tel: +32 2 764 73 97; Fax: +32 2 764 73 59; E-mail: patrice.cani@uclouvain.be or nathalie.delzenne@uclouvain.be

1381-6128/09 $55.00+.00

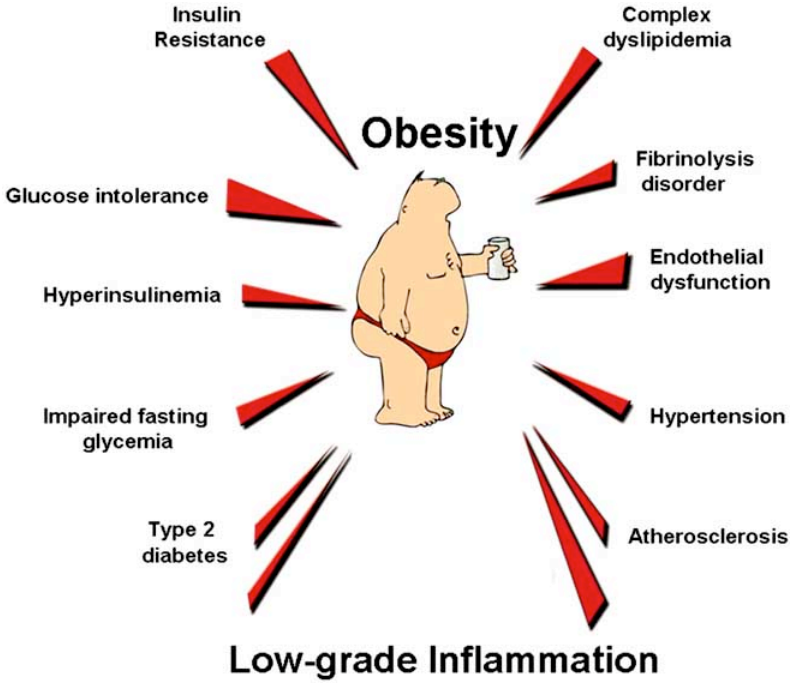

Fig. (1). Obesity and associated metabolic disorders.

Obesity is characterised by a cluster of metabolic disorders, related to the glucose homeostasis and to the development of cardiovascular diseases. Recently, the development of such pathologies has been associated with a low grade inflammatory tone.

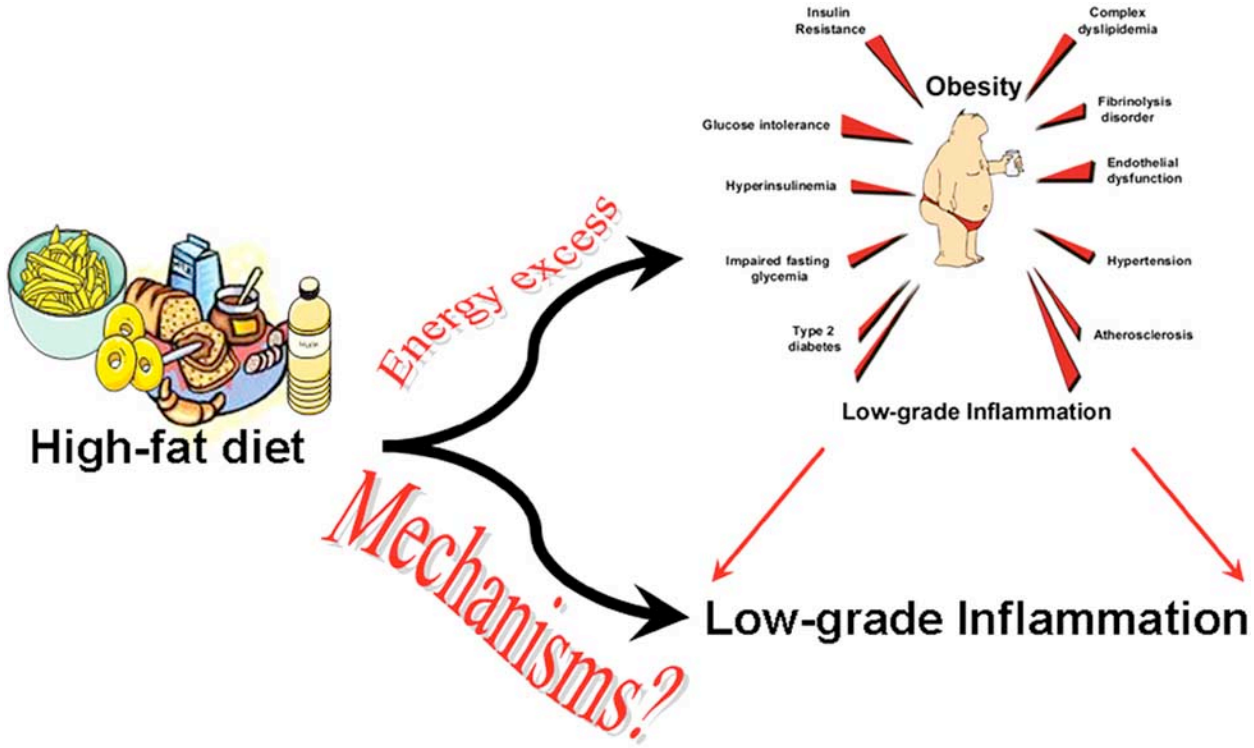

Fig. (2). Question: What are the mechanisms linking high-fat diet feeding to the development of a low grade inflammation?

GUT MICROBIOTA AND ENERGY METABOLISM

Gut Microbiota Regulates Fat Storage

Gut microbiota is involved in several intestinal biological functions such as defence against pathogens, immunity, the development of the intestinal microvilli, the degradation of non digestible polysaccharides (fermentation of resistant starch, oligosaccharides, inulin). Hence, the gut microbiota harvests energy for the host from dietary compounds ingested but not digested by the host. In the majority of adults,

the qualitative and quantitative composition of food intake varies considerably from meal to meal and from day to day, while adiposity and body weight are remarkably constant despite huge short-term variations in energy balance. When recording food intake and activity within a period including several meals, most individuals are able to compensate their cumulative energy intake with their energy expenditure with great precision [7]. Such an active process - energy homeostasis - stabilizes the amount of body energy stored as fat. However, an excess of energy intake by less than 1% compared to the daily energy expenditure, can lead to a detrimental increase of body weight and metabolic complications in the long term (several years) [8]. Consequently, all the mechanisms influencing calorie ingestion and subsequent harvesting should contribute to the balance of the body weight. Several recent studies from the group of J. Gordon (USA) highlighted that gut microbiota composition is involved in the regulation of energy homeostasis. Backhed, *et al.* found that the mice raised in the absence of microorganisms (germ free) had about 40% less total body fat than mice with a normal gut microbiota, eventhough the latter ate 30% less diet than did the germ free mice. To get more insight to those findings, the authors performed a key experiment: they conventionalized germ free mice with a normal gut microbiota harvested from the cecum of a "normal" mouse, and found that this conventionalization produced a 60% increase in body fat content and insulin resistance within two weeks, despite a significant lower food intake [9]. The mechanisms of the apparent weight gain implied an increase in the intestinal glucose absorption, energy extraction from non-digestible food component and concomitant higher glycemia and insulinemia, two key metabolic factors regulating lipogenesis. Moreover, glucose and insulin are also known to promote hepatic *de novo* lipogenesis through the expression of several key enzymes such as aceyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Strikingly, a two weeks conventionalization of germ free mice is accompanied by a two-fold increase in hepatic triglyceride content. Both ACC and FAS are controlled by ChREBP (Carbohydrate Responsive Element Binding Protein) and SREBP-1 (Sterol Responsive element Binding Protein) [10]. Accordingly, the conventionalized mice exhibited an increased hepatic ChREBP and SREBP-1 mRNA levels (Fig. **3**) [9]. In addition to a modulation of *de novo* lipogenesis, the authors found that germ free mice had a lower monosaccharide uptake from the intestine to the portal blood. This last phenomenon could be partly explained by the lower capillary density of the small intestine of germ free mice as compared to their conventionalized counterparts. Finally, all these data provide evidence that the digestion of polysaccharides by microbial enzymes and the increased saccharides delivery to the liver, participate in higher lipogenesis (Fig. **3**). However, in the adipose tissue, the adipocytes hypertrophy observed in the mice harbouring gut microbiota was not explained by the modulation of the

