LITERATURE REVIEW

Energy metabolism

The human body demands a continuous supply of chemical energy through energy metabolism to perform the biological work. An energy metabolism consists of a series of chemical reactions that break down foodstuffs and thereby produce energy. In general, the energy from food combustion becomes harvested and funneled through the energy rich-nucleotide compound adenosine triphosphate (ATP). Because phosphoric anhydride bonds of ATP "trap" a large of the original food molecule's potential energy, ATP is also referred as a high-energy phosphate compound. The hydrolysis of ATP which cleavages ATP's outermost phosphate bond, leads to release a phosphate anion (Pi) and liberate approximately 7.3 kcal of free energy per mole of ATP hydrolyzed to ADP. In the cell, ATP is by far the most important among other high energy phosphate compounds. The energy liberated during ATP hydrolysis powers all forms of biological work. Thus, ATP constitutes the cell's "energy currency", the cells energy-requiring process. ATP also serves as the ideal energy transfer agent, it readily transfers this energy to other compounds to raise them to a higher activation level (William *et al.*, 2000).

In skeletal muscle, energy from the hydrolysis of ATP by myosin ATPase activates specific sites on the force-developing elements, which allows a muscle's attempt to shorten. Also ATP is required for re-uptake of calcium ions by the sarcoplasmic reticulum, which consequently leads to muscle relaxation. The store of ATP in human skeleton is relatively small, and therefore, it must be continually resynthesized at its rate of use. This synthesis of ATP requires the potential energy which serves to phosphorylate ADP to re-from ATP. The body maintains a continuous ATP supply through different metabolic pathways. The cytosol contains the pathways for ATP production from the anaerobic breakdown of phosphocreatine (PCr), glucose, glycerol, and the carbon skeletons deaminated amino acids, whereas reactions that harness cellular energy to generate ATP aerobically reside within the mitochondria. Most energy for phosphorylation generally derives from the oxidation of dietary macronutrient (Maughan *et al.*, 1997). In essence, cellular oxidation-reduction constitutes the biochemical mechanism that underlies energy metabolism. The fuel sources that supply substrates for oxidation and subsequent formation of ATP consist primarily of 1) triacylglycerol and glycogen molecules stored within muscle cells, 2)

glucose derived from liver glycogen, 3) free fatty acid derived from triacylglycerol in adipocytes and 4) intramuscular and liver derived carbon skeletons of amino acids (Murrey *et al.*, 1993 and Mcardle *et al.*, 1999).

A number of factors are known to influence the selection of fuel for exercise, and there can be significant interactions between several of them. The four main factors include power, speed, strength and endurance and the most important factor influencing the selection of fuel for muscular work is the intensity of exercise (Romijn *et al.*, 1993 and Turcolte, 1999). During low intensity exercise (< 55% VO_{2max}) the majority of energy is derived from fatty acids mobilized from adipose tissue. As the intensity of exercise is increased, proportionally more of the energy is derived from carbohydrate (blood glucose and glycogen) and the contribution of intramuscular triacylglycerols (IMTG) is also higher. During moderate intensity exercise (55-69% VO_{2max}) carbohydrate (blood glucose and glycogen) and fat (plasma free fatty acids and muscle triacylglycerol) contribute equally to the total energy expenditure. If exercise is prolonged (>30 min), muscle substrates (glycogen and triacylglycerol) contribute less to total energy and blood glucose and fatty acids become more important (Maughan *et al.*, 1997). During high intensity exercise (70-89 % VO_{2max}), glycogen stored within muscle becomes the primary source of energy (William *et al.*, 2000). There has been long known that physical endurance training results in marked changes in musclular metabolism that influence selection of fuel for exercise. A well-accepted adaptation to endurance training is an increase in energy derived from fat (Martin, 1996).

