Metabolic Diseases: The Diversity at the Level of the Individual

Many socio-economic and medical issues are associated with the recent epidemic of obesity and diabetes. First, in addition to increasing incidence that characterizes the intensity of the epidemic, the mean age of the individuals with dysglycemia and/or who are overweight is coming down to include teenagers (22). Therefore, longer durations of the disease with more secondary complications are expected. Second, there is an increased impact morbidity and mortality of metabolic diseases on cardiovascular events (22) that is also linked to other factors such as smoking and a sedentary lifestyle. Third, the failure of the considerable preventive and therapeutic strategies to overcome the epidemic and corresponding comorbidities is due to the fact that the current drugs treat the consequences of yet unknown causal molecular origins. Excessive glucose production, reduced glucose utilization, or impaired insulin secretion are the consequence of molecular mechanisms that still remain to be determined and on which new therapeutic strategies could be generated. In effect, the vast heterogeneity of the disease further reduces the overall efficacy of any preventive or therapeutic strategy. It is now evident that genetic mutations account for no more than a few percent (17) of metabolic diseases, whereas the remaining diverse, non-genetic causes define the concepts of “metabolic diversity” and the need for a “personalized medicine.” For example, it is conceivable that two patients with similar HbA1c levels but different body weight, plasma insulin concentration, and fasted or fed glycemia most likely have different pathophysiological characteristics. Hence, the parameters used to qualify the potency of a therapeutic strategy are not appropriate with regard to the heterogeneity of the metabolic phenotypes. Altogether, there is a need to identify the causes before the onset of the first signs of diabetes and obesity, such as a slightly increased fasting glycemia or a transient body weight gain. Hence, new paradigms are needed, which should help to identify, first, biomarkers to predict the incidence of the disease before any clinical symptoms and to classify subgroups of metabolic similarities; second, causative targets of the early onset of the diseases; and third, preventive and therapeutic approaches based on vaccines and pharmacological strategies.

Metabolic Disease is Linked to a Change in the Intestinal Microbiota

In 2006 the scientific community recognized a new, metabolically active organ: the intestinal microbiota, which is composed of several trillions of commensal bacteria, that defines the microbiota normally present in the intestine of healthy individual. All coelomates, animals characterized by a cavity during their embryonic development that will generate a coelome, i.e., an intestine, carry a second genome, the microbiome, that constitutes the metagenome that is 100–400 times larger than the human genome, can evolve dynamically according to the nutrition of the individual, and is neither nation nor continent specific (4, 38). This catalog has now identified 4 and currently updated to 8 million nonredundant microbial genes and up to 2,000 prevalent bacterial species. Each individual has at least 160 shared species and a number of well balanced host-microbial molecular relationships that would define groups of individuals responding differently to diet and drug intake (4, 38). This conclusion about the metagenomic diversity addresses the concept of personalized medicine (FIGURE 1). The initial evidence is that the intestinal microbiota differs between obese and lean individuals (30). We validated this concept in a recent study where we showed that when a large cohort of >100 C57bl6 inbred mice were fed a fat-enriched diet for 3–6 mo, a yet not understood
process of metabolic adaptation occurs. It defines that some mice remain non diabetic, whereas others developed diabetes. Although the origin of the different phenotypes remains unknown, the sequencing of bacterial 16S rRNA genes in the diabetic-sensitive mice were distinguished from resistant mice, although they were in the same cage, fed the same diet (42), and were genetically identical. Analysis of the fecal microbial communities of 154 adult female monzygotic and dizygotic twin pairs concordant for leanness or obesity, and their mothers, showed that the human gut microbiome is shared among family members. This shared microbiome defines the “core microbiome” and was at the gene rather than the organism lineage level (48). However, each person’s gut microbial community varied in the specific bacterial lineages present, with a comparable degree of co-variation between adult monzygotic and dizygotic twin pairs. Two phyla mainly compose the gut microbiota in human and rodent models: the Bacteroidetes and the Firmicutes. Although obesity and leanness phenotypes were different at the phylum level through a change of the Bacteroidetes-to-Firmicute ratio, reduced bacterial diversity and an altered representation of bacterial genes and metabolic pathways were major traits of the obese phenotype. Similarly, obesity in the young is also characterized by a change in the intestinal microbiota even at the preschool stage (28). On the other hand, aging is characterized by a deterioration of energy homeostasis with a loss of muscle mass and in the occurrence of a certain degree of insulin resistance mimicking pre-diabetes. In this situation, the core microbiota of elderly subjects was distinct from that previously established for younger adults, with a greater proportion of Bacteroides spp. and distinct abundance patterns of the Clostridium groups (18) suggesting that the change in microbiota in the elderly could be provoking the onset of insulin resistance. However, although many metabolic situations are associated with changes in the intestinal microbiota, the causal molecular link still needs to be identified.

