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REVIEW

IMPLICATION OF GUT MICROBIOTA IN DIABETES MELLITUS AND OBESITY

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Abstract

Background and aims. Differences in the composition of the species of microorganisms in the gut may predict the evolution toward obesity and diabetes mellitus. We carried out a systematic review of the studies dedicated to the role of gut microbiota in diabetes mellitus and obesity.

Methods. A systematic literature search of electronic databases was performed, using the search syntax: "Gut microbiota and diabetes and obesity"; abstracts in English, with data about mechanisms of pathogenesis and treatment options by changing the gut composition were included (259 articles). Studies were excluded if they did not have an abstract, or they contained no data about the exact implication mechanism of microbiota.

Results. There are differences regarding the composition of the gut microbiota in healthy people and type 2 diabetes mellitus patients; the later proved to have significantly decreased Clostridium components, and increased Lactobacillus and Bifidobacterium populations.

The intestines of obese subjects are less rich in microbial genes, have a reduced amount of Bacteroidetes and an increased amount of Firmicutes. Fecal microbiota transplantation from obese subjects resulted in adoption of the donor somatotype. Early differences in gut microbiota composition (higher number of Bifidobacteria) function as diagnostic markers for the development of type 2 diabetes mellitus in high-risk patients.

The gut endotoxins contribute to metabolic syndrome manifestation. Experimental studies with prebiotic showed lower levels of cytokines and antiobesity potential.

Conclusion. Microbiota composition and its changes since childhood have an important role in the metabolic syndrome. Any intervention in order to prevent or treat obesity and diabetes mellitus should have as target the gut immune system.

Key words: microbiota, obesity, gut inflammation.

INTRODUCTION

The chronic inflammatory state represents an important link between obesity and insulin resistance. Dysbiosis is considered a modulator of pancreatic β-cell autoimmunity in the progression of the autoimmune process toward β-cell destruction. Differences in the composition of species of microorganisms of the gut and environmental factors may predict an individual's evolution towards obesity and diabetes mellitus even since childhood. The aim of this paper was to review available evidence on the role of microbiota in diabetes mellitus and obesity. The importance of microbiota implication in the metabolic syndrome has been described in different reviews up to now: Fallucca F (1), Hartstra AV (2), Tai N (3), Festi D *et al.* (4).

METHODS

A systematic literature search of electronic databases, including PubMed, ISI Web of Science, was performed (2007 to 31st May 2015) for all studies assessing the influence of gut microbiota on diabetes and obesity. The search strategy included text terms and MeSH headings for diabetes and obesity: "Gut microbiota and diabetes and obesity". The "related articles" function in PubMed was also used to identify articles not found in the original search.

Inclusion criteria

The inclusion criteria used were: full journal publication, references with abstracts, including data about molecular mechanisms of pathogenesis of gut microbiota in inducing obesity and diabetes, and treatment options in diabetes by changing the gut composition. Papers in English, French, Dutch, Polish and Japanese were included in the study, or in any language but with an English abstract. The titles and abstracts of all identified studies were reviewed by two independent authors (DDL, GI) according to the MOOSE criteria.

Exclusion criteria

Studies were excluded if they did not have any abstract available, or did not meet the inclusion criteria; these were abstracts/articles containing general

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literature data about gut microbiota and obesity or diabetes, but without mentioning any exact implication mechanism or without any experimental study.

RESULTS

The first search resulted in a total of 277 articles. After reviewing the abstracts, studies addressed the description of pathogenetic ways of the gut microbiota implication in diabetes and obesity and 259 met our inclusion criteria (Fig. 1). Included articles were published between 2007 and 31 May 2015. The 18 excluded articles mentioned either only about general implication of gut microbiota in the metabolic syndrome, or did not mention anything about the molecular pathways that would explain these correlations.

