CHAPTER 4

Discussion and Conclusions

In this study, our findings demonstrated that 1) obese-insulin resistance induced by HFD consumption is associated with high blood pressure, cardiac sympathovagal imbalance, and cardiac dysfunction, and these cardiac adverse effects are caused by dyslipidemia, systemic inflammation, oxidative stress, and cardiac mitochondrial dysfunction. 2) Probiotics treatment could ameliorate insulin resistance, oxidative stress, and systemic inflammation in HFD-fed rats. 3) Probiotics reduced blood pressure, cardiac sympathovagal imbalance, cardiac mitochondrial dysfunction, and LV dysfunction. 4) HFD and probiotics treatment did not alter cardiac apoptosis protein expression.

Previous studies demonstrated that long-term HFD induced obese-insulin resistance as indicated by increased body weight gain, visceral fat accumulation and hyperinsulinemia with euglycemia (Pipatpiboon, Pratchayasakul, Chattipakorn, & Chattipakorn, 2012; Samniang et al., 2016). HFD also induced gut dysbiosis and further contributed to higher LPS levels in the gut and blood circulation (Turnbaugh, Backhed, Fulton, & Gordon, 2008; Wu et al., 2011). The higher LPS levels in blood circulation, metabolic endotoxemia, was associated with low-grade systemic inflammation, and led to obesity as well as insulin resistance in various models (Cani et al., 2008; Ding et al., 2010). Consistent with these previous studies, our obese-insulin resistant rats had a higher levels of serum LPS.

An accumulation of LPS has been shown to trigger an inflammation response, and later disrupts metabolic function including insulin function and lipid metabolism (Lee et al., 2011; Osborn & Olefsky, 2012). The impairment of lipid metabolism caused an increased plasma total cholesterol and LDL levels. As a result, 12 weeks of HFD consumption caused a systemic inflammation, leading to an impairment of lipid

metabolism, which finally caused obesity in our obese-insulin resistant rats. Moreover, systemic inflammation has also been shown to impair insulin receptor function (de Luca & Olefsky, 2008), which is a leading cause of insulin resistance as shown in the insulin insensitivity during OGTT. Since gut dysbiosis induced low-grade systemic inflammation, contributed to obesity, and impaired insulin function (Festi et al., 2014), Attenuation of gut dysbiosis may provide beneficial effects in our obese-insulin resistant rats (Figure 4.1).

Probiotics are living microorganisms and have been shown to provide beneficial effects to the host when adequate amounts of probiotics are administered. Several studies found that probiotics could restore gut microbiota homeostasis, and their effects improved systemic inflammation in obese mice and high cholesterol diet induced obese rat models (Lim, Jeong, Woo, Han, & Kim, 2016; Wang et al., 2015). In the present study, our results demonstrated that *Lactobacillus paracasei* ST11 (HP4), which is probiotic from Thai pickles of leeks and red shallots isolation, could effectively reduce LPS levels, improve insulin resistance as well as metabolic disturbance, and that these beneficial effects are mainly due to a reduction of systemic inflammation and oxidative stress in our obese insulin resistant rats. Consistent with previous studies, our results showed that several strains of probiotics including *Lactobacillus reuteri* GMNL-263 and *Lactobacillus rhamnosus* GG improved insulin resistance in high fructose-fed rats, and obese mice models, respectively (Hsieh et al., 2013; Park, Kim, & Hyun, 2015). However, probiotic *Lactobacillus paracasei* ST11 (HP4), used in this study did not affect the body weight in our obese insulin resistant rats. It is possible that the anti-obesogenic effect of probiotics depends on the specific strain of probiotics (Fåk & Bäckhed, 2012; Million et al., 2012).

Endothelial dysfunction are known to cause by insulin resistance trough higher systemic inflammation and oxidative stress via impair endothelial nitric oxide synthesis and activity (Muniyappa & Sowers, 2013). This effects had contribute to increase blood pressure and cardiac dysfunction (Cai & Harrison, 2000; J. P. Sun et al., 2015) (Figure 4.1). Our results showed that higher blood pressure in HFD-fed rat. These results are consistent with previous studies (Aubin et al., 2008). Probiotics *Lactobacillus paracasei* ST11 (HP4) could improve insulin resistance, systemic inflammation and

oxidative stress as well as reduce blood pressure. These results are support by previous studies in SHR, obese mice and clinical models (Gomez-Guzman et al., 2015; Hariri et al., 2015; Toral et al., 2014).

