1993

Influence of diet composition and training on energy utilization by greyhound skeletal muscle

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Order Number 9321119

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Iowa State University, 1993

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Influence of diet composition and training on energy utilization by greyhound skeletal muscle

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Departments: Veterinary Physiology and Pharmacology
Animal Science
Majors: Physiology
Nutritional Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Departments

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1993

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
A-V difference	Arterial-venous difference
BCAA	Branched chain amino acids
CS	Citrate synthase
g	Gram(s)
ga	Gauge
GPT	Glutamate-pyruvate transaminase
kcal	Kilocalorie(s)
kg	Kilogram(s)
km	Kilometer(s)
LDH	Lactate dehydrogenase
µM	Micromolar concentration
µmol/l	Micromoles per liter
mM	Millimolar concentration
mmol/l	Millimoles per liter
ml	Milliliter(s)
mg/dl	Milligrams per deciliter
min	Minutes
NAD^+	Nicotinamide adenine dinucleotide
PFK	Phosphofrucktokinase
w:w	Weight to weight comparison
wwt	Wet weight

GENERAL INTRODUCTION

An Explanation of the Dissertation Organization

This dissertation begins with a general review of the literature focusing on the effects of a raw-beef mixed diet vs. a corn-soy based diet on skeletal muscle metabolism of exercised greyhounds. The literature review concentrates on (1) the effect of diet on key enzyme activities involved in energy production in trained dogs, (2) the thermodilution method of determining blood flow to the hindleg and (3) the effect of diet and exercise on alanine-glucose cycle substrate availability. Following the literature review are three papers presented in publication form. Paper 1 addresses the effect of diet on glycogen content and enzymes involved in energy production and included specifically citrate synthase, phosphofructokinase, and glutamate-pyruvate transaminase. Paper 2 describes an original application of a thermodilution technique used to determine blood flow in the external iliac vein of the conscious greyhound. This method was needed in order to determine the utilization of compounds across the hindleg of the greyhounds used in Paper 3. Paper 3 addresses the effect of test diets on concentrations of alanine, leucine, isoleucine, valine, glycine, glucose, and lactate in plasma and skeletal muscle. Following the papers is a general summary that addresses the effect of diet and training on greyhound skeletal muscle metabolism. The dissertation concludes with the list of references cited.

General Literature Review

Greyhounds

Greyhound diet Greyhound dogs have traditionally been fed a raw meat-mix diet (Lassen et al., 1986; Konke, 1983). Trainers believe racing performance is improved when meat is present in the diet, although this assumption may be due to psychological

factors in diet preference more than to physiological factors. Feeding pattern is basically uniform throughout the industry and consists of a single large meal, sometimes supplemented with a small "snack" prior to evening racing (Baumhover, 1986). This feeding pattern has recently been shown to result in increased hepatic storage of glycogen resulting in glycogen sparing during exercise in rats (Nagamatsu and Arao, 1990). In this study, rats that were fed a large meal then rested before a treadmill workout had lower plasma free fatty acid concentrations and higher liver glycogen concentrations than rats fed a large meal immediately before workout. Glycogen sparing has been associated with decreased muscle fatigue (Fitts et al., 1975).

Greyhounds utilize the phosphate generating system and glycogenolysis as primary sources of energy in a 30 second bout of exercise (Grandjean and Paragon, 1992) as do humans (Hirvonen et al., 1987; McCartney et al., 1986). Performance during short-term maximal exercise depends on muscle capacity to utilize high-energy phosphates at the beginning of a race and performance decreases when such phosphate stores are depleted, causing glycolysis to increase (Hirvonen et al., 1987). The bioavailability of muscle phosphagens is not affected by training or diet (Grandjean and Paragon, 1992); however, dietary enhancement of glycogen formation is possible (Bergstrom et al., 1967; Nagamatsu and Arao, 1990).

Most diet studies with dogs have concentrated on endurance rather than sprinting performance (Kronfeld, 1973; Hammel et al., 1977). A high fat diet improves performance of sled dogs engaged in severe endurance activity (Kronfeld, 1973). Most literature accounts of diets specific for greyhounds focus on which percentage of raw beef mixed with a corn-soy based food is ideal to the racing dog (Davis, 1977) rather than if raw beef is ideal to the sprinting dog at all. Dietary raw beef can increase greyhound packed cell volume (PCV), red blood cell count (RBC), and hemoglobin (Hb) content (Drisko,

1988), but this increase is caused by dehydration rather than by a hematinic effect (Engen, 1992), which is likely not to be beneficial to performance.

Greyhound muscle and serum chemistry values Baseline values for plasma lactic acid, glutamate-pyruvate transaminase (GPT) activity, and glucose concentration in greyhounds are 0.57 ±0.09 mM, 46 ± 16 U/L, and 6.0 ± 1.1 mM, respectively (Ilkiw et al., 1989). Greyhound blood lactate concentrations have been reported as 0.88 ± .004 mM (Bjotvedt, 1984) and 2.15 ± 0.263 mM (Dobson et al., 1988).

Greyhound muscle (GPT) activity has been reported as 56 ± 6 $\mu mol \cdot min^{-1} \cdot g^{-1}$ (Rose and Bloomberg, 1989). Greyhound resting biceps femoris muscle measurements are reported for lactate, 3.49 ± 0.13 μmol/g wwt; pyruvate, 0.173 ± 0.015 μmol/g wwt; and glycogen, 71.80 ± 8.52 μmol/g wwt (Dobson et al., 1988).

Dobson et al. (1988) did extensive work on quantifying greyhound biochemistry response to a sprint. In that study, greyhounds seemed to have a 25% higher glycogenolytic capacity per g muscle than did the Thoroughbred horse and 43% higher capacity than did humans. The increased ability to catabolize glycogen may account for the high lactic acid concentrations, up to 28.9 ± 3.0 mM, that can occur in greyhound muscle post-exertionally (Dobson et al., 1988; Ilkiw et al., 1989; Snow et al., 1988). The buffering capacity of greyhound muscle was not different from that of other animals, though; therefore, the authors contributed increased blood flow that physically removes lactate with combating acidosis in greyhounds. Greyhounds do have a greater ability to remove lactate and thus reestablish pre-race metabolite concentrations than do Thoroughbred horses or man (Harris et al., 1987; Dobson et al., 1988; Ilkiw et al., 1989), which may be a result of increased blood flow.

Fiber types There are inherent difference between type I, type IIa and type IIb skeletal muscle fibers. Functional differences are related to energy utilization and

production differences (Baldwin et al., 1972; Barnard et al., 1971; Bass et al., 1969; Lowry et al., 1978; Nelson and Tashiro, 1973; Opie and Newsholme, 1966; Pette and Staudte, 1973; Spamer and Pette, 1977). Type I fibers are high in myoglobin and enzymes of the citric acid cycle. Therefore, these fibers are best suited for oxidative metabolism required in endurance work. Type II fibers are primarily glycolytic, but are divided into two sub-groups based on oxidative ability within those fibers. Type IIa fibers are oxidative-glycolytic, and IIb fibers are purely glycolytic.

Greyhound hindleg skeletal muscle is 85-100% fast-twitch fibers which is a larger percentage of fast-twitch fibers than the 60% reported in similar muscles of non-greyhound breeds (Aguera et al., 1990; Gunn, 1978; Guy and Snow, 1981). Whether those fibers in greyhounds are primarily glycolytic (IIb) or oxidative-glycolytic (IIa) is controversial (Armstrong et al., 1982; Gunn, 1978; Gunn, 1975; Guy and Snow, 1981; Snow, 1985; Taylor, 1988). Greyhounds also have a greater number of fibers in cross-sectional areas of hindleg muscle than do other breeds of dogs (Gunn, 1979). Greyhound muscle has high creatine kinase and aldolase activities, which are typically associated with anaerobic capacity (Guy and Snow, 1981). These dogs also have an increased ability to withstand and clear lactate (Ilkiw et al., 1989), although lactate dehydrogenase (LDH) activity (Guy and Snow, 1981) or buffering capacity (Dobson et al., 1988; Harris et al., 1990) in greyhounds is not different from those of other breeds of dogs.

Although endurance training is typically associated with increases in citric acid cycle enzymes (Holloszy, 1967; Holloszy et al., 1970), it may also enhance glycolytic capacity in both type I and type II skeletal muscle fibers in horses (Essen-Gustavsson and Henriksson, 1984). Explanations for such exercise-induced increases in oxidative capacity of muscle are controversial. Baldwin et al. (1972) does not believe it is due to transformation of type I into type II fibers but rather because of enhanced enzyme activity within each fiber proportionately. A recent report, however, claims that type I fibers can be

transformed into type IIa with high-intensity training (Jansson et al., 1990). This study used ATPase staining to determine fiber type. The high intensity training was believed to increase stimulation frequency, and subsequently induce type II myosin.

Influence of exercise on glycogen and energy-producing enzymes

Several review articles have been written to provide a complete and understandable overview of the biochemical adaptations to endurance exercise (Felig, 1977; Holloszy, 1973; Holloszy, 1973b; Holloszy et al., 1973; Ruderman, 1975). Fewer reviews are available that illustrate the adaptations to acute exercise, however (Abernethy et al., 1990; Viru, 1987).

Glycogen Glycogen degradation in both type I and type IIb fibers is directly correlated with intensity and duration of exercise (Goldfarb et al., 1989) and is greatest with intermittent exercise (Ward et al., 1982). The magnitude of the glycogen sparing effect seen in training is directly correlated with increased muscle respiratory capacity in rats (Fitts et al., 1975). The availability of glycogen, however, has been determined not to limit high intensity exercise performance (Hermansen, 1981; Greenhaff et al., 1988), although selective glycogen depletion in type IIb fibers may cause fatigue (Tesch, 1980). Saltin and Karlsson (1971) did an extensive study that examined individual muscle fiber recruitment and glycogen content of those fibers with work intensities. They found that at work rates below 50% maximal oxygen uptake (VO_2 max), glycogen depletion does not occur and therefore does not contribute to exhaustion. At work rates between 65-89% VO_2 max, glycogen depletion is the limiting factor in exhaustion. At work loads above 90% VO_2 max, lactate accumulation, and not glycogen depletion, is the limiting factor in performance. Glycogen utilization and resynthesis is not limited within the exercised muscle, alone. Unexercised leg muscle can provide substrate to exercised arm muscle via the Cori cycle (Ahlborg et al., 1986).

Glycogen stores are highest in fast-twitch fibers because glycogen is the primary energy source in these fibers. Lactate production in rodents (Baldwin et al., 1977) and man (Schlicht et al., 1990) is primarily from these fast glycolytic fibers. In horses, also, muscle and blood lactate accumulation is positively correlated with amount of glycogen utilized (Valberg et al., 1985). The same correlations between glycogen and lactate found in other species most likely apply to greyhound fibers as well.

Glutamate-pyruvate transaminase Glutamate-pyruvate transaminase (GPT) is responsible for increased capacity to generate alanine from pyruvate and citric acid cycle intermediates from glutamate (Mole et al., 1973). Several compounds influence GPT activity, and their mechanisms have been theorized for many years. Glucose inhibits the activity of GPT and thus suppresses gluconeogenesis at the point of conversion of pyruvate to alanine (Exton and Park, 1967). Glucagon is thought to act by stimulating GPT activity because it stimulates the conversion of alanine, lactate and pyruvate to glucose by the isolated perfused rat liver with a concomitant rise of urea formation (Curry and Beaton, 1958). Recently, however, the extrapolation of GPT activity from rat to dog has been questioned. Glutamate-pyruvate transaminase activity is 7 times lower, and alanine production from lactate, pyruvate and glutamate is 3 times lower in rat kidney than in dog kidney (Lemieux et al., 1988).

Exercise training has been correlated with increased muscle and plasma GPT activity in men (Felig and Wahren, 1971b; Vukovich et al., 1992), rats (Mole et al., 1973), greyhounds (Ilkiw et al., 1989) and sled dogs (Hammel et al., 1977). The time it takes to see an elevated plasma GPT from exercise is controversial, however. Hammel et al. (1977) claims a plasma GPT increase within 30 minutes after exhaustive exercise, but Nosaka et al. (1992) states that plasma GPT does not increase immediately in peripheral blood after strenuous exercise because 24 to 48 hours are usually required for muscle protein release from damaged muscle fibers. Ilkiw et al. (1989) is in agreement with

Hammel and reports that immediately after racing, plasma concentrations of GPT, lactate dehydrogenase, lactic acid, and glucose were elevated, bicarbonate and pH were decreased and urea concentrations were unchanged in greyhound plasma. In this study all parameters had returned to baseline by three hours post-racing. The increased GPT was hypothesized to be caused by decreased splanchnic blood flow, which may occur in greyhounds because of extreme exercise intensity during racing, although decreased splanchnic flow in exercising dogs has not been reported in the literature. How decreased splanchnic flow could cause an increase in GPT was not theorized in Ilkiw's study, however, but most likely was a result of hepatic anoxia. Plasma GPT activity, therefore, is not as accurate as muscle GPT activity in indicating glycolytic potential of skeletal muscle.

It has been postulated that metabolic regulation during acute exercise may be controlled by the turnover rate of enzymes involved in energy production (Ji et al., 1985). The altered enzyme concentrations may affect flux through various metabolic pathways. In Ji's study, alanine aminotransferase and aspartate aminotransferase both increased in resting samples from trained individuals indicating exercise adaptation. In addition, both activities decreased immediately post-exercise indicating increased flux.

Phosphofructokinase Phosphofructokinase (PFK) is the rate-limiting enzyme of glycolysis during initial 30 second bursts of energy, but phosphorylase seems to be rate-limiting in successive bouts (McCartney et al., 1986). Because PFK is sensitive to shifts in pH (Halperin et al., 1969; Ui, 1966; Trivedi and Danforth, 1966), the drop in pH that occurs within the first 30-60 seconds of maximal exercise may be enough to inhibit PFK. However, with sprint training, PFK levels can increase (Troup et al., 1986), although an interruption in training, up to 7 weeks, does not seem to affect PFK activities, at least in humans (Simoneau et al., 1987).

Citrate synthase Citrate synthase (CS) is a key enzyme involved in the citric acid cycle and its activity parallels tissue respiratory capacity. Mitochondrial content of

exercised muscle, which is indicative of aerobic-oxidative potential, is important in determining substrate utilization during exercise. Exercise training results in increased CS activity in skeletal muscle of rats at 75% maximal oxygen consumption (Gosselin et al., 1992), at 90-95% maximal oxygen consumption (Powers et al., 1992), at maximal oxygen consumption (Moore et al., 1987) after 8 weeks of submaximal endurance training (Dohm et al., 1973) and when exercised to exhaustion after 13 weeks of training (Fitts et al., 1975). In men, 12 weeks of training increased resting muscle CS activity but not post-exercise CS activity (Coggan et al., 1991). The time course of changes in CS activity is controversial. Exercising to exhaustion, either after 8 weeks of submaximal endurance training or in untrained muscle, did not alter CS activity in rats in one study (Dohm, et. al., 1973); however, another study reported immediate post-exercise changes after 8 weeks of training (Ji et al., 1985). The increase in CS activity is directly correlated with intensity of training (Kirwan et al., 1990; Melichna et al., 1987; Fitts et al., 1975); maximal exercise, however, can actually inhibit mitochondrial respiration because of proton accumulation (Witt et al., 1987). The training pattern is important in maximizing oxidative potential of muscle. Continuous training is more effective at increasing oxidative capacity of skeletal muscle, as represented by increased CS activity than is interval training even when exercise intensity is equal between groups (Gorostiaga et al., 1991).

