DISCUSSION

Carbohydrate and fat are two major sources of energy used by skeletal muscle during exercise. A number of studies showed that the consumption of high carbohydrate diet were able to endure a high intensity work bout longer than those adapted to normal diet (Bergstrom, *et al.*, 1967; Wright, *et al.*, 1991; Williams, *et al.*, 1992). However, it has been claimed that the supply of acetyl CoA from glycolysis in high carbohydrate inhibits β-oxidation and minimizes the training shift toward lipid metabolism seen during endurance exercise performance (Brook and Fahey, 1984). The concept of high fat diet and endurance performance comes from the fact that animals and human adapt to high fat diet in much the same way as they did to exercise training (Conlee, *et al.*, 1990; Miller, *et al.*, 1984; Simi, *et al.*, 1991; Lambert, *et al.*, 1994; Okano, *et al.*, 1996; Goedecke, *et al.*, 1999). Previous studies on endurance performance compared between high fat and high carbohydrate diets yield inconsistent results. These may be due to the difference in period of dietary manipulation. In addition, the content of carbohydrate supply in the fat-rich diets is attributed to a conflicting picture when the effect of dietary fat on endurance performance has been investigated. Thus, the purpose of the present study was to investigate the effects of high carbohydrate and high fat diets on the endurance performance in exercise rats. All diets used in the current study were isocaloric. The fat content was zero and the carbohydrate content was 80% of total energy content in high carbohydrate (HC) diet. For high fat (HF) diet, the fat and

carbohydrate contents were 75% and 5% of total energy content in the diet, respectively. The rats were fed with high fat or high carbohydrate diet in concurrent with exercise training for 8 weeks.

The results demonstrated that there were no appreciable differences in growth rate between HC and HF groups although rats in HF group gained weight approximately 10% more than those in HC group. In accordance with previous studies (Scheuer and Tipton, 1977; Stone, 1977), the prominent adaptation as a result of exercise in both HC and HF groups were apparent decreases in resting heart rate and systolic blood pressure. These findings indicated that both HC and HF diet brought about similar effect on the fitness of trained rats. Although the mechanisms of these adaptations are still unclear, most frequently cited explanation involves alteration within the automatic nervous system (ANS) (Shephard, 1982). A number of investigations have stated that bradycardia following exercise training results from a predominant increase of vagal tone on the sinuatrial (SA) node (Scheuer and Tipton, 1977; Shephard, 1982; Clausen, 1977 ; Buttrick and Scheuer, 1994) as well as simultaneous decrease in sympathetic activity (Scheuer and Tipton, 1977; Shephard, 1982; Clausen, 1977). A reduction in heart rate leads to elevation in stroke volume which results in the greater capacity of oxygen delivery to tissues (Buttrick and Scheuer, 1994; Robergs and Roberts, 1996).

Data of blood borne substrates showed that high fat diet led to elevations of serum cholesterol and triglyceride concentrations in resting rats. Since exercise blunted hypercholesterolemia (Kronfeld, *et al.*, 1977;

Shephard, 1982; Wood, 1996, Brown and Cox, 1998) as well as induced a change in preferred substrate utilization in skeletal muscle toward a greater oxidation of lipids (Holloszy and Coyle, 1984; Phillips, *et al.*, 1996), it might be suggested that the absolute changes in both cholesterol and triglyceride concentrations in trained rats consuming high fat diets were much less than those without training. A change in blood triglyceride levels in rats consumed high carbohydrate diets was comparable to the previous report that isocaloric substitution of carbohydrates to fat whether the carbohydrates were simple sugars or starch, led to an elevation of blood triglyceride levels (Grundy, 1986). In addition, a slight increase of resting plasma glucose concentration in trained rats after 8 weeks of high carbohydrates feeding implied that excess carbohydrates were converted and stored as body fats (Arnheim, 1987; Whiney and Rolfes, 1996). High carbohydrate diet has shown to stimulate the production of hepatic very-low density lipoprotein (VLDL) (Thompson, *et al.*, 1984; Parks and Hellerstein, 2000). From the view point that VLDL plays role in triglyceride transports to the extrahepatic tissues, it is proposed that an increased blood triglyceride levels as a result of high carbohydrate intake is associated with the increase in VLDL production.