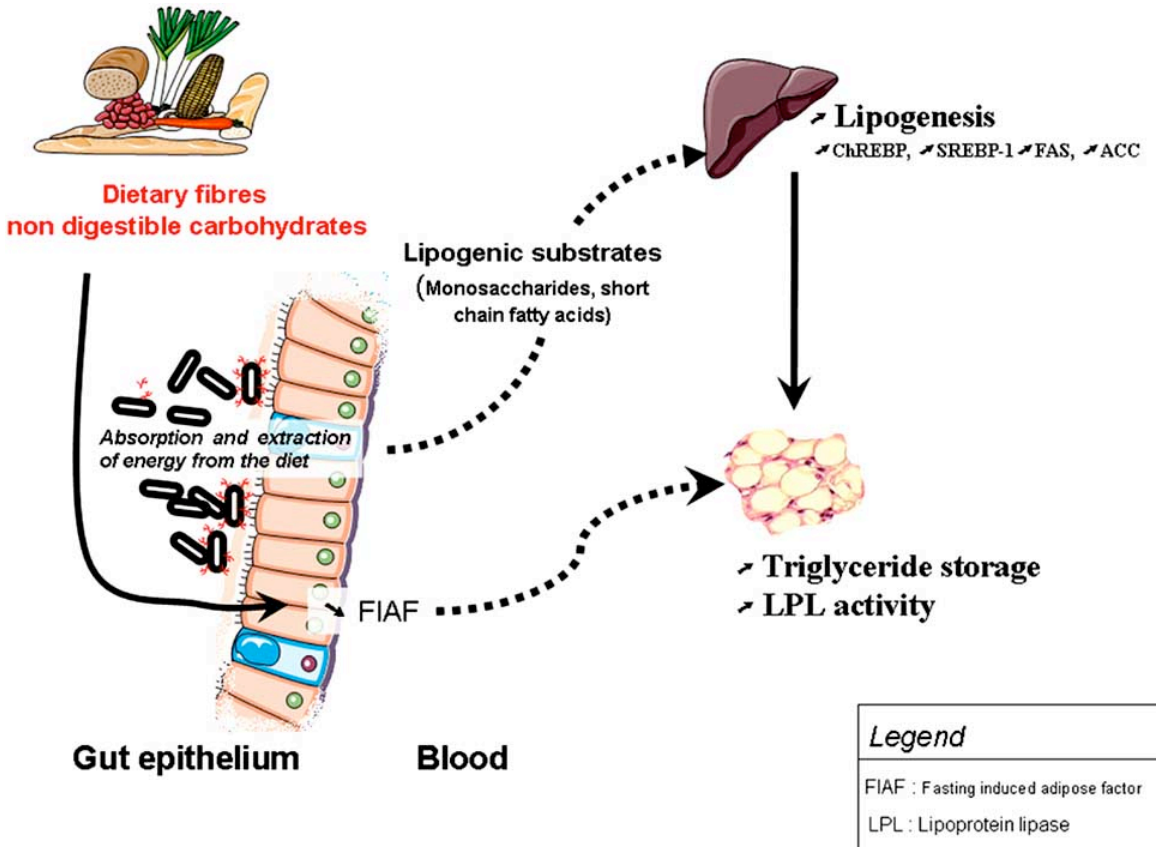

Fig. (3). Gut microbiota helps harvesting energy from the diet and increases lipogenesis. Environmental factor such as gut microbiota may regulate energy storage: 1) by providing lipogenic substrates (short chain fatty acids, monosaccharides) to the liver, 2) by increasing the enzyme lipoprotein lipase (LPL) activity (as a consequence of suppressing the Fasting-Induced Adipose Factor (FIAF) in the gut).

Both phenomenon, contribute to the release of fatty acids and triacylglycerol from circulating lipoproteins in muscle, and adipose tissue.

adipogenesis or the lipogenesis. Interestingly, the conventionalization also brought about a general increase in the activity of the enzyme lipoprotein lipase (LPL), catalyzing the release of fatty acids and triacylglycerol from circulating lipoproteins in muscle, and adipose tissue. The authors proposed that such an increase was the consequence of suppression of the Fasting-Induced Adipose Factor (FIAF) in the gut. FIAF inhibits the LPL activity. The blunted FIAF expression in conventionalized germ free mice could thus participate to the accumulation of triacylglycerol in the adipose tissue. This set of experiments demonstrated for the first time that an environmental factor such as gut microbiota may regulate energy storage Fig. (**3**) [9].

Obesity and Gut Microbiota

Ley, *et al.* demonstrated, in a rodent model, that obesity can be associated with an altered gut microbiota [11]. After the characterisation of more than five thousands bacterial 16S RNA gene sequences from gut microbiota of genetically obese *ob/ob* mice and their lean counterparts, they pointed out that *ob/ob* mice had a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes. The observed alterations in community may represent an unheralded contributing factor to the pattern of fuel partitioning between lean and obese subjects. Accordingly, these authors have also compared the distal gut microbiota of obese and lean human subjects [12]. To investigate the relation between gut microbial ecology and body fat mass in humans, they studied 12 obese subjects assigned to a fat restricted or a carbohydrate restricted low calorie diet. They found that before the dietary intervention, obese people had lower Bacteroidetes and more Firmicutes than did lean control subjects [12]. Whereas the ratio of Bacteroidetes to Firmicutes approached a lean type profile after 52 weeks of diet-induced weight loss.

Together, the results obtained in rodents and in humans, suggest that obesity alters the nature of the gut microbiota, but they did not prove that the relative difference of bacterial proportions leads to different body weights.

To determine if the gut microbial community from *ob/ob* mice can increase capacity for energy harvest from the diet, Turnbaugh, *et al.* transplanted caecal microbiota from lean and *ob/ob* mice to germ free wild-type recipients. They found that after only two weeks, mice harbouring the microbiota from obese mice had a modest fat gain, and extracted more calories from their food compared to the lean mice having received the gut microbiota from lean mouse donors [13]. Together, these data suggest that the characteristics of gut microbiota of obese mice participate per se to the accretion of fat and body weight gain.

GUT MICROBIOTA AND METABOLIC DISORDERS

Gut Microbiota Controls the Occurrence of High-Fat Diet Metabolic Disorders

The contribution of energy harvesting for the host due to bacterial colonization is not the sole and crucial metabolic exchange between the host and the intestinal bacteria. A recent study performed in germ free mice, has analyzed their resistance to diet-induced obesity [14]. The authors maintained germ free mice or conventionalized mice on a high-fat/high-carbohydrates diet (western diet). They found that conventionalized mice fed a high-fat diet gained significantly more weight and fat mass than the germ free mice. In addition, the germ free mice were also protected against the high-fat diet induced glucose intolerance and insulin resistance. Strikingly, and opposite to the results previously observed in germ free mice fed a normal chow diet, germ free mice consumed similar amounts of high-fat diet than the conventionalized mice and had a similar energy content in their feces. These last observations are not completely in favour of a better energy harvest from the high-fat diet in the conventionalized mice, as previously suggested in normal chow fed mice. The authors have proposed a mechanism dependent of the activation of a cellular energy-dependent protein kinase activated in response to metabolic stresses, namely AMP-activated protein kinase (AMPK) [14]. Comparisons of germ free mice and colonized mice fed a high-fat diet indicate that the gut microbiota can be involved in the regulation of AMPK activity and fatty acids oxidation. The resistance to diet induced obesity observed in germ free mice can be also explained by the following metabolic sequence : in the absence of gut microbiota, AMPK activity is constitutively higher in muscle, leading to a higher phosphorylation of its specific target acetylCoA carboxylase (ACC), reducing thereby malonyl CoA production. This drop in malonyCoA increases carnitine palmitoyl transferase-1 (CPT-1), and therfore promotes mitochondrial fatty acid oxidation.

Thus, these last experiments strongly suggest that a bacterially related factor/mechanism other than energy harvesting may be responsible for the development of diet-induced obesity and diabetes.

Although all these elegant studies revealed that the gut microbiota exerts a crucial role in the development of adiposity and the regulation of homeostasis, it remains to be demonstrated how the gut microbiota can be involved in the development of a low-grade inflammation classically associated with the metabolic disorders related to high-fat diet induced obesity [15,16].

Gut Microbiota-Related Factor Responsible for Low-Grade Inflammation

Experimental Data

Recently, a new hypothesis linking gut microbiota to the metabolic homeostasis has been proposed. High-fat diet-induced obesity and metabolic disorders are associated with an increased expression of several inflammatory related factors IL-1, TNF-α, MCP-1, and IL-6 in muscle, liver and adipose tissue [17-19]. These markers are involved in the development of impaired insulin action and induce insulin resistance. For instance, TNF-α phosphorylates serine residue substrate (IRS-1) from the insulin receptor, leading to its inactivation [20].

