

## The gut microbiota ecology: a new opportunity for the treatment of metabolic diseases ?

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## 1. ABSTRACT

In humans, the intestinal microflora is inherited from our parents and from the environment. It has established an ecological mutualism with the host, allowing each organism to benefit from the symbiotic relationship. Based on recent evidence, some molecular mechanisms for the role of intestinal microflora on the control of energy metabolism have been proposed. During metabolic diseases such as obesity and diabetes, it has been proposed that an imbalance between the two dominant groups of beneficial bacteria, the Bacteroidetes and the Firmicutes, generates signals controlling the expression of genes by the epithelial intestinal cells. Genes involved in lipid metabolism such as the Fast Induced Adipocyte Factor have been considered as putative targets. In addition, bacterial extracts such as the lipopolysaccharides control the tone of the innate immune system thus regulating the general inflammatory status, insulin resistance, and adipose tissue plasticity. Therefore, strategies aimed at controlling the ecological mutualism between intestinal microflora and the host should lead to a new era of therapeutic and health benefits.

## 2. INTRODUCTION

### 2.1. Initial concepts of ecological mutualism between intestinal microflora and the host

Ecological mutualism between organisms is a crucial advantage which allows selected species to survive and to take over other organisms, or to fight against every day aggressions (1, 2): an example is the host-bacteria ecology (3). Dissemination, struggling against sparse nutrients, dramatic and rapid changes in temperature, or hiding from enemies, are some of the problems that have been overcome by bacteria hosted by superior organisms. For example, plant polysaccharides that are not digestible by humans are the main substrates for microbial growth in the colon, whereas butyrate and other products of microbial fermentation are important energy sources for the host (4, 5). Within the concept of mutualism it should be considered that the symbiotic relationship allows protection against pathogens. As an example Bifidobacteria and Lactobacteria strains protect against pathogens (6). A recent work has also shown that the normal development and activity of the 'host' immune system is itself a result of mutualistic interactions (7).

Similarly, the host will contribute in determining which bacteria will be hosted (8, 9), by a mechanism involving the epithelial expression of RegIII gamma. Its expression is triggered by colonization with commensal microbes in germ-free mice (7). This secreted C-type lectin binds to intestinal bacteria but lacks the complement recruitment domains present in other microbe-binding mammalian C-type lectins. Hence, RegIII gamma molecules directly bind their bacterial targets via interactions with peptidoglycan carbohydrate (7). This molecular relationship represents an intestinal strategy for maintaining symbiotic host-microbial mutualism. Therefore, to envision a long term co-evolution, at first glance, the bacteria and the host should both benefit from this ecological mutualism (5). The advantage gained by the host could be an improved feeding efficiency and survival during starvation, increased fertility, or a better protection against enemies such as other diseases. However, the bacterial genome has evolved for a longer period of time than the human genome. Therefore, one could consider that, first at the surface of our planet, the bacteria would have “authorized” our development for one condition: that superior organisms would provide food, dissemination, and safety for the microorganisms. The only solution was to set up a long-term efficient ecological mutualism between bacteria and its host. One direct consequence of the microbiome for the host is the control of the host metabolism so that the bacteria will favor not only the feeding efficiency of the host to survive during period of starvation, but will also improve all the biological systems able to augment energy storage and utilization (10) and hence, the well-being of the host. Consequently, the organism will provide the microbiota with a healthy and safe environment. This co-evolutionary symbiotic ecosystem has evolved over million years and is probably still changing over time.

### 2.2. Recent evolution of the initial ecological mutualism concept linked to new social events

The ecological mutualism concept was that over the millennia the microbial consortia are acquired at birth and are more or less complete within a few days (11). This process of colonization will evolve through life according to antibiotic therapy, hygiene and infection (12). There is a growing awareness of the importance of variation in the gut microbiota as a factor that influences human and animal health, and there is increasing recognition that poor gut health and dysbiosis are related to a wide range of non-infectious disease processes (3, 13). Nowadays the symbiotic cooperation also depends on new social phenomena related to the new feeding habits in the Western world. Since the excess in available food has now changed the deal, the symbiotic ecological pact between the microflora and the human host now has to be renegotiated. Data and arguments from the literature now demonstrate that the intestinal microflora could be over-efficient and providing excessive feeding efficiency (reviewed below) This mechanism could lead to obesity or generate proinflammatory antigens that can overreact with the innate immune system and interfere with insulin action and secretion.

