# CHAPTER 1

# Introduction

## 1.1 Principle, Theory and Rational/Hypothesis

Obesity has a continued and increasing prevalence since the 1980s (Sowers, 2003a). In 2014, the WHO reported that over 1.9 billion adolescents and adults were overweight and more than 600 million men were obese (Organization, 2016). The factors involved in obesity include high-fat diet (HFD) feeding, low physical activity and low amounts of exercise (Organization, 2016). Obesity is not only a major risk factor of cardiovascular disease (CVD) (Poirier et al., 2006), but is also closely related with Metabolic syndrome (Mets) which includes insulin resistance, dyslipidemia, hyperglycemia and hypertension (Kaur, 2014; Ritchie & Connell, 2007). Moreover, previous studies found that long term obesity will develop into Mets (Reaven, 1988). Our previous studies have demonstrated that long-term HFD consumption leads to obese-insulin resistant conditions in animal models (Pipatpiboon, Pratchayasakul, Chattipakorn, & Chattipakorn, 2012). Insulin resistance induces oxidative stress (Evans, Maddux, & Goldfine, 2005), which then leads to cardiac mitochondrial dysfunction and cardiac dysfunction respectively (Kim, Wei, & Sowers, 2008). Moreover, obesity can result in inflammatory processes and cause CVD (Berg & Scherer, 2005). Furthermore, metabolic disturbance, cardiac mitochondrial dysfunction and cardiac dysfunction were observed followed by obese-insulin resistant conditions (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013c). Gut microbiota is associated with obesity and insulin resistance, leading to CVD (Shen, Obin, & Zhao, 2013). Alteration of gut microbiota composition has been shown to induce obesity (Musso, Gambino, & Cassader, 2010). Previous studies have shown that HFD consumption also alters intestinal microbiota composition and increases intestinal permeability. Altering these can contribute to metabolic endotoxemia, including low-grade systemic

inflammation, insulin resistance and increased CVD risk (Moreira & al., 2012; A. L. Neves, J. Coelho, L. Couto, A. Leite-Moreira, & R. Roncon-Albuquerque, Jr., 2013).

Probiotics are live microorganisms which, when adequately administered into the body, can cause health benefits on the host (Jones, Martoni, Parent, & Prakash, 2012; Klein, Sanders, Duong, & Young, 2010; Reid et al., 2011; Verbrugge et al., 2013). The consumption of probiotics can be produced from various grains, vegetables, beans, fish, and dairy products (Howell, 1988). Several microorganisms commonly used as probiotics include: *Lactobacillus, Bifidobacteria, Lactococci* as well as others. These are bacteria usually used in intestinal diseases such as gastrointestinal infections and inflammatory bowel diseases (Haenel & Bendig, 1975). One of the benefits of probiotics is the improvement of certain metabolic parameters. Yanping, et al. investigated the effects of *Lactobacillus plantarum* MA2, and they found that it significantly lowered serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL), triglycerides (TG) and also decreased liver total cholesterol (TC) and TG, but did not alter high-density lipoprotein cholesterol (HDL). They suggested that this cholesterol lowering-effect caused by altering gut microbiota, led to the incorporation of cholesterol into the cell and increased its excretion via feces, resulting in the inhibition of cholesterol reabsorption in the intestine (Y. Wang et al., 2009). Release of pro-inflammatory cytokines and pro-oxidative stress increased in obesity type. However, probiotics can reduce such parameters. The evidence showed that *Lactobacillus* attenuated the levels of serum malondialdehyde (MDA), oxidized low density lipoprotein (ox-LDL), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and increased superoxide dismutase (SOD) activities in apo$^{-E-}$ mice (L. Chen et al., 2013). Interestingly, it has been shown that gut microbiota alteration therapy may have potential benefits to cardio-protection (Gan et al., 2014a). Gan et al. showed that *Lactobacillus rhamnosus* GR-1 application improved Left Ventricle (LV) function and attenuated progressive development to cardiac hypertrophy and heart failure after myocardial infarction in rats (Gan et al., 2014a). Moreover, Lam showed that probiotics may be cardio-protective after administration. Probiotics reduced infarct size by 27% and improved LV function by 35% in an ischemia/reperfusion (I/R) model (Lam et al., 2012). However, the beneficial effects of probiotics in these therapeutics strategies are dependent on strain, amount, and duration of bacteria administration. The effect of the

probiotic, *Lactobacillus paracasei* ST11 (HP4) on cardiac function in obese-insulin resistant conditions has not been investigated. Therefore, we will determine the effects of probiotics on the cardiac function in high-fat diet induced insulin resistant rats.

