ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Diet and Cardiometabolic Risks: A Connection Through Gut Microbiota.

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ABSTRACT

Worldwide, obesity, diabetes, insulin resistance, hypertension, and dyslipidemia that characterize metabolic syndrome have increased considerably over the past two decades. Besides genetics and poor diet, several other risk factors may play a role in metabolic syndrome development. Gut microbiota has recently been proposed to exert a profound effect on host nutrition and energy metabolism impacting human physiology and pathology. In essence, gut microbiota has been identified as a potential risk factor for metabolic syndrome emergence; however, the underlying mechanisms of its pathogenesis remain poorly understood. This article is aimed at evaluating the current literature dealing with the connection among diet, gut microbiota and the pathogenesis of metabolic syndrome and its components, and suggesting future avenues of investigation. Gut microbiota along with metabolic syndrome components are introduced. Composition of gut microbiota, its methods of sampling and analysis, and effects of diet in normal states are also introduced. The link among diet, gut microbiota and pathogenesis of metabolic syndrome and its individual components is thoroughly reviewed. In animals, the available evidence indicates the presence of relationships between gut microbiota composition and emergence of obesity, insulin resistance, type 2 diabetes, and atherosclerosis. In humans, the evidence is highly controversial. However, no causal relationship is yet established. Controlled human research examining direct clinical and longitudinal effects of various dietary components or interventions on key microbial changes and their role in metabolic syndrome is needed.
Keywords: Gut microbiota, Cardiometabolic risks, Insulin resistance, Obesity, Dyslipidemia, Diabetes, Diet, Metabolic syndrome.

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ISSN: 0975-8585

INTRODUCTION

Metabolic syndrome is a complex of interrelated metabolic risk factors that increase the risk of cardiovascular disease and type 2 diabetes mellitus [1]. Visceral adiposity and its associated disorders including atherogenic dyslipidemia, hypertension, insulin resistance and/or glucose intolerance, as well as pro-inflammatory and pro-thrombotic states are the key elements characterizing the metabolic syndrome [2]. It is well appreciated that metabolic syndrome is the product of interaction between genetic and environmental factors; however, the underlying mechanisms of its pathogenesis remain poorly understood [3]. Metabolic syndrome has increased dramatically over the past two decades posing one of the greatest public health challenges worldwide [4]. Lifestyle changes particularly dietary patterns are thought to be the primary reason behind this problem [4]. However, recent reports have demonstrated the fundamental function of gut microbiota in host nutrition and energy metabolism with a major focus on its possible role in the regulation and pathogenesis of metabolic syndrome [5-8].

The relationship between microbes and human health seems to be more complicated than previously thought. Several trillions of microorganisms normally colonize the human gut [8]. The role of these microbes has been found to be far beyond digestion, fermentation, nutrients synthesis, and activation both innate and adaptive host immunity responses [8]. In fact, scientific evidence has pointed to the gut microbiota as a major modulator of host metabolism [8]. Gut microbiota performs its major functions in host nutrition and energy homeostasis through a number of mechanisms including increased energy harvest from diet through short chain fatty acids production [9], induction of metabolic endotoxemia [6], deconjugation of bile acids [10], modulation of gut-derived peptide secretion [11], and interaction with immune system triggering low grade inflammation [8].

After a decade of investigation, the contribution of human gut microbiota to metabolic syndrome and its components is still unclear. Several reasons have been suggested and examined that may explain such limitation. Most studies have pointed to the remarkable within an individual and between individuals fluctuation in the composition of gut microbiota [12], the use of different sampling and analytical techniques to explore gut microbiota [13], the inclusion of subjects with diverse ethnic origin and food habits [14], and the heterogeneous etiology of metabolic syndrome components particularly obesity, diabetes and insulin resistance that can be associated with different microbial communities [15,16]. Moreover, diet has been proposed as a potential modulator of the gut microbiota community, a matter that leads to emergence of a vicious cycle consisting of diet, gut microbiota and metabolic syndrome [17,18]. However, the impact of diet components on gut microbiota composition is not fully understood. In fact, manipulation of gut microbiota through dietary modification may represent a tool for controlling metabolic syndrome [19]. In this context, the so-suggested dietary modifications that include increase consumption of dietary fiber, plant foods, and modifying the quality and quantity of dietary fats is principally similar to the classical recommendations contained in the criteria of the healthful diet [19-21], which may not be enough to induce the required microbial changes [17,19-21]. Yet, studies have failed to identify a systematic change in the composition of gut microbiota that may contribute directly to human health and metabolic disease. This review is intended to examine and analyze the current scientific literature dealing with the connection among diet, gut microbiota and development and pathogenesis of the metabolic syndrome and its components, focusing on underlying mechanisms and suggesting future avenues of investigation.

