METABOLIC EFFECTS OF INCREMENTAL EXERCISE ON ARABIAN HORSES FED DIETS CONTAINING CORN OIL AND SOY LECITHIN

Kristen Alissa Kline

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D.S. Kronfeld, Chair
J.D. Jacobson
D.M. Denbow

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(Abstract)

Feeding a fat-containing diet to the exercising horse is a facile way to increase energy density without risking the complications associated with hydrolyzable carbohydrates. Fat adaptation may also result in increases in the utilization of free fatty acids for fuel during exercise and sparing of muscle glycogen. Phosphatidylcholine, the main component of lecithins, can influence muscle contraction and improve endurance capacity during exercise. When it is combined with corn oil in a total mixed ration, soy lecithin is both highly digestible and palatable to horses. Our objectives in this study were to compare the effects of incremental exercise and isocaloric control (CON), corn oil (CO), and a soy lecithin/corn oil (LE) diets on plasma free fatty acids (FFA), cholesterol, glycerol, triglyceride (TG), lactate, and glucose. Also three different statistical models were compared for goodness of fit to the lactate curve.

Plasma lactate and glucose both increased slowly early in the incremental exercise test (IET), then increased rapidly as the work intensity increased. Both decreased during recovery. No effects of IET or diet were found for either of these variables.

Plasma TG was unchanged during exercise, but increased rapidly during recovery. Plasma FFA decreased from resting early in the IET then remained steady throughout the remainder of exercise. During recovery a rapid increase was exhibited. Plasma glycerol was constant during exercise, but increased during recovery. Plasma cholesterol did not change during exercise or recovery.

Diet affected plasma FFA. Plasma FFA were lower for the CO and LE diets than the CON diet during the IET. Plasma glycerol was lower for the CO diet than the CON diet during the IET, with the LE diet intermediate between the two. Plasma cholesterol was higher for the CO and LE diets than the CON diet during the IET.

A segmented model and an exponential model were found to have a good fit to the lactate curve. A point of inflection for a rapid increase in plasma lactate during incremental exercise was discovered. When this model was applied to diet, no differences in lactate threshold were found between the diets.

Some criteria for fat adaptation were met, namely diet affected plasma FFA, glycerol, and cholesterol. However diet did not affect plasma TG, lactate, or glucose.

This indicates that the rate of fatty acid oxidation was increased following fat adaptation, but it did not affect the rate of glucose oxidation and glycolysis during exercise.

A lactate threshold for the equine can be obtained using a broken line model. Further studies using this approach are needed to establish its correlation with performance.

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General Introduction

Fat supplementation can favorably influence exercise performance in the horse (Potter et al., 1992; Kronfeld, 1996). Since fats are energy dense and decrease bulk of feed, they can be used to replace starch thereby decreasing the risk of digestive problems such as colic and laminitis (Clarke et al., 1990; Sprouse et al., 1987). Fats also reduce spontaneous activity and have a calming effect on horses, making them more tractable (Holland, 1996). Supplementation of fat in the diet in combination with training results in fat adaptation in the dog and horse (Kronfeld and Downey, 1981). Fat adaptation increases fatty acid oxidation and spares muscle glycogen (Griewe et al., 1989).

Lecithin or phosphatidylcholine is a phospholipid that is a basic component of the plasma membrane. Supplementation of choline improves endurance capacity in humans by preventing the depletion of choline caused by exercise (Sandage, 1992). It may also alter cell membrane fluidity allowing easier transport of fatty acids. Previous studies involving lecithin-containing diets have found them to be palatable and highly digestible by horses (Holland et al., 1997).

The lactate threshold was initially recognized as a point of inflection in the relationship between blood lactate and work intensity (Wasserman and McIlroy, 1964). It can be used to evaluate differences in individual performance due to variables such as diet or training (Kindermann et al., 1979; Custalow et al., 1993). A definitive model for determining the lactate threshold in the horse remains controversial. Currently a simple exponential model is commonly used (Vallette et al., 1996).

The purpose of this study was to determine the effects of corn oil and lecithin diets on responses of plasma free fatty acids, lactate, glucose, cholesterol, glycerol, and triglycerides to incremental exercise. Also three statistical models were compared for goodness of fit to lactate curves. The best models were used to test for differences in lactate threshold due to diet.

Review of Literature

Energy sources for exercise

The direct source of chemical energy that is converted to mechanical energy and heat in muscle contraction is adenosine triphosphate (ATP). It is replenished anaerobically from phosphocreatine (PCr) and by glycolysis (conversion of glycogen and glucose to lactic acid), and aerobically by the oxidation of glucose, lactic acid, fatty acids, and certain amino acids.

The power of these energy sources may be represented by the rates of ATP production (Figure 1), as discussed by Sahlin (1986). Anaerobic sources furnish power at 2- to 4-times the rate of aerobic sources, hence are used for more intense exercise. At rest the rate of glycolysis is regulated to compensate for use of lactate for oxidation (Connett et al., 1986). In incremental exercise tests, blood lactate tends to remain constant or to increase slowly at first with increasing work load, when the rates of lactate utilization and production are about equal. The lactate level then begins to rise sharply, when the rate of lactate utilization is exceeded by its production rate (Figure 2). This work load (speed or fraction of maximal oxygen consumption, VO_{max}) is called the anaerobic threshold (Wasserman and McIlroy, 1964).

ATP stores

The meager ATP stores are used during intense exercise in both horses and rats (Meyer and Terjung, 1979; Snow et al., 1985). Muscle ATP concentration has been depleted by about 30% during intense exercise in horses (Snow et al., 1985). Uric acid concentrations, which can be used to measure purine metabolism, have been negatively correlated with racing performances in Standardbreds. This finding indicates that better horses may regenerate their ATP more rapidly, hence retarding depletion (Rasanen et al., 1995).

Phosphocreatine stores

Phosphocreatine (PCr) is another relatively short-lived power source that is effective at generating ATP rapidly. It has a high-energy phosphate group that is donated to ADP to produce ATP. It is used at the beginning of exercise to maintain high ATP levels during muscle contraction. Creatine kinase is the enzyme that catalyzes the conversion of

phosphocreatine and ADP to creatine and ATP. Phosphocreatine stores are depleted more quickly in fast twitch muscle fibers since they have a higher rate of ATP utilization (Ivy et al., 1981). In the horse PCr levels are decreased about 50 to 70% during high intensity exercise (Valberg and Essen-Gustavson, 1987).

Anaerobic glycolysis

Production of ATP for muscle contraction can also be derived from glycolysis (Brooks, 1985). Glycogen stores and blood glucose are converted to pyruvate, generating 2 ATP per glucose. This reaction generates less power than that of ATP stores or phosphocreatine, but it is more sustained (Sahlin, 1986). Pyruvate is converted to either lactate anaerobically or acetyl CoA aerobically. Lactate levels in the blood are a good indicator of the rate of anaerobic glycolysis that is occurring. Lactic acid contributes to fatigue by lowering the pH which interferes with the contractile processes of muscles. Lactic acid is smaller than glucose and is readily transported across cellular membranes. Thus, for some time during moderate intensity exercise, lactate produced by glycolysis in fast twitch (Type IIa and IIb) fibers is a preferred fuel for highly oxidative (Type I) muscle fibers.

Some lactic acid is removed from the arterial blood and used by the heart for fuel. Shuttling of lactic acid to the liver, where it is converted to glucose, then to recovering muscle, helps replenish muscle glycogen. However as intensity and duration of exercise increase, these fibers are unable to use all the lactate being produced. At this point lactate levels in the blood rise rapidly, lowering the pH of the muscle and causing fatigue. This speed or level of work intensity is known as the lactate threshold. Carbohydrate is the main substrate that is oxidized once the lactate threshold is reached in rats and non-fat adapted humans (Coyle, 1995). Measurements of gas exchange and blood lactate concentration can both be used to identify increases in glycolytic activity in exercising skeletal muscle of horses (Gauvreau et al., 1996).

Carbohydrate oxidation

Most of the glucose-6-phosphate (G6P) that is utilized during exercise is derived from muscle glycogen or from blood glucose, which must be replenished by hepatic gluconeogenesis. In order to synthesize ATP, glycogen must first be split and phosphorylated to yield glucose-1-phosphate. Hexokinase (HK), a rate-limiting regulatory enzyme, phosphorylates intracellular glucose to yield G6P. Next G6P is isomerized to fructose-6-phosphate (F6P) which is phosphorylated by

phosphofructokinase (PFK), another key rate-limiting enzyme. The activity of PFK is influenced by ATP concentration and the availability of metabolites such as citrate and F6P. Therefore the rate of glycolysis is affected by both HK and PFK in response to feedback inhibition from excess acetyl CoA. The end products of glucose oxidation are CO_2 and water.

The catecholamines, epinephrine and norepinephrine, are released in response to exercise and low blood glucose and have a glycogenlytic effect (Snow et al., 1992). These hormones stimulate adenylate cyclase, an enzyme that converts ATP to cyclic AMP. Cyclic AMP then activates a protein kinase that stimulates phosphorylase kinase to begin glycogenolysis while inhibiting glycogen synthase. The response of the catecholamines to high-intensity exercise is considerably greater in the horse than in the human (Snow et al., 1992).