The training-induced increases in CS, malate dehydrogenase and GPT activities appears to be related to beta 2-adrenergic mechanisms (Ji et al., 1986). Down regulation, or enzyme activity suppression, of CS, malate dehydrogenase and GPT activities occurs in rats after a single bout of acute or exhaustive treadmill running and may be due to peroxides and oxygen free radicals produced in prolonged exercise, which alter the redox state of mitochondria (Ji et al., 1988). Training may enhance resistance to these oxygen species by increasing activity of glutathione peroxidase.

Type of training can affect skeletal muscle substrate utilization. Continuous training is more effective in increasing oxidative potential as determined by increased enzyme activities and in decreasing lactic acid build-up in vastus lateralis muscle in men; however, interval training is more effective in increasing maximal oxygen consumption and overall exercise capacity as determined by exercising work rates and power output (Gorostiaga et al., 1991). Tapering workouts has some beneficial effects also. When cyclists were rested 4- or 8- days in an exercise taper study, muscle glycogen concentration increased along with power output compared with that of those who exercised continuously (Neary et al., 1992). Increased training volume (30 $m \cdot min^{-1}$ at 6 degrees incline for up to 360 $min \cdot day^{-1}$), which correlates to 6 times the normal rate used in a study by Kirwan et al. (1990) resulted in increased liver glycogen but no change in muscle glycogen.

Endurance training causes a functional increase in CS activity in horses (Hodgson and Rose, 1987; Hodgson et al., 1985; Guy and Snow 1977), humans (Holloszy, 1967; Essen-Gustavsson and Henriksson, 1984) and rats (Fitts et al., 1975). Oxidative adaptability can affect other fiber types as well. Sprint training in rats caused increased CS activity in both fast- and slow-twitch skeletal muscle (Troup, et. al., 1986). Endurance-trained humans had significantly higher CS activity in all fiber types than did non-endurance trained controls (Essen-Gustavsson and Henriksson, 1984). Increased CS activity may be correlated with an increase in fatty acid synthesis also seen in endurance trained subjects. Pette et al. (1973) conducted an *in vivo* muscle stimulation trial in rabbits that showed sequential changes in enzyme activities. For 28 days, a slow-twitch pattern of stimulation was administered to rabbit fast-twitch muscle. This stimulation caused large initial increases in muscle enzyme activities of fatty acid activation at 4 days, followed by increases in glycogenolytic and glycolytic enzymes at 14 days, and lastly increases in CS and enzymes involved in fatty acid oxidation at 28 days.

Detraining effects on oxidative enzyme capacity seems related to duration of rest. In humans, detraining, as produced by 3 weeks of inactivity after 7 weeks of intense endurance exercise, caused a decrease in CS activity (Moore et al., 1987), but tapering exercise, by resting either 4- or 8- days, did not affect CS activity (Neary et al., 1992).

Citrate synthase activity is inversely proportional to the percentage of total energy derived from glucose oxidation in vastus lateralis muscle of men (Coggan et al. 1992). This conclusion that CS activity is higher in fibers that depend on oxidative metabolism rather than glycolytic metabolism seems logical if the premise that CS activity is a marker for oxidative potential in skeletal muscle is to be believed. Only muscles with an increased CS activity will show an increased insulin-stimulated glucose transport in rats (Cortez et al., 1991) and that may contribute to muscle recovery ability. Citrate synthase activity has been positively correlated with muscle recovery ability (Jansson et al., 1990) and may be related to potentiation of CS synthesis in low ADP states (Atkinson, 1968). This relationship is further illustrated in a study by Jansson et al. (1990) that showed peak torque is higher after recovery in men whose skeletal muscle has elevated CS activity, decreased lactate concentration, and higher creatine phosphate concentration than in men who did not have similar oxidative potential in their skeletal muscle (Jansson et al., 1990). Such torque was measured by an isokinetic device that monitored unilateral knee extensions, and was thought to accurately determine muscle power.

Dietary effects on citrate synthase activity

Most of what has been reported in the literature regarding the effect of diet on CS activity focuses on how dietary fat composition alters activity (Risse and Dargel, 1978; Risse et al., 1976; Guimaraes and Curi, 1991; Simi et al., 1991; Miller et al., 1984; Miller et al., 1983; Guimaraes et al., 1990).

Dietary effects on CS activity have been studied extensively in rats. Citrate synthase activity decreases in rat skin if a diet low in both energy and protein is fed, but recovery to control activities occurs after one week of normal diet replacement (Nguyen and Keast, 1991). A high fat diet containing 25% fat increased CS activity and decreased lactate and pyruvate concentrations in rat livers (Risse and Dargel, 1978; Risse et al., 1976) and skeletal muscle (Simi et al., 1991). The correlation between CS activity and fat content in the diet was apparent whether diet was changed from high fat to low fat or vice versa. The high-fat diet-induced effects are additive with the well-established training effect of CS activity. Neither high fat nor high carbohydrate diets affected citric acid cycle activities in trained rested or untrained rested rats (Dohm et al., 1973). A high-fat, low carbohydrate diet fed to male rats increased muscle CS activity 15-20% (Miller et al., 1984). Evidently, a high fat diet causes muscle to increase its ability to oxidize fat and therefore spare glycogen. Although a low carbohydrate diet increased CS activity in heart and soleus muscle of rats (Haddad et al., 1990), in actuality the diet used in that study may have had a high percentage of calories contributed by fat. The effect of high fat diets on CS activity may be species specific. Four weeks of a high-fat (54% of energy)-high carbohydrate diet or a lower fat (29% of energy)-high-carbohydrate diet did not affect CS activity in plasma or muscle of trained men (Kiens et al., 1987).

Dietary fat can modulate CS activity in tissues other than muscle. A high fat diet has been associated with decreased immune response in rats, which is correlated with CS activity of lymphoid tissue. When rats are fed an omega-3 polyunsaturated fatty acid-rich diet for 6 weeks CS activity decreased in the spleen and lymph nodes, but increased in the thymus (Guimaraes and Curi, 1991).

Substrates and pathways involved in exercise-induced gluconeogenesis

Gluconeogenesis Gluconeogenesis is the formation of glucose from non-carbohydrate sources. Among such sources are amino acids, lactate and pyruvate. The rates of gluconeogenesis in the perfused rat liver are high for alanine, lactate and pyruvate but unexpectedly low for most other amino acids, particularly glutamate and aspartate (Ross et al., 1967). The rate of conversion of lactate to glucose requires an average of 75 seconds when determined by injection of a pulse of radiolabeled lactate into a rat liver perfused for 20 minutes with 10mM lactate (Exton and Park, 1967). The rate-limiting factor seems to be the ability of precursors to penetrate into the cell where gluconeogenesis can occur (Ross et al., 1967). There may be species variation in transporters of gluconeogenic substrates. Based on literature values for hepatic uptake of glutamine and alanine, coupled with the fact that glutamine is produced in quantities twice that of alanine in isolated pup hepatocyctes, it has been theorized that glutamine is the main nitrogen and carbon transporter from muscle to liver for gluconeogenesis in dogs (Martin and Baverel, 1983). This is in contrast to rats where alanine is the main gluconeogenic transporter.

There is an increased gluconeogenic stimulus evoked by exercise. Glucose production from alanine increases with exercise because of increased hepatic extraction of alanine and increased intrahepatic conversion of alanine into glucose (Wasserman et al., 1988). The increase in gluconeogenesis continues into recovery primarily because alanine release from muscle continues to rise after exercise (Wahren et al., 1973). The protein catabolism observed during exercise, independent of caloric deficit (Mole and Johnson, 1971), may be partially the result of this outflow of alanine from exercised skeletal muscle, which suggests increased activity of the alanine-glucose cycle post-exercise (Wahren et al., 1973).

To establish gluconeogenic substrate utilization across any organ, the arterial-venous (A-V) difference technique is commonly employed (Fine, 1983; Felig and Wahren,

1971; Schulman et al., 1980; Wahren et al., 1973). This technique is based on the Fick principle which states that the difference between the arterial and venous concentrations of a compound multiplied by the blood flow of any tissue or organ with a single blood supply and drainage determines utilization or release of that compound (Ganong, 1985).

Alanine Alanine is a key integrator of metabolism. Alanine release from resting muscle is greater than that of all other amino acids in humans (London et al., 1965). Its primary regulatory role in gluconeogenesis, postulated as the alanine-glucose cycle, is well established in the literature (Felig et al., 1969; Felig, 1973; Felig and Wahren, 1971; Felig and Wahren, 1971b; Wahren et al., 1973; Elia et al., 1984). An overview of the cycle follows. Alanine, produced from the transamination of pyruvate from glutamate within muscle, diffuses into the blood where it is transported to the liver. Within the liver, alanine is transaminated back to pyruvate, which then reforms glucose. The amino group is eliminated as urea. The reformed glucose diffuses into the blood where it is transported back to muscle to serve as substrate in glycolysis.

Alanine production is not limited to skeletal muscle. Branched-chain amino acids (BCAA) are catabolized in intestinal tissue to produce alanine that is preferentially utilized by liver, along with glycine during the post-absorptive period, to provide carbon skeletons for gluconeogenesis (Barrett et al., 1986). Ingested amino acids are the major source of the absorbed amino acids following an amino acid load in dogs rather than digested endogenous protein. Ingested amino acids have similar effects in rats. Plasma BCAA remained elevated in rats fed a high protein diet (Anderson et al., 1967) and may contribute to protein sparing of muscle. Muscle amino acid release is the primary method of controlling resting arterial amino acid concentrations in the post-absorptive state, however (Pozefsky et al., 1969).

Glucose cycling does not increase net gain of glucose and seems to maintain a constant percentage of glucose recycling, regardless of activity or need (Weber et al.,

1990). The alanine-glucose cycle, therefore, has little role in regulating glucose flux, but may be more important in removing nitrogenous waste and in averting pyruvate away from lactate production. Originally, glucose and amino acids were thought to be equally important as sources of pyruvate to fuel the alanine-glucose cycle (Felig et al., 1969; Garber et al., 1976), but glucose is now thought to be the major source of pyruvate in alanine synthesis (Chang and Goldberg, 1977; Waterhouse and Keilson, 1978). Using the L_6 line of skeletal muscle cells, a cell line shown to be suitable for studying carbohydrate and amino acid metabolism, alanine production was determined to be a function of pyruvate overflow (Pardridge and Davidson, 1979). Although a direct correlation between alanine production and glucose utilization could not be established, it was concluded that the rate of alanine production is positively correlated with lactate production and dependent on a critical level of pyruvate concentration within a cell. This relationship between lactate and alanine production may indicate that alanine production is sensitive to acid -base balance more than simply to glucose utilization.

Alanine transport into liver cells is rate-limiting for its metabolism (Sips et al., 1980) and is increased in diabetic rats, but the mechanism is unknown (Rosenthal et al., 1985). Sodium-dependent transport does not seem to be involved, however. There is a direct correlation with glucagon, insulin (Cherrington 1981; Chiasson et al., 1975; Davis et al., 1985; Sestoft et al., 1977), catecholamine (Cherrington, 1981) and alanine load to the liver (Diamond et al., 1988; Sestoft et al., 1977) on hepatic alanine uptake. Some researchers believe the gluconeogenic effect of glucagon is by shunting more alanine already present in the liver toward new glucose formation rather than by increasing the amount of alanine extracted from the plasma into the liver, however (Chiasson et al., 1975). The gluconeogenic effect of cortisol operates by this mechanism (Goldstein et al., 1992). Increased alanine load to the liver can occur experimentally because of infusion

(Diamond et al., 1988) or naturally from exercise (Felig and Wahren, 1971; Mole et al., 1973; Poso et al., 1987).

Plasma and liver alanine concentrations have been inversely correlated with the state of gluconeogenesis in rats (Paleologos et al., 1968). This correlation is further illustrated by the attenuation of gluconeogenesis in prolonged fasting by decreased plasma alanine (Marliss et al., 1971). Further support is offered by a human study where plasma alanine concentrations were inversely proportional to changes in dietary energy, and corresponding alanine flux and de novo synthesis were inversely proportional to protein intake (Yang et al., 1986). Therefore, it seems gluconeogenesis is triggered when energy is low causing alanine to be produced and quickly removed from the circulation by the liver for tranformation into needed glucose. An increased need for gluconeogenesis is found in trauma situations also, but the increased alanine turnover is coupled with decreased plasma alanine and results in protein loss or wasting (Marliss et al., 1971). Cortisol is a likely factor in this flux contributing to protein utilization for glucose production rather than for tissue rebuilding.

In insulin-independent diabetic men, the post-exertional alanine increase normally associated with exercise is decreased and lactacidemia is increased when insulin is administered prior to ergometer workout (Czyzyk et al., 1989). This phenomenon illustrates the gluconeogenic role of alanine. As glucose is transported into cells by the action of insulin hypoglycemia results, thus causing alanine to be taken up by the liver at a greater rate for gluconeogenesis in an attempt to increase circulating glucose.

The idea of maximizing potential of the alanine-glucose cycle has been attempted commercially and a diet that is high in BCAA has been patented for humans (Brantman, 1987). The theory of this supplementation is that increased BCAA in the diet will increase glutamate production within skeletal muscle from a transamination reaction and, in another transamination with pyruvate, will produce alanine instead of lactate, thereby increasing

proton flux from muscle. Dietary supplementation with branched-chain amino acids during exercise has been speculated to prevent or decrease the net rate of protein degradation caused by endurance exercise (Blomstrand and Newsholme, 1992). This speculation is based on the observation that with BCAA supplements plasma and muscle concentrations of BCAA were elevated in subjects whereas the aromatic amino acids decreased. The aromatic amino acids are not metabolized, and therefore, are an indicator of muscle protein degradation. Oral administration of 25 g L-alpha-alanine to resting men, fasted overnight, has shown stimulation of the alanine-glucose cycle in both athletes and nonathletes, but the effect was significantly greater in the athletes three hours after oral dosing (Kita et al., 1990). Athletes had lower plasma alanine concentrations and increased glucose and pyruvate concentrations compared with non-athletes at three hours post-alanine dosing. The alanine was most likely being metabolized in the liver to glucose, thus decreasing circulating concentrations. Other approaches to dietary manipulation of muscle metabolism have been made. Increased BCAA metabolism has been correlated with increased ammonia production, and therefore, speculation was made that increased carbohydrate availability would be more beneficial to an athlete because it would decrease ammonia production that can be toxic (Graham and MacLean, 1992). Of interest is that exercise lessens the muscle protein breakdown common in renal insufficiency and is correlated with a decrease in muscle alanine release in uremic rats (Davis et al., 1985). Although the mechanism is unknown, this decrease in muscle protein catabolism may indicate a feedback control in uremic patients as an attempt to decrease ammonia production by BCAA metabolism.

