CHAPTER 2

Literature review

Alteration of Gut Microbiota Composition by High-Fat Diet Associates with an Increased Gut Inflammation

Changes in gut microbiota composition (dysbiosis) and development of low-grade inflammation after long-term consumption of high-fat diet are associated with obesity and insulin resistance (30-33). In the recent years, several studies in both human and animals suggested that prolonged consumption of high-fat diet causes alteration of gut microbiota leading to development of low-grade inflammation in both gastrointestinal tract and adipose tissues (20, 34-36). There are five major bacterial phyla found in both human and rodent intestinal tracts in a similar pattern for some extents including; Firmicutes, Proteobacteria, Bacteriodetes, Actinobacteria and Verrucomicrobia. High-fat diet induces dysbiosis by increasing the numbers of lipopolysaccharide (LPS) containing-Gram negative bacteria Enterobacteriaceae (phylum Proteobacteria) while decreasing in Gram positive Bifidobacteriaceae (phylum Actinobacteria) burdens (34, 37). Increased levels of LPS in plasma or serum (termed "metabolic endotoxemia") have also been reported in high-fat diet-fed obese animals (34, 38). Animal study showed that metabolic endotoxemia is an important event prior to the development of obesity and insulin resistance (31). There are some reports demonstrated the direct pathway of how high-fat diet can increase luminal LPS and gut permeability. High-fat diet directly inhibits gut epithelium cell production of intestinal alkaline phosphatase (IAP) which normally detoxify LPS in gut lumen (35). Decrease of IAP from the prolonged high-fat diet consumption resulting in increased gut luminal LPS levels. High levels of luminal LPS then trigger host innate immune response by binding to TLR4, an important host innate immune receptor, located on gut epithelial cell membrane. Recognition of bacterial LPS by host TLR4 receptor induces gut inflammatory response by promoting pro-inflammatory cytokine [tumor necrosis factor (TNF)-α, interleukin (IL)-1β or IL-6] production. The elevation of these pro-inflammatory cytokine levels (even in the low-

grade and chronic manner) in both local (gut) and systemic (serum and adipose tissues) sites has been reported in diet-induced obesity (34, 35, 38). High-fat diet can decrease expression of gut epithelium tight junction proteins (occludin and claudin-1) leading to increased gut permeability by unknown mechanism (34). These evidences suggesting that an elevation of luminal LPS levels from gut dysbiosis (increased growth of Enterobacteriaceae (phylum Proteobacteria) while decreased in Bifidobacteriaceae (phylum Actinobacteria) population) resulting in increased gut inflammatory tone. This low grade-inflamed gut together with increased gut permeability could be a potent contributor in development of metabolic endotoxemia or systemic LPS by promoting the influx of bacterial LPS from gut lumen into gut tissue and then go further to systemic sites (serum and adipose tissues, the major sources of inflammatory cells involved in metabolic syndrome and obesity). The presence of LPS in systemic sites has already been known as an important factor of insulin resistance in diet-induced obesity. In summary, recent reports suggesting that the long-term consumption of high fat diet induces change in host gut microbiota composition in the way that promotes chronic low-grade inflammation in both local and systemic sites prior to the development of systemic LPS or endotoxemia and insulin resistance in obesity (31, 34, 39) (*See Figure 1*)

Gut Dysbiosis: a Contributing Factor of Metabolic Endotoxemia

Gut dysbiosis, an alteration of gut microbiota composition, results from multiple factors including age, geographic location, diet also medical therapies (13, 40, 41). Prolonged high-fat diet consumption leads to gut dysbiosis in both human and animal studies (34-36, 42). Lecomte et al. (42) demonstrated that high-fat diet feeding decreases *Lactobacillus intestinalis* otherwise the predominant bacterial species found in rat's gut. Decreased numbers of *L. intestinalis* is associated with increased rat body weight. de La Serre and colleagues (35) also reported that rat fed with high-fat diet for a long-term (8 weeks) resulting in increased Bacteroidetes. Interestingly, the bloom of Enterobacteriaceae was significantly enhanced in high-fat diet-fed rats compared to the low-fat diet-fed rats. Moreover, gut (ileum) tissue of the obese rats were more inflamed (high levels of myeloperoxidase (MPO) activity and neutrophil infiltration) than the lean rats. The higher MPO activity was related to increased plasma LPS or endotoxin levels in high fat diet-fed rats. The blooming of Gram negative LPS-containing bacterial phyla such as Bacteroidetes and Proteobacteria is suspected as a major source of the elevated

