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ADAPTATIONS IN GLUCOSE KINETICS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
The Degree of Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Kevin Allen Jacobs, M.A.

*****

The Ohio State University
2000

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ABSTRACT

The purpose of this study was to examine the influences of dietary composition on short-term aerobic training-induced adaptations of whole body carbohydrate and fat metabolism, glucose kinetics, and exercise performance. Eight healthy, sedentary, males (27 ± 2 yr, 77.9 ± 2.8 kg, mean $\pm$ SE) with a peak oxygen consumption of 45.2 ± 2.3 ml $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ underwent two 10 d experimental phases separated by a two-week washout period. During the first 10 d phase subjects were randomly assigned to consume either a high carbohydrate (~ 7 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$) or high fat diet (~ 3 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$) while remaining sedentary (CHO or FAT). During the second 10 d phase subjects consumed the opposite diet and also performed daily aerobic training (CHO+T or FAT+T). CHO did not affect the pattern of whole body substrate metabolism during 90 min of moderate intensity exercise. FAT increased whole body fat oxidation at the end of exercise (9.6 ± 2.5 to 15.7 ± 2.4 μ mol $\cdot$ kg $^{-1}\cdot$ min $^{-1}$, FATpre vs. FATpost) and reduced glucose rate of appearance (R_a) and disappearance (R_d) at the end of exercise. CHO+T did not greatly alter whole body substrate metabolism during exercise. However, FAT+T produced a substantial increase in fat oxidation throughout exercise (11.6 ± 1.9 to 19.5 ± 3.9 μ mol $\cdot$ kg $^{-1}\cdot$ min $^{-1}$, 90 min average FATpre vs. 90 min average FATpost). Both CHO+T and FAT+T subjects had reductions in glucose R_a at the end of exercise. Only FAT+T subjects had reductions in glucose R_d at the end of exercise. There was a similar improvement in time trial performance for both groups. Thus, high carbohydrate and high fat diets alone do not substantially alter metabolism during exercise, except that high fat diets tend to increase fat oxidation late in exercise. The increase in fat oxidation late in exercise as a result of a 10 d high fat diet is magnified when accompanied by daily training for 10 d. High carbohydrate diets consumed during training eliminate the training-induced increase in fat utilization. Despite these differences in adaptation of substrate use, CHO+T and FAT+T subjects were equally capable of improving high-intensity time trial performance after 10 d of training.

Dedicated to my family and friends

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CHAPTER 1

INTRODUCTION

1.1 SUMMARY

The most pronounced adaptation in substrate metabolism to prolonged endurance training is the increased oxidation of fat during aerobic exercise (27, 100). This adaptation reduces the oxidation of limited carbohydrate stores, often referred to as “carbohydrate sparing”. While muscle glycogen was thought to be the source of “spared” carbohydrate, recent studies using tracer methodologies reveal that decreased plasma glucose oxidation accounts for 30-50% of the overall reduction in carbohydrate oxidation with training (32, 141). In other words blood glucose and muscle glycogen use are decreased with aerobic exercise training.

Short-term training studies demonstrate significant changes in plasma glucose metabolism during exercise. Ten days of intense training reduce the rate of plasma glucose oxidation by approximately 30% during prolonged moderate intensity exercise (124, 138, 141). This short-term adaptation may precede the increase in muscle respiratory capacity that is thought to be responsible for adaptations in substrate metabolism from prolonged training. While the mechanism for this early adaptation is not yet clear, it represents a pivotal step for further adaptations with continued aerobic training.

In addition to aerobic training, plasma glucose metabolism during exercise is also influenced by the composition of the diet. Martin et al. (113) found that a 3 d high fat diet (~90% of kcals from fat) reduced the oxidation of plasma glucose by nearly 50% during 30 min of moderate intensity exercise when compared with a 3 d high carbohydrate diet (~75% of kcals from carbohydrate). Chronic high fat diets

seem to induce a similar response. Phinney et al. (142) showed a 40% decrease in plasma glucose oxidation following a 4 wk high fat diet (~85% of kcals from fat) with trained subjects who continued exercise training.

The fact that aerobic training and dietary composition both individually influence substrate metabolism raises the possibility that interaction effects may exist. Several studies have examined the influence of dietary composition on training capability and performance of trained subjects (108, 109, 128, 142, 157, 160). However, the use of trained subjects does not improve our understanding of the influence of dietary composition on training-induced adaptations in substrate metabolism during exercise. To date, this issue has only been examined by Helge et al. (82). Healthy sedentary males were fed either a high or low carbohydrate diet while undergoing identical supervised 7 wk aerobic training programs. Despite identical training programs, those fed the low carbohydrate diet demonstrated less improvement in endurance performance after 7 wk (+ 68%) than those fed the high carbohydrate diet (+ 191%). These results, and the lack of concrete support of a chronic high carbohydrate diet in trained subjects, suggest that chronic high carbohydrate diets are the most valuable to untrained subjects undergoing adaptation to an aerobic training program. A possible mechanism for this difference in performance was that skeletal muscle plasma glucose uptake may have been impaired in those fed the low carbohydrate diet. This could not be confirmed because glucose kinetics were not measured. Therefore, the purpose of the current study was to examine whether dietary composition influences short-term aerobic training-induced adaptations of glucose kinetics.

1.2 RESEARCH HYPOTHESES

1. Consumption of a high fat diet for 10 d will reduce the rates of glucose appearance and disappearance at rest and during moderate intensity exercise compared with a high carbohydrate diet.
2. Ten days of training will reduce the rates of glucose appearance and disappearance during moderate intensity exercise. However, those fed a high fat diet will exhibit greater reductions in the rates of glucose appearance and disappearance due to an additive effect of training and the high fat diet.

3. **Ten days of training will significantly improve the aerobic capacity of both high carbohydrate and high fat fed subjects.**
4. **Subjects consuming the high carbohydrate diet will exhibit greater improvements in time trial performance after 10 d of training than those consuming the high fat diet.**

CHAPTER 2

REVIEW OF LITERATURE

2.1 EXOGENOUS CARBOHYDRATE DIGESTION AND ABSORPTION

2.1.1 Digestion

Limited digestion of exogenous carbohydrate is initiated in the mouth by salivary amylase before the low pH environment of the stomach inactivates this enzyme. Pancreatic amylase digests a majority of exogenous carbohydrate in the small intestine with subsequent absorption from the jejunum in the form of various mono- and disaccharides.

2.1.2 Absorption

The majority of exogenous carbohydrate absorption occurs by sodium-dependent active transport against a concentration gradient. Absorption of the remainder, primarily fructose, is reliant on much slower passive diffusion. Following absorption exogenous carbohydrate is transported to the liver by the hepatic portal vein. Upon reaching the liver the absorbed exogenous carbohydrate may be oxidized, synthesized into liver glycogen, or released into the circulation as glucose.

2.2 ENDOGENOUS CARBOHYDRATE STORES

Endogenous carbohydrate is the most limited bodily fuel source, representing approximately 2000 kcals of energy that is only 1-2% of the body's total energy stores (64). A majority of carbohydrate stores are in the form of glycogen located in skeletal muscle (82% of total carbohydrate energy) and the liver (14% of total) with the remainder in the form of plasma glucose (4% of total) (24).

Muscle glycogen concentration ranges from ~50 to 200 mmol glucose•kg muscle mass wet weight (ww)⁻¹ depending on nutritional and training status. Liver glycogen concentration averages ~490 mmol glucose and may range from ~67 mmol glucose with a low carbohydrate diet (6 g carbohydrate•d⁻¹ for 4-10 d) to ~900 mmol glucose with a high carbohydrate diet (~400 g carbohydrate•d⁻¹ for 2-5 d) (89, 131).

Plasma glucose represents the balance between carbohydrate absorption from the small intestine, hepatic glucose production, and peripheral glucose uptake. The normal plasma glucose concentration is ~5.5 mM (1.0 g•L⁻¹) amounting to a total of 8-10 g of carbohydrate in the blood. The importance of the contributions by the liver and exogenous sources of carbohydrate to plasma glucose is emphasized by the fact that the brain alone consumes ~4.0 to 6.0 g of glucose•h⁻¹; a rate that would quickly result in hypoglycemia if plasma glucose were not rapidly replenished (63, 131).

2.3 EXOGENOUS FAT DIGESTION AND ABSORPTION

2.3.1 Digestion

The digestive process in the stomach and small intestine takes place in an aqueous environment where dietary fats have a tendency to separate into an oily film. Therefore, the water soluble lipolytic enzymes of the stomach and small intestine have access to only the surface of large insoluble fat droplets, effectively reducing the surface area upon which enzymes can act. In order to counteract the insolubility of dietary fat, the body emulsifies the large lipid droplets with the combined actions of contractions in the stomach emulsification by bile in the duodenum. Bile is an emulsifying agent that contains bile acids, cholesterol, lecithin, and bile pigments, and is produced in the liver and stored in the gall bladder. Contractions in the stomach and the process of forcing fat through the pyloric sphincter produce strong shear forces. These combined processes sufficiently decrease the size of the lipid droplets, increasing the exposed surface area of the lipid droplets thousands of times to facilitate the actions of digestive enzymes.

The digestion of dietary triglyceride (TG) occurs in the stomach and upper small intestine. A class of lipolytic enzymes referred to as preduodenal lipases begin the limited digestion of fat in the stomach. Glands lying beneath the lining of the tongue secrete the enzyme lingual lipase, while gland cells lining the stomach secrete the enzyme gastric lipase. Most lipid hydrolysis, however, occurs in the small intestine by a group of enzymes secreted by the pancreas. These lipolytic enzymes are pancreatic

lipase, colipase, cholesterol esterase, and phospholipase A₂. Pancreatic lipase is considered the major enzyme of dietary lipid digestion, because it acts specifically upon TG and is secreted in large volumes.

2.3.2 Absorption

The products of the digestive process are mainly combinations of monoglycerides, lysolecithin, cholesterol, fatty acids, and bile that form particles called micelles. The water-soluble micelles cross the unstirred water layer that covers the intestinal mucosal cells, and are absorbed at the brush border of these cells. The lipid contents of the micelles, in turn, diffuse into the intestinal mucosal cells by moving down a concentration gradient.

Upon absorption into the intestinal mucosal cells, the lipid products are reesterified into TG, phosphatidylcholine, and cholesterol esters. Long chain fatty acids (LCFA) (≥ 16 carbons) that are reesterified in the intestinal mucosal cells are subsequently combined with fat soluble vitamins to form large fat particles. Due to the low solubility of these particles, transport in the liquid environment of plasma becomes problematic. These lipid droplets can be transported, however, if they are packaged with a soluble coating of phospholipids, cholesterol, and protein. These molecules made up of an insoluble core (TG and cholesterol esters) and a soluble coating (phospholipids, cholesterol, and protein) are referred to as lipoproteins. The class of lipoproteins that carry the reesterified LCFA and cholesterol esters from the gastrointestinal tract to the plasma are referred to as chylomicrons.

Once lipid particles have been packaged in the form of chylomicrons, they must first travel through the lymphatic circulation. Upon appearance in the bloodstream, chylomicrons undergo intravascular hydrolysis at different tissues throughout the body. This hydrolysis is catalyzed by the enzyme lipoprotein lipase (LPL), that exists on the endothelial surface of small blood vessels and capillaries within adipose and skeletal muscle. LPL acts extracellularly and releases free fatty acids (FFA) and glycerol that are absorbed by different tissues.

The entry of chylomicrons into the bloodstream from the lymphatic circulation can continue for approximately 14 h after the consumption of a high fat meal and prevents drastic changes in the plasma lipid content. Approximately 80% of the TG released from chylomicrons is taken up by adipose, heart, and skeletal muscle, while the remaining 20% is taken up by the liver (120). Chylomicrons are usually cleared from the bloodstream within 1 h of a fatty meal.

After hydrolysis and the release of approximately 90% of the TG carried by chylomicrons, they decrease in size and increase in relative composition of cholesterol and cholesterol esters. The resulting lipoproteins are referred to as chylomicron remnants. The liver then takes up the chylomicron remnants and the remaining cholesterol esters and TG are hydrolyzed and metabolized.

The absorption of the smaller and more soluble short chain fatty acids (SCFA) (2-4 carbons) and medium chain fatty acids (MCFA) (6-14 carbons) differs from that of LCFA. Short and medium chain fatty acids follow the same route of intestinal absorption as monosaccharides and amino acids. They are directly absorbed by the small intestine and passed on to the liver by the portal circulation bound to albumin. This direct route of absorption means that SCFA and MCFA appear in the bloodstream and can function as a substrate more rapidly than LCFA. Massicotte et al. (117) found that MCFA consumed 1 h before exercise are oxidized within the first 30 min of exercise.

2.4 ENDOGENOUS FAT STORES

Endogenous fat represents 90,000-100,000 kcals of energy or 70-80% of the body's total energy stores (24). Only 2-3% of endogenous fat is stored within skeletal muscle fibers as intramuscular TG (IMTG) (40). Intramuscular TG is important for endurance exercise because it is the only fat source stored in skeletal muscle that can be oxidized. Nearly all of endogenous fat is stored in adipose tissue as TG.

2.5 CELLULAR ENERGY BALANCE

Chemical energy in the form of adenosine triphosphate (ATP) is required for normal cellular function. The energy state of any cell is a function of how well ATP breakdown is matched by ATP production from metabolism of carbohydrate, fat, and protein sources. The major consumers of ATP in skeletal muscle are the actin-myosin ATPases (~70% of ATP consumed) and calcium ATPases of the sarcoplasmic reticulum (25-30%) (146). Exercise intensity determines the rate of ATP use in skeletal muscle and therefore dictates the required rate of ATP production as well as the selection of fuel sources used to produce ATP. The signals used by skeletal muscle to match ATP production with use are calcium, metabolites of cytoplasmic phosphorylation potential (ATP, adenosine diphosphate, inorganic phosphate, and adenosine monophosphate), and the mitochondrial state of respiratory control (163). These signals will be discussed below with the regulation of carbohydrate and fat metabolism.

2.6 REGULATION OF CARBOHYDRATE METABOLISM

2.6.1 Glycogen Synthesis

Glycogen synthesis occurs in both the liver and skeletal muscle and is catalyzed by the enzyme glycogen synthase. Glycogen synthase in the liver is activated by insulin and inhibited by glucagon and epinephrine. Glycogen synthase in skeletal muscle is activated by insulin and inhibited by epinephrine and the increase in intracellular calcium resulting from muscular contraction. There is also recent evidence that skeletal muscle glycogen limits its own synthesis by inhibiting glycogen synthase (110). A muscle glycogen loading protocol resulted in a ~30% decrease in insulin-stimulated glycogen synthesis during a euglycemic-hyperinsulinemic clamp. Greater intramuscular glucose 6-phosphate and glucose oxidation rates suggested that elevated muscle glycogen content resulted in a shunting of incoming blood glucose towards oxidation rather than storage. Glycogen synthase is most active in the fed state when insulin levels are high or after prolonged exercise when muscle glycogen has been reduced.

2.6.2 Hepatic Glucose Production

Hepatic glucose production, and exogenous carbohydrate absorption in the fed state, provides the only means of supplementing plasma glucose, a fuel source critical not only to obligatory organs such as the brain but also to skeletal muscle during prolonged exercise. Hepatic glucose production is a function of both hepatic gluconeogenesis and glycogenolysis. Gluconeogenesis occurs in the liver and kidneys and combines three carbon compounds such as lactate, glycerol, and amino acids to form glucose. Gluconeogenesis involves several key regulatory enzymes including phosphoenolpyruvate carboxykinase (PEPCK). Glucagon, epinephrine, and the glucocorticoids activate PEPCK and the other gluconeogenic enzymes. These enzymes are primarily inhibited by insulin. Gluconeogenesis is most active after a prolonged fast and after prolonged exercise when liver glycogen levels are low and gluconeogenic precursors are abundant.

Glycogenolysis involves the cleavage of glucose residues from glycogen and their phosphorylation to glucose-1 phosphate, which then enters the glycolytic pathway. The phosphorylation step is catalyzed by phosphorylase (PHOS) which exists in a less active *b* form and a more active *a* form. The transformation between PHOS *b* and PHOS *a* in the liver is dependent on the insulin/glucagon ratio. Glucagon released by the pancreas in response to decreases in plasma glucose will promote the transformation of PHOS *b* to PHOS *a*. The rate of glycogenolysis is then fine-tuned as the flux through

PHOS *a* is determined by the availability of substrate (glycogen and inorganic phosphate) (163). Thus, the rate of glycogenolysis will remain elevated under conditions of decreasing plasma glucose as long as substrate is available. When plasma glucose increases and insulin levels rise in response, the PHOS *b* form will dominate and rate of glycogenolysis will decrease.

2.6.3 Skeletal Muscle Plasma Glucose Uptake

Skeletal muscle plasma glucose uptake is considered the rate-limiting step of plasma glucose metabolism (107). The glucose transporter GLUT-4 regulates the uptake of plasma glucose by skeletal muscle and adipose tissue. GLUT-4 is stored in vesicles in an intracellular membrane pool. Insulin and muscular contraction stimulate movement of GLUT-4 to the plasma membrane and transverse tubules where it facilitates plasma glucose uptake. Interestingly, insulin and muscular contraction are thought to use separate signaling pathways and/or recruit separate pools of GLUT-4 transporters as additive effects of plasma glucose uptake are found with the combination of maximal contraction and insulin stimuli (20, 80, 179). There is evidence that the increases in cytosolic calcium concentration caused by muscular contractions may initiate a chain of signals that increase uptake of plasma glucose (188). It has also been suggested that GLUT-4 may be associated with glycogen and is released to facilitate glucose transport as glycogen is degraded during exercise (28). This idea is supported by the finding that contraction-induced glucose uptake and GLUT-4 translocation are inversely correlated to precontraction muscle glycogen content (47).

2.6.4 Skeletal Muscle Glycolysis

The regulatory enzymes of glycolysis are hexokinase, phosphofructokinase (PFK), and pyruvate kinase. Hexokinase may be stimulated by insulin and is inhibited by the accumulation of glucose-6 phosphate. PFK is stimulated by the products of ATP hydrolysis (AMP and inorganic phosphate) and insulin. High ATP/ADP ratios and possibly citrate inhibit PFK. Pyruvate kinase is activated by insulin and inhibited by high ATP/ADP ratios. Thus, glycolysis is activated in the fed state when insulin is high and during exercise when ATP/ADP ratios are lowered by accelerated rates of ATP hydrolysis.