Previous studies have shown that increasing fat intake, thus decreasing carbohydrate intake, resulted in a decrease in exercise performance (Blair, *et al.*, 1989; Bush, *et al.*, 1988). In contrast, current results demonstrated that in addition to markedly improved endurance capacity, trained rats consuming high fat diets had substantially increased Vo_{2max} as compared to those fed with high carbohydrate diets.

The proposed mechanisms behind these beneficial effects might be attributed to an increase in oxidative metabolic adaptation to high fat diets. A number of investigations found that the activities of citrate synthase, a Kreb cycle enzyme marker, 3-hydroxyacetyl-CoA-dehydrogenase, an enzyme used in fatty acid oxidation, and carnitine-palmitoyl-transferase, an enzyme responsible for the transport of fatty acid through mitochondrial membrane, increased dramatically in skeletal muscle in response to chronic fat consumption (Jansson, *et al.*, 1981; Fisher, *et al.*, 1983). This enzymatic adaptation indicated that mitochondrial metabolism in muscle cells shifts toward delivery of energy by fat. It has also been established that mitochondrial adaptations to high fat diets are similar to those observed after chronic exercise training (Holloszy and Coyle, 1984). Thus, it could be inferred that high fat diets increased the aerobic capacity of the body and the effect was greatest when high fat diets was superimposed on training (Boyadjiev, 1996). Correspondingly, Tayler, *et al.* (1995) showed that 6 weeks of high fat diet supplementation in trained dogs induced a large increase in mitochondrial volume density measured in the vastus lateralis muscle as well as a large increase in maximal fat oxidation rate. In addition to mitochondrial adaptation, several studies reported that high fat diet consumption reduced plasma insulin and resulted in a decreased inhibition of lipolysis (Kraegen, *et al.*, 1989; Storlien, *et al.*, 1991; Rosholt, *et al.*, 1994, Matsuo, *et al.*, 1999). Since physical exercise which promotes energy consumption, up-regulates lipoprotein lipase (LPL) expression in skeletal muscle (Vaziri, *et al.*, 1999), as a consequence, plasma free fatty

acid (FFA) increases. Elevation of plasma FFA spares muscle and liver glycogen, protects against hypoglycemia and increases endurance for prolonged strenuous exercise (Hickson, *et al.*, 1977). It has been proposed that the inhibitory effects of fatty acids on glucose oxidation, via glucose-fatty acid cycle, are due to the inhibition of pyruvate dehydrogenase mediated by an increased acetyl CoA, the inhibition of phosphofructokinase mediated by increase in citrate concentration and the inhibition of hexokinase, mediated by glucose-6-phosphate (Randle, *et al.*, 1963; Pitsiladis, *et al.*, 1999). Thus, it might be inferred that apart from training effects on stimulation of fat utilization with a proportion decrease in carbohydrate utilization during exercise (Karlsson, *et al.*, 1974; Phillips, *et al.*, 1996; Holloszy and Kohrt, 1996), the metabolic adaptation of muscle to high fat diet consumption could play important roles in increased in endurance capacity.

The second purpose of the present study was to study whether or not prolonged high fat or high carbohydrate diet, followed by the different proportion of fat to carbohydrate content in diet affected exercise endurance. In addition, the appropriate fat and carbohydrate contents in diet necessary for optimal exercise performance in trained rats was elucidated. The dietary regimen used in the study was isocaloric while the proportion of fat to carbohydrate content increased in the order of high carbohydrate < formula I diet < formula II diet < formula III diet < high fat diet. The proportion of fat and carbohydrate contents in formula I diet was closer to the value in normal diets. Results from this study showed that endurance time was the most extended when trained rats

were fed high fat diets following by diets containing carbohydrate 59%E and fat 20%E (formula III diet).