plasma LPS levels observed in high fat diet-fed group (35). To our knowledge, Cani et al. (31) is the first group who discovered that gut microbiota LPS plays a direct role in development of obesity and metabolic syndromes using mouse model. Their breakthrough study showed that high-fat diet consumption abrogates the normal physiological cycle of plasma LPS levels. Serum LPS levels of high-fat diet-fed mice were persistently elevated during the daytime and nighttime. The authors also showed that infusion of extracted bacterial LPS to mouse skin resulting in increased serum LPS levels in even lean mice. LPS-infused mice showed the significant increase in body weight, visceral and subcutaneous fat mass and glucose tolerance in spite of no change in their energy intake. They also found that high-fat diet consumption promotes the growth of Gram-negative LPS-containing bacteria in mouse gut. Moreover, CD14-deficient mice, mice without a co-receptor molecule for bacterial LPS recognition, were resistant to metabolic abnormalities when induced with LPS or fed with high-fat diet. The authors first time defined the increased plasma endotoxin (LPS) levels by high-fat diet consumption as a "metabolic endotoxemia".

Metabolic endotoxemia has been linked with low-grade inflammation in multiple tissues (43, 44). More evidences have been exhibited the crucial roles of changes in gut microbiota regulating metabolic endotoxemia-induced systemic inflammation in obesity (34, 36, 44). Relationship between gut dysbiosis, low-grade gut inflammation and metabolic endotoxemia has been shown in diet-induced obese mouse (36). The results revealed that both high-fat diet-fed and genetically obese *(ob/ob*) mice show the attenuation of metabolic endotoxemia as well as LPS concentration in cecal contents after the antibiotic treatment. Decreased obese mouse gut microbiota during antibiotic administration attenuated the metabolic syndrome features and increased glucose sensitivity. These effects were also associated with decreases in visceral adipose tissue inflammation. The contribution of host innate immune response in high fat diet-induced gut dysbiosis had been reported by Kim et al. (34). The authors showed that host innate immune receptor TLR4 signaling pathway plays a major role in gut dysbiosis-induced gut inflammation in high-fat diet-fed mouse. They found that high-fat diet induced both systemic and local (gut and adipose tissues) inflammations in mouse by observing the increased serum pro-inflammatory cytokine (TNF-α, IL-1β and IL-6) levels and increased expression of gut (colon) and adipocyte tissues pro-inflammatory cytokine

genes, respectively. Feeding mouse with high-fat diet also causes gut dysbiosis by increasing Gram-negative LPS-containing Enterobacteriaceae while reducing Bifidobacterium burdens (34).

Recent studies have elucidated the correlation between a shift in gut microbiota composition and metabolic endotoxemia in human subjects (40, 44, 45). Consistent with animal studies, Ghanim et al. (45) reported that healthy lean human subjects given a high-fat, high-carbohydrate (HFHC) meal exhibit significant higher plasma LPS concentrations at 3 hours after the meal by 47 ± 14% over the baseline (from 0.39 ± 0.07 to 0.58 ± 0.10 EU/ml, $P < 0.05$). The results were related to the increase in TLR2 and TLR4 protein expression in HFHC-treated subjects compared to standard meal-treated group. Radilla-Vázquez et al. (44) reported that the young obese (aged 18-25 years, BMI $\geq$ 30 kg/m^2) individuals have higher total cholesterol, triglycerides and low-density lipoprotein (LDL)-cholesterol levels than the normal-weight (BMI = 18-24.9 kg/m^2) subjects and have a greater number of *Clostridium leptum* and *Lactobacillus* (members of the Firmicutes phylum) together with a lower in *Prevotella* and *Escherichia coli* (members of the Bacteroidetes and the Proteobacteria phyla, respectively) numbers. Gut dysbiosis were correlated with the increased serum LPS concentrations in those obese subjects. These findings demonstrated that gut dysbiosis initiates metabolic endotoxemia then plays a role in pathogenesis of obesity and MS in human (44). Obviously, several studies both animal and human suggested that gut dysbiosis in obese phenotype contributes to metabolic endotoxemia and further lead to altered metabolic features in obesity and MS (34, 36, 44, 45).