2.6.5 Skeletal Muscle Glycogenolysis

Like the liver, skeletal muscle glycogenolysis is also catalyzed by PHOS that exists in both the less active *b* and more active *a* forms. Epinephrine and the increase in intracellular calcium from muscular contraction facilitate the transformation to PHOS *a* in skeletal muscle whereas H^+ accumulation may

inhibit this transformation. The inhibitory effect of H^+ may function to limit the rate of glycogenolysis to better coordinate glycolytic flux and pyruvate oxidation thereby avoiding excessive lactate accumulation (136). Flux through PHOS *a* is determined by the availability of substrate (glycogen and inorganic phosphate) as well as AMP, an indicator of cellular energy state (163). Thus the rate of glycogenolysis will remain elevated as long as substrate is available. AMP increases due to an imbalance between ATP use and production, and H^+ does not accumulate. Conditions that may activate phosphorylase include postabsorptive and fasting states as well as physical exertion such as exercise.

2.6.6 Pyruvate Dehydrogenase – Pivotal Point of Carbohydrate Entry into the TCA Cycle

Pyruvate dehydrogenase (PDH) catalyzes the conversion of pyruvate to acetyl-CoA. It exists in both a less active *b* form and a more active *a* form. Increases in intracellular calcium promote the transformation to the *a* form while increases in ATP/ADP and decreases in NAD/NADH ratios favor transformation to the *b* form. Flux through PDH *a* is maintained by the availability of substrate (pyruvate, CoASH, and NAD). The activity of PHOS and PDH are tightly coordinated, as PDH is reliant on the delivery of pyruvate that is initiated by PHOS. Peak PHOS activity is reached within 6 s of initiation of maximal exercise while peak PDH activity may not be reached until approximately 15 s (136). As discussed above the result of this initial mismatch in maximal activation time is an accumulation of H^+ that inhibits PHOS *a* transformation and leads to a better matching between glycolytic flux and pyruvate activation. PDH is considered a pivotal enzyme in fuel selection at rest and during exercise as it regulates the entry of carbohydrate into the mitochondria for oxidation.

2.7 REGULATION OF FAT METABOLISM

2.7.1 Lipogenesis

In humans, it is possible to synthesize fat from both FFA sources and non-FFA sources. Tissues such as the liver, kidney, brain, lung, mammary gland, and adipose can convert acetyl-CoA (from sources such as glucose) into palmitate in the cytosol of the cell. The liver and adipose tissue are considered the primary sites of lipogenesis in humans (1). In addition, the liver endoplasmic reticulum can perform fatty acid elongation which converts acetate into LCFA (106). Insulin is the primary promoter of lipogenesis, while glucagon and epinephrine inhibit this process.

The occurrence of lipogenesis is dependent upon the nutritional state of the individual. According to Acheson et al. (2), non-obese men that consumed a 500 g meal containing mostly carbohydrate only converted a minor portion of that meal (1-2%) into fat. Hellerstein et al. (83) confirmed these findings in a similar population that received a 260-380 g meal of mostly carbohydrate over a 9 h period. However, Aarsland et al. (1) demonstrated significant lipogenesis after a 4 d hyperenergetic diet (about 2.5 times caloric expenditure) containing 15 g carbohydrate·kg body weight⁻¹. However, it is reasonable to assume that under normal conditions, the conversion of dietary carbohydrate to fat is limited

2.7.2 Lipolysis and Transport

Lipolysis is the process by which TG is broken down into fatty acids and glycerol. This process is responsible for the mobilization of fatty acids in a variety of tissues including skeletal muscle, liver, and adipose. Lipolysis occurs both intracellularly and extracellularly. Triglycerides that travel bound to VLDL and chylomicrons are acted upon by LPL outside of the cell and the FFA can be taken up by adipose tissue, skeletal muscle, and the liver. Intracellularly, stored TG are acted on by hormone sensitive TG lipase. Fatty acids from adipose tissue may be released into the bloodstream, but due to their insoluble nature must be circulated bound to albumin. Circulating fatty acids are referred to as FFA. The term "free fatty acid" must be interpreted with caution, since fatty acids that remain completely unbound comprise less than 0.01% of the total plasma pool. Fatty acids mobilized from IMTG do not require transport in the bloodstream.

Glycerol released from lipolysis is also transported in the bloodstream, where it is converted to glycerol 3-phosphate and glucose in the liver. The enzyme glycerol kinase is required for the conversion of glycerol to glycerol 3-phosphate and glucose, but the activity of this enzyme in tissues other than the liver and kidneys is low in animals (130). Since glycerol cannot be reesterified by skeletal muscle or adipose tissue and must be released into the bloodstream to be transported into the liver, regardless of the site of lipolysis, the appearance of glycerol in the blood serves as a marker of whole body lipolysis. Romijn et al. (150) confirmed this hypothesis using stable isotope tracer techniques during exercise.

There are several processes that regulate lipolysis. The most potent inhibitor of lipolysis is insulin, but other factors such as adenosine, ketone bodies, and changes in blood flow may also have an effect (37). Epinephrine serves as the most potent activator of lipolysis. It likely acts on alpha adrenergic receptors at rest, and beta adrenergic receptors during exercise (10). It has recently been shown that

increases in exercise intensity may attenuate the effect of epinephrine on lipolysis (127). While the mechanism for this effect is unknown, the inhibitory effect of exercise intensity on epinephrine action may lead to a better matching between rates of lipolysis and fatty acid oxidation. Other factors that stimulate lipolysis include growth hormone and glucocorticoids (37). It is assumed that IMTG lipase is regulated by beta adrenergic stimulation, but this would be rare as most key enzymes that are involved in ATP supply in skeletal muscle are regulated by intramuscular signals (163).

Not all of the fatty acids mobilized from adipose tissue are released into the circulation. Fatty acids may remain in the adipocyte and be reesterified into TG. The glycerol backbone for the reesterification of TG is supplied by the conversion of glucose to glycerol 3-phosphate. The mobilization of TG and subsequent reesterification is referred to as the triglyceride-fatty acid cycle. Reesterification may occur in adipose tissue or other tissues such as the liver and is regulated by blood flow, albumin binding capacity, and the rates of lipolysis and fatty acid oxidation (184). The triglyceride-fatty acid cycle provides a mechanism for rapidly altering plasma FFA availability to match metabolic demand. Wolfe et al. (184) demonstrated that in conditions of reduced availability, the release of FFA into the bloodstream is increased due to a decrease in the rate of reesterification. At rest, approximately 70% of fatty acids released were reesterified, but during exercise at 40% maximal oxygen consumption (VO_{2peak}), the rate dropped to 25% with a concomitant increase in FFA release. This resulted in a 6-fold increase in FFA availability.

2.7.3 Skeletal Muscle Plasma Fatty Acid Uptake

Triglycerides that travel bound to lipoproteins and/or albumin cannot be incorporated into the muscle. The FFA must be released in the vascular space prior to incorporation into the cell. Traditionally, it was thought that the uptake of plasma FFA into muscle cells and adipocytes occurred via passive diffusion (9, 91), so that the uptake was directly proportional to the plasma FFA concentration. However, plasma FFA uptake demonstrates saturation kinetics at ~1.5-2.0 mM (173). It is currently thought that there may be a system of carrier proteins that transfer plasma FFA from the vascular space into the cell, and eventually into the mitochondria (102, 104, 173, 175). Once inside the cell, fatty acids can be oxidized in the mitochondria, used to replenish IMTG stores, or used to synthesize phospholipids. Little is currently known about the regulation of FFA uptake and whether it presents a limitation to fat oxidation.

2.7.4 Carnitine Palmitoyl-transferase I – Pivotal Point of Fat Entry into the TCA Cycle

Fatty acids from plasma or IMTG are oxidized in the same manner. Fatty acids of any chain length must move inside mitochondria to be oxidized. However, while SCFA and MCFA can diffuse into mitochondria freely, LCFA must be transported from the cytosol into the mitochondria by two bound enzymes, carnitine palmitoyl-transferase I and II (CPT I and II). Mitochondrial transport of LCFA may be regulated by malonyl-CoA, a product of lipogenesis that inhibits CPT I. Malonyl-CoA concentration may be high in the postabsorptive state when insulin is elevated and lipogenesis is stimulated. Malonyl-CoA concentration may also be elevated during high rates of glycolytic flux and carbohydrate oxidation that occur with exercise (44). Recent evidence (132, 134) downplays the influence of malonyl-CoA on CPT I activity during exercise and instead points to other factors such as pH (166). Regardless of the mechanism of CPT I regulation, like its PDH counterpart, it is considered a pivotal enzyme in fuel selection at rest and during exercise as it regulates the entry of fat into the mitochondria for oxidation.

2.7.5 Beta-Oxidation

Once inside the mitochondria, fatty acids of any chain length undergo a process referred to as beta-oxidation. The end product of beta-oxidation is acetyl-CoA that may be utilized three different ways. It may enter the TCA cycle and be oxidized completely to carbon dioxide and water. Secondly, acetyl-CoA may serve as a source of carbon atoms in cholesterol and/or steroid synthesis. Lastly, acetyl-CoA may be converted to ketone bodies in the liver. Substrate availability is thought to be the only factor that regulates the enzymes of beta-oxidation.

2.8 REGULATION OF THE TRICARBOXYLIC ACID CYCLE

The TCA cycle is primarily regulated by respiratory control, the ability to use the reducing equivalents produced by the TCA cycle to produce ATP by oxidative phosphorylation (119). Several TCA cycle enzymes including citrate synthase are activated by the increase in intracellular calcium resulting from muscular contraction. This activation assures that the TCA cycle will be upregulated to handle the increased flux of acetyl-CoA from oxidative glycolysis and beta-oxidation during exercise. It is important to remember that the TCA cycle itself does not regulate fuel selection, as it is a common destination of both carbohydrate- and fat-derived acetyl-CoA.

2.9 REGULATION OF THE INTERACTION OF CARBOHYDRATE AND FAT METABOLISM

The glucose-fatty acid cycle was the first widely accepted mechanism for the regulation of carbohydrate and fat metabolism (147). By increasing the availability of fat to perfused contracting heart muscle beta-oxidation increased resulting in an accumulation of acetyl-CoA and citrate. The accumulation of these two metabolites inhibited the activities of PDH and PFK, respectively, thereby reducing glycolytic flux and the oxidation of carbohydrate. While this mechanism may explain carbohydrate-fat interaction in contracting heart muscle there is little evidence of its existence in contracting skeletal muscle (35, 53, 54, 133). Instead, it is now believed that the regulation of fuel selection is determined at the level of carbohydrate and fat entry into the mitochondria by PDH and CPT I, respectively. The regulation of the interaction of carbohydrate and fat metabolism is affected not only by carbohydrate and fat availability, but also by diet, exercise intensity and duration, and aerobic training status and these factors will be discussed below.

2.10 CARBOHYDRATE TURNOVER AT REST

2.10.1 Postprandial State

In the resting postprandial state carbohydrate oxidation provides 5-10% of total energy turnover (7). The source of carbohydrate is plasma glucose and liver glycogen as there is little or no breakdown of muscle glycogen. Plasma glucose is metabolized by all tissues and is the sole source of energy for such tissues as erythrocytes, leukocytes, and the nervous system. The brain can also metabolize ketone bodies. The high insulin/glucagon ratio in the postprandial state promotes storage of carbohydrate as glycogen in the liver and skeletal muscle (25).

2.10.2 Postabsorptive State

This profile changes in the postabsorptive state. During the 12-16 h after a meal, plasma glucose is heavily supplemented by the breakdown of liver glycogen resulting from the lowering of the insulin/glucagon ratio. The basal rate of liver glycogen degradation ($32 \text{ mmol} \cdot \text{h}^{-1}$) depletes average liver glycogen in 10-15 h if food is not consumed (89). Lowering the insulin/glucagon ratio also decreases insulin-mediated uptake of plasma glucose by adipose tissue and skeletal muscle and reduces the inhibition of lipolysis that mobilizes fat from adipose tissue. This leaves skeletal muscle more reliant on plasma free

fatty acid (FFA) for energy. Plasma glucose is also supplemented by gluconeogenesis during this time with lactate from erythrocytes and skeletal muscle as well as alanine from the transamination of pyruvate in skeletal muscle serving as gluconeogenic precursors.

In the 24–48 h after a meal gluconeogenesis becomes the major means of plasma glucose supplementation as liver glycogen stores are lowered. The continued lowering of the insulin/glucagon ratio further reduces peripheral uptake of plasma glucose to maintain adequate glucose delivery to the brain. This effect is enhanced by increases in the plasma concentration of cortisol, norepinephrine, epinephrine, and growth hormone in response to decreases in plasma glucose. This neuroendocrine response also increases the rate of lipolysis and protein degradation to provide greater concentrations of the gluconeogenic precursors glycerol and amino acids. The accelerated rate of lipolysis may raise the plasma FFA concentration more than 10 times above the resting postprandial concentration (0.10 to 1.40 mM). This is normally followed by the formation of ketone bodies by the liver. Ketone bodies provide an alternative source of fuel for the brain and skeletal muscle when plasma glucose concentration may be low. While carbohydrate oxidation is not a large contributor to energy expenditure in the resting state, its importance is emphasized by the elaborate systems used to maintain plasma glucose supply to reliant tissues.

2.11 FAT TURNOVER AT REST

2.11.1 Postprandial State

In the resting postprandial and postabsorptive states fat supplies a majority of the energy used by the body (7). Most of this fat is in the form of plasma FFA. The high insulin/glucagon ratio in the postprandial state drives the metabolic systems towards storage of fat and lipogenesis while inhibiting lipolysis and beta-oxidation. A majority of the plasma FFA taken up by the periphery is reesterified into TG droplets while the remainder is oxidized.

2.11.2 Postabsorptive State

In the postabsorptive state the lowering of the insulin/glucagon ratio increases the mobilization of fatty acids from adipose tissue due to the attenuated inhibition of lipolysis. Fatty acid mobilization may be further enhanced by the strong counterregulatory response to the decrease in plasma glucose characterized by increases in epinephrine, norepinephrine, cortisol, and growth hormone. As the postabsorptive state continues, plasma FFA concentration may rise to more than 10 times the normal resting concentration

(0.10 to 1.40 mM). The increase in plasma FFA concentration will produce an increase in skeletal muscle FFA uptake and oxidation eventually leading to the formation of ketone bodies (acetoacetone, beta-hydroxybutyrate, and acetone) by the liver. The plasma concentration of beta-hydroxybutyrate may triple after a 12 h fast and increase nearly 50-fold after 3 d (25). With hypoglycemia likely during a prolonged fast, ketone bodies provide an alternative fuel source for peripheral tissue including the brain and skeletal muscle (49).

2.12 CARBOHYDRATE TURNOVER DURING EXERCISE

2.12.1 The Influence of Intensity, Duration, and Substrate Availability

The intensity and duration of exercise and the availability of carbohydrate and fat influence the metabolism of carbohydrate during exercise. Muscle glycogen is metabolized under conditions that require high rates of carbohydrate oxidation such as moderate to high intensity exercise. As exercise intensity increases linearly there is an exponential increase in the rate of muscle glycogen degradation (153). Muscle glycogen degradation averages $0.3 \text{ mmol glucose} \cdot \text{kg ww}^{-1} \cdot \text{min}^{-1}$ at 25% $\text{VO}_{2\text{peak}}$, and increases to 0.7, 1.4, and $3.4 \text{ mmol glucose} \cdot \text{kg ww}^{-1} \cdot \text{min}^{-1}$ at 50, 75, and 100% $\text{VO}_{2\text{peak}}$, respectively (153). Fatigue during prolonged exercise at 65–85% $\text{VO}_{2\text{peak}}$ is related to the "depletion" of carbohydrate stores including muscle glycogen (42, 84).

The rate of plasma glucose uptake and oxidation by skeletal muscle also increases with increasing exercise intensity. Romijn et al. (150) used stable isotope tracers and indirect calorimetry to profile the metabolism of endogenous fat and carbohydrate during exercise at 25, 65, and 85% $\text{VO}_{2\text{peak}}$. Five endurance trained cyclists exercised on separate days in random order for 120 min at 25 and 65% $\text{VO}_{2\text{peak}}$ and 30 min at 85% $\text{VO}_{2\text{peak}}$. The contribution of carbohydrate to total energy expenditure rose from ~10% at the lowest intensity to 40% and 73% at 65% and 85% $\text{VO}_{2\text{peak}}$, respectively. Fat contributed the balance of energy used at each exercise intensity. There was no detectable use of muscle glycogen at the lowest intensity, while at 65% and 85% $\text{VO}_{2\text{peak}}$ muscle glycogen contributed ~80–83% of the carbohydrate oxidized. This study clearly demonstrated that carbohydrate is the primary fuel that is oxidized at exercise intensities above 65% $\text{VO}_{2\text{peak}}$, that muscle glycogen is the major source of this oxidized carbohydrate. In addition, with increasing exercise duration plasma glucose and FFA oxidation increase as oxidation of muscle glycogen and IMTG diminishes.

The rate of muscle glycogen degradation is the most rapid during the first 20-30 min of moderate intensity exercise (84). After this time the rate of glycogen degradation slows. Significant depletion of muscle glycogen may occur after approximately 60 or 90 min of continuous moderate intensity exercise in untrained and trained subjects, respectively. As muscle glycogen stores decrease, plasma glucose uptake and oxidation increase to maintain high rates of carbohydrate oxidation. Liver glycogen degradation initially supplies the majority of plasma glucose, but the rate of gluconeogenesis increases as liver glycogen stores are lowered. The rate of gluconeogenesis ($\sim 2\text{-}10 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) cannot maintain plasma glucose during prolonged moderate intensity exercise when the rate of plasma glucose uptake may be as high as $40\text{-}50 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and eventually the plasma glucose concentration declines. Basal plasma glucose ($5.0\text{-}5.5 \text{ mM}$) can be maintained for up to 60 to 90 min of moderate intensity exercise without carbohydrate supplementation, but may decrease to $2.5\text{-}3.5 \text{ mM}$ after 180 min of exercise without carbohydrate supplementation in trained subjects (30, 31, 42).