Our previous studies found that both male and female gender of HFD-induced obese-insulin resistant models were imbalanced of autonomic nervous system as well as impaired cardiac function at the same time (Pongkan, Pintana, Sivasinprasasn, et al., 2016; Sivasinprasasn et al., 2015) This possible mechanism of these impairments might be through the increasing of systemic inflammation and oxidative stress (Figure 4.1). Regarding to cardiac dysfunction, oxidative stress could induce critical threshold level of mitochondria and its triggers the opening of the mitochondrial permeability transition pores (mPTP), contributed to induced mitochondrial membrane depolarization (Hausenloy et al. 2003). In addition, the prolonged opening of mPTP could lead to an increase in the matrix osmotic pressure, resulting in mitochondrial swelling and/or mitochondrial membrane rupture. This will cause cardiac dysfunction (Pongkan, Pintana, Jaiwongkam, et al., 2016). Regarding to sympathovagal imbalance, previous studies showed that metabolic syndrome also has been shown to have an inverse correlation with the vagal component of high-frequency component of the HRV (Licht et al., 2010). Interestingly, cardiac dysfunction and sympathovagal imbalance might related each other due to previous studies demonstrated that sympathovagal imbalance related to the decreasing of cardiac contractility and cardiac dysfunction (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; Liao, Carnethon, Evans, Cascio, & Heiss, 2002). Our results showed that the LF/HF ratio of HRV, which indicated cardiac sympathovagal imbalance, was higher as well as lower of the %FS and %EF, which indicated cardiac dysfunction, in HFD-fed rats. These results are consistent with our previous studies (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013; Pipatpiboon, Sripetchwandee, Chattipakorn, & Chattipakorn, 2015). Our data showed that probiotics reduced oxidative stress as well as inflammation, which could contribute to improved cardiac sympathovagal balance, and also improved cardiac performance (Malliani, 1999; Vinik & Ziegler, 2007). Thus, the improved LV function observed in the present study could be due to these mechanisms contributed by probiotics treatment.

Another factor that controls cardiac performance during the pathological state is cardiac mitochondrial function (M. G. Rosca & Hoppel, 2009; Wallace, 1999). The major role of mitochondria is to modulate oxidative stress generation and cellular energy. Several studies have shown that a greater amount of mitochondrial ROS levels could damage the integrity of mitochondrial membrane (Chen & Zweier, 2014). Our findings have shown that long-term HFD consumption impaired cardiac mitochondrial function indicated by increased mitochondrial ROS production, mitochondrial membrane depolarization, and mitochondrial swelling. These factors reduced mitochondrial ATP synthesis, and were associated with LV dysfunction (Boudina, 2009; Mariana G. Rosca & Hoppel, 2013) (Figure 4.1). Therefore, probiotics improved LV function could be via improving cardiac mitochondrial function in these obese-insulin resistant rats.

In addition, cardiac mitochondria also regulate cardiac apoptosis. Bax and Bcl-2 are keys of apoptosis markers in various conditions (Hasnan et al., 2010; Riva, Chevrier, Pasqual, Saks, & Rossi, 2001). Under the physiological condition, Bax resides in the cytosol and responds to stress stimulation by translocating onto the mitochondrial membrane. However, Bax activity is inhibited by Bcl-2 (Hasnan et al., 2010). In our study, apoptosis was not observed in our obese-insulin resistant rats. In contrast, other studies showed that HFD increased cardiac apoptosis in high fat diet-fed mice, and obese Sprague–Dawley rat models (Lin et al., 2016; W. Sun et al., 2014). This inconsistent finding could be due to the fact that the effect of HFD consumption on cardiac apoptosis is dependent on the amount of fat composition in the food, animal species, and diet consumption duration. In conclusion, our data indicated that probiotics improved insulin resistance, reduced dyslipidemia, attenuated cardiac sympathovagal imbalance, reduced blood pressure, and attenuated LV dysfunction. These beneficial effects through improving cardiac mitochondrial function, and reducing oxidative stress and inflammation that summarizing the effects of probiotics is shown in Figure 4.2. Moreover, the beneficial effects of probiotics on cardiac autonomic function as well as cardiac function were observed as early as 4 weeks after treatment.

