MINIREVIEW

Gut microbiota-based translational biomarkers to prevent metabolic syndrome via nutritional modulation

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Abstract

In the face of the global epidemic of metabolic syndrome (MetS) and its strong association with the increasing rate of cardiovascular morbidity and mortality, it is critical to detect MetS at an early stage in the clinical setting to implement preventive intervention long before the complications arise. Lipopolysaccharide, the cell wall component of Gram-negative bacteria produced from diet-disrupted gut microbiota, has been shown to induce metabolic endotoxemia, chronic low-grade inflammation, and ultimately insulin resistance. Therefore, ameliorating the inflammation and insulin resistance underlying MetS by gut microbiota-targeted, dietary intervention has gained increasing attention. In this review, we propose using dynamic monitoring of a set of translational biomarkers related with the etiological role of gut microbiota, including lipopoly-saccharide binding protein (LBP), C-reactive protein (CRP), fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR), for early detection and prevention of MetS via nutritional modulation. LBP initiates the recognition and monomerization of lipopolysaccharide and amplifies host immune responses, linking the gut-derived antigen load and inflammation indicated by the plasma levels of CRP. Fasting plasma insulin and HOMA-IR are measured to evaluate insulin sensitivity that is damaged by pro-inflammatory cytokines. The dynamic monitoring of these biomarkers in high-risk populations may provide translational methods for the quantitative and dynamic evaluation of dysbiosis-induced insulin resistance and the effectiveness of dietary treatment for MetS.

Introduction

Metabolic syndrome (MetS), a cluster of multiple cardio-vascular risk factors, is one of the most significant public health challenges worldwide (Alberti *et al.*, 2005). Although the clinical criteria of MetS have been defined differently by various organizations, all have come to an agreement on the core components: insulin resistance, central obesity, hyperglycemia, dyslipidemia, and hypertension (Kassi *et al.*, 2011). The International Diabetes Federation estimated that 20–25% of the world's adult population have MetS (Alberti *et al.*, 2006), but many are unaware that they are suffering from this syndrome. People with MetS are 3–5 times more likely to have heart attack/stroke or type 2 diabetes compared with unaffected individuals (Stern *et al.*, 2004; Ärnlöv *et al.*, 2010;

Grundy, 2012). The rise in comorbid chronic diseases accounts for much of the clinical and economic burden of MetS to individuals, their families, and society (Wild & Byrne, 2006; Scholze *et al.*, 2010). Early detection and intensive management of MetS, to reduce the long-term risk of cardiovascular disease and diabetes, are now possible and bring appreciable benefits (WHO, 2005; Després *et al.*, 2008). Because of the urgent need for strategies to prevent MetS, there has been increased interest in exploring translational methods for early diagnosis and prevention with biochemical or genetic markers.

Although the molecular mechanisms underlying MetS are not fully understood, the presence of MetS is commonly associated with inflammation (Haffner, 2006), insulin resistance (Lann & LeRoith, 2007), endothelial dysfunction (Hajer *et al.*, 2007), renal dysfunction (Servais
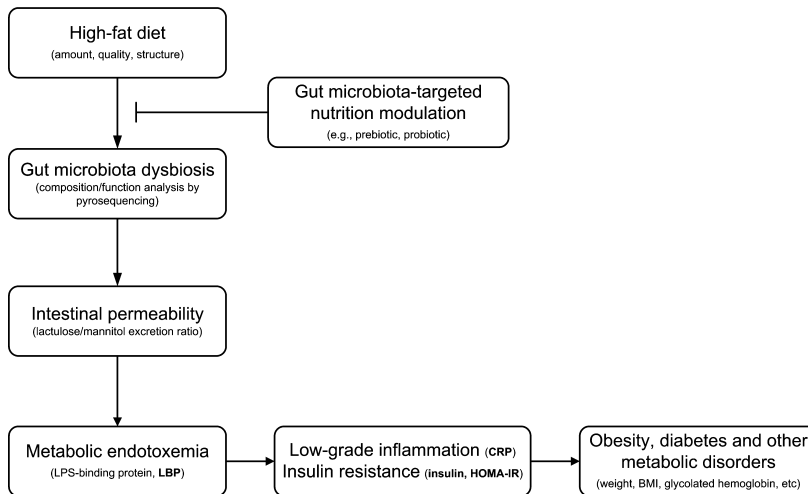
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┌─────────────────────────┐
│   High-fat diet          │
│ (amount, quality, structure) │
└─────────────────────────┘
                                  ┌──────────────────────────────┐
                                  │  Gut microbiota-targeted      │
                                  │    nutrition modulation       │
                                  │  (e.g., prebiotic, probiotic)  │
                                  └──────────────────────────────┘
┌─────────────────────────────┐
│ Gut microbiota dysbiosis     │
│ (composition/function analysis by │
│         pyrosequencing)       │
└─────────────────────────────┘