Carbohydrate oxidation during exercise is also influenced by carbohydrate availability. The rate of muscle glycogen degradation decreases as glycogen stores are lowered. Is the rate of muscle glycogen degradation during exercise affected by modifications of muscle glycogen concentration before the onset of exercise? Sherman et al. (156) observed that regardless of whether muscle glycogen was elevated or not before exercise, muscle glycogen concentration declined to similar levels after a 20.9 km run ($\sim 83 \text{ min}$). This suggested that there was a greater reliance on muscle glycogen during exercise when its concentration was elevated at the start of exercise. Hargreaves et al. (77) examined this issue by exercising 12 untrained men for 40 min at $65\text{-}70\% \text{ VO}_{2\text{peak}}$ with either elevated or lowered muscle glycogen at the onset of exercise. The results indicated that elevated preexercise muscle glycogen concentration significantly increased the rate of muscle glycogen degradation during 40 min of exercise while lowered preexercise muscle glycogen concentration had the opposite effect. The direct influence of glycogen on the PHOS α flux was thought to be the mechanism responsible for the differences in the rate of muscle glycogen degradation. Differences in muscle glycogen concentration did not affect plasma glucose uptake or oxidation as measured by [^3H]glucose tracer techniques.

Carbohydrate supplementation before and during exercise produces higher rates of carbohydrate oxidation likely by maintaining higher plasma concentrations of glucose and insulin (30, 31, 42, 43, 65, 125, 155, 158, 170, 186). The rate that exogenous carbohydrate can be oxidized is a function of the rates

of gastric emptying and intestinal absorption. Gastric emptying is influenced by many factors including the type and concentration of carbohydrate as well as the volume and temperature of the consumed meal. The maximal rate of exogenous carbohydrate oxidation during moderate intensity exercise is about $1.0 \text{ g} \cdot \text{min}^{-1}$ (17, 78, 94, 116, 126) and appears to be unaffected by training status (95, 176).

Fat availability also influences carbohydrate metabolism during exercise and the glucose-fatty acid cycle has been suggested as a possible mechanism. If the glucose-fatty acid cycle existed, the effectiveness of acute fat supplementation on sparing carbohydrate would depend upon whether there was a concomitant increase in fat oxidation. As pointed out by Romijn et al. (151), fat supplementation would likely provide a benefit if plasma FFA availability was a limiting factor to fat oxidation as it appeared to be during 20-30 min of exercise at 85% $\text{VO}_{2\text{peak}}$. There was a 15% reduction in muscle glycogen degradation when the plasma FFA concentration was elevated to 1.00-2.00 mM. A reduced muscle glycogen degradation was found in other studies where plasma FFA concentration was elevated to 0.57-2.00 mM by Intralipid/heparin infusion during 15-60 min of moderate to high intensity exercise (70-85% $\text{VO}_{2\text{peak}}$) (38, 53, 54, 133, 178). Plasma FFA concentrations without Intralipid/heparin infusion under these conditions were approximately 0.34 mM. By contrast, elevation of plasma FFA by Intralipid/heparin infusion did not significantly elevate fat oxidation or spare muscle glycogen at a low exercise intensity (44% $\text{VO}_{2\text{peak}}$) where plasma FFA availability was likely not limiting (148) or during one-legged knee extensions (76).

However, little or no research supports an existence of the glucose-fatty acid cycle during exercise. Romijn et al. (151) did not find a reduction in plasma glucose uptake with an elevated plasma FFA concentration as would be predicted by the glucose-fatty acid cycle. Dyck et al. (54) observed no difference in citrate or acetyl-CoA concentrations or PDH α activity between Intralipid/heparin infused and control conditions during high intensity, short duration exercise (15 min at 85% $\text{VO}_{2\text{peak}}$). The sparing of carbohydrate under these conditions was thought to be related to the posttransformational down-regulation of PHOS as increases in AMP and inorganic phosphate, an allosteric activator and substrate of PHOS α respectively, were attenuated by Intralipid/heparin infusion (53). The picture may be slightly different during exercise at moderate intensities. Intralipid/heparin infusion (133) and a 3 d low carbohydrate diet (145) resulted in decreased PDH α activity in addition to posttransformational down-regulation of PHOS

during 45-60 min of exercise at 65-75% $\text{VO}_{2\text{peak}}$. It appears that the down-regulation of PDH under these conditions may be due to the rapid increase in PDK kinase that is responsible for the transformation to the less active PDH *b* (137).

2.12.2 Adaptations in Carbohydrate Metabolism with Endurance Training

Endurance training stresses the balance between ATP production and utilization. This creates a stimulus for adaptation that results in tighter matching of this energy production and utilization balance. The mechanism behind training induced increases in TCA cycle enzyme activities is not well understood. It has been suggested that the increased flux of carbohydrate and fat through the TCA cycle associated with endurance training dictates the increased activity of TCA enzymes (81).

Before discussing the adaptations of substrate metabolism to endurance training, a note of caution is warranted. Most studies compare patterns of substrate use before and after training at the same absolute intensity (i.e., the same absolute workload). The reason for using the same absolute intensity is that it demands the same caloric expenditure and places equivalent metabolic stress on the muscle. However, many of the hormonal responses to exercise that influence substrate metabolism are coupled with relative rather than absolute intensity in the trained and untrained states (48). In addition, it is more applicable to compare subjects at the same relative intensity as this is likely the intensity to be chosen by athletes following a training regimen. Therefore, there is a tradeoff between keeping metabolism the same and keeping hormonal influence the same, both of which are desirable. A consensus has not been reached as to whether absolute or relative comparisons are more appropriate, but given the circumstances, testing using both approaches is the most desirable.

The most apparent change in substrate metabolism with training is the increased oxidation of fat. Evidence for the increase in fat oxidation came from the observation of a lower respiratory exchange ratio (RER) at the same absolute intensity after training (27, 92, 100). These results have been confirmed using tracer methodology (141). The advantage of this adaptation is that it reduces the oxidation of limited carbohydrate stores (69, 71, 84, 100). While much of the carbohydrate that was "spared" was assumed to be muscle glycogen, recent evidence has demonstrated the reduced oxidation of plasma glucose after training (32, 34, 36, 92, 124, 141). This may explain in part why trained individuals have a greater resistance to exercise-induced hypoglycemia.

In support of this contention, 12 wk of endurance training resulted in reduced plasma glucose turnover, clearance, and oxidation when subjects were tested at 60% pretraining $\text{VO}_{2\text{peak}}$ for 2 h (32). Subjects oxidized more fat as indicated by lower RER during the final 30 min of exercise and roughly half of the reduced carbohydrate oxidation during this period was due to the decreased oxidation of plasma glucose. The same training regimen reduced hepatic glucose production by 42% when subjects were tested at 60% pretraining $\text{VO}_{2\text{peak}}$ for 2 h (36). This reduction was due primarily to reduced liver glycogen degradation although significant reductions in liver gluconeogenesis were also reported. More recent evidence suggests that endurance training increases gluconeogenesis both at rest and during exercise (13). The contribution of gluconeogenesis to glucose rate of appearance (R_a) doubled at rest and tripled during exercise at the same absolute and relative intensity after 9 wk of training. It is likely that this occurs despite training-induced blunted glucagon responses and decreases in the gluconeogenic precursor lactate because of increases in hepatic glucagon sensitivity (51) and hepatic lactate metabolic clearance rate in trained subjects.

A shortcoming of much of the work in this area is its use of low to moderate intensity exercise ($\sim 60\% \text{VO}_{2\text{peak}}$) that may not produce a significant reduction in plasma glucose in either trained or untrained subjects (50). Plasma glucose is oxidized at a high rate in the later stages of prolonged exercise performed between 65–85% $\text{VO}_{2\text{peak}}$, providing an alternative source of carbohydrate oxidation when muscle glycogen is lowered. Coggan et al. (34) addressed this issue by measuring glucose kinetics in both trained and untrained subjects during 30 min of cycling at 80% $\text{VO}_{2\text{peak}}$. While glucose R_a was not different between the groups, the trained subjects had a 19% lower rate of plasma glucose rate of disappearance (R_d). Although the exercise intensity was higher than in previous studies, the 30 min exercise duration was not long enough to present any major challenge to the plasma glucose concentration.

The results of this study may also be questioned due to its cross-sectional design using subjects that may differ in their genetic potential of substrate metabolism (19, 57). However, the influence of training on glucose kinetics was recently examined in a longitudinal design that included both absolute and relative comparisons of appropriate intensity and duration (57). Healthy, sedentary males were tested for 1 h at 65% $\text{VO}_{2\text{peak}}$ before training, trained for 10 weeks, and then were retested at 65% of both their pre- and post-training $\text{VO}_{2\text{peak}}$ (i.e., 65% absolute and relative intensity). While training reduced glucose R_a , R_d , and plasma glucose oxidation at the same absolute intensity, there were no significant changes in these

variables at the same relative intensity. Similar results were found with female subjects (58). It has also recently been shown with the combination of tracer and limb-balance techniques that training-induced decreases in glucose flux at the same absolute intensity are primarily due to changes in active rather than inactive tissues (12).

Changes in lactate kinetics during exercise with endurance training are also reflective of adaptations in carbohydrate metabolism and occur rapidly with a half-time of 10.7 days at the same absolute workload (61). Unlike the measurement of glucose kinetics which does not need to account for any appreciable release of substrate from skeletal muscle, the measurement of lactate kinetics is complicated by the release of lactate by both active and inactive tissues during exercise (3). Thus, previous studies using tracer techniques cannot distinguish between these two lactate sources with measurements of whole body lactate R_e and R_d (139) and limb-balance studies of lactate release are confounded by simultaneous lactate uptake and oxidation (14). The recent combination of these two techniques allowed for a complete description of lactate kinetics adaptation to 9 wk of endurance training (14). Training resulted in significant reductions in arterial lactate concentration at both the same absolute and relative exercise intensities. At the same absolute intensity the reduced lactate concentration was a function of increased leg lactate clearance and decreased whole body and leg lactate production, while at the same relative intensity whole body and leg lactate clearance were increased and production was unaffected. The decreased lactate production was thought to be the result of reduced glycolytic flux while the increased clearance is likely the result of increases in sarcolemmal and mitochondrial lactate transporters responsible for cell-to-cell lactate exchange as well as intracellular lactate transport into mitochondria for oxidation (52).

An adaptation that seems contradictory to the results of the above studies is the 2- to 3-fold increase in skeletal muscle GLUT-4 concentration with training (86, 87) possibly in response to reductions in skeletal muscle ATP concentration (187). This coupled with greater insulin sensitivity (46), a higher insulin concentration at the same relative intensity, and greater hexokinase activity in the trained state would seem to favor plasma glucose uptake. However, glucose R_d at the same absolute intensity is clearly reduced after training (12, 57, 58, 124) as a result of blunted sarcolemmal GLUT-4 translocation (149, 182). There is evidence from human and animal models that adaptations in GLUT-4 play a key role in the greater rate and magnitude of muscle glycogen synthesis following glycogen depleting exercise (72, 123, 129).

2.12.3 Adaptations in Carbohydrate Metabolism with Short-Term Training

Short-term aerobic training studies were first introduced to elucidate the time course of metabolic adaptations to endurance exercise training. These studies have used training regimens of 3-14 d of training 45-120 min·d⁻¹ at 55-75% VO_{2peak} with some use of 6 x 1-5 min intervals at 85-100% VO_{2peak}. Consistent findings include increased phosphorylation potential (23, 66, 140), improved cellular energy state (23, 26, 66), reduced muscle glycogenolysis (26, 69, 71, 138, 140, 141, 144) and glucose R_a and R_d (124, 138, 141), increased GLUT-4 content (74, 86), decreased lactate production (144) and increased lactate clearance (139) and transport capacity (16), blunted catecholamine response (70, 124, 138, 141), better matching of glycolytic and PDH flux (144), and improved fatigue resistance (68). However, considerable debate exists regarding the mechanisms responsible for these adaptations. A recent study demonstrated that only 16 h of training (6 min·h⁻¹ at ~90% VO_{2peak}) reduced PCr and glycogen use 36-48 h after the last training session without any changes in maximal activities of citrate synthase or malate dehydrogenase (67). This study and others (66, 69, 71, 138, 140, 141) suggest that early adaptations take place before increases in mitochondrial oxidative potential and may be related to factors such as plasma volume expansion. They further suggest that the initial adaptation to the metabolic stress of endurance training is improved mitochondrial sensitivity to the cellular energy state. As training continues, further metabolic control occurs and is accompanied by increased mitochondrial volume and increased activity of enzymes involved in the TCA cycle. Others find evidence of increases in mitochondrial enzyme activity (26, 162) and mitochondrial ATP production rate (165) and have shown that acute volume expansion alone cannot explain early metabolic adaptations (180). While the mechanism for these early adaptations are not yet clear, they represent a pivotal step for further adaptations with continued training.

2.13 FAT TURNOVER DURING EXERCISE

2.13.1 The Influence of Intensity, Duration, and Substrate Availability

Intensity, duration, plasma FFA availability, and carbohydrate availability influence the oxidation of fat during exercise. Romijn et al. (150) performed the most extensive examination of the influence of both exercise intensity and duration on fat metabolism. Trained cyclists exercised for 120 min at 25 and 65% VO_{2peak} and 30 min at 85% VO_{2peak} on separate days in random order. Whole body fat oxidation, plasma FFA oxidation, IMTG oxidation, the R_a of plasma FFA, as well as the rates of both peripheral

(adipose tissue) lipolysis, and IMTG lipolysis were quantified through the combined use of indirect calorimetry and stable isotope tracer techniques. The R_a of glycerol was used as the marker of whole body lipolysis.

While glycerol R_a increased for all three conditions indicating increased whole body lipolysis, plasma FFA concentration only increased at 25 and 65% VO_{2peak} to 1.0–1.2 mM. The decrease in plasma FFA concentration from 1.0 to 0.5 mM at 85% VO_{2peak} in spite of an increased rate of lipolysis, indicated that fatty acid mobilization was impaired. While fatty acids were liberated by lipolysis, they were not being transported from the adipose tissue at a rate that matched that of lipolysis. This was confirmed by the failure of FFA R_a to increase above resting values at 85% VO_{2peak} . Thus, basal plasma FFA R_a coupled with increased skeletal muscle FFA uptake produced a decrease in plasma FFA concentration at 85% VO_{2peak} .

The rates of whole body lipolysis were greater for the 65 and 85% VO_{2peak} conditions. This was due to greater IMTG lipolysis because peripheral lipolysis was equivalent for each exercise intensity. Whole body fat oxidation was greater than could be accounted for by plasma FFA uptake and oxidation at 65 and 85% VO_{2peak} , suggesting that IMTG provided the additional source of fat oxidation at these intensities.

At 25% VO_{2peak} fat provided 86% of the energy expended and nearly all of this was accounted for by plasma FFA oxidation. IMTG oxidation only occurred at the two higher intensities of exercise. At 65% VO_{2peak} both plasma FFA and IMTG contributed 30% of the total energy expenditure and this decreased to 13% each at 85% VO_{2peak} . This study indicated that fat is the major substrate for low and moderate exercise intensities below 65% VO_{2peak} . The decreased contribution of plasma FFA between 25 and 65% VO_{2peak} was partially compensated by the increased oxidation of IMTG.

The effects of exercise duration on fat oxidation could only be examined at 25 and 65% VO_{2peak} because these workloads were maintained for 2 h. There were no significant fluctuations in substrate use at 25% VO_{2peak} from 30 min to the end of the test. At 65% VO_{2peak} there was a progressive increase in plasma FFA and glucose oxidation while total fat and carbohydrate oxidation did not change. Therefore, it was concluded that there was a progressive reduction in the use of IMTG and muscle glycogen as these stores became depleted. This was compensated for by the increased metabolism of plasma FFA and

glucose. It was estimated that the contribution of plasma FFA to total energy expenditure rose from 30% during the first 30 min to 49% by the end of the test. On the other hand, the contribution of IMTG decreased from 27% to 16% by the end of the test.

The effect of exercise duration on plasma FFA utilization can also be viewed as the effect of intramuscular substrate depletion on plasma FFA utilization. Evidence that contradicts the findings above was presented by Turcotte et al. (172). This study compared the uptake and oxidation of the FFA palmitate in contracting perfused rat skeletal muscle that was either glycogen depleted or supercompensated by prior exercise and dietary manipulations. Muscle glycogen concentration of the depleted rats was approximately 50% lower than the supercompensated rats while IMTG content was similar. Palmitate uptake and oxidation at rest and during electrically stimulated contractions were the same in both conditions. In contrast, glucose uptake was increased in the glycogen depleted condition at rest and during contractions. The authors suggested that the regulation of the pathways of carbohydrate metabolism took precedence over the regulation of the pathways between fat and carbohydrate metabolism. However, as IMTG content was similar between the two conditions there is the possibility that plasma FFA uptake and oxidation are also influenced by the availability of IMTG.

The availability of plasma FFA is thought to influence its uptake by skeletal muscle both at rest and during exercise. The minimal contribution of plasma FFA to energy expenditure during high intensity exercise is thought to be related to the fact that the mobilization, transport, and uptake of plasma FFA are simply too slow to support high rates of metabolism. Romijn et al. (150) indicated that the plasma FFA concentration decreased by 50% at 85% $\text{VO}_{2\text{peak}}$ and coincided with a lower rate of fat oxidation than at 65% $\text{VO}_{2\text{peak}}$. There is the possibility that part of the reason for the decrease in fat oxidation was decreased plasma FFA availability.

This was examined by Romijn et al. (151) by exercising trained subjects for 20-30 min at 85% $\text{VO}_{2\text{peak}}$ under control conditions when plasma FFA was low or when plasma FFA was maintained between 1.0-2.0 mM by the infusion of Intralipid and heparin. Total fat oxidation was measured by indirect calorimetry and plasma FFA kinetics were measured by stable isotope tracer techniques. Increasing plasma FFA availability increased total fat oxidation by 27% compared to control. However, the increased rate of fat oxidation was still lower than that measured at 65% $\text{VO}_{2\text{peak}}$. Therefore, it seems that part of the reason for lower fat oxidation rates during high intensity exercise is due to the failure to increase fatty acid

mobilization following lipolysis. The other factors responsible for the decreased rate of fat oxidation were not clear from this study. It has been suggested that fat entry into the mitochondria and oxidation at higher intensities of exercise may be limited due to increases in malonyl-CoA and its inhibitory effect on CPT I (44, 151). However, Odland et al. (134) reported no differences in malonyl-CoA during exercise at 35, 65, and 90% $\text{VO}_{2\text{peak}}$. Conversely, the increase in fat oxidation from rest to 35% $\text{VO}_{2\text{peak}}$ was not associated with a decrease in malonyl-CoA. This has been confirmed during prolonged submaximal exercise (132). It was concluded that the CPT I activity during exercise is not simply regulated by changes in malonyl-CoA concentration, but may be affected by other factors that inhibit CPT I or alter the effect of malonyl-CoA. Starritt et al. (166) examined the in vitro interaction of malonyl-CoA and CPT I in intact trained and untrained human subsarcolemmal mitochondria under simulated metabolic conditions of rest and exercise. A small decrease in pH from 7.0 to 6.8 resulted in a 40% decline in CPT I activity in both trained and untrained muscle samples. Therefore, decreases in muscle pH with increasing exercise intensity, rather than changes in malonyl-CoA, may explain the reduced oxidation of fat. This same mechanism may explain the increased oxidation of fat during prolonged exercise as muscle glycogen becomes depleted and glycolytic flux is decreased.