Fatty acid oxidation

Most fats in the body are stored as triglycerides (TG) in adipocytes, and to a lesser extent, in muscle cells. During prolonged exercise intramuscular TG are decreased (Holloszy et al., 1986). Triglycerides are composed of three fatty acids attached to one glycerol. Hydrolysis of TG in adipose tissue produces glycerol and free fatty acids (FFA), the main source of energy during low intensity exercise. Free fatty acids are bound to albumin for transport in blood (Coyle, 1995). This process is stimulated by hormone-sensitive lipase, lipoprotein lipase, and catecholamines.

Fatty acids are a crucial fuel source for several reasons. Most importantly their supply is inexhaustible as fatigue never results from depletion of triglyceride stores. Also oxidation of fatty acids produces less carbon dioxide than glucose oxidation, thus reducing respiratory effort. Unfortunately the power output from FA oxidation is very low primarily due to the slow entry of FA into the mitochondria (Sahlin, 1986). This process may be facilitated following fat adaptation (Holloszy et al., 1986).

Effects of training

Aerobic conditioning

Aerobic training alters the composition of muscle fibers, increasing the number of Type I and IIA muscle fibers in the horse (Hodgson et al., 1987). Muscles have increased blood supply through capillarization and numbers of mitochondria increase. Each of these changes results in an increased capacity to utilize oxygen. Lactic acid produced by fast

twitch glycolytic fibers can be used for fuel by slow twitch oxidative fibers (Brooks, 1985). The rate of lactate formation is decreased with aerobic training while lactate removal is increased. Muscle glycogen storage capacity may increase up to 150% following aerobic conditioning in humans (Gollnick et al., 1972). Following fat supplementation and training, mitochondrial capacity is greater resulting in increased use of FFA in sled dogs (Reynolds et al., 1994).

Exercise training enhances the lipolytic response of adipose tissue to epinephrine and norepinephrine. Training also facilitates the oxidation of intramuscular fatty acids, and trained individuals exhibit lower plasma FFA concentrations. Trained people usually also have lower blood glycerol concentrations during exercise than untrained people (Holloszy et al., 1986). Increased fatty acid oxidation serves to spare muscle glycogen and decrease lactate accumulation in muscle. Trained horses have lowered resting muscle lactate levels, probably due to glycogen sparing (Griewe et al., 1989; Lindholm and Piehl, 1974). Aerobically conditioned horses also require less glycolytically derived energy, have a lowered respiratory exchange ratio, and thus increased overall aerobic capacity (Gauvreau et al, 1996).

Anaerobic conditioning

Training which stresses anaerobic systems can also cause profound effects. Many enzymes in the glycolytic pathway such as HK and PFK increase their rate of activity. Muscle fiber composition may also be affected. Fast-twitch muscle fibers are more glycolytic and therefore are more developed by anaerobic training (Takekura and Yoshioka, 1990). All of the short-term energy stores, ATP, PCr, and muscle glycogen are increased after prolonged training (Houston and Thompson, 1977). Most of these changes are a result of alterations in enzyme activity and substrate utilization.

For the equine interval training has been found to be effective for bettering anaerobic performance (Roneus et al., 1994). Interval trained horses experience an eight to ten-fold increase in muscle glucose and G6P levels, and their blood glucose levels are lower than their muscle glucose levels (Lindholm and Saltin, 1974). These results illustrate that interval training maximizes enzyme activity and anaerobic capability in the horse.

Effects of diet

Feeding the exercising horse

As discussed in the previous section, the major sources of energy during exercise are plasma free fatty acids, muscle glycogen, and blood glucose. With this in mind,

altering the diet to maximize availability of all of these energy sources is crucial to performance.

Carbohydrates

Carbohydrates are either fermented or hydrolyzed during digestion. Each type yields different products when digested, and therefore they should be considered separately from one another. Most forages such as grass and hay are high in fermented carbohydrates. These are converted into short chain fatty acids (SCFA). Thus fermented carbohydrates are often replaced with hydrolyzable carbohydrates that are more efficiently utilized than SCFA. Hydrolyzable carbohydrates, which include oats and corn, yield glucose when hydrolyzed. This is an efficient system metabolically, but becomes problematic if the system is overloaded. Laminitis, exertional rhabdomyolysis, and colic, have all been associated with hydrolytic overload (Sprouse et al., 1987; Clarke et al., 1990).

Also, although in humans carbohydrates are very effective for repletion of glucose and glycogen loading post-exercise (Costill, 1985), horses do not demonstrate similar benefits (Frape, 1994). Horses already have the ability to store more glycogen in their muscle than humans (Lindholm and Piehl, 1974; Snow et al., 1992).

In short, for high performance horses a high energy substitute for hydrolyzable carbohydrate is needed to minimize the risk of hydrolytic overload without compromising performance. Fat sources such as corn oil may provide the solution.

Fat adaptation

Fat adaptation refers to a combination of training and fat supplementation in the diet (Kronfeld and Downey, 1981). There is some question as to the period of time necessary to reap the full effect of adaptation. Some studies have indicated that a diet period of three weeks is sufficient for adaptation, if horses are already conditioned (Oldham et al., 1990; Scott et al., 1992). Others have found that at least eleven weeks of combined training and fat supplementation are necessary (Custalow et al., 1993; Ferrante et al., 1993). Perhaps the duration of training and feeding fat required for the development of fat adaptation depends on the criteria used.

Adaptation results in some metabolic changes that appear to facilitate fatty acid oxidation. When fatty acids are used for fuel, citrate and acetyl Co-A levels build up causing two key regulatory enzymes of glycolysis, PFK and pyruvate dehydrogenase, to be inhibited. This causes a decrease in carbohydrate metabolism and enhancement of fatty

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acid oxidation in muscle. Increased utilization of fatty acids may result in sparing of muscle glycogen stores. Following fat adaptation horses exhibit elevated plasma glucose concentrations during aerobic exercise possibly due to glycogen sparing (Hambleton et al., 1980). Blood lactate levels during aerobic exercise are lower due to an increased rate of fatty acid oxidation which in turn decreases muscle glycogen utilization (Griewe et al., 1989).

Fat adapted horses also have higher resting muscle glycogen levels indicating that fatty acids are being utilized not only during exercise but for maintenance as well (Meyers et al., 1987). High muscle glycogen levels enable a greater rate of glycolysis during anaerobic exercise since more substrate is available for ATP production. More glycogen was used during anaerobically stressful work in fat adapted horses and lactate levels were higher (Oldham et al., 1990).

Horses are able to digest fat well when it represents up to 30% of digestible energy in the diet (Kane et al., 1979). Most diets are supplemented at a rate of 10% by weight (Potter et al., 1992; Ferrante et al., 1993). Fat-containing diets produce less heat when digested than carbohydrate diets, resulting from a diminished heat load from fermentation (Scott et al., 1992). This leaves more energy available for exercise work.

Increased plasma cholesterol develops during fat adaptation (Taylor et al., 1995). Therefore it is a useful index for determining whether fat adaptation has occurred. Glycerol levels change during exercise and can be used to index the rate of lipolysis. Lactate levels in fat adapted horses may also be higher during sprint exercise, perhaps due to an increased rate of glycolysis (Oldham et al., 1990; Ferrante et al., 1993).

Lecithins

Choline is a component of all biological membranes and a precursor of acetylcholine, a neurotransmitter. Most of the body's choline is derived from dietary sources of phosphatidylcholine, also known as lecithins, which are phospholipids. During exercise, plasma choline levels decrease, and may slow contraction transmission in muscle in humans (Sandage, 1992). Lecithin supplementation has also been shown to reduce plasma cholesterol and triglyceride levels in rats (Jiminez et al., 1990). The opportunity to increase power output from FA oxidation is limited because transport across the mitochondrial membrane is slow. Phosphatidylcholine is an integral component of the plasma membrane of cells. Supplementing lecithin in the diet could conceivably influence the fluidity of the plasma membrane. With increased membrane fluidity transport is facilitated, and fatty acids may be more available to the cell. During exercise this change

has been associated with decreased blood lactate responses during repeated sprints in horses fed a lecithin-containing diet (Taylor et al., 1995).

Lactate threshold

The term anaerobic threshold was first used to describe a rapid increase in blood lactate during incremental exercise by Wasserman and McIlroy (1964). This concept can be explained in terms of the rate of lactate production versus its rate of utilization (Figure 2). It has been shown to be highly correlated with running performance in marathon runners (Farrell et al, 1979) and Standardbred trotters (Casini and Greppi, 1996). However, despite its utility as a predictor of performance there are arguments that blood lactate changes throughout exercise, and that the rate of lactate appearance and removal is not reflected using blood samples. Also, some have argued that the equine lactate curve exhibits no point of inflection (Wilson et al., 1983).

Human Studies

Most of the research involving the anaerobic threshold (AT) has been focused in human studies. Once the AT has been identified for an individual it can be used to tailor the duration and intensity of exercise regimens for maximum benefit (Kindermann et al., 1979). There are a variety of ways that this threshold is defined such as by ventilatory threshold, lactate threshold, onset of blood lactate accumulation (OBLA), highest exercise intensity reached prior to a rapid elevation in blood lactate (0.2 mmol/L per 1.0 m/s), and a fixed plasma lactate level, such as 4mmol/L. Each of these methods has been proven to be valid for predicting performance when directly compared, although rapid lactate elevation was the best predictor (Yoshida et al., 1990).