### 2.3. Mutualism ecology in numbers

The human genome has ~30 000 genes whereas the microbial genome hosted by humans has on average 200 000 to 300 000 genes hosted by a trillion individuals (14). The distribution of microorganisms in and on the human body reflects adaptations to life on land, which took place about 400 million years ago. Now the human intestine has the largest colony of bacteria since humans carry on average 1.5 kg of symbiotic and commensal organisms i.e.  $10^{14}$  individuals (14). This large microbiome is shared by more than a 1000 different species. Most of the phylogenetic diversity is found in shallow, wide radiations in a small subset of the known deep lineages (15). Specifically, there are more than 50 bacterial phyla on Earth, but human-associated communities are dominated by 4 main phyla (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria), along with 9 others (Chlamydiae, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Fusobacteria, Spirochaetes, Verrucomicrobia (8, 16). Such a large microbiome therefore has a tremendous capacity to produce molecules able to interact with its host. The challenge is certainly to determine the role of these molecules.

### 2.4. Consequences of the rapid change in mutualism ecology

Due to the dramatic changes that have occurred over that last decades with regards to human feeding behavior there is speculation as to whether the human intestinal microbiome has been able to evolve as rapidly as the new feeding habits and hence to properly allow the host to adapt to this new nutritional situation. Recent data from the literature have demonstrated that the human microbiome from obese patients was different from that of lean subjects and was producing some of the messengers (molecular signals) controlling body weight gain (17-19). The relative proportion of Bacteroidetes is decreased in obese people by comparison with lean people, and this proportion increases with weight loss on two types of low-calorie diet (20). The striking point was that the extensive sequencing of the bacterial 16S rRNA revealed that 70% of the sequences identified were unique to each person. Hence, the proportion of the flora changed that was common to all obese patients under caloric restriction was certainly small but it could still be responsible for the metabolic changes. In addition, animal studies in mice showed that the relative abundance of the two predominant bacterial divisions or super-kingdoms differs between lean and genetically obese animals (*ob/ob*) mice. These animals have 50% fewer Bacteroidetes, and correspondingly more Firmicutes, than their lean (+/+) siblings (19). Similarly, we proposed and have shown that the gut microflora from mice feeding on a fat-enriched diet was dramatically different from their chow-fed counterparts (20, 21). Only four weeks of dietary treatment was necessary to impact on the microbial ecology. The molecular mechanisms linked to the change in microflora was related to the release into the blood of an extract from Gram negative bacteria: lipopolysaccharides (LPS) (22). These molecules can trigger a metabolic inflammatory state that is an important

negative regulator of insulin action leading to insulin resistance and diabetes (23, 24). Importantly, LPS is also a required factor for adipose tissue plasticity leading to obesity (Luche *et al.*, 2008, personal communication). Altogether, the actual rapid changes in feeding behavior have allowed the human intestinal microbiome to adapt but not in tandem with the human genome and physiology. Therefore, the metagenome and genome are no longer in a symbiotic relationship and the human body can no longer benefit from the symbiotic ecology. A therapeutic challenge is now to reconstitute an intestinal microflora that would restore well-being to prevent the over activity of intestinal microflora generated by an excess of food or by nutrients too rich in fat. Certainly, a symbiosis between appropriate probiotics and their corresponding nutrient prebiotics would be an interesting strategy to control the intestinal microflora and its consequences for human health.

