CHAPTER IV

DISCUSSION

The morbidity and mortality in cardiac disease has been increased in pararell with a growing prevalence of obesity. According to the report of relationship between sympathetic nervous system activity and obesity, it has been suggested that an increase of sympathetic nervous system activity might be linked with the high incidence of cardiac death in obesity (Karason *et al*., 1999; Scherrer *et al*., 1994; Park *et al*., 2006). An elevated plasma lipid has been correlated with many cardiovascular diseases, and is also associated with an increase in sudden death (Jouven *et al.*, 2001; Wyne, 2003). Moreover, most of obese subjects have an increase plasma free fatty acid levels. Study of Paolisso *et al*. (2000) showed that elevated plasma free fatty acid levels could exaggerate cardiac SNS activity. In addition, previous studies have reported that an imbalance of sympathetic/para- sympathetic activity is responsible for the many cases of sudden death (Kleiger *et al*., 1987; Tsuji *et al.*, 1994; Ryan *et al*., 1976). Curcuminoids, an active principle of *Curcuma longa* Linn., has shown to exert diverse physiologic function including decrease blood triglyceride, cholesterol and phospholipids in diabetic animals (Babu and Srinivasan, 1997). Nevertheless, there are few reports about the effects of curcuminoids on plasma free fatty acid concentrations. Thus, the main objective in the present study was to investigate the cardiac autonomic status in obese rats induced by high-fat feeding. In addition, we also determined whether or not curcuminoids administration could decrease plasma

free fatty acid concentrations and if so this might be associated with changes in cardiac autonomic status in obesity.

Chronic high-fat feeding in rats has been well accepted as animal model of obesity equivalent to human abdominal obesity-insulin resistance syndrome. The amounts of fat in normal and high-fat diets were about 20 and 60% of total energy in diet, respectively. Accordingly, the caloric intakes of high-fat and normal diets were 4899.6 and 3040 kcal/kg, respectively. The results showed that rats fed with high-fat diet alone or in combination with curcuminoids administration developed central obesity as indicated by significant increase of body weight with substantially higher visceral fat mass (Kim *et al*., 2000; Ali and Crowther, 2005) after 12 wk dietary intervention. Data from analysis of dietary records showed the greater amount of daily energy intake in high-fat group compared with normal diet group. In addition, there were no difference in energy intake between high-fat content and those of curcuminoids treated rats. Thus, it was suggested that visceral obesity in rats induced by high-fat feeding resulted from an increase of both energy intake and fat intake. The surplus energy will be stored as fat in the adipose tissue (Ali and Crowther, 2005; Srinivasan *et al*., 2005).

An apparent finding in the present study was a significant correlation between plasma free fatty acid levels and visceral fat mass. It has been well recognized that visceral adipose tissue is less responsive to the antilipolytic action of insulin and more responsive to lipolytic action catecholamines than subcutaneous adipose tissue (Bjorntorp *et al*., 1969; Jenseen *et al*., 1989). An increase of lipolysis from visceral adipocyte resulted in an elevated plasma free fatty acid levels which further exerted multiple effects on glucose metabolism. The present study showed that high-fat

induced obese rats exhibited an obviously elevated plasma free fatty acid levels in parallel with fasting hyperglycemia. It has been evidence that elevated level of plasma free fatty acids, particularly under obese condition, plays an important role in the overproduction of glucose in the diabetic state (Bergman and Ader, 2000). Also, in type 2 diabetic patient, fasting hyperglycemia is apparently associated with an inappropriated increase hepatic glucose production (Massillon *et al.*, 1997; Roden *et al*., 2000). It is most likely that an increase of portal free fatty acids leads to an increased hepatic glucose output (Bergman and Ader, 2000). Free fatty acids potentially influence hepatic glucose metabolism by several other metabolisms. The ability of free fatty acids to stimulate gluconeogenesis has been reported both *in vitro* (Williamsson *et al.*, 1966; Gonzalez-Manchon *et al.*, 1989) and *in vivo* (Fanelli *et al.*, 1993; Chen *et al.*, 1999) studies. The proposed mechanisms included an enhanced fatty acid oxidation which provides ATP, and acetyl CoA which activates pyruvate carboxylase, the important key enzyme in the gluconeogenesis pathway (Williamsson *et al.*, 1966). Free fatty acids not only increase gluconeogenesis but also upregulate glucose-6-phosphate (G-6-P) system (Massillon *et al*, 1997). This causes a greater proportion of the resultant G-6-P to dephosphate and release into system. In addition, most studies have been reported that elevated plasma free fatty acid levels impair insulin-induced suppression of endogenous glucose production (i.e., cause hepatic insulin resistance) in normal control or in patients with NIDDM (Bevillacqua *et al*., 1990; Saloranta *et al*., 1993; Boden and Chen, 1995; Rigalleau *et al.*, 2001; Clore *et al.*, 1991). This effect is due to the inhibition action of free fatty acids on the suppression of glycogenolysis via insulin (Boden *et al*., 2003). Moreover, an additional effect of free fatty acids, the inhibition on insulin-stimulated glucose uptake