SCIENTIFIC EVIDENCE: LITERATURE SEARCH

An up-to-date literature review was conducted on the relationship among diet and its components, cardiometabolic risks, and gut microbiota composition. The search was limited to English publications from a 15-year period (2002–2016). Relevant articles were principally identified through the PubMed search using the following keywords or their combinations: gut microbiota, cardiometabolic risks, diet, insulin resistance, obesity, dyslipidemia, diabetes, and metabolic syndrome. Only original experimental or clinical research articles on humans or animals were included. For further search accuracy, the reference lists of original works were checked for additional PubMed publications.

GUT MICROBIOTA IN NORMAL MAN

The neonate is born sterile, nonetheless during and after birth, bacterial colonization starts by either infants encounter with their maternal vagina or by cutaneous microorganisms depending on the type of delivery [22]. New microbes enter the gastrointestinal tract with foods, primarily, breast milk. The breast milk itself has a highly diverse and complex bacterial community which may include *staphylococcus, streptococcus, Serratia, Corynebacteria*, propionibacteria and bifidobacteria [23]. As an infant grows up, gut microbiota composition changes significantly with any health- or diet- related events, such as introducing solid foods and antibiotic treatment, where more complex and stable communities similar to the adult microbiota become established at 2-3 years of age [24]. Thus, intestinal tract is a dynamic ecosystem affected by numerous external and internal host-related factors particularly foods and feeding habits.

Indeed, adult gut microbiota has been found to be highly diverse. Results of an early study using molecular analytical techniques to assess fecal microbial communities in 16 healthy individuals have revealed that each person has his own unique fecal microbiota [25]. Actually, it has been established that intestinal microbiota in healthy adult humans is dominated by members of two bacterial phyla: the *Bacteroidetes* and the *Firmicutes* [26]. Analysis of 91 fecal samples from healthy humans have shown that both *Clostridium coccoides- Eubacterium rectale* group and the *Clostridium leptum* group which belong to *Firmicutes* phylum represent more than 50% of total bacteria, followed by the *Bacteroides* group (*Bacteroidetes* phylum) which represents less than 10% [27].

However, more recent studies have looked for shared features among gut microbial communities in humans. Arumugam et al [28] have suggested that the microbiota of most individuals is predominated by three enterotypes: *Bacteroides*, *Prevotella*, and *Ruminococcus* which are independent of age, gender, ethnicity, or body mass index. These results are based on the microbial analysis of fecal samples taken from only 39 subjects from 6 different nationalities [28]. On the other hand, a larger cohort study consisting of 238 individuals have failed to identify these three distinct categories, but rather combined gradients of the three enterotypes with substantial overlap between them have been expressed [12].

Thus, identifying a predominant gut microbiota enterotype for the same subject seems to be a difficult task; this could be attributed to the significant variation in microbiota composition that has been detected between fecal and mucosal samples obtained from the same person [26,29]. Moreover, results of daily analysis of fecal microbiota for more than 6 months have revealed a pronounced temporal variation in individuals' microbiota over time [30]. In fact, identification of a full profile of gut microbiota genera in healthy individuals is still a scientific debate. Further investigation is also needed to identify the composition of microbiota community in relation to specific disease.

SAMPLING AND ANALISIS OF GUT MIROBIOTA

The use of advance culture- independent molecular methods has created a new understanding for gut microbiota. These methods with their bioinformatics advance systems are primarily used to identify the microbial community by sequencing of 16S ribosomal RNA gene, and sometime the whole-genome providing a huge amount of data and information [30]. However, these methods are not without problems that may influence the results. Several inaccuracies have been detected through sampling methods, DNA extraction procedures, and molecular analytical techniques. Primarily, conditions of sample storage and transportation have a major effect on sample quality. Storing samples at room temperature for more than 12 hours reduces significantly their bacterial counts [29,31]. Moreover, marked reduction in the detected numbers of Gram negative *Bacteroides* spp. has been recorded in samples stored at −20 °C for one week as compared with fresh samples [13]. In essence, in human studies, it is impractical to process and analyze fresh fecal samples, thus the impact of storage and transportation conditions on gut microbiota must be taken into consideration.