### 3. HYPOTHESES ABOUT MANIPULATING THE HUMAN MICROBIOME FOR THE CONTROL OF METABOLIC DISEASES

#### 3.1. Why should it be possible to manipulate the human intestinal microbiota?

The human microbiome is the consequence of a fine balance between environmental factors, such as food, bacteria from external origin, and the host genome. Over millennia the microbiome itself and the human genome have both co-evolved and hence imprinted their mark on their respective counterparts. However, the precise mechanisms that are responsible for such evolution are unknown. Whereas changing the human genome by means of gene therapy is still a long way off, it is a different matter when it comes to manipulating the human microbiome. What is required now is to understand how the genetic engineering of intestinal flora would affect the human genome and its function. The idea of microbiome-derived therapy is conceivable. This conclusion stems from the fact that new-born babies are germ-free beings. During natural delivery the new-born is immediately colonized by the mother's vaginal flora and then by the environmental flora. This first microflora evolves over the first days and weeks and then progressively with the change in feeding habits from suckling to weaning (25). Therefore, it is conceivable to engineer the human intestinal microflora at birth with a given set of bacteria able to protect the host from some diseases and maintain it in good health. As detailed above some molecular mechanisms are involved to determine the type of microflora that will colonize the intestine (7) and conversely, microflora will install a given ecology to prevent other members to become established (3, 13). At the adulthood, the microflora will then remain mostly stable. It is important to note that from the first days of life to the weaned state the organism will be ongoing dramatic hormonal and environmental changes (26, 27) that should impact on the evolution of the intestinal microflora. Later on during adulthood, the intestinal microflora remains stable over years within minimal changes linked to rapid and reversible events such as a change in food intake or sickness for example (3). However, a period of major

hormonal changes such as the menopause could involve new changes not yet documented. Altogether, it is clear that the microbiota of an individual is unique and can be considered as a fingerprint. The difference when compared to a simple identity card is that microflora could provide information regarding not only the genomic profile and hence the absolute identity of the being, but also some ongoing developmental processes such as the slow development of metabolic diseases. Although one could propose the manipulation of the intestinal microflora to control health it should be noted that the physiological endocrine evolution of the body throughout life will impact on the original manipulation of the intestinal microflora.

The respective contribution of the human genome i.e. gene expression, metabolic, neural and other factors versus the role played by the intestinal microbiome on the control of human development is a challenge that will certainly help the understanding of the development of metabolic diseases and more broadly, human health.

#### 3.2. Why should it be impossible to manipulate the human intestinal microbiota?

The molecular engineering of intestinal microflora necessitates that one should first have some solid molecular knowledge of the microbiome. However deciphering the full extent of the human microbiota is at present almost impossible. New technologies involving large scale genomic sequencing are certainly promising (28) and will produce valuable information in the next few years. However, the usefulness of such techniques is definitively limited by the notion of site-specific communities on the mucosal and luminal compartments of the intestine which are most likely different (16). To gain some knowledge one could study the ecology in other vertebrates, that while being different from human still remains related to the human flora (16). This is probably due to the co-evolution of species living during the same period of time. As an example, human beings living in a similar social environment tend to share similar microbiota that could be responsible for a certain degree of susceptibility to some diseases like colon cancer (29). This impact of the flora could also control dietary preferences such as individuals who crave chocolate compared with those who are indifferent (30). Recent nutrimentalomic studies showed that metabolomic profiles and phenotypes are associated with behavioral preferences. Urinary and plasma components partly issued from microflora are providing a specific imprint for each behavior. Therefore, the gut microbiome could in turn have a long-term influence on the host with regards to metabolic diseases. One could suggest that intestinal related factors would control feeding behavior by changing the neural circuits of the brain, as proposed in obese animal models (31) or animals treated with neurohormones (32). This conclusion suggests that indeed, the engineering of intestinal microflora should be able to dramatically affect human health and in general its development. To interfere with the way that the microbiota impact on our body one has to consider the

following concept. The number of phyla is restricted, and the number of species is large, suggesting that at the beginning of the host bacteria co-evolution only a few members colonized the intestine and then the bacterial community evolved in the human intestine to increase diversity and to allow an improved symbiotic relationship responsible for a better survival of the host. Therefore, the regulatory mechanisms from intestinal microflora that could impact on our body health should be derived from precise bacterial species subtly generating signals that would target specific functions in the host. The strategy to modify human health will then depend on several more concepts including the fact that in addition to the host/bacteria symbiotic relationship, each member of the bacterial community can be considered as symbiotic to each other since the survival of one bacterial strain depends on numerous other strains. For example, the oxygen gradient needs to be eliminated by aerobic bacteria so that anaerobic strains develop (33). This has been suggested in studies where an increased proportion of anaerobic/aerobic bacterial strains have been determined along the intestinal tract (33). Another example is that nutrients are partly degraded by some strains and the products released will be used by others (34, 35). The elimination or the increase survival of lactobacilli in the intestinal microflora by antimicrobial therapy may also impact on other bacterial species since Lactobacilli produce lactic and acetic acids, hydrogen peroxide, and antimicrobial substances, that contribute to the maintenance of colony resistance (35). This is one reason why an antibiotic can significantly affect a large spectrum of the intestinal microbiota even if not all the species are sensitive to the antibiotic (36). Conversely, it is not yet understood how the typical flora can return to the pretreatment situation within a few weeks. Therefore, given the complexity and the integrated ecological mutualism between the host and the intestinal microflora it is most likely that engineering the microflora would not be manageable. Although the recent sets of metagenomic data available, while still limited, have showed that there is only a small group of bacterial lineages that constitute 90% of intestinal flora (16), the manipulation of this flora for the control of health is yet not possible.