The kidney is another site of alanine metabolism and helps to maintain plasma alanine homeostasis. At normal plasma alanine concentrations, the kidney produces alanine as a substrate for gluconeogenesis more than it utilizes alanine in ammonia formation. When plasma alanine concentration is elevated, such as with exercise, the reverse is true and ammonia produced from skeletal muscle metabolism is excreted (Pitts and Stone,

1966). Alanine protects rabbit kidney proximal tubules against anoxic injury in vitro (Garza-Quintero et al., 1990). Although the mechanism is unknown, research has determined that tubule protection is not attributed to ATP preservation as originally speculated, but rather the observed ATP elevation in protected tubules may be a result of cell protection instead. Earlier work with glycine, which is structurally similar to alanine, showed protection against anoxic injury also (Weinberg et al., 1987). The protective mechanism of glycine is also unknown but is independent of tubule cell ATP preservation as well and to changes in glutathione metabolism (Weinberg et al., 1989).

Alanine exerts a hypoketonemic effect in rats. The mechanism may be attributed to altering the redox equilibrium of the liver from the mitochondria to the cytosol, thus decreasing the ability of cells to reduce acetoacetate to 3-hydroxybutyrate, which typically occurs in the mitochondria (Pinar et al., 1977). An alternate possibility suggests that the antiketogenic effect is secondary to an increase in hepatic oxaloacetate formation resulting in increased citrate formation and therefore decreased availability of acetyl-CoA for ketogenesis (Nosadini et al., 1980). Whether this is similar in dogs is not known; dogs, however are known to be resistant to ketogenesis. In general, alanine production and flux through the alanine-glucose cycle is increased with exercise.

Leucine Leucine flux is a measure of protein turnover in the body (Carraro et al., 1990; Millward et al., 1982; Mole and Johnson, 1969; Picou and Taylor-Roberts, 1969; Rennie et al., 1981; Stein et al., 1989). Recommended dietary intake of leucine in humans has been challenged as inadequate based on research that shows measured whole-body leucine catabolism to be greater than that recommended for replacement; therefore, dietary supplementation of BCAA may be warranted (Hood and Terjung, 1990). It is likely that the erroneous leucine recommendation in humans occurred because it was based on data in rats, which may not be directly extrapolated to other species.

Exercise decreases whole-body incorporation of leucine into protein in humans suggesting a decreased amount of protein synthesis during exercise (Rennie and Edwards et al., 1981; Rennie and Halliday et al., 1981; Hagg et al., 1982). Exercise effects on protein degradation are not as well established, however. Exercise has been suggested to increase protein degradation (Dohm et al., 1985; Rennie and Edwards et al., 1981; Wolfe et al., 1981), decrease protein degradation (Rennie and Halliday et al., 1981) or not affect protein degradation (Evans et al., 1981). Acute exercise in trained and untrained rats caused a decrease in protein synthesis but did not affect protein degradation (Davis and Karl, 1986).

Exercise increases lactate, pyruvate and alanine release from skeletal muscle in man unless 200 g of glucose is administered prior to workout (Ahlborg and Bjorkman, 1987). This observation would verify that lactate, pyruvate and alanine are released from skeletal muscle when glucose uptake by skeletal muscle is inadequate. Leucine degradation decreases pyruvate oxidation in cardiac and skeletal muscle and adipose tissue (Tischler and Goldberg, 1980). Evidently, leucine acts as a fuel in the fasted state by undergoing transamination and thereby sparing glucose oxidation. The acetyl-CoA produced from leucine degradation is comparable with the acetyl-CoA lost from decreasing pyruvate oxidation, and so energy production remains the same.

Glutamate Glutamate, the source of amino groups for alanine production, is formed within muscle from a transamination reaction involving alpha-ketoglutarate and the BCAA, valine, leucine, and isoleucine. Glutamate also acts as a substrate for glutamine production in muscle in another transamination reaction, and therefore, is a source of eliminating nitrogenous waste in a similar capacity as alanine because the glutamine is subsequently removed by the splanchnic bed and kidney (Marliss et al., 1971). There are conflicting reports examining the effect of stress on glutamate/glutamine concentrations in rats. Stress was shown to regulate muscle amino acid transport during exercise and result

in increased glutamine and glutamate in epitrochlearis muscle in rats (Nie et al., 1989). Contrastingly, both glutamate and glutamine were decreased in liver, skeletal muscle, and kidney of rats during acute exercise and was contributed to enhanced glutaminase activity resulting from acidosis (Christophe et al., 1971)

Glycine The diffusion of glycine from plasma to muscle is impaired by a permeability barrier but can the amount of glycine transported into muscle can be increased with higher blood flow (Henriques et al., 1955). Glycine is elevated in plasma in 2-week starved and isocaloric protein-deprived humans and is thought to be caused by a decrease in nucleic acid synthesis, which requires glycine as a substrate (Adibi, 1968). In 5 to 6 week starved humans, glycine utilization in kidney increases, along with increased alanine production, and is thought to be secondary to increased renal gluconeogenesis (Felig et al., 1969).

Exercise effects on alanine and the branched-chain amino acids

The relationship between protein metabolism and energy production during exercise is of great interest to the scientific world as evidenced by numerous review articles written (Henrikkson, 1991; Dohm et al., 1985; Viru, 1987; Brooks, 1987). During exercise, carbohydrate and free fatty acids are the major energy sources, but some amino acids also contribute to energy requirements. Because protein synthesis is depressed during exercise, amino acids are available for catabolic processes (Dohm et al., 1985). The BCAA (Lemon and Nagle, 1981) and alanine (Favier et al., 1987) are deemed the most important amino acids which contribute to exercise energy because of their activity in the alanine-glucose cycle.

There is disagreement on the fate of alanine in exercised muscle, and it may be species related. Alanine decreases in muscle and plasma in rats when exercised (Dohm et al., 1981), but, in humans, alanine release from exercised muscle increases proportionately

with intensity and is directly correlated with arterial pyruvate concentration (Felig and Wahren, 1971; Felig and Wahren, 1971b). In humans after ergometer exercise, forearm alanine and lactate release doubled 2 hours post-exercise and BCAA concentrations increased coincident with increased forearm uptake (Devlin et al., 1989). In rats, the BCAA are generally elevated by exercise (Dohm et al., 1981). There is general agreement that prolonged exercise increases gluconeogenesis from alanine (Wasserman et al., 1988; Dohm et al., 1985; Ahlborg and Felig, 1976; Lemon and Nagle, 1981; Dohm et al., 1981; Galbo et al., 1977). Few references are available evaluating the alanine-glucose cycle in short-term, maximal exercise. The alanine-glucose cycle is important in short-term exercise, however, as a means of gluconeogenesis and nitrogenous waste removal (Henrikkson, 1991). The potential of the alanine-glucose cycle in decreasing lactate build-up in exercising muscle was postulated by Felig and Wahren (1971b) when they estimated that conversion of pyruvate to alanine occurs at 35-60% of the lactate formation rate in human leg muscle undergoing strenuous, submaximal exercise. Christophe et al. (1971) determined that exercise had no effect on plasma alanine or BCAA concentrations, but their sampling method involved peripheral blood collection after decapitation and their rat subjects were varied in their physical fitness abilities and body compositions. Christophe's conclusions , therefore, are questionable.

There seems to be significant differences between trained and untrained individuals in metabolic and hormonal responses to exercise (Bloom et al., 1976). In trained men, glucose, glycerol and free fatty acids in plasma were higher, while, lactate, pyruvate, alanine and catecholamines in plasma were lower during exercise than in plasma from untrained men. Resting BCAA concentrations and post-exercise alanine concentrations were higher in trained athletes (Einspahr and Tharp, 1989). Knowing BCAA are precursors for alanine production, it was postulated that the higher post-exercise alanine concentrations in endurance-trained athletes may develop to accommodate metabolic

demands of exercise such as acidosis and ammonia accumulation. When comparing the relationship between glucose and alanine metabolism in Japanese athletes, it was found that the increase in plasma alanine is the same between trained and untrained men during exercise, but the athletes had a higher plasma glucose concentration and lower circulating lactate during exercise than did untrained men. This difference may be attributed to enhanced alanine-glucose cycle activity in trained subjects. In trained rats, ratios of BCAA to their branched-chain keto acids were dramatically lowered by exercise (Ji et al., 1987), indicating increased conversion of the BCAA to their alpha-keto form, thus increasing the supply of substrate for the alanine-glucose cycle.

Alanine formation is not limited to exercise, but occurs in any hypoxic/anoxic situation (Dohm et al., 1985) even in cold-blooded animals (Carlsson and Gaede, 1986; Lallier and Walsh, 1992; Meinardus-Hager and Gade, 1986) where the pathway is speculated to be similar to mammalian alanine-glucose cycling between skeletal muscle and liver utilizing GPT and glutamate dehydrogenase. Alanine also increases in femoral blood in patients with peripheral arterial occlusive disease whose affected muscles depend on anaerobic energy production to a larger degree than do unaffected people (Rexroth et al., 1989). Thus, alanine formation is correlated with anaerobic conditions, which could potentially result in lactate production.

Effect of diet on the alanine-glucose cycle

In the postabsorptive state, skeletal muscle is the main source of plasma alanine and lactate, although diet can affect substrates and enzyme activities that regulate flux rates through the alanine-glucose cycle (Consoli et al., 1990). As previously stated, de novo synthesis of alanine increases when protein intake is restricted and changes proportionately with dietary energy from carbohydrate (Yang et al., 1986). Protein supplementation in exercised humans has been associated with decreased lactate formation and increased GPT

and is thought to reflect increased transamination of pyruvate, resulting in decreased lactate accumulation (Vukovich et al., 1992). Dietary protein supplementation has been associated with decreased activity of the first of three reactions common to BCAA catabolism, which is the transaminase reaction that converts BCAA into their alpha-keto acids (Exton and Park, 1967). The same protein supplementation, however, increased activity of those enzymes that control the second and third common reactions in BCAA catabolism, which are the BCAA alpha-keto acid dehydrogenase and BCAA acyl-CoA thioester dehydrogenase.

In exercised humans, a low carbohydrate (3%), high fat (73%), high protein (24%) diet induces metabolic acidosis immediately pre-exercise (Greenhaff et al., 1988). This same diet caused lower plasma alanine and glutamine concentrations and lower blood lactate concentrations immediately prior to exercise, which may signify an increased removal of alanine, glutamine and lactate by the liver and, thus increased gluconeogenic potential. Evidently, the high protein, high fat diet decreases pre-exercise muscle buffering capacity. When men fed this diet were exercised, a 104% greater decrease in muscle pH occurred compared with results of another group of men who were fed a high carbohydrate, low fat, low protein diet. In addition, the high carbohydrate, low fat, low protein diet increased muscle glycogen content by 23% in men fed this diet and, when exercised, their muscle glutamine was 17% lower than concentrations in men fed the high fat, high protein diet.

Acid-base influence on gluconeogenic substrates

Acid-base balance can regulate alanine-related energy metabolism. Diet (Patience, 1989) and exercise can affect acid-base balance. Although acidosis is typically associated with the exercised state, alkalosis from ammonia production does occur simultaneously. Ammonia production within skeletal muscle occurs via the purine nucleotide cycle

(Goodman and Lowenstein, 1977; Lowenstein and Goodman, 1978; Graham and MacLean, 1992). Blood ammonia concentration is directly correlated with blood lactate concentrations (Itoh and Ohkuwa, 1991) because, with acidosis, waste nitrogen is diverted from the excretion of urea to ammonia (Cersosimo et al., 1986; Oliver and Bourke, 1975). The bicarbonate buffering system has been hypothesized to almost entirely neutralize the lactic acid produced from exercise (Beaver et al., 1986), but the ammonia produced may be cleared ultimately by the alanine-glucose cycle. Plasma alanine elevation is important in transporting ammonia and carbon skeletons out of muscle tissue in racehorses (Poso et al., 1987). This was concluded because plasma alanine remained elevated whereas lactate, pyruvate and glucose concentration decreased post-exercise. This concept is supported by a study in humans that showed that, during isometric contraction of the quadriceps femoris muscle, muscle ammonia, lactate, and alanine increased significantly (Katz et al., 1986). During exercise recovery, however, ammonia concentrations remained elevated whereas alanine increased and glutamate and lactate decreased. This substrate pattern can be explained by branched-chain amino acid metabolism that utilizes glutamate to produce alanine and produces ammonia as a by-product. It is hypothesized that increasing carbohydrate availability may suppress ammonia accumulation by decreasing the need for BCAA metabolism (Graham and MacLean, 1992).

Ammonia accumulation occurs only during strenuous exercise and seems to be of minor importance in regulating acid-base balance in body fluids during exercise (Katz et al., 1986); however, acid-base balance does seem to strongly influence alanine metabolism. Elevated ammonia concentration in human quadriceps femoris muscle caused a decrease in glutamate, an increase in alanine (Katz et al., 1986) and increased deamination of that alanine (Bhonensack and Fritz, 1991). At physiological concentrations, ammonia stimulated gluconeogenesis in perfused rat liver also (Fritz and Bohnensack, 1988).

Hyperthermia, also associated with exercise, results in increased plasma ammonia and glutamate concentrations as well as an uptake of glutamine and alanine by skeletal muscle, thus indicating a nitrogen-sparing effect (Jacob et al., 1989). There seems to be no increase in ammonia accumulation up to 50% VO_2 max, but a threefold increase occurs up to maximal exertion (Babi et al., 1983). In Babi's study, ammonia increased concomitantly with lactate and blood glutamine and alanine rose linearly with power output. A linear regression has also been demonstrated between ammonia, lactate and alanine concentrations both at rest and during exercise (Eriksson et al., 1985). It was concluded that the ammonia formed during exercise is primarily from muscle and is used in the synthesis of glutamine and probably alanine. The alanine-glucose cycle would, indeed, unite activities of these metabolites and provide a nontoxic alternative to ammonia production (Felig and Wahren, 1976). As exercise work increases, ammonia and lactate production increases. The formation and release of ammonia provides additional nitrogen for alanine production. The augmented alanine production results in decreased lactate accumulation. Glutamine is important in removal of that ammonia and can also provide nitrogen for alanine production (Matsutaka et al., 1973).

Conditioning can affect lactate and ammonia metabolism. When male sprinters were compared to long-distance runners in a study that measured plasma ammonia concentrations at 50%-, 75%-, 100%- and supra-maximal heart rates, peak concentrations were greatest at supramaximal exertion in the sprinters, but only at maximal exertion with endurance runners (Itoh and Ohkuwa, 1990). Sprinters in this study produced higher ammonia concentrations than long-distance runners at the same exertion level also. Improvement in sprint performance in humans is accompanied by an increase in post-exercise muscle lactate, suggesting improved ability to produce and withstand a lower pH in trained athletes (Nevill et al., 1989). This capacity may be explained by the increased muscle buffering capacity that occurs with sprint training in humans (Sharp et al., 1986).

However, in conditioned horses run on a treadmill, blood lactate and plasma ammonia concentrations were significantly decreased post-exercise, when compared with that of the same horses tested previously in an unconditioned state (Miller and Lawrence, 1986). It was not clear if these measurements were taken immediately after exercise or after one hour of recovery. If samples were taken immediately post-exercise, then either the horses were not truly run at maximal exertion or horses respond differently than humans. If samples were taken one hour post-exercise, then the importance of improved metabolite clearance in conditioned horses becomes a factor. In this case, the alanine-glucose shuttle may be regarded as central to ammonia and lactate removal from exercising muscle because degree of acidosis can be a factor in stimulating the alanine-glucose cycle. Indeed, alanine and amino-N release by muscle does increase in acidosis in the dog (Fine, 1983). It seems that the accumulation of hydrogen ions caused by muscular work inhibits glycolysis at the reaction catalyzed by PFK (Sahlin et al., 1981) and may thus stimulate gluconeogenic cycles to maintain glucose homeostasis. Because the alanine-glucose cycle is also a nitrogen removal cycle (Felig and Wahren, 1975; Poso et al., 1987), the ammonia can be removed. Severe acidosis appears to be the stimulant, however, because stable, moderate acidosis and alkalosis has little effect on glucose and lactate kinetics and gluconeogenesis from lactate, at least in normal and diabetic dogs (Hetenyi et al., 1987).