## 1.2 Literature Review

### 1.2.1 Obesity and Insulin Resistance

The World Health Organization (WHO) has stated that being overweight or obese is defined as an "abnormal or excessive fat accumulation that presents a risk to health" (Sowers, 2003b). The prevalence of obesity in children and adults since 1980 to 2013 has increased from 28.8% to 36.8% in men and 29.8% to 38.0% in women. However, the rate of obesity is predicted to rise in future (Ng & al., 2014). The factors of obesity are energy expenditure imbalance, low physical activity and sedentary occupation (S. M. Grundy, 2004) , and lead to several diseases such as diabetes mellitus (DM), atherosclerosis, and non-alcoholic fatty liver disease (NAFLD) (Parekh, Arusi, Vinik, & Johnson, 2014). Growing evidences reported that 8-12 weeks of HFD consumption induced obese-insulin resistance in rat models (Buettner et al., 2006). Obesity is a cause of insulin resistance, while insulin resistance can also worsen the effects of obesity (Scott M. Grundy, 2004). Under normal physiological conditions, the pancreas releases appropriate amounts of insulin leading to normal glucose uptake via the activated insulin signaling pathway (Wilcox, 2005b). After the insulin molecule binds to the insulin receptor of target cells, activation of the insulin receptor occurs by phosphorylation at the tyrosine site of the insulin receptor substrate (IRS). This activates the phosphatidylinositol 3-kinase (PI3K) pathway respectively. PI3K can recruit the PKB/Akt signaling pathway that regulates the translocation of glucose transporter 4 to the plasma membrane for glucose uptake (Mehta, Rasouli, Sinha, & Molavi, 2006). The degree of cellular insulin resistance was not stimulated by insulin at standard physiological insulin levels (Ghanadian, Lewis, & Chisholm, 1975; Kahn, Hull, & Utzschneider, 2006). Adipocytes enlargement leads to altered free fatty acid mechanisms and inhibits insulin facilitated glucose uptake. Subsequently, muscle and glycogen synthesis reducing the sensitivity for insulin activation of the insulin receptor or insulin receptor substrate phosphorylation at the serine/threonine site, resulting in the dampening of downstream insulin signaling pathways (Draznin, 2006). This can cause

a decrease in glucose transporter 4 trafficking to the plasma membrane, resulting in a diminished cellular glucose uptake and increased circulating glucose levels (Cook et al., 2010; Kyriakis & Avruch, 1996). Under this condition the islets β-cells show compensatory responses to the high circulating glucose levels by releasing large amounts of insulin into the circulation - leading to high levels of circulating insulin (Wilcox, 2005a). Therefore, the symptoms of insulin resistance impair glucose tolerance, high blood insulin concentrations, and reduce insulin sensitivity (Kotsis, Stabouli, Papakatsika, Rizos, & Parati, 2010). Moreover, infiltration of the macrophages into adipose tissue leads to activation and adipocytokine release - including pro-inflammatory cytokines, glycerol, plasminogen activator inhibitor-1 (PAI-1) and C-reactive protein (CRP). Specifically, these are the cause of inflammation (Lau, Dhillon, Yan, Szmitko, & Verma, 2005). The inflammatory process is not localized to only adipose tissue, it can develop into systemic inflammation and cause organ dysfunction (Tsimikas et al., 2009). The pro-inflammatory cytokine tumor necrosis factor alpha (TNF-$\alpha$) affects insulin function by reducing insulin sensitivity (Xydakis et al., 2004). Equally interleukin-10 (IL-10), an anti-inflammatory cytokine, can facilitate the tissue healing process post-inflammation (Ouyang, Rutz, Crellin, Valdez, & Hymowitz, 2011). Moreover, previous studies have found that IL-10 could inhibit the effect of TNF-α on the endothelial cells of murine aorta (Zemse, Chiao, Hilgers, & Webb, 2010).