Role of the Low-Grade Inflammation in Insulin Resistance and Obesity

It has been previously known that chronic low-grade inflammation plays a crucial role in insulin resistance as well as obesity (13, 19, 43). Metabolic endotoxemia associates with enhanced inflammation-related cytokine gene expressions, such as TNF-α, IL-1β as well as IL-6 (36). Recently, mechanisms of inflammation inducing an impaired glucose sensitivity and obese features have been proposed (13, 19, 46). An obesity-related inflammation differs from *"the classic inflammation*" in which characterized by edema, erythematous, warmth and tenderness (47). In contrast to *"the classic inflammation*" response which focusing on the immune function to protect cell damages as well as

infection, obesity-associated inflammatory response is defined as a signaling from metabolic cells by the excess consumption of nutrients which results in trigger not only metabolic, but also inflammatory responses (46). Interestingly, Ding et al. showed that high-fat diet interacts with gut microbiota then resulting in gut inflammation, insulin resistance and obesity in mice (32). The authors used conventionally gut microbiota-raised mice and no gut microbiota (germ free) mice, then feeding mice with either high-fat or low-fat diet for 2-16 weeks. They found that gut inflammation only found in mice with the intact gut microbiota not the germ free mice (32). Prolonged inflammatory state in metabolic tissues such as adipose tissue, muscle and liver resulting in development of insulin resistance and disrupting their cellular insulin and cytokine signaling pathways (13, 46, 48). The molecular origins of chronic systemic inflammation found in metabolic tissues and serum are still poorly understood. However, recent several findings in both animal and human studies suggested that gut immune system (activation of low-grade inflammatory tone from high fat diet consumption and gut dysbiosis) could be a potential source of the low-grade systemic inflammation found in obesity and MS individual.

Low-grade systemic inflammation in obesity contributes to adipose tissue inflammation, a key source of metabolic inflammation, and further induces various metabolic tissue inflammations as well as the impairment of insulin sensitivity in those organs, including fatty liver, reduced glucose uptake in skeletal muscle, islet inflammation in pancreas and hypothalamic inflammation in brain which mainly initiates from gut inflammation (39, 46-48). Mechanisms by which the inflammatory signaling molecules worsen the insulin sensitivity by inhibiting insulin and cytokine signaling pathways (48). These findings emphasized the important relationship between gut dysbiosis and low-grade systemic inflammation from prolonged high-fat diet consumption, in the initiation of obesity and MS.