┌─────────────────────────────┐
│ Intestinal permeability       │
│ (lactulose/mannitol excretion ratio) │
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┌──────────────────────┐  ┌──────────────────────────────┐  ┌──────────────────────────────┐
│ Metabolic endotoxemia │  │ Low-grade inflammation (CRP)   │  │ Obesity, diabetes and other    │
│ (LPS-binding protein, LBP) │  │ Insulin resistance (insulin, HOMA-IR) │  │      metabolic disorders       │
└──────────────────────┘  └──────────────────────────────┘  │ (weight, BMI, glycolated hemoglobin, etc) │
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Fig. 1. The biomarkers represent key links to the potential causal pathway from high-fat diet to initiation of metabolic endotoxemia, insulin resistance, and MetS and the restoration of dysbiosis by gut microbiota-targeted nutritional approach can abolish obesity-associated metabolic features.

et al., 2008), oxidative stress (Onat et al., 2006), disturbed hemostasis (Mansfield et al., 1996), and neurohormonal activation (Prasad & Quyyumi, 2004; Olsen et al., 2005). Several biological markers of these physiologic and pathological phenomena have been proposed as risk factors for MetS and its associated complications, such as white blood cell count, high-sensitivity C-reactive protein (hs-CRP), homeostasis model assessment insulin resistance index (HOMA-IR), homocysteine, cystatin C, uric acid, plasminogen activator inhibitor-1, fibrinogen, aldosterone, renin, and B-type natriuretic peptide (Lee et al., 2009). These biomarkers are readily measured in clinical practice, and altered levels could serve as diagnostic criteria to guide clinical management. However, generally, they are applied individually or independently, and it is less clear whether variations of biomarker levels are pathogenetically related to the incidence of MetS. In addition, although impaired fasting glycemia or impaired glucose tolerance can predict the risk of developing diabetes, it can only indicate an individual's glycemic state at a single point in time (Stern et al., 2002; Meigs et al., 2003). It is most likely that the risk of developing diabetes is present at the stage when glucose concentration is still at the 'normal range' (Rydén et al., 2007). Therefore, monitoring early biomarker profiles that participate in the pathogenesis of MetS will help identify patients at risk and allow them to take effective preventive interventions.

Low-grade, chronic activation of the innate immune system is regarded as an important underlying condition for the pathogenesis of obesity and associated metabolic disorders (Wellen & Hotamisligil, 2005). The process might partly depend on the immunomodulatory effects exerted by dietary compounds in the gut and beyond (Zeyda & Stulnig, 2007). Particularly in recent years, gut microbiota shaped by day-to-day dietary changes is considered a possible causative factor of metabolic disorders,

as well as a therapeutic target (Cani & Delzenne, 2011; Vrieze et al., 2012; Everard et al., 2013; Kimura et al., 2013; Zhao, 2013). Modulating gut microbiota by designed dietary schemes has become a promising strategy to help manage obesity and metabolic abnormalities (Sanz et al., 2010). In the current review, we propose that the measurement of longitudinal changes in gut microbiota-based, translational biomarkers could be applied to monitor insulin sensitivity alterations during MetS development and progression. This multimarker approach includes lipopolysaccharide binding protein (LBP), CRP, fasting insulin, and HOMA-IR, all of which represent key links to the potential causal pathway from unhealthy diet to initiation of insulin resistance and MetS (Fig. 1): diet-induced gut microbiota dysbiosis, namely, an increase in opportunistic pathogens and a decrease in gut barrier protectors (Zhang et al., 2010), leads to increased gut permeability, increased lipopolysaccharide in the bloodstream to provoke low-grade inflammation, which can increase insulin resistance. An effective intervention would reverse this pathway, as indicated by these biomarkers. This new biomarker profile could not only be used for the early detection of insulin resistance, but also for assessing the degree of success in treating MetS.

