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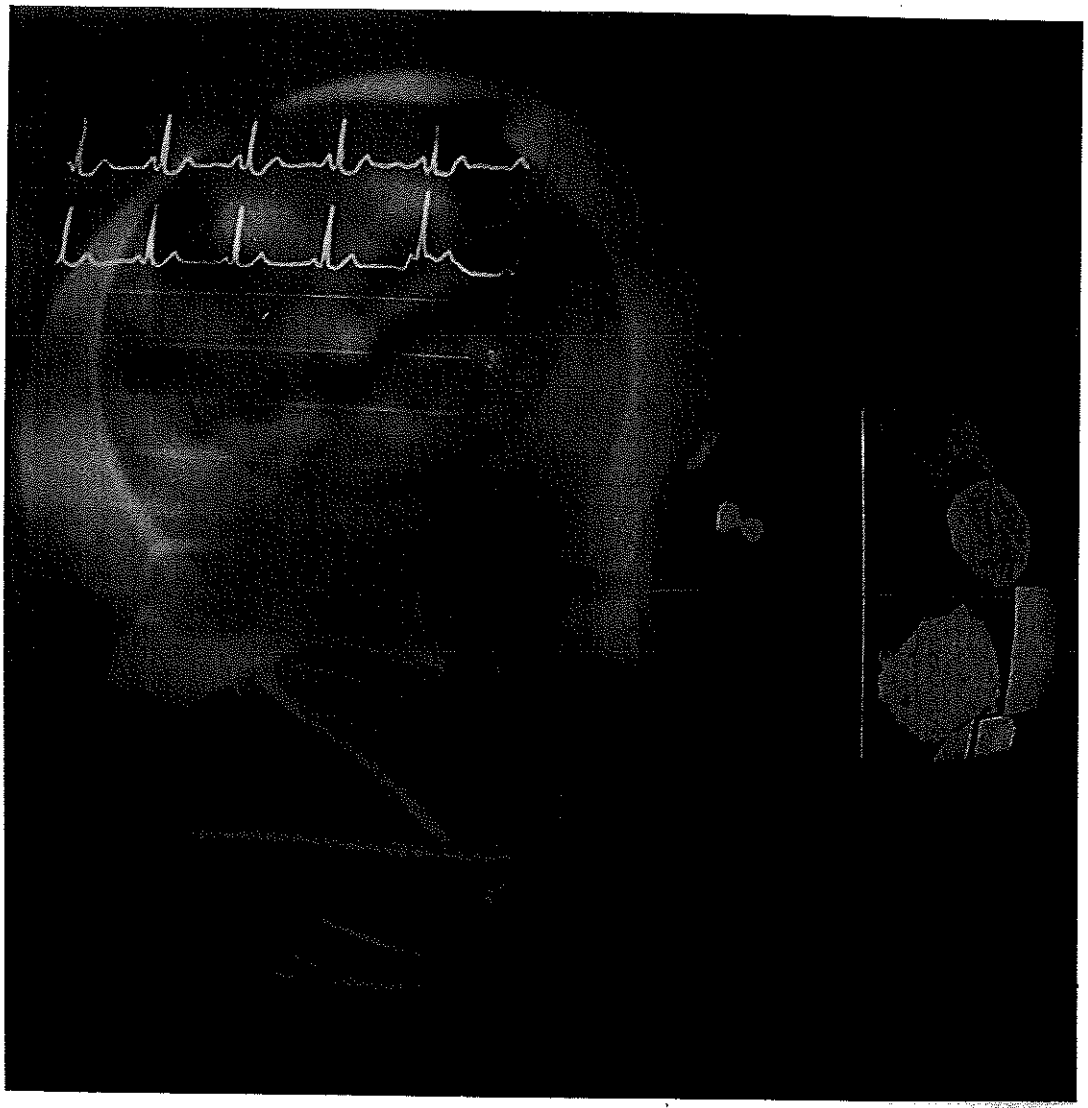

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Gut Microbiota, Diet, Endotoxemia, and Diseases

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The evidence reviewed in this chapter, has largely been made possible by the recent development of powerful tools (high-throughput sequencing methods) that help to delineate the complexity of the gut microbiota. Overall, germ-free mice, probiotic and prebiotic studies suggest that the gut microbiota play a pivotal role in the regulation of energy balance and the development of metabolic disorders. Moreover, the discovery of the role of gut-derived factors, such as lipopolysaccharides (LPSs), in the physiopathology of low-grade inflammation, type 2 diabetes, insulin resistance, and liver diseases is promising. It will be important, nevertheless, to determine whether metabolic endotoxemia induced by high-fat feeding contributes to the development of metabolic disorders associated with obesity. Another interesting point to unravel is whether and how changing gut microbiota impacts on metabolic endotoxemia.