As summarized in Table 5 and Figure 4, the endurance performance was apparently enhanced in high carbohydrate pre-fed rats received diets formula II and III (HCII and HCIII subgroups) than in high carbohydrate fed rats (HC subgroup). The explanation for the enhanced endurance capacity in HCII and HCIII subgroups might be associated with the greater fat oxidation capacity as indicated by the utilization rate of blood triglyceride (Table 10 and Figure 8). Recent study reported that increased concentrations of plasma epinephrine and norepinephrine were induced by exercise (McArdle, *et al.*, 1999). In addition, cathecholamine secretion was significantly higher with intake of high fat diet (Matsuo and Suzuki, 1999). Consequently, these hormones stimulate lipase activation and increase fat utilization (Brouns and Vusse, 1998; McArdle, *et al.*, 1999). This was partly supported by the magnitude increase of Vo_{2max} as shown in Figure 6. An increase in fat oxidation plays an important role in slowing glycogen depletion and results in increased endurance (Costill, *et al.*, 1977; Rinnies, *et al.*, 1976; Brouns and Vusses, 1988). The mechanism by which raising fat utilization slows the depletion of carbohydrate stores probably include an inhibition of anaerobic glycolysis (Hickson, *et al.*, 1977; Holloszy and Khort, 1996). The high utilization rate of blood triglyceride in HCIII subgroup confirmed the reports that an amount of fat oxidation was associated with the amount of dietary fat consumed (Mollica, *et al.*, 1999; Smith, *et al.*, 2000). Accordingly, the apparent decrease in blood glucose utilization rate was

seen in rats in the HCIII subgroup. Thus, it is reasonable to propose that HC pre-fed rats consumed formula III diet has endurance performance better than other HC's subgroups.

In contrast, a high utilization rate of blood glucose in parallel with the production rate of blood lactate in HC subgroup implied that energy production during exercise was primarily from glycogenolysis. As the glycogen store in the body is limited, during prolonged exercise the depletion of hepatic glycogen stored leads to development of hypoglycemia, resulting in dysfunction of central nervous system (Pruett, 1970; Shephard and Astrand, 1992; Holloszy and Khort, 1996). In addition, when muscles contract vigorously for long periods, the circulatory system begins to lose ground in delivery of oxygen. In these conditions most the pyruvic acid produced in the breakdown of glucose is converted to lactic acid (Robergs and Roberts, 1996). High levels of lactate and dysfunction of central nervous system induce rapid exhaustion (Pruett, 1970; Shephard and Astrand, 1992; Holloszy and Khort, 1996).

It was noteworthy to note that endurance performance in rats in HFI, HFII and HFIII subgroups were superior to those of HF and HC's subgroups. This was consistent with the previous report that exercise endurance time was optimized in trained rats received a carbohydrate load after adaptation to a high fat diet (Lapachet, *et al.*, 1996). Data of blood triglyceride utilization rate from rats in HFI, HFII and HFIII subgroups implied that the effects of high fat diet persisted throughout the 12 days of dietary manipulation with increasing carbohydrate contents. In addition, markedly increased magnitudes of Vo_{2max} were seen

in these subgroups. Based on these findings, it might be inferred that the enhanced aerobic metabolism in high fat pre-fed rats and then switched to diet with increasing carbohydrate contents resulted from a combined effect of increased Vo_{2max} and increased utilization rate of triglyceride.

The endurance performance in HF's subgroups increased in the order of HF<HFI<HFII<HFIII. These findings indicated the essence of dietary carbohydrate availability for exercise endurance performance. It is generally accepted that exercise endurance is directly related to the glycogen concentration in muscle prior to beginning work (Conlee, *et al.*, 1990). The study of Saitoh, *et al.* (1996) demonstrated that short-term supplementation of high carbohydrate diets to high fat pre-fed rats increased glycogen stores of soleus and plantaris muscles and liver while the muscle triglyceride stores were still high. Thus, the explanation for the extended endurance time in rats pre-fed high fat and thus switched to diets with increasing proportion of fat to carbohydrate contents (HFI, HFII and HFIII subgroups) might attributed to the elevated muscle glycogen. To prove this explanation, determination of muscle glycogen must be further investigated.