Implication of microbiota in diabetes

Two large studies on human subjects (Table 1), metagenome-wide association studies, regarding the faecal metagenome changes in type 2 diabetes mellitus (T2DM) patients were reported in Europe and China and both showed that composition and function of gut microbiota change in the state of disease; metagenomic markers for T2DM differ between the European and Chinese cohorts, and therefore age and geographical location could be the cause (5). The Chinese T2DM patients were characterized by a moderate degree of gut microbial dysbiosis, enrichment of various opportunistic pathogens, and by microbial functions conferring sulphate reduction and oxidative stress resistance, but a decrease of some butyrate-producing bacteria (6).

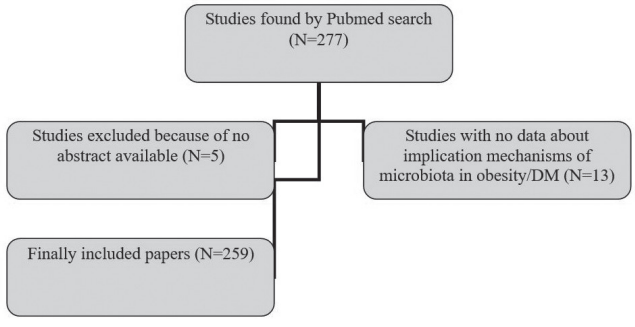

Figure 1. The selection of papers for this analysis.

Bacterial diversity of microbiota in the first year of life is influenced by breast-feeding duration, caesarean section and the age of solid food introduction. These factors mentioned above are correlated to induction of anti-islet cell autoimmunity in type 1 diabetes mellitus (T1DM). Breast-fed infants have different composition of microbiota than formula-fed infants. Children in whom anti-islet cell autoantibodies developed (with T1DM), had lower *Veillonella* abundances and higher *Enterococcus* abundances than children who remained autoantibody-negative (7).

Sasaki M *et al.* showed that transglucosidase treatment represents another possibility of modulating gut microbiota composition by inducing the production of oligosaccharides; blood glucose levels decreased and body weight regulation in the T2DM patients was achieved (8).

Han J *et al.* started from the hypothesis of modulating gut microbiota without systemic anti-infective activity by using berberine, one constituent of a Chinese traditional herb used to treat bacterial diarrhea, and achieving finally an antidiabetic effect

Table 1. Diabetes studies on humans

Study	No. of patients	Microbiota	Outcome	Influence/modulation on/of microbiota	DM type
Karlsson FH *et al.* (5)			Fecal mutagenomic markers		T2DM
Qin J *et al.* (6)	345	butyrate-producing bacteria ↑ various opportunistic pathogens			T2DM
Endesfelder D *et al.* (7)	44		gut microbiome differences in children with T1DM development	Breastfeeding, food type and birth delivery mode	T1DM
Fallucca F *et al.* (1)	NR		Rich diet în pre-and probiotics influence T2DM	Ma-Pi 2 diet	T2DM
Sasaki M *et al.* (8)	60	↑Bacteroidetes/Firmicutes		transglucosidase	T2DM
Han J *et al.* (9)	NR		Antidiabetic effect	Berberine	T2DM

NR= not reported

(9). Berberine prevents insulin-resistance in obese mice and the decrease of serum adiponectin levels corrected for body fat in high-fat diet rats (P<0.01). It also downregulates lipogenic genes and upregulates genes involved in energy expenditure, restores the impaired insulin sensitivity in rats with T2DM (mechanism of protein kinase C-dependent elevation of insulin receptor gene expression), suppresses the expression of pro-inflammatory genes (TNF-α, IL-1β, IL-6, MCP-1), and cyclooxygenase-2, in the white adipose tissue of db/db mice. Administration at a dose of 100 mg/kg body weight, berberine prevented the weight gain and development of insulin resistance induced by long-term high-fat diet feeding (10).

Recent data suggest that fecal microbiota transplantation (FMT) might be beneficial for insulin resistance (11), especially when it is done from lean donors in patients with metabolic syndrome resulting in a significant improvement in insulin sensitivity in conjunction with an increased intestinal microbial diversity, including a distinct increase in butyrate-producing bacterial strains (2).