in skeletal muscle (Boden and Chen, 1995; Boden *et al.*, 1994; Roden *et al.*, 1996) is another possibility to be considered. The mechanism for this effect of free fatty acids is via insulin signaling cascade (Schmitz-Peiffer *et al.*, 1997), resulting in an inhibition of GLUT-4 translocation (Shulman, 2000) and a decreasing muscle glycogen synthase activity (Boden, 1997).

However, the present results showed that despite of fasting hyperglycemia, there was no increase of fasting plasma insulin levels in high-fat fed rats compared with normal diet fed rats. It is likely that the extent of an increase in hepatic glucose production stimulated by elevated plasma free fatty acid levels was antagonized by the free fatty acid-mediated stimulation of insulin secretion. Acute stimulation of insulin secretion by free fatty acids has been established (Pelkonen *et al.*, 1968; Malaisse *et al.*, 1968; Crespin *et al.*, 1973; Goberna *et al.*, 1974). As an elevated plasma free fatty acid levels which related with these high-fat fed rats had occurred for month, its effect on insulin response might have different effect from acute elevated plasma free fatty acid levels. A chronically elevated plasma free fatty acid levels in high-fat induced obese rats and in obese individuals who are genetically predisposed to develop NIDDM may eventually lose their ability to increase insulin secretion in response to elevated plasma free fatty acid levels (Boden, 1997).

An interesting finding in this study was the substantial triglyceride accumulation in liver of high-fat fed rats. Triglyceride in liver could originate from exogenous or endogenous free fatty acids or free fatty acids could be synthesized through de novo lipogenesis from carbohydrate (Tiikkainen *et al.*, 2002). This result, in agree with study of Samuel *et al*. (2004), demonstrated that hepatic triglyceride contents increased in high-fat fed rats without significant alteration of peripheral fat contents.

The present results of fasting plasma triglyceride levels showed a trend toward an increase in high-fat fed rats. It was most likely that an increased hepatic triglyceride contents might partly result from lipid intake overload due to high-fat feeding. However, study in mice with liver-specific over expression of lipoprotein lipase (Kim *et al.*, 2001) demonstrated that an increased portal free fatty acids delivery to the liver resulted in an increase of hepatic triglyceride contents. Although the exact mechanism was unclear, triglyceride accumulation in the liver in rats fed high-fat diets might also be a direct effect of elevated free fatty acids from visceral adipose tissue delivery to the liver.

It has been proposed that the triglyceride content in the target tissues of insulin and in the pancrease determines the level of both insulin resistance and insulin production (Koyama *et al*., 1997). There was evidence that hepatic triglyceride contents were closely correlated with hepatic insulin sensitivity (Samuel *et al*., 2004; Ryysy *et al*., 2000). In the study of fat-fed rats, Samuel *et al*. (2004) showed that an increase in liver triglyceride accumulation decreased insulin activation of glycogen synthase and increased gluconeogenesis, whereas preventing hepatic fat accumulation improved hepatic insulin responsiveness. Furthermore, associated with elevated plasma free fatty acid levels, an increased liver triglyceride content had been linked with an impairment of insulin's ability to suppress endogenous glucose production. This might be attributed in part to decrease insulin-stimulated tyrosin phosphorylation of IRS-1 and IRS-2 which in turn blocked the ability to activate glycogen synthase and diminished the ability of the liver to store as glycogen (Samuel *et al*., 2004, Kim *et al*., 2001). Together with these findings, it was suggested that in rats fed high-fat diets, hepatic triglyceride accumulation associated with elevated plasma free fatty

acid levels led to hepatic insulin resistance, ultimately resulting decreased insulin's action on the liver to suppress glucose production by elevated free fatty acids. This might be supported by data of fasting hyperglycemia in high-fat fed rats.