Since type 2 diabetes and obesity are closely associated to a low-grade inflammatory state when feeding a high-fat diet, we have been seeking a bacterially related factor able to trigger the development of high-fat diet-induced obesity, diabetes and inflammation. The eligible candidate should be an inflammatory compound of bacterial origin, continu-

ously produced within the gut and its absorption/action should be associated with high-fat diet feeding. We hypothesized that the bacterial lipopolysaccharide (LPS) could be the eligible candidate, for the following reasons : 1) LPS is a constituent of Gram negative bacteria present in the gut microbiota, 2) LPS triggers the secretion of proinflammatory cytokines when it binds to the complex of CD14 and the toll-like receptor 4 (TLR4) at the surface of innate immune cells [21] , 3) LPS is continuously produced within the gut by the death of Gram negative bacteria and is physiologically carried into intestinal capillaries through a TLR4 dependent mechanism [22], 4) LPS is transported from the intestine towards target tissues by a mechanism facilitated by lipoproteins, notably chylomicrons freshly synthesized from epithelial intestinal cells in response to fat feeding [23-26]. We have recently demonstrated that mice fed a high-fat diet for as short a term as 2 to 4 weeks, exhibited a significant increase in plasma LPS (Fig. **4**) [27]. This can be considered as a "metabolic endotoxemia", since, the LPS plasma concentrations were very much lower than those obtained during a septic shock [28]. We have demonstrated that high-fat diet feeding changed gut microbiota profile. Indeed, the population levels of *Bifidobacterium* spp. and *E. rectale/Cl. coccoides* group were significantly reduced in high fat fed animals versus mice receiving the standard high carbohydrate diet (Fig. **4**) [27]. Importantly, *Bifidobacterium* spp. have been shown to reduce intestinal endotoxin levels in rodents and improve mucosal barrier function Fig. (**4**) [29-31]. In order to determine the role of metabolic endotoxemia as a triggering factor in the development of metabolic disorders associated with obesity, we mimicked the metabolic endotoxemia by developing a mouse model chronically infused with a very low dose of LPS to reach the same plasma LPS levels as the one measured in the high-fat diet fed mice [27]. The four weeks chronic low dose LPS infusion mimicked the high-fat diet fed mice phenotype namely, fasting hyperglycemia, obesity, steatosis, adipose tissue macrophages infiltration, hepatic insulin resistance and hyperinsulinemia Fig. (**4**). Finally, in order to demonstrate the causative link between LPS and the development of metabolic diseases, we challenged LPS receptor knock out mice (CD14 knock out mice-CD14KO) with a high-fat diet and/or a chronic low dose LPS infusion. CD14 is a key molecule involved in the innate immune system [32]. CD14 is a multifunctional receptor constituted by a phosphatidyl inositol phosphate-anchored glycoprotein of 55kDa expressed on the surface of monocytes, macrophages and neutrophils [33-36]. We have shown that CD14KO mice were completely resistant to the development of the inflammation induced by both, high-fat feeding or following the chronic low dose LPS administration in the visceral and subcutaneous adipose depots, the liver and the muscle. Moreover, CD14KO mice are hyper-

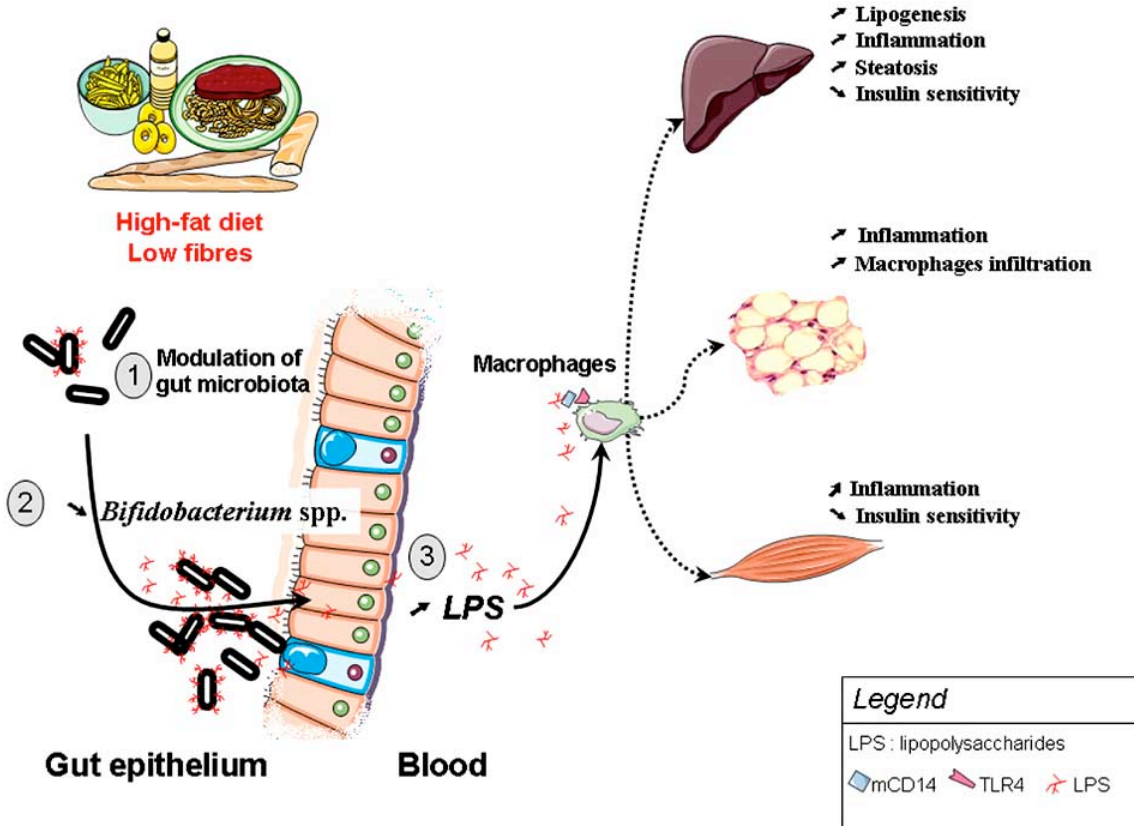

Fig. (4). High-fat diet feeding changes gut microbiota, promotes metabolic endotoxemia and triggers the development of metabolic disorders, *via* a CD14/TLR4 dependent mechanism.

(1) High-fat diet feeding changes gut microbiota in a complex way and (2) specifically decreases *Bifidobacterium* spp. (3) This phenomenon is associated with a higher plasma LPS content (metabolic endotoxaemia), a LPS-dependent secretion of proinflammatory cytokines. High-fat feeding and LPS promotes low-grade inflammation-induced metabolic disorders (insulin resistance, diabetes, obesity, steatosis, adipose tissue macrophages infiltration).

sensitive to insulin, even when they are fed a normal diet, suggesting that CD14 could be a modulator of insulin sensitivity in physiological conditions [27]. As a matter of fact, CD14KO mice were completely resistant to the insulin resistance induced by the high-fat diet and chronic LPS treatment. In these sets of experiments, we showed that high-fat feeding induced a low-grade inflammation which originates from the intestinal absorption of the LPS. Thus taken together our data support the key idea that the gut microbiota can contribute to the pathophysiology of obesity and type 2 diabetes (Fig. **4**).

Importantly, the real mechanism by which in the absence of the complex CD14/TLR4 receptor the mice are resistant to the high-fat diet induced metabolic disorders remains a matter of debate. Several studies have demonstrated that the TLR4 receptor could also be activated by specific saturated fatty acids [37-39]. Hence, TLR4KO mice fed a high-fat diet are resistant to the development of a high-fat diet induced obesity and related disorders (obesity, inflammation, insulin resistance,…) [37,40-43]. However, none of these studies have investigated the putative modulation by the dietary intervention of either the gut microbiota nor the putative metabolic endotoxemia. Therefore, it is difficult to conclude whether the protective effect linked to the invalidation of the TLR4 receptor is a mechanism dependent of the high-fat diet induced endotoxemia and/or a direct effect of the fatty acid pattern of the diet. Previous experiments performed in germ free mice fed a high-fat diet helped to answer this question. The fact that the germ free mice resist the deleterious effects of a high-fat diet supports the idea that the phenomenon is not exclusively mediated through a fatty acids/TLR4 dependent mechanism. To ascertain this hypothesis and to assess the contribution of gut microbiota to the development of high-fat diet-induced metabolic disorders, we used intestinal-focused antibiotic treatment in high-fat fed mice. The antibiotic treatment completely abolished the high-fat diet-induced metabolic disorders, namely metabolic endotoxemia, the development of visceral adipose tissue inflammation, macrophages infiltration, oxidative stress and metabolic disorders [44]. These last experiments clearly demonstrate the contribution of the gut microbiota to the metabolic endotoxemia.