DNA extraction is also a critical step in the procedures involving gut microbiota analysis [13, 32, 33]. The use of different DNA extraction methods has led to varied results. A significant increase in the bacterial diversity has been observed after mechanical DNA extraction compared with enzymatic DNA extraction methods [13,32]. Furthermore, the use of different commercial DNA extraction kits has produced significant variability in the DNA yield and composition [33]. The quality and quantity of extracted DNA is considered the key determinant for the next step of analyses. For instance, the bacteria belonging to the family

Coriobacteriaceae have not been detected as part of the normal gut microbiota by using enzymatic DNA extraction and 16S rDNA amplification [26], whereas these bacteria constitute 1–8% of the total human gut microbiota in studies using fluorescent *in situ* hybridization methods which do not need the DNA extraction step [27, 34]. Thus, results of various studies using different methods of analysis could be incompatible. Nonetheless, in studies investigating gut microbiota, it is important to point out the DNA extraction procedures particularly the molecular analytical techniques whenever a comparison of studies' results is made.

DIET-GUT MICROBIOTA CONNECTION

Although full identification of gut microbiota composition in healthy individuals has not been yet established, diet and dietary pattern have been studied as major environmental modulators of the gut microbiota community [35]. Cross- sectional observational studies have investigated the association between dietary pattern and components of gut microbiota in healthy population [14,17]. A comparison between fecal microbiota of 15 European children consuming a typical Western diet, and that of 15 rural African children consuming a vegetarian diet has indicated a higher microbial richness and biodiversity in African than in European children [14]. The high-fiber vegetarian diet ingested by the African children has associated with a high level of gut *Bacteroidetes,* with the abundance of *Prevotella* and *Xylanibacter* species, whereas members of the *Firmicutes* phylum has predominated the gut of the European children ingesting the Western diet [14]. It may be noted that apart from the diet, other environmental factors such as ethnicity, hygiene, climate and geography that could influence the formation of gut microbiota have not been investigated [14].

Dietary intervention trials have demonstrated variable degrees of changes in the composition of gut microbiota [17, 21, 36]. In an attempt to establish a link between dietary components and specific enterotype, a cross sectional analysis of fecal microbiota of 98 healthy volunteers with their habitual diet has shown that the *Prevotella* predominance is associated with consumption of low amounts of saturated fats and animal proteins, and high amounts of carbohydrates and simple sugars, whereas *Bacteroides* dominance is associated with high intakes of saturated fats and animal proteins [17]. Indeed, *Prevotella* abundance has shown good association with diets rich in carbohydrates and poor in saturated fats and animal proteins [14]. However, provision of high- fat, low- fiber diets or low- fat, high fiber- diets to 98 healthy subjects for 10 days has caused rapid but limited changes in subjects' gut microbiota composition; these changes are not sufficient to overcome inter-subject variations or changing subject predominant enterotype [17].

The diet- related alterations in gut microbiota have been shown to be more detectable in studies comparing obese versus lean subjects. A significant increase in fecal microbial richness has been observed in obese, but not lean, subjects ingesting high-protein, energy-restricted diet for 6 weeks [21]. Deceased *Firmicutes* and increased *Bacteroidetes* abundance have been shown in 6 obese subjects following strict vegetarian diet for one month [19]. On the other hand, in a 5 days intervention experiment involving 10 subjects, provision of animal- based diet has enhanced the abundance of bile tolerant organisms namely *Alistipes, Bilophila*, and *Bacteroides* but inhibited the level of *Firmicutes* phylum including *Roseburia, Eubacterium rectale*, and *Ruminoccus bromi* that metabolize dietary plant polysaccharides [36]. Interestingly, provision of plant- based diet to the same subjects and under similar experimental conditions has not induced significant changes in the intestinal microbial community [36]. In fact, changing gut microbiota diversity and richness seems to be a complicated task. Sonnenburg et al [37] have found that gut microbiota faces a progressive loss of its diversity after feeding four generation of mice a low- fiber diet that is not recoverable even after the reintroduction of dietary fiber. Instead, to restore gut microbiota to its original state requires the administration of missing microbiome taxa in combination with dietary fiber [37].