Experimental studies in animals (Table 2) showed that increase of *Akkermansia spp.* population enhances the metformin effect in T2DM mice (12). A good influence on T2DM is shown by the pre- and probiotics, like the macrobiotic Ma-Pi 2 diet, by influencing the microbiota (1). Prebiotics act favorably on the gut barrier, improving metabolic disorders (13). VSL3 probiotic prevents and treats obesity and diabetes in mice by modulating the microbiota-SCFA (short-chain fatty acids) - hormones axis (12). *Lactobacillus plantarum* DSM 15313, a probiotic, has anti-diabetic properties when fed together with a high-fat diet in mice (14).

Ampicillin and neomycin improve metabolic disorders in DM and obesity, like serum glucose levels, by decreasing inflammation in adipose tissue, decreasing oxidative stress, endotoxemia, preventing adipocytes hypertrophy induced by high-fat diet (15). Administration of trimethoprim and sulfamethoxazole reduced the number of B cells in Peyer's patches in rats, leading to downmodulation of immune responses (14). Experimental studies on ob/ob mice showed that the combination of norfloxacin and ampicillin, at a dose of 1g/L, suppressed the numbers of cecal aerobic and anaerobic bacteria; significant improvement of fasting glycemia and oral glucose tolerance, independent of food intake or adiposity, were noticed after 2 weeks of this antibiotic administration both in ob/ob and diet-induced obese and insulin-resistant mice (16).

Implication of microbiota in obesity

Differences in the microbial gut composition between obese and lean persons are related to dietary factors independent of obesity (17). The gut of obese subjects have reduced numbers of *Bacteroidetes* and increased numbers of *Firmicutes* compared with lean people.

The nutrient load (kcal/d) seem to be a key variable, which influences the structure of the microbacterial community (18). Human studies (Table 3) proved the existence of positive correlation between serum glucose levels and *Bacteroidetes/Firmicutes* ratio, *Bacteroidetes-Prevotella/C.coccioides-E.rectale* ratio, but a negative correlation of these ratios with the BMI was demonstrated (19).

A new mechanism involved in the regulation of host metabolism by microbiota is represented by the decrease of food intake by means of butyrate and propionate (20).

A study performed on pregnant women showed a quantitative difference consisting in the increase of the number of bacteria during the progression of pregnancy trimester in both groups (normal, overweight women). Overweight women have increased numbers

Table 2. Diabetes studies on animals

Study	Animal	No. of animals	Microbiota spp	Outcome	Influence/modulation on/of microbiota	DM type
Yadav H *et al.* (12)	mice	16	*Akkermansia*	Increased effect of Metformin		T2DM
Axling U *et al.* (36)	mice	40	*Lactobacillus plantarum* DSM 15313	antidiabetic		
Hara N *et al.* (14)	rats	NR			trimethoprim and sulfamethoxazole	T1DM
Membrez M *et al.* (16)	ob/ob mice	NR			norfloxacin and ampicillin	
Zhang X *et al.* (10)	mice	NR		Prophylaxis of DM	berberine	

NR= not reported

of *Enterobacteriaceae* in general and of *E. coli* in particular. Any kilogram of weight gain correlated with a corresponding increase in *Bacteroides* numbers by 0.006 log units. Correlations were also found between different biochemical analyses and increase of specific types of bacteria: hypercholesterolemia-*Staphylococcus*; increased serum ferritin, saturation transferrin index and decreased levels of transferrin-*Enterobacteriaceae* and *E. coli*; reduced levels of ferritin, saturation transferrin index and increased levels of transferrin and folic acid- *Bifidobacterium*; increased HDL-cholesterol, folic acid and low levels of triacylglycerol (TAG)-*Bacteroides* (21).

Obese subjects had significantly higher levels of F. prausnitzii, a representative of the *Firmicutes* (22).

Overweight 10 year old children had lower bifidobacterial numbers in their feces compared to their assessing at the age of 3 months, while 10 year old normal weight children had higher mean concentrations of serum-soluble innate microbial receptor (sCD14) than overweight children. Differences were also noticed regarding the mean concentration of adiponectin in maternal colostrum in mothers of children who were normal weight at the age of 10 years: significantly higher compared to those mothers of overweight children (23). Compared to breast-fed neonates, formula-feeding increases basal blood glucose and decreases plasma ketone body concentrations; breast-feeding is correlated to lower incidence of inflammatory bowel diseases, T2DM and obesity later in life; conclusions on postprandial glycaemia, insulin and incretin responses in both human and experimental studies are inconclusive yet (24).