Thermodilution method of blood flow determination

Blood flow determination is required in many research and clinical settings. Although several methods are available, indicator dilution methods of determining blood flow have been widely used. This technique involves injecting a known amount of indicator into a vessel and determining the indicator concentration in timed serial samples collected a distance away. Common indicator choices such as dye (Wahren, 1966; Rowell et al., 1964; Zierler, 1961; Andres et al., 1953) or radioiodinated human serum albumin

(Hobbs et al., 1962) were limiting because of indicator recirculation that made repeated determinations difficult, if not impossible. Para-amino hippuric acid is an endogenous indicator that has been successfully used, but a constant infusion is needed (Katz and Bergman, 1969; Roe et al., 1966). The search for an endogenous indicator that would not recirculate and could be injected as a bolus led to the thermodilution technique first described in determination of cardiac output in 1954 by Fegler. This method requires saline to be injected intravenously at a temperature different from body temperature. The temperature difference can be recorded by a thermistor previously placed in the blood stream. Blood flow is then inversely proportional to the area under the thermodilution curve.

In 1960, local blood flow by thermodilution was described by Fronek and Ganz and was later applied to the femoral artery of man (Ganz et al., 1964). Thermal indicators chosen were either 5 % glucose or physiological saline at 18-22 degrees C. The technique was further modified in 1985 when ice-cold physiological saline was used as the thermal indicator to determine cardiac output in dogs (Pendergast et al., 1985). The thermodilution method has advantages over other methods of blood flow determination in that measurements can be repeated 3-4 times per minute and can be performed at rest and during exercise. This technique has since been used in conscious humans to determine leg blood flow (Jorfeldt and Wahren, 1971; Hlavova et al., 1965), in conscious dogs to determine cardiac output (Pendergast et al., 1985) and in unanesthetized rabbits to determine portal and renal blood flow (White et al., 1967). Recent accounts of blood flow determinations in dogs were performed under anesthesia using a variety of techniques, but each had drawbacks. The microsphere technique requires whole muscle removal and, therefore is unrepeatable (Musch, 1988). Blood flow determination using an electromagnetic flowmeter typically requires a vessel to be cuffed and, therefore impedes physiological flow (Ballard et al., 1989).

Most hindleg blood flow determinations in dogs have been measured in the femoral artery or vein rather than the intra-abdominal external iliac vessels because of ease of accessibility (Hobbs et al., 1962), even though determination of blood flow in the external iliac vessels is more representative of entire hindleg flow (Evans and Christiansen, 1979). External iliac flow has been accomplished by using a thermostromuhr in conscious dogs (Baldes et al., 1941; Linton et al., 1941). A thermostromuhr is a resistor that encompasses the vessel, heats the blood and records temperature change. The method is similar in principle to thermodilution; however, the resistor can restrict vessel diameter. Reported flows of 4.8 (Baldes et al., 1941) and 12.0 $ml \cdot min^{-1} \cdot kg^{-1}$ body weight (Linton et al., 1941) are reported in anesthetized dogs with this method. One report of thermostromuhr determination of external iliac arterial flow in a conscious dog indicated a flow of 100-200 ml/min, considered low by the authors, although a body weight was not given (Linton et al., 1941). The dog was given 1/2 grain of morphine to calm him down during the procedure, which most likely contributed to the low flow. Fluctuations in blood flow can occur because of emotion (Linton et al., 1941) or vascular reaction to catheterization (Cori et al., 1935), further complicating attempts at blood flow determination in conscious animals.

Hindleg blood flow has been determined with an electromagnetic flowmeter in the left, femoral artery and vein of seven anesthetized greyhounds and averaged 18.5 ± 5.0 $ml \cdot min^{-1} \cdot 100g^{-1}$of hindleg tissue (Ballard et al., 1989). The only other report of canine (non-greyhound) hindleg flow using an electromagnetic flowmeter listed flows of 4.0- and 4.5-$ml \cdot min^{-1} \cdot kg^{-1}$ body weight. A body weight of 27 kg was associated with the rate of 4.0-$ml \cdot min^{-1} \cdot kg^{-1}$ body weight. If one assumes a non-greyhound hindleg is 5% of bodyweight, calculated from weight tables in the literature (Hobbs et al., 1962), a flow of 7 $ml \cdot min^{-1} \cdot 100g^{-1}$ hindleg tissue can be calculated. Interestingly, although bone weight and volume increase with regular strenuous exercise in rats (Saville and Whyte, 1969) and

would, therefore, be expected to do so in dogs, the proportion of greyhound bone to live weight is not different than other more sedate dog breeds (Gunn, 1980b). Adult greyhounds possess more hindlimb muscle mass than do other breeds of adult dogs (Gunn, 1980; Gunn, 1980b), which may contribute to the elevated hindleg blood flow rates reported in that breed. The higher blood flows reported in greyhounds may be a result of breed difference. Adult greyhounds are hemodynamically different, almost hypertensive, and possess lower peripheral resistance compared to mongrel counterparts (Cox et al., 1976).

PAPER 1. EFFECT OF DIET ON GLYCOGEN CONTENT AND ACTIVITIES OF PHOSPHOFRUCTOKINASE, CITRATE SYNTHASE, AND GLUTAMATE-PYRUVATE TRANSAMINASE IN GREYHOUND SKELETAL MUSCLE.

Effect of Diet on Glycogen Content and Activities of Phosphofructokinase, Citrate Synthase, and Glutamate-Pyruvate Transaminase in Greyhound Skeletal Muscle.

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ABSTRACT

The effect of diets on energy related compounds in skeletal muscle was examined in 15 adult, exercised greyhounds. Group 1 was fed a daily diet comprised of 75:25 (w:w) raw beef and a corn-soy base dry food that contained 1960 kcal, 169 g protein, and 96 g fat. Group 2 was fed a daily diet of 100% corn-soy base dry food that contained 1925 kcal, 139 g protein, and 57 g fat. Group 1 diet was significantly higher in protein and fat than the Group 2 diet. Exercise consisted of one 5/16 mile sprint around a track twice a week. Glycogen content and enzyme activities of phosphofructokinase, citrate synthase, and glutamate-pyruvate transaminase were examined at 0, 8, and 16 weeks in resting biceps femoris muscle. Citrate synthase activity in corn-soy fed dogs was significantly elevated at 8 weeks and was still elevated at 16 weeks ($P \leq 0.06$). Activities measured were $29.1 \pm 4.2 \mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 0 weeks, 36.1 ± 5.8 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 8 weeks, and 34.2 ± 4.4 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 16 weeks. Comparable values in the meat-mix fed dogs were 27.8 ± 7.3 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 0 weeks, 27.1 ± 4.6 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 8 weeks, and 26.5 ± 6.4 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt at 16 weeks. No other parameters were significantly different within or between diet groups. A corn-soy base diet enhanced oxidative potential in greyhound skeletal muscle as evidenced by an increase in activity of citrate synthase, but did not alter glycolytic potential.

INTRODUCTION

Raw beef has been an integral part of the racing greyhounds diet throughout the history of the breed.[1,2] The importance of raw beef to greyhound performance is advocated by trainers, but questioned by nutritionists. Recent studies have shown that dietary raw beef causes an increase in greyhound packed cell volume, red blood cell count, and hemoglobin content[3] when compared to similar measurements in greyhounds fed a corn-soy-based diet. This increase is due to dehydration rather than a hematinic effect[4].

The hypothesis that diet can affect the fate of pyruvate in muscle was addressed. Specifically questioned was whether dietary raw beef provides a preferential energy substrate to greyhound muscle. Greyhound hindleg skeletal muscle is 85 - 100% fast-twich fibers.[5,6] Whether those fibers are strictly glycolytic or actually oxidative- glycolytic is controversial.[5-8] It has been postulated that diet can affect muscle metabolism.[9-11] A low carbohydrate diet increases CS activity in both heart and soleus muscle of rats.[12]

To test the hypothesis that dietary raw beef enhances skeletal muscle energy production more than a corn-soy base diet, greyhounds were fed these diets and skeletal muscle activity of key energy producing enzymes and substrate content was compared. For this reason, phosphofructokinase (PFK) activity was measured as a marker for glycolytic capacity because it is the rate-limiting enzyme of glycolysis. Citrate synthase (CS) activity was measured as a marker for oxidative capacity because its activity closely correlates with maximum oxygen consumption of tissues and increases in response to endurance exercise training.[13] Glycogen content was measured to determine if either diet altered carbohydrate availability.

Glutamate-pyruvate transaminase (GPT) activity was measured as a marker of muscle ability to withstand high intensity exercise. GPT activity reflects muscle's capacity to dispose of excess pyruvate while producing less lactate, regenerating NAD^+, and

perhaps minimizing ammonia accumulation.[14] Glutamate-pyruvate transaminase activity controls the transamination of pyruvate within muscle to alanine. Alanine release from muscle increases with exercise[15,16] and protein supplementation[9]. The increase in alanine has been estimated to be 35-60% of the lactate production.[15] The fact that GPT activity, and not lactate dehydrogenase activity, is increased in endurance trained muscle would suggest the possibility that alanine is produced as an alternative to lactate production thus reducing acidosis.[16] Diet alone, can increase GPT activity, as well.[9]

The objective of the study was to determine if the raw-meat mixed diet could alter activity of key enzymes involved in energy production. If the raw meat-mix diet could increase PFK activity, CS activity, and/or GPT activity, then training-like performance improvements may result.

MATERIALS AND METHODS

A study which incorporated diets and a training regimen that simulated field conditions was designed. Fifteen, 2-5 year old, male and female greyhounds were randomly divided into two diet groups. Dog body weights were approximately 30-40 kg. All dogs were previously track trained, most within 3-4 months of this study. One female dog, however, was 7 years old and had not been formally trained for one year prior to this study. A more uniform distribution of sex, weight and age among dogs used in this study was not possible because of limited availability of greyhounds. Dogs were housed outside in 50-foot-long pens for the duration of the study. To acclimate dogs to surroundings and maintenance-training protocol, all dogs were fed a control cereal diet[a] that was different from test diets, and exercised at the track used in this study, for at least one month before start of study.

Group 1 contained 7 dogs (4 females and 3 males) which were fed a 75:25 (w:w) raw beef and corn-soy base dry food diet.[b] Group 2 contained 8 dogs (5 females and 3 males) fed a 100% corn-soy base dry food diet. Composition of each diet is shown in Table 1. The 800 g meat-mix diet contained 1960 kcal with protein, fat and carbohydrate contributing approximately 35, 44 and 21% of kcal, respectively. The corn-soy diet weighed approximately 500 g and contained 1945 kcal with protein, fat and carbohydrate contributing 28, 27 and 45% of kcal, respectively. The disparity in diet composition between groups was unavoidable since the funding source required actual diets used by trainers. All dogs were fed one meal daily at approximately 10 to 12.a.m. in the morning. The volume of corn-soy base dry food was the most a greyhound would consume in a single meal. Water was given ad libitum.

Exercise was similar to race length and field training procedures. Two mornings per week from 8-10 a.m. (Mondays and Thursdays or Tuesdays and Fridays) dogs were

transported 20 miles in a trailer to and from a practice track where they were exercised. On three separate occasions, inclement weather forced all dogs to be exercised only one day per week. Once at the track, two or three dogs at a time were placed in mechanically-gated starting boxes that opened simultaneously. The dogs chased an electronic lure around the 1/4 mile track so that a total distance of 5/16 mile (.52 km) was run. Because of the dogs' willingness to run it was assumed that maximal speed was attained.

Procedures for sample collection were performed on fully conscious dogs lying in left lateral recumbency on a surgical table. A heating pad set on the lowest heat level was placed between dog and table and a small pillow was provided for head support. Restraint consisted of the animal handler gently laying a hand on the greyhound's chest and occassionally rubbing the thorax. All dogs were acclimated in this position for approximately 15 minutes before any procedure was performed.

Approximately 25 mg of resting muscle was taken from the biceps femoris muscle of each dog at 0, 8, and 16 weeks using a 6 mm biopsy needle using a method described by Bergstrom.[17] Lidocaine®, a local anesthetic, was used prior to skin incision.[c] Biopsies were analyzed fluorometrically for PFK activity using the technique described by Mansour et al.[18] and for glycogen content using a method described by Passoneau and Lauderdale[19]. Biopsies were also analyzed spectrophotometrically for CS activity using the technique by Srere[20] and for GPT activity using the method described by Mole et al.[16].

The statistical method used was a two-way ANOVA for repeated measures (Diet x Week) followed by post-hoc analysis (Scheffe) when a significant F ratio ($P \leq 0.05$) was obtained.[21]

RESULTS

There was a significant difference in CS activity at 8 weeks when comparing the 100% corn-soy fed dogs (group 2 = 36.1 ± 5.8 μmol • min^{-1} • g^{-1} wwt) to the meat-mix fed dogs (group 1 = 27.1 ± 4.6 μmol • min^{-1} • g^{-1} wwt). The dogs fed the corn-soy diet had a significantly ($P \leq 0.05$) elevated CS activity at this time point. At 16 weeks CS activity was still elevated ($P \leq 0.06$) for the corn-soy fed dogs (34.2 ± 4.4 μmol • min^{-1} • g^{-1} wwt) when compared to the meat-mix fed dogs (26.5 ± 6.4 μmol • min^{-1} • g^{-1} wwt).

There were no significant differences for any other parameter measured. Glutamate-pyruvate transaminase activity (Table 2) was remarkably similar between diet groups and time periods, although the lowest activities were measured at the 16 week sample. Phosphofructokinase activity (37.6 ± 6.2 μmol•min^{-1}•g^{-1}wwt) was remarkably similar between diet groups and sampling periods (Table 3). Glycogen content was not different for any diet group or sampling period. There was a trend over time, however, in glycogen content of the meat-mix fed dogs. Those dogs fed the meat-mix diet had the lowest glycogen concentration (57.5 ± 9.9 μmol glycosly unit • g^{-1}) at the 8 week sampling period compared to other sampling periods (65.9 ± 3.6 μmol glycosly unit • g^{-1}).

Body weights were remarkably consistent throughout the 16 week study (Table 4). Although there was a slight difference between diet groups at the begining of the study (28.3 ± 2.7 kg, meat-mix diet; 31.4 ± 4.9 kg, corn-soy diet), the difference was consistent throughout the study. Within diet groups, however, there was no difference in body weights over the 16 week study.

DISCUSSION

The hypothesis that diet could enhance cellular potential for energy metabolism in trained dogs, as measured by substrate and enzyme activities, was examined. Oxidative potential was examined by measuring the marker for oxidative metabolism. Glycolytic potential was examined by measuring the substrate concentration and rate-limiting enzyme activity for glycolysis.

