DISCUSSION

During exercise, energy for biological and chemical functions is obtained from the hydrolysis of ATP. In muscle, the amount of ATP is limited, ATP must be regenerated from ADP and phosphate through the oxidation of fuels. Fat and carbohydrate are the primary fuel sources for aerobic ATP production during exercise (Maughan *et al.*, 1997 and McArdle *et al.*, 1999) and the pathway that metabolize these fuels must be heavily up- regulated to meet the increased demand for energy. In comparison with fat, body carbohydrate stores are not large and can be exhausted following 1-2 hours of intense exercise (Maughan *et al.*, 1997). Thus, a shift to an increased fat oxidation and concomitant decrease of carbohydrate oxidation during exercise is desirable. In addition to exercise training, prolonged high fat diets have been reported to induce enzymatic and metabolic changes which favor fat oxidation and are attained considerable interest as a potential tool to improve endurance performance. However, the relationship between fat diet with an increased fat deposition has been warned to use high fat diet as an ergogenic aid (Miller *et al.*, 1984 and Oscai *et al.*, 1984). In addition, conflicting results of metabolic adaptation to fat diet due to differences in dietary fat content have been reported. Previous study showed that supplementation of diet containing moderate fat content (20.14% E) to trained rat prefed with either high fat (74.85% E) or high carbohydrate (79.85% E) diets led to increase of endurance performance as well as VO_{2max} (Veerapun, *et al.*, 2002). In the present study, the effects of moderate fat diet on metabolic adaptation in aerobically trained rats were investigated. In addition, the effect of moderate fat diet on endurance performance was determined.

Growth and Lipid profile

It has been widely accepted that the obesity is related with increase in fat intake and decrease in physical activity. Correspondingly, Oscai *et al.* (1984) demonstrated that a diet of 40% fat by energy value led to obesity in sedentary rats in the absence of hyperphagia. In the present study, data from analysis of dietary records revealed similar energy intake over 10 weeks in all trained rats regardless of diet composition. However, there were no appreciable differences in weight gain between the experimental groups. This finding indicated that prolonged

consumption of increasing dietary fat had no adverse impact on normal growth rate and the exercise training kept the animals from becoming obesity despite of increase in fat intake.

High fat diet is claimed to have potential negative health consequences, including increased coronary heart disease risk. showed that there were no difference in the data of lipid profiles between dietary groups except for triacylglycerol (Table 3). High fat diet led to a substantial increase of plasma triacylglycerol level compared with MF and NF groups. Because the regular endurance exercise has consistently been shown to decrease total blood cholesterol, triacylglycerol and LDL concentrations and to increase HDL concentration (Maughan *et al.*, 1997), the increase of plasma triacylglycerol concentration in trained rats consuming HF diet might be much less than those without training. Thus, during period of endurance training when energy requirements are high, increasing fat intake dose not result in adverse changes to the plasma lipoprotein profiles (Holloszy and Coyle, 1984).

Energy Metabolism

The results of resting triacylglycerol storage, in agreement with the previous studies, demonstrated that after consumption of high fat diet intramuscular triacylglycerol concentration was elevated (Straczkowski *et al.*, 2001; Starling *et al.*, 1997 and Kiens *et al.*, 1987). The present study also showed that in addition to intramuscular triacylglycerol storage, liver triacylglycerol concentration was apparently increased in trained after 10 weeks of HF and MF diets. According to the close relationship of the adipose tissue triacylglycerol storage and the higher concentration of plasma triacylglycerol (Frayn *et al.*, 1995), it has been proposed that the increased muscle triacylglycerol concentration was related to the activity of enzyme LPL. Several investigators have demonstrated an increased activity of skeletal muscle LPL after several days (Jacobs *et al.*, 1982) or weeks (Kiens *et al.*, 1987 and Thompson *et al.*, 1984) of a high fat diet. An increased LPL activity indicates a higher capacity for uptake of fatty acids from the circulating serum triacylglycerols into the muscle cell in association with greater capacity for triacylglycerol storage in the muscle (Kiens *et al.*, 1987). In addition, skeletal LPL activity has been shown to be increased after training (Oscai *et al.*, 1990 and Turcotte *et al.*, 1995). Thus, 10 weeks exercise training together with supplementation of diet with increasing fat content have synergistic effect with regard to increasing muscle triacylglycerol storage in this study. In addition, the