Skeletal muscle triglyceride contents, like hepatic triglyceride accumulation, have been found to inversely correlate with insulin sensitivity (Storlien *et al*., 1991). Clinical studies also suggested that muscle insulin resistance correlated with muscle triglyceride contents in Pima Indians (Pan *et al*., 1997) and type 2 diabetic subjects (Jacob *et al*., 1999). The lack of an effect of free fatty acids on triglyceride contents in muscle, both soleus and red gastrocnemius muscles, was intriguing. These results, in agreement with some studies, indicated that hepatic fat accumulation and hepatic insulin resistance occurred in the absence of the development of peripheral insulin resistance (Kim *et al.*, 2001; Kraegen *et al.*, 1991). Previous results regarding the effects of high-fat feeding on insulin sensitivity were mixed. Study with long-term (10 months) high-fat fed rats showed severe insulin resistance with mild fasting hyperglycemia (Chalkley *et al.*, 2002). Also, one study of prolonged high-fat feeding (32 weeks) produced insulin resistance with a wider variation in fasting plasma glucose levels (Han *et al*., 1997). In contrast, feeding with isocaloric moderate-fat diet in rats for 12 weeks found that the development of hepatic insulin resistance is relatively with minor changes in peripheral insulin resistance (Kim *et al*., 2003). In the present study, a slightly higher HOMA index in rats fed high-fat diet than those fed normal diet might reflect a mild defect in peripheral insulin sensitivity. It is possible that peripheral insulin resistance may develop if the period of high-fat feeding is extended beyond the 12 weeks. The present study, along with Kraegen *et al*. (1991) suggested that hypercaloric-fat feeding produced an increase of visceral

adiposity with initial hepatic insulin resistance while the development of peripheral insulin resistance required longer exposure of high-fat diets.

Curcuminoids, the phenolic yellowish pigment of turmeric, are known to have various beneficial effects on human health (Ammon and Wahl, 1991). With respect to lipid metabolism, previous study demonstrated that curcumin and its analog effectively reduced the levels plasma of cholesterol, triglyceride and free fatty acids in alcohol and polyunsaturated fatty acid-induced hyperlipidemia (Rukkumani *et al*., 2002; 2005). The present results showed that an increased plasma free fatty acid levels developed in high-fat induced obese rats were significantly countered by curcuminoids administration at all doses (30, 60 and 90 mg/kg BW). Although the exact mechanism by which curcuminoids lowers lipid levels were not investigated, study of Shishodia *et al*. (2005) had revealed that curcumin, the active component of curcuminoids, mediated its effects by modulation of several important molecular target, including transcription factors such as PPAR-γ. PPARs are ligand-activated transcription factors that regulate gene expression of a variety of lipid metabolizing protein (Schoonjans *et al*., 1996). Among PPAR isofroms as PPAR-α, PPAR-δ and PPAR-γ, PPAR-γ is widely found within white and brown tissue, but it is expressed in small levels in cardiac and skeletal muscles. In general, PPARs regulate gene involved in fatty acid uptake and storage, inflammatory and glucose homeostasis. Activation of PPAR-γ promotes adipocyte differentiation, free fatty acid uptake and storage in subcutaneous adipose rather visceral adipose tissue (Staels and Fruchart, 2005). Curcumin has also been reported to activate PPAR-γ in rat hepatic stellate cells activated by oxidative stress (Xu *et al*., 2003). Furthermore, study of Nishiyama *et al*. (2005) demonstrated that curcuminoids exhibited PPAR-γ ligand-binding

activity in GAL4-PPAR-γ chimeria assay and accelerated triglyceride accumulation in adipocytes. Together with these above findings, it was suggested that decrease of plasma free fatty acid levels with curcuminoids supplementation in high-fat fed rats might be mediated through activation of PPAR-γ as one of the mechanism.