Substrate utilization is very different above and below the lactate threshold, and is also influenced by training. Elevations in blood lactate above the threshold have been shown to inhibit lipolysis and increase carbohydrate utilization (Boyd et al., 1974). However lactate thresholds in humans have been increased slightly by raising blood FFA levels before an incremental exercise test (Ivy et al., 1981). Increasing the fat content of the diet was thought to cause an increased rate of fatty oxidation with a corresponding reduction in lactate production. Increased recruitment of Type II muscle fibers, which have a greater capacity for glycolysis, occurs at the lactate threshold too (Brooks, 1985).

The human AT was found to occur at a blood lactate concentration of 4 ± 1 mmol/L, and , working backwards, this level was taken to indicate a speed that could serve as the AT, the VLa4. Incremental exercise testing provides a very reliable measurement of lactate threshold. Increments varying from 1 to 4 min in duration have been found to provide similar threshold values (Wasserman et al., 1973).

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Horse Studies

As is the case in humans, a variety of methods are used to define the AT in horses. These include using percent of maximum heart rate as an indicator of work intensity (Dwyer and Bybee, 1983). In horses, no point of inflection lactate threshold was found in the initial studies, and the data were fit to an exponential. Later the exponential was interpolated to find VLa4 (Wilson et al., 1983; Snow et al., 1985). The VLa4 was found to be inconsistent with an LT determined by graphical analysis (VLaL) in ponies (Vallette et al., 1989) and with an LT determined as a point of inflection in Arabians (Custalow et al., 1993). Use of IETs has been proven to be a valid method of obtaining lactate thresholds and predicting performance in horses (Seeherman and Morris, 1990; Custalow et al., 1993; Casini and Greppi, 1996).

A broken-line model that is commonly used for growth data has been previously applied to equine lactate thresholds (Robbins, 1986; Custalow et al., 1993). Its advantage lies in the fact that it is easy to interpret the exact velocity at which a rapid increase in lactate occurs.

Plasma lactate may be better than blood lactate for determining lactate threshold, because erythrocyte lactate concentration is correlated better with plasma lactate than with blood lactate (Pösö et al., 1995). Erythrocytes act as a reservoir for excess lactate which working muscle is unable to use. Horses that are identified as good runners tend to have a high cell lactate level probably due to an increased rate of lactate transport into erythrocytes (Pösö et al., 1995).

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Objectives

The main objective of this research was to improve understanding of fat adaptation in the horse. The study had four specific objectives:

1) to establish whether 21 days is a sufficient period for adaptation to a high fat diet;

2) to examine the effect of a fat-containing diet and interval training on mixed venous plasma lactate, glucose, free fatty acids, glycerol, triglyceride, and cholesterol at rest and during an incremental exercise test;

3) to determine the best statistical model for determining the lactate threshold in the horse;

4) to utilize that model to discern differences in lactate threshold caused by diet.

Journal Article

Metabolic responses of plasma lactate, glucose, triglyceride, free fatty acids, glycerol, and cholesterol to an incremental exercise test in Arabian horses fed a control, corn oil, or soy lecithin containing diet and comparison of three statistical models for goodness of fit to the lactate curve.

Introduction

Dietary fat supplementation has been proposed to improve athletic performance and to reduce risks of colic, laminitis, and exertional rhabdomyolysis in the horse (Potter et al., 1992; Kronfeld, 1996). Most studies have used 10% by weight of corn oil or tallow in the concentrate. Corn oil and combinations of corn oil, soybean oil, or soy lecithins have reduced spontaneous activity up to 25% and had a calming effect on horses, making them more tractable (Holland et al., 1996).

Accumulation of lactate in the blood was among the first metabolic changes associated with fatigue during exercise. As work intensity increases, blood lactate concentration rises slowly at first then more rapidly. The point of inflection in the lactate/power curve was called the anaerobic threshold (Wasserman and McIlroy, 1964) or lactate threshold (Ivy et al., 1981). The lactate/power curve has prompted various mathematical analyses (Vallette et al., 1996), rival physiological interpretations (Davis, 1985; Stainsby and Brooks, 1991), and several empirical predictors of performance (Farrell et al., 1979; Casini and Greppi, 1996).

Previous studies in our laboratory have determined the lactate threshold (LT) as a point of inflection (POI) in the blood lactate/speed curve generated by an incremental exercise test in Arabian horses (Custalow et al., 1993). The LT and peak blood lactate were increased by 12 weeks of training and by dietary supplementation with corn oil. Blood lactate elevations during repeated sprints in these horses were raised by supplementation with corn oil (Ferrante et al., 1993) but not by a combination of corn oil and soy lecithin (Taylor et al, 1995). The corn oil effect on blood lactate during repeated sprints and other findings were taken to indicate that fat adaptation enhanced the regulation of glycolysis, including less glycolysis during slow work and more rapid glycolysis during sprints (Kronfeld et al., 1995). The lecithin effect may indicate facilitation of fatty acid transport through cell membranes and augmented fatty acid oxidation, which would spare the need for glycolysis during sprints.

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Previous studies in our laboratory have indicated that a period of at least 12 weeks of corn oil supplementation and interval training of previously untrained horses was necessary for full metabolic adaptation (Greiwe et al., 1989; Ferrante et al., 1993; Taylor et al., 1995). In contrast, several studies using tallow have indicated complete digestive and metabolic adaptation within 3 weeks provided the horses were already fully trained (Potter et al., 1992). In the present experiment, periods of 3 week adaptation were used to compare the effects of supplementary corn oil or a combination of corn oil and soy lecithin on the LT of already fully fit horses.

The POI in the lactate/power curve was determined previously by means of a segmented or broken line model (Custalow et al., 1993). Most studies of LT in horses have used an exponential model (with no POI) and interpolated the speed at a blood lactate concentration of 4 mmol/L (Wilson et al., 1983; Casini and Greppi, 1996). This form of LT, known as the VLa4, was developed from human data and may not apply broadly to the horse (Vallette et al., 1989; Custalow et al., 1993). The segmented and exponential models for determining the LT have been compared in the present study.

The purpose of this study was to determine the effects of corn oil and soy lecithin supplementation on plasma free fatty acids, lactate, glucose, cholesterol, glycerol, and triglyceride during incremental exercise and recovery. Also, three different statistical models were compared for goodness of fit to the lactate curve and the effects of diet and IET on LT were examined.

Materials and Methods

The experimental plan consisted of a 3x3x3 Latin square in triplicate, with 9 horses, 3 diets, and 3 experimental periods of 21 days. The protocol was approved by the institutional animal care committee.

Animals

Nine Arabian horses, five geldings and three mares, ranging in age from four to eight years were conditioned on the treadmill, then used in this study. Horses were fed twice daily in box stalls (3.5 x 2.5 m) and were housed together in a dirt paddock (60 x 78 m) when not eating. Water was available ad libitum. Horses were exercised twice weekly for 10 weeks on a Mustang 2200 high speed treadmill (Kagra Ag Mustang 2200, Switzerland) and walked 1 h/day at 1.5 m/s twice weekly on a hot walker. This was done to achieve a standard level of fitness before starting the experimental diet period. The

conditioning protocol was designed for interval training to maximize anaerobic capacity (Table 1). Body weight was monitored weekly using an electric large animal scale (E-Z Weigh, Cave Creek, AZ), and individual intakes were adjusted to maintain body weight.

Diets

Each diet formed a total mixed ration so that no additional hay was fed. Horses were randomly assigned to one of three diet groups: a control diet (CON) consisting of orchard grass hay, soybean meal, molasses, limestone, and corn, or an isocaloric amount of a similar diet with 10% fat added in either the form of 100% corn oil (CO) or 50% corn oil and 50% soy lecithin (LE) (Tables 2 and 3).   Diets were formulated in order to meet or exceed requirements for working horses (NRC, 1989).   Each diet was fed for a 21 day adaptation period.

Exercise Test

An incremental exercise test (IET) was performed at the end of each 21 day diet period. The test was continued according to the protocol until horses failed to maintain pace with the treadmill or developed an uneven gait (Table 4). Feed was withheld for at least 14 hours overnight prior to performing the exercise test, but water was available. Catheterization of the left jugular vein was performed one hour before the test. A sterile catheter was introduced into the pulmonary artery through the left jugular vein. Pressure waves from the tip of the catheter were analyzed using a blood pressure monitor (Propaq 140, Protocol Systems Inc.) to assure placement of the catheter in the pulmonary artery.

Sample collection and assays

Resting blood samples of about 30 ml were collected from the pulmonary artery while the horse stood quietly prior to beginning the test. Additional samples were collected 30 seconds prior to the end of each 3 min. incremental step, and at 5, 10, 20, and 30 minutes of recovery.   Samples were placed in heparinized tubes and immediately centrifuged. The plasma was drawn off and stored at -5° C until assays were done.

Plasma was assayed for lactate (Proc. # 826-UV, Sigma), triglyceride and glycerol (Proc. #337, Sigma), glucose (Proc. #16-UV, Sigma), cholesterol (Proc. #352, Sigma), and FFA (Wako NEFA-C, Biochemical Diagnostics). All analyses were performed within 3 weeks after each test.