Gut microbiota, inflammation, and insulin resistance

Insulin resistance, in which the physiologic concentration of insulin cannot produce a blood glucose lowering effect, is the most accepted hypothesis to describe the pathophysiology of MetS (Eckel et al., 2005). This pathological phenomenon is often seen in obesity, stress, infection, or severe illness. Simultaneously, obesity and metabolic abnormalities are closely associated with chronic low-grade inflammation characterized by abnormal cytokines

production and activation of inflammatory signaling pathways (Wellen & Hotamisligil, 2005). Increasing evidence directly implicates the abundance of pro-inflammatory cytokines in insulin resistance in the liver, muscle, and adipose tissue (Neuschwander-Tetri & Caldwell, 2003), which have multifaceted effects on insulin resistance susceptibility genes, including those regulating lipid synthesis, gluconeogensis, and adipogenesis (Kim, 2010). Increased levels of inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, can facilitate serine phosphorylation of insulin receptor substrate-I, contributing to insulin resistance (Reaven, 1988). Thus, inflammation suppresses insulin-signaling pathways and makes the human body less responsive to insulin, increasing the risk for insulin resistance (Xu et al., 2003; Cai et al., 2005). Therefore, low-grade chronic inflammation is viewed as an important underlying condition for the pathogenesis of MetS and a possible target of prevention and/or therapy.

Diet is considered the key determinant influencing the overall chronic inflammatory response (Bulló et al., 2007); however, the primary mediator(s) between diet and inflammation remain to be elucidated. Some dietary compounds in high-fat meals, such as the saturated fatty acids frequently consumed in substantial quantities by obese individuals, have been demonstrated to bind to Toll-like receptor 4 (TLR4) to activate nuclear factor kappa-B, leading to over-expression of pro-inflammatory cytokines. These increases are completely absent after a meal rich in fiber and fruit (Ghanim et al., 2009).

Gut microbiota, consisted of trillions of microorganisms (c. 1×10^{13}–1×10^{14}, biomass > 1 kg) and modulated mainly by diet (Scott et al., 2012), has recently been recognized as a primary mediator for human health (Cani & Delzenne, 2009; Ley, 2010; Monteiro & Azevedo, 2010; Kelly et al., 2012; Everard & Cani, 2013). Current evidences support that alterations in composition and/or metabolic activity of gut microbiota play pivotal role in the pathogenesis of obesity and related disorders (Musso et al., 2011). Lipopolysaccharide, the cell wall component of Gram-negative bacteria living in the gut, has been identified as an important factor for inducing this metabolically triggered chronic inflammation associated with obesity (Cani et al., 2007a). High-fat diet-disrupted gut microbiota, with the increase in the proportion of lipopolysaccharide producers and the decrease in intestinal barrier protectors Bifidobacterium spp., released lipopolysaccharide into the host bloodstream through a partially impaired gut barrier to act as a primary mediator for inflammation, leading to insulin resistance and obesity (namely metabolic endotoxemia) (Cani et al., 2007a). The plasma concentration of lipopolysaccharide increased 2–3 times in high-fat diet-fed obese mice, comparable to what has been found in human subjects with MetS (Cani et al.,

2007a; Creely et al., 2007). Subcutaneous injection of lipopolysaccharide in otherwise lean and healthy mice fed on normal chow evoked systemic inflammation and eventually induced insulin resistance and obesity, whereas knock-out of CD14, a co-receptor of TLR4, abolished these responses to lipopolysaccharide treatment (Cani et al., 2007a). Supporting normal levels of bifidobacteria by adding oligofructose maintained a gut barrier less permeable to lipopolysaccharide and prevented high-fat diet-fed mice from developing insulin resistance and obesity (Cani et al., 2007b). In human subjects, increased lipopolysaccharide content was also associated with an increased BMI and high-fat diet (Erridge et al., 2007; Lajunen et al., 2008). Thus, metabolic endotoxemia induced by intestinal dysbiosis has been proposed as a possible contributor to the inflammatory state associated with MetS.