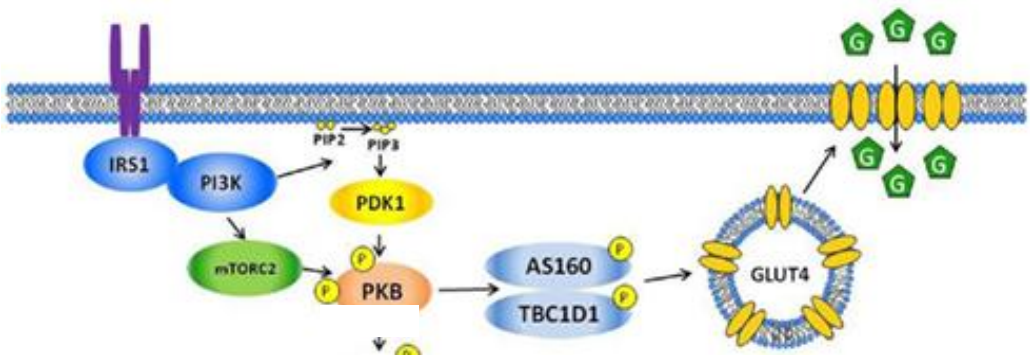


**Figure 1.1** Insulin signaling pathway modulating glucose uptake in skeletal muscle cells (Jensen, Rustad, Kolnes, & Lai, 2011).

### 1.2.2 Obese-insulin resistant and Heart

Cardiovascular diseases are the leading cause of death globally. In 2012, an estimated 17.5 million people died from CVD - 29% of all global deaths (Yusuf, Reddy, Ôunpuu, & Anand, 2001). The WHO predicted that by 2030 there will be an increase in the prevalence of CVD induced mortality. Specifically, they state that about 23.6 million people will die annually from CVD (2015, 2015). Higher insulin levels and insulin resistance condition cause defective nitric oxide synthesis, increase free fatty acid (FFA) in blood circulation, influence insulin receptor in hypothalamus, increase oxidative stress, decrease anti-oxidants and lead to sympathetic over-activity (Canale et al., 2013; Xydakis et al., 2004). Increased sympathetic drive or sympathetic over-activity is typically found in many pathologic states, including Mets and CVD (Grassi et al., 2004; Grassi et al., 1998). Looking at waist hip ratio, a previous study found that 2,042 adult men and women have lower LV ejection fraction (%EF), and LV diastolic dysfunction (Ammar et al., 2008). Patients with congestive heart failure have an increased variability of venous plasma norepinephrine, heart rate variability (HRV) and cardiac norepinephrine - suggesting sympathetic over activity (Bristow et al., 2004). The obese-insulin resistant rats were found to have a decreased HRV – indicating an increased cardiac sympathovagal imbalance (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013b). Moreover, sympathetic overactivity can facilitate the development of high blood pressure via increased heart rate (Grassi, 1998).

### 1.2.3 Insulin resistance and mitochondrial function

Cardiomyocytes need adequate energy for cardiac contraction and relaxation (Kolwicz, Purohit, & Tian, 2013). Mitochondria are important organelles that supply cellular energy by special mechanisms localized to the inner mitochondrial membrane. Specifically, this is called the electron transport chain (ETC) (Sivitz & Yorek, 2010). The ETC contains five complexes: complex I, II, III, IV and V which produce chemical energy in the form of adenosine triphosphate (ATP) for cellular metabolism. However, it is found that the complexes I and III can produce reactive oxygen species (ROS) and lead to increased oxidative stress. In turn, this can trigger stress-activated kinases, including the JNK/SAPK pathway and p38 MAPK, leading to mitochondrial dysfunction and insulin signaling inhibition (Montgomery & Turner, 2015).

Oxidative stress can also trigger NF-kB activity, which is a mediator of immune and inflammatory responses that can contribute activation of cellular apoptosis (Morgan & Liu, 2011). High levels of ROS production cause reduced oxygen consumption resulting in decreased mitochondrial function, and cellular apoptosis (Madamanchi & Runge, 2007). Moreover, previous studies have demonstrated that HFD consumption can lead to development of insulin resistance. Also, under this condition, they found that impaired cardiac and brain mitochondrial function lead to impaired cardiac and brain function respectively (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2012). These models also increased the cardiac mitochondrial dysfunction - as shown by increased cardiac mitochondrial ROS, cardiac mitochondrial membrane depolarization ($\Delta\Psi m$) and cardiac mitochondrial swelling (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013a).