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The data was summarized as least squares mean ± standard error. One-way analysis of variance, analysis of variance for repeated measures, and contrasts were used to evaluate effects of horse, diet, period (IET), and interactions, using computer software (SAS/STAT User's Guide 6.03, SAS Institute, Inc., Cary, NC). The *t*-test was used for differences between means when the overall F-test was significant (P < 0.05).

Three statistical models were applied initially to mean lactate/speed points for 9 horses at each IET and to means for 9 horses fed each diet. Data obtained from each of 9 horses in the first IET were also used to compare models. The first model consisted of a simple exponential line (exp) with an intercept: $y = a + be^{cx}$ (Vallette et al., 1996). The second and third models were both derived from the broken-line model developed by Robbins (1986), and are referred to as one-slope and two-slope (Custalow et al., 1993). For the one-slope model, the first slope is fixed at zero, and the second slope is allowed to adjust to a least squares best fit by an iterative procedure. In the two-slope version, both slopes are allowed to adjust. Goodness of fit of each model was evaluated by the coefficient of determination (r^2) and the mean square error (MSE).

Results

No overall differences were found between IETs for any variable. Resting values were similar for the three IETs. No interactions were found. Exercise and recovery affected all variables except cholesterol. The responses to exercise were also affected by diet for plasma FFA, glycerol and cholesterol but not lactate, glucose or TG.

Plasma lactate was 0.46 ± .09 mmol/L, and plasma glucose was 107.5 ± 3.0 mg/dl at rest. Both increased slightly through the walk and trot then more rapidly, starting about 4.5 m/s for glucose and 5.5–6.5 m/s for lactate (Figures 3 and 4). During recovery both decreased.

Plasma TG was 14.8 ± 1.4 mmol/L at rest. It was unchanged during exercise but increased about 2-fold ($P = .036$) during recovery (Figure 5).

Plasma FFA was 536.3 ± 57.5, 435.6 ± 57.5, and 435.7 ± 57.5 mg/dl for CON, CO and LE, respectively, at rest.   After an initial drop at a walk and slow trot, FFA levels remained constant during exercise. During recovery, plasma FFA increased to 562.1 ± 55.4, 484.5 ± 61.0, and 498.8 ± 64.8 mg/dl for CON, CO and LE, respectively. Diet affected plasma FFA at rest and during exercise and recovery ($P = .049$). Compared to the control diet, plasma FFA were lower ($P < .05$) at most of the speed increments during the exercise phase for the CO and LE diets (Figure 6).

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Plasma glycerol was 1.26 ± .15, .594 ± .15, and .811 ± .15 mg/dl for CON, CO and LE, respectively, at rest. It remained fairly constant during exercise, then increased two-fold ($P < .044$) during recovery (Figure 7). Diet affected plasma glycerol ($P = .059$). During exercise and recovery, plasma glycerol was lower ($P < .05$) at most points for the CO diet than for CON (Figures 7). Plasma glycerol was intermediate for the LE diet.

Plasma cholesterol was 87.3 ± 2.1, 101.8 ± 2.1, and 103.6 ± 2.1 mg/dl for CON, CO and LE, respectively, at rest. Compared to the control diet, plasma cholesterol levels were higher ($P < .05$) for 8 of the 10 exercise phase increments for both CO and LE (Figure 8).

Each IET yielded 10 to 12 points for modeling of the lactate curve. All three models demonstrated good fits ($r^2 > .98$) to the data (Figures 9 to 11). The r^2 and MSE values were lower for the one slope model than for the two slope and exponential models (Table 5). The exponential model yielded an unphysiological negative value for plasma lactate at zero speed (Figure 11).

In the first IET, the LT was 6.24 ± .18, 6.71 ± .15 and 7.02 ± .28 mmol/L or the one slope, two slope and exponential models, respectively. The values for the one and two slope models were different ($P = 0.011$) but correlated ($P = .083$) (Figure 12). The values for the one slope model and the exponential model were different ($P = .026$) and not correlated. The values for the two slope model and the exponential model were not different ($P = .32$) but not correlated ($P = .58$) (Figure 13). With the two slope model, mean values of LT was not affected by diet ($P > .61$) or IET ($P > .34$).

Discussion

The lack of differences in these variables between IETs confirms that the horses were already fit and were maintained at the same degree of fitness for the 9 week period. The time period needed to achieve fat adaptation has been widely debated. Previous studies in our laboratory have indicated that a period of at least 12 weeks of corn oil supplementation and interval training of previously untrained horses was necessary for full metabolic adaptation   (Greiwe et al., 1989; Ferrante et al., 1994; Taylor et al., 1995). In contrast, several studies using tallow have indicated complete digestive and metabolic adaptation within 3 weeks provided the horses were already fully trained (Potter et al., 1992). These present results confirmed that a 21 day period was sufficient to demonstrate dietary differences in plasma glycerol, FFA and cholesterol during exercise and recovery. Plasma cholesterol has been used previously as an index of fat adaptation (Taylor et al.,

1995). On the other hand, diet had no effect on plasma lactate, glucose or TG in these experiments, so the possibility exists that a longer observational period may allow more complete metabolic adaptation. Thus whether metabolic adaptation to different dietary fats was fully achieved in 21 days may depend on the criteria—affirmative for cholesterol, glycerol and FFA, but negative for lactate, glucose and TG.

Interpretation of plasma concentrations of metabolites during exercise and recovery relates to the net balance between entry and removal rates. The abrupt increases in glycerol, FFA and TG during recovery (Figures 5 to 7) are suggestive of continuing increased entry rates after increased removal rates have ceased.   Lipolysis of triglycerides in adipose tissue yields glycerol and, to a lesser extent, FFA, because some of the latter is re-utilized for triglyceride synthesis (Pösö et al., 1989).   Plasma glycerol is removed largely by the liver and converted to glucose, especially during exercise when blood glucose needs replenishing. Plasma FFA are taken up by the liver in the horse and converted largely to triglycerides that are released into plasma incorporated into very low density lipoproteins (Kurcz et al., 1991).   The plasma TG are split by lipoprotein lipase on the muscle cell membrane, releasing fatty acids into the cell. Oxidation of fatty acids derived from FFA and TG approach a maximal rate at slower speeds. Thus the fall in plasma FFA early in exercise reflects an increase in entry rate that is less than the removal rates by liver and muscle. The lack of such a decline in plasma glycerol and TG indicates that the increases in entry rate more closely matched the increases in removal rates.

The relatively slow increases in plasma glucose and lactate at the walk and trot represent increased removal rates matched by entry rates. Glucose is presumably contributed to the blood mainly from the liver in these overnight fasted horses and the increase in its removal rate is mainly due to the active muscles. The "lactate shuttle" is more complex, because lactate is both produced and utilized by working muscles, and it is removed from blood by many organs, notably the liver and heart (Brooks, 1985).   The removal of both metabolites probably increases up to a saturation rate. On the other hand, lactate production is geared to work intensity and catecholamine release rather than to oxygen availability (Stainsby and Brooks, 1990), and it increases in a curvilinear manner (Stanley et al., 1985). Catecholamine release occurs in horses during sprinting (Snow et al., 1992). Catecholamine release probably occurred at about 4.5 m/s in this study and could explain the observed increase in plasma glucose, due to hepatic glycogenolysis, and subsequent increase in plasma lactate due to muscle glycolysis.

Dietary fat supplementation decreased plasma FFA and glycerol concentrations and increased plasma cholesterol at rest and during recovery and exercise (Figures 6 to 8). Plasma cholesterol has been found previously to increase during dietary fat

supplementation (Taylor et al., 1995). It was used as an index of fat adaptation, which was considered to be complete when plasma cholesterol ceased to increase or maintained a stable value. The lower plasma concentrations of FFA and glycerol may be attributed partly to increased rates of utilization attained through fat adaptation (Simi et al., 1991; Reynolds et al., 1996).

Plasma lactate, glucose, and TG concentrations were unaffected by dietary fat supplementation. Fat adapted horses have been found to have higher lactate responses and utilize more muscle glycogen during anaerobic exercise (Oldham et al., 1990). The lack of response in plasma lactate and glucose in this study may be a result of incomplete adaptation.   Plasma TG may have been replenished rapidly in the fat-adapted horses, therefore causing an absence of affect.

Exponential curves and VLa4 are both popular methods for examining lactate response to exercise (Wilson et al., 1983; Vallette et al., 1996). Using a segmented line model to discover a POI where a rapid accumulation of lactate occurs has been shown to have a good fit to the lactate curve (Custalow et al., 1993). When these methods were compared, there was no correlation between values for lactate threshold and VLa4 (Figure 13). Lactate threshold has been found to have a higher correlation with race time than VLa4 (Casini et al., 1996). VLa4 has also been found to be variable according to the type of training done prior to testing (Vallette et al., 1989). Although both models demonstrated good fit to the curve, the lack of correlation and previous findings that VLa4 is a less accurate predictor of performance, lead to the conclusion that the segmented model is preferable.