The type and quantity of fat in the diet have been considered an important factor in modulation gut microbiota [20]. Evidence for possible effect of different dietary fat on gut microbiota composition is relatively limited and debatable. The high- fat diet containing palm oil, but not that containing either olive oil or sunflower oil, has increased body weight gain and liver triglycerides content in mice, and has associated with reduced microbial diversity and increased the *Firmicutes: Bacteroidetes* ratio [38]. In another study in mice, high- fat diets supplemented with flaxseed oil or fish oil have positively shaped the host microbial ecosystem, whereas those supplemented with olive oil have associated with increased *Bacteroidetes* at the phylum level compared with those supplemented with palm oil, flaxseed, or fish oil [39]. Significant increase in the abundance of *Firmicutes* and *Proteobacteria* and marked decrease in that of *Bacteroidetes* have been described in Apo E deficient mice given the Western diet [40]. Only one controlled, randomized and single

blind clinical trial is available that has correlated the consumption of dietary fats with gut microbiota composition in humans [20]. The high- monounsaturated fatty acid diets consumed by 39 subjects have not been able to change individuals' bacterial population but have reduced total bacteria, plasma total cholesterol, and low-density lipoprotein cholesterol [20].

So far, in contrast to animal studies, there is scarcity of human studies that link diet with gut microbiota. The available studies suffer from several major flaws particularly small sample size, short intervention periods, and presence of confounders, a matter that hinders getting feasible results or establishing reliable connection between dietary components and alterations in gut microbial composition. Large, well- designed controlled trials are required to identify the key microbial changes that respond to different dietary components and their connection with the cardiometabolic risks.

CARDIOMETABOLIC RISKS-GUT MICROBIOTA CONNECTION

Obesity-Gut Microbiota Connection

Obesity and its associated disorders including atherogenic dyslipidemia, hypertension, insulin resistance and diabetes are the key risk factors defining the metabolic syndrome [2]. Obesity is classically presented as a result of increased food intake and reduced physical activity [4]. Interestingly, recent evidence has proposed the potential role of gut microbiota as a contributor in host nutrition and energy homeostasis regulation [41]. In fact, gut microbiota modulates energy metabolism by the production of several biofuels and vitamins, fermentation of the indigestible food components, and maintenance of intestinal epithelial metabolic balance [8-10,].

It is well appreciated that different gut microbial community may promote energy extraction from the diet [42], or may induce adiposity through signals that affect host genes and metabolism [7]. The association between of gut microbiota and adiposity was first reported by Backhed et al in 2004 [9]. The study has shown that, compared to the germ-free mice, the conventional raised mice consume 29% less food, as a standard chow diet, and contain 42% more body fat [9]. Furthermore, there has been 57% increase in body weight of the germ-free mice after colonization with gut microbiota harvested from conventional raised mice [9]. In another study, significant differences in intestinal microbiota phenotypes between obese and lean mice have been documented [41]. These differences have been characterized by a reduction in the abundance of *Bacteroidetes* phylum by 50% and an increase in the abundance of *Firmicutes* phylum in obese *ob/ob* mice compared to their lean littermates [41]. Indeed, there has been a marked increase in the total body fat of the germ-free mice upon colonization with gut microbiota harvested from the obese mice [43]. This increase has been remarkably greater than colonization of gut microbiota of germ-free mice with that of the lean mice [43]. In this context, it seems that research efforts intended to induce obesity in germ-free mice using of high- fat, high- sugar diets are not successful [42].

While gut microbiota confers numerous metabolic and health benefits [9-11], the mechanisms whereby this microbial community affects body weight homeostasis and controls obesity have not been fully elucidated. However, several mechanistic role of the gut microbiota composition corresponding to weight-related effects have been studied. One of the proposed mechanisms involves the role of gut microbiota in the regulation of lipoprotein lipase and adipocyte fatty acid storage [9]. It has been shown that the intestinal expression of production of a lipoprotein lipase inhibitor known as fasting-induced adipose factor is suppressed in conventional raised mice compared to germ-free mice with induced intestinal expression of fasting-induced adipose factor [9]. Given the differences between the two mice groups in terms of gut microbiota community and body fat content [9,42], these connotations may imply that the gut microbiota promotes adipocyte fatty acid storage through the regulation of lipoprotein lipase [9].