Together, these findings strongly suggest that the gut microbiota contributes to the metabolic endotoxemia related to high-fat diet feeding. In the same line of our results, recent studies report that plasma LPS is increased in *ob/ob* and *db/db* mice [45]. Furthermore, polymyxin B treatment, which specifically eliminates Gram-negative bacteria and further quenches LPS, diminishes hepatic steatosis [46]. However, these studies did not demonstrate that the gut bacteria determine the threshold at which metabolic endotoxemia occurs and that the modulation of gut microbiota in obese and diabetic *ob/ob* mice controls the occurrence of metabolic and inflammatory disorders.

Therefore, we asked the following question.

What is the Contribution of Gut Microbiota to the Development of Metabolic Endotoxemia, Inflammation, Oxidative Stress and Metabolic Disorders in ob/ob Mice?

To test this hypothesis, we changed the gut microbiota of *ob/ob* mice using antibiotic treatment for four weeks. Antibiotic treatment dramatically changed the gut microbiota; reduced the *Lactobacillus* spp., *Bifidobacterium* spp.; and *Bacteroides-Prevotella* spp. All these features were associated with a strong decrease of metabolic endotoxemia. Furthermore, these parameters were associated with a significantly lower inflammatory tone in *ob/ob* antibiotic-treated mice [44]. Macrophages infiltration, inflammatory markers and oxidative stress were reduced in the visceral adipose depots and to a lesser extent in the subcutaneous fat. This experiment demonstrates that the gut microbiota is an important factor involved in the development of the metabolic disorders in *ob/ob* mice. Finally, we wanted to demonstrate that the metabolic endotoxemia *per se* was the triggering factor of the inflammatory tone characterizing these obese and diabetic leptino-deficient mice. Therefore, we used two different approaches to block the endogenous LPS action, the first one consisted of a pharmacological administration of a LPS-quencher molecule inactivating the circulating LPS, and the second one consists of a genetic model of obese mice lacking the LPS receptor CD14, the double knock out mice *ob/ob-CD14*$^{-/-}$. In both models, impairing the endogenous LPS action, recapitulated the phenotype observed during the modulation of gut microbiota by antibiotics [44]. In addition to the improved inflammatory status, all the models were also characterized by a significant improvement of glucose tolerance and insulin resistance [44]. These last results have been confirmed in a study using a similar approach [47]. Altogether, this set of data confirms that the gut microbiota and the consequent increased bacteria-related factor LPS exert a key role in the development of adipose depots and inflammation in *ob/ob* mice.

Several studies have shown that bifidobacteria, seen as beneficial members of the gut microbiota, lower intestinal endotoxin levels and improve mucosal barrier function [29-31]. Conversely, we reported that high-fat feeding alters the intestinal microbiota composition where *Bifidobacterium* spp. were reduced. Therefore, we addressed the following question.

Could the Selective Increase of Bifidobacteria in Gut Microbiota Improve High-Fat Diet-Induced Diabetes in Mice?

To answer this question, we used prebiotic dietary fibres (oligofructose, OFS) [48] to specifically increase the gut bifidobacteria content in high-fat fed mice. We confirmed that mice fed a high-fat diet exhibit a higher endotoxemia, a phenomenon completely abolished through dietary supplementation with the prebiotic dietary fibres (Fig. **5**). In prebiotic treated-mice, *Bifidobacterium*-spp. significantly and positively correlated with improved glucose-tolerance, glucose-induced insulin-secretion, and normalized low-grade inflammation (decreased endotoxemia, plasma and adipose tissue proinflammatory cytokines) (Fig. **5**) [49]. We also found that metabolic endotoxemia correlated negatively with *Bifidobacterium* spp [49].

Thus, it would be useful to develop specific strategies for modifying gut microbiota to favour specific gut microbiota (i.e. bifidobacteria) to prevent the deleterious effect of high-fat or obesity-induced metabolic diseases.

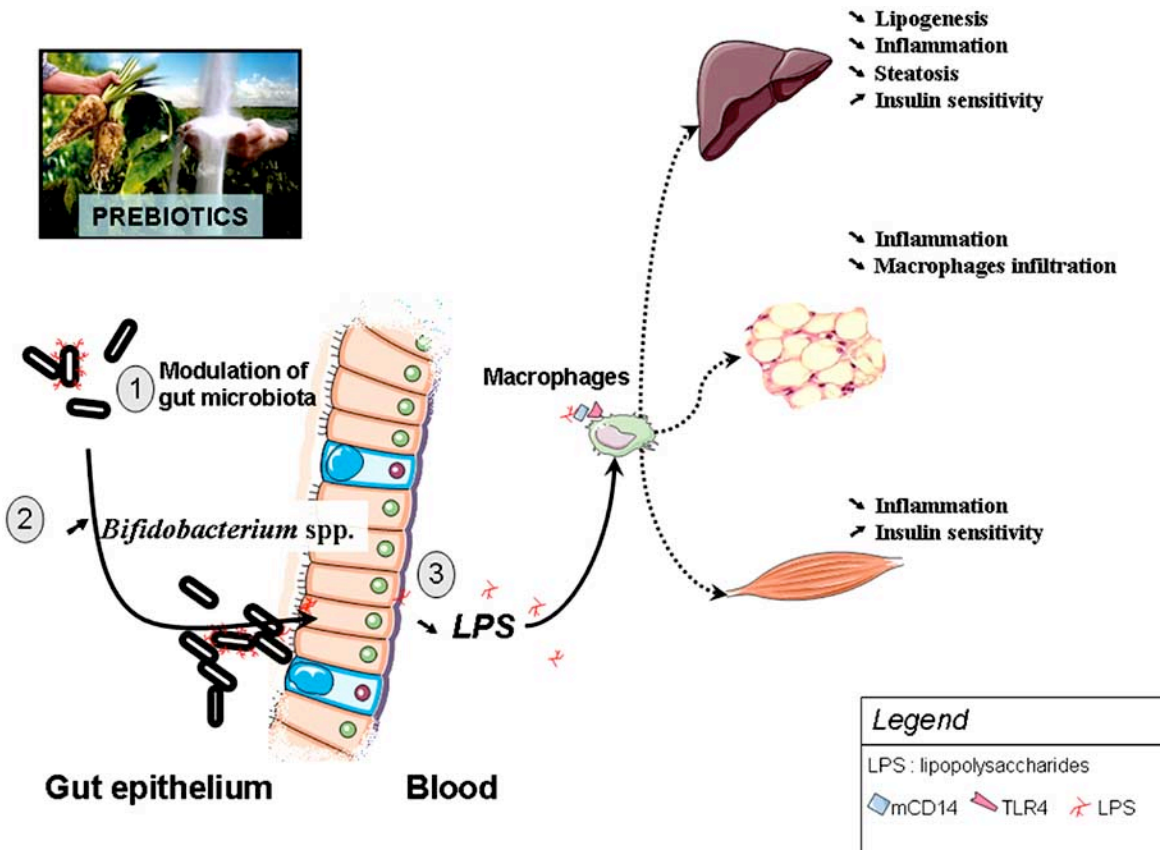

Fig. (5). Changing gut microbiota by the mean of prebiotics protects against high-fat diet induced metabolic endotoxemia and the development of metabolic disorders. Prebiotic treatment increases *Bifidobacterium*-spp., decreases plasma LPS levels and improved insulin sensitivity, steatosis, and normalized low-grade inflammation (decreased endotoxemia, plasma and adipose tissue proinflammatory cytokines).

Human Evidence

Even if from a mechanistic point of view, the results obtained in rodent models are very encouraging, it remains to be demonstrated that such a mechanism is also observed in humans.

Is a High-Fat Meal Associated with Metabolic Endotoxemia in Humans?