Dysbiosis is involved in childhood obesity, demonstrated also on a large Swiss study (25). Association of changes in microbiota with high-fat diet led to obesity, being demonstrated in a study on 1042 children from Mexico (26).

Table 3. Obesity studies on humans

Study	No. of patients	Microbiota	Outcome	Influence/modulation on/of microbiota
Jumpertz R *et al.* (18)	21	Bacteroidetes/Firmicutes, Bacteroidetes-Prevotella/C.coccioides-*E.rectale* ratios	Correlations between ratios and seric glucose levels, BMI	Caloric load
Larsen N *et al.* (19)	?			
Lin HV *et al.* (20)	NR		Decrease of food intake	Butyrate and propionate
Furet JP *et al.* (22)	43	*F. prausnitzii*		Pregnant women
Luoto R *et al.* (23)	?	Faecal bifidobacterial numbers, adiponectin in maternal colostrum		Children, mothers
Payne AN *et al.* (25)	?	dysbiosis		Children (Switzerland)
Estrada-Velasco BI *et al.* (26)	?	dysbiosis+high fat diet		Children (Mexico)

NR= not reported

Table 4. Obesity experimental studies on animals

Study	Animal	No. of animals	Microbiota spp	Outcome	Influence/modulation on/of microbiota
Duca FA *et al.* (29)	rats+mice	31		FMT	Adopting phenotype of donor
Kimura I *et al.* (30)	mice	NR		Supraexpression GPR43	obesity
Cowan TE *et al.* (31)	rats	NR	↓ Firmicutes/ Bacteroidetes ratio ↑ Enterobacteria		
Engevik MA *et al.* (32)	mice	NR	↓ Firmicutes ↑Bacteroidetes		loss of NHE3
Cani PD *et al.* (17)	mice	24	Bacteroides/Prevotella, *E. coli*, Bifidobacterium	Correlations with body weight, BMI, body fat mass and leptin concentration	TNF$f\alpha$, IL1b, IL1$f\alpha$, IL6 and INF$f\gamma$
Everard A *et al.* (34)	mice	NR	Akkermansia muciniphila	prebiotics	
Neyrinck AM *et al.* (35)	mice	NR		arabino-xylan oligosaccharides	
Axling U *et al.* (36)	mice	NR	*Lactobacillus plantarum*	Green tea	

NR= not reported

Fecal microbiota transplantation (FMT) from obese and lean human, and from mouse donors to gnotobiotic mice, result in the adoption of the donor somatotype by the formerly germ-free rodents. More studies are needed to determine whether the microbiota plays a similarly potent role in human body-weight regulation and obesity (27). FMT from diseased persons (or mice) to germfree mice transfers some aspects of disease phenotype, indicating that altered microbiota plays a role in disease onset and manifestation; the mechanism by which an aberrant microbiota negatively impacts health is by driving chronic inflammation. Host-microbiota relationship can be perturbed by instigator bacteria or dietary components, which may prove to play a role in promoting chronic inflammatory disease states (28).

While performing the transfer of microbiota from obese-prone (*vs*. obese-resistant) mice (Table 4), both being fed with high-fat diet, a specific phenotype was obtained, with differences regarding some characteristics: low intestinal permeability, reduced intestinal and hypothalamic satiation signaling, hyperphagia, increased weight gain and adiposity, and enhanced lipogenesis and adipogenesis (29).

Mice with deficit of GPR43 (receptor for SCFA) are obese at a normal diet, while those with over-expression of this receptor remain lean even at high-fat diet (30). Diet-induced obesity in animal models leads to increased *Mollicutes* (a class of *Firmicutes*); this is reversible with diet. Coffee is associated in rats with decreasing the increased *Firmicutes/Bacteroidetes* ratio in high-fat diet and with enhancing the *Enterobacteria* percentage (31). Decreased *Firmicutes* and increased *Bacteroidetes* amount can be achieved by alterations of the intestinal environment determined by loss of NHE3 (Na-H-exchanger isoform 3), which is a target of *C.difficile* (32).