Finally, fat oxidation during exercise may be directly regulated by carbohydrate availability (44). Compared to an overnight fast, glucose ingestion before 40 min of exercise at 50% $\text{VO}_{2\text{peak}}$ elevated plasma glucose and insulin concentrations and reduced fat oxidation by 34%. Stable isotope tracer techniques revealed that LCFA oxidation was reduced while MCFA oxidation was unchanged. These results indicate that increases in glycolytic flux due to elevated plasma glucose and insulin concentrations during exercise selectively inhibit long chain fatty acid oxidation. Medium chain fatty acid oxidation is not affected, however, due to its unregulated mitochondrial transport. These effects may be mediated by the inhibition of CPT I by decreases in pH associated with the high glycolytic flux (166).

2.13.2 Adaptations in Fat Metabolism with Endurance Training

Endurance training results in a greater reliance on fat oxidation during exercise at the same absolute intensity (27, 92, 141). This shift in substrate utilization is thought to be the result of the increase in the oxidative capacity of skeletal muscle.

Just as there is disagreement with respect to the source of carbohydrate sparing with training, there are three hypotheses regarding the source of increased fat utilization after training. Some investigators believe that training leads to an increased oxidation of plasma FFA (102, 103, 174) possibly in females (56) and not males (11, 59). The fact that training increases the potential for rapid plasma FFA oxidation at the beginning of exercise (152) and that the expression of a fatty acid binding protein thought to mediate skeletal muscle FFA uptake increases with training (104, 171) supports this perspective. Many of the studies in this area have employed one-legged testing and training that results in much lower fluctuations in hormones that influence substrate utilization.

The other hypothesis proposes that the increased reliance on fat is due to increased IMTG oxidation (90, 114, 115, 141). Hurley et al. (90) found that two-legged exercise at 64% of pretraining $\text{VO}_{2\text{peak}}$ after 12 wk of training produced an increased oxidation of fat, a 41% decrease in muscle glycogen use, and a doubling of IMTG degradation during exercise. Martin et al. (115) later reported reduced plasma FFA uptake and oxidation despite an increase in total fat oxidation after training. The increased oxidation of IMTG was thought to account for the difference.

Regardless of the source, aerobic training-induced increases in LCFA oxidation must be accompanied by increases in LCFA transport into the mitochondria by CPT I. Recent evidence suggests that muscle pH is a more potent regulator of CPT I activity during exercise than malonyl-CoA and may explain these training adaptations (166). The improved respiratory control of aerobic trained muscle and resulting attenuated lactate production at the same absolute intensity should lessen the inhibition of decreased pH on CPT I allowing for greater rates of LCFA mitochondrial transport and oxidation.

The third and more recent hypothesis proposes that inactive tissue is solely responsible for the increased reliance on fat in males (11). Endurance training resulted in reduced RER at the same absolute intensity (65% pretraining $\text{VO}_{2\text{peak}}$) with unchanged leg respiratory quotient, leg FFA fractional extraction, leg net glycerol release, IMTG degradation rates (11) and whole body glycerol R_a (59) at the same absolute and relative intensity. These results suggest that the use of plasma FFA and IMTG by active tissue is unaffected in males by training and that inactive tissues increase their use of fat thereby creating a “shunt” of valuable carbohydrate to dependent active skeletal muscle. The divergence of these results from previous studies is likely the result of two methodological differences. Limb-balance techniques were used in

addition to tracer techniques to improve measures of active tissue FFA and IMTG use and it is suggested that the use of RER and FFA R_d overestimates IMTG degradation. Additionally, subjects were 4.5-5 rather than 12 h fasted to better mimic normal practices and conditions.

2.13.3 Adaptations in Fat Metabolism with Short-Term Training

Little is known of the early adaptations in fat metabolism to endurance training. Training $2 \text{ h} \cdot \text{d}^{-1}$ at 59% $\text{VO}_{2\text{peak}}$ for 10 consecutive days significantly increased whole body fat oxidation during 90 min of exercise at 59% $\text{VO}_{2\text{peak}}$ (138). Tracer methodology revealed that increased IMTG use was likely responsible for the increased fat oxidation and that glycerol R_d , a measure of whole body lipolysis, was also significantly increased after only 5 d of training (141). This rapid increase in whole body fat oxidation seems to be supported by the increased maximal activities of enzymes involved in β -oxidation (162).

2.14 CARBOHYDRATE SUPPLEMENTATION AND ENDURANCE PERFORMANCE

2.14.1 Carbohydrate Supplementation During Exercise

The fact that prolonged moderate intensity exercise is limited by carbohydrate availability has led to studies of the effects of carbohydrate supplementation on endurance performance. During the 1970's fluid replacement was thought to be more beneficial to endurance performance than carbohydrate consumption during exercise (78) due to reports that even low concentration carbohydrate solutions delayed gastric emptying (39). Later research demonstrated that these results may have been due to the low volumes of solution consumed (126). However, unequivocal evidence of the benefits of carbohydrate supplementation during exercise were realized by Coyle et al. (43). Trained cyclists who consumed $1.0 \text{ g carbohydrate} \cdot \text{kg body weight}^{-1}$ (50% solution) after 20 min and then consumed $0.25 \text{ g carbohydrate} \cdot \text{kg}^{-1}$ (6% solution) after 60, 90, and 120 min of exercise at 74% $\text{VO}_{2\text{peak}}$ maintained plasma glucose and extended time to exhaustion by 23 min when compared to the placebo condition.

The hypothesis that plasma glucose uptake and oxidation was greater with carbohydrate supplementation coupled with the fact that the percentage of energy derived from carbohydrate was not different between the carbohydrate and placebo conditions was thought to indicate that the rate of muscle glycogen degradation was lowered by carbohydrate consumption during exercise. This "sparing" of muscle glycogen presumably accounted for the improved performance. However, examination of this interpretation

later showed that carbohydrate supplementation during exercise did not “spare” muscle glycogen, but instead prevented the decline of plasma glucose (42). The maintenance of this alternative source of carbohydrate allowed subjects to maintain high rates of carbohydrate oxidation late in exercise when muscle glycogen was lowered. What remained unanswered was whether carbohydrate supplementation “spared” liver glycogen.

Bosch et al. (17) found that trained, carbohydrate loaded subjects had significantly lower rates of hepatic glucose production when consuming a 10% carbohydrate drink during 180 min of exercise at 70% $\text{VO}_{2\text{peak}}$ compared to placebo. The reduced hepatic glucose production was likely contributed to by reduced liver glycogen degradation and gluconeogenesis and the effect could delay the decline in liver glycogen. Consistent with the findings of Coyle et al. (42), muscle glycogen degradation was not different between conditions. McConnell et al. (121) reported similar results with trained, non-carbohydrate loaded subjects during a 120 min ride at 69% $\text{VO}_{2\text{peak}}$. Ingestion of a 10% carbohydrate solution during exercise reduced hepatic glucose production by 51% compared to placebo. Hepatic glucose production was reduced due to its inhibition by elevated plasma glucose and insulin resulting from carbohydrate consumption.

2.14.2 Carbohydrate Supplementation Before Exercise

The evaluation of the effectiveness of preexercise carbohydrate supplementation is similar to that of the evaluation of the effectiveness of carbohydrate supplementation during exercise. Initially preexercise carbohydrate consumption was proposed to produce hormonal changes that would decrease endurance performance. Costill et al. (38) found that ingesting 75 g of glucose 45 min before 30 min of running at 70% $\text{VO}_{2\text{peak}}$ resulted in increased carbohydrate oxidation including increased muscle glycogen degradation, elevated insulin concentrations before and throughout exercise, and a premature lowering of plasma glucose from 5.0 to 3.5 mM by the tenth minute of exercise. It was proposed the higher insulin concentration produced the drastic decrease in plasma glucose that, when combined with the lack of an increase in plasma FFA concentration, placed a greater demand on muscle glycogen as a substrate. The authors noted, however, that a 30 min exercise test was not sufficient to assess the effects of these changes on endurance performance.

To evaluate the effects of preexercise carbohydrate consumption on endurance performance, Sherman et al. (155) fed moderately trained cyclists 0, 45, 156, or 312 g of carbohydrate 4 h before 95 min of cycling that was followed by a performance ride. Consuming 312 g of carbohydrate produced a 15%

improvement in performance compared to placebo while the 45 and 156 g trials did not affect performance. Consuming 312 g of carbohydrate produced a decrease in plasma glucose in the first 15 min of exercise, but this was only temporary because it then increased and remained constant at “normal” levels thereafter (Figure 2). The improved performance was thought to be due to the ability to maintain a high rate of carbohydrate oxidation by the continued absorption of the large carbohydrate meal during exercise. In support of this conclusion, Montain et al. (125) found that carbohydrate oxidation during exercise remained elevated for up to 6 h after consuming a 125-150 g carbohydrate meal.

Later results have demonstrated that carbohydrate feedings (1.1 and $2.2 \text{ g}\cdot\text{kg}^{-1}$) 1 h before exercise also improved time trial performance following a 90 min ride at 70% $\text{VO}_{2\text{peak}}$ (158). Moreover, combining carbohydrate feedings before and during exercise provided further improvements in performance than either manipulation alone (186).

The mechanisms responsible for the transient decrease in plasma glucose, increased carbohydrate oxidation, and improved performance with preexercise carbohydrate consumption were outlined by Marmy-Conus et al. (112). Trained subjects consumed either 75 g of glucose or placebo 30 min before 1 h of exercise at 71% $\text{VO}_{2\text{peak}}$ on two separate occasions. Glucose kinetics were assessed with the use of a primed continuous infusion of $[6,6\text{-}^2\text{H}]\text{glucose}$. Ingestion of carbohydrate resulted in increased plasma glucose uptake and suppression of hepatic glucose production compared to placebo. This imbalance initially caused a decrease in plasma glucose to 4.2 mM during the first 20 min of exercise. However, this was counteracted by further increases in the absorption of exogenous carbohydrate from the small intestine causing plasma glucose to increase and subsequently be maintained at ~ 5.5 mM after 40 min. The reduced hepatic glucose production compared to placebo was likely the result of higher plasma glucose and insulin concentrations. The initial higher plasma glucose uptake probably resulted from the combined stimulatory effects of higher plasma insulin concentration and muscle contraction.

2.14.3 Muscle Glycogen Supercompensation

The idea that high carbohydrate diets are beneficial for endurance training and performance comes not only from the overwhelming evidence of the effectiveness of carbohydrate supplementation before and during exercise, but also from short-term training and dietary manipulations (3-7 d) that significantly increase muscle glycogen concentration (15, 79, 111, 156, 169) and in some cases improve performance (15, 169). Bergstrom et al. (15) found that the combination of two glycogen depleting exercise sessions

interspersed by a 3 d high fat/protein diet followed by a 3 d high carbohydrate diet resulted in muscle glycogen concentrations of ~ 220 mmol glucose $\cdot$ kg ww $^{-1}$ compared to ~ 40 -50 and 115 mmol $\cdot$ kg ww $^{-1}$ after high fat/protein and mixed diets, respectively. The differences in initial muscle glycogen concentration were thought to be a major determinant in the vast differences in time to exhaustion at 75% $\text{VO}_{2\text{peak}}$ observed after each dietary manipulation (59, 126, and 189 min for high fat/protein, mixed, and high carbohydrate diets, respectively).

In a subsequent study it was demonstrated that muscle glycogen could be supercompensated with less drastic exercise and dietary manipulations (156). Three days of a low (15% carbohydrate) or moderate (50% carbohydrate) carbohydrate diet followed by 3 d of a high carbohydrate diet (70% carbohydrate) with a tapered exercise sequence (90 min on day one to rest on day six) resulted in a muscle glycogen concentration of ~ 205 -210 mmol glucose $\cdot$ kg ww $^{-1}$. Six days of the moderate carbohydrate diet and the same tapered exercise sequence resulted in a muscle glycogen concentration of ~ 159 mmol glucose $\cdot$ kg ww $^{-1}$. Despite these differences in muscle glycogen concentration, no difference was found in 20.9 km run time (~ 83 min) suggesting that muscle glycogen supercompensation would only benefit longer duration exercise. This has been confirmed in subsequent studies (79, 111), however, others suggest that muscle glycogen supercompensation may improve short duration, high intensity exercise performance (118, 143).

Although others have found muscle glycogen supercompensation to benefit endurance performance (15, 169), the reasons for this improvement are not entirely clear. The exercise and dietary manipulations consistently produce a greater muscle glycogen concentration that would seem to prolong its depletion. However, the rate of muscle glycogen degradation is higher with higher initial muscle glycogen concentrations (77, 156). It is unlikely this completely counteracts the benefits of increased muscle glycogen stores because improvements in endurance performance have been observed (15, 169). Rather, the benefit of muscle glycogen supercompensation may be derived from an increase in liver glycogen concentration as it is known to increase with high carbohydrate diets (89, 131). Muscle glycogen supercompensation has also been found to reduce the relative contribution of plasma glucose to total carbohydrate oxidation (18). These effects may improve performance by delaying the decline in plasma glucose allowing a higher rate of carbohydrate oxidation late in exercise compared to the non-supercompensated state. Others suggest that previously reported ergogenic effects of muscle glycogen supercompensation may be due to a placebo effect (22). Trained cyclists completed two separate 100-km

time trials after 3 d carbohydrate loading ($9 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and placebo-controlled moderate carbohydrate ($6 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) diets in random crossover fashion. Time trial performance was not different (147.5 vs. 149.1 min) despite differences in preexercise muscle glycogen content. The applicability of these results is strengthened by the fact that subjects were fed carbohydrate before and during exercise.

2.14.4 Chronic Carbohydrate Supplementation, Adaptation, and Endurance Performance

Does dietary composition influence the adaptive response to training that will ultimately influence performance? To address this question, the effect of consuming either a moderate ($5 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) or high carbohydrate diet ($10 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) on training and performance was examined over a 7 d period (157). Subjects were trained runners and cyclists and training consisted of 1 h of running or cycling at 75% of mode-specific $\text{VO}_{2\text{peak}}$ followed by five 1 min sprints at 100% $\text{VO}_{2\text{peak}}$ with 1 min rest intervals. Muscle biopsies were obtained on four separate days before exercise. Subjects consuming the high carbohydrate diet maintained basal muscle glycogen concentration over the 7 d training period, while those on the moderate carbohydrate diet exhibited a 30-36% reduction by day five. Despite these differences all of the subjects successfully completed all training sessions and performed similarly during a test to exhaustion at 80% $\text{VO}_{2\text{peak}}$ on day seven.

While Bergstrom et al. (15) found a close association between initial muscle glycogen concentration and endurance performance, the differences in concentration were more extreme under two of the conditions. The high fat/protein diet resulted in a muscle glycogen concentration of $40\text{-}50 \text{ mmol glucose} \cdot \text{kg ww}^{-1}$ and a 3-fold lower time to exhaustion compared to the high carbohydrate diet that produced a muscle glycogen concentration of $\sim 220 \text{ mmol glucose} \cdot \text{kg ww}^{-1}$. Muscle glycogen concentrations in the Sherman et al. (157) study ranged from 104 to 140 $\text{mmol glucose} \cdot \text{kg ww}^{-1}$ for cyclists on the moderate and high carbohydrate diets, respectively, and from 84 to 122 $\text{mmol glucose} \cdot \text{kg ww}^{-1}$ for runners on the moderate and high carbohydrate diets, respectively. While muscle glycogen was different between the two diets (157), the magnitude of that difference may have been too small to influence exercise capacity or the variability in performance was too large to detect the effect of the smaller difference in muscle glycogen concentration. The other possibility is that the dietary manipulation was too brief to have an effect on endurance performance as significant differences in muscle glycogen concentration between the diets did not occur until after days three and five of the protocol.

Very few studies have manipulated both diet and exercise to determine the long term effects of dietary composition on endurance performance (Table 2). The effects of four weeks of consuming either 5 or 10 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ on rowing training ability and performance was investigated by Simonsen et al. (160). Male and female collegiate rowers were subjects and trained intensely twice daily over the four weeks. Those consuming 5 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ maintained a muscle glycogen concentration of 119 mmol glucose $\cdot$ kg ww $^{-1}$, while those consuming 10 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ had a progressive 65% increase to 155 mmol glucose $\cdot$ kg ww $^{-1}$ over the four weeks. While all subjects completed the training sessions successfully, those consuming the high carbohydrate diet showed greater improvements in mean power output in time trials performed three times weekly (+11%) than those consuming the moderate carbohydrate diet (+2%). This study provides evidence that a high carbohydrate diet consumed chronically optimizes improvements in performance of trained subjects compared to a moderate carbohydrate diet. However, it appears that a moderate carbohydrate diet does not reduce one's ability to complete rigorous training sessions for up to one month.

Previous studies that have manipulated the diets of trained subjects did not "greatly" alter training habits. While this allows investigation of the influence of dietary composition on training ability and performance, it does not allow determination of the influence of diet on the adaptations associated with endurance training and subsequent improvements in performance. Recently (82), 20 untrained males underwent eight weeks of identical aerobic training while consuming either a high carbohydrate diet (CHO) (65% carbohydrate, 20% fat, 6.8 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$) or a high fat diet (FAT) (62% fat, 22% carbohydrate, 2.4 g carbohydrate $\cdot$ kg $^{-1}\cdot$ d $^{-1}$). During the eighth week both groups consumed the high carbohydrate diet. Although both groups had increased $\text{VO}_{2\text{peak}}$ 11% by the seventh week of training, the carbohydrate group increased time to exhaustion at 81% of pretraining $\text{VO}_{2\text{peak}}$ by nearly 3-fold while the FAT group only increased 1.8-fold. Therefore, although both groups had the same baseline time to exhaustion and followed the same training program, the difference in diet accounted for a 37 min difference in time to exhaustion after seven weeks of training.