#### 4. THE BASIS OF FOOD INDUCED METABOLIC DISEASES AND THE LINK WITH THE SOCIAL EVOLUTION OF FEEDING HABITS

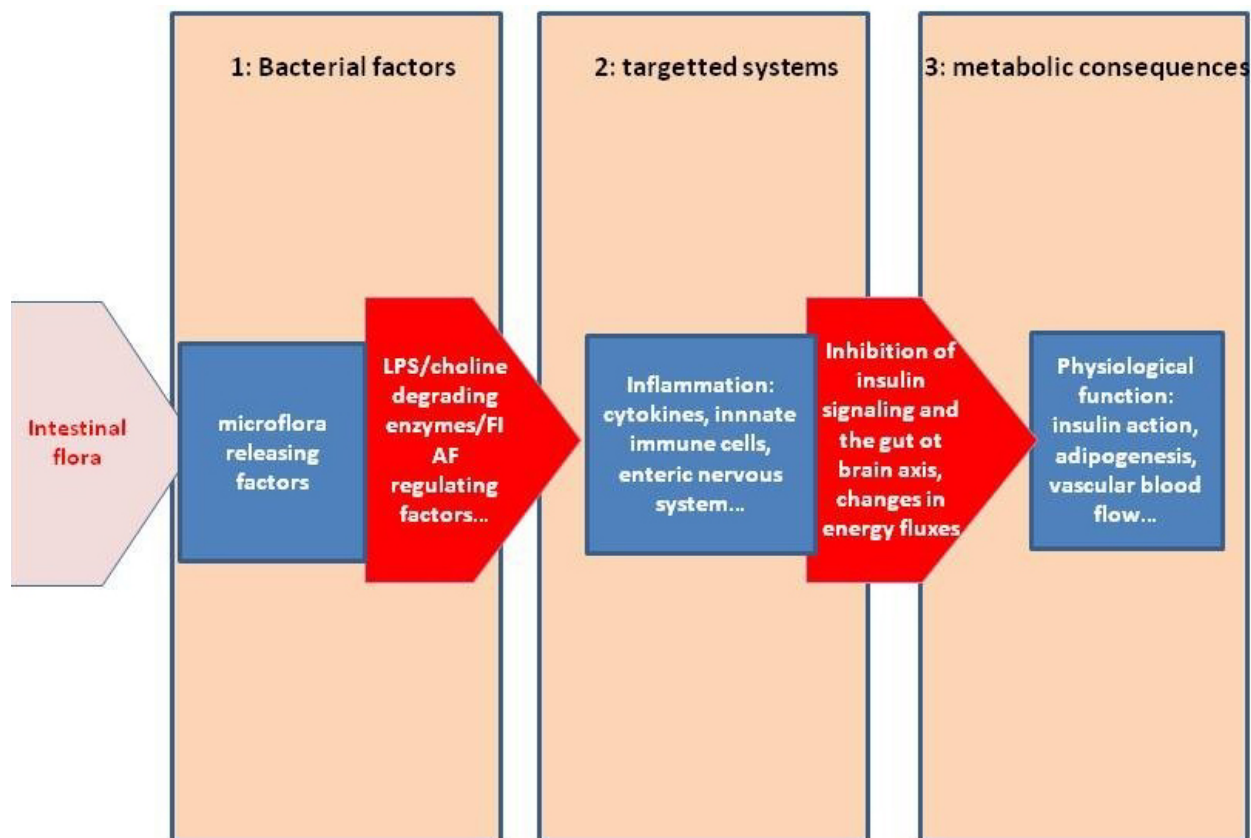
##### 4.1. From the social problem to the microbiota hypothesis