No diet or IET effects were noted for lactate threshold. This follows along with the lack of diet effect found for lactate or glucose level. Even though cholesterol, glycerol, and FFA levels changed with diet, there were no effects found for metabolites associated with glycolysis. These results are in contrast to other studies which found lactate threshold to be increased following fat adaptation (Custalow et al. 1993, Ferrante et al. 1993), and decreased with a lecithin-containing diet (Taylor et al., 1995). This study may indicate that a fat adaptation period of 21 days does not influence anaerobic performance. Although an increased rate of fatty acid production and utilization was exhibited following fat adaptation for 21 days, it was not sufficient to affect the processes of glucose oxidation and glycolysis during exercise.

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General Discussion

There was conflicting evidence of fat adaptation after a 21 day diet period in this study. Lactate and glucose levels were not affected by diet or IET. Thus the rate of glycolysis was most likely unaffected by the 10% fat-containing diets after 21 days of fat adaptation. Also muscle glycogen levels were either not spared or did not reach a level of depletion that would affect blood glucose during exercise. Apparently a plateau of fitness or anaerobic conditioning was reached as these variables did not change over time despite continued training throughout the study.

Metabolites associated with fat adaptation were affected by diet and in some cases by IET. Plasma FFA, glycerol, and cholesterol were increased in the fat diets during exercise and recovery. There was probably an increased rate of fatty acid oxidation that was caused by training and fat supplementation. This change caused more plasma FFA to be utilized, glycerol to be taken up at a rapid rate, and cholesterol concentration in the plasma to stay high during exercise.

A distinct lactate threshold was identified in the two slope segmented model. Up to 12 points were obtained for the lactate curves using an IET. This was a sufficient number of points to produce a distinct point of inflection (POI). The POI represents the velocity at which lactate production exceeds the rate of utilization due to saturation. Therefore a rapid increase in lactate level becomes evident.

A broken-line segmented model and an exponential model were both found to have excellent fit. No correlation between lactate threshold for the segmented model and VLa4 was found. Lactate threshold was unaffected by either corn oil or lecithin supplementation. After three weeks of fat adaptation there was no delay in the onset of lactate threshold.

Overall it was determined that after 21 days of adaptation to a 10% fat added diet, some criteria of fat adaptation were met. Metabolites such as plasma FFA, glycerol, TG, and cholesterol were all affected by the two fat-containing diets. Each of these have been shown to be changed following fat adaptation. However, lactate levels did not increase with the corn oil diet as was expected. The lecithin diet did not decrease lactate level as was found in a previous study done in our lab. Thus, the 21 day period was insufficient to produce these changes. Furthermore no changes in the onset of lactate threshold were found between diets. Both a segmented model and an exponential model provided a good fit to the lactate curve, but it is likely that the segmented model provides a better prediction of performance.

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Tables

Table 1. **Conditioning protocol for interval training.**

EXERCISE PROTOCOL - Weeks 1- 3

TIME (MIN)	SPEED (M/S) / SLOPE (%)	GAIT
0 - 2.5	1.2 / 0	Walk
2.5 - 5	1.2 / 6	Walk
5 - 9	3.2 / 6	Trot
9 - 12	7 / 6	Canter
12 - 15	1.2 / 0	Walk

EXERCISE PROTOCOL - Weeks 4-7

TIME (MIN)	SPEED (M/S) / SLOPE (%)	GAIT
0 - 2.5	1.2 / 0	Walk
2.5 - 5	1.2 / 6	Walk
5 - 9	3.2 / 6	Trot
9 - 12	7 / 6	Canter
12 - 15	3.2 / 6	Trot
15 - 18	8 / 6	Canter / Gallop
18 - 21	3.2 / 6	Trot
21 - 24	1.2 / 0	Walk

EXERCISE PROTOCOL - Weeks 7 - end of study

TIME (MIN)	SPEED (M/S) / SLOPE (%)	GAIT
0 - 2.5	1.2 / 0	Walk
2.5 - 5	1.2 / 6	Walk
5 - 9	3.2 / 6	Trot
9 - 12	7 / 6	Canter
12 - 15	3.2 / 6	Trot
15 - 18	8 / 6	Canter / Gallop
18 - 21	3.2 / 6	Trot
21-24	9 / 6	Canter / Gallop
24-27	3.2 / 6	Trot
27 - 30	1.2 / 0	Walk

Table 2. Composition of control, corn oil, and lecithin diets.

CONTROL DIET COMPOSITION

FEED	AS FED	LBS. DM
Cane Molasses	1.000	0.745
Orchardgrass hay	10.000	9.240
Limestone	0.060	0.059
Corn grain	6.500	5.720
44% Soybean meal	1.500	1.366
Minerals	0.500	0.499

CORN OIL & CORN OIL/SOY LECITHIN DIET COMPOSITION

FEED	AS FED	LBS. DM
Cane molasses	1.000	0.745
Corn oil/ Lecithin	1.650	1.648
Orchardgrass hay	9.000	8.316
Limestone	0.060	0.059
Corn grain	3.000	2.640
44% Soybean meal	1.750	1.559
Minerals	0.500	0.449

Table 3. Chemical composition of control, corn oil, and lecithin diets.

Item	CONTROL 100% DM	As Fed	CORN OIL 100% DM	As Fed	LECITHIN 100% DM	As Fed
Dry Matter, %	89.00	----	91.29	----	90.39	----
Composition of DM, %						
Crude Protein	13.19	11.73	10.89	9.94	12.50	11.29
Digestible Protein	8.73	7.76	6.59	6.01	8.09	7.31
Ether extract	8.25	----	13.82	----	13.25	----
Acid detergent fiber	22.00	19.58	25.79	23.54	25.59	23.13

Table 4. Incremental Exercise Test protocol.

RUNNING TIME (MINUTES)	SPEED (METERS/SECOND); GAIT
Rest: 0	0 ; standing
3	1.5; walk
6	1.5 (+ increase to 6% slope); walk
9	2.5; trot
12	3.5; trot
15	4.5; trot
18	5.5; trot/canter
21	6.5; canter
24	7.5; canter
27	8.5; canter
30	9.5; canter/gallop
33	10.5; canter/gallop
Max	Varied; point of exhaustion
5 min. recovery	1.2; walk
10	1.2; walk
20	1.2; walk
30	1.2; walk

Table 5. Comparison of x-intercept, y-intercept, r^2, MSE between the one-slope, two-slope, and exponential models using lsm $\pm$ SE of diet values.

MODEL	X-INT (M/S)	Y-INT (MMOL/L)	R^2	MSE
One-slope	6.03	.71	.9808 ± .002	.599 ± .101
Two-slope	6.95	2.28	.9895 ± .002	.302 ± .101
Exp	7.3	4.0	.9882 ± .002	.291 ± .101