20.1
Introduction

Obesity is a growing epidemic in developed countries and constitutes a major health problem. Obesity is now classically associated with a cluster of metabolic disorders including glucose intolerance, insulin resistance, type 2 diabetes, hypertension, dyslipidemia, fibrinolysis disorders, epithelial dysfunction, atherosclerosis, cardiovascular diseases, nonalcoholic fatty liver diseases (NAFLDs), and nonalcoholic steatohepatitis (NASH) [1, 2]. Most of them are related to glucose homeostasis and to the development of cardiovascular diseases, and probably result from a combination of variable associations of genetic and environmental factors [3–5].

Over the past decade, the physiological processes regulating body weight and energy metabolism, including appetite signals, the central mechanisms of the appetite integration, and gastrointestinal responses to food intake, have received intense investigation [6–9]. Excessive energy intakes and the reduction of physical activity are certainly two environmental factors classically associated with the development of metabolic diseases. The analysis of the nutritional disorders associated with obesity reveals that the adverse health consequences of weight gain

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and obesity are especially prominent following prolonged periods of positive energy balance and are mostly associated with a high-fat diet ingestion in our Western countries. A fat-enriched diet generates features of metabolic disorders leading to the diseases and is considered the most common triggering event. Finally, combining an increased energy intake with a reduced physical activity surely contributes to the development of obesity and metabolic disorders. However, the existence of complex systems that regulate energy homeostasis requires that this paradigm be considered in a larger context.

Over the last years, unequivocal epidemiological, clinical, and/or experimental evidence have causally linked inflammatory signaling responses to the development of the metabolic disorders associated with obesity. Now it becomes clear that obesity and type 2 diabetes are characterized by a low grade inflammation associated with the development of insulin resistance [10–12]. However, it is more difficult to understand why and how metabolic diseases are so commonly linked to inflammatory processes. *What are the mechanisms by which high-fat diet feeding promotes low grade inflammation? What is the molecular link between high-fat or high-energy feeding and the development of this particular response?* 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 [3]. Numerous attempts have been made to determine the triggering factor, which (i) would depend on fat feeding, (ii) trigger a low grade inflammation, and (iii) contribute over a long term period to progressive disease. These questions constitute the core of this chapter.

20.2
Gut Microbiota and Energy Homeostasis

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. To date, the human gut microbiota has not been fully described, but it is clear that human gut is home for a complex consortium of 10^{13}–10^{14} bacterial cells and up to 1000 different species. As a whole, the microorganisms that live inside humans are estimated to outnumber human cells by a factor of 10 and represent genomes overall more than 100 times the size of the human genome to become the so-called "metagenome" [13, 14].

20.2.1
Gut Microbiota and Energy Harvest

The gut flora has been recently proposed as an environmental factor involved in the control of body weight and energy homeostasis [15–20]. Thus, the gut

microbiota can be considered as an "exteriorized organ" which contributes to our homeostasis with multiple metabolic functions largely diversified [21]. More specifically, the biological functions controlled by the gut microbiota are related to the effectiveness of energy harvest, by the bacteria, of the energy ingested but not digested by the host. This mechanism facilitates the extraction of calories from the ingested dietary substances and helps to store these calories in host adipose tissue for later use. Among the dietary compounds escaping digestion in the upper part of human gastrointestinal tract, polysaccharides constitute the major source of nutrient for the bacteria. Part of these polysaccharides could be transformed into digestible substances such as sugars or short chain carboxylic acids (SCFAs), providing energy substrates. The control of body weight depends on mechanisms subtly controlled over time, and a small daily excess, as low as 1–2% of the daily energy needs, can have important consequences in the long term on body weight and metabolism [22]. Consequently, it has been assumed that all mechanisms modifying the food-derived energy availability should contribute to the balance of the body weight.