Could inadequate carbohydrate stores explain the poor performance of the FAT group? At the same absolute intensity, the FAT group had a significant decrease in RER indicating a greater reliance on fat as a substrate during exercise. Despite the low carbohydrate intake of the FAT group, subjects completed training sessions successfully and muscle glycogen concentration did not significantly change

between baseline and week seven ($\sim 118 \text{ mmol glucose} \cdot \text{kg ww}^{-1}$). Both groups had similar rates of muscle glycogen degradation before and after training and demonstrated a significant sparing of muscle glycogen as degradation rates decreased by $\sim 51\%$ in both groups as a result of seven weeks of training. The FAT group also maintained a higher plasma glucose concentration during most of exercise. Moreover, when the FAT group was switched to the high carbohydrate diet and exhibited a 73% increase in muscle glycogen concentration, their time to exhaustion was still less than the CHO group.

Therefore, the fact that the FAT group maintained or increased muscle glycogen content and maintained plasma glucose during exercise suggested that their attenuated improvement in endurance performance was not due to a lack of carbohydrate availability, but rather may have been due to suboptimal adaptations in the ability to utilize carbohydrate during exercise creating a greater reliance on fat. The reduction in carbohydrate oxidation was likely the result of decreased oxidation of plasma glucose because the rate of muscle glycogen degradation was similar between groups.

Animal studies suggest that insulin resistance may be a mechanism for reduced plasma glucose uptake and oxidation with a high fat diet. Rats fed high fat diets developed insulin resistance and consequent reduced rates of insulin stimulated muscle glucose uptake (21, 73) that were only partially reversed by exercise training (101). Mechanisms responsible for the development of insulin resistance with high fat diets include reduced insulin stimulated recruitment of GLUT-4 (189) and inhibition of insulin stimulated glucose metabolism (60, 105). Interestingly, contraction mediated GLUT-4 recruitment and glucose uptake were unaffected by a high fat diet (189). There is evidence, however, that physically active humans may be able to prevent high fat diet induced insulin resistance by increasing their ability to dispose of plasma glucose as muscle glycogen (45).

Thus it seems that both trained and untrained individuals benefit from the consumption of high carbohydrate diets during endurance training. Not only has it been shown to result in greater improvements in power output of trained athletes over a four week period, it also appears to be a necessary ingredient for the optimal adaptation by untrained individuals to a typical endurance training program.

2.15 FAT SUPPLEMENTATION AND ENDURANCE PERFORMANCE

2.15.1 Acute Fat Supplementation

Although supplementing plasma FFA by Intralipid/heparin infusion spares muscle glycogen (38, 53, 54, 133, 151, 178), the use of infusion techniques are not practical. More importantly, performance was not measured in these studies. Therefore, although these techniques may result in glycogen sparing they have not been shown to alter endurance performance in humans.

Studies that have examined the effects of acute fat supplementation with high fat meals on exercise performance have not found an effect on time to exhaustion or self-paced cycling performance (135, 154, 164). Starling et al. (164) fed endurance trained athletes either a high carbohydrate (83% carbohydrate) or high fat (68% fat) diet for 12 h following a 120 min ride at 65% $\text{VO}_{2\text{peak}}$. A 12 h overnight fast and a self-paced cycling task followed the 12 h feeding. Although the high fat diet increased IMTG concentration by 36%, it only replaced 13% of the muscle glycogen used during the 120 min ride. The high carbohydrate diet, on the other hand, did not significantly raise IMTG concentration, but did replace 93% of muscle glycogen used. Following the high fat diet, subjects had a lower respiratory exchange ratio (RER) indicating greater oxidation of fat, and a higher plasma FFA concentration during the self-paced cycling task compared to the high carbohydrate diet. During the high fat trial subjects took significantly longer to perform the prescribed cycling task and exhibited a significant decrease in the percentage of $\text{VO}_{2\text{peak}}$ they were able to maintain by the end of the task. Although the high fat diet increased the availability of plasma FFA and enhanced fat oxidation during exercise, self-paced cycling performance was decreased likely as a result of inadequate muscle glycogen synthesis in the 24 h preceding the task.

Okano et al. (135) fed endurance trained athletes either a high carbohydrate or high fat meal 4 h before a ride to exhaustion at 65% (0-120 min) and 85% $\text{VO}_{2\text{peak}}$ (120 min to exhaustion). The high fat meal produced a lower RER suggesting a greater reliance on fat and the possible sparing of carbohydrate. However, times to exhaustion were not significantly different between the conditions and the possible sparing of muscle glycogen could not be assessed because muscle biopsies were not taken.

The rapid absorption and unregulated mitochondrial transport of MCFA has led to the investigation of their efficacy as an ergogenic aid. Coingestion of medium chain TG (MCT) and carbohydrate increased the amount of MCT oxidized during exercise (98). One study has shown

improvements in endurance performance with MCT and carbohydrate coingestion (177) while another has not (8). Others, however, have found that the amount of MCT that can be tolerated without gastrointestinal complaints only contributes 3-7% of total energy expenditure during prolonged exercise (98) even when muscle glycogen is reduced prior to exercise (99). In addition, MCT supplementation does not decrease muscle glycogen utilization during exercise (97).

2.15.2 Chronic Fat Supplementation, Adaptation, and Endurance Performance

Starling et al. (164) demonstrated that acute high fat feedings after prolonged exercise increased fat oxidation during exercise performed 24 h later. Exercise performance was decreased and was likely the result of inadequate muscle glycogen repletion. Although acute fat supplementation may enhance fat oxidation during exercise, the low muscle glycogen stores that result from lower carbohydrate consumption may counteract this. The next question is whether humans can adapt to chronic high fat diets by improving their ability to oxidize fat during exercise, sparing carbohydrate stores, and thereby improving endurance training and possibly performance.

Muoio et al. (128) fed trained runners a 7 d diet containing either 15, 24, or 38% of the energy intake from fat. Carbohydrate was 73, 61, and 50% of the total energy intake, respectively. Running time to exhaustion at 75-85% $\text{VO}_{2\text{peak}}$ and $\text{VO}_{2\text{peak}}$ were highest after the fat diet (38% fat) compared to the other two conditions. It was suggested that increased plasma FFA availability produced the improved performance and $\text{VO}_{2\text{peak}}$. However, RER values were equivalent between the conditions during the runs to exhaustion suggesting that fat oxidation was the same despite differences in plasma FFA concentration. The results of this study must be viewed with caution because the order of the trials was not randomized and the percentage of fat provided to the subjects (38% total energy intake) was not enough to be considered a high fat diet.

Lambert et al. (109) fed trained cyclists either a high carbohydrate (74% carbohydrate) or high fat (67% fat) diet for 2 wk. At the end of 2 wk subjects performed a Wingate test of maximal anaerobic power, a time to exhaustion test at 90% $\text{VO}_{2\text{peak}}$, and finally a time to exhaustion test at 60% $\text{VO}_{2\text{peak}}$ with rest periods between each test. There were no differences in maximal anaerobic power or time to exhaustion at 90% $\text{VO}_{2\text{peak}}$. The high fat diet resulted in significantly longer time to exhaustion at 60%

$\text{VO}_{2\text{peak}}$ despite lower muscle glycogen levels at the onset of exercise. It was suggested that the cyclists were able to adapt to the high fat diet after 2 wk and that this adaptation allowed them to spare endogenous carbohydrate during moderate intensity exercise in a glycogen depleted state.

Phinney et al. (142) found no difference in time to exhaustion at 64% $\text{VO}_{2\text{peak}}$ for trained cyclists before and after 4 wk of consuming a high fat diet despite a 47% decrease in resting muscle glycogen concentration. It was suggested that the subjects adapted to the high fat diet and oxidized fat to a greater extent during exercise and this spared carbohydrate. However, the extremely low carbohydrate intake (< 20 g carbohydrate $\cdot\text{d}^{-1}$) and ketogenic nature of this diet brings into question its applicability for athletes.

Helge et al. (82) is the only study to examine the effects of a high fat diet on the training induced adaptations in fat metabolism of untrained subjects. Untrained subjects were fed either a high carbohydrate (CHO) (65% carbohydrate, 20% fat, 6.8 g carbohydrate $\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) or high fat diet (FAT) (62% fat, 22% carbohydrate, 2.4 g carbohydrate $\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) over an 8 wk period during which both groups underwent identical endurance training. At the end of 7 wk of training both groups increased $\text{VO}_{2\text{peak}}$ by 11%. However, the CHO group increased time to exhaustion at 81% of pretraining $\text{VO}_{2\text{peak}}$ by nearly 3-fold while the FAT group only increased time to exhaustion by 1.8-fold.

The fact that the FAT group had a lower RER and similar rates of glycogen degradation during exercise as the CHO group after 7 wk of training suggested that plasma glucose uptake and oxidation was decreased in the FAT group. In addition to a lower RER during exercise, the FAT group also had similar plasma FFA concentrations as the CHO group despite greater glycerol levels. This implies that plasma FFA uptake and oxidation were greater in the FAT group resulting in similar plasma FFA levels despite greater rates of lipolysis. Alternatively, this may suggest that there was a greater contribution of IMTG oxidation in the FAT group that increased plasma glycerol levels without increasing plasma FFA concentration. The exact pattern of fat utilization cannot be elucidated without the use of tracer techniques.

This study demonstrated that chronic fat supplementation while training resulted in the increased reliance on fat and sparing of carbohydrate during exercise. However, the source of the spared carbohydrate was likely liver glycogen and plasma glucose as the rates of muscle glycogen degradation were not different between groups. While the sparing of plasma glucose for use as an alternate source of carbohydrate late in exercise is likely to improve endurance performance (30, 31, 42, 65), the chronic high fat diet may have given rise to a suboptimal adaptation in the ability of skeletal muscle to take up plasma glucose for

oxidation. The FAT group may have not had the ability to oxidize plasma glucose late in exercise to the same degree as the CHO group, possibly explaining their attenuated improvement in endurance performance.

CHAPTER 3

METHODS

3.1 SUBJECTS

Eight healthy, nonsmoking, weight stable, sedentary males aged 22-34 were recruited for the study. Only males were chosen because it has been demonstrated that females oxidize fat to a greater extent than equally trained males at the same relative workload (168). Such differences may present a confounding variable. Subjects were considered sedentary if they engaged in less than 2 h·wk⁻¹ of aerobic activity for at least 1 y and had an aerobic capacity less than 50 ml·kg⁻¹·min⁻¹. To reduce the chance of including a subject with abnormal glucose kinetics, only subjects with normal body composition ($\leq$ 18% body fat), with normal 8 h fasting blood glucose ($<$ 110 mg·dl⁻¹), and no family history of non-insulin-dependent diabetes mellitus were enrolled. The procedures and risks were thoroughly explained to the subjects and their written informed consent was obtained. The study was approved by the Biomedical Sciences Institutional Review Board of The Ohio State University (#98H0354).

3.2 EXPERIMENTAL DESIGN

Subjects underwent two 10 d experimental phases during which they were fed either a high carbohydrate or high fat diet in randomized fashion. Glucose kinetics at rest and during 90 min of exercise were assessed before and after each experimental phase. The initial 10 d intervention was designed to examine the influence of dietary composition alone on glucose kinetics at rest and during exercise. This was followed by a 2 wk washout period during which subjects consumed their habitual diets and remained

inactive. The second 10 d intervention phase was designed to examine the influence of dietary composition on short-term aerobic training-induced adaptations in glucose kinetics at rest and during exercise.

3.3 PRELIMINARY TESTING

All exercise testing and training sessions were performed on an electronically braked bicycle ergometer (Lode Excalibur Sport). Aerobic capacity ($\text{VO}_{2\text{peak}}$) was assessed by progressively increasing an initial workload of 50 W by 25-50 W every 2 min until voluntary exhaustion. Due to the novelty of this task to sedentary subjects, two aerobic capacity tests separated by at least 3 d were performed at baseline to assure a true maximal effort. True $\text{VO}_{2\text{peak}}$ was defined as the achievement of at least two of the following three criteria: 1) $\text{RER} \geq 1.10$, 2) plateau in VO_2 despite increases in workload, and 3) maximum heart rate within 10% of age predicted value. Aerobic capacity measurements were repeated before and after the second experimental period to assess training-induced changes. Lactate threshold was assessed within 48 h of the determination of $\text{VO}_{2\text{peak}}$. Subjects reported to the laboratory 3-4 h fasted, well hydrated (500 ml of water within 3 h of testing), and abstained from caffeine consumption for 8 h and rigorous exercise for 48 h. A Teflon catheter (21 gauge, 1.25 inch; Angiocath, Becton Dickinson) was secured in an antecubital or forearm vein and subjects rested in a seated position for 5-10 min before the resting blood sample was taken on the bicycle ergometer. Subjects initially exercised for 6 min at a workload corresponding to approximately 30-40% $\text{VO}_{2\text{peak}}$. The workload was progressively increased by 25 W every 6 min until five stages were completed. Blood drawn at the end of each stage was assayed for lactate concentration and lactate threshold was defined as the breakpoint in the lactate concentration-intensity curve. This information was used to set the workload during the glucose kinetics trials to one that corresponded with the lactate threshold. Workloads were set relative to lactate threshold rather than $\text{VO}_{2\text{peak}}$ to minimize intersubject variability in glucose kinetics during exercise (33). Lactate threshold measurements were repeated before and after the second experimental period to assess training-induced changes in lactate threshold. Body density was measured by underwater weighing and body composition was calculated using the Siri equation (161). Subjects completed a 4 d dietary recall (3 weekdays and 1 weekend day) at baseline to document habitual daily energy intake and composition.

3.4 SUBJECT PREPARATION

Subjects were instructed to consume a mixed diet containing at least 250 g carbohydrate $\cdot$ d⁻¹ for 3 d before both baseline trials (trials 1 and 3) to assure similar endogenous carbohydrate stores. In addition, subjects recorded items consumed the evening before their baseline trial and repeated this meal the evening before their second baseline trial. Subjects were instructed to not exercise for 24 h before their trial, avoid caffeine for 8 h before their trial, and consume at least 500 ml of water within 3 h of testing. They reported to the laboratory 6 h after consuming a small standardized meal (4.2 kcal $\cdot$ kg⁻¹; ~290 kcal, 66% carbohydrate, 15% fat, 19% protein). Small meals of this type more closely resemble preexercise eating habits than overnight fasting and have been shown to result in similar metabolic responses during exercise as after an 8-12 h fast (125).

3.5 GLUCOSE TRACER INFUSION PROTOCOL

Prior to isotope infusion, Teflon catheters (21 gauge, 1.25 inch; Angiocath, Becton Dickinson) were placed in veins of the left and right forearms for blood collection and isotope infusion, respectively. Dorsal veins of a hand placed in a thermoregulated heat box are optimally used to secure arterialized venous samples for the determination of substrate kinetics. However, this technique is not amenable to exercise on a bicycle ergometer. Therefore, the right forearm was covered with a flexible heating pad for the arterialization of venous blood and was secured with a large elastic bandage. This adapted technique was used recently (127) and pilot data indicated that it resulted in suitable skin temperatures (40–45 °C) for arterialization of venous blood. A blood sample was taken before infusion to determine background isotopic enrichment. Subjects then received a primed (17 μ mol $\cdot$ kg⁻¹) continuous (0.22 μ mol $\cdot$ kg⁻¹ $\cdot$ min⁻¹) infusion of sterile, pyrogen-free [6,6²H]glucose (99% enriched; Cambridge Isotopes, Cambridge, MA) that was maintained with a calibrated autosyringe (PHD 2000, Harvard Apparatus, South Natick, MA). The glucose tracer solution was dissolved in 0.9% sterile saline by a licensed pharmacist and was infused through a 0.2- μ m filter. To assure isotopic equilibrium this infusion was continued for 2 h of rest. Pilot data indicated that isotopic equilibrium was attained within 75 min at rest. Subjects then sat on the cycle ergometer for 5 min before exercise and the tracer infusion rate was doubled to 0.44 μ mol $\cdot$ kg⁻¹ $\cdot$ min⁻¹ to maintain an appropriate isotopic enrichment during exercise.

3.6 METABOLIC CHALLENGE RIDE

The metabolic challenge ride consisted of 90 min of continuous exercise at a workload that corresponded to the lactate threshold. During this time subjects were encouraged to consume 1200-1600 ml of water to maintain euhydration (4) and were cooled by a fan. Ambient conditions remained constant across all trials (23.5 ± 0.3 °C, 744 ± 1 mm Hg, 62 ± 2 % relative humidity).

3.7 BLOOD SAMPLING

After a background blood sample was taken immediately before the start of infusion, blood samples (10 ml) were drawn at 15 min intervals at the end of rest (-30, -15, and 0 min) and throughout exercise (15, 30, 45, 60, 75, and 90 min). The catheter was maintained patent by intermittent flushing with heparinized sterile saline. Blood samples were distributed into EDTA tubes for the assessment of hematocrit and glucose isotopic enrichment, into sodium fluoride-potassium oxalate tubes for glucose and lactate, and into serum separation tubes for non-esterified fatty acids (NEFA), glycerol, insulin, and glucagon. EDTA and sodium fluoride-potassium oxalate tubes were kept on ice while the serum separation tubes were allowed to clot at room temperature for at least 15 min. All tubes were then centrifuged (3000 rpm, 15 min, 4°C) for the separation of plasma and serum. All samples were stored at -80°C until analysis.

3.8 RESPIRATORY GAS-EXCHANGE MEASUREMENTS

Respiratory measurements were made at minutes 10-20, 40-50, and 80-90 during the metabolic challenge ride. Expired gases were analyzed with a mixing-chamber metabolic measuring system (Ametek S-3A/I oxygen and CD-3A carbon dioxide analyzers, heated pneumotach and pressure transducer, Consentius Technologies metabolic software) that was calibrated with gases of known concentration and a 3-liter syringe prior to each collection period. Calibration procedures were repeated after each collection period to document analyzer drift and respiratory values were adjusted accordingly.

3.9 EXERCISE PERFORMANCE

Exercise performance was assessed with a time trial test following the metabolic challenge ride and a 15 min rest. Subjects completed a target amount of work on an electronically braked ergometer in pedal dependent mode in the fastest time possible (93). The target amount of work was based on 65% of a subject's maximum workload ($W_{\max}$) and an approximate target time of 25 min (1500 s).

$$\text{Target Work (J)} = 0.65 \cdot W_{\max} \cdot 1500$$

The time it took a subject to complete the target amount of work was used as the measurement of exercise performance. The only feedback provided to the subjects during the time trial was elapsed distance and power output. Subjects were not told their performance times until the study was completed. Heart rate, power output, and accumulated work were recorded at 1 min intervals. Respiratory measures were not taken during the time trial to minimize disturbances to subjects' pacing strategies. A familiarization time trial was completed after a previous lactate threshold test to minimize learning effects.