Interesting data suggest that high-fat feeding is associated with a higher endotoxemia in humans. Erridge, *et al.* have highlighted the putative role of a high-fat meal and development of metabolic endotxemia. The study is the first to examine the kinetics of baseline endotoxemia concentrations in healthy human subjects. Even if, in humans plasma endotoxin levels are classically associated with sepsis, many studies have also reported that in healthy subjects plasma endotoxin concentrations range from 1 to 200 pg/ml [50-53]. In this study, the authors found that a high-fat meal induces a metabolic endotoxemia which fluctuates rapidly in healthy subjects, from a very low concentration at baseline (between 1 to 9 pg/mL) to concentrations that may be sufficient to induce some degree of cellular activation in *in vitro* experiments [54]. They found that the metabolic endotoxemia observed following a high-fat meal is sufficient to activate cultured human aortic endothelial cells, and that this endothelial cell activation is likely to be due to the release of soluble inflammatory mediators, such as TNF-α, from monocytes. Along the same line, in a large sample of men (n=211) from a population based-study, we found a link between energy (food) intake and metabolic endotoxemia [55]. Furthermore, a similar metabolic endotoxemia has been shown to increase adipose TNF-α and IL-6 concentrations and insulin resistance in healthy volunteers [56]. By linking energy intake and endotoxemia in a large sample of healthy men, the study adds important information to this body of evidence. This study shows for the first time that the confounding factor of the relation between fat intake and metabolic endotoxemia is likely to be energy intake. Taken together, both human studies suggest that diet-induced changes in endotoxemia may bridge the gap between food intake behaviour and metabolic diseases in humans.

What is the Contribution of Gut Microbiota to the Development of Metabolic Disorders in Humans?

Creely, *et al.* recently reinforced the hypothesis that metabolic endotoxemia might act as a gut microbiota related factor involved in the development of type 2 diabetes and obesity in humans [57]. The authors found that endotoxemia was 2-fold higher in the BMI-, sex-, and age-matched type 2 diabetes patients group than in the non diabetic subjects. Furthermore, they found that fasting insulin significantly correlated with metabolic endotoxemia in the whole non diabetic population, and this correlation persisted when controlled for sex, age, and BMI [57]. The quest for the gut–dependent source of endotoxemia in these patients remains unanswered.

Specific Modulation of Gut Microbiota by Prebiotic Nutrients : A Rationale to Support Nutritional Advices in the Context of Obesity?

Current recommendations for the management of obesity and diabetes propose an increase in dietary fibre which may contribute to the control of several metabolic disorders (*i.e.* lower fasting glycemia, an improved glucose tolerance, lower body weight gain, decreased food intake,…) [58-60]. Among the dietary fibres which seem to be effective in this context, prebiotics dietary fibres are now well described in the literature [48,61,62]. Prebiotics can be used as a tool to modulate the gut microbiota. A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [63] and probiotic are live bacteria given in oral quantities that allow for colonization of the colon [64]. Inulin-type fructans, namely inulin and fructooligosaccharides are prebiotic dietary fibres well studied and clearly effective in humans to stimulate growth of health-promoting species belonging to the genera *Bifidobacterium spp*. and *Lactobacillus spp.* The daily amount taken in the diet necessary to exert a prebiotic effect is relatively small (5–20 g/day) [61,65].

Besides their effect on metabolic endotoxemia previously described, prebiotic dietary fibers may also modulate other targets prone to influence metabolic disorders associated with obesity, such as gut peptides.

Are Gut Peptides Involved in the Effect Of Prebiotics Dietary Fibres on Energy Metabolism and Metabolic Disorders Associated with Obesity?

The modulation of gut peptides involved in the control of energy and glucose homeostasis could be one of the mechanisms by which the modulation of gut microbiota *via* specific dietary fibres is associated with an improvement of metabolic disorders. Endocrine cells present in the intestinal mucosa secrete peptides involved in the regulation of energy homeostasis, and/or pancreatic functions - the later being called incretins (GLP-1 and GIP) [66-69]. Among those peptides, GLP-1, PYY, Ghrelin and oxyntomodulin have recently been proposed as important modulators of food intake and energy expenditure (Fig. **6**) [70-74]. Several experimental data suggest that those peptides could constitute a link between the outcome of gut microbiota fermentation in the lower part of the gut and systemic consequences.

The putative link between gut microbiota fermentation of non digestible carbohydrate and the modulation of gut peptides secretion was proposed in 1987 by Goodlad, *et al.*, demonstrating that inert bulk fibre cannot stimulate colonic epithelial cell proliferation, but that fermentable fibres were capable of stimulating proliferation in the colon, linking these effects to the increased enteroglucagon plasma levels [75,76]. And along the 20 years, other reports suggesting new mechanisms of such a dietary compound have appeared in the literature. In 1996, the first study demonstrating a role of the fermentation occurring in the lower part of the gut was associated with an increase of GLP-1 synthesis, secretion and insulin metabolism. The study demonstrated that rats fed a high fiber diet (300 g/kg of diet) had a higher plasma GLP-1, insulin and c-peptide 30 min after an oral glucose load [77]. Two years later, Kok, *et al.* observed that feeding rats with a prebiotic fibre oligofructose (OFS) lead to an increase in total caecal GLP-1 and jejunum GIP concentrations [78]. Several data show that prebiotics containing short chain oligosaccharides reduce food intake, body weight gain and fat mass development. All these features are associated with a significant 2 fold increase of the portal plasma levels of two gut peptide GLP-1 and PYY (anorexigenic) and a decrease in Ghrelin (orexigenic) (Fig. **6**) [79,80]. Prebiotic feeding promotes GLP-1 synthesis (mRNA and peptide content) in the proximal colon by a mechanism linked to the differentiation of precursor cells into enteroendocrine cells [81]. Moreover, in another set of experiments performed in high-fat diet-induced obesity and type 2 diabetes, the modulation of gut microbiota using prebiotics protects against body weight gain, fat mass development (visceral, epidydimal and subcutaneous), glucose intolerance, and hepatic insulin resistance [82-84]. Accordingly, prebiotics like fructans added in the diet are able to counteract diabetes when given in streptozotocin-treated diabetic rats [85]. Studies showing similar effects to those observed in fructans studies, for example, with lactitol or resistant starch (both fermentable carbohydrates) added into the diet of rats, lowers food intake, body weight gain and increases plasma GLP-1 and PYY (Fig. **6**) [86-88]. Nevertheless, the putative role of a specific gut microbiota profile has not been studied.

What is the Relevance of Prebiotics-Dependent Gut Microbiota Modulation and Energy Metabolism in Humans?

To date, only a few studies have reported the effects of prebiotics on energy homeostasis and metabolism in humans. Interestingly, one study reported that oligofructose feeding (20g/d) significantly increased plasma GLP-1 after a mixed meal [89]. Moreover, in healthy humans, feeding 16g/d OFS promotes satiety following breakfast and diner, and reduces hunger and prospective food consumption after the diner. This was accompanied by a significant 10% lower total energy intake [90]. Along the same lines, Archer, *et al.* have demonstrated that of fructans, added in food as fat-replacer, were able to lower energy intake during a test day [91]. The role of fermentable dietary fibres in the management of appetite in healthy human has been recently confirmed [92]. Finally, in the quest for the role of prebiotic in the control of body weight and fat mass development, a recent study demonstrated that supplementation with a prebiotic, in addition to its benefit to bone mineralization, had a significant benefit in the maintenance of an appropriate BMI, and fat mass in primarily nonobese young adolescents [93]. Altogether, these human studies provide evidence that the modulation of gut microbiota by using prebiotics impacts on energy homeostasis and body weight gain.

What is the Contribution of Bifidobacterium spp. in the Prebiotic-Improved Metabolic Status?

A recent study has shown for the first time in human that differences in the gut microbiota may precede overweight development [94]. The authors found that *Bifidobacterium* spp., affecting both the quantity and quality of the microbiota during the first year of life, was higher in number in children who exhibited a normal weight at 7 years than in children developing overweight. More importantly and according to the results obtained in experimental models, they found that