20.2.1.1 Gut Microbiota and Adipose Tissue Development

Gordon and colleagues have demonstrated in an elegant series of experiments [13, 15–20, 23] that the mice raised in the absence of microorganisms (germ free or gnotobiotic) had about 40% less total body fat than mice with a normal gut microbiota, even though the latter ate 30% less diet than did the germ-free mice [17, 19]. When the distal gut microbiota from the mice was transplanted into the gnotobiotic mice, this conventionalization produced a 60% increase in body fat content and insulin resistance within two weeks, without any clear changes in food consumption or obvious differences in energy expenditure [19]. These data support the hypothesis that the composition of the gut microbiota affects the amount of energy extracted from the diet.

20.2.1.2 Lipogenesis

To account for the effect of gut microbiota to increase fat mass, the authors proposed two leading theories. The first one supports the role of an increase in the intestinal glucose absorption and energy extraction from nondigestible food and concomitantly higher glycemia and insulinemia, two key metabolic factors regulating lipogenesis. The second theory supports the role of a SCFA receptor (GPR41), which could somehow promote deposition of fat [24–26].

Glucose and insulin are known to promote hepatic *de novo* lipogenesis through the expression of several key enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Backhed *et al.* found that a two weeks conventionalization of germ-free mice is accompanied by a twofold increase in hepatic triglyceride content [19]. Both ACC and FAS are controlled by ChREBP (carbohydrate responsive element binding protein) and SREBP-1 (sterol responsive element binding protein) [27]. Accordingly, the conventionalized mice exhibited an increased hepatic ChREBP and SREBP-1 mRNA levels [19].

Strikingly, the development of the adipose tissue observed in the mice harboring gut microbiota was not explained by the modulation of adipogenesis or lipogenesis. The authors proposed that the adipocyte hypertrophy was merely due to a general increase in the activity of the enzyme lipoprotein lipase (LPL), catalyzing the release of fatty acids from circulating triacylglycerol in lipoproteins, which was taken up by muscle and adipose tissue. The researchers proposed that this phenomenon was the consequence of suppression of the fasting-induced adipose factor (FIAF) in the intestine. FIAF inhibits LPL activity, and a blunted FIAF expression in conventionalized germ-free mice could thus participate to the accumulation of triacylglycerol in the adipose tissue.

20.2.1.3 Specific SCFA Receptors

SCFA have been proposed as signaling molecules; propionate, acetate, and to a lesser extent butyrate and pentanoate have been described as ligands for at least two G protein-coupled receptors GPR41 and GPR43, largely expressed in the distal small intestine, the colon, and adipocytes. Very recently, Samuel *et al.* demonstrated that both conventionalized raised and germ-free GPR41−/− mice were leaner than their wild-type counterparts and developed less adipose tissue after colonization with a model of fermentative microbial community (composed of *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*) [28]. These data support the role of a microbiota-dependent metabolic flux in the regulation of the flow of calories between the diet and the host.

20.3 Energy Harvest, Obesity, and Metabolic Disorders: Paradoxes?

This particular original idea, that the bacteria can contribute to the maintenance of the host body weight, is characterized by several paradoxes: first it is not clear that the small increase in energy extraction can actually contribute to a meaningful body weight gain within a short period, as suggested in the gut microbiota transplantation studies; second we and others have clearly shown that a diet enriched with specific nondigestible fibers decreases body weight, fat mass, and the severity of diabetes [29–33]. These specific nondigestible fibers are known as prebiotics: "a selectively fermented ingredient that allows specific changes in the composition and/or in the activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [34]. Moreover, these prebiotics increase the strains of bacteria able to digest the polysaccharide compounds and provide extra energy for the host as they increase the total mass of bacteria in the colon [35–37].

The results, obtained both in rodents and human, suggested that obesity is associated with an altered composition of gut microbiota, with obese subjects or animals characterized by lower *Bacteroidetes* and more *Firmicutes* than lean [15, 16]. To investigate the relation between gut microbial ecology and body fat mass in humans, Ley *et al.* studied 12 obese subjects assigned to a low calorie

diet (fat or carbohydrate restricted), and found that the ratio of *Bacteroidetes* to *Firmicutes* approached a lean type profile after 52 weeks of diet-induced weight loss. However, this study did not demonstrate that the relative changes in bacterial strain profile lead to the different fates of body weight gain. Very recently, Duncan *et al.* performed a similar study, and found data that do not support the hypothesis that the proportions of *Bacteroidetes* and *Firmicutes* are different between obese and lean subjects [38]. The authors did not detect difference between obese and nonobese individuals in the proportion of *Bacteroidetes* measured in fecal samples, and no significant change in the percentage of *Bacteroidetes* in feces from obese subjects on weight loss diets. These data lend credence to the hypothesis that smaller changes or more specific modulation of the gut microbiota community are involved in the development of obesity. The important consequences of such a study warrant the further investigation of this hypothesis.