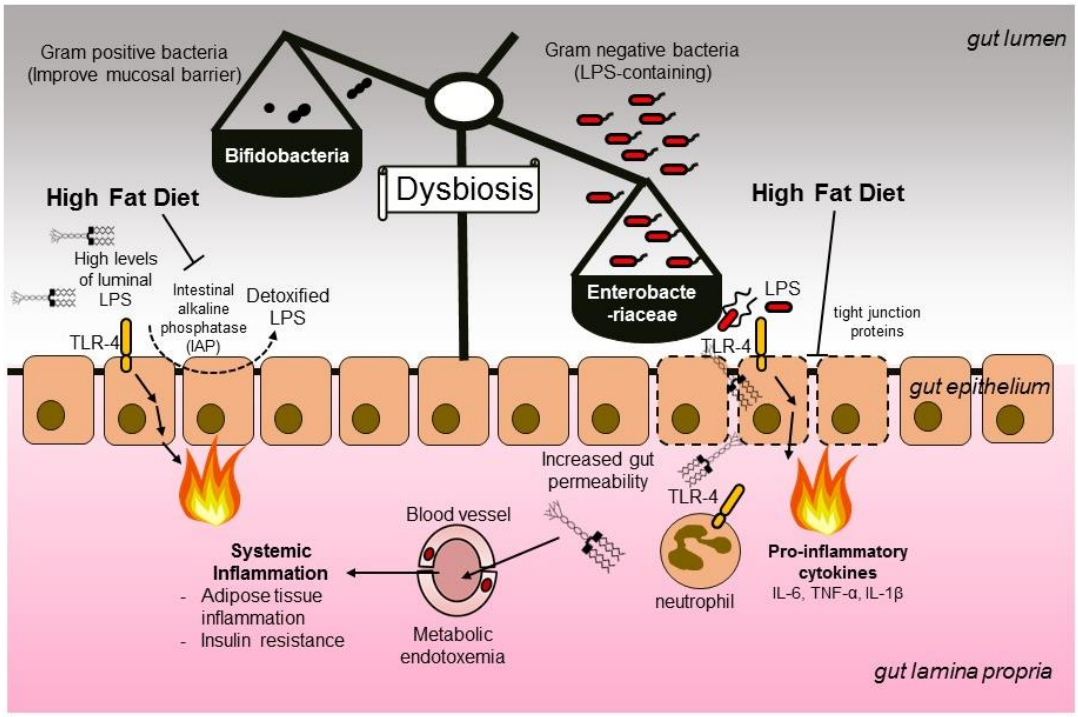

Figure 1 Prolonged consumption of high-fat diet resulting in altered gut microbiota composition (gut dysbiosis) and increased gut inflammatory tone. High-fat diet-consumption is associated with increased levels of Gram negative lipopolysaccharide (LPS)-containing bacteria group such Enterobacteriaceae (phylum Proteobacteria) while decreased numbers of mucosal-protective Gram positive Bifidobacteriaceae (phylum Actinobacteria) in gut lumen. This shift of gut microbiota composition has been linked to increased serum LPS levels (metabolic endotoxemia), adipocyte inflammation and insulin resistance found in obese individual.

Gut Microbiota Manipulation: a Potential Way for Diet-Induced Obesity Treatment

Although, there have been various intrinsic host factors involved in shaping and modulating the gut microbiota composition, such as host genetic background, age, host immunological status, the extrinsic (environmental) factors still have a crucial role (1, 49, 50). The well-recognized extrinsic factors contributing to changes in gut microbiota composition and activity such as the exposure to the microbes during early life, dietary components, antibiotic or some medications and exercise (24, 49, 51, 52). Many studies have mainly focused on regulating controllable environmental factors, especially dietary intervention, which provide a possibility of improving the gut dysbiosis, including

probiotic, prebiotic, and synbiotic consumptions (24, 49, 52). Probiotic administration is widely considered nutritional supplement which is defined by the Food and Agricultural Organization and the WHO as *"living organisms that confer a beneficial health effect on the host when administrated in adequate amounts"* (23). Prebiotics are non-digestible oligosaccharides of natural intermediate between simple sugar and polysaccharides which have the potential effects to enhance the growth of selective and beneficial gut microbes providing a beneficial health to the host (24, 49). Synbiotic approach is termed as the combination of probiotic and prebiotic administration to mediate the survival and implantation of probiotics together with stimulating growth, activity and metabolism of beneficial gut microbes simultaneously (24).

The possible mechanisms of action of probiotics in gut health including; (a) providing antimicrobial effects of gut microbiota in gut, (b) enhancing mucosal barrier integrity of the gastrointestinal tract and (c) modulating mucosal immune system and maintaining gut immune system homeostasis (50). Some probiotic bacteria especially *Lactobacillus* can produce lactic acid and SCFA (acetate, butyrate and propionate) from the fermentation of the complex carbohydrates such as dietary fiber. Butyrate confers a reduction in gut luminal bacterial translocation, induces mucin production from goblet cells in gut tissue, strengthen the gut barrier integrity by induction of tight junction proteins (50). The additional studies provided more evidences that several Lactobacilli exhibit capacity of antimicrobial peptide production, such as defensins and bacteriocins, which can directly inhibit some groups of Gram-positive, Gram-negative bacteria and viruses (24). More recently, Flynn and colleagues (53) reported that a human isolated strain of *Lactobacillus salivarius* subsp. *salivarius* UCC118 produces bacteriocin to inhibit growth of enteropathogens including *Enterococcus*, *Bacillus*, *Listeria* and *Salmonella* species in gut. The beneficial role of probiotic bacteria *Lactobacillus* species in promoting mucosal barrier integrity and prevention of gut inflammation also shown by Madsen et al. (54). The authors observed the preventive effects of *Lactobacillus* on the development of spontaneous colitis (inflammation of colon) using IL-10-deficient mice. Administration of probiotic *Lactobacillus* improved gut barrier integrity and ameliorated the severity of colitis in these mice (54). Moreover, probiotic *Lactobacillus* regulating gut adaptive immunity by enhancing the production and secretion of specific antibody that can neutralize invading pathogens (24, 55).