Greyhounds fed a 100% corn-soy base diet had significantly increased CS activity in biceps femoris muscle when compared to greyhounds fed a raw-meat mix diet at the 8 week sample. Greyhounds have higher levels of CS activity in gluteus medius and semitendinosus muscles than do crossbreds.[5] Earlier research has shown large increases in muscle citric acid cycle enzyme activities as a consequence of prolonged endurance training.[15] In the present study, there was no increase in CS activity in the meat-fed group. Because all dogs were exercised identically, two explanations of the data are possible. Either the exercise regimen was sufficient to elicit a functional increase in all dogs, but meat-feeding interfered with the expected increase in CS activity in the meat - fed dogs, or the exercise was not adequate to elicit a functional adaptation in either group, and the corn-soy diet, itself, increased CS activity in those dogs fed the dry diet. Because the exercise regimen in the present study would not qualify as endurance training, and thus cause a functional increase in CS activity,[22-25] the latter possibility that a corn-soy diet increased CS activity is favored.

Increased activity of CS may indicate increased capacity for oxidative metabolism and could be extrapolated to mean oxidative capacity of greyhound skeletal muscle increased with a corn-soy diet. This enzyme induction within skeletal muscle most likely occurs in response to an increased need for ATP.[25] Citrate synthase synthesis is potentiated by ADP and inhibited by ATP.[26] Skeletal muscle in a glucose deprived state is

characterized by high ADP and low ATP concentrations. It may be possible that fatty acid metabolism within the skeletal muscle might increase resulting in an accumulation of acetyl-CoA available for oxidation in the citric acid cycle.[14] An increase in CS activity might be expected in an animal oxidizing fatty acids because acetyl-CoA and oxaloacetic acid are substrates for citrate formation. Increased activity of this reaction would provide fuel for the citric acid cycle. Such a sequential change in muscle enzyme activity was shown by Pette et al. when intermittent long term stimulation of rabbit fast-twitch muscle caused large initial increases in enzymes of fatty acid activation followed later by increases in CS and enzymes involved in fatty acid oxidation.[27] There is a positive correlation between higher oxidative capacity and increased fatty acid oxidation. It has been shown that greyhounds have a higher activity of 3-hydroxyacyl CoA dehydrogenase, the enzyme marker for fatty acid metabolism, than man eventhough greyhound body fat composition is considerably lower than man.[13] Although a high fat diet can increase CS activity in some rats[28], other rats[29] and trained men[30] do not show higher CS activity when fed a high fat diet. Although both diets in this study had similar caloric content the corn-soy base diet had 40% less fat by weight than the meat-mix diet. Body weights were consistent throughout the study with the exception of one meat-mix fed dog indicating caloric content and protein level were adequate. Dietary fat content may not be the stimulus to increased CS activity in greyhounds fed the corn-soy diet. Dogs that were fed the higher fat, meat diet in the present study did not have an increased CS activity. Perhaps greyhounds fed the corn-soy diet were more active in their 50-foot-long pens than the meat-mix fed dogs. If the corn-soy diet was less satisfying or palatable the dogs fed this diet may have moved around the outdoor pen more to seek more appealing food. The corn-soy diet was fed to dogs in portions that were 300 g less per day than the meat-mix diet portions. It is possible that the dogs fed the corn-soy diet felt less full than the meat-mix fed dogs and were, therefore, more active. The increased CS activity then might be due to an exercise effect rather than a

true dietary effect. Whether increased activity of CS is advantageous to a sprinting greyhound has yet to be determined, but it is possible that the corn-soy diet optimizes physical conditions that favor activity.

Glycolytic capacity in greyhound skeletal muscle was evaluated by measuring glycogen, the preferred substrate of anaerobic metabolism, and PFK activity, the rate-limiting enzyme activity of glycolysis. Since diet and sampling period did not affect these levels, it was concluded that the diets used in this study did not alter glycolytic potential of greyhound skeletal muscle.

Muscle GPT activity was not different between diet groups or sampling period and, therefore, may indicate that alanine production would not be expected to be different between diets. Muscle alanine concentration is elevated with exercise[16], and acidosis[31] and postexertionally with protein supplementation.[9] The possibility of an immediate post-exercise or post prandial enzyme activity difference between diets used in this study cannot be ruled out.

Reported values for greyhound glycogen, PFK, GPT, and CS activities are sparse and varied. The two reports which cited values measured in this study used measurement techniques different from each other and some were different from those used in this study, therefore direct comparison of data is difficult. For example, the present study reported glycogen as 65.9 ± 3.6 μmol • g^{-1} glucosyl units, however, one report expressed glycogen concentration as 33 μmol • g^{-1} of muscle (determined from graph)[5] and the other study reported glycogen phosphorylase activity as 130 ± 11 μmol • min^{-1} • g^{-1} dry wt[32]. The value for greyhound GPT in the literature, 56 ± 6 μmol • min^{-1} • g^{-1} dry wt, is comparable to the measurement of 13.6 ± 3.2 μmol • min^{-1} • g^{-1} wwt reported in this study.[32] Typically, dry weight determinations of a compound within greyhound skeletal muscle are about fourfold higher than wet weight determinations in the same muscle (unpublished data). However, this same reference used the identical technique used in the present study

to determine CS activity, but the value reported, 19 ± 2 μmol • min^{-1} • g^{-1} dry wt, is lower than the value presented in the present study, 27.8 ± 7.3 μmol • min^{-1} • g^{-1} wwt. More studies are needed to verify accurate values.

In conclusion, a raw meat-mixed diet fed to exercised greyhounds did not enhance glycolytic or oxidative energy production. On the contrary, it appeared that the 100% corn-soy base diet may have increased oxidative potential in greyhound skeletal muscle as indicated by the significantly elevated CS activity in those dogs fed a 100% corn-soy base diet. This increase in CS activity may be related to increased physical activity levels in dogs fed the corn-soy diet and may indicate that the corn-soy diet optimizes physical conditions that favor activity. The traditional meat-mixed diet does not provide greyhounds with an advantage in terms of oxidative or glycolytic capacity. Use of this diet, therefore cannot be supported on this basis. Whether dogs fed a meat-mixed diet perform better in competition was not addressed in this study, but the lack of significant effects on either oxidative or glycolytic potential argues against a performance advantage as a result of a meat-mixed diet.

FOOTNOTES

[a]K-9 Maintenance, Science Diet ®; Hill's Pet Products, Division of Colgate-Palmolive Co., PO Box 148, Topeka, KS.

[b]Purina Hi-Pro®, Ralston-Purina Co., 1 Checkerboard Square, St. Louis, MO; corn soy ratio

[c]Lidocaine HCl, Norden Laboratories, Lincoln, NE

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Table 1. Composition of daily diets fed to greyhounds

	Group 1 = Meat-Mix Diet[a]		Group 2 = Corn-Soy Diet[b]	
Parameter	%[c]	g	%	g
Protein	21.1	169	27.8	139
Fat	12.0	96	11.4	57
Fiber	1.5	12	2.2	11
Nitrogen Free Extract	13.1	105	43.9	219
Water	49.7	398	8.7	43
Phosphorus	0.4	2.9	0.9	4.3
Calcium	0.4	3.4	1.3	6.4

[a]Meat-mix diet = 75% raw beef: 25% Purina Hi-Pro® (w:w).

[b]Corn-soy diet = 100% Purina Hi-Pro®.

[c]Percentage of diet, by weight, on a dry-matter basis.

Table 2. Effect of diet on citrate synthase (CS) and glutamate-pyruvate transaminase (GPT) activities in resting biceps femoris muscle of trained greyhounds

Enzyme	Sample (weeks)	Meat-Mix Diet[a] (n)	Corn-Soy Diet[b] (n)
CS ($\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt[c])	0	27.8 ± 7.3(5)[d]	29.1 ± 4.2(7)
	8	27.1 ± 4.6*(7)	36.1 ± 5.6*(8)
	16	26.5 ± 6.4(7)	34.2 ± 4.4(8)
GPT ($\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt)	0	13.6 ± 3.2(5)	12.9 ± 2.9(7)
	8	12.8 ± 2.2(7)	13.8 ± 2.9(8)
	16	10.6 ± 3.3(7)	10.2 ± 2.6(8)

*Significantly different between diet groups at this time point ($P \leq 0.05$).

[a]Meat-mix diet = 75% raw beef: 25% Purina Hi-Pro®(w:w).

[b]Corn-soy diet = 100% Purina Hi-Pro.®

[c]Wwt represents wet weight of muscle tissue.

[d]Values are means ± SD; the (n) represents number of dogs.

Table 3. Effect of diet on phosphofructokinase (PFK)activity and glycogen content in biceps femoris muscle of trained greyhounds

Parameter	Sample (weeks)	Meat-Mix Diet[a] (n)	Corn-Soy Diet[b] (n)
PFK ($\mu mol \cdot min^{-1} \cdot g^{-1}$ wwt[c])	0	37.6 ± 6.2(6)[d]	36.1 ± 6.7(7)
	8	34.4 ± 7.2(7)	38.6 ± 4.9(7)
	16	36.2 ± 3.0(7)	36.1 ± 6.7(8)
Glycogen (μmol glycosyl units $\cdot g^{-1}$)	0	65.9 ± 3.6(5)	64.7 ± 8.9(7)
	8	57.5 ± 9.9(7)	67.5 ± 8.5(7)
	16	62.8 ± 7.5(7)	65.0 ± 5.8(8)

[a]Meat-mix diet = 75% raw beef: 25% Purina Hi-Pro® (w:w).

[b]Corn-soy diet = 100% Purina Hi-Pro®.

[c]Wwt represents wet weight of muscle tissue.

[d]Values are means ± SD; the (n) represents number of dogs.

Table 4. Body weights of greyhounds (kg) pooled for diet at 0, 8 and 16 weeks

Diet	Length of exercise period		
	0 weeks	8 weeks	16 weeks
Meat-mix diet (7)[a]	28.3 ± 2.7[b]	28.8 ± 3.2	28.4 ± 2.6
Corn-soy base diet (8)	31.4 ± 4.9	30.5 ± 4.2	31.5 ± 4.5

[a]Number in parentheses is number of dogs.

[b]Mean ± SD.

PAPER 2. THERMODILUTION METHOD OF BLOOD FLOW DETERMINATION IN THE EXTERNAL ILIAC VEIN OF THE CONSCIOUS GREYHOUND

Thermodilution Method of Blood Flow Determination in the External Iliac Vein of the Conscious Greyhound

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Lyle Kesl,[2] MS; Donald C. Beitz,[3] PhD; and Wolfgang Weber[4]

ABSTRACT

The thermodilution technique was applied through a percutaneous approach to produce valid, reliable estimates of hindleg blood flow in the external iliac vein of 9 conscious, trained, adult greyhound dogs. The total flow through the external iliac vein averaged 595 ± 50 ml/min. This same total flow, when expressed on a dog-weight basis, was 19.5 ± 2.6 ml • min^{-1} • kg^{-1} body weight or, when expressed on a hindleg-weight basis, was 19.5 ± 3.4 ml • min^{-1} • 100 g^{-1} hindlimb tissue (195.0 ± 34.0 ml • min^{-1}• kg^{-1} hindlimb weight). These flows were higher than previously published results for canine hindlimb flows where femoral arterial flows were evaluated. The external iliac vein, which includes flow from the femoral vein and the deep femoral vein, more truly represents total hindleg blood flow than does flow measured in the femoral vein alone. Hindleg flow rates presented in this study, therefore, are more representative of actual hindleg blood flow than are values previously reported.

INTRODUCTION

Evaluation techniques to measure hindlimb blood flow have been addressed often in the literature.[1-8] Use of established methods to quantify this flow in conscious, non-surgically intervened dogs, however, has not been determined. Of the several procedures available, thermodilution seems the most promising for percutaneous, repeatable application in conscious dogs.

Thermodilution is a valid method of blood flow determination in single vessels.[4,9] The technique is based on injecting ice-cold physiological saline into a vessel and measuring the temperature change recorded by a thermistor placed in that same vessel. Both arterial and venous flows can be determined with this method. The choice of which to use is dependent on protocol. Perhaps most important for venous flow validity is that arterial manipulation can cause immediate and drastic collateral circulation, which greatly decreases blood flow in the catheterized artery.[7,10]

Of the several veins available in the hindleg, the external iliac vein is ideal for assessment of total hindlimb blood flow. This intra-abdominal vein receives blood from the femoral vein, the deep femoral vein, and the pudendoepigastric trunk.[11,a] The femoral vein drains most of the hindlimb musculature and has been the vessel of choice of others for determining hindlimb blood flow.[1,2,4-8] The deep femoral vein, however, drains the skin of the upper caudal thigh and the proximal hamstring musculature (biceps femoris, semitendinosus, and semimembranosus).[11] Therefore, the external iliac vein truly represents total blood flow from all major muscles of the hindlimb. A slight overestimation of hindleg flow may occur because the pudendoepigastric trunk, which drains adipose tissue inside the pelvic inlet and a portion of the rectus abdominus muscle, will be included in any blood flow measurement of the external iliac vein. This degree of error is less than the underestimation of flow assumed by eliminating the deep femoral venous contribution

altogether. The intra-abdominal location of the external iliac vein, however, has precluded any blood flow measurement attempts on conscious, non-surgically intervened dogs prior to development of the thermodilution technique. The purpose of this study was to present a percutaneous approach to and use of the thermodilution technique to determine total hindleg blood flow in the external iliac vein of the conscious greyhound.

MATERIALS AND METHODS

Blood Flow Determination

Blood flow in the external iliac vein was determined for 9 trained greyhounds in a cross-over study examining a meat-mix diet vs. a dry, grain-based diet. Procedures were performed on fully conscious dogs lying in left lateral recumbency on a surgical table. A heating pad set on the lowest heat level was placed between dog and table and a small pillow was provided for head support. Restraint consisted of the animal handler gently laying a hand on the greyhound's chest and occassionally rubbing the thorax. All dogs were acclimated in this position for approximately 15 minutes before any procedure was performed. Blood flow rates were determined from an average of six measurements taken per dog within a 15 minute period.

The distal end of an Edslab® balloonless thermodilution catheter[b] was marked with the length of the external iliac vein that had been predetermined from greyhound cadavers. This catheter, which contained a temperature-sensitive thermistor at its tip, was placed percutaneously into the left femoral vein at the inguinal canal by using the following technique. An 18 ga, 1 1/2 inch long, Cathlon IV® catheter[c] was first placed into the femoral vein. The thermodilution catheter was threaded through this IV catheter into the femoral vein. The IV catheter then was removed over the thermodilution catheter, leaving the thermodilution catheter in place. The preplaced marks were then used as a guide to ensure thermistor placement in the external iliac vein.

The temperature indicator chosen was 0.5 ml of ice-cold physiological saline. The saline was injected into the femoral vein through a 20 ga IV catheter[e] placed either 13.3, 15.2, or 17.0 cm up-stream (toward the patella) from the thermistor tip. Thermistor leads were interfaced with an analog-to-digital computer[d] that recorded area of temperature

change. The area under the resulting thermodilution curve was indirectly proportional to the rate of blood flow.

Standardization of Thermodilution Catheters

Standard curves had to be established for each of 3 thermal probes used, at each distance the 0.5 ml indicator traversed to reach the probe. To prevent a plateaued thermodilution curve due to indicator speed exceeding blood flow at shorter distances, 13.3, 15.2, and 17 cm distances were used routinely throughout the experiment. Physiological flows ranging from 96 ml/min to 800 ml/min were assessed. A constant flow water pump[f] attached to approximately 160 cm of 0.64 cm diameter latex tubing was used to simulate venous flow. The thermistor and injection catheters were introduced into the tubing in a manner identical to live animal preparation. Standard curves were determined from measurements of thermodilution curves produced in triplicate from each of the 3 flow rates tested at the specified injection to probe distance. Each standard curve, therefore, is a result of a minimum of 9 thermodilution curves. An X-Y planimeter[g] was used to determine area under the curve. Regression lines for each catheter at both distances were calculated and used for subsequent blood flow determinations (Table 1). All attempts were made to use the same thermistor in the same dog for each sampling period.