In the same context, another proposed mechanism focuses on the role of short chain fatty acids produced by the gut microbiota [42]. It has been suggested that the production of short chain fatty acids by the gut microbial action would increase energy harvest from the food, a matter that could explain obesity resistance in germ free mice [42]. This theory could also explain the increase of *Firmicutes* phylum, the butyrate producing bacteria, at the expense of *Bacteroidetes* phylum in obese population [43,44]. Schwiertz et al [45] have confirmed the detection of higher short chain fatty acids concentrations in fecal samples of 68 obese and overweight subjects compared to 30 lean subjects. However, this increase in these fatty acids

concentrations has been found to associate with a reduction in *Firmicutes* abundance in obese and overweight subjects [45], the case that contradicts the proposed theory [42-44]. Likewise, high consumption of vegetables, fruits and legumes has been shown to associate with increased short chain fatty acids production and positively correlate with the abundance *of Lachnospira* (*Firmicutes* phulum) and *Prevotella* (*Bacteroidetes* phylum) in vegetarian and vegan subjects [46]. Besides being a substrate for hepatic lipogenesis, short chain fatty acids may affect host metabolism through a number of metabolic routes.

They could act as signaling molecules that bind and activate G- protein-coupled receptors (GPR41 and GPR43) which would induce peptide YY secretion, and thus suppress gut motility, increase intestinal transit time and reduce energy harvest from the diet [11]. In fact, butyrate supplementation has been reported to prevent insulin resistance and obesity development in mice fed high- fat diet [47]. Moreover, Butyrate and propionate have been found to inhibit food intake and stimulate anorexigenic gut hormones, and thus prevent obesity [48].

Experimental animal studies have provided indicators for the scientific evidence to correlate gut microbiota with obesity development, especially in germ- free model [9,42-44]. Nonetheless, human gut microbiota has been found to be highly diverse and continuously changing [12,30], which raises the question of how real is the relation between gut microbiota and obesity development. Tracking early microbial colonization during childhood has suggested that a higher *Bifidobacterium* genus colonized in infant gut may provide protection from obesity development during childhood [49]. However, a large scale analysis for 154 obese and lean twins and their mothers has demonstrated a strong association between obesity and gut microbiota at phylum level [15]. This association has been characterized by reduction in the bacterial diversity and in the proportion of *Bacteroidetes*, an increase in the proportion of *Actinobacteria*, and no change in the level of *Firmicutes* in obese compared with lean individuals [15]. However, this association fails to identify specific bacterial abundance in the gut microbiota of the 154 subjects [15]. In another study, a reduction in *Bacteroidetes* abundance, and an increase in *lactobacillus* species (*Firmicutes*) abundance in 20 obese patients compared to 20 lean subjects have been found [44]. More precisely, in a study involving 68 obese and lean subjects, *lactobacillus reuteri* has associated with obesity and the two groups have not exhibited significant differences in the abundance of *Bacteroidetes* and *Firmicutes* at the phylum level [50]. In contrast to these results, findings of a cross sectional analysis of 68 obese and overweight subjects has revealed a higher abundance of *Bacteroidetes* and lower proportion of *Firmicutes* in obese and overweight subjects compared to lean controls [45].

Dietary intervention studies have been designed to investigate the gut microbiota changes in response to weight loss. Results of one intervention study involving 12 obese subjects randomly assigned to two different low calorie diets have revealed that the composition of fecal microbiota changes toward increasing *Bacteroidetes* and decreasing *Firmicutes* phyla after 1 year on the diet [5]. It has been evident that the changes in bacterial community correlate with percentage loss of body weight rather than changes in dietary calorie content [5]. There has been also a wide range in these changes that are not related to specific bacterial species [5]. Moreover, in 16 obese subjects, changes in gut microbiota community induced by dietetic and lifestyle modifications undertaken for 52 weeks have been shown to return to their initial situation at the end of the intervention period [51]. On the other hand, no significant differences have been detected between 23 obese and 14 lean individuals in the proportion of gut *Bacteroidetes* [35]. There have been also no significant changes in *Bacteroidetes* percentage in the obese subjects after 8 weeks on weight loss diets [35]. However, a reduction in *Roseburia* and *Eubacterium rectale* within the *Firmicutes* phylum has been observed in response to decreasing carbohydrates quantity in the weight loss diets [35]. In a study involving gastric bypass surgery, gut microbiota richness has been shown to increase after weight reduction induced by the surgery as indicated by the remarkable increase in *Proteobacteria* (37%) and *Bacteroidetes* [52]. It is worth noting that the word richness could be a key point here where the more the bacterial richness and diverse in human gut is the less tendency to weight gain and adiposity [53]. Of note, the recorded level of detection of gut *Bacteroidetes* by different studies is highly variable [5,15,35,42-45].