While a negative correlation of body weight, BMI, body fat mass and leptin concentration with *Bacteroides/Prevotella* and *E. coli* was found, all the parameters mentioned above correlated positively with Bifidobacterium populations. Experimental studies on mice fed with prebiotic (oligofructose) showed lower levels of the cytokines TNFα, IL1b, IL1α, IL6 and INFγ, important reduction of gut permeability because of increased *Bifidobacterium spp* and decrease in markers of oxidative and inflammatory stress in liver tissue (13, 33).

Administration of prebiotics in mice had as effect the normalisation of abundance of *Akkermansia muciniphila*, which improves the metabolic profile; therefore treatment including this colonizer might be a variant in treating or preventing obesity (34). Neyrinck *et al.* showed in mice that non-digestible carbohydrates produced by cereals (AXOS= arabino-xylan oligosaccharides) represent a promising treatment for the control of obesity (35). Green tea together with *Lactobacillus plantarum* decreased high-fat induced inflammation in mice (36).

Elevated proinflammatory gene expression as an expression of a "low-grade" inflammation associated with the metabolic syndrome represent an important link in the pathophysiology of the correlation microbiota-metabolic syndrome (28).

In a study on 93 Gambian lean and obese (with and without DM) women, obesity and DM proved to correlate with high levels of IL6, LPS being maximal in obese subjects and IgM Endo-Cab being low in obese diabetic patients (37). *F. prausnitzii* species are linked to the reduction (bariatric surgery) in low grade inflammation state in obesity and DM, independently of caloric intake (22).

DISCUSSION

Gut microbiota has an important influence on the intestinal peristalsis, and also on the expression of various host genes implicated in the regulation of metabolism, angiogenesis, mucosal barrier function, the development of the enteric nervous system and maturation of mucosal immunity. The endotoxins derived from the gram negative bacteria of the gut can be one of the causes/mediators of low-grade systemic inflammation; their circulation, especially in T2DM patients, is influenced by alterations in diet.

Although possible correlations between microbiota, diabetes and obesity have already been searched in other reviews, the distinct character of our review consists in the fact that our study comprises both theoretical and experimental studies on human and animal models, and also particular situations such as pregnant women, small children; furthermore, it enclosed, besides pathophysiological mechanisms, also therapeutic methods of treating the components of the metabolic syndrome.

Triggers of the metabolic inflammation are saturated fatty acids, glucose and changes in gut microbiota; also involved are toll-like receptors (TLR), inflammasome, and nucleotide oligomerization domain (38).

It has been noticed that there are differences regarding the composition of the gut microbiota in

healthy people and T2DM patients; the latter proved to have significantly decreased *Clostridium* cluster IV and subcluster XIVa components, and increased *Lactobacillales* and *Bifidobacterium* populations. In T1DM, *Bacteroidetes*-to-*Firmicutes* ratio increased over time, whereas it decreased in children who remained nondiabetic (39). Another argument for the role of microbiota in T1DM etiology is the diminished amount of genus *Faecalibacterium* and *Prevotella* in diabetes patients, and the *Bacteroides* was somewhat less than 2-fold more common in diabetics than in control patients (40).

The increase in the number of gram-negative bacteria in gut microbiota and the consumption of high-fat diets increase the plasmatic lipopolysaccharide (LPS) levels (41).

Daily intake of some fruit/drinks rich in polyphenols (3 apples/pears/grapefruit, green tea), significantly reduce body weight in obese people. Glycans are necessary for survival of the intestinal microbiota; *Firmicutes* possess a smaller number of glycan-degrading enzymes than *Bacteroidetes* (42). Higher proportion of the *Firmicutes* classes *Erysipelotrichi* and a lower proportion of *Bacteroidetes* and *Bacillus* was present experimentally in mice that were consuming a "western" diet, compared to the microbiota of those having a low fat/plant polysaccharide diet (21). Thus the imbalance between *Firmicutes/Bacteroidetes* ratio could be explained in humans when using an unhealthy western diet.