Fat Metabolism

The two primary substrates available for use by working skeletal muscle during exercise are carbohydrate and fat (Brooks *et al.*, 1994; Coggan *et al.*, 1990; Green *et al.*, 1991 and Romijn *et al.*, 1993). Compared to carbohydrate, fat contains more than twice the energy per gram as carbohydrates. In addition, fat stores in the body are abundant and in terms of the amount available, not limiting to the performance of prolonged exercise. It is well established that fat represents a major fuel for skeletal muscle during prolonged exercise. The principal storage form of fats in the body is triacylglycerol, most of which is located in adipose tissue. Triacylglycerol stores are also found in the liver and muscle and as lipoproteins in blood (Maughan *et al.*, 1997).

Fat metabolism begins with the breakdown of triacylglycerol into glycerol and water-insoluble fatty acid molecules. This process is called lipolysis and is catalysed by a hormone-sensitive lipase (Murrey *et al.*, 1993). During exercise, an activation of the hormone-sensitive lipase results from an increase release of catecholamines and glucagon hormones. Free fatty acids (FFAs) are released from storage sites bound to blood albumin and delivered to muscle and triacylglycerol within the muscle itself (IMTG) provides the major energy substrates during exercise. The first step in intracellular metabolism of fat is the activation of fatty acid in preparation for the β-oxidation. This activation process raises the fatty acids to a high energy level and involves ATP. That is, fatty acid is converted to a CoA derivative which is termed fatty acyl-CoA (Maughan *et al.*, 1997).

The β–oxidation is the major pathway for fat catabolism. This process occurs in the mitochondrial matrix, involves the sequential removal of 2-C units from the fatty acid chain in the form of acetyl-CoA which can then enter the citric acid or tricarboxylic acid cycle (TCA cycle). As the site of fatty acyl-CoA formation is the cytoplasm, the fatty acyl-CoA must bind with intramuscular protein carnitine in order to enter the mitochondria. The enzyme regulating the transport of FFAs via carnitine is called carnitine acyltransferase (Murrey *et al.*, 1993). During exercise, the activity of this enzyme is an extremely important step in overall control of fat oxidation. The activity of this enzyme is inhibited by malonyl-CoA, a precursor for fatty acid synthesis (Maughan *et al.*, 1997). In side the mitochondria, free fatty acyl-CoA is first oxidized to enoyl-CoA by fatty acyl-CoA dehydrogenase. Hydration of the enoyl-CoA subsequently occurs via enoyl-CoA hydratase to form hydroxyacyl-CoA which is then oxidized to ketoacyl-CoA by 3-hydroxyacyl-CoA dehydrogenase (3-HAD) (McArdle *et al.*, 1999). This reaction is the rate-limiting step in the β-oxidation pathway. The activity of 3-HAD is altered in response to dietary manipulation and by exercise, the basis of this control is through substrate availability. The final step in the pathway is catalysed by acetyl-CoA acetyltransferase and results in the cleavage of ketoacyl-CoA, releasing a molecule of acetyl-CoA and a fatty acyl-CoA. This fatty acyl-CoA can now repeat the cycle, while the acetyl-CoA formed can enter the TCA cycle (Maughan *et al.*, 1997).

In TCA cycle, acetyl-CoA substrate is degraded to carbon dioxide and hydrogen atoms, and this occurs within the mitochondria. The hydrogen atoms are then oxidized via the electron

transport (respiratory) chain allowing oxidative phosphorylation with the subsequent regeneration of ATP from ADP. A key regulation point in the TCA cycle is the reaction catalysed by citrate synthase (CS). The activity of this enzyme is inhibited by ATP, nicotinamide-adenine dinucleotide (reduced form) [NADH], succinyl-CoA and acyl-CoA derivatives of fatty acids and citrate availability. In general, for each 18 carbon fatty acid molecule, 147 molecules of ADP are phosphorylated to ATP during β–oxidation and TCA cycle metabolism (Murrey *et al.*, 1993 and Maughan *et al.*, 1997).