### The Change in Microbiota Could be at the Origin of the Onset of Metabolic Diseases and the Metabolic Inflammation

To clarify the role of microbiota in metabolic diseases, it is important to point out that the intestinal microbiota is inherited at birth and has a dynamic composition throughout life (35). The evidence is that the adult intestinal microbiota is relatively stable over time (58); therefore, the question can be posed as to whether the specific microbiota profile observed in the adult could be inherited at birth and could hence program the development of the metabolic phenotypes. At birth, a dominance of species of Bifidobacterium, Clostridium, and Bacteroides is observed in the early microbiota (36) in the infant intestine. The microbial colonization is influenced by many factors (18) including mode of delivery (23), type of feeding (34), and the widely used antibiotic therapy. The composition of the gut microbiome and the temporal patterns of the microbial communities varied widely from infant to infant, whereas individually each retained a microbial composition that could be recognized for intervals of weeks to months (35). Importantly, this early colonization programs the development of the intestine such as the intestinal vascular bed. Adult germ-free mice had arrested capillary network formation, which corresponds to the development of endothelial capillaries that will be in contact with nutrients and bacterial-released factors (44). This blood capillary network could restart and be completed within 10 days after colonization with a complete microbiota harvested from conventionally raised mice. The mechanism is through a remodeling process that involves the glycosylation of a tissue factor associated with the localization of protein on the cell surface and the activation of
coagulation proteases (39). Therefore, one could suggest that the colonization of the intestine by the microbiota in early life could secrete bacterial factors that directly interfere with the coagulation proteases to regulate their activity and hence the development of the capillary network. Another hypothesis would be that, through the activation of an inflammatory process, the bacteria would stimulate the proliferation of endothelial precursor cells leading to the development of the intestinal capillaries. The microbiota also regulates a pro-angiogenic factor angiopoietin-1 (Ang-1) in the small intestine. Therefore, one could suggest that the colonization of the intestine in early life could also program the future relationship with the metabolic phenotype. In fact, the early gut colonization also shapes future immune responses of the host, which is important with regard to the role of immune system and metabolic inflammation on the control of metabolic diseases (24). Therefore, it is noteworthy that, during the first years of life, the individual microbial ecosystems in each baby remained distinct but converged toward a profile characteristic of the adult gastrointestinal tract, suggesting that incidental environmental exposures may play a major role in determining the distinctive characteristics of the microbial community. An inadequate gut microbiota composition and function in early life seems to account for the deviant programming of later immunity and overall health status (10). In this regard, probiotics and prebiotics, which have the potential to restore the intestinal microbiota balance, may be effective in preventing the development of chronic, immune-mediated diseases such as metabolic diseases and bowel diseases (10, 20). However, in human and animal models, the use of probiotics and prebiotics in the treatment of metabolic disease is still controversial since both beneficial (2, 13, 14) and deleterious (3, 29) effects have been reported on metabolism, although the animal models were different. Altogether, the shaping of the intestinal microbiota, essentially at birth and even in adulthood by the means of pre-probiotic strategy, seems to influence the development of metabolic diseases at the early age and could explain, at least in part, the recent occurrence of the diseases among teenagers.