This result could be due to the sensitivity of this bacterial phylum to the storage temperature and duration [13] which would necessitate the use of fresh stool samples. Furthermore, the relatively short duration (≤ 8 weeks) used by various intervention studies may not be sufficient to cause the required changes in the microbial community and obtain feasible associations with the dietary components or patterns [17]. Thus, it seems that a conclusive correlation between microbial phyla that inhabit human gut and obesity is not

yet established. In fact, the relatively limited and conflicting research in humans linking gut microbiota composition with obesity and leanness urges the need for further investigations.

Diabetes and Insulin Resistance-Gut Microbiota Connection

Insulin resistance refers to reduced insulin action in peripheral tissues and impaired suppression of endogenous glucose production, a state which is critical for maintaining normal glucose homeostasis [1]. Insulin resistance is associated with obesity and increased body fat deposition, and is a principal risk factor of developing type 2 diabetes and metabolic syndrome [2]. The low grade inflammation is triggered by increasing fat accumulation in the adipose tissues which would induce insulin resistance development [54]. Although obesity and insulin resistance are epidemiologically related, the mechanistic nature of the relation between gut microbiota and each of them is quite different.

Lipopolysaccharide, the major component of the membrane of Gram negative bacteria, is a triggering factor for several cytokines which are the key inducer for insulin resistance [55]. It has been demonstrated that feeding experimental mice a high- fat diet (70% of total calories as fat) would increase plasma lipopolysaccharide concentration and trigger metabolic endotoxemia, as well as would induce obesity and insulin resistance [6]. Noticeably, this increase has been shown to associate with significant reductions in the abundance of the *Bacteroides*– like bacteria (the predominant Gram negative bacteria in the mouse intestine), the *Eubacterium rectale-Clostridium coccoides* group (the dominant Gram-positive group) and the bifidobacteria [6]. Besides, it has been observed that the antibiotic treatment reduces metabolic endotoxemia and the cecal content of lipopolysaccharide in the high- fat fed mice [56]. This reduction has been shown to correlate with reduction of systemic inflammation and improvement of insulin sensitivity [56]. Nevertheless, gut barrier disruption has been shown to enhance gut permeability and facilitate lipopolysaccharide translocation; such changes may correlate with the composition of gut microbiota [57]. Certain bacterial groups such as *Eubacterium rectale- Clostridium coccoides* have been reported to affect gut barrier integrity [6]. In this regard, reduction in some bacterial species such as *Akkermansia muciniphila* which is a mucus degrading bacteria, representing 1-4% of the colon bacterial population, has been shown to increase gut permeability [58]. *A. muciniphila* abundance has been shown to inversely correlate with body fat and insulin resistance. *A. muciniphila* administration to high- fat diet obese mice has been shown to enhance gut barrier integrity, improve metabolic profile, and reduce fat mass, metabolic endotoxemia and insulin resistance [59]. It may be noted that the available scientific evidence provided by the animal studies seems to be not sufficient to identify specific composition of gut microbiota that may induce insulin resistance.