In the process of aerobic energy metabolism (Figure 12), plasma fatty acid is oxidized via the β-oxidation pathway giving rise to a product, acetyl CoA. By the action of citrate synthase, acetyl CoA is condensed with oxaloacetate (OAA) forming citrate. Citrate is then oxidized in the tricarboxylic acid cycle (TCA) cycle producing two moles of CO_2 per mole of acetyl CoA and 1 mole of OAA is recycled. Glucose from glycogen store in muscle cell is also a source of acetyl CoA. Within

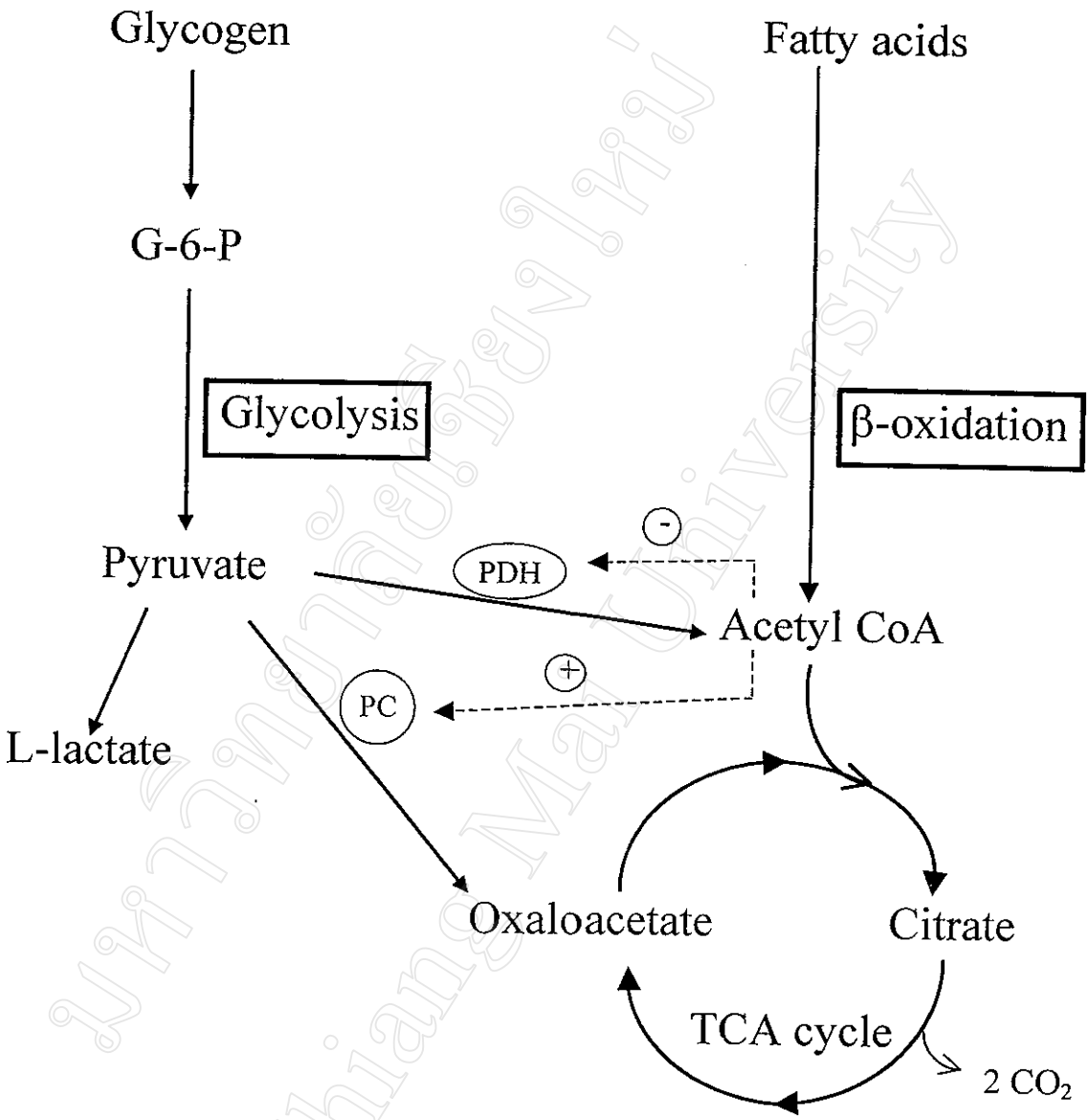

Figure 10 Relationship of carbohydrate and fat utilizations for energy during exercise