The oxidation of fat during exercise is influenced by intensity, duration, plasma FFAs availability and carbohydrate availability. Study in trained cyclists who exercised between 25 and 85% VO_{2max} demonstrated that during light to mild exercise (< 40% VO_{2max}), fat provided the main energy source, predominantly as plasma FFAs from adipose tissue depots. The availability of plasma FFAs is thought to influence its uptake by skeletal muscle both at rest and during exercise. At moderate exercise intensity (below 65% VO_{2max}), the IMTG provided the additional source of fat oxidation. With exercise intensity at 85% VO_{2max}, total energy from fat oxidation remained essentially unchanged, but required added energy from blood glucose and muscle glycogen (Romijn *et al.*, 1995). Exercise duration also influences fat oxidation. Romijn *et al.* (1995) found that during moderate exercise intensity for 2 hours, plasma FFAs and glucose oxidation progressively increased. On the other hand, there was a progressive reduction in the oxidation of IMTG and muscle glycogen as these stores became depleted. The minimal contribution of plasma FFAs to energy expenditure during high intensity exercise is related to the fact that the mobilization, transport and uptake of plasma FFAs are simply too slow to match high rate of metabolism. This was supported by the study of demonstrating that the plasma FFAs concentration decreased by 50% during exercise intensity at 85% VO_{2max} and coincided with a lower rate of fat oxidation than at 65% VO_{2max} (Romijn *et al.*, 1995). Fat oxidation during exercise is also directly regulated by carbohydrate availability (Coyle *et al.*, 1997). Compared to an overnight fast, glucose ingestion before 40 minutes of exercise intensity at 50% VO_{2max} elevated plasma glucose and insulin concentrations and reduced fat oxidation. It is suggested that increases in glycolytic flux due to elevated plasma glucose and insulin concentrations during exercise selectively inhibit long chain fatty acid oxidation, possibly due to increases in malonyl CoA (Maughan *et al.*, 1997)

Exercise Training and Energy Metabolism

A shift toward greater total fat utilization during submaximal exercise has been observed after endurance training in several animals and humans (Holloszy *et al.*, 1984; Turcotte, 1999; Hambleton *et al.*, 1980 and Oldham and Sipe, 1990). This is evidence by a lower respiratory exchange ratio (RER) or respiratory quotient (RQ) in the trained subjects compared to untrained controls (Coggan *et al.*, 1993 and Phillips *et al.*, 1996). The mechanisms underlying a greater reliance on fat metabolism during exercise are due to a number of structural and metabolic adaptations to endurance training. An increase in the capacity to take up and oxidize plasma FFAs of trained muscles has been reported. This occurs via the increased capillary density in muscle, allowing a greater surface area for FFAs uptake from blood (Gollnick and Saltin, 1982) and an increase in activity of lipid-mobilizing enzyme. An increase of the activity of lipoprotein lipase (LPL) in the capillary endothelium of trained muscle has been reported (Turcotte, 1999). Furthermore, the expression of a sarcolemmal fatty acid binding proteins which thought to mediate skeletal muscle free fatty acid uptake has been shown to be increased by 49% after 3 weeks of training (Kiens *et al.*, 1997). Several studies demonstrated an increased mitochondrial volume as well as mitochondrial density enzyme activity in trained muscle (Maughan *et al.*, 1997). For example, when rats were trained by daily treadmill running, the cytochrome c of the gastrocnemius was elevated above control value by 102% whereas the activity of various TCA cycle enzymes increased by 34-101% (Holloszy, 1973). This suggests that a change in the mitochondrial composition, as well as increase in the activities of enzymes of the TCA cycle and the electron transport enzymes, occurs as a result of endurance training (Holloszy and Coyle, 1984 and Turcotte, 1999). An increased level of activities of enzymes involved in the TCA cycle and electron transport chain confers a greater capacity to generate ATP in the presence of oxygen. Thus, trained individuals are able to oxidize more fatty acids which is also expressed in an increased oxygen consumption at maximal exercise intensities (Holloszy and Booth, 1976). Interestingly, it has also been reported a considerably higher IMTG content in trained individuals compared with untrained controls (Morgan *et al.*, 1969). Whether or not training leads to an increased contribution of IMTG for energy provision during exercise remains uncertain. Study of Hurley and colleagues (1986) demonstrated that two-legged exercise at 64% VO_{2max} after 12 weeks of training produced an increased oxidation of fat, a 41% decrease in muscle glycogen use,