Clinical studies in humans have been more concerned in detecting the gut microbiota differences between diabetic and non-diabetic population rather than exploring the composition that may trigger insulin resistance. Compared to non-diabetic subjects, type 2 diabetic patients have been found to have a lower *Firmicutes* proportion and a higher *Bacteroidetes* proportion [16]. The ratio of *Bacteroidetes* to *Firmicutes* has also been shown to positively correlate with the plasma glucose concentrations rather than body mass indices [16]. Reduced levels of *Bifidobacterium* spp. (anti-inflammatory bacteria) and *Bacteroides vulgatus* have been detected in 16 diabetic patients as compared to healthy individuals [60]. In the metagenome-wide associated study, analysis of the gut microbiome of 345 Chinese individuals comprising of type 2 diabetes and non-diabetic control subjects has been documented [61]. Opportunistic pathogens, such as *Bacteroidescaccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum, Eggerthella lenta*, and *Escherichia coli* have been reported to be the abundant species, with relative diversity, among the Chinese diabetic patients [61]. In contrast to diabetic patients, non- diabetic subjects have been shown to have a higher proportion of butyrate-producing bacteria, including *Clostridiales* sp. SS3/4, *E. rectale, Faecalibacterium prausnitzii, Roseburia intestinalis* and *Roseburia inulinivorans* [61]. However, *A. muciniphila* has been reported to be more abundant in Chinese patients with diabetes compared to normal controls, a finding that contrasts the results of other studies [62].

Pre-diabetes mellitus is an important stage in the progression from normal glucose metabolism to diabetes; thus, microbial dysbiosis may be used as predictor for type 2 diabetes development. It has been reported that the relative abundances of *Bacteroides* and *Clostridium* change markedly with the progression of glucose intolerance [62]. Additionally, the relative abundances of *Verrucomicrobiae* and *Betaproteobacteria* from normal glucose tolerance stage to pre-diabetes mellitus and type 2 diabetes stages have shown an opposite trend [62]. Contrary to other findings [61], the relative abundances of butyrate-producing bacteria

(*Faecalibacterium prausnitzii*) and *A. muciniphila* have been reported to decrease along with decreasing glucose tolerance [62]. More recently, elevated levels of serum branched-chain amino acids have been described in 277 insulin-resistant non-diabetic individuals after analysis of their serum metabolome [63]. This increase has been found to correlate with a gut microbiome that is enriched with a biosynthetic potential for the branched-chain amino acids and is deprived of genes encoding bacterial inward transporters for these amino acids [63].

However, the apparent inconsistency of the results of the different studies could not exclude the role of microbial dysbiosis in the development of type 2 diabetes. The high diversity and the continuous dynamic changes in human gut microbiota community, and the different analytical techniques used by various studies seem to be among many reasons behind the production of these conflicting findings.

Atherogenic Dyslipidemia-Gut Microbiota Connection

Insulin resistance is central to atherogenic lipid disturbances that are the primary indicators for the development of atherosclerosis and subsequent cardiovascular disease [4]. It has been proposed that gut microbiota have a protective role against atherosclerosis development in mice [64]. There are marked differences in lipid metabolism between the conventional raised mice and the germ- free mice as indicated by a number of lipid classes particularly those in the serum, adipose tissue and liver [65]. Given the great variations in gut microbiota composition between the conventional raised mice and the germ- free mice [9,42], the impact of gut microbiota on lipid metabolism and assimilation such as *de novo* lipogenesis and blood lipid clearance is becoming a major scientific issue, stimulating further research [65].

In fact, some intestinal microbial genera such as *Lactobacillus, Bifidobacterium, Enterobacteria* and *Bacteroides* have been found to act on bile acid conversions and formation of secondary bile acids [10]. Thus, changes in the gut microbiota composition are likely to affect enterohepatic circulation, *de novo* synthesis of bile acids, lipid emulsification, and cholesterol absorption [10]. In this regard, it has been recently found that atherosclerosis lesions in Apo E deficient mice fed the western diet can be treated by administration of *A. muciniphila* [40]. Moreover, cholic acid administration to rats has been shown to induce marked changes in gut microbiota at phylum level causing an increase in *Firmicutes* abundance and a decrease in the diversity of microbial genera [66]. These results may provide a strong link among high- fat diets, their profound modifying effects on gut microbial community, and the development of metabolic disease [20,22,38,39].