The present study also showed that curcuminoids supplement at all doses (30, 60 and 90 mg/kg BW), particularly in high-fat induced obese rats, decreased hepatic triglyceride accumulation. In addition, the plasma triglyceride levels in high-fat fed rats with curcuminoids supplement at dose of 90 mg/kg BW was significantly lowered. Asai and Miyazawa (2001) demonstrated that curcuminoids treatment increased activity of hepatic Acyl-CoA oxidase (ACO) in high-fat fed rats, suggesting that curcuminoids affected fatty acid catabolizing in the liver. This assumption was based on the fact that ACO performs the first catalytic step enzyme of peroxisomal fatty acid β-oxidation, and its gene expression was regulated by the PPARs (Schoonjans *et al*., 1996). Although few study explained entirely how curcuminoids lowered the triglyceride levels, it was proposed that triglyceride lowering effect of curcuminoids might be through multiple induction of fatty acid catabolism and utilization pathway (Asai and Miyazawa, 2001). Furthermore, this effect might be mediated through activation of PPARs.

Besides lipid-lowering effects, curcuminoids have been reported to be effective in controlling blood glucose level and enzymes of glucose metabolism in alloxan-induced diabetic rats (Arun and Nalini, 2002). In agreement with the previous reports, this present study demonstrated that curcuminoids supplement at all doses (30, 60 and 90 mg/kg BW) suppressed an increase in fasting blood glucose in high-fat diet induced obese rats. Study of Nishiyama *et al*. (2005) in genetically type 2

diabetic mice indicated that one of the mechanisms responsible for antihyperglycemic effect of curcuminoids is mediated via PPAR-γ activation. A decreased hepatic triglyceride accumulation with curcuminoids treatment might improve hepatic insulin resistance which in turn led to a decrease of fasting hyperglycemia. This antihyperglycemic effect could be not a direction action of curcuminoids supplement but may resulted from a decreased influx of plasma free fatty acids into the liver. In addition to effect on lipid metabolism, activation of PPAR-γ has also been reported to increase the expression and translocation of the glucose transporters GLUT-1 and -4, thus increasing glucose uptake into liver and skeletal muscle cells and reducing plasma glucose levels (Kramer *et al*., 2001).

Electrocardiographic abnormality with prolongation of the QT interval has been found to be associated with both obesity (Blunberg, 1992; El-Gamal *et al*., 1995) and abdominal fat deposition (Peiris *et al*., 1991; Park and Swan, 1997). Importantly, it has been considered as the risk of potentially malignant arrhythmic and sudden death (Vlay *et al*., 1984; Moss, 1993). Although the mechanism for such association remains elusive, the significant relationship between QTc interval, waist to hip ratio, plasma free fatty acid levels, epinephrine and norepinephrine concentrations may suggest autonomic nervous system dysfunction as a possible mechanism (Corbi *et al*., 2002). This might be supported with the report of chronic sympathetic overstimulation and increased catecholamines in obesity (Laederach-Hofmann *et al*., 2000).

Cardiac autonomic activity, assessed by HRV measurement, indicates that reduced HRV, expression of exaggerated cardiac sympathetic nervous system, is associated with increased cardiovascular mortality (Kleiger *et al*., 1987; Ryan *et al*.,

1976; Tsuji *et al*., 1994). In the present study, there were significantly enhancing of LF component and LF/HF ratio accompanied with significant decrease of HF component. These findings indicated that high-fat feeding was associated with cardiac autonomic imbalance. Correspondingly, visceral fat mass had significantly positive correlation with LF component and LF/HF ratio whereas it had significantly negative correlation with HF component. The association between visceral obesity and impaired cardiac autonomic activity had been reported by several studies although the data were variety. Study in obese women, Gao *et al*. (1996) found that impaired cardiac autonomic activity varied depending on the regional body fat distribution. Significant increase of cardiac sympathetic and parasympathetic activity was pronounced in women with combined upper body obesity and visceral obesity. On the other hand, Peterson *et al*. (1988) reported an inverse correlation of sympathetic and parasympathetic activities with increasing percentage of body fat. The present study, in agreement with Zahorska-Markiewicz *et al.* (1993), demonstrated that cardiac autonomic disturbance with high-fat diet induced obesity was characterized by an overactivity of cardiac sympathetic nervous system with relative reduction of parasympathetic nervous system. Such disturbance might be an independent risk factor for sudden death and other cardiac mortality in obesity (Laederach-Hofmann *et al.*, 2000).