Hindleg Volume

Hindleg volume was determined in order to establish blood flow/100 g hindleg tissue. Direct volume was determined by water displacement of the dismembered left leg after euthanasia at the end of the experiment. The leg was cut with a band-saw parallel to the vertebral column and perpendicular to the femur on a line that transected the top fourth of the femoral head. The leg was weighed before water displacement to determine density.

In addition, volume was determined indirectly by using the formula for cone volume: $V = 1/3bh$, where b equaled the cross-sectional area (πr^2) of the widest part of the

thigh and h equaled the distance from the femoral head to mid-lateral condyle to lateral process of the talus. Radius of the widest aspect of the thigh, measured while the living dog was standing, was calculated from a circumference measurement of the thigh applied to the circumference formula $2\pi r$.

An example of calculations used in this formula follows. Radius (r) can be calculated from the circumference measurement taken from the thigh and the formula for circumference (C), $C = 2\pi r$. If 50.8 cm = 6.28r, then r = 8.1 cm. From this, r can be utilized in the formula for area (A), $A = \pi r^2$, where $A = 3.14 \cdot 8.1 cm^2$. Therefore, A = 206 cm^2. Volume can then be determined by using the formula V = 1/3 b h, where b = 206 cm^2 (calculated) and h = 48.3 cm (measurement). Finally, volume = (1/3) • 206 cm^2 • 48.3 cm, or 3317 cm^3.

All values are presented as means ± standard deviations.

RESULTS

Standard Curves

Three catheters were used in blood flow determinations. Both the A and the B catheters were standardized for 2 different injection to thermistor distances. The C catheter was evaluated at one distance only. The correlation coefficient comparing the actual flow of warm water through the constant-flow water pump with the area produced under the thermodilution curve was greater than 0.92 in all cases (Table 1).

Blood Flow

Resting hindleg blood flow rates in greyhounds were higher than comparable blood flow rates reported for non-greyhound dog breeds. Greyhound resting blood flows were determined from the external iliac vein and had a mean (± SD) value of 595 ± 50 ml • min^{-1} total flow. This blood flow value can also be expressed as an average of 19.5 ± 2.6 ml • min^{-1} • kg^{-1} body weight or 19.5 ± 3.4 ml • min^{-1} • 100 g^{-1} hindleg tissue (Table 2). All blood flow values were averaged from a minimum of 6 flow determinations per dog.

Hindleg Volumes

Actual hindleg volume determinations averaged 2711 ± 308 ml. Calculated hindleg volume measurements averaged 2638 ± 314 ml. There was no significant difference between direct hindleg volume and indirect volume measurements (Table 3). A correlation coefficient of 0.83 demonstrates that the indirect method of determining volume closley approximates that determined by actual methods.

Density of greyhound hindlegs was determined by dividing the actual weight of each hindleg determined by scale measurement with the actual volume of the same hindleg

as determined by water displacement of the dismembered leg. Mean density of greyhound hindlegs determined in this manner was 1.14 ± 0.04 g/ml.

DISCUSSION

Local thermodilution has been established as a valid method for determination of single vessel blood flow in humans.[4,9] This study reported successful use of the thermodilution method, as determined by reproducibility of data, to determine external iliac vein blood flow in conscious greyhounds without surgical intervention. The external iliac vein was chosen to assess total hindlimb blood flow. Venous blood flow can be used instead of arterial blood flow to evaluate hindlimb blood flow. Arterial blood flow is assumed to equal venous blood flow in a limb fed and drained by a single vessel provided there is no edema development. The lower pressures in the venous system coupled with larger diameter vessels make venous blood flow determinations preferable to arterial flow determinations in conscious dogs. Hematoma development is minimal and cannot be disregarded when working with unanesthetized animals. A conscious dog will not tolerate 30 to 40 minutes of constant, heavy pressure applied to the catheter site as is necessary when an artery is catheterized. In addition, catheter impingement on flow is minimal with venapuncture because vessel radius is relatively large.

Values reported in this study for total hindleg blood flow in greyhounds were higher than previous flows presented in a literature review by Hobbs et al.(1962) that compared different methods of flow determination in the femoral artery of non-greyhound dog breeds that were under barbiturate anesthesia. Three accounts of canine external iliac artery flow determinations by using a thermostromuhr[2,7] were also lower than flow results in the present study. A single report of anesthetized, surgically manipulated greyhounds indicated hindleg blood flow values similar to the present study.[12]

The higher hindleg flows in the present study could be attributed to several factors. Blood flow measurement in the external iliac vein should be higher than femoral vein flows in the same animal because the latter excludes flow from the deep femoral vein. Inclusion

of this vessel is necessary for assessing total hindleg flow. In addition, venous flow measurements may be higher and more accurate than comparable arterial flow measurements because arterial rerouting may occur in an artery when catheterized.[3,4] Only one account of external iliac arterial flow determination in a conscious dog is reported and the flow of 100-200 ml/min could not be associated with body weight as none was indicated (Linton et al., 1941). The authors commented on this value as low, but, did not associate the 1/2 grain of morphine administered to calm the dog as a factor. Two reports of external iliac arterial flow determination in anesthetized dogs using a thermostromuhr indicated flows of 4.8 and 12.4 mls • min^{-1} • kg^{-1} body weight, which are lower than the comparable venous flows reported in this study.[2,7] The thermodilution technique itself may provide higher, and perhaps more accurate, flow values than other methods of blood flow analysis. In humans where thermodilution was used to measure femoral artery flows, the resulting values were higher than values from other methods.[1,4]

Genetically, the greyhound breed may have a higher hindleg blood flow than do other breeds of dogs. The only report of greyhound hindleg blood flow in the literature states a value of 18.5 ± 5.0 mls • min^{-1} • 100 g^{-1} gracilis muscle.[12] The 7 anesthetized greyhounds used in that study were surgically manipulated so that the left gracilis muscle was vascularly isolated in a femoral artery and vein perfusion circuit. Both arterial and venous flows were determined by an electromagnetic flowmeter. Even though the animal preparation and flow measurement technique was vastly different from the present study, there was remarkable similarity in reported resting flows emphasizing that greyhounds may have genetically higher flows than other breeds of dogs. Additional evidence of genetic advantage in the greyhound breed is that greyhounds possess more hindlimb muscle mass per total body weight and have higher hindlimb flows/100 g hindlimb weight than do other canine breeds.[13] Table 3 shows greyhound left hindleg weight to be 10% of its body weight as determined from direct weighing of the dismembered left hindlegs. In non-

greyhound breeds of dogs, the single hindleg weight is only 5% of body weight. This percentage was calculated from the literature[5,6], by the author, by comparing the ratio of a dog's total hindleg blood flow to its body weight with the ratio of the same total hindleg blood flow to its hindleg weight. In non-greyhound breeds, a 1:20 ratio exists indicating a non-greyhound hindleg is 1/20th, or 5%, of its body weight. Therefore, total blood flow, in ml/min, through the external iliac vein of a greyhound would be 2-fold higher than total hindleg blood flow in a similarly sized non-greyhound dog because a greyhound hindleg weighs twice as much. That additional muscle tissue needs to be perfused. However, the external iliac blood flow per unit hindleg weight would not be different between greyhounds and other breeds unless greyhounds had a genetically higher blood flow per unit weight. Data from this study show such a genetic advantage. One hundred g of greyhound hindleg tissue receives 38% more blood flow than does 100 g of a non-greyhound breed's hindleg tissue. This increase in hindleg blood flow may actually be greater than 38% because of calculation errors based on increased bone weight in exercised animals. Rats that received regular strenuous exercise had heavier bones with greater volume.[14] Because bone has a lower perfusion need, but still contributes to the total hindleg weight, the actual blood flow to the muscle is probably higher than the calculated ratio, blood flow/100 g hindleg tissue, would indicate. Another indicator of muscle perfusion or drainage potential is the mean area of muscle supplied by a capillary. This area is remarkably similar between greyhounds and other breeds of dogs.[13] The increased blood flow in greyhounds, therefore, may be caused by higher mean arterial pressure and lower calculated peripheral resistance that is common to the greyhound breed.[15]

Lastly, dogs used in this study were conscious, thus anesthetic cardiovascular depression was not a factor for greater hindleg flow rates measured in these greyhounds. Barbiturate anesthesia was used in most previous accounts of canine hindlimb flow. Because anesthesia may have decreased peripheral resistance and caused erroneously

elevated flows, those reported flows may have been even lower in the unanesthetized state.[2,4,6,7,9]

The concern that epinephrine elevation due to apprehension in conscious dogs might contribute to higher flows is unwarranted. Circulating epinephrine concentrations high enough to increase systemic blood pressure cause a decrease in blood flow through the external iliac vein.[3] The external iliac vein drains muscle, skin, and bone marrow of the entire hindlimb. Upon an epinephrine surge, the vasoconstriction of vessels in non-muscle tissue such as skin and splanchnia, compensates for the vasodilatation of vessels in muscle. The result of this skin and splanchnic blood pooling is an overall decrease in blood flowing through the external iliac vein. In addition, the greyhounds used in this study showed no signs of apprehension and most would lie on the table with eyes closed throughout the procedure. Data were collected over a 15 to 30 minute post-catheterization period when dogs were calm. Furthermore, the values reported in the present study were averaged from at least 6 determinations at 2 separate collection periods 6 weeks apart. External iliac vein flow values for individual dogs varied little from one measurement to another as shown by a typical collection range of 70 ml (544, 550, 590, 603, 614 ml recorded in dog 6).

An accurate mathematical estimation of hindleg volume was determined that closely predicted actual volume. Together with hindleg density of 1.14 ± 0.04 g/ml, these calculations can be used clinically or experimentally to easily assess accurate hindleg size and weight.

Based on these results, the thermodilution technique can be used percutaneously to accurately determine external iliac vein blood flow in the conscious greyhound. Because of breed variation, values reported in the present study represent total hindleg blood flow in the greyhound better than do previously reported canine values. In any breed, however,

the external iliac vessels should be used to evaluate total hindleg blood flow because these measurements include blood flow from the entire hindleg.

FOOTNOTES

[a]Nani Goshal, DVM, PhD. 1991. Personal communication. Iowa State University, Ames, IA 50010.

[b]Baxter International Inc., Edwards Divisions, Santa Ana, CA 92711-5686.

[c]Cathlon IV®, Johnson and Johnson Company, Tampa, FL 33630.

[d]Analog-to-digital computer program designed by Dr. Richard L. Engen, College of Veterinary Medicine, Iowa State University.

[e]Angiocath®, Deseret IV, Deseret Medical Inc., Becton Dickinson and Company, Sandy, UT 84070.

[f]Haake FJ, Haake Instruments Inc., 244 Saddle River Rd., Saddle Brook, NJ 07662.

[g]Lasico Polar Compensating Planimeter, Lasico, 2451 Riverside Dr., Los Angeles, CA 90039.

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Table 1. Standard curves for thermodilution catheters as determined from a constant-flow water pump

Catheter[a]	Distance[b] (cm)	Warm Water Flow (ml/min)	Area Under Curve[c] (cm^2)	CC[d]	Regression Line
A	17	96	35.7	-.981	Y = -.053x+39.3
		384	15.8		
		616	8.4		
A	13.3	96	41.1	-.972	Y = -.063x+45.0
		384	16.2		
		616	8.8		
B	17	96	45.1	-.931	Y = -.052x+43.8
		156	34.4		
		384	15.0		
		800	5.6		
B	13.3	96	38.8	-.975	Y = -.046x+40.7
		384	18.7		
		800	5.6		
C	15.2	146	34.4	-.925	Y = -.041x+36.2
		373	14.7		
		800	5.9		

[a]Three identical catheters. The same catheter was used on the same dog throughout the procedures.

[b]Distance between indicator injection and thermistor tip.

[c]Area under the thermodilution curve recorded by an x-y planimeter.

[d]Correlation coefficient between water flow and corresponding area under the curve.

Table 2. Resting blood flows from the external iliac vein in greyhounds

Dog	Total Flow ($ml \cdot min^{-1}$)	Total Flow/ kg BW[a] ($ml \cdot min^{-1} \cdot kg^{-1}$)	Total Flow/ 100 g Hindleg ($ml \cdot min^{-1} \cdot 100g^{-1}$)
1	625	19.3	19.6
2	561	16.3	15.9
3	600	18.3	17.2
4	613	21.7	22.7
5	506	15.7	14.0
6	570	19.6	20.2
7	688	23.6	25.0
8	577	19.1	19.9
9	614	21.6	21.0
Mean	595	19.5	19.5
SD[b]	50	2.6	3.4

[a]BW = body weight.

[b]SD = Standard deviation. Values for each dog were an average of at least 6 determinations taken within 15 minutes of each other. Values were determined on three dogs per day, three days in a row.

Table 3. Hindleg volume and density determinations in greyhounds

Dog	Body Weight (kg)	Actual Volume[a] (ml)	Calc. Volume[b] (ml)	Hindleg Wt (g)	Density (g/ml)
1	32.3	2810	2844	3180	1.13
2	34.3	3190	3012	3523	1.10
3	32.7	3125	2749	3482	1.11
4	28.2	2400	2357	2700	1.12
5	32.3	2910	3115	3604	1.24
6	29.1	2450	2285	2819	1.15
7	29.1	2430	2269	2752	1.13
8	30.2	2605	2467	2904	1.11
9	28.4	2480	2642	2919	1.18
Mean	30.7	2711	2638	3098	1.14
SD[c]	2.21	308	314	356	.04

[a]Actual volume was determined from water weight displacement of leg severed from body after euthanasia. Correlation coefficient comparing actual vs. calculated volumes = 0.83.

[b]Calculated volume of hindleg determined from indirect measure ment using formula V = 1/3 bh. V = volume where b = cross-sectional area of the widest part of the thigh measured on the living, standing dog and h = the distance from femoral head to mid-lateral condyle to lateral process of the talus.

[c]SD = Standard deviation.

PAPER 3. EFFECT OF DIET COMPOSITION AND EXERCISE ON ENERGY PRODUCING AMINO ACIDS IN GREYHOUNDS.

Effect of Diet Composition and Exercise on Energy Producing Amino Acids in Greyhounds.

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ABSTRACT

The effect of diet and training was examined in 10 adult, male, previously track trained, greyhounds to determine if either treatment altered the de novo production of alanine from pyruvate as an alternative to lactate formation. A double cross-over study was designed that included a 24 week diet/no exercise challenge followed by a 14 week diet/exercise challenge. During the diet/no exercise challenge, group 1 was fed diet 1 for 12 weeks and then switched to diet 2 for the next 12 weeks. Simultaneously, group 2 was fed diet 2 for 12 weeks and then switched to diet 1 for the next 12 weeks. Diet 1 was a 100% corn-soy based dry food providing 134 g protein and 1966 kcal. Diet 2 was 74% raw beef, 10% corn-soy-based dry food, and 16% sucrose, providing 139 g protein and 2076 kcal. In the diet/exercise portion of the study, the same dogs were fed a similar diet regimen and exercised three times a week for 14 weeks. Diet 2, however, included 150 g corn starch in place of the sucrose supplement used before. Samples were collected at times that corresponded with diet-switching at 12, 24, 31, and 38 weeks. Samples included femoral arterial and venous whole blood and plasma and a biopsy of the left biceps femoris muscle. Plasma and muscle samples were analyzed for alanine, glycine, leucine, isoleucine, valine, and glutamate concentrations. Plasma samples were also analyzed for glucose concentrations. Whole blood has analyzed for lactate concentration.