The increasing occurrence of excessive weight gain in Western countries is associated with metabolic and cardiovascular diseases. The maintenance of normal body weight and the prevention of the associated co-morbidities remains a difficult task despite the improvement of prevention methods. Furthermore, the control of excessive body weight gain lacks the input of new efficient therapeutic molecules, and therefore new strategies need to be developed. The World Health Organisation has estimated that 600 million people will be obese in 2025, a doubling of the current population. For example, more than 15% of the population in Europe are

considered overweight. Due to the growing number of newly-diagnosed obese patients as well as the existence of non-diagnosed patients (1/3 are not aware of the disease) the treatment of excessive body weight gain remains a major and therapeutic challenge that society has to face. A growing body of evidence has shown that changes in body weight are due to combined genetic and environmental factors. Although no clear unifying concept has yet emerged, with regards to environmental factors, an original hypothesis has been recently proposed whereby the intestinal microflora would be a causative factor in obesity (10, 19, 20-22, 37, 38) (Figure 1). Therefore, an initial hypothesis is that a gut-to-adipose tissue axis exists but the molecular regulators are not yet identified. A second hypothesis would be that the recent advances of gut peptides such as GLP-1 in the therapeutic field for the treatment of diabetes (39, 40) strongly indicated that the intestine plays a crucial part in the control of glucose homeostasis. Therefore, one could suggest that the ecology of the intestinal microbiota would impact on the host's gut physiological functions such as the secretion of intestinal hormones. Consequently, numerous therapeutic approaches could derive from these concepts.

##### 4.2. From the microbiota hypothesis to the pathophysiological concept of metabolic diseases

Metabolic diseases originate from an intricate network of genetic and environmental factors (41). Certainly, the genetic impact is leading most of the metabolic phenotypes such as hyperglycemia, dyslipidemia, or increased body weight gain. Insulin resistance is central to these phenotypes and is considered an important risk factor for the development of cardiovascular complications. However, the genetic mechanisms responsible for impaired energy homeostasis are mostly monogenic and are almost never associating all the phenotypes characterizing the metabolic syndrome. A more pleiotropic hypothesis needs to be validated. Environmental factors could have a broader range of effect affecting many functions of energy metabolism (41). The arguments supporting the environmental hypothesis could be coming from data which showed that dietary fiber improves energy homeostasis (42). Dietary fiber is indeed of particular interest regarding the putative role in the management of metabolic syndrome by the means of the control of food intake, body weight, glucose homeostasis, plasma lipid profile, and associated cardiovascular diseases (43, 44). Some soluble dietary fiber reduces postprandial glycemia by delaying gastric emptying (45). Others such as dietary fructans and particularly oligofructose (OFS) (46), suggest in animal studies that their dietary consumption might enhance satiety, thereby resulting in reductions in energy intake in streptozotocin-treated diabetic rats (47). A key point linking the metabolic effect of dietary fiber and gut microflora is that the prebiotics are major regulators of certain bacterial strains of intestinal flora such as the bifidobacteria (48). Since dietary fiber has been largely replaced in Western diets by fat one could suggest that a fat-enriched diet would have an opposite impact on microflora profiles. Indeed, 16S RNA analyses showed that one month of high-fat feeding dramatically changed the bacterial profile



**Figure 1.** Molecular relationship between intestinal microflora and metabolic diseases. In a first series of events intestinal microflora releases factors that will interact with targets. In a second series of events the intestinal targetted systems such as the gut hormones, the enteric nervous system and the innate cells from the intestine will transfer the microbial signal towards processes such as insulin action, adipogenesis, and vascular blood flow. Consequently, insulin resistance, obesity, and vascular resistance could develop.

of the intestine reducing resulting the number of bifidobacteria (20-22, 48, 49). The mechanisms related to the effect of changes in feeding habits on gut microflora are not yet understood and will be probably very difficult to analyse since the metagenome is so large. However, there already some hypotheses concerning the mechanisms by which the microflora control energy metabolism, as detailed below (Figure 1).

#### 4.3. The intestinal microflora controls energy harvesting and body weight gain

It has recently been showed that a given intestinal microflora could increase the biodisponibility of energy intake and hence energy storage, by transforming non-digestible fiber into absorbable nutrients (50). The authors showed that obesity is characterized by a change in intestinal microflora. A functional analysis of this flora showed that it favoured the production of short chain fatty acids by the means of their own bacterial metabolism. Such molecules are activators of triglyceride synthesis. This could explain why axenic mice are leaner than their conventional mice counterparts (10). In addition, the colonization of axenic mice with conventional microflora rapidly increased body weight (10). This was associated

with the increased expression of genes involved in glucose and lipid metabolism (38) confirming the role of intestinal microflora in the control of energy metabolism. Precisely, the authors identified that Fast Induced Adipocyte Factor is released under the control of intestinal microflora. This protein then controls the lipoprotein lipase activity and hence the release of free fatty acids from the lipoproteins. Although this hypothesis is seductive, it does not include an important feature of metabolic disease, that is the control of a low inflammatory status (24, 51).