The present study showed that curcuminoids administration improved the cardiac autonomic disturbance induced by high-fat feeding. Although the mechanism responsible for the effect of high-fat diet on cardiac autonomic activity remains unresolved, the results of significane correlation between plasma free fatty acid levels and HRV measurement might indicate a strong relation between free fatty acids and

cardiac autonomic activity. Elevated plasma free fatty acid levels has shown to be associated with increase of ventricular premature complex in both nondiabetic (Paolisso *et al*., 1996a) and nonischemic diabetic patients (Paolisso *et al*., 1997). It has been also reported that elevated plasma free fatty acid levels stimulate cardiac sympathetic autonomic activity in healthy subjects (Paolisso *et al*., 2000) and in NIDDM patients (Manzella *et al*., 2001). Paolisso *et al*. (2000) suggested that stimulatory effect of free fatty acids on cardiac sympathetic activity was indirectly but was mediated in part, through an increase in plasma catecholamine concentrations which in turn stimulated myocardial β_1–receptors. However, results of negative correlation between plasma free fatty acid levels and HF component indicated that parasympathetic activity was inversely associated with elevated plasma free fatty acid levels. This was in agreement with spectral analysis findings demonstrating that elevated plasma free fatty acid levels suppressed myocardial vagal tone (Shaltout and Abdel-Rahman, 2005). This result suggested that decrease of parasympathetic counteraction to sympathetic activation resulted in the shift toward the sympathetic dominance (Sgoifo A. *et al*., 1999). At the cellular level, Shaltout and Abdel-Rahman (2005) showed that elevated plasma free fatty acid levels increased caveolar sequestration of cardiac muscarinic cholinergic receptors (M_2-AChR) causing M_2-mAChR inactivation and attenuation of parasympathetic control on the heart. Based on the above findings, the effect of curcuminoids supplement on cardiac autonomic activity was in parts related to a decrease plasma free fatty acid levels, which in turn led to an improved cardiac autonomic disturbance in high-fat fed rats.

Several studies have shown that curcuminoids is a potent antioxidant (Ammon and Wahl, 1991; Araújo and Leon, 2001; Maheshwari *et al*., 2006). An increased

plasma free fatty acid concentrations has been found to be pro-oxidant factor (Paolisso *et al*., 1996b). Manzella *et al*. (2002) demonstrated that increased post-prandial plasma free fatty acid concentrations was associated with an enhanced degree of oxidative stress and of LF/HF ratio which is an index of cardiac sympathovagal balance. It has also been reported that an impaired cardiac autonomic nervous system activity in type 2 diabetic is linked to the elevated oxidative stress (Dhalla *et al*., 1998). This evidence suggested that changing in cardiac sympathovagal balance was correlated with the degree of oxidative stress (Manzella *et al*., 2001). Although the oxidative stress had not been determined in the present study, the additional antioxidant effect of curcuminoids can not be excluded. Thus, it should be pointed out that an addition effect of curcuminoids might be partly due to decrease in oxidative stress which in turn may have beneficial effects on the cardiac autonomic nervous system activity. Whether or not the effect of curcuminoids is mediated via decrease in plasma free fatty acid levels or via antioxidant action needs to be addressed in future studies.

In conclusion, this investigation reported several novel findings. First, it demonstrated that 12 weeks of high-fat feeding in adult rats induced not only an increase in fat storage, enhanced plasma free fatty acids and fasting plasma glucose levels but also decreased HFnu component and increased LFnu component and LF/HF ratio indicating an impairment in cardiac sympathovagal balance. Second, elevated plasma free fatty acid levels appeared to relate to depressed HRV which indicated the cardiac sympathovagal disturbance. Third, curcuminoids supplement (30, 60 and 90 mg/kg BW/day) significantly reduced plasma free fatty acid and fasting plasma glucose levels in high-fat fed rats. Fourth, curcuminoids supplement

ameliorated the cardiac sympathovagal imbalance in high-fat fed rats. These findings suggested that curcuminoids supplement improved the cardiac autonomic imbalance in high-fat fed rats, partly due to its lipid-lowering effect.