However, the hyper cholesterolemic model in relation to gut microbiota was rarely studied. The characterization of the gut microbiota in a hamster model of hypercholesterolemia has shown strong positive associations between Bifido bacteria population and plasma levels of high-density lipoprotein cholesterol [67]. Furthermore, several bacterial taxa within the families *Coriobacteriaceae* (*Actinobacteria* phylum) and *Erysipelotrichaceae* (*Firmicutes* phylum) have been shown to be significantly reduced in hamster models in response to dietary addition of plant sterol esters [68]. It has been reported that in hamsters, plant sterol esters could reduce cholesterol absorption and increase cholesterol excretion, thus the host cholesterol excretion itself would modulate gut microbiota structure through its antibacterial activity [68]. On the other hand, an altered gut metagenome has been reported in patients with symptomatic atherosclerosis [69]. Fecal microbiome of 12 patients with atherosclerosis has been shown to be enriched with the genus *Collinsella* without any significant association with serum cholesterol levels, whereas the *Roseburia* and *Eubacterium* genera have dominated that of 13 healthy subjects [69]. It is worth noting that the *Roseburia* and *Eubacterium* genera have been shown to associate with high carbohydrate consumption as discussed earlier [35].

Beside cholesterol and triglycerides, phospholipids may be involved in the risk of cardiovascular disease. In a large clinical cohort, three phospholipid-associated molecules: choline, betaine, and trimethylamine-N-oxide have been reported to promote atherosclerosis, and proposed to be used as a biomarker for predicting the risk of cardiovascular disease [70]. Choline catabolism by the gut microbiota has been described to produce trimethylamine that can be then metabolized in the liver to form atherogenic trimethylamine-N-oxide [70]. L-carnitine, a trimethylamine abundant in red meats, can also be metabolized by the gut microbiota and converted in the liver to trimethylamine-N-oxide augmenting atherosclerosis in mice [71]. Plasma trimethylamine level has been reported to positively correlate with the *Prevotellaceae* family and the genus *Prevotella* in mice fed L-carnitine supplemented diet [71]. Moreover, plasma trimethylamine-N-oxide levels in subjects with a *Prevotella* enterotype have been shown to be significantly higher than those

with *Bacteroides* enterotype [71]. Significantly higher plasma trimethylamine-N-oxide levels have been also detected in omnivore subjects than in vegetarians and vegans [71]. Such findings contradict the previously described association between *Prevotella* abundance and low animal protein and fat consumption [17]. On the other hand, urinary trimethylamine-N-oxide levels have been reported to be significantly lower in vegetarians with strict adherence to the Mediterranean diet compared to omnivores, and to positively correlate with L-*Ruminococcus* which is known to be associated with high intake of fats and animal proteins [46]. Interestingly, supplementation of the polyphenol resveratrol has been shown to decrease the levels of trimethylamine-N-oxide in the C57BL/6J mice through manipulating gut microbiota [72]. Furthermore, this supplementation increases the levels of *Lactobacillus* and *Bifidobacterium* genera, which result in increasing bile salt hydrolase activity, thus enhancing bile acid deconjugation and fecal excretion in this animal model [72].

Similar to other cardiometabolic risks, studies that investigate atherogenic dyslipidemia and its relationship with the gut microbiota are very limited and their results are inconsistent. Studies that link diet or its components with these biological measures are generally lacking. Thus, the connection between gut microbiota and atherogenic dyslipidemia is yet to be established.

CONCLUSIONS

Evidences from experimental animal studies certainly establish new understanding in the mechanisms underlying the pathogenesis of metabolic syndrome through suggesting a link among diet, gut microbiota, and cardiometabolic risks. However, exploring the world of gut microbiota in humans is a quite difficult task. Human gut microbiota is highly diverse and continuously changing. Diet and dietary pattern are indeed a major determinant for gut microbiota diversity and composition. In essence, diet is the primary modulator of gut microbiota that potentially interferes in nutrition and energy metabolism through a variety of homeostatic mechanisms. Though limited, small-scale and short duration, and controversial, human studies do investigate the interrelation among diet, gut microbiota and the individual cardiometabolic risks. However, the role of gut microbiota in the pathogenesis of abdominal obesity, atherogenic dyslipidemia, insulin resistance, and type 2 diabetes mellitus are not yet well elucidated. Controlled, large-scale and long-term clinical studies that link among diet, gut microbiota and the metabolic syndrome as a single entity disorder in particular are generally needed. Great efforts should be exerted to identify key changes in gut microbiome community in response to different dietary interventions and their connection with cardiometabolic risks.

ACKNOWLEDGEMENTS

This work is supported by the Deanship of Academic Research at The University of Jordan, Amman, Jordan.

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