Involvement of the gut microbiota in the development of low grade inflammation associated with obesity : focus on this neglected partner

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Abstract

Nowadays, the literature provides evidence that obesity, type 2 diabetes and insulin resistance are characterized by a low grade inflammation. Among the environmental factors involved in such diseases, the gut microbiota has been proposed as a key player. This neglected "organ" has been found to be different between healthy and or obese and type 2 diabetic patients. For example, recent data have proposed that dysbiosis of gut microbiota (at phyla, genus, or species level) affects host metabolism and energy storage. Among the mechanisms, metabolic endotoxemia (higher plasma LPS levels), gut permeability and the modulation of gut peptides (GLP-1 and GLP-2) have been proposed as putative targets. Here we discuss 1° the specific modulation of the gut microbiota composition by using prebiotics and 2° the novel findings that may explain how gut microbiota can be involved in the development or in the control of obesity and associated low-grade inflammation. (Acta gastroenterol. belg., **2010**, 73, **267-269**).

Key words : obesity, gut microbiota, inflammation, LPS, endotoxemia, adipose tissue, GLP-2.

Introduction

Obesity is classically associated with a cluster of metabolic diseases including glucose homeostasis alteration (insulin resistance, glucose intolerance, type 2 diabetes) and cardiovascular diseases (and/or risk factors such as hypertension, fibrinolysis disorders, dyslipidaemia, ...). Now, epidemiological, clinical and or experimental studies have causally linked a tight relationship between theses diseases and the development of a low grade inflammation (1). However, the mechanisms linking a low inflammatory tone and the onset of metabolic diseases are poorly defined. Along this line, we have discovered that the gut microbiota initiates inflammatory disorders associated with obesity and type 2 diabetes. In that context, experimental evidence highlights potential mechanisms connecting gut microbiota to host metabolism (2,3). Until the recent development of powerful molecular biology methods and gnotobiotic animals the role of the gut microbiota was limited to the investigation of specific pathogens or infectious diseases. However, more studies are becoming essential to unravel whether specific and selective modulation of the gut microbiota may have an impact on the development of obesity and related metabolic alterations. Among the potential tools to target the gut microbiota, probiotic and prebiotic (4) approaches appear as interesting treatments to reverse host metabolic disorders linked to gut microbiota dysbiosis (5,6).

Obesity and related disorders : an inflammatory state directly linked with changes in gut microbiota composition ?

Over the last years, we have demonstrated that both nutritional and genetic obese mice models are characterized by a significant increase in plasma lipopolysaccharide (LPS), defined as "metabolic endotoxemia" (7-10). This constituent of the Gram negative bacteria present within the gut is transported from the gut lumen towards target tissues by a mechanism facilitated by the chylomicrons freshly synthesized from epithelial intestinal cells in response to fat feeding (11,12). We and others, have confirmed these data in human subjects, hence, this phenomenon could participate to the higher plasma LPS levels and low grade inflammation found following high fat diet feeding (7,13-15). However, we may not exclude that these specific changes occur only at the level of fat absorption, since, genetic obese models fed with a normal chow diet are also stigmatized by a higher plasma LPS levels (10,16). Hence, we hypothesized that metabolic endotoxemia could also bring about a modulation in the gut microbiota. Therefore, we characterized and quantified several bacterial families and demonstrated for the first time that high-fat diet-induced obesity was associated with changes in the gut microbiota (7,8). More specifically, we found, that diet-induced obesity strongly altered gut microbiota composition with reduced *Bifidobacterium* spp. and *Bacteroides*-related bacteria, *Eubacterium rectale-Clostridium coccoides* group content (7,8).

To decipher the role of the metabolic endotoxemia in the onset of insulin resistance and metabolic disorders associated with obesity, we used a model of specific alteration in the host-gut microbiota interaction such as genetic invalidation of the LPS co-receptor CD14 (CD14/TLR4 receptor complex). In this model, we found that LPS plays a key role in driving insulin resistance, systemic inflammation, fat mass development and steatosis because CD14-receptor knock out animals completely resist to the high-fat diet induced metabolic

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disorders (7). In addition, these metabolic features were abolished in high fat or leptin-deficient animals (*ob/ob*) treated with large spectrum antibiotics, a treatment blunting intestinal LPS content (9). Finally, we generated double knock out *ob/ob-CD14*$^{-/-}$ mice or *ob/ob* mice treated with a specific LPS antagonist and confirmed these findings (9). In summary, we have shown that these specific approaches (antibiotic treatment or genetic disruption) abolished the onset of metabolic disorders associated with obesity and dependent of the metabolic endotoxemia (i.e., glucose tolerance and insulin sensitivity, the development of visceral adipose tissue inflammation, macrophages infiltration, and oxidative stress) (9). Altogether, these sets of experiments strongly support the role of the gut microbiota and more specifically the LPS as a triggering molecule in the onset of metabolic disorders associated with obesity and type 2 diabetes.