#### 4.4. The intestinal microflora controls the inflammatory tone and insulin action

An increased metabolic inflammatory tone characterizes metabolic diseases (23, 24). The inflammatory mediators are then deleterious for numerous functions such as insulin action. In models of fat-enriched diets, adipose depots express several inflammatory factors such as IL-1, TNF- $\alpha$  and IL-6 (24, 51). These cytokines impair insulin action and induce insulin resistance. Cells from innate immune cells, such as macrophages, and dendritic cells, secrete TNF- $\alpha$  which phosphorylates serine residues of the insulin receptor substrate (IRS-1) leading to its inactivation (52). Hence, the mechanisms leading to

the activation of the inflammatory profiles must be included in the overall picture. Recently, we showed that the intestinal microflora regulates the inflammatory tone of metabolically active tissues such as adipose depots, muscles, and liver. We and others have shown that the increased size of adipose depots, induced by a high-fat diet, is the consequence of the induction of a moderate inflammation as characterized by the tissue content of cytokines (22, 23, 53). We could show causally in mice and confirm in humans that a fat-enriched diet increased endotoxemia within a metabolic range of 2-3 fold that correlated with inflammation (22, 54). When this increased endotoxemia was mimicked by a continuous LPS infusion (by means of an indwelling osmotic pump) in normal chow-fed mice, the body weight and the size of adipose depots increased within a month. This was not observed in mice lacking CD14, the main LPS receptor (22). Eventually we demonstrated the development of adipose tissue and the inflammatory status was linked to specific microflora (characterized by a 100 fold reduction of the Bifidobacteria) (22). The role of other LPS targeted proteins has been proposed. The Toll Like Receptor 4 (TLR4) is the co-receptor for the lipopolysaccharides and it has been proposed that nutritional fatty acids trigger inflammatory responses by acting via the TLR4 signaling in adipocytes and macrophages. The authors showed that the capacity of fatty acids to induce inflammatory signaling following a high-fat diet is blunted in TLR4 knockout mice (53) although they did not characterize the gut microbiota following a high fat diet.

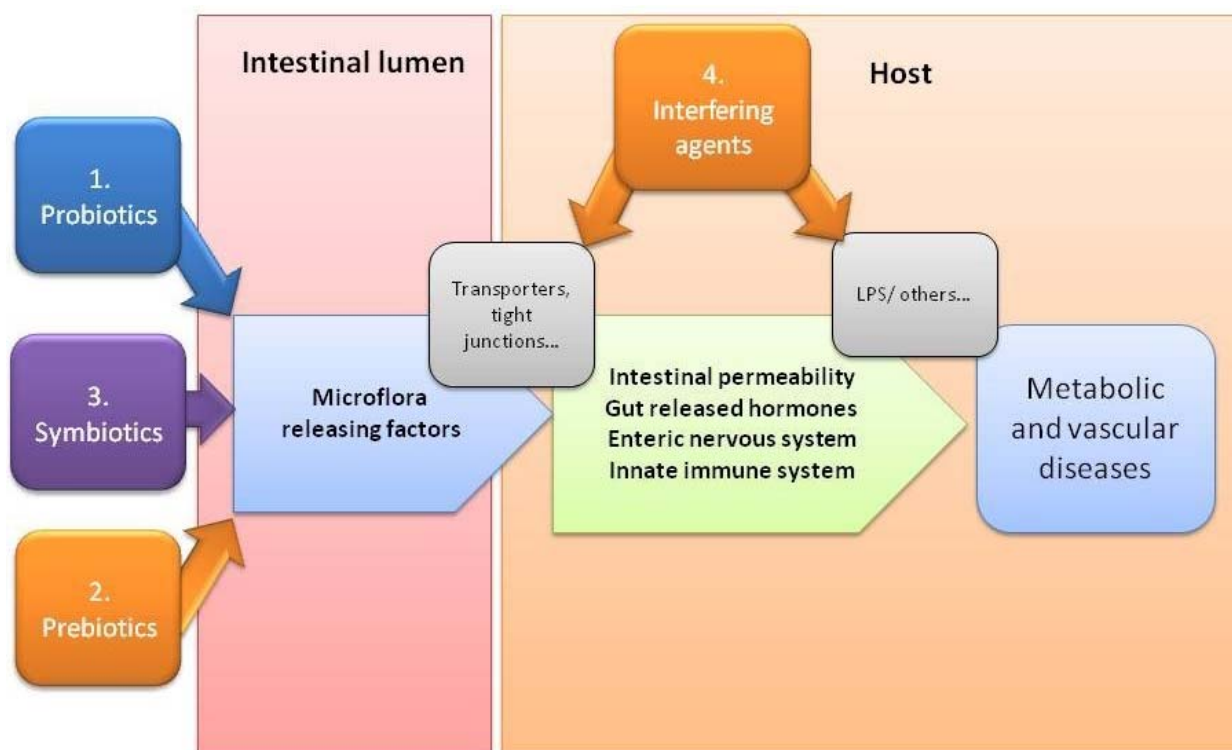
## 5. THERAPEUTIC APPROACHES AND HYPOTHESES FOR THE CONTROL OF INTESTINAL FLORA AND METABOLISM