triacylglycerol storage in muscle as well as liver was enhanced as dietary fat content increased. The amount of tissue triacylglycerol was higher in HF compared with MF groups implied. It might be suggested that the apparent increase of plasma triacylglycerol concentration together with an increased activity of lipoprotein lipase subsequent to high fat diet resulted in the greater capacity for tissue triacylglycerol storage. In contrast to triacylglycerol storage, the glycogen concentrations in both muscles and liver were proportionally decreased with increasing dietary fat content. The proposed mechanism behinds the decreased glycogen storage after fat diet has been linked to the change of hormonal activity. Study of Kim *et al.* (2000) found that the insulin-stimulated whole body glycogen synthesis was impaired after 3 weeks of high fat feeding. This impairment was accompanied by reduced glycogen synthase (GS) activity and reduced accumulation of glycogen in skeletal muscle (Oakes *et al.*, 1997). Although the precise mechanism needs to be further determined, it was suggested that the increased storage of muscle and liver triacylglycerol in either trained rat received MF or HF diet might be apparently substitute as fuel source in associated with the decreased glycogen deposition.

The present study demonstrated a substantial increase of resting plasma glycerol level in MF and HF groups which suggested an increase in the rate of whole body lipolysis (Willian *et al.*, 2000). As lipolysis mostly occurring in adipose tissue provides the vast majority of circulating lipid fuel in the post-absorptive state (Jensen, 2003), this finding indicated that metabolic adaptation to increase of dietary fat might be related with adipose tissue lipolysis. Such adaptation appeared to compensate for lower carbohydrate availability (Willian *et al.*, 2000). It has been proposed that an enhanced lipolysis during increasing fat intake is associated with insulin response. Insulin is known to be an important inhibitor of lipolysis (Cambell *et al.*, 1992). A lower insulinemia at rest and during exercise has been found during high fat diet (Maughan *et al.*, 1997). Thus, a lower insulin level might lead to less inhibition of lipolysis, resulting in increased plasma glycerol level and higher circulating FFA. In the rat, glycerol has been reported to be a highly gluconeogenic substrate (Maughan *et al.*, 1997). The plasma glycerol is mostly converted to glycerol-3-phosphate and glucose in the liver (Willian *et al.*, 2000). It was likely that during fat diet with a low carbohydrate availability glycerol from adipose tissue lipolysis was directed toward gluoneogenesis. This was reflected by the similar resting plasma glucose concentration in all groups regardless of diet composition,.

One of the most prominent findings provided by the present study was an increased utilization of intramuscular fat during initial stage of endurance exercise. This was indicated by the substantial decrease of triacylglycerol concentrations in both vastus lateralis and soleus muscle as well as liver after exercise test in MF and HF groups. Although the substrate utilization rate per se was not measured periodically throughout the exercise time period in the present study, the calculated average rate of substrate utilization can be used as an approximation of substrate utilization rate throughout the entire exercise period (Lapachet *et al.*, 1996). Data summarized in Table 6 shows a strong relationship between the dietary fat content and fat oxidation. The total triacylglycerol (muscle and liver) utilization rate was in order of HF > MF > NF. These findings, in agreement with previous studies, demonstrated that adaptation to fat diet led to change of substrate utilization toward higher fat oxidation during exercise (Helge *et al.*, 1996; Jansson and Kaijser, 1982 and Phinney *et al.*, 1983). Further evidence to support the substrate utilization shift to fat oxidation was the activities of two key mitochondrial enzymes, CS, a key enzyme in TCA cycle and 3-HAD, a key enzyme in β-oxidation. The current study found, in harmony with other reports (Fisher *et al.*, 1983; Jansson, 1980 and Miller *et al.*, 1984), that activity of these two enzymes increased dramatically in soleus and vastus lateralis muscles with HF diet. There were also increased activities of both CS and 3-HAD in muscles obtained from MF group while these changes were apparent in soleus muscle. These findings implied that an increase of triacylglycerol utilization in MF group actually resulted from the increased aerobic capacity of muscle cell. In addition, the magnitude of increased activities of both enzymes was greater in HF than in MF group which suggested that the enzymatic adaptation to fat diet to some extent was dependent on the amount of dietary fat.

It has been established that transport of fatty acyl CoA from cytoplasm to mitochondria matrix is the main-rate-limiting step in the utilization of fatty acids for energy production in muscle. This transport process is catalyzed by the enzyme carnitine palmitoeyl transferase I (CPTI). This enzyme is reversibly inhibited by malonyl CoA (Maughan *et al.*, 1997). Study in rodent skeletal muscle demonstrated the decreased malonyl CoA level during muscle contraction when energy production from fat oxidation was increasing (Winder *et al.*, 1989). In addition, a significant increase of CPTI activity has been found in muscles of trained rats fed high fat diet (78% E). Accordingly, an alternative mechanism which might be attributed to the increase of total

triacylglycerol utilization was linked to the change of enzyme CPTI activity in response to fat diet feeding. The total rate of triacylglycerol utilization was lower in MF group compared with HF group. This was in accordance with the hypothesis that carbohydrate availability rather than that of fat controls the rate of fat oxidation (Saha *et al.*, 1995 and Sidossis *et al,* 1996). They demonstrated that an increased carbohydrate availability resulted in increase acetyl CoA and malonyl CoA concentration in muscle and liver. Thus, the increased carbohydrate amount in MF diet resulted in some extent inhibition of CPTI which reduced uptake of fatty acids into the mitochondria and thus lowered triacylglycerol-derived fatty acid oxidation.