and a doubling of IMTG degradation during exercise. Likewise, Martin *et al.* (1994) reported the reduced plasma FFAs uptake and oxidation despite an increase in total fat oxidation after training. This difference was thought to be accounted by the increased oxidation of IMTG. However, study of Kiens *et al.* (1998) found unchanged IMTG concentration after 90 minutes of exercise. Although the effects of endurance training on the contribution of IMTG to energy provision during exercise is controversial, an increase in fat oxidation after training plays an important role in reducing the rate of utilization of muscle glycogen and blood glucose and decreasing the rate of accumulation of lactate during exercise. These adaptations contribute to the marked improvement in endurance capacity following training (Jansson and Kaijser, 1987; Phillips *et al.*, 1996 and Carter *et al.*, 2001).

Fat Diet and Energy Metabolism

It is well accepted that an increase in fat oxidation during exercise can significantly attenuate the oxidation of glycogen and blood glucose, consequently spares muscle glycogen. According to the concept of glycogen store as a limiting factor in the performance of prolonged submaximal exercise, many strategies have been used by endurance athletes in an attempt to promote fat oxidation. It has been suggested that a contribution of fat oxidation to energy metabolism during exercise. It has been reported to depend on the nutritional status and the composition of fat and carbohydrate in the diet prior to exercise in addition to endurance training (Martin *et al.*, 1994 and Holloszy and Kohrt, 1996). Dietary manipulation demonstrated that consumption of fat-rich diet has consistently been shown to change substrate utilization toward higher fat oxidation during exercise (Helge *et al.*, 1996; Jansson, 1982 and Phinney *et al.*, 1983). Studies in rats and humans have been demonstrated physiologic and metabolic adaptations that account for this change include a decreased muscle glycogen utilization during exercise (Lapachet *et al.*, 1996; Miller *et al.*, 1984; Phinney *et al.*, 1983 and Simi *et al.*, 1991), an increased plasma FFA concentration during exercise (Conlee *et al.*, 1990; Kronfeld *et al.*, 1973 and Simi *et al.*, 1991), and/or increased β-oxidative capacity (Fisher *et al.*, 1983; Helge *et al.*, 1996; Miller *et al.*, 1984 and Simi *et al.*, 1991). It has been also reported that fat oxidation only gradually increases on a high fat diet, but can be increased rapidly when glycogen stores are lowered (Schrauwen *et al.*, 1997). The mechanism by which consumption of a high fat diet and/or decrease in glycogen

stores leads to an increase in fat oxidation is not clear. Insulin, an important inhibitor of lipolysis, might play a role (Campbell *et al.*, 1992). Flatt *et al.* (1987) has proposed that glycogen stores which are maintained in a lower range on a high fat diet leads to lower glucose and insulin concentrations between meals, and hence higher fatty acid concentration and higher rates of fat oxidation. In addition, expansion of the adipose tissue mass after adaptation to high fat diets leads to enhanced fat oxidation. Studies in humans (Schrauwen *et al.*, 2000) in accordance with findings of Turcotte (1999) in rats showed that on a high fat diet, an increase in fat oxidation was accounted for by an increase in triacylglycerol-derived fatty acid oxidation (intramuscular and or very low density lipoprotein (VLDL) triacylglycerol). An increase of IMTG concentrations has been reported after consumption of a high fat diet (Lapachet *et al.*, 1996; Conlee *et al.*, 1990; Kiens *et al.*, 1987 and Starling *et al.*, 1997), and this may allow an increased IMTG oxidation. Furthermore, the increased of VLDL triacylglycerol oxidation after adaptation to high fat diet could be linked to the increased activity of LPL (Kiens *et al.*, 1987 and Jacobs *et al.*, 1982). A higher LPL activity on a high fat diet facilitates the release of fatty acids from VLDL triacylglycerol, which might be directly oxidized in the muscle, especially in the postabsorptive state and during exercise. In addition, it is also possible that the higher LPL activity on a high fat diet facilitates the storage of fatty acid derived from VLDL triacylglycerols into IMTG, and that this IMTG is oxidized in the postabsorptive state and during exercise (Schrauwen *et al.*, 2000). High fat diets have also shown to alter the activity of several enzymes. These includes increased carnitine acyl transferase, a key enzyme involved in fatty acid transport in to the mitochondria (Fisher *et al.*, 1983); increased 3-HAD, a key enzyme in mitochondrial oxidation (Cheng *et al.*, 1997; Miller *et al.*, 1984; Simi *et al.*, 1991 and Helge and Kiens, 1997) and CS, a key enzyme in TCA cycle (Orme *et al.*, 1997). The enzymatic adaptation which is indicative of greater potential for fat metabolism may be important in allowing the athletes to sustain a higher percentage of aerobic capacity (VO_{2max}) during prolonged exercise.