### 1.2.4 Obese-insulin resistance and gut microbiota

Human gut microbiota or microflora have trillions ($10^{13}$) of cells of bacteria (Tilg & Kaser, 2011). The main bacterial phylum found include: Firmicutes, Actinobacteria, Bacteroidetes in intestines (Zoetendal, Vaughan, & de Vos, 2006). Factors such as antibiotics , environment, and food consumption are involved with decreased numbers of bacterial colonies in the gut microbiota (Manco, Putignani, & Bottazzo, 2010a). Previous studies have demonstrated that, in humans older than 2 years of age, the gut microbiota increases in size. Eventually, this reaches a colony concentration similar to a human adult (Penders et al., 2006). Gut microbiota is related to host energy balance and energy storage (Gerritsen, Smidt, Rijkers, & de Vos, 2011). This includes energy extracted from diet, harvested energy, suppression of fasting-induced adipocytes and adenosine monophosphate-activated protein kinase (Parekh et al., 2014). Alterations in gut microbiota are also associated with metabolic endotoxemia and inflammation (Cani, Bibiloni, Knauf, Waget, Neyrinck, Delzenne, & Burcelin, 2008). Obesity may be associated with an alteration in the proportion of bacterial phylum, by increased *Firmicutes* and reduced *Bacteroidetes* (Manco, Putignani, & Bottazzo, 2010b). Bäckhead and colleagues investigated the role of microbiota in germ-free mice. They showed that alterations in gut microbiota increased body fat by 60%, reduced food intake by 27% and caused the development of insulin resistance (Backhed et al., 2004).

A Previous study found that chronic HFD consumption was related to alterations in gut microbiota and led to increased systemic inflammation and obesity (Cani, Bibiloni, Knauf, Waget, Neyrinck, Delzenne, & al., 2008). Consumption of HFD increased the production of bile which was associated with reduced expression of occludin, a junctional adhesion molecule-1, in small intestinal epithelial cells (Camilleri, 2016). Moreover, HFD caused lipopolysaccharide(LPS)-induced inflammation in the intestinal barrier such as in Zona-1 cells, by a c-Src-, TLR4-, LBP-, and myosin light chain kinase-dependent mechanism (Sheth, Delos Santos, Seth, LaRusso, & Rao, 2007).
In addition, HFD induced gut microbiota composition changed the influence of the immune system by decreased T-cell activity and proliferation, activated innate immune cells such as B cell product inflammatory cytokines including TNF-a, and interleukin-6 (IL-6) and decreased anti-inflammatory cytokines such as IL-10. Specifically, LPS is a component of the outer membrane of Gram-negative bacteria (Elin & Wolff, 1976) and plays an important role by activating low-grade systemic inflammation via increased activity of the immune response (Herder et al., 2007; Stoll & Bendszus, 2006). LPS has negative effects on the host cells under pathological conditions such as obesity (Bayston & Cohen, 1990; Laugerette et al., 2011). Previous studies suggest that, when the increased intestinal permeability of the intestinal barrier is changed by absorption across enterocytes during chylomicron secretion, LPS can leak into the blood circulation - causing systemic inflammation (Juskewitch et al., 2012). A two or three fold concentration of LPS in serum is called “Metabolic endotoxemia” (A. L. Neves, J. Coelho, L. Couto, A. Leite-Moreira, & R. Roncon-Albuquerque, 2013). Metabolic endotoxemia causes an increase in low-grade inflammation levels via toll-like receptor 4 (TLR-4) activation and can lead to inflammation of multiple organs and damage. This, in turn, contributes to an increased risk of CVD (Neves & al., 2013).