Except the fact that the gut microbiota and most likely bacterial components (such as LPS) may play a role in low grade inflammation and obesity, recent results obtained both in rodents and humans, have suggested that obesity is associated with an altered composition of gut microbiota (17). In accordance with this concept of dysbiosis, obesity has also been characterized by a division wide shifts of the two major phyla (more *Firmicutes*, and even if this later is more controversial, less *Bacteroidetes* characterize obese versus individuals) (18-20). More recently, the level of fecal *Bifidobacterium* spp. was shown to be higher in children with normal weight at the age of 7, but not in overweight children. In addition, *Staphylococcus aureus* counting was lower in children who maintain a normal weight than in overweight (21).

For that reason, we and others have proposed that changes in specific bacteria and/or activity could be involved in the development of obesity, and that targeted changes in specific bacteria – through the prebiotics or probiotics approach - could be of utmost interest to help managing obesity and related diseases.

What are the mechanisms linking specific changes in the gut microbiota and the development of metabolic endotoxemia ?

Several studies have shown that *Bifidobacterium* spp. may be involved in the regulation of gut barrier function and in the reduction of gut lumen endotoxin levels as well as in the improvement of mucosal barrier function (22-24). Among the potential mechanisms explaining the development of metabolic endotoxemia, we found that obese and diabetic mice display enhanced intestinal permeability, that participate to the occurrence of LPS-induced inflammation and metabolic disorders (9,10,16). More specifically, we have shown that fat feeding participate to the disruption of the gut barrier by mechanisms including tight-junction proteins (ZO-1 and Occludin), a phenomenon directly dependent of the gut microbiota (9,10). In accordance with this hypothesis, we found that selective modulation of the gut microbiota by using prebiotics improves gut barrier, reduces metabolic endotoxemia, lowers inflammatory and glucose intolerance (8,10).

Among the mechanisms, we found that the selective change in gut microbiota increases endogenous glucagon-like peptides production (GLP-1 and GLP-2), involved in glucose homeostasis and gut barrier function respectively, both peptides being important in the metabolic effects occurring upon prebiotics-induced modulation of the gut microbiota (8,10,25-29). In obese *ob/ob* mice, we further defined the GLP-2 has a key hormone explaining the metabolic effects of prebiotics such as the improvement of gut barrier, the decreased plasma LPS levels, as well as systemic and hepatic inflammation. Indeed, GLP-2 receptor antagonist has been shown to completely abolish the positive effect of prebiotics (10). Altogether, these data are in favour of a role played by the gut peptide GLP-2 and appear as a novel mechanism contributing to the decrease in inflammation and metabolic disorders during obesity and diabetes.

Finally, in addition to the modifications of inflammatory tone, several studies have also proposed that the gut microbiota participate in the control of food intake and fat mass development via several mechanisms including endogenous gut peptides production involved in food intake and energy homeostasis (5,6,30,31). Therefore, we may not exclude the potential involvement of such mechanisms in the development of obesity and related disorders. Nevertheless, both experimental and human data support the hypothesis that specific change in the gut microbiota (with prebiotics) may participate in the control of glucose tolerance and the development of metabolic diseases associated with obesity. Thus, it would be useful to decipher specific strategies aiming at modifying gut microbiota in order to impact on the occurrence of metabolic diseases.

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References

1. HOTAMISLIGIL G.S., ERBAY E. Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.*, 2008, **8** : 923-34.
2. BACKHED F. Changes in intestinal microflora in obesity : cause or consequence ? *J. Pediatr. Gastroenterol. Nutr.*, 2009, **48** Suppl 2 : S56-S57.
3. BACKHED F., CRAWFORD P.A. Coordinated regulation of the metabolome and lipidome at the host-microbial interface. *Biochim. Biophys. Acta*, 2010, **1801** : 240-5.
4. GIBSON G.R., ROBERFROID M.B. Dietary modulation of the human colonic microbiota : introducing the concept of prebiotics. *J. Nutr.*, 1995, **125** : 1401-12.
5. CANI P.D., DELZENNE N.M. Interplay between obesity and associated metabolic disorders : new insights into the gut microbiota. *Curr. Opin. Pharmacol.*, 2009, **9** : 737-43.