Specific bacteria, which are positively associated with MetS, have been identified. In our previous work, we found that the endotoxin-producing Enterobacter decreased in relative abundance from 35% of a morbidly obese volunteer's gut bacteria to non-detectable, during which time the volunteer lost 51.4 kg of 174.8 kg initial weight and recovered from hyperglycemia and hypertension after 23 weeks on the whole grains, traditional Chinese medicinal foods, and prebiotics diet (WTP diet) (Fei & Zhao, 2013). The strain Enterobacter cloacae B29 isolated from the volunteer's gut induced germfree C57BL/6J mice to develop obesity and insulin resistance on a high-fat diet, suggesting that the endotoxin-producing bacterium may be a causative factor in the development of obesity in its human host (Fei & Zhao, 2013). After the Enterobacter population reduced to almost non-detectible, the human host started to reduce endotoxin load in his serum, alleviate his inflammation and recover from insulin resistance and other metabolic deteriorations. In B29-induced obese mice, we observed increased endotoxin load in the serum, increased systemic and local inflammation and significantly increased insulin resistance (Fei & Zhao, 2013). Thus, there seems to be a causal path between endotoxin producers in the gut and obesity/insulin resistance disease endpoints.

Gut bacteria, which are negatively correlated with MetS, have also been identified. Akkermansia muciniphila, an obligate mucin degrader that grows almost fully dependent on the mucin (Derrien et al., 2004), inversely correlates with body weight (Santacruz et al., 2010; Karlsson et al., 2012) and type 1 diabetes (Hansen et al., 2012), and increases by c. 100-fold with prebiotic (oligofructose) treatment (Everard et al., 2011). Very recently, Everard et al. (2013) demonstrated that the abundance of A. muciniphila decreased in genetic and high-fat-fed obese and type 2 diabetic mice, which was restored and

correlated with the improved metabolic profile after prebiotics administration. In addition, introduction of *A. muciniphila* by daily oral gavage reversed high-fat diet-induced metabolic disorders, indicating the potential application of *A. muciniphila* in prevention or treatment of obesity and related complications.

MetS can thus be driven by structurally aberrant gut microbiota with increased pathobionts such as *E. cloacae* B29 and decreased gut barrier protectors such as *A. muciniphila,* which may have been perturbed by host gene defect (Vijay-Kumar *et al.*, 2010), dietary change (Turnbaugh *et al.*, 2008), antibiotics administration (Ajslev *et al.*, 2011), or gastrointestinal tract illnesses/surgery (Liou *et al.*, 2013).

Gut microbiota-targeted nutritional modulation in the management of MetS

Due to its key role in the initiation, propagation, and development of insulin resistance and obesity, which is associated with increased risks for other metabolic abnormalities, the pro-inflammatory state is a possible target of prevention and/or therapy for MetS (Hotamisligil *et al.*, 1993; Cai *et al.*, 2005). Directly inhibiting the inflammation by pharmacological interventions, such as inhibitors of inflammatory kinases and agonists of relevant transcription factors/cytokines, prevents insulin resistance (Moller & Berger, 2003; Kaneto *et al.*, 2004). But the problem is that by blocking the action of individual inflammatory mediators, other redundant components may be sufficient to continue propagating the inflammatory signal (Wellen & Hotamisligil, 2005). On the other hand, weakening or even inhibiting inflammation in the first place may compromise the immune response, leaving the system vulnerable to subsequent injury or infection (Gregor & Hotamisligil, 2011). Therefore, the most effective anti-inflammatory therapy strategies should address the root causes of inflammation, which include metabolic endotoxemia (Monteiro & Azevedo, 2010; Gregor & Hotamisligil, 2011; Moreira *et al.*, 2012).