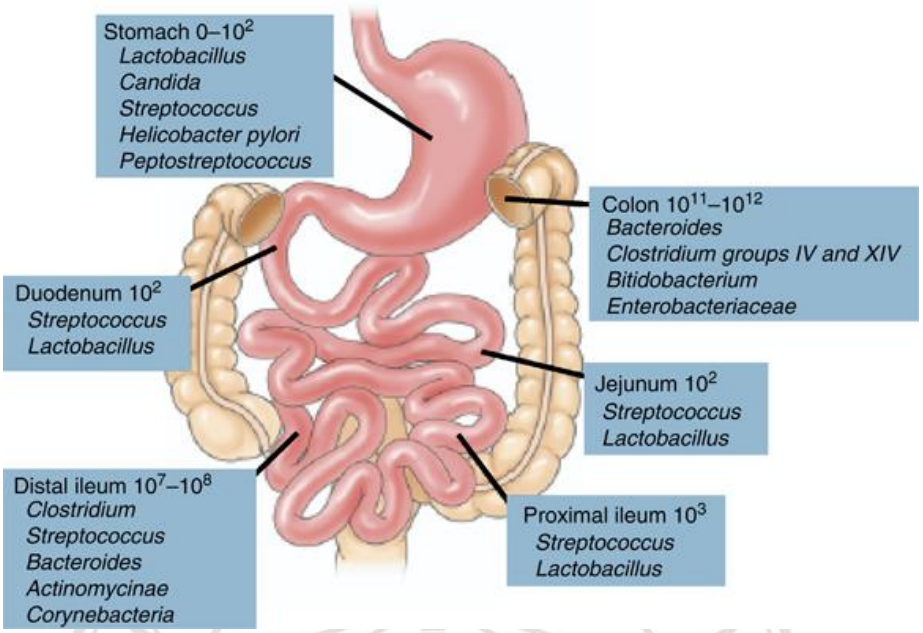


**Figure 1.2** Composition of dominant microbial species in the human gastrointestinal tract (Sartor & Mazmanian, 2012).

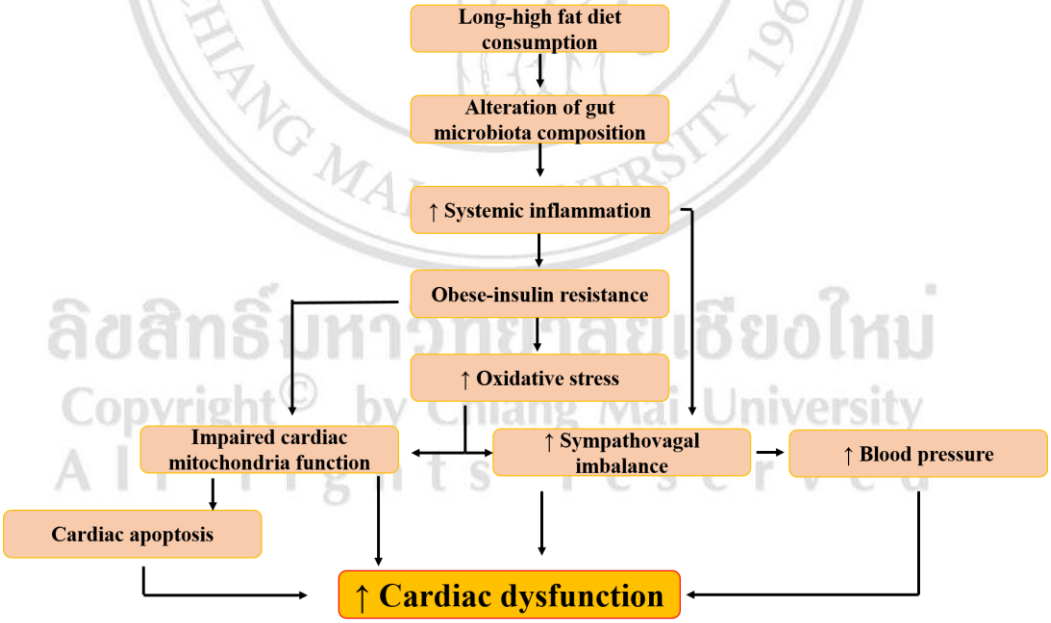


**Figure 1.3** A diagram summarizing the association of long-term HFD, obese-insulin resistance and cardiac dysfunction.

### 1.2.5 The probiotics

WHO has defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Gobbetti, Cagno, & De Angelis, 2010). In 1907, Metchnikoff, an immunology researcher introduced the first probiotics concepts. He found that *Lactobacilli*, a beneficial microorganism, might counteract the putrefactive effects of gastrointestinal metabolism. These effects are the reason that Bulgarian peasants had a long life span. In 1992, Metchnikoff's concepts were accepted worldwide by scientist and consumers. Probiotics have since been used in functional foods, and to prevent and treat disease (Mackowiak, 2013). The product of probiotics can be found in fermented foods, milk, vegetables, grains and beans. The main phyla of bacterial probiotics are Firmicutes (*Clostridium, Lactobacillus, Lactococcus, Streptococcus*), Bacteroidetes (*Bacteroides*), and Actinobacteria (*Bifidobacterium*) (Eckel, Grundy, & Zimmet, 2005). In order for microorganisms to be defined as effective probiotics they must have certain criteria; 1) resistance to gastric acid and bile salt, 2) adherence to mucus and epithelial