Figures
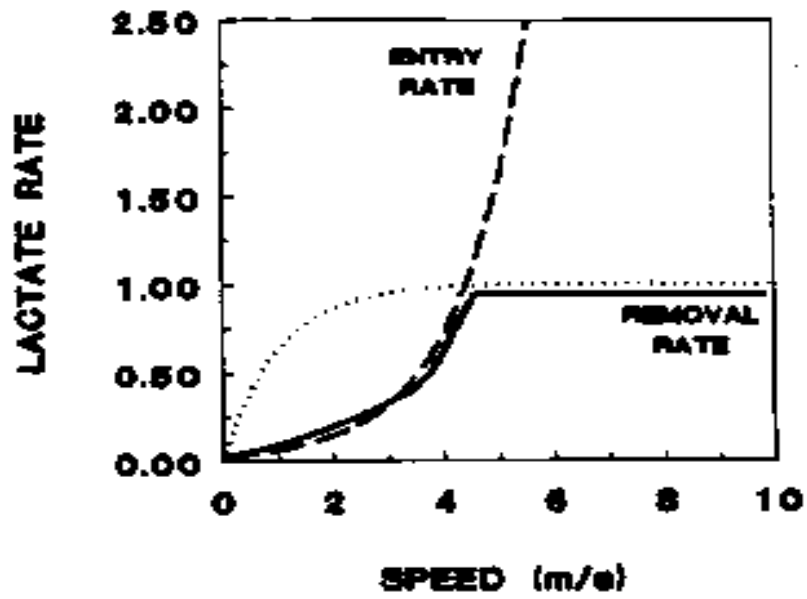
Figure 1. Lactate removal rate versus lactate entry rate during incremental exercise.
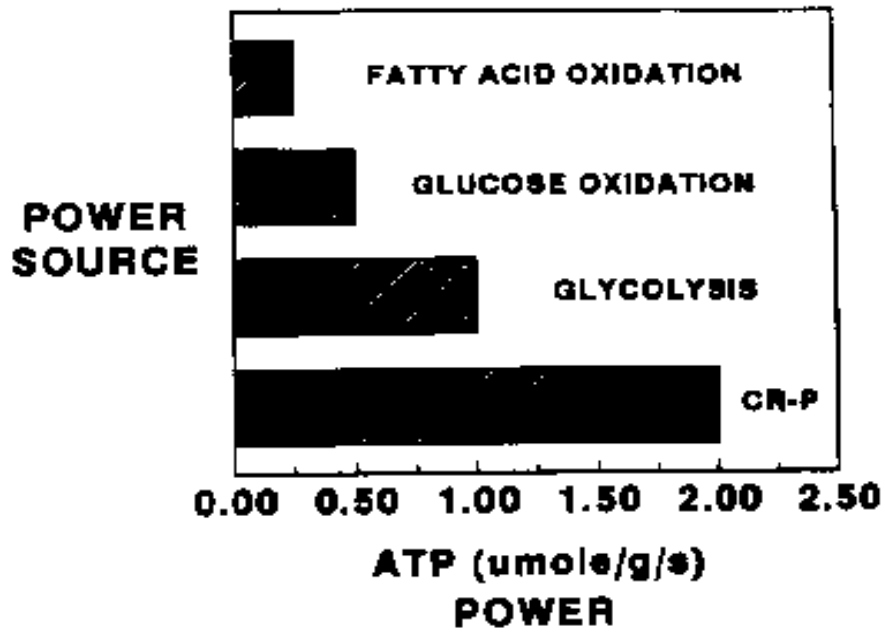
**Figure 2.** Relative power output of fatty acid and glucose oxidation, glycolysis, and phosphocreatine (Cr-P).
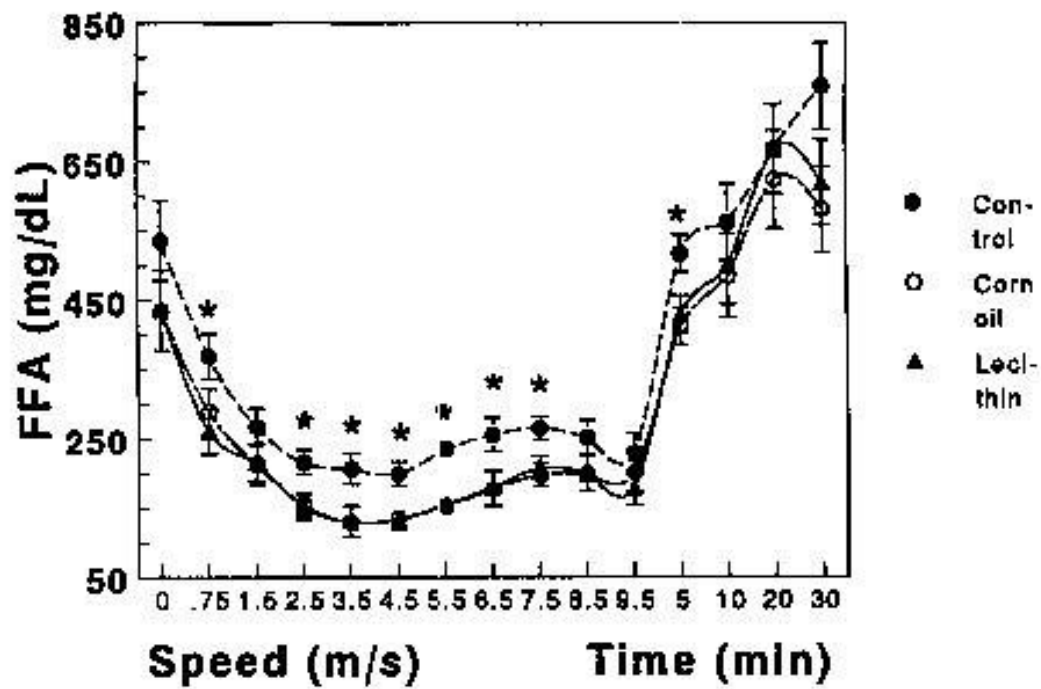
Figure 3. Changes in plasma FFA during rest, incremental exercise, and recovery in conditioned horses. Decreased plasma FFA were found in the CO and LE diets (*P < .05) during exercise.
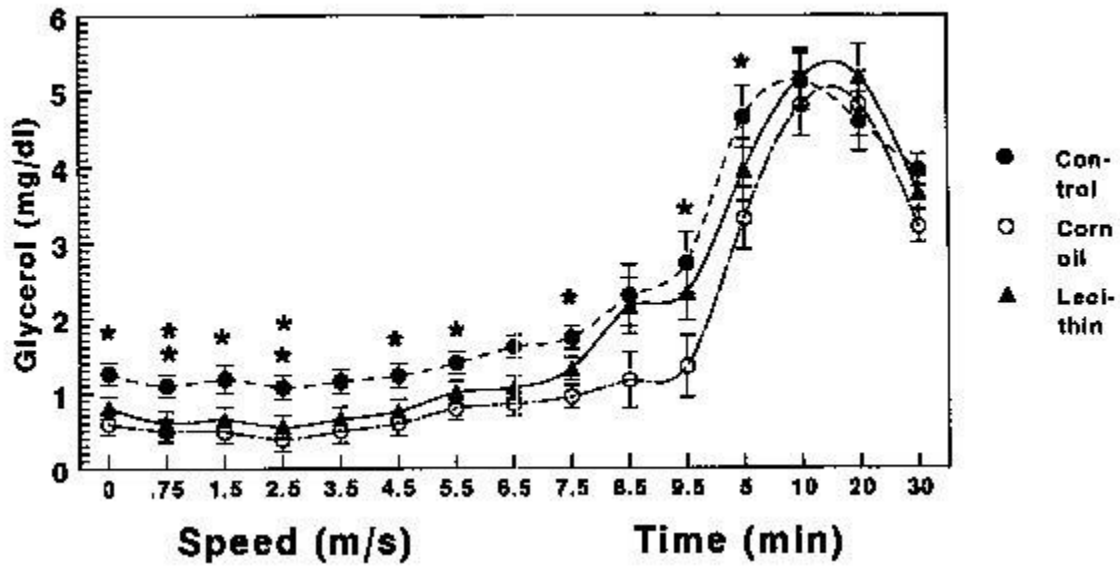
Figure 4. Changes in plasma glycerol during rest, incremental exercise, and recovery in conditioned horses. Decreased plasma glycerol were found in the CO and LE diet (**$P < .05$) from the CON diet and in the CO diet (*$P < .044$) from the CON diet.