During exercise, fat for oxidation in muscle is recruited from plasma fatty acid, plasma triacylglycerol and muscle triacylglycerol (Holloszy *et al.*, 1998). Study of Turcotte (1999) in rats found that the increase in fat oxidation on high fat diet was accounted for by an increase in triacylglycerol-derived fatty acid oxidation. In addition to IMTG oxidation, another source for triacylglycerol-derived fatty acid oxidation could be the plasma triacylglycerol presenting in the form of lipoprotein. Previous results regarding to the contribution of VLDL triacylglycrol oxidation to total fat oxidation during exercise were not clear (Kiens *et al.*, 1987 and Havel *et al.*, 1976). In the present study, the contribution of plasma triacylglycerol to the triacylglycrol-derived fatty acid oxidation during high fat diet was evident by the significant decrease of plasma triacylglycerol level after exercise test. Furthermore, an increased LPL activity on a high fat diet facilitated the release of fatty acids from lipoprotein triacylglycerol which might be directly oxidized in the muscle especially during exercise. On the other hand, there was a trend of increased plasma triacylglycerol concentration after exercise test in MF and NF groups. The changes of plasma triaclyglycerol concentrations in MF and NF groups were associated with the mobilization of liver triacylglycerol in response to exercise stimulus. Although the contribution of plasma triacylglycerol to fat oxidation during exercise test in MF group can not be excluded, further studies are needed to examine whether its contribution is responsible for the increased fat oxidation with moderate fat diet.

It is remarkable that total glycogen utilization during exercise test was decreased in trained rat consumed diet with increasing fat content (Table 5). This indicated that concomitant with increasing triacylglycerol utilization during exercise after fat adaptation was a reduced reliance on glycogen. Alternatively, the proportional increase in fat oxidation in MF and HF

group is compensated by the decreased utilization of carbohydrate during exercise. The explanation for this reciprocal relationship between fat and carbohydrate metabolism was based on the glucose-fatty acid cycle theory (Randle *et al.*, 1963). In skeletal muscle, fatty acid is oxidized via the β–oxidation pathway giving rise to a product acetyl CoA. By the action of CS, acetyl-CoA is condensed with oxaloacetate (OAA) forming citrate. Citrate is then oxidized in the TCA cycle producing two moles of CO_2 and one mole of OAA. In addition to fatty acid, glucose from glycogen store in muscle cell is also a source of acetyl-CoA. Within the cytoplasm, glucose is oxidized via glycolysis to pyruvate and the product is metabolized to acetyl-CoA by mitochondrial pyruvate dehydrogenase (PDH) (Murrey *et al.*, 1993). Study of Jeukendrup (2002) found that an increase of fat oxidation resulted in the accumulation of acetyl-CoA, citrate and G-6-P contents. Thus, it was suggested that the inhibitory effects of increased fat oxidation on carbohydrate utilization was due to the inhibition of PDH mediated by an increased acetyl CoA, the inhibition of phosphofructokinase (PFK) mediated by increase in citrate concentration and the inhibition of hexokinase (HK) mediated by G-6-P (Randle *et al.*, 1963; Pitsiladis *et al.*, 1999 and Spiet and Watt 2003). Similarly, the rate of liver glycogen utilization was low in trained rats consumed diets with increasing fat content. The low hepatic glycogenolysis during exercise in HF group might be a rheologic mechanism to compensate for a reduced availability of carbohydrate in liver. However, Rennie *et al.* (1976) found an accumulation of citrate concentration in the liver of the exercised animals with increased FFA. Although the mechanism for decreased hepatic glycogenolysis in MF and HF groups is currently not clear, it might be related with down-regulation of PDH and PFK activities resulting from an increase of citrate. The current result demonstrated that the increased glycogen breakdown was proportional to the amount of tissue glycogen storage. The total glycogen utilization rate was higher in MF group compared with HF group whereas it was highest in NF group. It might be suggested that an increased amount of carbohydrate in MF diet attenuated the decreased in liver and muscle glycogen storage and possibly utilization during exercise that was observed with HF diet.