cells, 3) do not display pathogenic and antimicrobial activity, 4) reduce pathogen adhesion to the surface, and 5) resistance to spermicides (Orlando et al., 2012). Several studies show that bacterial strains, especially *Lactobacillus* and *Bifidobacteria*, have strong beneficial effects on health (Festi et al., 2014). The direct effects of probiotics on the host include: 1) expressing microorganism-associated molecular patterns (MAMPs) that can bind to host pattern recognition receptors (PRRs) located on the cell surface of intestinal epithelial cells (IEC) and dendritic cells, 2) increasing both the diversity of commensal bacteria and availability of nutrients for intestinal epithelial cells (IECs) usage through its metabolite production, 3) increasing tight- and adherent junction (TJ and AJ) protein production, 4) improving gut permeability and inhibiting LPS circulation in the blood (Sirilun, Chaiyasut, Kantachote, & Luxananil, 2010), 5) inducing activation/ inhibition of signaling pathways, 6) inhibiting pro-inflammatory CD4+ cell proliferation and activation of anti-inflammatory pathways though stimulating dendritic cells, 7) protecting mucus layers by releasing IgA, which is an immunoglobulin, and stimulating goblet cells, leading to activate mucin gene expression and production of mucin glycoproteins, and 8) acting as a barrier against pathogen colonization (Figure 1.4) (Le Barz et al., 2015).