6. CANI P.D., DELZENNE N.M. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr. Pharm. Des.*, 2009, **15** : 1546-58.
7. CANI P.D., AMAR J., IGLESIAS M.A., POGGI M., KNAUF C., BASTELICA D., NEYRINCK A.M., FAVA F., TUOHY K.M., CHABO C., WAGET A., DELMÉE E., COUSIN B., SULPICE T., CHAMONTIN B., FERRIÈRES J., TANTI J.F., GIBSON G.R., CASTEILLA L., DELZENNE N.M., ALESSI M.C., BURCELIN R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 2007, **56** : 1761-72.
8. CANI P.D., NEYRINCK A.M., FAVA F., KNAUF C., BURCELIN R.G., TUOHY K.M., GIBSON G.R., DELZENNE N.M. 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.
9. CANI P.D., BIBILONI R., KNAUF C., WAGET A., NEYRINCK A.M., DELZENNE N.M., BURCELIN R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 2008, **57** : 1470-81.
10. CANI P.D., POSSEMIERS S., VAN DE WIELE T., GUIOT Y., EVERARD A., ROTTIER O., GEURTS L., NASLAIN D., NEYRINCK A., LAMBERT D.M., MUCCIOLI G.G., DELZENNE N.M. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*, 2009, **58** : 1091-103.
11. VREUGDENHIL A.C., ROUSSEAU C.H., HARTUNG T., GREVE J.W., VAN 'T VEER C., BUURMAN W.A. Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *J. Immunol.*, 2003, **170** : 1399-405.
12. GHOSHAL S., WITTA J., ZHONG J., DE VILLIERS W., ECKHARDT E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J. Lipid. Res.*, 2009, **50** : 90-97
13. AMAR J., BURCELIN R., RUIDAVETS J.B., CANI P.D., FAUVEL J., ALESSI M.C., CHAMONTIN B., FERRIÉRES J. Energy intake is associated with endotoxemia in apparently healthy men. *Am. J. Clin. Nutr.*, 2008, **87** : 1219-23.
14. LAUGERETTE F., VORS C., GÉLOËN A., CHAUVIN MA., SOULAGE C., LAMBERT-PORCHERON S., PERETTI N., ALLIGIER M., BURCELIN R., LAVILLE M., VIDAL H., MICHALSKI M.C. Emulsified lipids increase endotoxemia : possible role in early postprandial low-grade inflammation. *J. Nutr. Biochem.*, 2010 Mar 19 (PMID : 20303729).
15. ERRIDGE C., ATTINA T., SPICKETT C.M., WEBB D.J. A high-fat meal induces low-grade endotoxemia : evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.*, 2007, **86** : 1286-92.
16. BRUN P., CASTAGLIUOLO I., DI LEO V., BUDA A., PINZANI M., PALÙ G., MARTINES D. Increased intestinal permeability in obese mice : new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am. J. Physiol. Gastrointest. Liver. Physiol.*, 2007, **292** : G518-G525.
17. LEY R.E. Obesity and the human microbiome. *Curr. Opin. Gastroenterol.*, 2010, **26** : 5-11.
18. LEY R.E., BACKHED F., TURNBAUGH P., LOZUPONE C.A., KNIGHT R.D., GORDON J.I. Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. USA*, 2005, **102** : 11070-5.
19. LEY R.E., TURNBAUGH P.J., KLEIN S., GORDON J.I. Microbial ecology : human gut microbes associated with obesity. *Nature*, 2006, **444** : 1022-3.
20. TURNBAUGH P.J., LEY R.E., MAHOWALD M.A., MAGRINI V., MARDIS E.R., GORDON J.I. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 2006, **444** : 1027-31.
21. 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.
22. GRIFFITHS E.A., DUFFY L.C., SCHANBACHER F.L., QIAO H., DRYJA D., LEAVENS A., ROSSMAN J., RICH G., DIRIENZO D., OGRA P.L. 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.
23. 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.
24. WANG Z.T., YAO Y.M., XIAO G.X., SHENG Z.Y. Risk factors of development of gut-derived bacterial translocation in thermally injured rats. *World J. Gastroenterol.*, 2004, **10** : 1619-24.
25. CANI P.D., HOSTE S., GUIOT Y., DELZENNE N.M. Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *Br. J. Nutr.*, 2007, **98** : 32-7.
26. DELZENNE N.M., CANI P.D., NEYRINCK A.M. Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose : experimental data. *J. Nutr.*, 2007, **137** : 2547S-51S.
27. CANI P.D., DEWEVER C., DELZENNE N.M. 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.
28. CANI P.D., NEYRINCK A.M., MATON N., DELZENNE N.M. Oligofructose promotes satiety in rats fed a high-fat diet : involvement of glucagon-like Peptide-1. *Obes. Res.*, 2005, **13** : 1000-7.
29. CANI P.D., KNAUF C., IGLESIAS M.A., DRUCKER D.J., DELZENNE N.M., 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.
30. PARNELL J.A., REIMER R.A. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am. J. Clin. Nutr.*, 2009, **89** : 1751-9.
31. CANI P.D., LECOURT E., DEWULF E.M., SOHET F.M., PACHIKIAN B.D., NASLAIN D., DE BACKER F., NEYRINCK A.M., DELZENNE N.M. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am. J. Clin. Nutr.*, 2009, **90** : 1236-43.