3.10 DIETARY MANIPULATION

Subjects were fed 10 d high carbohydrate and high fat diets (CHO and FAT) in randomized order during the two experimental periods. The energy content of the diets was designed to match subjects' individual daily energy needs using the World Health Organization metabolic equations (185) and an activity factor of 1.5. These figures were adjusted for exercise energy expenditure during training calculated from individual workload- VO_2 regression equations and changes in body weight. The diets were designed to be different in carbohydrate and fat content, but similar in protein and fiber content as well as the ratio between polyunsaturated and saturated fats. The CHO diet reflects the current recommendations of the American Dietetic Association and the Canadian Dietetic Association for physically active adults (5). Both diets are similar to those used by Helge et al. (82).

A 3 d menu that included breakfast, lunch, dinner, and snacks was repeated during each experimental period. Subject's preferred foods were included as much as possible. Subjects were provided with food on a daily basis and were instructed to not deviate from the prescribed diets. Compliance was

improved with the inclusion of extra snacks in case of hunger and a list of low caloric “free foods” that could be added to meals. The menus were prepared on five different energy levels (2400, 2800, 3200, 3600, and 4000 kcal) and snacks were used to precisely adjust energy intake (6). Subjects were not allowed to consume alcohol or caffeine during the study. Subjects recorded food not provided to them and omissions from the prescribed diet in journals if necessary.

3.11 AEROBIC EXERCISE TRAINING PROGRAM

All subjects completed identical 10 d bicycle ergometer training programs that were supervised by the primary investigator. The 10 d training protocol consisted of three workouts: 1) 4 d of 45 min at 60-65% $\text{VO}_{2\text{peak}}$, 2) 2 d of 90 min at 55-60% $\text{VO}_{2\text{peak}}$, and 3) 4 d of 6 x 1 min intervals at 85-100% $\text{VO}_{2\text{peak}}$ separated by 3 min of recovery. This training program resembles those that have resulted in adaptations in glucose kinetics and improvements in $\text{VO}_{2\text{peak}}$ in 10 d (124, 138). Subjects were instructed to warm-up and stretch before each exercise session. A day of rest was included after the last day of training to allow for adequate rest before the final metabolic challenge ride.

3.12 ANALYTIC TECHNIQUES

3.11.1 Energy expenditure and whole body carbohydrate and fat oxidation

Energy expenditure values were derived from respiratory data averaged over the last 8 min of each 10 min collection period. Total energy expenditure (TEE), whole body carbohydrate and fat oxidation were calculated using the following stoichiometric equations (55, 183) assuming a whole body nitrogen excretion rate of $135 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$

$$\text{TEE (kcal}\cdot\text{min}^{-1}) = 3.9 \text{ VCO}_2/\text{RER} + 1.11 \text{ VCO}_2$$

$$\text{Carbohydrate oxidation (mmol}\cdot\text{min}^{-1}) = 25.196 \text{ VCO}_2 - 17.749 \text{ VO}_2 - 0.21349 \text{ n}$$

$$\text{Fat oxidation (mmol}\cdot\text{min}^{-1}) = 1.9357 \text{ VO}_2 - 1.9357 \text{ VCO}_2 - 0.031978 \text{ n}$$

where VO_2 and VCO_2 are expressed in $\text{L}\cdot\text{min}^{-1}$ and n is the nitrogen excretion rate.

3.11.2 Isotopic enrichment

Plasma samples were deproteinized and derivatized using an adapted technique (62) prior to analysis. Plasma samples (0.5 ml) were deproteinized with the addition of 0.86 ml of ice cold 0.3 M ZnSO₄ and 0.86 ml of ice cold 0.3 M Ba(OH)₂, vortexed, and placed in an ice bath for 20 min. Samples were then centrifuged (3000 rpm, 20 min, 4°C) and the resulting supernatant was lyophilized by vacuum centrifugation in 10 x 75 mm borosilicate tubes. Dried samples were then capped and stored at 4°C until derivatization. The pentaacetate derivative of glucose was formed with the addition of 100 µl of a 2:1 mixture of acetic anhydride and pyridine and 60 min incubation at 60 °C. Samples were then partitioned with the addition of 1.5 ml of distilled water and 400 µl of dimethylchloride and centrifugation (3000 rpm, 10 min). The water phase was removed by suction and the dimethylchloride phase was dried under a stream of N₂ gas. The dried pentaacetate glucose derivative was suspended in 50 µl of ethyl acetate and transferred to airtight chromatography vials with glass pipettes.

Glucose isotopic enrichment was measured by gas chromatography-mass spectrometry (Hewlett-Packard 5989A). Duplicate 1 µl injections passed through a gas chromatograph (30 m x 0.25 mm DB-5 capillary column) and were then ionized in the mass spectrometer by electron impact ionization at an electron beam energy of 70 eV. Duplicate injections were used rather than duplicate samples due to pilot data indicating similar intra-assay coefficients of variation (CV) of 0.16-0.32% (see Appendix X). The resulting data was processed by selected ion monitoring at mass-to-charge ratios (*m/e*) of 200, 201, and 202 and correction was made for the contribution of *m/e* 201 to the apparent enrichment of *m/e* 202 (183). Samples were analyzed in sets grouped by subject to reduce intra-subject variability. Intra- and inter-assay CVs averaged 1.3 and 5.3%, respectively, as determined by control specimens analyzed within each run.

3.11.3 Glucose kinetics

Glucose R_i and R_d at rest were calculated using the steady state tracer dilution equation (183)

$$R_i (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = R_d = F \cdot [(IE_i/IE_p) - 1]$$

where F is the infusion rate of the isotope, IE_i and IE_p are isotopic enrichments of the infusate and plasma, respectively, and -1 accounts for the tracer's contribution to the turnover rate of glucose. The glucose

concentration of the infusate was determined within 24 h before each trial and was used with the subject's weight to calculate F. This was confirmed with the later analysis of a sample of the infusate taken immediately prior to infusion and stored at -80 °C.

Glucose kinetics during exercise were calculated using Steele's non-steady state equations modified for use with stable isotope tracers (167, 183)

$$R_a = \frac{F - V_d \frac{C_m}{1 + E} \frac{dE}{dt}}{E}$$

$$R_d = R_a - V_d \frac{\frac{dC_m}{dt}(1 + E) - C_m \frac{dE}{dt}}{(1 + E)^2}$$

where V_d is the effective volume of distribution, E is the plasma isotopic enrichment, C_m is the measured plasma concentration of the tracee, and dE/dt and dC_m/dt are maximum rates of change in enrichment and glucose concentration, respectively. The V_d was assumed to be 100ml•kg⁻¹.

3.11.4 Substrates and metabolites

Plasma glucose, lactate, NEFA, and glycerol were all assayed spectrophotometrically in duplicate with a microplate reader using commercially available kits. Plasma glucose was measured using a hexokinase enzymatic kit (Sigma 16-UV) with intra- and inter-assay CV of 2.2 and 1.9%, respectively. Plasma lactate was measured using a lactate dehydrogenase enzymatic kit (Sigma #735) with intra- and inter-assay CV of 2.0 and 1.5%, respectively. Plasma NEFA was measured using an enzymatic colorimetric kit (Wako NEFA-C) with intra- and inter-assay CV of 3.1 and 2.5%, respectively. Plasma glycerol was measured using a portion of a triglyceride kit (Sigma GPO Trinder) with intra- and inter-assay CV of 2.0 and 1.6%, respectively.

3.11.5 Statistical analyses

The significance of between-group differences in subject characteristics and dietary composition were analyzed by unpaired t-tests. Differences in aerobic fitness and time trial performance variables between groups and over time were analyzed by two-factor ANOVA with repeated measures. Between-group, within-trial differences (i.e. CHOPre vs. FATpre) and within-group, between-trial differences (i.e. CHOPre vs CHOpst) were analyzed by separate two-factor ANOVA with repeated measures. Post hoc comparisons were made with the Tukey test of significant differences. Significance was set at $p < 0.05$.

CHAPTER 4

RESULTS

4.1 DIET ONLY PHASE

The goal of the diet only phase was to determine the influences of 10 d of consuming either a high carbohydrate or a high fat diet on whole body carbohydrate and fat oxidation as well glucose kinetics during 90 min of moderate intensity exercise. CHO_{pre} and CHO_{post} will represent values of dependent variables for the high carbohydrate fed subjects at baseline and after the 10 d treatment, respectively. FAT_{pre} and FAT_{post} will represent values of dependent variables for the high fat fed subjects at baseline and after the 10 d treatment, respectively.

4.1.1 Subject Characteristics – Diet Only Phase

Subject characteristics are displayed in Table 4.1. There were no significant differences for any of the variables for subjects in the CHO and FAT groups.

Variable	CHO	FAT
n	3	3
Age (yr)	25.0 ± 3.0	28.0 ± 1.2
Height (cm)	180.7 ± 1.8	177.0 ± 4.6
Weight (kg)	71.5 ± 2.0	77.4 ± 0.8
Body fat %	11.8 ± 3.4	16.5 ± 1.6
VO _{2peak}		
(L•min ⁻¹)	3.42 ± 0.47	3.34 ± 0.18
(ml•kg ⁻¹ •min ⁻¹)	47.58 ± 5.56	42.78 ± 1.89
Lactate threshold (% VO _{2peak})	56.0 ± 2.1	51.3 ± 2.3

Table 4.1: Subject characteristics for the diet only phase. Values are means ± SE. CHO = high carbohydrate diet only; FAT = high fat diet only; VO_{2peak} = peak oxygen consumption.

4.1.2 Dietary Composition – Diet Only Phase

The compositions of the habitual and experimental diets are detailed in Table 4.2. Habitual diets of the subjects in the CHO and FAT groups were grouped because there were no differences in these diets among the subjects. The daily energy intake for the experimental diets successfully maintained body weight because the average weight change during the diet only phase was + 0.1 ± 0.3 kg. The lower energy intake for the habitual diet is likely due to under-reporting of energy intake and inaccuracy of the 4 d dietary recall. Analysis of subjects' food diaries indicated near complete adherence to the experimental diets. The composition of the experimental diets differed substantially from the habitual diet. The carbohydrate content of the CHO diet was 60% higher than the habitual diet. The FAT diet contained 147% more fat than the habitual diet. Protein content was similar for the habitual and CHO and FAT diets.

Variable	Habitual	CHO	FAT
Kcal•d ⁻¹	2298 ± 197	2760 ± 93	2904 ± 76
g Carbohydrate•d ⁻¹	313.4 ± 43.4	490.7 ± 20.2	208.0 ± 3.5*
%E Carbohydrate	53.8 ± 4.1	70.7 ± 1.7†	28.3 ± 0.3*†
g Carbohydrate •kg ⁻¹ •d ⁻¹	4.2 ± 0.6	6.8 ± 0.2†	2.7 ± 0.0*
g Fat•d ⁻¹	71.8 ± 10.1	44.3 ± 1.9	187.3 ± 4.7*†
%E Fat	28.0 ± 3.6	13.7 ± 0.9†	57.7 ± 0.3*†
g Protein•d ⁻¹	102.8 ± 12.7	104.3 ± 3.8	105.0 ± 4.1
%E Protein	17.4 ± 1.5	14.7 ± 0.9	14.3 ± 0.3

Table 4.2: Habitual and experimental dietary composition for the diet only phase. * Significantly different from CHO at p<0.05. † significantly different from Habitual at p<0.05. Values are means ± SE. %E = percent of total energy intake.

4.1.3 Heart Rate Response – Diet Only Phase

Heart rate during the 90 min metabolic challenge ride was not different between groups at baseline and was not affected by either diet (Table 4.3).

Group	Pre-Diet	Post-Diet
CHO	156 ± 7	154 ± 10
FAT	157 ± 9	155 ± 5

Table 4.3: Average heart rate during 90 min metabolic challenge ride before and after diet only phase. Values are means ± SE.

4.1.4 Respiratory Exchange Ratio, Whole Body Carbohydrate and Fat Oxidation – Diet Only Phase

The RER and whole body carbohydrate and fat oxidation results (Table 4.4) reveal the typical increase in fat oxidation during prolonged exercise. CHO diet did not alter this pattern of increased fat oxidation during prolonged exercise. There were no significant differences between CHO_{pre} and CHO_{post} for RER, whole body carbohydrate or fat oxidation during exercise. FAT increased fat oxidation at the end of exercise. Compared to FAT_{pre}, at 80-90 min of exercise the RER, whole body carbohydrate and fat oxidation were significantly different from FAT_{post}. There was a 22% reduction in whole body carbohydrate oxidation and a 64% increase in whole body fat oxidation after 10 d of the FAT diet.

Variable	Group	Pre-Diet			Post-Diet		
		Exercise Time (min)			Exercise Time (min)		
		10-20	40-50	80-90	10-20	40-50	80-90
VO ₂ (ml•kg ⁻¹ •min ⁻¹)	CHO	26.8 ± 3.9	28.2 ± 3.5	29.4 ± 4.1	26.6 ± 3.7	27.8 ± 3.8	28.6 ± 3.7
	FAT	23.2 ± 0.5	24.2 ± 1.3	25.8 ± 1.0	22.8 ± 1.2	24.3 ± 0.9	25.1 ± 0.5
RER	CHO	0.98 ± 0.00	0.93 ± 0.01	0.91 ± 0.01	0.98 ± 0.01	0.94 ± 0.02	0.91 ± 0.00
	FAT	0.98 ± 0.01	0.93 ± 0.02	0.93 ± 0.02	0.97 ± 0.02	0.92 ± 0.01	0.88 ± 0.02*
Whole Body CHO Oxidation (μmol•kg ⁻¹ •min ⁻¹)	CHO	185.3 ± 29.2	162.0 ± 22.1	153.0 ± 26.9	178.5 ± 20.1	156.0 ± 11.5	146.6 ± 18.0
	FAT	162.2 ± 9.9	132.7 ± 11.5	142.7 ± 9.1	146.1 ± 7.0	130.3 ± 7.1	111.0 ± 13.2*
Whole Body Fat Oxidation (μmol•kg ⁻¹ •min ⁻¹)	CHO	1.9 ± 0.2	9.7 ± 1.3	13.8 ± 1.0	3.3 ± 2.3	10.1 ± 4.7	13.7 ± 2.4
	FAT	2.3 ± 1.2	9.4 ± 2.7	9.6 ± 2.5	3.8 ± 2.9	9.8 ± 2.3	15.7 ± 2.4*

Table 4.4: Respiratory measures and whole body carbohydrate and fat oxidation during 90 min metabolic challenge before and after the diet only phase. RER = respiratory exchange ratio. Values are means ± SE. * Significantly different from corresponding value of FATpre.

4.1.5 Glucose Kinetics – Diet Only Phase

The tracer infusion protocol produced isotopic equilibrium by the end of the rest period and maintained steady enrichment during exercise (Figure 4.1). Plasma glucose homeostasis was maintained during exercise as there were no significant differences in the plasma glucose concentration (Figure 4.2). Resting glucose R_a and R_d were not different for either CHO or FAT. In general, glucose R_a and R_d had increased approximately threefold above the resting R_a and R_d by the end of exercise (Figure 4.3 and 4.4). Glucose R_d began to exceed glucose R_a after 30 min of exercise producing a gradual decline in the plasma glucose concentration. There was no effect of CHO on glucose R_a or R_d during exercise. The average glucose R_a during 90 min of exercise was lower for FATpost compared to FATpre (23.38 ± 1.88 vs. $20.42 \pm 1.44 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, FATpre vs. FATpost, respectively) that was significant between 15-30 and 60-75 min of exercise. The average glucose R_d during 90 min of exercise was lower for FATpost compared to FATpre (23.67 ± 1.38 vs. $20.67 \pm 1.79 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, FATpre vs. FATpost, respectively) that was significant between 15-30 and 45-75 min of exercise. Glucose metabolic clearance rate (MCR) had similar patterns as glucose R_d for FATpre and FATpost (Figure 4.5).

4.1.6 Percent Substrate Oxidation – Diet Only Phase

It was assumed that ~95-100% of glucose R_d was oxidized (33, 96) allowing the estimation of muscle glycogen (and lactate) oxidation (whole body carbohydrate oxidation minus glucose R_d). Carbohydrate oxidation provided a majority of energy during 90 min of exercise (Figures 4.6, 4.7, 4.8, and 4.9). The contribution of fat oxidation increased with exercise duration. Before the dietary treatments, carbohydrate and fat oxidation accounted for ~ 77 and 23% of total substrate oxidation, respectively, at the end of exercise (Figure 4.8). Plasma glucose oxidation accounted for ~20% of total substrate oxidized at the end of exercise. CHO did not alter this pattern. FAT resulted in increased fat and decreased carbohydrate oxidation at the end of exercise.

Figure 4.1: Glucose isotopic enrichment at rest and during 90 min metabolic challenge ride before and after diet only phase. Values are means $\pm$ SE.

Figure 4.2: Plasma glucose concentration at rest and during 90 min metabolic challenge ride before and after diet only phase. Values are means $\pm$ SE.

Figure 4.3: Glucose rate of appearance (R_a) at rest and during 90 min metabolic challenge ride before and after diet only phase. * Significantly different from FATpre at $p < 0.05$. Δ significantly different from CHOpost at $p < 0.05$. Values are means $\pm$ SE.

Figure 4.4: Glucose rate of disappearance (R_d) at rest and during 90 min metabolic challenge ride before and after diet only phase. * Significantly different from FATpre at $p < 0.05$. Values are means $\pm$ SE.

Figure 4.5: Glucose metabolic clearance rate (MCR) at rest and during 90 min metabolic challenge ride before and after diet only phase. Δ Significantly different from CHOpre at $p < 0.05$. * significantly different from FATpre at $p < 0.05$. Values are means $\pm$ SE.

Figure 4.6: Percent substrate oxidation during min 10-20 of exercise before and after diet only phase. Values are means $\pm$ SE.

Figure 4.7: Percent substrate oxidation during min 40-50 of exercise before and after diet only phase. Values are means $\pm$ SE.

Figure 4.8: Percent substrate oxidation during min 80-90 of exercise before and after diet only phase. * Significantly different from FATpre. Values are means $\pm$ SE.

Figure 4.9: Summary of substrate oxidation during 90 min metabolic challenge ride. A. Before high carbohydrate diet only. B. After high carbohydrate diet only. C. Before high fat diet only. D. After high fat diet only.