the cytoplasm, glucose is oxidized via glycolysis to pyruvate and the product is transported into mitochondria. In mitochondria, pyruvate is metabolized either to acetyl CoA by pyruvate dehydrogenase or to OAA by pyruvate carboxylase. Acetyl CoA is an allosteric effector of the two enzymes. As acetyl CoA concentration is increased by β-oxidation of fatty acids, pyruvate dehydrogenase is inhibited and pyruvate carboxylase is activated. Anaplerotic reaction of pyruvate carboxylase serves to produce stoichiometric concentration of OAA so that acetyl CoA can be completely oxidized in TCA cycle (Champe and Harvey, 1994; Murrey, *et al.*, 1993). In addition, it has been demonstrated that the total concentration of tricarboxylic acid intermediates increase several fold during exercise (Spencer, *et al.*, 1991; Gibala, *et al.*, 1998). Based on these reasons it is possible to propose that the significant function of muscle glycogen in endurance exercise is for anaplerotic supply of OAA. The lowest endurance performance of rats in the HF subgroup suggests to the low efficiency of acetyl CoA oxidation by TCA cycle due to insufficient supply of glucose.

According to Lapachet, *et al.* (1996), dietary carbohydrate availability and/or initial glycogen concentration was an effective regulator of glycogen utilization rate. Although muscle glycogen was not determined in the present study, the value of blood glucose utilization rate suggested that the rats in HFIII subgroup oxidized plasma glucose with slower rate than in HFI and HFII subgroups. In addition to muscle glycogen, it has been reported that muscle triglyceride are significantly depleted during relatively high intensity, submaximal exercise and

skeletal muscle endurance is diminished when the availability of endogenous triglyceride is reduced (Conlee, *et al.*, 1990; Brouns and Vusse, 1998). It has also reported that ingestion of high fat diet increases muscle triglyceride concentrations (Starling, *et al.*, 1997). Whether or not there are different in the muscle triglyceride stores among HF pre-fed rats consuming diet with different proportion of fat to carbohydrate contents must be further investigated. However the utilization rates of blood triglyceride were similar among HFI, HFII and HFIII subgroups. The current results suggested that appropriate proportion of fat to carbohydrate contents (59%E CHO, 20%E fat) in formula III diet was such a potent stimulator for metabolic adaptation that its effects were manifest in rats which had already obtained high fat diet together with exercise induced metabolic adaptation. The appropriate proportion of fat to carbohydrate contents that fits for exercise program should be further studied.

In conclusion, the present study has shown that consumption of a high fat diet during prolonged exercise training leads to improved exercise performance in rats as illustrated by increases in endurance time and Vo_{2max}. It seems probably that interrelated metabolic adaptations to endurance training and high fat diets are largely responsible for the increased endurance in the trained stated. After a training period and consumption of a high carbohydrate diet, supplementation of diets with decreasing carbohydrate and increasing fat contents for 12 days leads to an alteration in substrate utilization during exercise. That is the utilization rate of blood glucose is decreased whereas that of blood

triglyceride is increased. An apparent extension of endurance time in HC pre-fed rats consuming formula III diet is primarily due to an increased utilization rate of blood triglyceride. Accordingly, a decreased utilization rate of blood glucose results in a decreased production rate of lactate. In HF pre-fed rats, supplementation of diets with different proportion of fat to carbohydrate contents leads to an increased aerobic metabolism and endurance time which appears to be greater than in HC pre-fed rats. The most improved endurance performance was seen in HF pre-fed rats consuming formula III diets. There were similar increases in blood triglyceride utilization rate in all HF's subgroups. This indicates that the high fat diet's effects persisted throughout the dietary manipulation. However, the utilization rate of blood glucose in HF pre-fed rats consumed formula III diets was apparently lower than those consumed formula I and II diets, Thus, it is suggested that the formula III diet containing 59%E carbohydrate and 20%E fat gives rise to optimum efficiency for endurance exercise than diets containing only carbohydrate or fat. The more effective increase in endurance performance is manifested after adaptation to a high fat diet than to a high carbohydrate diet.