### 5.1. Targeting lipopolysaccharides

Several approaches can be envisaged. Firstly, a dramatic destruction of intestinal flora by the means of antibiotherapy could be considered. We and our collaborators recently showed that different sets of antibiotics had an impact on glucose metabolism in high-fat diet-fed diabetic mice and ob/ob obese mice (20, 49). Chronic treatment with broad spectrum antibiotics dramatically destroyed intestinal flora. As a consequence oral glucose tolerance was improved, and furthermore, this phenotype was associated with a reduction of tissue inflammation. Cytokine synthesis and macrophage inflammation was reduced. The reduced inflammatory status was correlated with a decrease in metabolic endotoxemia. The strong correlation suggested that the decreased plasma LPS concentration i.e. endotoxemia could be the cause of the reduced inflammatory profile and hence, improved insulin action and glycemic control. The molecular mechanisms responsible for the change in endotoxemia are unknown but could be related to increased intestinal permeability. Among that the factors promoting a leaky gut and increasing endotoxemia levels, could be alcohol consumption (25-29), immobilization stress (29, 30), and radiation (31) have been put forward. Therefore, we showed that the modulation of gut bacteria following high-fat diet strongly increased intestinal permeability with the molecular targets seeming to be

proteins of the tight junctions: high fat feeding reduced the expression of genes coding for zonula occludens-1 and occludin. *In vivo* measurement of intestinal permeability by FITC-dextran absorption showed that a change in the microflora resulting from antibiotic use or a change in diet impacted on the rate of LPS absorption. Hence, we could suggest that the luminal LPS would be similarly absorbed and given that LPS is transported by lipoprotein, a fat-enriched diet will favour this mechanism (55). We showed that the change in feeding habits dramatically impacts on intestinal microflora with the bifidobacteria species dramatically reduced (22, 48). Conversely, other strains were unaffected. Importantly, the bifidobacteria have been shown to reduce intestinal LPS levels in mice and to improve the mucosal barrier function (32-34). Therefore, one goal would be to overcome this reduction in bacteria number. Dietary fibers do so and have an impact on glucose metabolism (43, 46, 48, 56) and the metabolic effect of these prebiotics may be by them changing the intestinal microflora. We could indeed, show that chronic treatment with oligofructose improve glucose metabolism by increasing GLP-1 action (57). This improved intestinal hormone secretion was also attributable to an increase in bifidobacteria induced by the dietary treatment, further highlighting the importance of the intestine and the corresponding hormones as a major organ controlling of glucose metabolism. Therefore, the intestine should be considered as a new therapeutic target for the treatment of metabolic diseases.