4.1.7 Plasma Lactate Concentration – Diet Only Phase

Before dietary treatment plasma lactate increased nearly sixfold in the first 30 min of exercise and gradually fell to ~ 3 mM by the end of exercise (Figure 4.10). CHO did not change this pattern, as there were no significant differences between CHOpre and CHOpost at any time during exercise. FAT significantly reduced the increase in lactate at the beginning of exercise. The plasma lactate concentration of FATpost was significantly lower than FATpre at 15-30 min of exercise.

Figure 4.10: Plasma lactate concentration at rest and during 90 min metabolic challenge ride before and after diet only phase. *Significantly different from FATpre at $p < 0.05$. Values are means $\pm$ SE.

4.1.8 Plasma NEFA Concentration – Diet Only Phase

Plasma NEFA concentration decreased in the first 15 min of exercise and then gradually rose throughout the remainder of exercise (Figure 4.11). There were no significant differences in plasma NEFA concentration from pre to post for either CHO or FAT.

Figure 4.11: Plasma nonesterified fatty acid (NEFA) concentration at rest and during 90 min metabolic challenge ride before and after diet only phase. Values are means $\pm$ SE.

4.1.9 Plasma Glycerol Concentrations – Diet Only Phase

Plasma glycerol concentration more than doubled during exercise. Plasma glycerol concentration was significantly different between FATpost and CHOpst at the end of exercise (Figure 4.12).

Figure 4.12: Plasma glycerol concentration at rest and during 90 min metabolic challenge ride before and after diet only phase. † Significantly different from CHOpst at $p < 0.05$. Values are means $\pm$ SE.

4.2 DIET + TRAINING PHASE

The goal of the diet and training phase was to determine the influences of 10 d of consuming either a high carbohydrate or a high fat diet on training-induced adaptations of whole body carbohydrate and fat oxidation, glucose kinetics, and exercise performance. CHO+Tpre and CHO+Tpost will represent values of dependent variables for the high carbohydrate fed subjects at baseline and after the 10 d dietary/training treatment, respectively. FAT+Tpre and FAT+Tpost will represent values of dependent variables for the high fat fed subjects at baseline and after the 10 d dietary/training treatment, respectively.

4.2.1 Subject Characteristics – Diet + Training Phase

Subject characteristics are displayed in Table 4.5. There were no significant differences for any of the variables between CHO+T and FAT+T subjects.

Variable	CHO+T	FAT+T
n	4	4
Age (yr)	29.5 ± 1.7	24.3 ± 2.3
Height (cm)	179.0 ± 3.8	181.8 ± 1.7
Weight (kg)	81.6 ± 4.3	74.2 ± 3.0
Body fat %	18.6 ± 2.4	12.6 ± 2.6
VO _{2peak} (L•min ⁻¹)	3.47 ± 0.18	3.60 ± 0.38
(ml•kg ⁻¹ •min ⁻¹)	42.24 ± 1.44	48.24 ± 3.98
Lactate threshold (% VO _{2peak})	51.5 ± 1.7	56.5 ± 1.6

Table 4.5: Subject characteristics for the diet and training phase. Values are means ± SE. CHO+T = high carbohydrate diet and training; FAT+T = high fat diet and training; VO_{2peak} = peak oxygen consumption.

4.2.2 Dietary Composition – Diet + Training Phase

The compositions of the habitual and experimental diets for the subjects are detailed in Table 4.6. Habitual diets of the subjects in both the CHO+T and FAT+T groups were grouped because there were no differences in these diets among the subjects. The daily energy intake for the experimental diets successfully maintained body weight because the average weight change during the diet and training phase was only -0.2 ± 0.4 kg. Energy intake was increased by ~20% to compensate for increased energy expenditure from training. The energy caloric intake for the habitual diet is likely due to under-reporting of energy intake and inaccuracy of the 4 d dietary recall. Analysis of subjects' food diaries indicated near complete adherence to the experimental diets. The composition of the experimental diets differed substantially from the habitual diet. The carbohydrate content of the CHO diet was 60% higher than the habitual diet. The FAT diet contained 147% more fat than the habitual diet. Protein content was similar for the habitual and CHO+T and FAT+T diets.

Variable	Habitual	CHO+T	FAT+T
Kcal•d ⁻¹	2345 ± 167	3253 ± 99†	3193 ± 118†
g Carbohydrate•d ⁻¹	307.3 ± 36.0	582.0 ± 18.7	230.8 ± 8.0*
%E carbohydrate	52.0 ± 3.8	71.0 ± 0.0	28.5 ± 0.3*
g Carbohydrate •kg ⁻¹ •d ⁻¹	4.1 ± 0.5	7.2 ± 0.1	3.1 ± 0.1*
g Fat•d ⁻¹	75.8 ± 9.2	51.8 ± 1.0	204.3 ± 8.0*†
%E Fat	30.5 ± 3.0	13.8 ± 0.3†	57.0 ± 0.4*†
g Protein•d ⁻¹	105.5 ± 10.7	121.0 ± 4.3	113.5 ± 4.3
%E Protein	17.5 ± 1.3	14.5 ± 0.3	13.5 ± 0.3

Table 4.6: Habitual and experimental dietary composition during the diet and training phase.

* Significantly different from CHO at $p < 0.05$. † significantly different from Habitual at $p < 0.05$. Values are means ± SE. %E = percent of total energy intake.

4.2.3 Heart Rate Response – Diet + Training Phase

Heart rate during the 90 min metabolic challenge ride was not different between groups at baseline (Table 4.7). A significant training-induced reduction in average exercise heart rate was only evident for the carbohydrate fed subjects.

Group	Pre-Diet + Training	Post-Diet + Training
CHO +T	154 ± 7	140 ± 3*
FAT +T	152 ± 7	146 ± 6

Table 4.7: Average heart rate during 90 min metabolic challenge ride before and after diet and training phase. * Significantly different from within-group pretraining value at $p < 0.05$. Values are means $\pm$ SE.

4.2.4 Respiratory Exchange Ratio, Whole Body Carbohydrate and Fat Oxidation – Diet + Training Phase

The RER and whole body carbohydrate and fat oxidation data (Table 4.8) reveal the typical increase in fat oxidation during prolonged exercise. CHO+T did not alter this pattern of increased fat oxidation during prolonged exercise. There were no significant differences between CHO+T_{pre} and CHO+T_{post} for RER, whole body carbohydrate or fat oxidation during exercise. FAT+T increased oxidation of fat throughout exercise. The RER of FAT+T_{post} was significantly lower than FAT+T_{pre} throughout exercise. Whole body carbohydrate oxidation of FAT+T_{post} was significantly higher than FAT+T_{pre} between minutes 10-20 and 40-50. Whole body fat oxidation for FAT+T_{post} was significantly higher than FAT+T_{pre} between minutes 10-20 and 40-50. These changes represented a 190, 64, and 36% increase in whole body fat oxidation for FAT+T_{post} compared to FAT+T_{pre} at 10-20, 40-50, and 80-90 min of exercise, respectively.

Variable	Group	Pre-Diet + Training			Post-Diet + Training		
		Exercise Time (min)			Exercise Time (min)		
		10-20	40-50	80-90	10-20	40-50	80-90
VO ₂ (ml•kg ⁻¹ •min ⁻¹)	CHO+T	22.2 ± 1.1	23.3 ± 1.5	25.0 ± 1.4	21.9 ± 1.1	23.2 ± 1.2	24.0 ± 1.2
	FAT+T	27.4 ± 3.0	28.9 ± 2.9	28.8 ± 3.0	25.7 ± 2.7	26.9 ± 2.0	28.4 ± 2.7
RER	CHO+T	0.95 ± 0.00	0.92 ± 0.01	0.89 ± 0.02	0.96 ± 0.02	0.92 ± 0.02	0.91 ± 0.02
	FAT+T	0.96 ± 0.02	0.92 ± 0.01	0.89 ± 0.01	0.90 ± 0.02*†	0.87 ± 0.02*†	0.86 ± 0.02*†
Whole Body CHO Oxidation (μmol•kg ⁻¹ •min ⁻¹)	CHO+T	130.6 ± 6.5	118.3 ± 5.9	110.3 ± 7.6	136.5 ± 8.1	121.9 ± 8.0	121.1 ± 8.8
	FAT+T	155.2 ± 5.6	138.2 ± 10.2	114.6 ± 8.6	124.7 ± 8.7*	105.3 ± 13.2*	93.5 ± 9.3
Whole Body Fat Oxidation (μmol•kg ⁻¹ •min ⁻¹)	CHO+T	5.5 ± 0.2	9.4 ± 1.8	14.3 ± 2.4	4.2 ± 2.2	9.9 ± 3.0	11.3 ± 2.5
	FAT+T	5.0 ± 2.9	12.9 ± 1.6	16.8 ± 1.2	14.5 ± 4.5*	21.1 ± 4.2*†	22.8 ± 2.9†

Table 4.8: Respiratory measures and whole body carbohydrate and fat oxidation during 90 min metabolic challenge ride before and after the diet and training phase. Values are means ± SE. * Significantly different from corresponding value of FATpre, † significantly different from corresponding value of CHOpost.

4.2.5 Glucose Kinetics – Diet + Training Phase

The tracer infusion protocol produced isotopic equilibrium by the end of the rest period and maintained steady enrichment during exercise (Figure 4.13). Plasma glucose homeostasis was maintained during exercise, as there was no significant decline in plasma glucose concentration during exercise (Figure 4.14). Resting glucose R_a and R_d were not different for either CHO+T or FAT+T. In general, glucose R_a and R_d increased approximately threefold above resting R_a and R_d values by the end of exercise (Figure 4.15 and 4.16). The average glucose R_a for CHO+Tpost was lower than CHO+Tpre during the last 30 min of exercise (34.12 ± 2.60 vs. $28.45 \pm 3.81 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, CHO+Tpre vs. CHO+Tpost, respectively) and reached significance between 75-90 min of exercise. The average glucose R_d for CHO+Tpost was lower than CHO+Tpre during the last 30 min of exercise (35.45 ± 3.01 vs. $28.40 \pm 3.65 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, CHO+Tpre vs. CHO+Tpost), but this difference was not significant. The average glucose R_a for FAT+Tpost was lower than FAT+Tpre during the last 30 min of exercise (34.64 ± 3.26 vs. $27.06 \pm 2.75 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, FAT+Tpre vs. FAT+Tpost, respectively) and reached significance between 75-90 min of exercise. The glucose R_d for FAT+Tpost was lower than FAT+Tpre during the last 45 min of exercise (33.25 ± 2.31 vs. $26.18 \pm 2.87 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, FAT+Tpre vs. FAT+Tpost, respectively). This difference significant between 45-60 and 75-90 min of exercise. Glucose metabolic clearance rate (MCR) showed similar patterns as glucose R_d for FATpre and FATpost (Figure 4.17).

4.2.6 Percent Substrate Oxidation – Diet + Training Phase

Carbohydrate oxidation provided a majority of energy during 90 min of exercise (Figures 4.18, 4.19, 4.20, and 4.21). The contribution of fat oxidation increased with exercise duration. CHO+T did not greatly alter the pattern of whole body substrate oxidation during exercise. FAT+T increased fat oxidation throughout exercise and reduced carbohydrate oxidation primarily by reducing muscle glycogen oxidation.

Figure 4.13: Glucose isotopic enrichment at rest during 90 min metabolic challenge ride before and after diet training phase. Values are means $\pm$ SE.

Figure 4.14: Plasma glucose concentration at rest and during 90 min metabolic challenge ride before and after diet and training phase. Values are means $\pm$ SE.

Figure 4.15: Glucose R_a at rest during 90 min metabolic challenge ride before and after diet and training phase. § Significantly different from CHO+Tpre at $p < 0.05$. * significantly different from FAT+Tpre at $p < 0.05$. Values are means $\pm$ SE.

Figure 4.16: Glucose R_d at rest during 90 min metabolic challenge ride before and after diet and training phase. * Significantly different from FAT+Tpre. Values are means $\pm$ SE.

Figure 4.17: Glucose MCR at rest during 90 min metabolic challenge ride before and after diet and training phase. * Significantly different from FAT+Tpre at $p < 0.05$. Values are means $\pm$ SE.

Figure 4.18: Percent substrate oxidation during min 10-20 of exercise before and after diet and training phase. * Significantly different from FAT+Tpre. † significantly different from CHO+Tpost. Values are means $\pm$ SE.

Figure 4.19: Percent substrate oxidation during min 40-50 of exercise before and after diet and training phase* Significantly different from FAT+Tpre. † significantly different from CHO+Tpost. Values are means $\pm$ SE.

Figure 4.20: Percent substrate oxidation during min 80-90 of exercise before and after diet and training phase. † Significantly different from CHO+Tpost. ‡ Significantly different from FAT+Tpre. Values are means $\pm$ SE.

Figure 4.21: Summary of substrate oxidation during 90 min of moderate intensity exercise. A. Before high carbohydrate diet and training. B. After high carbohydrate diet and training. C. Before high fat diet and training. D. After high fat diet and training.

4.2.7 Plasma Lactate Concentration – Diet + Training Phase

Before treatment with CHO+T or FAT+T, plasma lactate increased nearly fourfold in the first 30 min of exercise and gradually fell to ~ 2.2 mM by the end of exercise (Figure 4.22). The CHO+T did not change this pattern, as there were not significant differences between CHOpre and CHOpost at any time. The FAT+T significantly reduced the increase in lactate throughout exercise. The plasma lactate concentration of FATpost was significantly lower than FATpre at 15-75 min of exercise.

Figure 4.22: Plasma lactate concentration at rest and during 90 min metabolic challenge ride before and after diet and training phase. * Significantly different from FAT+Tpre at $p < 0.05$. Values are means $\pm$ SE.

4.2.8 Plasma NEFA Concentration – Diet + Training Phase

Plasma NEFA concentration decreased in the first 15 min of exercise and then gradually rose throughout the remainder of exercise (Figure 4.23). There were no significant differences in plasma NEFA concentration for either treatment during exercise.

Figure 4.23: Plasma non-esterified fatty acid (NEFA) concentration at rest and during 90 min metabolic challenge ride before and after diet and training phase. Values are means $\pm$ SE.

4.2.9 Plasma Glycerol Concentration – Diet + Training Phase

Plasma glycerol concentration more than doubled during exercise (Figure 4.24). No significant differences were found in plasma glycerol concentration. There were no significant differences in plasma glycerol concentration for either treatment during exercise.

Figure 4.24: Plasma glycerol concentration at rest and during 90 min metabolic challenge ride before and after diet and training. Values are means $\pm$ SE.

4.2.10 Aerobic Capacity and Lactate Threshold – Diet + Training Phase

Aerobic capacity and lactate threshold are displayed in Table 4.9. The CHO+T group significantly increased VO_{2peak} and the percent of pretraining VO_{2peak} and power output corresponding to lactate threshold. The only improvement for FAT+T was a significant improvement in lactate threshold power output.

Variable	Group	Pretraining	Posttraining	% Change
VO_{2peak} ($L \cdot min^{-1}$)	CHO+T	3.47 ± 0.18	$3.95 \pm 0.21^*$	13.8 ± 2.9
	FAT+T	3.60 ± 0.38	3.64 ± 0.40	1.1 ± 1.1
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	CHO+T	42.24 ± 1.44	$48.13 \pm 1.61^*$	14.1 ± 3.5
	FAT+T	48.24 ± 3.98	49.14 ± 4.13	1.9 ± 1.9
Lactate threshold (% pretraining VO_{2peak})	CHO+T	51.5 ± 1.7	$61.8 \pm 2.2^*$	20.0 ± 3.3
	FAT+T	56.5 ± 1.6	60.8 ± 1.7	7.7 ± 3.8
Lactate threshold (W)	CHO+T	116.8 ± 6.5	$133.8 \pm 8.8^*$	14.5 ± 3.9
	FAT+T	130.3 ± 16.0	$149.0 \pm 18.0^*$	15.1 ± 4.7

Table 4.9: Aerobic capacity and lactate threshold before and after diet and training phase. Values are means $\pm$ SE. W = power output in watts. *Significantly different from within-group pretraining values at $p < 0.05$.

4.2.11 Time Trial Performance

Effects of training on time trial performance are displayed in Table 4.10. There were significant improvements in time trial performance and average power output for both CHO+T and FAT+T.

Variable	Group	Pretraining	Posttraining	% Change
Time trial time (min)	CHO+T	26.1 ± 1.4	23.1 ± 0.5*	-11.3 ± 3.3
	FAT+T	23.7 ± 1.3	21.4 ± 1.7*	-10.1 ± 2.6
Average time trial power output (W)	CHO+T	151.8 ± 12.8	172.0 ± 8.4*	14.3 ± 4.0
	FAT+T	165.0 ± 14.0	185.8 ± 17.6*	12.4 ± 4.2

Table 4.10: Time trial performance before and after diet and training phase. Values are means ± SE.

*Significantly different from within-group pretraining values at $p < 0.05$.

CHAPTER 5

DISCUSSION

The purpose of this study was to examine the influences of dietary composition on changes in whole body fat and carbohydrate oxidation, glucose kinetics, and exercise performance after 10 d of aerobic training. The inability to washout the effects of prior training prevented the use of a true crossover approach in which subjects would train while consuming both experimental diets in a randomized order. Subjects completed only two of four possible experimental treatments (CHO, FAT, CHO+T, and FAT+T). This required the separate analysis of the two experimental phases (CHO vs. FAT and CHO+T vs. FAT+T). The diet only phase revealed that a 10 d high fat diet increased the reliance on fat towards the end of 90 min of moderate intensity exercise. The 10 d high carbohydrate diet did not alter substrate metabolism during 90 min of moderate intensity exercise. Ten days of training while consuming a high fat diet resulted in greater fat oxidation throughout exercise. In contrast, 10 d of training while consuming a high carbohydrate diet neither increased nor decreased carbohydrate or fat oxidation. Despite the differences in substrate metabolism resulting from the diets during 10 d of training, both groups equally improved high intensity time trial performance.

5.1 DIETARY CONTROL

To evaluate the influences of dietary composition on substrate oxidation it was necessary to have adherence by the subjects to the two experimental diets that differed greatly in carbohydrate and fat contents. This was achieved by providing subjects with every meal over the 10 d experimental period rather than asking them to follow a prescribed menu. Compliance was improved by including as many

preferred foods as possible, providing extra food in case of hunger, and allowing the addition of certain listed low calorie food items to add variety in food selection. Analysis of subjects' food diaries indicated that this approach was very successful, as there was little variation from the designed diets.