Diet modification is an integral part of lifestyle intervention to reduce MetS risk factors (Grundy *et al.*, 2006; Bulló *et al.*, 2007). Numerous studies of weight loss have demonstrated that improvement in metabolic parameters due to dietary intervention is often associated with a decrease in circulating levels of inflammatory markers (Imayama *et al.*, 2012; Fayh *et al.*, 2013; Nicklas *et al.*, 2013). However, not all dietary weight loss interventions lead to reduced inflammation; for example, inflammatory markers increased in overweight children after they effectively lost weight on a low carbohydrate, high-fat diet (Alvarez *et al.*, 2009). Therefore, when choosing an intervention for weight management, priority should be given

to schemes that can decrease the inflammatory tone, eventually decreasing the risk of progression to metabolic diseases, not just reducing the body weight temporarily.

The homeostasis of gut microbiota depends on host physiology and environmental conditions; moreover, it also relies on day-to-day dietary changes (Turnbaugh *et al.*, 2009). The composition and/or activity of gut microbiota is a factor that characterizes obese vs. lean individuals, and diabetic vs. non diabetic patients (Bäckhed *et al.*, 2004; Caesar *et al.*, 2010; Qin *et al.*, 2012; Everard *et al.*, 2013; Karlsson *et al.*, 2013). More importantly, the changes associated with the above disorders in gut microbes can be reversed by nutritional intervention (Ley *et al.*, 2006; Cani *et al.*, 2007b; Zhang *et al.*, 2012a; Everard *et al.*, 2013). Because of the dominating role of diet in shaping the composition and gene transcription network of gut microbiota (Sonnenburg *et al.*, 2005; Zhang *et al.*, 2010), modulation of gut microbiota by nutrients with prebiotic properties is a promising strategy for managing obesity and metabolic diseases (Delzenne & Cani, 2010; Sanz *et al.*, 2010; Delzenne *et al.*, 2011). This dietary scheme should not only meet human nutritional needs, but also balance gut microbiota. A diet protecting against MetS should be rich in whole grains, fruits, vegetables, lean meats, and fish, and low-fat or fat-free dairy products, and avoid processed foods (Bulló *et al.*, 2007).

Following the encouraging results from experimental models aimed at affecting gut microbiota, both probiotic (live microorganisms beneficial to the host organism, such as *Bifidobacterium* and *Lactobacillus* spp.) (An *et al.*, 2011; Chen *et al.*, 2011; Fåk & Bäckhed, 2012) and prebiotic (non-digestible food ingredients that stimulate the growth and/or activity of probiotic, such as oligofructose, galactooligosaccharide, etc.) (Everard *et al.*, 2011; Neyrinck *et al.*, 2012) approaches were effective in managing the metabolic diseases associated with obesity. A few clinical studies using dietary interventions to manipulate gut microbiota and host metabolism have succeeded in linking the intervention to beneficial phenotypic changes (Cani *et al.*, 2009; Parnell & Reimer, 2009; Bays *et al.*, 2012). Targeted approaches, such as fluorescence *in situ* hybridization and real-time PCR, have been used to evaluate the gut microbiota in overweight or obese patients after dietary treatment (Musso *et al.*, 2010). Weight loss was associated with an increase in *Bacteroides fragilis* and *Lactobacillus* and a decrease in *Bifidobacterium longum* and *Clostridium coccoides* in overweight adolescents after 10 weeks on a calorie restriction diet (Santacruz *et al.*, 2009). Ley *et al.* (2006) found that the relative proportion of *Bacteroidetes* over *Firmicutes* increased in obese subjects after weight reduction on low carbohydrate or low fat diets, indicating that modulating gut

microbiota can be an effective means for weight management. However, it remains obscure how these reported changes may lead to weight loss or improved metabolic health (Everard *et al.*, 2013; Liou *et al.*, 2013). Our recent study showed that after a 23-weeks WTP dietary intervention (9 weeks strict intervention followed by a 14-week maintenance period), 89 central obese volunteers (BMI $\geq$ 28 kg m^{-2}) lost 5.79 $\pm$ 4.64 kg (6.62 $\pm$ 4.94%) weight. The incidence of MetS decreased from 60.67% (baseline) to 31.46% (9 weeks later) and 29.21% (23 weeks later), in addition to the improvement in insulin sensitivity, lipid profiles, and blood pressure. Plasma endotoxin load as LBP was also significantly reduced, with concomitant decrease in CRP, tumor necrosis factor-α, interleukin-6, and an increase in adiponectin, indicating a significant alleviation of the inflammatory condition. Pyrosequencing of fecal samples showed that phylotypes related to endotoxin-producing opportunistic pathogens of *Enterobacteriaceae* and *Desulfovibrionaceae* were reduced significantly, while those related to gut barrier-protecting bacteria of *Bifidobacteriaceae* increased. These results suggest that modulation of the gut microbiota via dietary intervention may enhance the intestinal barrier integrity, reduce circulating antigen load, and ultimately ameliorate the inflammation, insulin resistance, and metabolic phenotypes (Xiao *et al.*, 2013). This also indicates that there is a possible causal linkage from dietary alteration of gut microbiota to reduced endotoxin load in serum and alleviation of inflammation in human body.