A question remains regarding the vast majority of obese and Type 2 diabetic patients who developed metabolic disease in adulthood and mostly in response to an inappropriate diet such as a fat-enriched, low-fiber diet. The molecular mechanisms through which the microbiome regulates metabolic diseases have been studied recently and show some initial promising insights. In the first set of experiments to identify the bacterial members, i.e., the taxons and the molecular mechanisms responsible for the onset of obesity, it was shown that the human distal gut from obese patients harbors a vast ensemble of microbes that provide important metabolic capabilities, including the ability to extract energy from otherwise indigestible dietary polysaccharides (49). The proof of principal of the causal demonstration was validated in seminal work by the transfer of obesity to germ-free mice colonized with the microbiota from obese but not from lean mice (47, 49). Furthermore, the “conventionalization” of adult germ-free mice with a normal microbiota harvested from the cecum of conventionally raised animals produced a 60% increase in body fat content and insulin resistance within 14 days despite reduced food intake (5). The microbiota promoted absorption of monosaccharides from the gut lumen, with resulting induction of de novo hepatic lipogenesis. The molecular mechanism was elucidated since germ-free knockout mice lacking fasting-induced adipose factor (FIAF), a circulating lipoprotein lipase inhibitor whose expression is normally selectively suppressed in the gut epithelium by the microbiota, are not protected from diet-induced obesity. These data show that intestinal microbiota increase the storage of triglycerides in adipocytes and the liver by repressing the lipoprotein lipase inhibitor FIAF (5, 6). However, these data could not explain the role of metabolic inflammation that characterizes metabolic diseases (27, 43). In Type 2 diabetes and obesity, there is an increased production of inflammatory cytokines that impairs muscle, liver, and adipose tissue insulin signaling (27, 43). The innate and adaptive immune systems are associated with the cytokine production since macrophages and lymphocytes accumulate in the adipose tissue of fat-enriched diet fed mice and ob/ob mice (7, 21, 54, 55). However, the origin of the corresponding antigens remains unknown. The answer to this question could be linked to the molecular mechanisms accompanying the change in the microbiota that causes metabolic diseases. We first suggested that a major risk factor such as a high-fat diet could impact on the intestinal microbiota first leading to the onset of a new molecular relationship with the host genome (FIGURE 2).

We showed that the intestinal microbiota produces lipopolysaccharides (LPS) that enter directly into the blood of humans (1) and animal models to induce inflammation (2, 11, 12) and most features of metabolic diseases. Briefly, the change in diet increases the gut proportion of gram negative bacteria producing inflammatory LPS (11). The latter are absorbed and accumulate in the blood to activate CD14/TLR4-positive immune cells that secrete deleterious cytokines. The liver is mostly affected by a chronic low-grade LPS infusion. This procedure activates the innate immune system to
release cytokines, which, through the binding to their receptors, interfere with the insulin receptor signaling pathway to generate a state of insulin resistance as suggested in a recent publication (9). Thus LPS may not be an obligatory factor linking high-fat diet to the development of metabolic disease (9). Other bacterial molecules might be involved, such as peptidoglycans, which are components of the bacterial wall. The critical point is that the triggering of the inflammatory process in response to a change in diet-induced microbiota modulation precedes metabolic impairment. One recent example related to this hypothesis has been shown in the context of non-alcoholic hepatic steatosis where the accumulation of fat in the hepatocytes and a concomitant inflammation impair liver cell homeostasis leading to apoptosis and fibrosis (51). In this case, the gut microbiota has profound effects on host gene expression in the enterohepatic system, including genes involved in immunity and metabolism (51). For example, the gut microbiota affects expression by the gut cells of secreted proteins, which modulate lipid metabolism in peripheral organs (6). However, in addition to the impact of the intestinal microbiota on metabolism such as on fat metabolism, the gut microbiota must initiate inflammation to impair insulin signaling and generate insulin resistance in the liver (50). Recently, it has been demonstrated that the microbiota-dependent activation of the leucine-rich repeat containing protein NLRP3 and NLRP6 inflammasome sensing regulate hepatic steatosis and altogether may govern the rate of progression of multiple metabolic syndrome-associated abnormalities (25). Hence, we suggested that the complex interaction between bacteria, the epithelium, and the gut immune system could be a prerequisite for the development of metabolic diseases (FIGURE 3).