The dietary fat induced changes toward glycogen sparing and/or higher fat oxidation during exercise have most likely contributed to the improved endurance performance. Most animal studies have shown that a high fat diet can significantly enhanced endurance performance in rats (Conlee *et al.*, 1990; Miller *et al.*, 1984 and Simi *et al.*, 1991), dogs (Kronfeld *et al.*, 1994; Hammel *et al.*, 1997 and Kronfeld *et al.*, 1997) and horses (Kronfeld *et al.*, 1994; Harkin *et al.*,

1992; Oldham and Sipe, 1990 and Webb *et al.*, 1987). Although Helge (1998) failed to detect a significant benefit of fat adaptation to endurance in either trained or untrained rats after consumption of high fat diet (65% E fat). Study in trained cyclists demonstrated that a high fat diet (65% E fat), in combination with high volume intense training, increased rates of fat oxidation and spared muscle glycogen during 2 hours cycling at 70% of peak O2 uptake (VO_{2max}) (Burke *et al.*, 2000). Despite muscle glycogen sparing, fat adaptation did not provide a clear benefit to the performance of a 30 minutes time trial undertaken after 120 minutes of submaximal cycling (Burke *et al.*, 2000). Phinney *et al* (1983) reported a preservation of submaximal exercise capability in endurance trained humans fed during 4 weeks with fat rich diet (85% E fat). In contrast, other studies (Helge *et al.*, 1996) reported decreased endurance performance after both 2 and 7 weeks adaptations to a fat rich diet (62% E fat) compared with a carbohydrate rich diet (9% E fat). Differences among these studies in species, training status and dietary composition may explain the discrepant findings. It seems that the effects of adaptation to fat diet on endurance capacity to some extent depend on the dietary fat content. In most animal studies, the dietary fat was ~ 75-85 % E whereas dietary fat was 65% E in the study of Helge *et al.* (1998).

According to a strong relationship between dietary fat and body fat deposition (Oscai *et al.*, 1984 and Miller *et al.*, 1994), this is one of the drawbacks in promoting dietary fat as an ergogenic aid. Study of Lapachet *et al.* (1996) demonstrated a higher body fat percentage in trained fat diet rats despite reducing energy intake by 12% compared with the trained carbohydrate diet rats. Although the optimal fat intake to induce positive adaptation in energy metabolism and exercise performance is not known, it has been suggested that moderate increases in dietary fat might be beneficial for overall health and physical performance. This is supported by the finding that increasing dietary fat from 15% to 42% E increased VO_{2max} and endurance capacity (Leddy *et al.*, 1997 and Muoio *et al.*, 1994) without compromising either immune functioning (Venkatraman *et al.*, 1997) or blood lipoprotein profiles (Leddy *et al.*, 1997). In addition, animal studies demonstrated that 2 weeks supplementation of a diet with fat content 21% E to trained rats prefed with either high fat or high carbohydrate diet significantly increased endurance as well as VO_{2max} (Veerapun *et al.*, 2002). In addition, data of blood borne substrates demonstrating an increased utilization rate of blood triacylglycerol and decreased utilization rate of blood glucose could indicate a shift of energy substrate from carbohydrate to fat during exercise. However, the

changes in tissue substrate store and utilization have not been investigated. Thus, the mechanism underlying adaptation in energy metabolism to different contents of dietary fat and whether or not these adaptations impact the endurance performance remains to be further studied.