Probiotic *Lactobacillus* treatment, a Long History of the Promising Therapy for Obesity and Metabolic Syndrome

Consumption of probiotic bacteria in the genus *Lactobacillus* shown a great beneficial role in obesity and MS therapies (29, 55-58). The effects of candidate probiotic *Lactobacillus* strains targeting gut microbiota on diet-induced obese mice were studied by Wang et al. (56). The authors fed probiotic *L. paracasei* CNCM I-4270 and *L. rhamnosus* I-3690 to high-fat diet-induced mice for 12 weeks. They found that both probiotic *Lactobacillus* strains attenuate the obesity and insulin resistance in mice. Obese mice fed with *L. paracasei* CNCM I-4270 and *L. rhamnosus* I-3690 showed the significant decrease in homeostasis model assessment (HOMA) index, a potential marker of insulin sensitivity state, as well as epididymal tissue macrophage infiltration. Supplementation with these probiotic *Lactobacilli* significantly elevated gut acetate levels and restoration gut microbiota population (56). Interestingly, Sirilun et al. (59) performed the *in vitro* study of 4 strains of non-human origin isolated probiotic *Lactobacillus plantarum* including; TGCM 15, TGCM 26, TGCM 33, and TGCM 128 in cholesterol-lowering effects. The results demonstrated that all selected *L. plantarum* strains have an intensive antimicrobial activity against important human pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* Typhi. For the cholesterol-lowering effect, they showed that only the viable *L. plantarum* TGCM 15 and TGCM 33 decrease cholesterol levels in the culture media not the resting nor dead cells (the cholesterol levels were reduced approximately 50%). This finding indicated that a non-human isolated *L. plantarum* TGCM 15 and TGCM 33 have a probiotic and cholesterol-lowering properties *in vitro* (59). Consistent with Sirilun's report, Salaj et al. found that probiotic *L. plantarum* LS/07 decreases serum total cholesterol and low density lipoprotein (LDL) cholesterol and *L. plantarum* Biocenol LP96 decreases serum triglyceride (TG) and very low density lipoprotein (VLDL) levels without change in rat's body weight (60). The results indicated that the distinct *L. plantarum* strains demonstrate the beneficial and different effects on host lipid metabolism (60). Wu CC et al. reported the effect of *L. plantarum* K21 in obesity treatment using mouse model (61). They found that *L. plantarum* K21 supplementation decreases body weight, epididymal fat accumulation and dyslipidemia in obese mice. Administration of *L. plantarum* K21 attenuated gut dysbiosis by enhancing

Bifidobacterium numbers and improved gut barrier integrity in obese mice (61). Aronsson et al. (62) showed that probiotic *L. paracasei* ssp. *paracasei* F19 significantly decreases fat accumulation and improves serum lipoprotein profiles through the reduction of angiopoietin-like 4 protein (ANGPTL4), a lipoprotein lipase inhibitor in the circulation (62). Moreover, Tanida et al. investigated the therapeutic effect of probiotic *L. paracasei* ST11 (NCC2461) on obesity in rodents and found that long-term (11 weeks) consumption of probiotic *L. paracasei* ST11 (NCC2461) reduces rat body weight and abdominal fat mass (63). Although these findings suggested the beneficial role for using probiotic *Lactobacillus* as a new therapeutic way for obesity and MS, there are still inconsistency outcomes in which most likely arise from the strain-dependent factor of the probiotic bacteria (1, 29, 49, 64).