The raw meat-mixed diet did not significantly alter utilization of any amino acid examined, but glycine concentration was significantly increased in arterial and venous plasma and muscle when dogs were fed the corn-soy diet. Though not statistically significant ($P > 0.05$), more alanine was released from dogs hindlegs when they were fed the corn-soy diet coupled with training than when they were fed the meat-mixed diet and trained. In addition, when dogs were fed the corn-soy diet regardless of whether or not they were trained, there was less lactate accumulation in both arterial and venous plasma.

Trends in the alanine and glutamate utilization data as well as lactate, glucose, and glutamate concentrations in arterial and venous plasma indicate that the corn-soy based diet may increase glycolytic ability in exercising greyhounds by increasing potential substrate flux through the alanine-glucose cycle.

INTRODUCTION

Greyhounds are sprinting animals that depend primarily on glycolytic metabolism, which results in production of lactic acid and subsequent muscular acidosis. Historically, greyhound trainers routinely have fed a high percentage raw beef diet to their racing dogs in the belief that raw beef increases glycolytic performance and recovery ability.[1] The breed has evolved genetically to tolerate the stresses of glycolytic exercise. Maximal oxygen consumption is elevated in greyhound muscle and has been associated with a predominance of intermediate fibers present in greyhound skeletal muscle.[2] Greyhound muscle has higher enzyme activities associated with anaerobic capacity[2] and an increased ability to withstand and remove lactate[3].

Physiologically, there are three ways of removing from muscle the lactate that has been produced by glycolytic metabolism. The simplest method is to physically remove the compound by increasing blood flow to the area of concentration. Greyhounds have higher hindleg blood flow rates per weight of hindleg than other breeds of dogs.[4] Another method of removing lactate is by oxidizing lactate to pyruvate. This conversion is limited by the concentration of lactate dehydrogenase (LDH). Lactate dehydrogenase activity is not elevated in greyhounds, relative to other breeds of dogs, even though greyhounds have a higher proportion, relative to other breeds of dogs, of high myosin ATPase activity fibers, which typically have greater LDH activities than do other muscle fiber types.[5] The final method is to decrease lactate production by funneling its substrate, pyruvate, into alternate pathways before lactate is formed. Alternate routing of pyruvate results in production of (a) alanine, which diffuses into the blood and is utilized by the liver for gluconeogenesis[6], or (b) acetyl-CoA, which is oxidized in the citric acid cycle[2].

The focus of this study was to study the de novo production of alanine from pyruvate as an alternative to lactate formation. Specifically questioned was whether a raw

meat-mix diet could increase muscle amino acid concentrations, which then could cycle through the alanine-glucose cycle. The alanine-glucose cycle is an important gluconeogenic pathway.[7] In skeletal muscle, the branched-chain amino acids, leucine, isoleucine, and valine, preferentially transaminate α-ketoglutarate to form glutamate. Glutamate preferentially transaminates the pyruvate produced from muscle glycolysis to form alanine and thus regenerate α-ketoglutarate. The alanine diffuses into the blood where it is transported to the liver and transaminated back to pyruvate as an initial step in glucose formation. The nitrogen may be recycled to other amino acids and converted to urea for elimination from the body. Additional benefits to this pathway include removal of nitrogen produced during muscular catabolism, cytoprotection against anoxic injury[8], and an anti-ketogenic effect[9]. These benefits are also important to a racing animal.

Glycine was first shown to protect rabbit kidney tubules *in vitro* against anoxic injury.[10] Because alanine, which has a structure closely related to glycine, was also shown to protect kidney tubules against anoxic injury, it was thought that glycine may be metabolically similar to alanine in other ways, too. Specifically, because diet can affect alanine production, it was postulated that diet may also affect glycine concentrations and, therefore, glycine was included in the present study.

Dietary influence on alanine production in skeletal muscle has been implicated. A protein supplement has been suggested to increase the amount of alanine released from muscle into circulation and to increase glutamate-pyruvate transaminase (GPT) activity in exercised, previously sedate humans.[11] That study suggested that the elevated GPT allowed an increased conversion of pyruvate to alanine, thus decreasing the acidosis of exercise by removing conversion of pyruvate to lactate. It, therefore, can be implied that diet can determine the end product of pyruvate metabolism. This idea is supported further by a study that showed plasma branched-chain amino acids, which are a substrate for

glutamate production, stay elevated in rats when fed a high protein diet.[12] In addition, diet can affect acid-base balance, which ultimately affects amino acid metabolism.[13]

The purpose of this study was to determine if a raw meat-mixed diet could increase the release or utilization of the amino acids involved in the alanine-glucose cycle, therefore aiding in the removal of nitrogenous waste and potential substrate for lactate production.

MATERIALS AND METHODS

A double cross-over study comparing two amounts of diet and of exercise was conducted with 10 male, 2 to 4 year-old greyhounds randomly divided into two groups. All dogs had been raced at a local track for the preceeding racing season and were donated to the College of Veterinary Medicine, Iowa State University because of slow race times. All dogs were delivered to the research facilities at the end of the racing season approximately 4 to 6 weeks before start of the present study and were deemed to be in good health. Dogs were housed indoors in individual 15-foot long runs within a climate controlled building for the duration of the study. On 4 occassions, dogs were transported in a trailer, 4 miles each way, to the laboratory for blood and muscle collections. All dogs were housed at the laboratory for 24 hours before collections in individual 10-foot runs. Water was given ad libitum throughout the study.

The 38-week continuous study included a 24-week diet/no exercise challenge followed by a 14-week diet/exercise challenge. During the diet/no exercise challenge, group 1 was fed diet 1 for 12 weeks. At 12 weeks, blood and muscle samples were collected and then the diet was switched to diet 2 for the next 12 weeks, at which time blood and muscle samples were again collected. Simultaneously, group 2 was fed diet 2 for 12 weeks and after blood and muscle sample collections, group 2 was switched to diet 1 for the next 12 weeks and blood and muscle samples were collected again. Diet 1 was a 100% corn-soy base diet[a] that was approximately 500 g total, providing 134 g protein and 1966 kcal. Diet 2 was 74% raw beef, 10% corn-soy base dry food[a] (w:w), and 16% sucrose added for caloric supplementation that weighed approximately 950 g, providing 139 g protein and 2076 kcal. Kilocalorie content of diets was calculated from proximate analysis measurements of diets, and dietary amino acid composition was determined by gas

chromatography mass spectrometry analysis of diet samples determined by a commercial laboratory[b]

The dogs that were studied in the diet/no exercise challenge were also used for the diet/exercise challenge that lasted an additional 14 weeks. Exercise consisted of a maximal speed (approximately 15 m/sec), counterclockwise run for 30 seconds in a outdoor whirligig[c] three times a week. All dogs were transported in a trailer for 20 minutes before and after exercise. The diet-switching and blood and muscle sampling pattern followed in the exercise portion of the study was similar to the pattern followed in the no exercise part of the study, except the time between diet-switching was shorter in the exercise study than in the diet only study. Group 1 was fed diet 1 for 7 weeks, blood and muscle samples were collected, the dogs' diet was then switched to diet 2 for an additional 7 weeks, and blood and muscle samples were collected, again. Group 2 was fed diet 2 for 7 weeks, blood and muscle samples were collected, the dogs' diet was then switched to diet 1 for an additional 7 weeks, and blood and muscle samples were collected. Diet 1, fed during the exercise portion of this study, was identical to diet 1 fed during the no exercise portion of the study. Diet 2, however, included 150 g corn starch[d] in place of the sucrose supplement used before.

Procedures for sample collection were performed on fully conscious dogs lying in left lateral recumbency on a surgical table. A heating pad set on the lowest heat level was placed between dog and table and a small pillow was provided for head support. Restraint consisted of the animal handler gently laying a hand on the greyhound's chest and occasionally rubbing the thorax. All dogs were acclimated in this position for approximately 15 minutes before any procedure was performed.

Because utilization and release of gluconeogenic substrates in the hindleg were the measurements of interest, simultaneous blood samples and blood flow from the vessels feeding and draining the hind limb were obtained. These samples were based on the Fick

principle, which states that utilization or release = (arterial concentration - venous concentration) x blood flow.[14] Blood samples and blood flow rates were collected after a 24 hour fast at 12, 24, 31, and 38 weeks, which corresponded with time of diet-switching. In the exercise portion of the study, dogs were blood sampled 24 hours after eating a post-exercise meal, therefore, the 24 hour fast before blood sampling also corresponded to a 24 hour post-exercise period.

Total blood flow to the hindleg was determined in the external iliac vein of the left leg of conscious dogs by using a percutaneous, thermodilution technique adapted previously.[4] This whole blood flow was converted to plasma flow by multiplying whole blood flow in ml/min x (1.0 - PCV/100). This plasma flow was then used to calculate hindleg utilization/release of plasma substrates examined in this study.

Twelve ml of blood was simultaneously collected from the femoral artery and vein immediately after blood flow was determined. One ml was immediately placed into ice-cold 8% perchloric acid solution and refrigerated until plasma could be harvested by refrigerated centrifugation. Plasma was then stored at 4 C until it could be analyzed for lactate concentration which usually occurred within 1 to 2 weeks. Remaining blood was placed on ice for 1 to 3 hours until hematocrit could be determined. Plasma was then harvested by centrifugation and a 1 to 2 ml aliquot was separated and stored at 4 C for 1 to 2 weeks until glucose concentration could be determined. The remaining 3 to 4 ml of plasma was stored at -70 C for 1 to 3 months until amino acid analysis could be determined. Approximately 25 mg of the left biceps femoris muscle was collected by using the muscle biopsy method described by Bergstrom.[15] Muscle samples were quickly frozen in liquid nitrogen and then stored at -70 C for 1 to 3 months, also, until amino acid analysis could be determined.

Amino acid analysis was determined on a gas chromatographic mass spectrometer[f] using 100 microliter plasma samples and 25 mg portions of muscle. All samples were

lyophilized[e] then heated for one hour at 60 C and allowed to sit overnight. Derivatization of samples was continued with acetonitrile[g] and BSTFA[g]. Concentration of individual amino acids was based on selected ion monitoring that utilizes mass to charge ratios (m/z) of the individual amino acids. The m/z ratios used were glycine, 102.2; alanine, 116.2; valine, 144.2; leucine, isoleucine, valine, 144.2; and glutamate, 246.3. Norleucine was used as an internal standard. Initial injection and transfer line temperatures were set at 50, 240 and 290 C, respectively. After injection, the oven was ramped from 50 C to 240 C at a rate of 20 C/min. Temperature was held at 240 C for one minute. A second ramp was initiated from 240 to 290 C at a rate of 60 C/min. Arterial and venous plasma samples from the same collection period were analyzed at the same time. All muscle samples were analyzed at the same time. Arterial and venous lactate and glucose concentrations from the same collection period were determined enzymatically, in duplicate, using a glucometer.[h]

Data was analyzed by using analysis of variance with the general linear model and uneven sample distribution.[16] Parameters were analyzed for diet effects, exercise effects, and diet x exercise interactions throughout the 38 week study. Means are expressed as least-squares means. Significance levels were set at $P \leq 0.05$.

RESULTS

All values are presented as least-squares means. There were no significant differences in any utilization or release measured (Table 1). There were significant ($P \leq 0.05$) dietary differences in arterial and venous glycine concentrations (Table 2). Arterial and venous glycine concentrations were 20-25% higher when dogs were fed the corn-soy diet (199 and 173 μM vs. 199 and 157 μM, respectively) compared to concentrations when dogs were fed the meat-mixed diet (146 and 142 μM vs. 147 and 133 μM, respectively). There were similarly significant ($P \leq 0.05$) dietary differences in muscle glycine concentrations, also (Table 1). Dogs fed the corn-soy diet had muscle glycine concentrations averaging 5826 and 5962 μM compared to concentrations averaging 5385 and 4163 μM when dogs were fed the meat-mixed diet

Venous lactate concentrations were significantly greater ($P \leq 0.05$) when dogs were fed the meat-mixed diet (1.20, 1.12 μM) compared to when dogs were fed the corn-soy base diet (1.08, 0.90 μM). Arterial lactate concentrations were approaching a significant increase ($P \leq 0.06$) when dogs were fed the meat-mixed diet (1.04, 1.04 μM) compared to when dogs were fed the corn-soy base diet (1.02, 0.85 μM; Table 1).

When dogs were exercised, arterial and venous leucine concentrations averaged 87 and 90 μM. However, when the dogs were unexercised, corresponding arterial and venous leucine concentrations were 148 and 142 μM, respectively.

Arterial and venous glucose concentrations were significantly increased when dogs were exercised (120, 121 mg/dl and 115, 117 mg/dl, respectively) compared to arterial and venous concentrations when dogs were unexercised (111, 109 mg/dl and 96, 88 mg/dl, respectively). There was no effect of diet on the plasma concentrations of glucose.

Body weight was significantly decreased when dogs were trained (30 and 31 kg) compared to when dogs were not trained (31 and 33 kg) and when fed the meat-mix diet

(31 and 31 kg) compared to when dogs were fed the corn-soy diet (33 and 31 kg). Total hindleg blood flow and hematocrit did not change throughout the study (Table 2). Plasma flow was determined from total blood flow and hematocrit measurements that were determined for each dog at each sampling time.

DISCUSSION

The fact that no gluconeogenic substrate measured showed a significant difference in utilization or release in the hindleg between diets or training regimen suggests that release and utilization of glucose and lactate, and of branched-chain and gluconeogenic amino acids in the hindleg are unaffected by diets and training regimen used in this study. More alanine was released from greyhound skeletal muscle than was any other amino acid measured, however. This observation is in agreement with human resting muscle utilization trends reported in the literature.[17,18] There were significant differences in paired arterial and venous amino acid concentrations when dogs were fed one diet, but not when fed the other diet, or when dogs were trained compared to when they were not trained. This observation, combined with insignificant utilization/release measurements may indicate that the A-V difference measurement techniques used in this study are too variable, as indicated by the large standard errors. This may have resulted from non-simultaneous sampling of artery and vein when exactly simultaneous sampling of artery and vein are required in a rapidly changing amino acid environment in order to determine utilization or release of amino acids. It is possible that both diets used in this study resulted in similar amino acid utilization or release from the hindleg, but may have produced differences in intestinal absorption rates, and therefore, in plasma levels of amino acids. Calculations of amino acids determined from steady state infusions of labelled amino acids would have been more definitive in determining if turnover rate of amino acids was different for either diet.