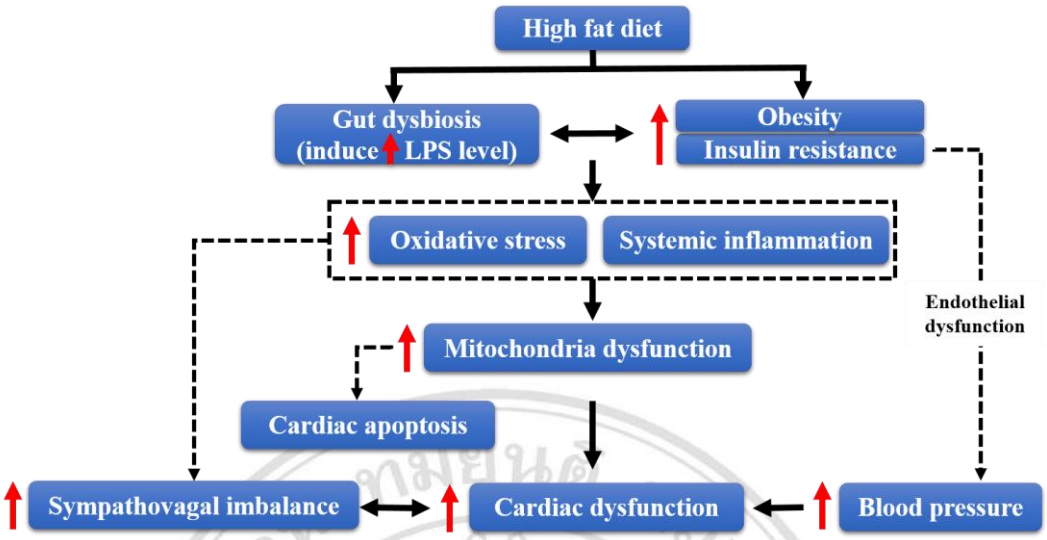

Figure 4.1 A diagram summarizing the effects of chronic HFD consumption on cardiac function in HFD-induced obese-insulin resistant rat model.

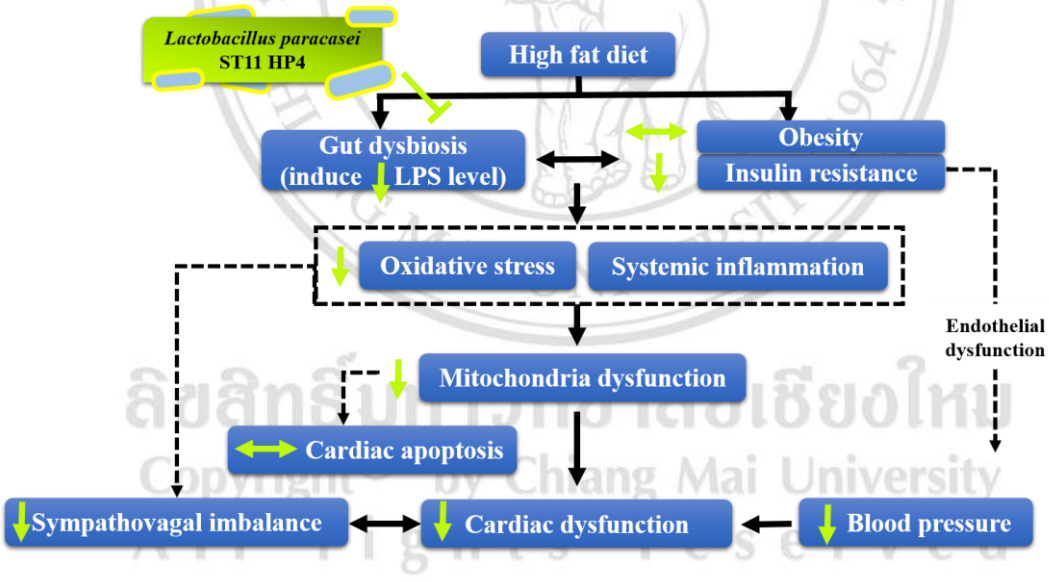

Figure 4.2 A diagram summarizing the effects of probiotic *Lactobacillus paracasei* ST11 (HP4) on cardiac function in HFD-induced obese-insulin resistant rat model.