Although all the data showing the role of the gut microbiota in the extraction of energy from the diet and the development of obesity and related metabolic disorders are convincing, this theory has never unraveled the interplay between obesity and the obesity-related metabolic disorders and the development of a low grade inflammation. It does not explain the result of experiments in which germ-free or conventionalized mice were maintained on a high-fat/high-refined carbohydrate diet (Western diet). Such a study found that conventionalized mice fed the Western diet not only gained significantly more weight and fat mass but also had higher glycemia and insulinemia than the germ-free mice [17]. Not only were the results opposite to those previously observed with germ-free mice fed a normal chow diet but also the amount of Western diet consumed by germ-free and conventionalized mice was similar and hence had similar fecal energy output. All these data suggest that a bacterially related factor is responsible for the development of diet-induced obesity and diabetes.

We thus turn to the question: *What are the mechanisms by which high-fat diet feeding promotes low grade inflammation? Can we attribute the low grade inflammatory process observed during metabolic diseases to the gut microbiota?* This question will now be considered to understand how the gut microbiota may play an even more important role in the development of metabolic disorders associated with obesity.

20.4 Role of the Gut Microbiota in the Inflammatory Associated with Obesity

On the basis of the recent demonstration that obesity and insulin resistance are associated with a low grade inflammation [10–12], we have postulated another mechanism linking gut microbiota to the development of obesity and metabolic disorders. In the models of high-fat diet–induced obesity, adipose depots express several inflammatory factors such as IL-1, TNF-α, MCP-1, iNOS, and IL-6 [39, 40]. These factors have been causally related with the development of impaired insulin action and insulin resistance. The proinflammatory effect of high-fat diets

has mainly been attributed to the inflammatory properties of dietary fatty acids (i.e., palmitic acid). Recently, it has been proposed that such fatty acids trigger inflammatory response by acting via the toll-like receptor-4 (TLR4) signaling in the adipocyte and macrophage, which might contribute to inflammation of adipose tissue in obesity [41–43]. Because high-fat diet–induced type 2 diabetes and obesity are closely associated with a low grade inflammatory state, 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, continuously 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: (i) LPS is a constituent of gram-negative bacteria present in the gut microbiota; (ii) triggers the secretion of proinflammatory cytokines when it binds to the complex of CD14 and TLR4 at the surface of innate immune cells [44]; (iii) continuously produced within the gut by the death of gram-negative bacteria and is physiologically carried into intestinal capillaries through a TLR4-dependent mechanism [45]; (iv) transported from the intestine toward target tissues by a mechanism facilitated by lipoproteins, notably chylomicrons freshly synthesized from epithelial intestinal cells in response to fat feeding [46–50].

20.4.1 Metabolić Endotoxemia and Metabolic Disorders

Therefore, we identified LPS as the triggering factor of the early development of metabolic diseases, the *primum movens* in the cascade of inflammation [51]. Excess dietary fat not only increases systemic exposure to potential proinflammatory free fatty acids and their derivatives, but, as we have recently demonstrated, also facilitates the absorption of highly proinflammatory bacterial LPS from the gut [51, 52]. This new hypothesis provides a new insight into the role played by key gut microbiota-derived products, because the LPS absorbed could affect whole body inflammation and interfere with both metabolism and the function of the immune system. In a series of experiments in mice fed a high-fat diet, we showed that (i) a high-fat diet increases endotoxemia two- to threefold, a level defined as "metabolic endotoxemia"; (ii) fat feeding changes the bacterial populations, which are predominant in the intestinal microbiota, with a marked reduction in *Bifidobacterium* spp. number, and also a reduced *Bacteroides*-related bacteria and *Eubacterium rectale–Clostridium coccoides* group content [51]. We also demonstrated that chronic metabolic endotoxemia, produced by subcutaneously infusing a chronic low dose LPS with an osmotic mimipumps, induces obesity, insulin resistance and diabetes, which was measured by the euglycemic-hyperinsulinemic clamp and oral glucose tolerance test. With LPS receptor knockout mice (CD14−/−) fed a high-fat diet, we showed that metabolic endotoxemia triggers the expression of inflammatory cytokines (TNF-α, IL-1, IL-6, and PAI-1) via a CD14-dependent mechanism. In a study to ascertain the role