Gut microbiota-based host biomarkers to prevent MetS

Gut microbiota dysbiosis (mainly caused by high-fat/energy diet) affects host gut barrier permeability, increases endotoxin load, and evokes inflammation, which lead to downstream metabolic consequences (Cox & Blaser, 2013). Manipulating the gut microbiota composition with diet becomes a promising 'pharmaco-nutritional' approach to reverse the dysbiosis and host metabolic disorders (Cani & Delzenne, 2011; Kovatcheva-Datchary & Arora, 2013). However, assessing qualitative and quantitative alterations of gut microbiota is still not feasible in the conventional healthcare system. Developing a set of host biomarkers related to changes in gut microbiota should be an alternative for MetS prevention and management at the population level. Here, we propose a set of biomarkers for the dietary management of MetS in a public health project: diet modulates the composition of gut microbiota to suppress opportunistic pathogens and promote gut barrier protectors and decreases gut permeability, thus alleviating metabolic endotoxemia (LBP reduces) and inflammatory tone (CRP reduces), and

improving insulin sensitivity (fasting insulin and HOMA-IR decrease).

LBP

Various toxins produced by particular members of gut microbiota may enter the bloodstream via enterohepatic circulations or the impaired gut barrier to affect host immunity and metabolism (Manco *et al.*, 2010; Zhao & Shen, 2010). Experimental data indicated that lipopolysaccharide, derived from Gram-negative bacteria in the gut, plays a key role in driving systemic inflammation, insulin resistance, and fat mass development (Cani *et al.*, 2007a). However, because of its short half-life, low concentration, and high susceptibility to interfering substances (Novitsky, 1998), the utility of lipopolysaccharide detection via the Limulus lysate assay is limited in routine clinical setting and large-scale studies. The development of more reliable methods for lipopolysaccharide detection in blood samples is needed. Furthermore, individual endotoxin preparations from various Gram-negative bacteria vary widely in their capacity to mediate activation of alternative pathways (Morrison & Ryan, 1987; Erridge *et al.*, 2002; Coats *et al.*, 2011; Matsuura, 2013). The lipopolysaccharide produced from *Escherichia coli*, *Salmonella minnesota,* and other *Enterobacteriaceae* has potent inflammation-inducing capacity, usually nearly 100- to 1000-fold higher than the lipopolysaccharide from *B. fragilis* (Lindberg *et al.*, 1990; Erridge *et al.*, 2002; Hakansson & Molin, 2011). The total amount of bacterial lipopolysaccharide from different sources varies dramatically in the capacity to lead to metabolic endotoxemia and the inflammatory response.

LBP is an acute-phase protein mainly produced in the liver that circulates in the blood, which can be conveniently detected by commercial ELISA kits. LBP initiates the recognition of lipopolysaccharide and amplifies host immune responses to lipopolysaccharide (Schumann *et al.*, 1990), which indicates the amount of effective lipopolysaccharide and could be a reliable biomarker linking lipopolysaccharide load and the induced innate immune response (Lepper *et al.*, 2007). LBP also binds to other bacterial compounds, including glycolipids of spirochetes, lipoteichoic acid, lipomannan of mycobacteria, at least two types of lipopeptides, and elements of the pneumococcal cell wall to modulate their ability to stimulate the innate immune system and therefore can be a more general indicator of the exogenous antigen load in the host (Schröder & Schumann, 2005; Cesaro *et al.*, 2011).