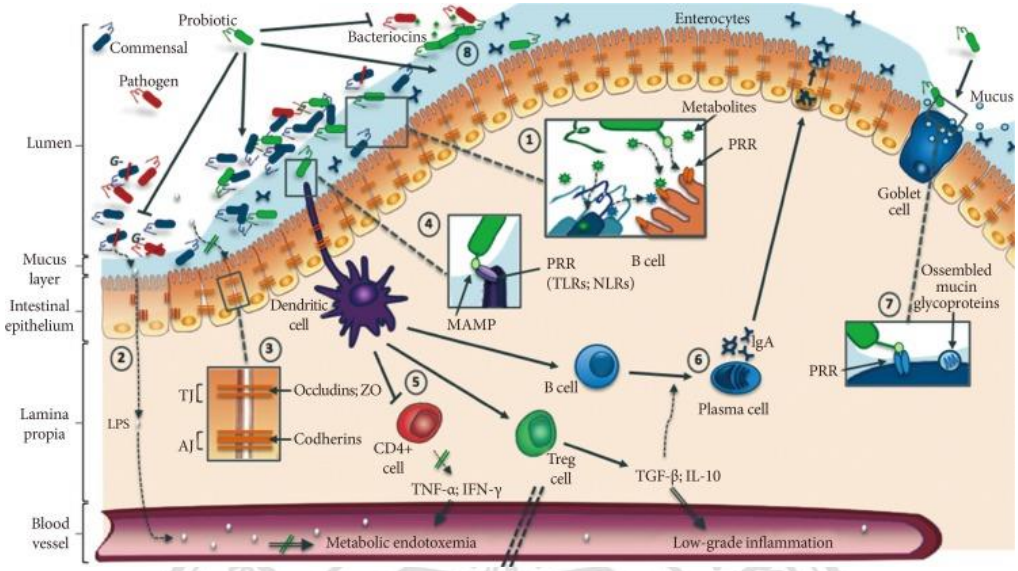


**Figure 1.4** Potential direct effects of probiotics (Le Barz et al., 2015).

The effects of probiotics have been evident but are considered to be strain specific (Ebel et al., 2014). Probiotic *lactobacillus paracasei* is a gram-positive bacterium and commonly used in daily products (Orlando et al., 2012). Previous studies found that active cells of *Lactobacilli* had lowering-cholesterol effects via exhibiting bile salt hydrolase (BSH) activity (Sirilun et al., 2010). In addition, HFD-induced obese mice had improved blood sugar, insulin resistance, and reduced body weight following *Lactobacillus paracasei* CNCM I-4270 (LC) administration (J. Wang et al., 2015). Strong evidence shows that probiotics are clinically effective at preventing symptoms of lactose intolerance, as well as treating acute diarrhea and inflammatory bowel disease. In addition, they can be used to prevent allergy manifestations, attenuating antibiotic-linked gastrointestinal tract side effects and more (Doron & Gorbach, 2006). Several studies showed that probiotics changed gut microbiota composition to a normal physiological condition in the HFD model, and activated the immune system in HFD-induced diabetes mice and hyperlipidemia mice (Cani et al., 2007; L.-X. Wang, Liu, Gao, & Hao, 2013).

### 1.2.6 Effects of probiotics on obesity and metabolic syndrome

Several studies have shown that probiotics can improve metabolic profile, fat attenuation and body weight (Tomaro-Duchesneau et al., 2014). Jinjin C, et al. investigated *Bifidobacterium* supplementation in rat adolescents who were receiving

a HFD. The results showed a significantly decreased fat mass and body weight and improvement in insulin signaling (J. J. Chen, Wang, Li, & Wang, 2011). Moreover, the studies also reported that *Bifidobacterium longum* can improve immune system function in metabolic syndrome in rats fed a HFD via the reduction of metabolic endotoxin levels and intestinal inflammation (J. J. Chen et al., 2011). Tanida demonstrated that *Lactobacillus paracasei* ST11 (NCC2461) supplementation in rats fed a HFD caused attenuation of body weight and fat mass (Tanida et al., 2008). However, some studies reported that probiotics attenuated serum cholesterol levels without any effects on body weight in apo$^{-E-}$ mouse models (London et al., 2014). Other studies compared different probiotic strains (*Lactobacillus plantarum* LS/07 or *Lactobacillus plantarum* LP96) on metabolic parameters, including body weight, body fat mass, HDL, LDL, very low-density lipoprotein (VLDL), serum TC and TG. The results showed that *Lactobacillus plantarum* LS/07 decreased LDL and TC while *Lactobacillus plantarum* LP96 reduced VLDL and TG. However, there were no significant changes in body weight, body fat mass, HDL and liver lipids (Salaj et al., 2013).

### 1.2.7 Effects of probiotics on the inflammatory process and oxidative stress

Previous studies have described probiotic microorganisms as being associated with modulation of the host immune system (Corthésy, Gaskins, & Mercenier, 2007). Administration of *Lactobacillus casei* CRL 431 in a mouse model fed a high-fat diet resulted in an anti-inflammatory response in the mice and a reduction in the pro-inflammatory cytokine IL-6, IL-17, and TNF-$\alpha$(Novotny Nunez, Maldonado Galdeano, de Moreno de LeBlanc, & Perdigon, 2015). Recently, Yi Quao investigated the effects of the various strains of the same probiotic species on the inflammatory responses in obese mice. In addition to their beneficial effects on the metabolic parameters, *Lactobacillus reuteri* L3 decreased IL-6, IL-12 and TNF-$\alpha$. In contrast, *Lactobacillus reuteri* L10 did not reduce LPS and TNF-$\alpha$(Qiao et al., 2015). They suggested that a different strain of the probiotics had a different inflammatory response. Beside the anti-inflammation, probiotics; *Lactobacillus acidophilus* ATCC 4356 exerted an anti-oxidative function. These studies reported that *Lactobacillus acidophilus* increased serum superoxide dismutase (SOD) levels and MDA level. Moreover, their treatment

improved inflammation by increasing IL-10 and decreasing TNF-$\alpha$ on aorta tissue in apo$^{-E-}$ mouse model when fed a HFD (L. Chen et al., 2013).