The activation of the innate host defense mechanisms depends on specific pattern recognition receptors (PRRs) that recognize highly conserved microbial signature molecules called “microbe-associated molecular patterns” (MAMPs). The PRRs include the family of toll-like receptors (TLRs). At least 9 TLRs have been described and notably the 2 [lipoproteins-linked peptidoglycan ligand (46)], 4 (LPS ligand), 5 (Flagellin ligand), and 9 (unmethylated CpG DNA ligand), which are the most relevant in gram-positive and gram-negative bacteria (33). Besides the role of TLR4 on metabolic diseases described above, recent data demonstrated the importance of TLR2 on the control of the gut microbiota to host relationship for the control of insulin resistance (15). TLR2 knockout mice were characterized by features of metabolic syndrome, i.e., glucose intolerance and insulin intolerance. Furthermore, a reduction in regulatory T-cell in visceral fat was observed. Since a pro-diabetogenic role of adipose tissue lymphocytes has been recently showed (21, 56, 57), it is suggested that TLR2 would favor the cross talk between gut microbiota and adipose tissue lymphocyte for the control of inflammation and hence insulin resistance. Importantly, the microbiota from the TLR2 knockout mice was modified with a threefold increase in Firmicutes and a slight increase in Bacteroidetes, suggesting that TLR2 was also involved in the microbiota to immune system equilibrium.

Another class of bacterial receptors, the NOD-like receptors (NLRs), is considered as key mediators of inflammatory and immune responses (40). It recognizes intracellular MAMPs and would hence be an important sensor of microbes when...
these would be intracellular, such as when phagocytized. The NLR receptors that includes Nod1, Nod2, Naip5, Ipaf, and also Nalp3 would signal a danger for the host when intracellular bacterial fragments are detected (40). Precisely, peptidoglycan fragments from gram-negative bacteria D-glutamyl-meso-diaminopimelic acid (meso-DAP) is recognized by NOD1 receptors, whereas NOD2 detects muramyl dipeptide (MDP) present in all bacteria, although more abundant in gram-positive strains (16). The stimulation of TLRs by MAMPs leads to the activation of Myd88 and nuclear factor kappa B (NFkB) that, in turn, results in the transcription of inflammatory cytokines, chemokines, and antimicrobial genes, which further enhances the risk of diabetes developing (26). Altogether, we suggested that a change in the intestinal repertoire of MAMPs following a change in diet such as a fat-enriched diet was responsible for the activation of the intestinal and then the systemic immune system to generate insulin resistance and promote diabetes. To validate our hypothesis, we and others have analyzed metabolism in mice deleted for some of the MAMPs receptors. Wild-type and CD14 and TLR4 knockout mice where fed a fat-enriched diet to induce insulin resistance, excessive hepatic glucose production, and fasted hyperglycemia. The data showed

**FIGURE 3.** A complex interaction between bacteria and the gut and tissue immune systems could be a prerequisite for the development of metabolic diseases

Upon a change in diet, the intestinal microbiota change, leading to the production of new bacterial factors named the metafactors, such as short-chain fatty acids (SCFA), that could regulate eukaryotic cell functions. Host factors such as the fasting-induced adipocyte factor (FIAF) regulated by the intestinal microbiota will then control cell functions. In addition, the new microbiota ecology represents a change in the antigenic repertoire. Lipopolysaccharides (LPS), peptidoglycans (PGN), Pilin, Flagellin, Fimbriae, and even bacterial DNA (defined as MAMPs) will be recognized by PRRs on host cells and notable intestinal immune cells. In some instances, such as in response to a high-fat diet or in obese ob/o mice, intestinal bacteria can translocate toward tissues and meet the corresponding immune cells.
that, in response to a hyperinsulinemic glucose clamp during which insulin is infused at a constant rate and glucose utilization is recorded throughout the insulin infusion, the CD14/TLR4 knockout mice were shown to be more hypersensitive to insulin compared with wild-type controls (11, 37). This impaired insulin action was affecting mostly the liver since hepatic glucose production remained higher in the wild-type mice than in the knockout mice in response to the insulin infusion (11, 37). Interestingly, this state of hypersensitivity to insulin detected in the CD14 knockout mice was also observed in normal chow-fed mice, suggesting that the LPS receptor was considered as a master physiological switch of insulin action. Similar observations were made in NOD2 knockout mice.