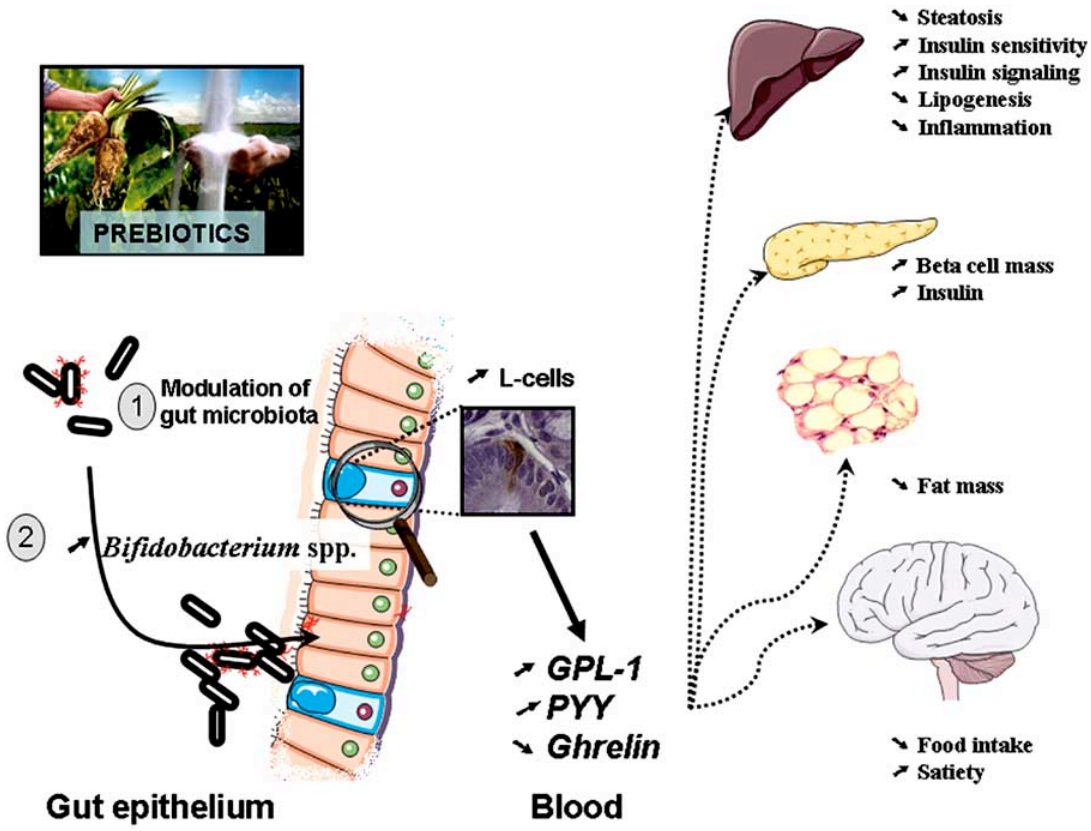

Fig. (6). The modulation of gut microbiota by prebiotics treatment modulates the endogenous production of gut peptides associated with energy homeostasis.

Prebiotics change gut microbiota, increase portal plasma levels of two gut peptide GLP-1 and PYY (anorexigenic) and decrease Ghrelin (orexigenic). Prebiotics feeding promotes GLP-1 synthesis in the proximal colon by a mechanism linked to the differentiation of precursor cells into enteroendocrine L-cells. All these features are associated with a reduced food intake, body weight gain and fat mass development, a restored beta cell mass and glucose-induced insulin secretion.

the fecal numbers of *Staphylococcus aureus* were lower in children remaining normal weight than in children who became overweight several years later. These results unequivocally imply that the gut microbiota profile in favour of a higher bifidobacteria and a lower number of *S. aureus* in infancy may provide protection against overweight and obesity development. The authors proposed that *S. aureus* may act as a trigger of low-grade inflammation [95], contributing to the development of obesity [27].

GUT MICROBIOTA AND CARDIOVASCULAR RISK

The link between periodontal diseases and cardiovascular diseases is now well established [96-99]. The accumulated evidence supports that periodontal infections and atherosclerosis are causally linked by the metabolic endotoxemia. Several marker related to the metabolic endotoxemia (LPS binding protein, soluble CD14, and antibodies to LPS of periodontal pathogens) have been reported to be also elevated in the plasma of affected patients. *Porphyromonas gingivalis* systemic exposure appears to predispose to incident stroke [100].

Metabolic endotoxemia is positively correlated with total cholesterol, diastolic blood pressure, waist to hip ratio, BMI, and antibody levels to *P gingivalis*, and a negative correlation with HDL cholesterol [101]. Whether the metabolic endotoxemia measured in these studies derives from periodontal pathogens alone or not remains to be demonstrated.

What is the Contribution of the Gut Microbiota to the Development of Atherosclerosis?

Experimental Evidence

Several authors have demonstrated that the link between metabolic endotoxemia and the development of systemic low grade inflammation and cardiovascular diseases is mediated through a LPS receptor dependent mechanism [102-105]. In accordance with the recent evidence suggesting that inflammatory process induced by high-fat diet feeding causes insulin resistance *via* a mechanism involving CD14 and TLR4, two recent studies have proposed that inflammation can be activated in the vasculature of mice fed a high-fat diet [43, 106]. Mice fed a high-fat diet for 8 weeks developed vascular inflammation (higher thoracic aorta IκBα-phosphorylation, ICAM, IL-6) and vascular insulin resistance (lower thoracic aorta insulin dependent AKT-phosphorylation and eNOS-phosphorylation). All these features were completely absent in mice lacking the TLR4 receptor. Furthermore, deficiency of either TLR4 or Myd88 attenuates the high-fat diet induced atherosclerosis, chemokine secretion and macrophage infiltration in apolipoprotein E deficient mice

(ApoE-/-) [107-111]. These studies support the idea that TLR4 and Myd88 likely contribute to atherosclerosis progression *via* a fatty acid dependent mechanism. In addition, it has been proposed that modulation of gut microbiota in ApoE-/- also contribute to the reduction of inflammation and atherosclerosis development [112]. The authors found that changing the gut microbiota of atherosclerotic prone ApoE-/- mice by feeding mice with prebiotics for 16 weeks, significantly reduce the development of atherosclerotic lesions by about 35% as compared to the mice fed a control diet [112]. However, the authors did not propose any putative mechanisms related to the modulation of gut microbiota, inflammation or metabolic endotoxemia.

Human Evidence

The notion that gut microbiota may participate in the prevention of coronary artery disease has been already investigated and proposed several years ago. Based on previous animal studies demonstrating that probiotic feeding participate in the improvement of atherogenic markers (LDL-cholesterol, fibrinogen), Bukowska, *et al.* decided to test this interesting possibility in human subjects. The authors investigated in a double blind cross over study with 30 male subjects the role of both a fermentable carbohydrate (fermentable oat fraction) and a probiotic (*Lactobacillus plantarum*) supplementation on two key atherogenic parameters, namely LDL-cholesterol and fibrinogen. After 6 weeks of treatment, levels of LDL-cholesterol and fibrinogen were significantly reduced [113]. This study showed for the first time that the modulation of gut microbiota may participate to the modulation of two key risk factors.

Along the same line, the same group documented the influence of *L. plantarum* in a controlled double-blind study with placebo on 36 smokers [114]. The authors found that a 6 weeks treatment reduces several proatherogenic markers. Plasma fibrinogen concentrations decreased by 21%, plasma IL-6 concentrations decreased by 41% and F_2-isoprostanes (markers of lipid oxidant stress) decreased by 31%. Moreover, the authors found that *L. plantarum* administration in smokers markedly decreased the adherence of monocytes to resting (40%) and tumor necrosis factor–activated (36%) endothelial cells [114]. These studies demonstrate that supplementation of the diet with *L. plantarum* may contribute to the prevention and treatment of metabolic disorders in smokers. The authors proposed that this positive effect may be directly associated with the production of propionic acid through the bacterial fermentation of fiber [114]. In accordance with these studies, Kullissar, *et al.* demonstrated that changing gut microbiota by means of probiotic lactobacilli fermented goat milk feeding impacted on several atherogenic markers. The authors found that a 3 weeks treatment significantly improved the oxidative status (lower conjugated diene level in plasma lipoprotein fraction, diminished the level of oxidized LDL and suppressed production of 8-isoprostanes) [115]. Altogether, these data suggest that the modulation of gut microbiota may positively impact on several atherogenic markers. However, systemic investigations are needed to clarify the molecular mechanisms linking the modulation of gut microbiota by pre/probiotics and the positive effect observed.

CONCLUSIONS

The evidence that the gut microbiota composition can be different between healthy and/or obese and type 2 diabetic patients has led to the study of this environmental factor as an important contributor to the pathophysiology of metabolic diseases. Different and complementary mechanisms have been recently proposed. The gut microbiota may participate to the regulation of energy metabolism by several mechanisms, i.e. energy harvest from the diet, regulation of fat storage (FIAF expression), regulation of lipogenesis (ACC, FAS, chREBP and SREBP-1 expression), or regulation of fatty acid oxidation (AMPK activity). Moreover, the development of obesity and metabolic disorders following a high-fat diet may be associated to the innate immune system. Indeed, high-fat diet feeding triggers the development of obesity, inflammation, insulin resistance, type 2 diabetes and atherosclerosis by mechanisms dependent of the LPS and/or the fatty acids activation of the CD14/TLR4 receptor complex. Importantly, fat feeding is also associated with the development of metabolic endotoxemia in human subjects and participates in the low-grade inflammation, a mechanism associated with the development of atherogenic markers. Among the mechanisms linking the gut microbiota to the control of body weight, insulin secretion and appetite, the modulation of gut peptides (i.e. GLP-1, PPY, …) by prebiotics seems to be of interest. Several data obtained in experimental models and human subjects are in favour of the fact that changing the gut microbiota by the means of prebiotics and/or probiotics may participate in the control of several parameters involved in the development of metabolic diseases associated with obesity. Nevertheless, progress in understanding the mechanisms by which the gut microbiota interact with the host will, provide new basis for putative pharmacological or dietary intervention. Moreover, the tremendous lack of data limits our current knowledge of the complexity of gut microbiota-host interactions and proposal of exact mechanisms linking dietary habits, gut microbiota and metabolic disorders. Multidisciplinary research in this field will be helpful to provide evidence-based data, which will be taken into account to consider the gut microbiota as a putative target to prevent metabolic disorders.