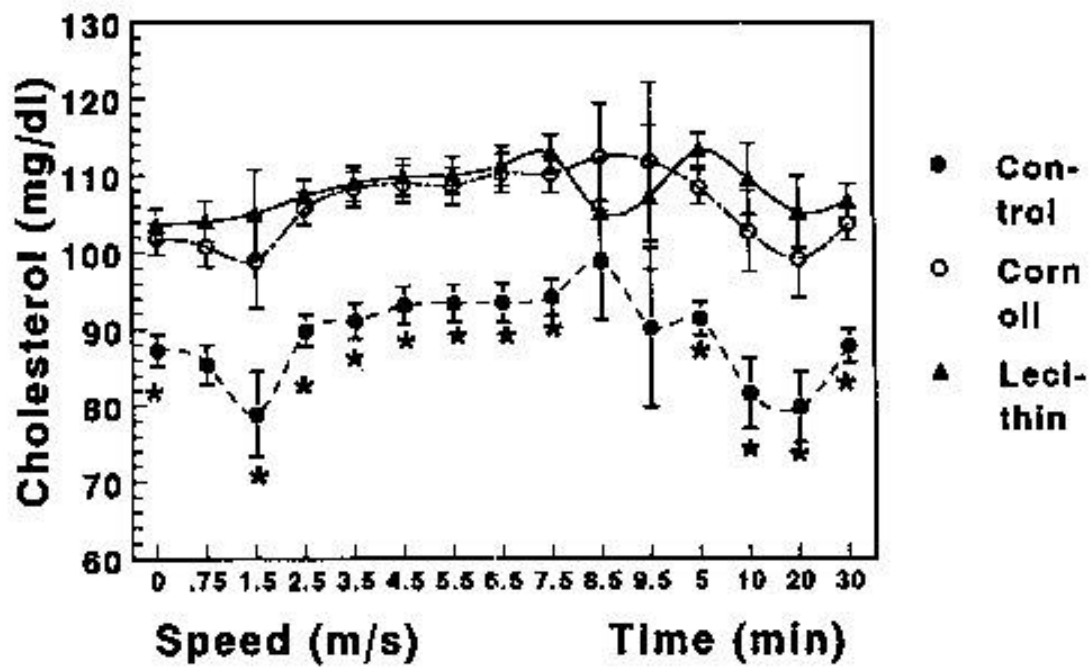
Figure 5. Changes in plasma cholesterol during rest, incremental exercise, and recovery in conditioned horses. Increased plasma cholesterol was found in the CO and LE diet (*$P < .05$) from the CON diet during rest, exercise, and recovery.
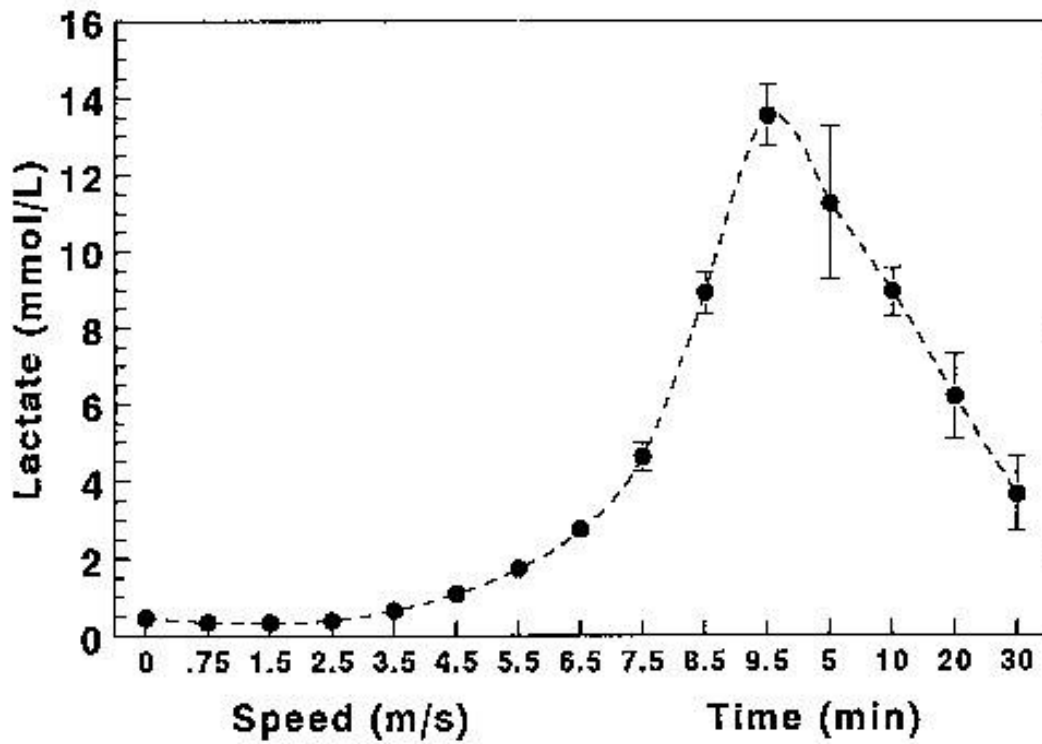
Figure 6. Representative example of changes in plasma lactate during rest, incremental exercise, and recovery in conditioned horses.
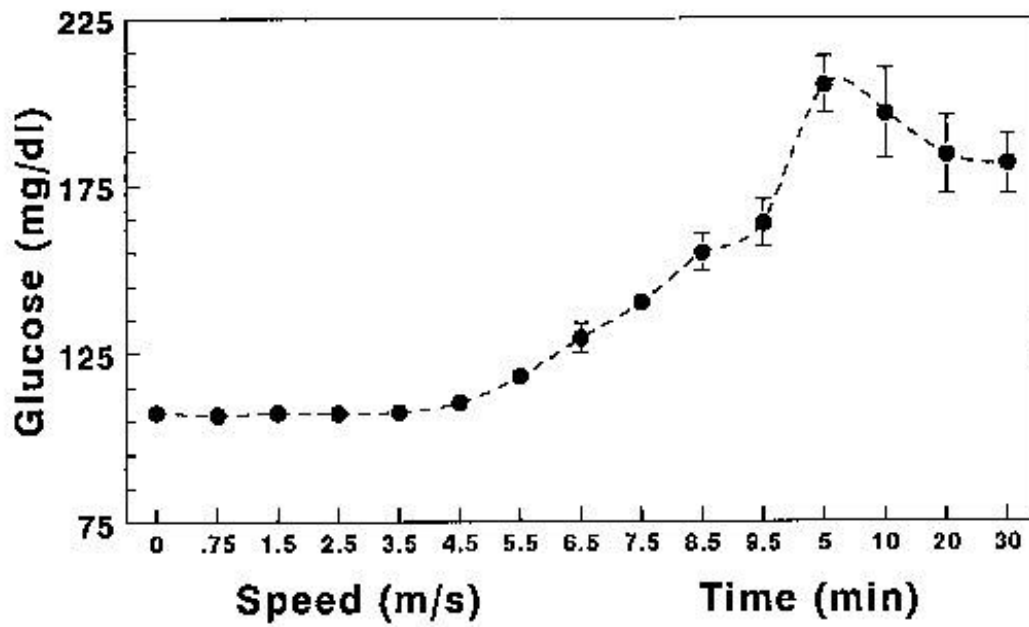
Figure 7.   Representative example of changes in plasma glucose during rest, incremental exercise, and recovery in conditioned horses.
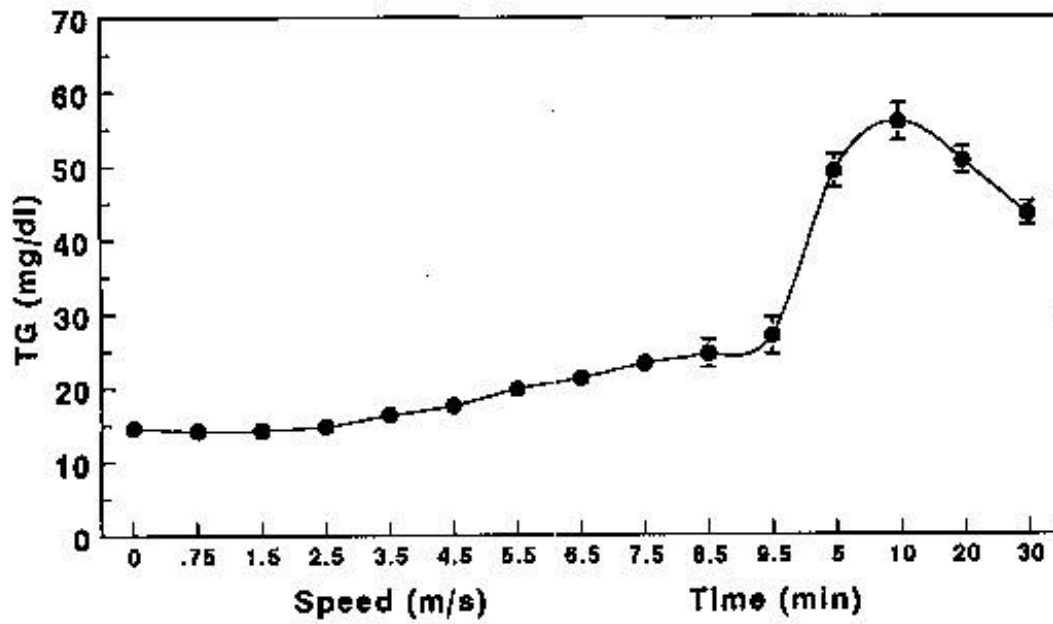
Figure 8. Representative example of changes in plasma triglyceride during rest, incremental exercise, and recovery in conditioned horses.