Circulating LBP concentration is significantly increased in non-alcoholic fatty liver disease patients (Guerra *et al.*, 2007), glucose-intolerant men (Gubern *et al.*, 2006), and coronary artery disease patients (Lepper *et al.*, 2007), and

becomes an inflammatory marker associated with obesity-related insulin resistance (Moreno-Navarrete et al., 2012). Sun et al. (2010) found that increased circulating LBP is associated with obesity, MetS, and type-2 diabetes in apparently healthy Chinese people in a cross-sectional cohort study. These findings suggest a potential association between LBP and metabolic disorders, and the possibility of using LBP as a biomarker for early detection, diagnosis, and progression of MetS. Furthermore, our previous work found that increased serum LBP levels in Wistar rats after high-fat diet was essentially prevented by berberine co-administration, which accompanied with significant reductions in bacterial diversity and the total bacterial population resulting in a decrease in the free antigen load in the host (Zhang et al., 2012b). Another report showed that C57BL/6J mice with 30% calorie restriction had higher abundance of gut barrier-protecting bacteria, such as *Lactobacillus* and *Bifidobacterium*, but markedly reduced opportunistic pathogens, such as *Enterobacteraceae* and *Streptococcaceae*. These changes in the gut microbiota were also concomitant with significantly reduced serum levels of LBP (Zhang et al., 2013).

CRP

Metabolic endotoxemia, evoked by antigens that originate from gut microbiota (e.g. lipopolysaccharide, peptidoglycans, and flagellin) through the dysfunction intestinal barrier, induces chronic inflammation (Cani et al., 2007a, 2008; Everard & Cani, 2013). CRP, a stable downstream biomarker of inflammation, is the most widely measured acute-phase reactant responding to tissue injury, infection, and inflammation in clinical practice (Pepys & Baltz, 1983). A chronically increased level of CRP is associated with obesity (Hiura et al., 2003; Piéroni et al., 2003), atherosclerosis (Libby, 2002), and MetS (Fröhlich et al., 2000). In patients with MetS, CRP increases linearly with the number of abnormalities that comprise this syndrome (Pradhan et al., 2001; Devaraj et al., 2004). Therefore, a significant reduction in CRP levels through weight loss could reduce the risk of cardiovascular disease and other obesity-associated chronic diseases. Large studies have been conducted to investigate the association of weight loss by a dietary approach and CRP levels (Dietrich & Jialal, 2005). Heilbronn et al. (2001) found a 26% reduction in CRP plasma concentrations in 83 healthy obese women who performed 12 weeks of energy restriction using a low-fat diet with an average weight loss of 7.9 kg. The weight loss achieved by dietary programs ranged from 3 to 15 kg and was accompanied by a 7–48% reduction in CRP levels (Dietrich & Jialal, 2005). Gut microbiota alterations in obese subjects are associated with local and systemic inflammation, for example, plasma CRP was

also increased in these subjects ($P = 0.0005$) and correlated with the *Bacteroidetes/Firmicutes* ratio ($r = -0.41$, $P = 0.03$) (Verdam et al., 2013). Brignardello et al. (2010) observed an inverse correlation between CRP concentrations and G + C abundance, suggesting that bacterial populations with high DNA GC contents may modulate inflammatory processes in the host. High-cocoa flavanol intervention in healthy human volunteers significantly reduced CRP concentrations, which correlated with the amounts of specific bacteria (*Bifidobacteria*: $r = -0.438$, $P < 0.05$; *Lactobacilli*: $r = -0.492$, $P < 0.01$) (Tzounis et al., 2011). The CRP level, which is downstream of LBP in the metabolic endotoxemia causal pathway, can thus be employed as a biomarker for inflammation in managing MetS (Fig. 1).

Fasting insulin

Insulin is a hormone central to regulating carbohydrate and fat metabolism, which causes cells in the liver, muscle, and fat tissue to take up glucose from the blood. Fasting plasma insulin (FPI) concentration is one of the most practical ways to estimate insulin resistance from the clinical perspective (Monzillo & Hamdy, 2003). Fasting insulin has been used in population-based studies, high values of which reflect the presence of insulin resistance (Bo et al., 2012; Gagnon et al., 2012; Zuo et al., 2013). There is a good correlation between FPI and insulin sensitivity derived from the hyperinsulinemic euglycemic clamp (Yeni-Komshian et al., 2000). Despite some limitations, such as the pulsatile mode of insulin secretion (Seino et al., 2011) and the lack of established standards for insulin assays (Staten et al., 2010; Kalathil et al., 2013), FPI level can still be an important biomarker for monitoring the trend of increasing insulin resistance.