ACKNOWLEDGEMENT

PDC is postdoctoral researcher from the FRS-FNRS (Fonds de la Recherche Scientifique, Belgique). NMD and PDC are recipients of subsides from fonds spéciaux de recherche, UCL (Université catholique de Louvain) and FNRS.

REFERENCES

[1] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet 2005; 366: 1059-62.

[2] Matarese G, Mantzoros C, La CA. Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. Curr Pharm Des 2007; 13: 3676-80.

[3] Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. Curr Pharm Des 2008; 14: 1225-30.

[4] Kahn SE, HulRFl RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006; 444: 840-6.

[5] Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, *et al.* Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol 2007; 5: e156.

[6] Xu J, Gordon JI. Inaugural Article: Honor thy symbionts. Proc Natl Acad Sci USA 2003; 100: 10452-9.
[7] Edholm OG. Energy balance in man studies carried out by the Division of Human Physiology, National Institute for Medical Research. J Hum Nutr 1977; 31: 413-31.
[8] Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. Endocr Rev 2006; 27: 750-61.
[9] Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, *et al.* The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004; 101: 15718-23.
[10] Denechaud PD, Dentin R, Girard J, Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. FEBS Lett 2008; 582: 68-73.
[11] Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 2005; 102: 11070-5.
[12] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444: 1022-3.
[13] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444: 1027-31.
[14] Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA 2007; 104: 979-84.
[15] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005; 115: 1111-9.
[16] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444: 860-7.
[17] Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, *et al.* Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005; 11: 183-90.
[18] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.
[19] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-808.
[20] Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996; 271: 665-8.
[21] Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990; 249: 1431-3.
[22] Neal MD, Leaphart C, Levy R, Prince J, Billiar TR, Watkins S, *et al.* Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. J Immunol 2006; 176: 3070-9.
[23] Tomita M, Ohkubo R, Hayashi M. Lipopolysaccharide transport system across colonic epithelial cells in normal and infective rat. Drug Metab Pharmacokinet 2004; 19: 33-40.
[24] Moore FA, Moore EE, Poggetti R, McAnena OJ, Peterson VM, Abernathy CM, *et al.* Gut bacterial translocation *via* the portal vein: a clinical perspective with major torso trauma. J Trauma 1991; 31: 629-36.
[25] Vreugdenhil AC, Rousseau CH, Hartung T, Greve JW, van ', V, Buurman WA. Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. J Immunol 2003; 170: 1399-405.
[26] Black DD, Tso P, Weidman S, Sabesin SM. Intestinal lipoproteins in the rat with D-(+)-galactosamine hepatitis. J Lipid Res 1983; 24: 977-92.
[27] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-72.
[28] Mitaka C. Clinical laboratory differentiation of infectious versus non-infectious systemic inflammatory response syndrome. Clin Chim Acta 2005; 351: 17-29.
[29] Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, *et al. In vivo* effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. Dig Dis Sci 2004; 49: 579-89.
[30] Wang Z, Xiao G, Yao Y, Guo S, Lu K, Sheng Z. The role of bifidobacteria in gut barrier function after thermal injury in rats. J Trauma 2006; 61: 650-7.
[31] Wang ZT, Yao YM, Xiao GX, Sheng ZY. Risk factors of development of gut-derived bacterial translocation in thermally injured rats. World J Gastroenterol 2004; 10: 1619-24.
[32] Kitchens RL, Thompson PA. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. J Endotoxin Res 2005; 11: 225-9.
[33] Haziot A, Ferrero E, Kontgen F, Hijiya N, Yamamoto S, Silver J, *et al.* Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. Immunity 1996; 4: 407-14.
[34] Haziot A, Ferrero E, Lin XY, Stewart CL, Goyert SM. CD14-deficient mice are exquisitely insensitive to the effects of LPS. Prog Clin Biol Res 1995; 392: 349-51.
[35] Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. Science 1988; 239: 497-500.
[36] Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. J Immunol 1988; 141: 547-52.
[37] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-25.
[38] Suganami T, Mieda T, Itoh M, Shimoda Y, Kamei Y, Ogawa Y. Attenuation of obesity-induced adipose tissue inflammation in C3H/HeJ mice carrying a Toll-like receptor 4 mutation. Biochem Biophys Res Commun 2007; 354: 45-9.
[39] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006; 346: 739-45.
[40] Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, *et al.* Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007; 56: 1986-18.
[41] Poggi M, Bastelica D, Gual P, Iglesias MA, Gremeaux T, Knauf C, *et al.* C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. Diabetologia 2007; 50: 1267-76.
[42] Davis JE, Gabler NK, Walker-Daniels J, Spurlock ME. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. Obesity (Silver Spring) 2008; 16: 1248-55.
[43] Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, *et al.* Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circ Res 2007; 100: 1589-96.
[44] Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, *et al.* Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008; 57: 1470-81.
[45] Brun P, Castagliuolo I, Leo VD, Buda A, Pinzani M, Palu G, *et al.* Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2007; 292: G518-25.
[46] Pappo I, Becovier H, Berry EM, Freund HR. Polymyxin B reduces cecal flora, TNF production and hepatic steatosis during total parenteral nutrition in the rat. J Surg Res 1991; 51: 106-12.
[47] Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, *et al.* Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 2008; 22: 2416-26.
[48] Tuohy KM, Rouzaud GC, Bruck WM, Gibson GR. Modulation of the human gut microflora towards improved health using prebiotics--assessment of efficacy. Curr Pharm Des 2005; 11: 75-90.
[49] Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, *et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007; 50: 2374-83.
[50] Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, *et al.* Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. J Am Coll Cardiol 1999; 34: 1975-81.
[51] Bolke E, Jehle PM, Storck M, Nothnagel B, Stanescu A, Orth K. Endotoxin release and endotoxin neutralizing capacity during colonoscopy. Clin Chim Acta 2001; 303: 49-53.

[52] Goto T, Eden S, Nordenstam G, Sundh V, Svanborg-Eden C, Mattsby-Baltzer I. Endotoxin levels in sera of elderly individuals. Clin Diagn Lab Immunol 1994; 1: 684-8.

[53] Hasday JD, Bascom R, Costa JJ, Fitzgerald T, Dubin W. Bacterial endotoxin is an active component of cigarette smoke. Chest 1999; 115: 829-35.

[54] Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr 2007; 86: 1286-92.

[55] Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MC, *et al.* Energy intake is associated with endotoxemia in apparently healthy men. Am J Clin Nutr 2008; 87: 1219-23.

[56] Anderson PD, Mehta NN, Wolfe ML, Hinkle CC, Pruscino L, Comiskey LL, *et al.* Innate immunity modulates adipokines in humans. J Clin Endocrinol Metab 2007; 92: 2272-9.

[57] Creely SJ, McTernan PG, Kusminski CM, Fisher M, da Silva NF, Khanolkar M, *et al.* Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 2007; 292: E740-7.

[58] Vinik AI, Jenkins DJ. Dietary fiber in management of diabetes. Diabetes Care 1988; 11: 160-73.

[59] American Diabetes Association. Nutrition recommendations and principles for people with diabetes mellitus. Diabetes Care 2000; 23(Suppl 1): S43-6.

[60] Delzenne NM, Cani PD. A place for dietary fibre in the management of the metabolic syndrome. Curr Opin Clin Nutr Metab Care 2005; 8: 636-40.

[61] Macfarlane S, Macfarlane GT, Cummings JH. Review article: prebiotics in the gastrointestinal tract. Aliment Pharmacol Ther 2006; 24: 701-14.

[62] Tuohy KM, Probert HM, Smejkal CW, Gibson GR. Using probiotics and prebiotics to improve gut health. Drug Discov Today 2003; 8: 692-700.

[63] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995; 125: 1401-12.

[64] Hord NG. Eukaryotic-microbiota crosstalk: Potential mechanisms for health benefits of prebiotics and probiotics. Annu Rev Nutr 2008; 28: 215-31.