The mutation does not protect against the deleterious impact of a high-fat diet on metabolic parameters such as glucose tolerance and fasted insulin concentration (2, 41), suggesting a protective effect of NOD2 ligands (2, 41). This set of data is in agreement with the observation that lacking NOD2 may predispose one to higher bacterial load, which could drive inflammation and promote insulin resistance as shown (2). However, in vitro recent data show that NOD2 is expressed as well in metabolic tissues such as muscle cells (45). The incubation of the L6 muscle cell line with peptidoglycan motifs that selectively activate the intracellular receptor induces the phosphorylation of serine/threonine MAPK kinases, leading to the production of proinflammatory cytokines. These results suggest that NOD2 alone can also induce insulin resistance, at least in vitro, possibly by activating endogenous inflammatory signals that will impair the insulin signaling pathway. This discrepancy could be linked to the integrated role of inflammation, which in an appropriate situation, such as in acute phase, could prevent a low-grade chronic inflammatory state. Conversely to what was observed in vivo for NOD2, the activation of CD14/TLR4 or NOD1 enhances insulin resistance (11, 41), which is supported by data reporting that the lack of NOD1 or both NOD1 and NOD2 (in a double knockout mouse) protects against diet-induced insulin resistance. Interestingly, some PPR such as TLR5 were shown to protect against metabolic diseases (52). TLR5 is expressed by the innate immune system in the gut mucosa and helps defend against infection. Its deletion favors hyperphagia and many hallmark features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance, and increased adiposity (52). The mechanism could involve the recognition of flagellin that would educate the immune system and prevent an overt inflammation (53). The mechanism through which a change in gut flora induced the activation of these MAMPs could also be related to an original observation whereby bacteria and bacterial fragments translocate from the intestine toward the lymph nodes of the adipose tissue (2). This tissue microbiome could activate innate and adaptive immune cells to recognize the bacterial fragments as non-self and trigger the inflammation process. A co-localization of intestinal bacteria with dendritic cells has been observed (2), although their causal role is not yet established. Importantly, the mechanism of intestinal bacterial translocation could be controlled by leptin. In ob/ob mice, the lack of hormone was associated with a dramatic 10- to 100-fold increase in the microbiota content of adipose tissue. This mechanism seemed to depend on the action of leptin on the intestine since the use of a probiotic-producing leptin partly reversed the metabolic phenotype, suggesting that some intestinal leptin-sensitive cells were controlled and prevented the translocation process. The control of intestinal permeability appears as an important regulatory factor linked to inflammation and metabolic disease (12, 32). Reduced expression of proteins such as Clonidine and Zonula Ocludens would favor tight junction loosening, allowing paracellular bacteria translocation (12) and hence metabolic endotoxemia. This permeability is controlled by numerous regulatory factors, among them the endocannabinoid system that impacts on adipogenesis (32). Consequently, a blood microbiota was also described in humans by pyrosequencing analyses and was mostly composed of the Proteobacteria phylum (85–90%). Importantly, since the major phyla in the feces are the Bacteroidetes and the Firmicutes, we suggest that there should be a specific filter that samples mostly Bacteroidetes and Proteobacteria that then accumulate in the blood. The data also showed that human subjects destined to develop diabetes and control subjects shared a core blood microbiota and that the blood concentration of bacterial 16S rRNA DNA was shown to be an independent marker of the risk of diabetes.

A major question concerned the respective contribution of the diet and the microbiota to the phenotype. Related to this question is the notion of metabolic diversity, which is a prerequisite for the concept of personalized medicine. We suggested that a change in microbiota itself, independent from the diet and the genetic background, could be associated to different metabolic phenotypes as observed in homozygous twins (31, 48). A similar concept is observed in mice. When fed a fat-enriched diet for 3–9 mo, each individual mouse develops diabetes and obesity to a different extent (8). This metabolic adaptation was characterized by a specific hepatic lipid profile (19) and a specific intestinal microbiota (42). Similarly, intestinal permeability was differently modified according to the
diabetic-resistant and diabetic-sensitive status, further emphasizing the importance of intestinal function in the control of metabolism. Altogether, the epidemic and diversity of metabolic disease could have its origin in the change in the microbiota. Its interaction with the host immune system is most likely a key component situated at the origin of metabolic programming and diversity.

Conclusion and Perspectives

The recent discovery that metabolic diseases are associated with a change in intestinal and tissue microbiota opens a new era of putative therapeutic and preventive strategies to reduce the economic and social burden of diabetes and obesity. The millions of genes encompassed by the microbiome is a major area for discovery that should certainly help our understanding of the molecular origin of metabolic diversity. Probiotics and prebiotics able to directly modify the gut microbiota and its interaction with the immune system could be promising avenues to modify metabolic disease. Hence, the importance of the immune system to microbiota relationship suggests that new immunomodulatory strategies could be conceived to reduce the metabolic inflammation associated with obesity and Type 2 diabetes.

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References