At exhaustion, the triacylglycerol concentration in both vastus lateralis and soleus muscles substantially decreased in all dietary groups. The decrease of intramuscular triacylglycerol was greater and the utilization rate of muscle triacylglycerol was higher in the MF and HF groups as compared with the NF group. These results implied that during endurance

exercise rats consumed diets with increasing fat contents relied more heavily on muscle triacylglycerol stores for energy. In addition to intramuscular triacylglycerol, a significant decrease of plasma triacylglycerol levels in both MF and HF groups after endurance exercise suggested the increased contribution of triacylglycerol-derived fatty acid oxidation in trained rats received increasing fat intake. By contrast, the proportion of tissue glycogen utilization during endurance exercise as shown in Table 7 was in order of HF < MF < NF. The decrease of carbohydrate oxidation subsequent to HF or MF diet was accompanied by the apparent decrease of plasma lactate concentration. These findings, in accordance with the results observed in exercise test, indicated that the increase of dietary fat did have an impact on the metabolic adaptations. The differential proportion of fat adaptation was clearly dependent on the dietary fat content.

The dietary fat induced changes toward higher fat oxidation during exercise and concomitant glycogen sparing was considered to favor endurance performance. Previous studies regardings the effects of dietary fat on endurance performance provided a conflicting pictiure. Helge *et al* (1998) did not found enhanced endurance in trained or untrained rats consumed fat diets (65%E) despite of a shift toward increased fat utilization during exercise. The positive effect of fat diet on endurance performance in rats, however, was demonstrated when dietary fat content was ~ 75-85%E (Lapachet *et al.*, 1996; Miller *et al.*, 1984 and Simi *et al.*, 1991). The current study clearly showed that the endurance performance was enhanced in trained rats received either MF (20.14% E) or HF (74.85% E) diet (Figure 1). The most marked improvement in endurance performance was seen in the HF group whereas the increase of endurance performance in MF group was significantly different from the NF group. From the present data it could be speculated that the degree of enhanced endurance capacity in trained rats consumed diets with increasing fat content was related to the proportion of metabolic adaptations.

The cause of fatigue in the current study is not entirely clear. There is considerable evidence that the development of exhaustion is associated with hypoglycemia and/or depletion of muscle glycogen during prolonged strenuous exercise (Sherman, 1995 and Maughan *et al.*, 1997). Liver glycogen values fell in all dietary groups after endurance exercise but to lower absolute values in the rats received fat diets (MF and HF groups). Even so, all dietary groups did not experience hypoglycemia. The intramuscular glycogen significantly decreased in all groups at exhaustion. Although it has been reported that during exercise a marked reduction in performance

was observed when muscle glycogen approached low values (Karlsson and Saltin, 1971), it did not appear that muscle glycogen depletion played a significant role as a cause of fatigue. Lactate accumulation might have been the cause of fatigue except that this explanation did not seem to apply to all groups. At exhaustion, the lactate concentrations in the MF and HF groups were similar to the values obtained after exercise test. It was possible that the increased keto-acid resulting from fat utilization in MF and HF groups together with the presence of lactate could give rise to low pH, consequently leading to fatigue. Alteration in intracellular handling Ca^{2+} linked to activity of the sarcoplasmic reticulum Ca^{2+}dependent ATPase (SR Ca^{2+}-ATPase) might be a contributing factor. This was based on the finding that the maximal force developed by muscle declined and its time to peak tension and half-relaxation time were prolonged (Bigland-Ritchite *et al.*, 1983 and Fitts *et al.*, 1976). There has also been reported depression of sarcoplasmic reticulum function in rat heart and skeletal muscles after prolonged exhaustive running or swimming (Fitts *et al.*, 1982). In addition, study in trained rats fed diets with increasing fat content found that the activity of SR Ca^{2+}–ATPase apparently reduced after fatigue stimulation (Kaoien, 2003).

In conclusion, the data in this study showed that chronic consumption of diet containing increase of fat content during exercise training has been shown to be associated with metabolic adaptations that enhanced muscle triacylglycerol storage and the activities of enzymes involved in aerobic and fat metabolism. These fat adaptations resulted in an increased utilization of fat as an energy source during early stage of exercise as well as prolonged submaximal exercise. The triacylglycerol-derived fatty acid oxidation (plasma and/or muscle triacylglycerol) played an important role in the increase of fat oxidation. The proportion of increased fat oxidation correlated with the dietary fat content. The average utilization rate of tissue triacylglycerol during exercise test as well as endurance exercise was in the order of HF>MF>NF. On the contrary, the decrease of tissue glycogen utilization rate in trained rats consuming diets with increasing fat contents appeared to conpensate for a low carbohydrate availability. These glycogen sparing effects of fat adaptation resulted in improved endurance performance. The endurance capacity apparently enhanced as the dietary fat content increased.