### 1.2.8 Effects of probiotics on cardiovascular disease

There is a lot of evidence published supporting the cardio-protective effects of probiotics on the heart in several *in vivo* models. Gan and colleagues had investigated the effects of *Lactobacillus rhamnosus* GR-1 in a myocardial infarction rat model (Gan et al., 2014b). In that study, rats received *Lactobacillus rhamnosus* GR-1 in their drinking water for 6 weeks. The results showed that *Lactobacillus rhamnosus* GR-1 could improve LV dysfunction by improving cardiac functions as indicated by improved %EF and fractional shortening (%FS) (Gan et al., 2014b). Moreover, the probiotics also maintained LV function for two weeks after treatment ended (Gan et al., 2014b). Additionally, probiotics can reduce lesion size of atherosclerosis in hypercholesterolemia in a rabbit model (Cavallini, Bedani, Bomdespacho, Vendramini, & Rossi, 2009). On the other hand, some studies have reported probiotics having the opposite effect. Fak and Bäckhed reported that *Lactobacillus reuteri* reduced body weight but did not reduce insulin resistance, inflammatory markers, and blood cholesterol as well as the lesion size of atherosclerosis in apo$^{-E-}$ mice fed with a high-fat diet (Fak & Backhed, 2012). In addition, long-term probiotic administration could reduce systolic blood pressure and improve cardiac function by decreasing pro-inflammatory cytokines and pro-oxidative stress in spontaneous hypertensive rats (Gomez-Guzman et al., 2015).

## 1.3 Purposes of the study

Probiotics have been shown to exert several cardio-protective effects such as anti-inflammation, anti-oxidants and preserved mitochondrial function in several cardiovascular pathological models. In addition, probiotics can modulate several metabolic parameters to control body weight and appetite. However, the potential effects of the probiotics *Lactobacillus paracasei* ST11 HP4 on cardiac function in obese-insulin resistant rats have never been investigated. Therefore, the objectives of the present study are as follows:

**Aim 1** To examine the effects of probiotic *Lactobacillus paracasei* ST11 (HP4) on metabolic parameters, oxidative stress and bacteria endotoxin levels in obese-insulin resistant rats

**Aim 2** To examine the effects of probiotics on blood pressure (BP), heart rate variability (HRV), cardiac function, cardiac mitochondrial function, and the cardiac anti-apoptosis signaling pathway in obese-insulin resistant rats

### 1.4 Hypotheses of the study

**Hypothesis 1:** A probiotic *Lactobacillus paracasei* ST11 (HP4) can attenuate metabolic disturbance by decreasing plasma TC, LDL, TG and insulin levels, HOMA-index, body weight, visceral fat mass and food intake, and also by increasing plasma HDL levels. Moreover, this probiotics can attenuate oxidative stress by decreasing plasma and cardiac tissue levels of malondialdehyde (MDA), attenuating bacteria endotoxin by decreasing serum LPS in obese-insulin resistant rats.

**Hypothesis 2:** A probiotic *Lactobacillus paracasei* ST11 (HP4) can improve blood pressure (BP) by decreasing systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) in obese-insulin resistant rats.

**Hypothesis 3:** A probiotic *Lactobacillus paracasei* ST11 (HP4) can improve heart rate variability (HRV) by decreasing the LF/HF ratio in obese-insulin resistant rats.

**Hypothesis 4:** A probiotic *Lactobacillus paracasei* ST11 (HP4) can improve cardiac dysfunction including fractional shortening (%FS), stroke volume (SV), left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP) and maximum and minimum dP/dt by increasing LVEDP and minimum dP/dt and decreasing LVESP, maximum dP/dt and SV in obese-insulin resistant rats.

**Hypothesis 5:** A probiotic *Lactobacillus paracasei* ST11 (HP4) can improve cardiac mitochondrial function by decreasing cardiac mitochondrial reactive oxygen species (ROS) production, mitochondrial membrane depolarization ($\Delta\psi_m$), and mitochondrial swelling. Moreover, the probiotics can attenuate mitochondrial apoptosis by decreasing the Bax/Bcl-2 ratio in obese-insulin resistant rats.