An increased proportion of fecal *Bacteroidetes* was observed to parallel weight loss in obese humans after a hypocaloric diet of one year (43). Environmental factors that affect the establishment of the gut microbiota during childhood may lead to obesity later in adulthood. The richness of microbial genes in the gut is lower in obese patients compared to non-obese persons (44) and the benefit of Roux-en-Y gastric bypass consists in the increased richness of microbiota (45), especially of *Proteobacteria* (46), *Escherichia coli* and *Faecalibacterium prausnitzii*. It has been observed that the composition of the gut microbiota is more similar between family members than unrelated individuals.

Demonstration of causality between different phyla of the microbiota and specific diseases remains an important challenge. Early differences in fecal microbiota composition in children may predict overweight: decreased fecal *S. aureus* number was linked to normal weight development and a higher number of *Bifidobacteria* to overweight children (47). The intestines of obese subjects have reduced richness of microbial genes, reduced amount of *Bacteroidetes* and increased amount of *Firmicutes*.

Diet seemed to have a stronger influence than microbes on T1D development in rats, and the gut inflammation induced by it could be a prerequisite for gut pathogen–induced islet autoimmunity; Patrick *et al* found that low-antigen hydrolyzed casein diet had a protective effect against diabetes by changing the microbiota (48). Well-controlled diets would represent the main non-pharmacologic option for the prevention of T2DM, by creating a balance of the gut microflora and through it, also of the metabolic functions.

Changes of microbiota composition, actually of the endotoxin's levels, may be achieved by means of probiotics (49). Immunostimulation by probiotic and vaccination against common human enterovirus might decrease the risk of developing autoimmune diseases, including T1DM (50).

Small intestinal bacterial overgrowth (SIBO) in diabetic patients is thought to be caused by delayed orocecal transit time (51). SIBO is also more frequently encountered in people with autonomic neuropathy and implies higher daily insulin requirements (52). Disorders of the intestinal motility in T2DM patients, implying diarrhea, constipation, flatulence, and abdominal pain, are often followed by SIBO.

The dysbiosis might act as a regulator of β-cell autoimmunity in the progression of the autoimmune process toward β-cell destruction and clinical disease, but direct causality has not been proven yet; this would plead for the correlation of certain bacterial findings with the number of positive autoantibodies (53).

Environmental factors such as microbial stimulation are, in part, implied in the pathogenesis of T1DM. Constant microbial stimulation offers protection against T1DM, the higher the bacterial diversity the higher the probability of not developing diabetes (40, 54, 55). Another hypothesis regarding the islet destruction, associated with β-cell autoimmunity, involves the cross-talk between the gut microbiota and the innate immune system (56): a low abundance of lactate-producing and butyrate-producing species. An increased abundance of the Bacteroides genus was observed in the children with β-cell autoimmunity (53). This exposure to environmental bacteria from birth onward decreases the risk for other autoimmune diseases as well (50).

The pathogenic way of insulin resistance implies also high plasmatic lipopolysaccharide (LPS) levels, causing metabolic endotoxemia; LPS represents

an endotoxin derived from the membrane of gram-negative bacteria and binds to Toll-like receptor 4, so that inflammation is activated (41).

Chronic endotoxinemia contributes to insulin resistance, weight gain and T2DM manifestation (41). The obesity-inducing Enterobacter-derived endotoxin in mice might contribute to the development of obesity in humans (57).

Any intervention, in order to prevent or treat T1DM in humans, should have as target the gut immune system (58). Adiponectin provides protection against the metabolic syndrome. Diabetics and obese individuals have low levels of adiponectin (23).

Arora *et al.* present the probiotics antiobesity potential by implication in energy homeostasis, fat accumulation and alteration of the properties of resident bacterial members already present in the gut (59).

In conclusion, the chronic inflammatory state represents an important link between obesity and insulin resistance. Any intervention in order to prevent or treat T1DM in humans should have as target the gut immune system. Constant microbial stimulation, well-controlled diets and use of probiotics and prebiotics would represent the main non-pharmacologic option for the prevention of T2DM.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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