5.2 INFLUENCE OF DIET ALONE ON WHOLE BODY SUBSTRATE METABOLISM DURING EXERCISE

It is well established that carbohydrate fed before and during exercise increases whole body carbohydrate oxidation during exercise likely as a result of elevated carbohydrate availability and higher plasma insulin concentrations (41, 112, 121, 122, 155, 158, 186). It is also known that 3-5 d of consuming a high carbohydrate diet increases both muscle glycogen (15, 156) and liver glycogen concentrations (131). An increased glycogen concentration may increase the rate of glycogen oxidation during exercise (77). While it is likely that the 10 d high carbohydrate diet of the current study resulted in increased carbohydrate availability, no evidence was found to indicate there was increased whole body carbohydrate oxidation (Table 4.4). This is may due to the fact that subjects were exercising at an intensity that resulted in high RER values (0.91-0.98) that demanded a high rate of carbohydrate (~94% at min 15 and ~70% at min 90) that could not be further increased even by increased availability of glycogen. Nearly identical plasma lactate responses during exercise before and after the high carbohydrate diet (Figure 4.10) further suggests no increase in carbohydrate oxidation following the high carbohydrate diet. Thus, in untrained subjects, consuming a high carbohydrate diet for 10 d does not increase carbohydrate oxidation during exercise.

An increase in fat oxidation during exercise after the 10 d high fat diet of this study has been found in studies involving preexercise fat feedings (135, 178), a 12 h high fat diet (164), and 14-28 d high fat diets (109, 142). However, not all have observed this effect (128, 181). The increased fat availability promoted by high fat diets increases fat oxidation during exercise. Preexercise high fat meals (135, 178), 12 h high fat diets (164), and 28 d high fat diets (142) elevate serum NEFA concentration during exercise. There is no evidence for this effect from a 14 d high fat diet (109) or from the current study (Figure 4.11). Increased fat availability and oxidation may result from increased IMTG concentration (164). The variability associated with the currents methods used to measure IMTG prevent conclusions about the effect of dietary fat on IMTG concentration (85). In the present study fat oxidation may have been increased at the end of exercise due to the decreased availability of carbohydrate. A 14 d high fat diet resulted in lower

resting muscle glycogen concentrations when compared to a high carbohydrate diet (68.1 ± 3.9 vs. 120.6 ± 3.8 mmol $\cdot$ kg⁻¹ wet weight, respectively) (109). It is also possible that the high fat diet promoted fat oxidation by influencing the activity of enzymes that are associated with fat oxidation. The flux of substrates associated with changing diets may alter exercise metabolism by promoting adaptation of muscle enzymes (81). Thus, a high fat diet alone may promote increases in beta-oxidative enzyme activity simply through chronic elevations in the oxidation of fatty acids. Concurrently, the high fat diet may have reduced the activity of enzymes such as phosphorylase (53), phosphofructokinase (133), and pyruvate dehydrogenase (137) resulting in decreased glycolysis and increased fat oxidation. However, this mechanism would seemingly elevate fat oxidation throughout exercise rather than just at the end of exercise unless this effect is only observed when carbohydrate stores are lowered.

5.3 INFLUENCE OF DIET ALONE ON GLUCOSE KINETICS

It is clear that preexercise carbohydrate feedings and carbohydrate supplementation during exercise suppress hepatic glucose production and enhance glucose R_d in humans (88, 112, 121) and animals (62). No study has examined the effect of a high carbohydrate diet on glucose kinetics during exercise. As with whole body carbohydrate oxidation, the current study found that the high carbohydrate diet had no detectable influence on glucose kinetics during 90 min of exercise at lactate threshold (Figure 4.3 and 4.4). While the diet may have increased carbohydrate availability by increasing muscle and liver glycogen concentrations, the fact that the exercise intensity used already demanded a large proportion of energy from carbohydrate might have prevented any further increases in the oxidation of carbohydrate. In addition, subjects were tested following an 8 h fast that may have also lowered the liver glycogen concentration to pre-manipulation levels precluding any changes in glucose kinetics.

Marginal reductions in glucose R_a and R_d were found especially towards the end of exercise following the high fat diet (Figure 4.3 and 4.4). Using the limb-balance technique, Hargreaves et al. (76) demonstrated a 33% reduction in glucose uptake during 1 h of high intensity one-legged exercise following Intralipid/heparin infusion. Martin et al. (113) found that a 3 d high fat diet (~90% of kcals from fat) reduced the oxidation of plasma glucose by nearly 50% during 30 min of moderate intensity exercise compared with a 3 d high carbohydrate diet (~75% of kcals from carbohydrate). Consumption of high fat diets for a longer period seem to induce a similar response. A 28 d high fat diet (~85% kcals from fat, <20

g carbohydrate·d⁻¹) produced a 40% decrease in plasma glucose use as determined by ¹³C glucose infusion (142). The mechanism for these changes is unclear. There is little support for the existence of the glucose-fatty acid cycle in contracting skeletal muscle (35, 53, 54, 133). Instead, it is believed that increased fat availability may reduce increases in allosteric activators of key glycolytic enzymes thereby decreasing muscle glycogen and plasma glucose oxidation (53, 133, 137). Reduced glucose oxidation during exercise following a high fat diet may be due to impaired glucose transport. High fat diets consumed for 8 wk impair insulin- and contraction-stimulated glucose transport in an animal model (75). There was no evidence of a change in membrane lipid composition that would affect glucose transporter function at the sarcolemma while the down-regulation of insulin receptors was thought to play a minor role. The 8 wk high fat diet reduced glucose transport by impairing the GLUT-4 translocation process (75). It is not known if such a mechanism exists in humans who consume a high fat diet.

5.4 TRAINING CONTROL AND ABILITY OF SUBJECTS TO COMPLETE TRAINING

In addition to assuring adherence by the subjects to the diets it was also necessary to insure supervised that all subjects completed identical aerobic training protocols. This was achieved successfully as every training session was completed as scheduled by every subject. Regardless of diet, subjects were completed each training session with encouragement from the investigator.

5.5 INFLUENCE OF DIET AND 10 D AEROBIC TRAINING ON WHOLE BODY SUBSTRATE METABOLISM DURING EXERCISE

Despite consistent evidence that short-term aerobic training reduces carbohydrate oxidation and increases fat oxidation the results of this study indicate that whole body carbohydrate and fat oxidation during exercise are relatively unaltered when training while consuming a high carbohydrate diet for 10 d. Similar effects occur after 7 wk of training on a high carbohydrate diet (82). It may be that consuming a high carbohydrate diet while training produces a significant increase in muscle glycogen concentration that increases glycogen oxidation during exercise. This may have eliminated the typical increase in fat oxidation observed after training. Estimations of muscle glycogen and lactate oxidation during exercise suggests that these sources represented a larger percent substrate oxidation at the end of exercise after the

CHO+T treatment (Figure 4.20). Identical plasma lactate levels during exercise and a tendency for higher lactate levels late in exercise also support oxidation of a carbohydrate source that was likely muscle glycogen.

Training while consuming the high fat diet produced a significant increase in fat oxidation throughout exercise (Table 4.8). A 15% decrease in whole body carbohydrate oxidation throughout exercise and a significant reduction in plasma lactate concentration from 15-75 min of exercise accompanied the increased fat oxidation. The source of the increased fat oxidation may be IMTG as found by others after only 5 d of training (141). Alternatively, inactive tissue may be responsible for the increased oxidation of fat after training at the same absolute workload (11). While it cannot be tested statistically with the current design, the more pronounced increase in whole body fat oxidation compared to the fat diet alone suggests an interaction between the high fat diet and short-term training. Similar interactions have been suggested by others using rat models (159). Increased oxidative enzyme activity with intensive endurance training was further magnified when training was combined with a high fat diet. This led to significant improvements in endurance performance (159). However, these results may not be applicable to humans given the substantial differences in substrate metabolism and adaptation to endurance training between rats and humans (29). In addition, Helge et al. (82) clearly demonstrated that even if such interaction effects exist in humans, improvements in endurance performance are attenuated after 7 wk of training while consuming a high fat diet. Further work is necessary to determine the mechanism for increased fat oxidation following training when consuming a high fat diet.

5.6 INFLUENCE OF DIET AND 10 D AEROBIC TRAINING ON GLUCOSE KINETICS

The high carbohydrate diet alone did not influence glucose R_e and R_d during exercise (Figure 4.3 and 4.4). There were significant reductions in glucose R_e (-19%) and reductions in glucose R_d towards the end of exercise when the high carbohydrate diet was consumed while training (Figure 4.15 and 4.16). This suggests that the mechanisms responsible for decreased glucose R_e and R_d after exercise training, such as blunted catecholamine response (70, 124, 138, 141) and reduced sarcolemmal GLUT-4 translocation (149, 182), outweighed the effects of increased carbohydrate availability on increasing carbohydrate oxidation.

Training while consuming a high fat diet also produced a significant reduction in both glucose R_d and R_d towards the end of exercise (Figure 4.15 and 4.16). The greater reduction in glucose R_d at the end of exercise for FAT+T (-26%) compared to FAT (-15%) suggests that the effects of a high fat diet on substrate metabolism during exercise are magnified when combined with daily aerobic training.

5.7 INFLUENCE OF DIET AND 10 D AEROBIC TRAINING ON AEROBIC CAPACITY, LACTATE THRESHOLD, AND EXERCISE PERFORMANCE

The few short-term training studies that have reported VO_{2peak} data found either no change (69, 144) or a 9% increase in VO_{2peak} (162) when subjects trained daily for 10 d. The current study found a significant 14% increase in the VO_{2peak} of the CHO+T group and no change in the VO_{2peak} of the FAT+T group (Table 4.9). This may simply be due to the degree of variability in responses. It is likely that the not statistically different higher VO_{2peak} is not a confounding variable, as the initial aerobic power does not influence metabolic adaptations to short-term training (66). Changes in lactate threshold with 10 d training have not been previously reported. This study indicates that the training protocol produced significant increases in the power output associated with lactate threshold. This adaptation was likely responsible for the improved time trial performance in both groups (Table 4.10). Before training subjects were capable of maintaining a power output that was 35 W above their lactate threshold. After training this increased to 38 W above their posttraining lactate threshold. Despite drastic differences in dietary composition and training-induced adaptations in steady state substrate use, both groups were equally capable of improving high intensity time trial performance. These results are incongruent with those of Helge et al. (82) who showed an attenuated improvement in endurance performance after a 7 wk of training on a high fat diet compared to a high carbohydrate diet. This suggests that suboptimal adaptations in carbohydrate use that may compromise exercise performance after 7 wk of training do not occur within the first 10 d of consumption of a high fat diet while training.

5.8 EVALUATION OF HYPOTHESES

1. Consumption of a high fat diet for 10 d will reduce glucose R_a and R_d at rest and during moderate intensity exercise compared with a high carbohydrate diet.

This hypothesis was partly supported. The high fat diet did not reduce glucose R_a and R_d at rest. However, the high fat diet resulted in reduced glucose R_a and R_d at varying points during moderate intensity exercise.

2. Ten days of training will reduce glucose R_a and R_d during moderate intensity exercise. However, those fed a high fat diet will exhibit greater reductions in glucose R_a and R_d due to an additive effect of training and the high fat diet.

This hypothesis was partly supported. Consumption of the high carbohydrate diet while training resulted in a significant reduction in glucose R_a and a nearly significant reduction in glucose R_d at the end of exercise. Consumption of the high fat diet while training resulted in significant reductions in glucose R_a and R_d at the end of exercise.

3. Ten days of training will significantly improve the aerobic capacity of both high carbohydrate and high fat fed subjects.

This hypothesis was partly supported. Only the high carbohydrate fed subjects improved VO_{2peak} with training. The lack of a training-induced increase in VO_{2peak} of the high fat fed subjects may be due to the tendency for their pretraining VO_{2peak} values to be higher than high carbohydrate fed subjects.

4. Subjects consuming the high carbohydrate diet will exhibit greater improvements in time trial performance after 10 d of training than those consuming the high fat diet.

This hypothesis was not supported. Both groups exhibited similar improvements in time trial performance.

5.9 FUTURE STUDIES

Future studies should examine the effects of 7-8 wk high carbohydrate and high fat diets on aerobic training-induced adaptations of whole body substrate metabolism, glucose kinetics, and exercise performance. Results of such studies may elucidate the mechanisms responsible for the incongruent results of the current study and those of a 7 wk study (82) that demonstrated attenuated improvements in exercise performance when subjects trained while consuming a high fat diet compared to a high carbohydrate diet.

APPENDIX A

Reliability of Determination of Glucose Isotopic Enrichment

Purpose: The purpose of this pilot work was to determine 1) intra-assay preparation and injection reliability of unenriched specimens, 2) intra-assay preparation and injection reliability of enriched specimens, and 3) inter-assay injection reliability of enriched specimens.

Methods: Unenriched specimens were prepared at glucose concentrations of 50, 75, 100, 125, and 150 mg·dl⁻¹. Enriched specimens were prepared at enrichments of 2, 4, and 6%. All samples were prepared using 0.9% saline and were derivatized as detailed in the methods section.

Results:

Intra-assay preparation reliability of unenriched samples:	1.2%
Intra-assay injection reliability of unriched samples:	1.0%
Intra-assay preparation reliability of enriched samples:	0.2-0.3%
Intra-assay injection reliability of enriched samples:	0.2-0.3%
Inter-assay injection reliability of enriched samples:	3.9%

Conclusions: Intra-assay preparation and injection reliability were similar for both unenriched and enriched samples. These results justify the use of duplicate injection analysis for the determination of glucose isotopic enrichment. Inter-assay injection reliability was high enough to provide confidence in comparing samples analyzed on different days.

APPENDIX B

Sample High Carbohydrate Menu

Breakfast

Entrees (Choose 3)

Cold cereals (suggest 2-3)

Hot cereals (suggest 1)

Waffles

Pancakes

Other suggestions:

Beverages (Choose 1)

Chocolate milk

Orange juice

Skim milk

Other suggestions:

Extras

Butter - sweet, salted

Butter - sweet, unsalted

English muffin and jam

Regular syrup

Maple syrup

Other suggestions:

Lunch

Breads (Choose 1-2)

Blueberry bagel

Cinnamon raisin bagel

Egg bagel

Beverages (Choose 1)

Chocolate milk

GatorLode

GatorPro

Lunch Meat (Choose 1)

Turkey breast

Smoked ham

Honey ham

Everything bagel

Onion bagel

Plain bagel

Soft plain bagel

Whole wheat bread

Other suggestions:

Orange juice

Skim milk

Other suggestions:

Other suggestions:

Condiments (Choose 2)

Cheese

Low fat cream cheese

Mayo

Mustard

Other suggestions:

Snacks (Choose 3)

Fruit newtons

Granola bars

Pretzels

WOW chips

Yogurt

Other suggestions:

Vegetables (Choose 1)

Baby carrots

Broccoli

Cauliflower

Vegetable Dip (Choose 1)

Blue cheese

Creamy 1000 island

Garlic ranch dressing

Parmesan peppercorn

Ranch dressing

Sweet and sour

Other suggestions:

Dinner

Entrees (Choose 3)

Beef stroganoff and pasta

Cheese lasagna

Chicken carbonera over linguine

Chicken enchilada w/ mexican rice

Fettucini alfredo

Grilled chicken with penne pasta

Pepperoni french bread pizza

Salisbury steak and whipped potatoes

Sun dried tomatoes french bread pizza

Vegetable eggroll w/ long grain rice

Other suggestions:

Side Dishes (Choose 2)

Mashed potatoes with margarine

Potato wedges

Smoky cheddar mashed potatoes

Other suggestions:

Vegetables (Choose 2)

Broccoli

Cauliflower

Corn

Green beans

Mixed vegetables

Other suggestions:

Beverages (Choose 2)

Chocolate milk

GatorLode

GatorPro

Orange juice

Skim milk

Other suggestions:

Dessert

Frozen yogurt

Other suggestions:

.

APPENDIX C

Sample High Fat Menu

Breakfast

Entrees (Choose 3)

Egg and cheese sandwich

Pancakes with bacon

Pancakes with sausage

Scrambled eggs and sausage

Scrambled eggs, bacon, and potatoes

Toaster Scramble - Cheese and egg

Toaster Scramble - Cheese, egg, and bacon

Toaster Scramble - Cheese, egg, and ham

Toaster Scramble - Cheese, egg, and sausage

Toaster Strudel with cream cheese

Other suggestions:

Beverages (Choose 1)

Chocolate milk

Whole milk

Other suggestions:

Extras

Butter - sweet, salted

Butter - sweet, unsalted

Regular syrup

Maple syrup

Other suggestions:

Lunch

Breads (Choose 1-2)

Blueberry bagel

Cinnamon raisin bagel

Egg bagel

Everything bagel

Onion bagel

Plain bagel

Soft plain bagel

Whole wheat bread

Other suggestions:

Beverages (Choose 1)

Chocolate milk

Strawberry milk

Whole milk

Other suggestions:

Lunch Meat (Choose 1)

Bologna

Chopped ham

Cooked salami

Turkey

Other suggestions:

Condiments (Choose 2)

Apple cinnamon cream cheese

Blueberry cream cheese

Cheesecake cream cheese

Chile and onion cream cheese

Cream cheese

Creamy peanut butter

Crunchy peanut butter

Extra crunchy peanut butter

Honey nut cream cheese

Garden vegetable cream cheese

Pineapple cream cheese

Strawberry cream cheese

Snacks (Choose 3)

Caramel chocolate chip cookies

Coconut chocolate chip cookies

Chocolate chip cookies

Chocolate donuts

Glazed donuts

Mini muffins

M&M's

Nutter butters

Oreos

Powdered donuts

Other suggestions:

Vegetables (Choose 1)

Baby carrots

Broccoli

Cauliflower

Vegetable Dip (Choose 1)

Blue cheese

Creamy 1000 island

Garlic ranch dressing

Parmesan peppercorn

Ranch dressing

Sweet and sour

Dinner

Entrees (Choose 3)

Beef pot pie
Chicken pot pie
Country fried beef steak
Creamed sauce, croutons, & shredded beef
Lasagna alfredo
Meatloaf
Salisbury steak
Sausage, pepperoni, bacon pizza
Sausage, pepperoni pizza
Turkey pot pie
Vcal parmigiana
Other suggestions:

Side Dishes (Choose 3)

Cheese and pepperoni bites
Cream cheese bites
Mashed potatoes with butter
Monterey jack and jalapeno bites
Smoky cheddar mashed potatoes
Other suggestions:

Vegetables (Choose 2)

Broccoli
Cauliflower
Corn
Green beans
Mixed vegetables
Other suggestions:

Beverages (Choose 1)

Chocolate milk
Strawberry milk
Whole milk
Other suggestions:

Dessert Shake

Specify ice cream flavors: _____

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