HOMA-IR

Because fasting insulin does not provide an accurate evaluation of insulin sensitivity in MetS risk populations *per se*, this biochemical parameter has been incorporated in a formula for better estimation of insulin sensitivity. The HOMA is another method used to quantify insulin sensitivity and islet β-cell function (Matthews et al., 1985). HOMA is calculated from FPI and fasting plasma glucose (FPG), using the following mathematic formula: HOMA-IR = FPI × FPG/22.5. HOMA-IR correlates well with the glucose disposal rate derived from the hyperinsulinemic euglycemic clamp (Emoto et al., 1999; Katsuki et al., 2001), a low value of which indicates high insulin sensitivity. Therefore, HOMA-IR is most useful for the evaluation of insulin sensitivity in euglycemic individuals and in persons with mild diabetes for large population-

based studies that require a simple method to assess insulin sensitivity. As it has become increasingly evident that gut microbiota plays a significant role in the development of insulin resistance (Delzenne & Cani, 2011; Shen *et al.*, 2012; Vrieze *et al.*, 2012), insulin sensitivity indicators, fasting insulin, and HOMA-IR index were widely used in the nutritional modulation of gut microbiota in the context of obesity and insulin resistance (Gao *et al.*, 2009; Dewulf *et al.*, 2013; Vulevic *et al.*, 2013).

Trajectory analysis

Developmental trajectories of individuals entering MetS or in the process of interventions are poorly understood (Franco *et al.*, 2009). The trend in an individual person is more important than a single point value that stays at the 'normal range'. Tabák *et al.* (2009) analyzed data from a prospective occupational cohort study of 6538 British civil servants without diabetes mellitus as the baseline. During a median follow-up period of 9.7 years, 505 diabetes cases were diagnosed. In this group, a linear increase in fasting glucose was followed by a steep quadratic increase starting 3 years before the diagnosis of diabetes, and HOMA insulin sensitivity decreased steeply during the 5 years before diagnosis. This suggests that a description of biomarker trajectories leading to diabetes diagnosis could contribute to more-accurate risk prediction models that use repeated measures available to patients through regular checkups.

In fact, except for LBP, the biomarkers we propose have been widely used in clinical practice. However, both doctors and patients regard these biomarkers as diagnostics and pay more attention to the exact value of the biomarkers relative to the normal range at a single time point. To support a preventive public health program, these biomarkers should be continuously monitored through regular checkups. A trajectory for each biomarker in each individual over a long period of time should be used to monitor the increase in disease risk long before real disease symptoms become manifest. An 'inflection point' in the curve could indicate increased risks of illness, even though the exact value is still below the diagnostic threshold. A continued increase in LBP, CRP, and HOMA-IR in an individual is a strong indication that the endotoxin level is increasing in the bloodstream and that the immune system is responding by increasing inflammation, leading to increased insulin resistance. Conversely, continued decrease in these biomarkers in an individual over a period of dietary intervention may be a good indication that the intervention is effectively reducing disease risk. Due to the fact that these parameters can be routinely checked in community clinics, an eHealth database could be established for the local population to construct each individual's health trajectory for the predictive, preventative, preemptive, and personalized management of metabolic diseases.

Conclusion

The nutritional modulation strategy for gut microbiota causes changes in biomarker profiles indicative of metabolic endotoxemia, systemic inflammation, and reduced insulin resistance in MetS patients. Monitoring the trajectories of biomarker profiles will provide quantitative, dynamic, and translational methods aimed at the root cause for evaluating dysbiosis-induced insulin resistance, as well as the degree of success in preventing MetS by dietary treatment. Prospective studies in different populations are needed to further confirm the association between the gut microbiota-based biomarker panel and the development of MetS, and to evaluate the success of applying trajectory analyses of related biomarkers in managing MetS for the prevention of metabolic diseases.

Acknowledgements

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