of the gut microbiota in the development of metabolic endotoxemia following a high-fat diet treatment, we chronically treated high-fat fed mice with antibiotic. The results confirmed the role of LPS in the development of metabolic diseases associated with obesity because antibiotic treatment completely abolished the high-fat diet–induced disorders: metabolic endotoxemia, the development of visceral adipose tissue inflammation, macrophages infiltration, oxidative stress, and metabolic disorders (e.g., glucose intolerance and insulin resistance) [53]. These last experiments clearly demonstrate the contribution of the gut microbiota to the metabolic endotoxemia.

Consistent with our results, recent studies reported that plasma LPS was increased in *ob/ob* and *db/db* mice [54]. Furthermore, polymyxin B treatment, which specifically eliminates gram-negative bacteria and further quenches LPS, diminished hepatic steatosis [55]. However, these studies did not demonstrate that the gut bacteria determine the threshold at which metabolic endotoxemia occurs or that the modulation of gut microbiota in obese and diabetic *ob/ob* mice controls the occurrence of metabolic and inflammatory disorders. To test this hypothesis, we changed the gut microbiota of *ob/ob* mice using antibiotic treatment and found that macrophage infiltration, inflammatory markers, and oxidative stress were reduced in the visceral adipose depots and to a lesser extent in the subcutaneous fat [53]. Together with the earlier results, these findings strongly suggest that the gut microbiota contributes to the metabolic endotoxemia related to high-fat diet feeding.

20.4.2
Metabolic Endotoxemia and Nutritional Intervention

Among the possibilities for selectively modulating gut microflora, prebiotics and probiotics (live bacteria given in oral quantities that allow for colonization of the colon [56]) are the most important. We found that *Bifidobacterium* spp. were markedly reduced during high-fat diet treatment. This is likely important because several studies have shown that these bacteria reduced the intestinal endotoxin levels and improved mucosal barrier function [57–59]. We therefore used prebiotic dietary fibers [60] to specifically increase the gut bifidobacteria content of high-fat diet–treated mice. We found that among the different gut bacteria analyzed, metabolic endotoxemia correlated negatively with the bifidobacteria count [61]. We also found that in the prebiotic-treated mice, *Bifidobacterium* spp. significantly and positively correlated with improved glucose homeostasis and decreased markers of systemic inflammation (decreased metabolic endotoxemia and plasma and adipose tissue proinflammatory cytokines) [61]. All these data reinforce the role of the gut microbiota in the pathophysiological regulation of endotoxemia, and the development of inflammation and related metabolic abnormalities occurring through diabetes/obesity.

A similar recent study reported that changing gut microbiota with the use of probiotics improved the development of high-fat diet–induced obesity and insulin

resistance. Unfortunately, the authors did not investigate the relationship between gut microbiota and metabolic endotoxemia in that context [62].

20.4.3 Metabolic Endotoxemia, Gut Microbiota, and Fatty Liver Diseases

The concept that the gut microbiota matters for the pathogenesis of liver disease is not novel. An extensive body of literature, mostly derived from animal studies, highlights the concept that a gut-derived bacterial product can target the liver and cause systemic diseases [63–68]. However, the notion that selective changes in the gut microbiota occurring during obesity might prevent the development of NAFLD and NASH is relatively recent. The specific mechanisms linking gut microbiota, obesity, and liver diseases has thus been more specifically investigated. The gastrointestinal tract appears to play a key role in the pathogenesis of fatty liver diseases and inflammation. LPS constitutes an important component, which is believed to stimulate proinflammatory cytokines. More specifically, it has been suggested that portal endotoxemia is a major risk for inducing hepatic inflammation in alcoholic liver diseases [69] and NAFLD [54]. It has also been suggested that bacteria growth in the small intestine of NASH patients could be promoted by a decreased sIgA (a factor inhibiting adherence of bacteria to the intestinal mucosa). A small bacterial overgrowth can produce endotoxin in the enteric cavity, leading to intestinal mucosal barrier damage and higher endotoxin absorption, finally leading to metabolic endotoxemia [70].