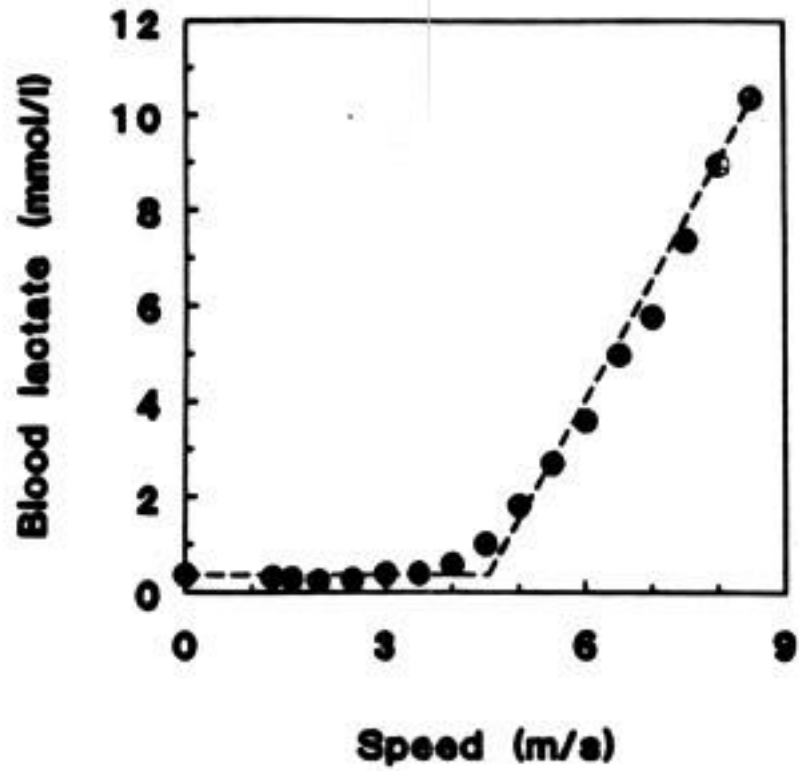
Figure 9. Representative example of one slope segmented line model when fit to the lactate curve.
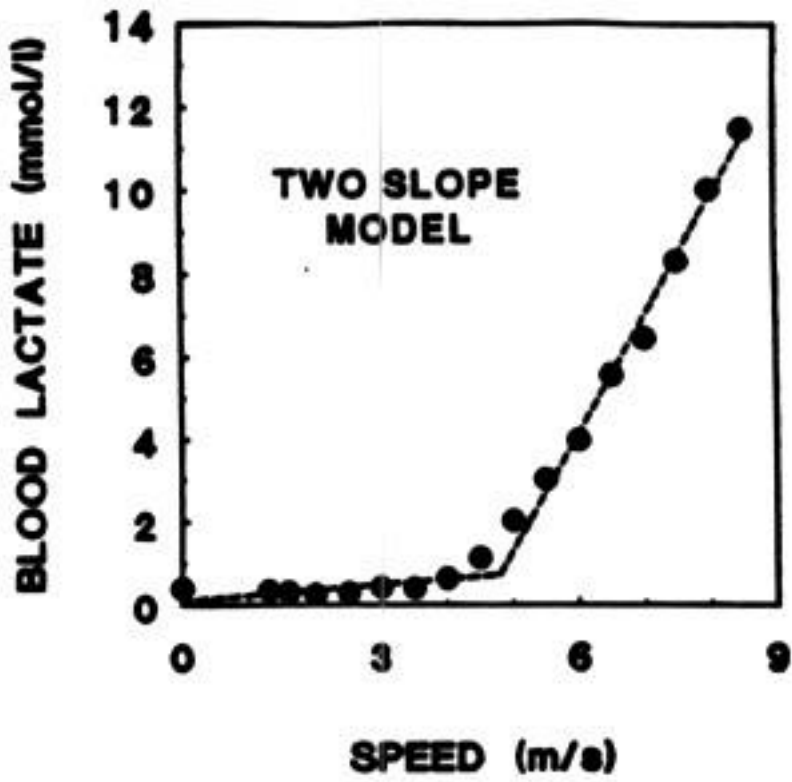
Figure 10. Representative example of two slope segmented line model when fit to the lactate curve.
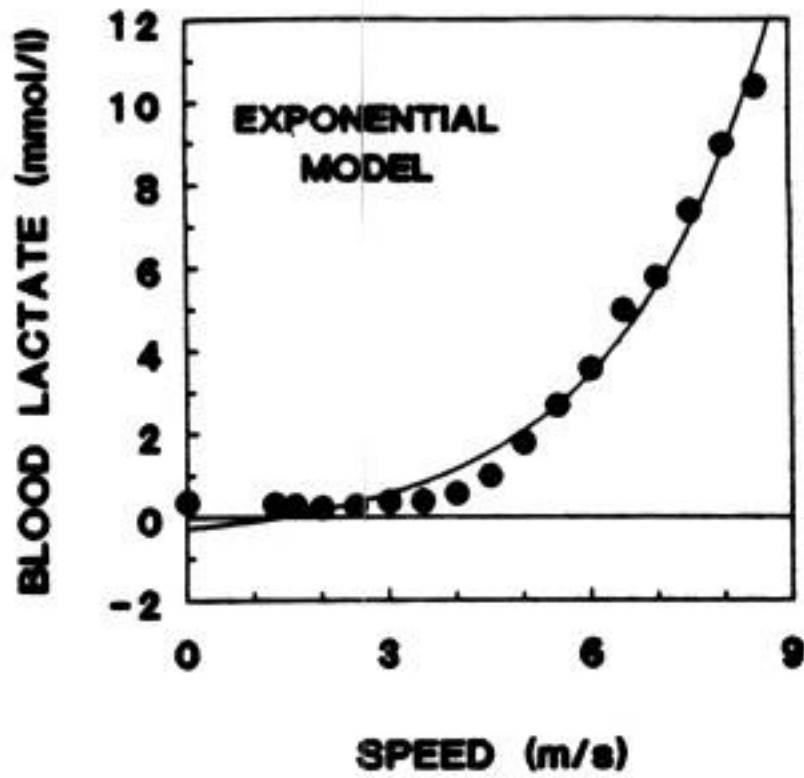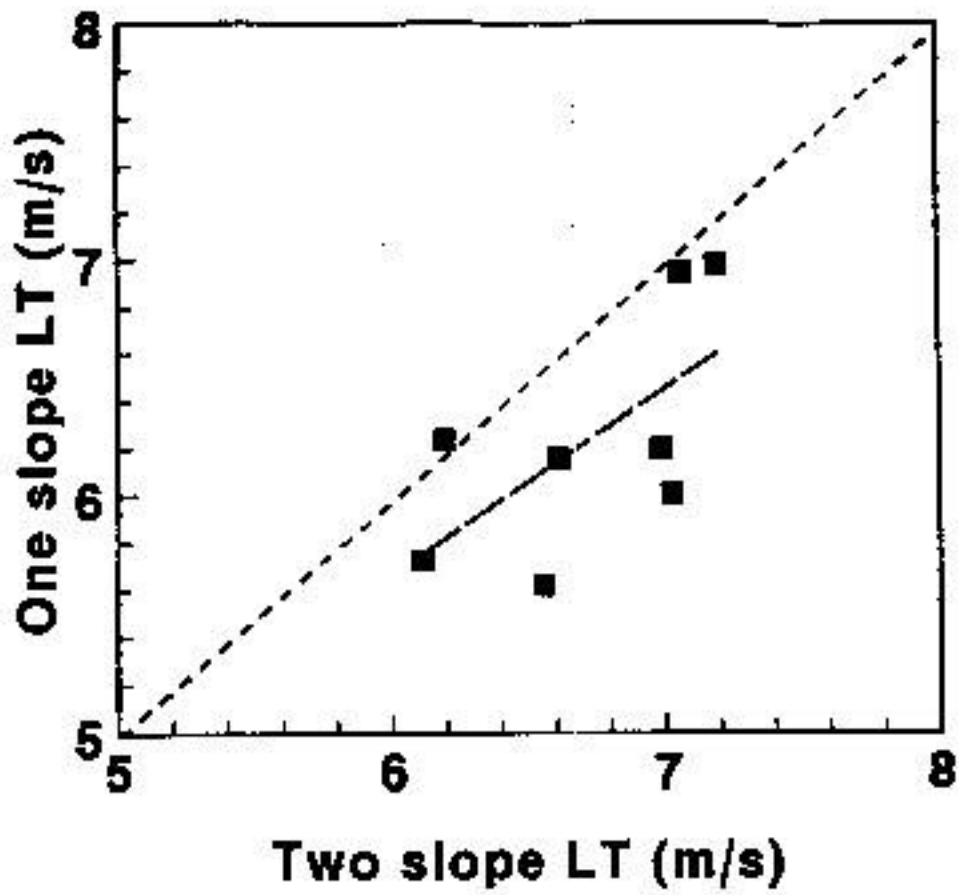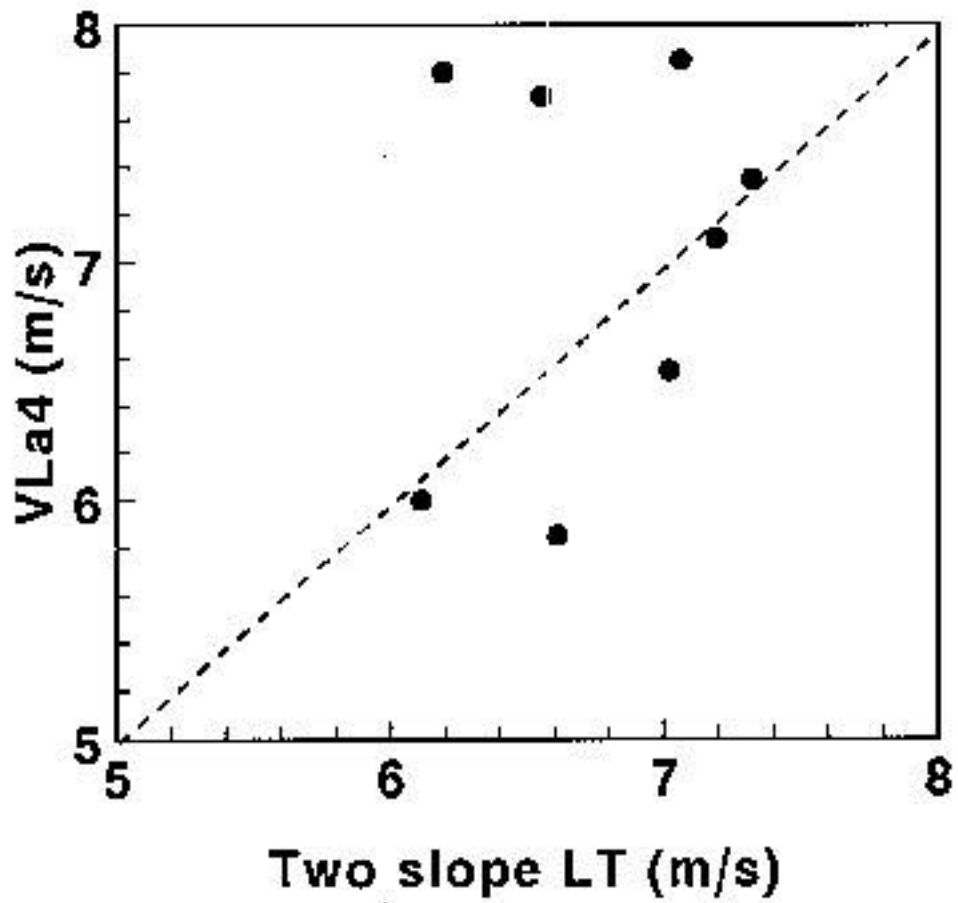
Figure 11. Representative example of exponential model when fit to the lactate curve.

Figure 12. Positive correlation of lactate threshold values obtained from the one and two slope segmented line models (P = .083)

Figure 13. Lack of correlation between lactate threshold from two slope segmented line model and VLa4 from exponential model (P = .58).