[65] Flamm G, Glinsmann W, Kritchevsky D, Prosky L, Roberfroid M. Inulin and oligofructose as dietary fiber: A review of the evidence. Crit Rev Food Sci Nutr 2001; 41: 353-62.

[66] Burcelin R, Cani PD, Knauf C. Glucagon-like peptide-1 and energy homeostasis. J Nutr 2007; 137: 2534S-8S.

[67] Cani PD, Holst JJ, Drucker DJ, Delzenne NM, Thorens B, Burcelin R, *et al.* GLUT2 and the incretin receptors are involved in glucose-induced incretin secretion. Mol Cell Endocrinol 2007; 276: 18-23.

[68] Knauf C, Cani PD, Ait-Belgnaoui A, Benani A, Dray C, Cabou C, *et al.* Brain glucagon-like peptide 1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. Endocrinology 2008; 149: 4768-77.

[69] Knauf C, Cani PD, Kim DH, Iglesias MA, Chabo C, Waget A, *et al.* Role of central nervous system glucagon-like Peptide-1 receptors in enteric glucose sensing. Diabetes 2008; 57: 2603-12.

[70] Chaudhri OB, Salem V, Murphy KG, Bloom SR. Gastrointestinal satiety signals. Annu Rev Physiol 2008; 70: 239-55.

[71] Druce MR, Small CJ, Bloom SR. Minireview: Gut peptides regulating satiety. Endocrinology 2004; 145: 2660-5.

[72] Wynne K, Stanley S, McGowan B, Bloom S. Appetite control. J Endocrinol 2005; 184: 291-318.

[73] Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, *et al.* The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 2003; 37: 649-61.

[74] Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, *et al.* Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. J Clin Invest 2005; 115: 3554-63.

[75] Goodlad RA, Lenton W, Ghatei MA, Adrian TE, Bloom SR, Wright NA. Effects of an elemental diet, inert bulk and different types of dietary fibre on the response of the intestinal epithelium to refeeding in the rat and relationship to plasma gastrin, enteroglucagon, and PYY concentrations. Gut 1987; 28: 171-80.

[76] Goodlad RA, Lenton W, Ghatei MA, Adrian TE, Bloom SR, Wright NA. Proliferative effects of 'fibre' on the intestinal epithelium: relationship to gastrin, enteroglucagon and PYY. Gut 1987; 28(Suppl): 221-6.

[77] Reimer RA, McBurney MI. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology 1996; 137: 3948-56.

[78] Kok NN, Morgan LM, Williams CM, Roberfroid MB, Thissen JP, Delzenne NM. Insulin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide and insulin-like growth factor I as putative mediators of the hypolipidemic effect of oligofructose in rats. J Nutr 1998; 128: 1099-103.

[79] Cani PD, Dewever C, Delzenne NM. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br J Nutr 2004; 92: 521-6.

[80] Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. Impact of inulin and oligofructose on gastrointestinal peptides. Br J Nutr 2005; 93(Suppl 1): S157-61.

[81] Cani PD, Hoste S, Guiot Y, Delzenne NM. Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. Br J Nutr 2007; 98: 32-7.

[82] Cani PD, Neyrinck AM, Maton N, Delzenne NM. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1. Obes Res 2005; 13: 1000-7.

[83] Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R. Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes 2006; 55: 1484-90.

[84] Delmee E, Cani PD, Gual G, Knauf C, Burcelin R, Maton N, *et al.* Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. Life Sci 2006; 79: 1007-13.

[85] Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM. Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol 2005; 185: 457-65.

[86] Gee JM, Johnson IT. Dietary lactitol fermentation increases circulating peptide YY and glucagon-like peptide-1 in rats and humans. Nutrition 2005; 21: 1036-43.

[87] Keenan MJ, Zhou J, McCutcheon KL, Raggio AM, Bateman HG, Todd E, *et al.* Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. Obesity (Silver Spring) 2006; 14: 1523-34.

[88] Zhou J, Hegsted M, McCutcheon KL, Keenan MJ, Xi X, Raggio AM, *et al.* Peptide YY and proglucagon mRNA expression patterns and regulation in the gut. Obesity (Silver Spring) 2006; 14: 683-9.

[89] Piche T, des Varannes SB, Sacher-Huvelin S, Holst JJ, Cuber JC, Galmiche JP. Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. Gastroenterology 2003; 124: 894-902.

[90] Cani PD, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr 2006; 60: 567-72.

[91] Archer BJ, Johnson SK, Devereux HM, Baxter AL. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. Br J Nutr 2004; 91: 591-9.

[92] Whelan K, Efthymiou L, Judd PA, Preedy VR, Taylor MA. Appetite during consumption of enteral formula as a sole source of nutrition: the effect of supplementing pea-fibre and fructo-oligosaccharides. Br J Nutr 2006; 96: 350-6.

[93] Abrams SA, Griffin IJ, Hawthorne KM, Ellis KJ. Effect of prebiotic supplementation and calcium intake on body mass index. J Pediatr 2007; 151: 293-8.

[94] Kalliomaki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 2008; 87: 534-8.

[95] Lundell AC, Adlerberth I, Lindberg E, Karlsson H, Ekberg S, Aberg N, *et al.* Increased levels of circulating soluble CD14 but not CD83 in infants are associated with early intestinal colonization with Staphylococcus aureus. Clin Exp Allergy 2007; 37: 62-71.

[96] Pischon N, Heng N, Bernimoulin JP, Kleber BM, Willich SN, Pischon T. Obesity, inflammation, and periodontal disease. J Dent Res 2007; 86: 400-9.

[97] Saito T, Hayashida H, Furugen R. Comment on: Cani, *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance: Diabetes 56: 1761-1772. Diabetes 2007; 56: e20.

[98] Saito T, Shimazaki Y, Kiyohara Y, Kato I, Kubo M, Iida M, *et al.* The severity of periodontal disease is associated with the development of glucose intolerance in non-diabetics: the Hisayama study. J Dent Res 2004; 83: 485-90.

[99] Saito T, Shimazaki Y, Sakamoto M. Obesity and periodontitis. N Engl J Med 1998; 339: 482-3.

[100] Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J. Systemic exposure to Porphyromonas gingivalis predicts incident stroke. Atherosclerosis 2007; 193: 222-8.

[101] Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. Arterioscler Thromb Vasc Biol 2007; 27: 1433-9.

[102] Smith BJ, Lightfoot SA, Lerner MR, Denson KD, Morgan DL, Hanas JS, *et al.* Induction of cardiovascular pathology in a novel model of low-grade chronic inflammation. Cardiovasc Pathol 2009; 18(1): 1-10.

[103] Smith BJ, Lerner MR, Bu SY, Lucas EA, Hanas JS, Lightfoot SA, *et al.* Systemic bone loss and induction of coronary vessel disease in a rat model of chronic inflammation. Bone 2006; 38: 378-86.

[104] Joyee AG, Yang X. Role of toll-like receptors in immune responses to chlamydial infections. Curr Pharm Des 2008; 14: 593-600.

[105] Stoll LL, Denning GM, Weintraub NL. Endotoxin, TLR4 signaling and vascular inflammation: potential therapeutic targets in cardiovascular disease. Curr Pharm Des 2006; 12: 4229-45.

[106] Timmers L, Sluijter JP, van Keulen JK, Hoefer IE, Nederhoff MG, Goumans MJ, *et al.* Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction. Circ Res 2008; 102: 257-64.

[107] Michelsen KS, Arditi M. Toll-like receptor signaling and atherosclerosis. Curr Opin Hematol 2006; 13: 163-8.

[108] Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, *et al.* Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci USA 2004; 101: 10679-84.

[109] Bjorkbacka H. Multiple roles of Toll-like receptor signaling in atherosclerosis. Curr Opin Lipidol 2006; 17: 527-33.

[110] Bjorkbacka H, Fitzgerald KA, Huet F, Li X, Gregory JA, Lee MA, *et al.* The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades. Physiol Genomics 2004; 19: 319-30.

[111] Bjorkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, *et al.* Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med 2004; 10: 416-21.

[112] Rault-Nania MH, Gueux E, Demougeot C, Demigne C, Rock E, Mazur A. Inulin attenuates atherosclerosis in apolipoprotein E-deficient mice. Br J Nutr 2006; 96: 840-4.

[113] Bukowska H, Pieczul-Mroz J, Jastrzebska M, Chelstowski K, Naruszewicz M. Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with Lactobacillus plantarum in subjects with moderately elevated cholesterol. Atherosclerosis 1998; 137: 437-8.

[114] Naruszewicz M, Johansson ML, Zapolska-Downar D, Bukowska H. Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. Am J Clin Nutr 2002; 76: 1249-55.

[115] Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 2003; 90: 449-56.