20.4.4 Selective Changes in Gut Microbiota and NASH

We and others have demonstrated that changing the gut microbiota by using prebiotics has a salutary effect on the development of NASH in several animal models (i.e., high-fat diet–induced obesity and genetically obese Zucker *fa/fa* rats) and in human subjects [30, 71–74]. More recently, we have demonstrated that changing the gut microbiota of *ob/ob* mice by using antibiotic significantly reduces hepatic triglyceride accumulation, improves liver function, improves glucose tolerance, and increases adiponectin. Importantly, all these changes were associated with a significantly lower metabolic endotoxemia [75].

Similarly, probiotics protect against high-fat diet–induced NAFLD and hepatic inflammation. It has also been found that changing the gut microbiota by using probiotics significantly suppressed high-fat diet–induced activation of the TNF-α/IKK-β signaling, which is the critical signaling for diet-induced insulin resistance [62]. Along the same line in a model of alcohol-induced metabolic endotoxemia and liver disease, rats fed a probiotic lactobacilli were characterized by a reduced plasma endotoxin levels in addition to a lower liver pathology score [76]. A probiotic mixture (*Bifidobacterium*, *Lactobacillus*, and *Streptococcus thermophilus*) has also been found to decrease liver inflammation in *ob/ob* mice [77]. The administration of TNF-α antibodies has also been found to result in a comparable

decrease in inflammation to the prebiotics-induced decrease. While probiotics may prevent further damage to the liver by modulating the gut microbiota, they may also help to stimulate the immune system to produce noninflammatory cytokines and reduce proinflammatory markers. Among the mechanisms, several authors have proposed that changing the gut microbiota can effectively attenuate liver damage and maintain/restore gut barrier function and epithelial function. Other authors have elegantly discussed the role of probiotics in the liver health and overall health [78].

20.5 Metabolic Endotoxemia and High-Fat Feeding: 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 similar mechanisms are observed in humans. Recent data suggest, however, that high-fat feeding induces a metabolic endotoxemia similar to the one observed in rodents. The first study examining the kinetics of baseline endotoxemia in healthy human subjects was recently published by Erridge *et al.* The authors highlighted the putative role of a high-fat meal on the development of metabolic endotoxemia. They 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 and 9 pg ml^{-1}) to concentrations that would be sufficient to induce some degree of cellular activation even in *in vitro* experiments [79]. In addition, they found that the metabolic endotoxemia was sufficient to activate cultured human aortic endothelial cells, and that this endothelial cell activation was likely due to the release of soluble inflammatory mediators, such as TNF-α, from monocytes. Circulating endotoxin levels also increased in patients with type 2 diabetes [80]. The authors found that metabolic endotoxemia was twofold higher in the BMI-, sex-, and age-matched type 2 diabetic patients group than that in the nondiabetic subjects. Furthermore, they found a positive correlation between fasting insulin and metabolic endotoxemia in the whole nondiabetic population, and this correlation persisted after controlling for sex, age, and BMI [80]. Along the same line, we have recently demonstrated that in a large sample of men ($n = 211$) from a population-based study, a positive correlation existed between plasma endotoxin levels and energy/or fat intake [81]. Furthermore, a similar metabolic endotoxemia was shown to increase adipose TNF-α and IL-6 concentrations and insulin resistance in healthy volunteers [82]. 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 behavior and metabolic diseases in humans. Finally, a pancreatic and gastric lipase inhibitor has been shown to reduce metabolic endotoxemia in individuals with impaired glucose tolerance. These findings reinforce the role of fat feeding (and absorption) in the development of metabolic endotoxemia [83].

20.6 Conclusion

The evidence reviewed in this chapter has largely been made possible by the recent development of powerful tools (high throughput sequencing methods) that help to delineate the complexity of the gut microbiota. Overall, germ-free mice, probiotic, and prebiotic studies suggest that the gut microbiota play a pivotal role in the regulation of energy balance and the development of metabolic disorders. Moreover, the discovery of the role of gut-derived factors, such as LPS, in the physiopathology of low grade inflammation, type 2 diabetes, insulin resistance, and liver diseases is promising. It will be important, nevertheless, to determine whether metabolic endotoxemia induced by high-fat feeding contributes to the development of metabolic disorders associated with obesity. Another interesting point to unravel is whether and how changing gut microbiota impacts metabolic endotoxemia.

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