A growing body of evidence supports the influence of the endotoxin pathway in metabolic syndrome and beyond to cardiovascular outcomes in humans. A population-based study first demonstrated that significant levels of endotoxin were detectable in the plasma from healthy subjects (58). In another representative sample of a general population, we showed that endotoxin levels were influenced by food intake (54) with a high fat intake correlating with high plasma concentrations of LPS. Importantly, a positive link has been established in a prospective epidemiological study, between plasma endotoxin levels and the occurrence of atherosclerotic plaques (58). Also the deleterious influence of high plasma endotoxin concentrations on insulin sensitivity has been repeatedly observed in septic patients (59). Furthermore, correlations have been described between the CD14 receptor, the main endotoxin receptor in humans, and both insulin sensitivity (60) and acute coronary syndrome (61-63). Recently, we evaluated whether LPS related data were relevant in humans (54) by looking at the relationship between endotoxemia and food intake in healthy men selected from polling lists and surveyed for dietary habits. A positive and significant relation was observed with energy and fat and intakes with all fat types ingested, saturated, monounsaturated, and polyunsaturated showed a similar statistical trend. Conversely, no significant correlation was observed with carbohydrate and protein intakes showing the specificity of fat in the occurrence of metabolic endotoxemia. As the origin of the endotoxemia is related to the intestinal microflora one would consider that controlling the intestinal flora and enteric LPS permeability would be a



**Figure 2.** Putative therapeutic strategies developed from the ecology of intestinal microflora. Probiotics, prebiotics and symbiotics could be used to directly influence intestinal microflora. Interfering molecules could target released bacterial factors or directly the functions controlled by the released factors.

key target for the control of the onset of inflammation-induced metabolic diseases. Taken together these data suggest that the LPS/CD14 pathway may be the bridge between metabolic syndrome and cardiovascular outcomes. Thus in the face of the burgeoning obesity epidemic, modulating LPS levels may represent a new and innovative therapeutic approach.

## 5.2. Intervening in the prebiotic-probiotic relationship

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 (64, 65). Based on this concept very large amounts of data have been generated regarding the role of dietary fibers such as oligosaccharides on the control of metabolism (66, 67). The ingested prebiotic stimulates the whole indigenous population of bifidobacteria to growth (68). The fermentation of dietary fibers produces short-chain fatty acids, that acidify the colonic contents, increase bacterial biomass and, consequently, fecal mass, and modify the composition of the microflora, especially by stimulating the growth of bifidobacteria (68). Less data are available on probiotics, but lactobacilli and bifidobacteria are the strains that are the most used (69, 70). The control of numerous physiological functions has been attributed to the prebiotics-probiotic association. In the case of metabolic diseases intestinal permeability is an important physiological mechanism that could impact on the

inflammatory tone and hence glucose homeostasis (Figure 2). Recent data showed that transepithelial passage and uptake into dendritic cells of non-pathogenic *E. coli* were occurring in chronic inflammatory diseases such as the Crohn's disease (71), that was associated with an increased numbers of adherent bacteria. Although the transcellular pathway was also observed in healthy conditions, microscopy revealed both transcellular and intercellular uptake of *E. coli*. Therefore, a change in intestinal permeability leads in inflammatory disease to an increased absorption of highly inflammatory agents such as *E. coli*, though whether such a mechanism occurs in metabolic diseases still remains to be determined. Another mechanistic hypothesis would be that bacteria would metabolize the prebiotics and generate secondary molecules important for the control of energy homeostasis. As an example, phytoestrogens improve glucose homeostasis in diabetic rats (72) by a mechanism that could be related to the activation of the AMP-activated kinase. However, the circulating amount of phytoestrogen required to activate this enzyme is not known and might not be circulating in concentrations high enough in the blood of these treated rats to ensure a direct physiological action. It is hence suspected that gut flora would be important to fully activate or transform the phytoestrogens into a biologically active set of compounds. Indeed, in germ free animal the effect of lignans was totally absent (73) strongly suggesting that a pro-prebiotic strategy would efficiently beneficiate the host for the control of its

metabolism (74). However, more clinical data are required to validate this concept.

## 6. CONCLUSIONS

Certainly over the next years manipulating intestinal ecology will be a means for the treatment of metabolic diseases. Strategies will involve 1: The direct use of probiotics able to interfere with intestinal functions, 2: The consumption of prebiotics able to favour the proliferation of a given flora, 3: Symbiosis between pro- and prebiotics, 4: Molecules interfering with molecules secreted by intestinal flora or intestinal receptor/transporters, 5: or molecules interfering with bacterial compounds that are absorbed like LPS. Combinations of such strategies should control intestinal function and all other related functions such as hormone secretion, and the enteric nervous system.

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## **Metabolic diseases and intestinal microbiota**

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