There was a significant influence of diet on glycine concentration. Dogs had a 25% higher concentration of glycine in arterial and venous plasma and a 19% increase in glycine concentration of biceps femoris muscle when they were fed the corn-soy base diet than when fed meat. The diffusion of glycine from plasma to muscle is impaired by a permeability barrier, but total amount diffused can be increased with higher blood flow.[19]

Plasma glycine concentrations are elevated in starvation and isocaloric protein deprivation in man and are thought to be due to a decrease in nucleic acid synthesis for which glycine is a substrate.[20] It is improbable that the corn-soy diet used in this study would mimic either of those conditions because protein and calorie content were approximately equal between diets and adequate (Table 4). Although glycine content was virtually identical between diets given, there was an obvious trend in glycine utilization between diet groups when dogs were trained, although it was not physiologically significant. Glycine utilization was higher when dogs were fed the corn-soy diet and trained compared to any other sampling period. Glycine protects rabbit kidney tubules against hypoxic injury *in vitro.*[10,21] It may be speculated that glycine offers the same protection against anoxia to exercising skeletal muscle, as well. Although a 30 second bout of exercise would not necessarily produce hypoxic conditions equal to the one hour of oxygen deprivation produced in the *in vitro* study, the idea of a potential mechanism to protect against hypoxia is intriguing. Alanine has been shown to be protective against anoxia in kidney tubules, as well.[8] Perhaps the training-induced increase in alanine production by way of the alanine-glucose cycle has additional benefits to exercising muscle other than just decreasing lactate accumulation.

Although alanine and lactate utilization was not significantly different between any sampling period in this study, there are some interesting trends between these compounds when dogs were fed the corn-soy diet and trained. More alanine was released from dogs hindlegs when they were fed the corn-soy diet and trained than when the same dogs were fed the meat-mixed diet and trained. In addition, when dogs were fed the corn-soy diet, regardless of whether or not they were trained, they had less lactate accumulation in both arterial and venous plasma samples. This difference may indicate that, with training, a corn-soy diet is more beneficial in decreasing lactate production by increasing the flux of pyruvate through the alanine-glucose cycle. The causal relationship between increased alanine production and decreased lactate accumulation in skeletal muscle has been

proposed[6,7] and has been postulated to increase with protein supplementation[11]. In that study, the decrease in lactate concentration was associated with a concomitant increase in the enzyme glutamate-pyruvate transaminase, which is theorized to cause increased conversion of pyruvate to alanine and thereby decrease circulating lactate. Diets used in the present study each contained 134-139 g of protein, and although diets did differ in amino acid composition, there was not necessarily a difference in ratio of amino acids (Table 4). This difference in protein composition even though amount of protein is similar may indicate that a corn-soy protein source provides a different circulating amino acid profile than does a raw meat protein source.

Arterial and venous leucine concentrations were both significantly lower when dogs were trained, although the measured utilization was not significantly different between training groups. Leucine flux is a measure of protein turnover in the body.[22-27] This unchanged utilization with decreased circulating leucine may indicate a down regulation of protein metabolism when the dogs are trained. A similar conclusion about leucine flux was observed during exercise using constant infusions of l-^{13}C leucine and 6,6-^{2}H glucose in exercising humans and stated the leucine flux decreased during the first 4 hours of exercise, then reached a new plateau 20% lower than the pre-exercise value.[24] There were no differences in venous plasma amino acid concentrations in that reported study, and arterial plasma was not collected.

Average body weight decreased 1.3 kg over each 7-week period when dogs were trained. This loss was expected because calories consumed were not increased, even though activity increased. This was not an unacceptable weight loss in dogs on a 38-week study, however. A similar weight loss occurred when dogs were fed the meat-mix diet. This may be a result of decreased water consumption or increased water loss due to urea excretion in those dogs fed a meat-mixed diet compared to dogs fed the corn-soy diet.

Although hematocrit did not change throughout the study, a change in body water can occur before significantly changing hematocrit.[28]

Training caused an increase in resting circulating glucose concentration, regardless of diet consumed. Although not significantly different, a trained state also caused an increase in resting glutamate release from the hindleg when dogs were fed the corn-soy based diet. These two events may be related. It can be assumed that an increase in glutamate release from skeletal muscle means an increase in glutamate production within skeletal muscle. Glycolytic exercise, which depends on glucose from glycogen catabolism, results in pyruvate formation within skeletal muscle. The pyruvate can be either dehydrogenated to lactate, thus resynthesizing NAD^+ to perpetuate glycolysis, or it can be transaminated to alanine and, through the gluconeogenic alanine-glucose cycle, decrease lactate accummulation and increase glucose production. Glutamate is the amino acid that provides an amino group for transamination of pyruvate to form alanine. Therefore, an increase in glutamate production may result in increased substrate for the alanine-glucose cycle, which could increase circulating glucose concentration necessary to glycolytic exercise. This increase in glutamate release from trained muscle may be extrapolated to mean that the corn-soy based diet may allow more production of glutamate from trained muscle.

Increased gluconeogenic potential has been suggested in studies using human runners. Increased plasma glucose was noted in elite Japanese runners during exercise compared with untrained controls and was attributed to alanine, pyruvate, and lactate conversion to glucose in excess of what untrained individuals were capable of converting.[29] Post-exercise plasma alanine concentrations were the same and plasma lactate concentrations were higher in the untrained men. An increase in alanine-glucose cycling, resulting in less pyruvate available for lactate production, could explain why the

athletes had lower lactate concentrations and higher circulating glucose concentrations than did the untrained men. Diet composition was not mentioned in that study.

In conclusion, it seems that the raw meat-mixed diet did not significantly alter hindleg release or utilization of alanine, glycine, leucine, isoleucine, valine, glutamate, glucose or lactate as determined by simultaneously collected arterial and venous blood samples, and gas chromatographic mass spectrometry analysis of amino acid determination. The large standard deviations observed in this study may indicate that (a) substrate utilization/release studies requiring simultaneous blood sampling and blood flow determination is subject to error when used on conscious dogs, (b) gas chromatography, mass spectrometry analysis of plasma samples is unpredictable, or (c) individual amino acid fluxes vary considerably in conscious dogs. In any case, constant infusion studies with labelled compounds may be the method of choice in determining fluxes through cyclic pathways of amino acid metabolism. It cannot be ruled out that the diets used in this study simply were not metabolized differently. In this case, the observation by the greyhound racing industry that a raw meat diet improves performance may be due to psychological factors rather than to metabolic factors. However, the fact that glycine concentration in the biceps femoris muscle of the greyhounds was significantly elevated when dogs were fed the corn-soy diet would indicate a true metabolic difference.

By examining trends in the alanine and glutamate utilization data as well as glucose and glutamate arterial and venous concentrations, it may be postulated that the corn-soy based diet increases glycolytic ability in exercising greyhounds by increasing the potential flux through the alanine-glucose cycle. Performance comparison of dogs fed these same diets would need to be done to truly assess dietary impact on racing time.

FOOTNOTES

[a]Purina Hi-Pro®, Ralston-Purina Co., 1 Checkerboard Square, St. Louis, MO

[b]Woodsen-Tenent Laboratories, Inc., Des Moines, Iowa

[c]A whirligig is a circular track with a centrally attached fixed diameter arm to which a lure is affixed. The arm is either manually or electronically controlled.

[d]Harlan-Teklad®, Madison WI

[e]Vac-stop® tray dryer, serial number 54744, Labconco Corp., Kansas City, MS

[f]Hewlett Packard - 5970B series, mass selective detector, 5890A gas chromatograph, HP-1 crosslinked methyl silicon gum column (25m x 0.2mm x 0.11 μm film thickness).

[g]Acetonitrile, BSTFA = bis (tri-methylsilyl)-tri-fluoroacetamide; Regis Chemical Company, 8210 Austin Ave, PO Box 519, Morton Grove, IL 60053

[h]YSI Model 2800 Glucose-Lactate Analyzer

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Table 1. Least squares means of arterial and venous plasma and muscle (biceps femoris) concentrations (μM), and of exchange (μmol/min) of glucose, lactate, and branched-chain and gluconeogenic amino acids in greyhound hindleg.

Parameter		Sampling Period[a]				P ≤ Values[b]		
		M-N	C-N	M-T	C-T	D	T	D x T
Arterial	Alanine	282[c]	341	388	323	0.91	0.17	0.05
	Glutamate	96	88	74	68	0.34	≤0.01	0.90
	Glycine	146	199	143	173	≤0.01	0.25	0.34
	Isoleucine	79	73	80	68	0.16	0.81	0.59
	Leucine	148	142	87	91	0.91	≤0.01	0.60
	Valine	234	201	209	173	0.05	0.15	0.94
	Glucose	111	109	120	121	0.80	≤0.01	0.64
	Lactate	1.04	1.02	1.04	0.85	0.06	0.13	0.14
Venous	Alanine	306	327	387	348	0.77	0.11	0.33
	Glutamate	105	89	72	82	0.82	0.15	0.35
	Glycine	148	199	133	157	0.02	0.09	0.40
	Isoleucine	81	73	76	76	0.63	0.91	0.59
	Leucine	148	134	88	92	0.72	≤0.01	0.51
	Valine	166	176	167	159	0.96	0.76	0.72
	Glucose	108	109	115	117	0.70	0.02	0.81
	Lactate	1.20	1.08	1.12	0.90	≤0.01	0.04	0.45
U/R[d]	Alanine	-5.71	2.44	-3.09	-8.15	0.83	0.58	0.35
	Glutamate	-1.38	-0.65	0.87	-4.62	0.41	0.77	0.28
	Glycine	0.28	-1.07	-1.60	4.64	0.63	0.70	0.45
	Isoleucine	-0.44	-0.27	1.59	-2.68	0.21	0.91	0.17
	Leucine	0.36	1.52	-0.18	-0.44	0.88	0.68	0.81
	Valine	19.8	3.65	12.65	3.30	0.19	0.70	0.72
	Glucose	0.75	0.09	1.46	1.24	0.36	0.06	0.65
	Lactate	-0.04	-0.01	-0.02	-0.01	0.32	0.66	0.65
Muscle	Alanine	5335	5574	4778	5146	0.31	0.11	0.83
	Glutamate	16362	16152	15775	18395	0.32	0.51	0.25
	Glycine	5386	5829	4163	5962	≤0.01	0.19	0.09
	Isoleucine	221	191	226	215	0.35	0.53	0.67
	Leucine	92	113	106	103	0.51	0.90	0.42
	Valine	543	635	614	632	0.26	0.48	0.45

[a]MN=meat-mix diet, not trained; CN=corn-soy diet, not trained; MT=meat-mix diet, trained; CT=corn-soy diet, trained.

[b]P values expressed for D=diet effect, T=training effect, and D x T=diet by training interaction.

[c]Values based on 10 dogs in the "not trained" portion of the study, and 9 dogs in the "trained" portion of the study. One dog death occurred that was unrelated to treatments.

[d]U/R=utilization or release of parameter as determined by arterial minus venous concentration difference multiplied by plasma flow.

Table 2. Greyhound body weight, and hindleg blood flow and hematocrit determinations.

Parameter	Sampling Period[a]				P ≤ Values[b]		
	M-N	C-N	M-T	C-T	D	T	D x T
Body weight (kg)	31	33	30	31	0.01	0.01	0.01
Hematocrit (%)	50c	49	48	46	0.29	0.18	1.0
Total blood flow[e] ($ml \cdot min^{-1}$)	583	582	568	601	0.90	0.39	0.37

[a]MN=meat-mix diet, not trained; CN=corn-soy diet, not trained; MT=meat-mix diet, trained; CT=corn-soy diet, trained.

[b]P ≤ values expressed for D=diet effect, T=training effect, and D x T=diet by training interaction.

cValues based on 10 dogs in the "not trained" portion of the study, and 9 dogs in the "trained" portion of the study. One dog death occurred that was unrelated to treatments.

[d]Blood flow measured in the left external iliac vein of conscious dogs in left lateral recumbency.

Table 3. Amino acid content of diets fed to greyhounds

Amino acid[a]	Corn-soy base diet		Raw beef-mixed diet	
	%[b]	g/day	%	g/day
Alanine	2.0	11.4	1.3	10.4
Arginine	1.4	7.8	1.1	8.8
Aspartate	2.8	15.8	1.7	13.4
Cystine	.4	2.2	.2	1.7
Methionine	.6	3.1	.5	4.3
Glycine	1.5	8.7	1.1	8.6
Histidine	.7	4.2	.6	4.6
Glutamate	4.9	27.9	2.7	21.6
Leucine	3.0	17.0	1.4	11.6
Lysine	1.1	6.5	1.3	10.7
Isoleucine	1.1	6.4	.8	6.6
Phenylalanine	1.9	10.8	1.0	7.9
Proline	2.2	12.4	1.0	8.0
Serine	1.4	7.7	.7	5.8
Threonine	1.0	5.9	.8	6.5
Tryptophan	.2	1.1	.2	1.7
Tyrosine	.7	3.9	.5	4.2
Valine	1.3	7.6	.9	7.3

[a]Amino acid analysis was determined on diet samples by using gas chromatography mass spectrometry at Woodsen-Tenent Laboratory, Des Moines, Iowa.

[b]Percentage of diet, by weight, on a dry-matter basis.

Table 4. Composition of diets fed to greyhounds

Parameter[a]	Diet 1=corn-soy		Diet 2[b]=meat-mix	
	%[c]	g/day	%	g/day
Grams/day[d]	--	500	--	950
Kcals/day (calculated)	--	1921	--	2076
Protein (Kjeldahl)	26.8	134	14.7	139
Fat (acid hydrolysis)	11.0	54.8	3.8	35.6
Nitrogen-free extract (calculated)	44.6	223	31.5	300
Moisture (forced draft oven)	8.7	44	48.4	460
Fiber (crude)	1.8	9	0.3	3.2
Calcium	1.4	6.8	0.1	1.1
Phosphorus	1.0	4.8	0.1	1.0
Ash	7.1	35.4	1.2	11.7

[a]Woodson-Tenent Laboratories, Inc. Des Moines, IA.

[b]Diet 2 carbohydrate supplementation was 150 g sucrose during the untrained phase of the trial and was replaced with 150 g corn starch during the training portion of the trial.

[c]Percentage of diet, by weight, on a dry-matter basis.

[d]Grams fed per day is approximate because measuring cups were used daily to quantify amount of diets fed to dogs.rather than actual weights of diets. Volumes of each diet were measured in cups and weighed in triplicate before study began to estimate quantity needed per day.to provide approximately 2000 kcals. These weight to volume ratios were verified at 3 time points throughout the study.

GENERAL CONCLUSIONS

The optimal diets fed to running greyhounds have been controversial. Traditionally, racing greyhounds have been fed a predominantly raw-beef diet, but recently the benefits of a corn-soy based diet have been considered. This research was designed to compare if either diet was more beneficial in producing energy or in removing metabolites produced in a racing greyhound. This two-part study examined key energy producing enzymes, and substrates of the alanine-glucose cycle.

Examined were glycogen content and activities of phosphofructokinase, citrate synthase, and glutamate-pyruvate transaminase in trained greyhounds fed either a meat-mixed diet or a corn-soy based diet for 16 weeks. Because CS activity in skeletal muscle increased significantly at 8 weeks when dogs were fed a corn-soy diet and remained elevated at 16 weeks ($P \leq 0.06$) and the meat-mixed diet had no effect on any enzyme activity measured, it was concluded that the corn-soy diet may increase oxidative potential in greyhounds. This increase in CS activity may be related to increased physical activity levels in dogs fed the corn-soy diet, also, and may, therefore, indicate that the corn-soy diet optimizes physical conditions that favor activity. This elevation of CS activity may be important regardless of reason for elevation because CS is a key regulator of the citric acid cycle, and increased activity suggests increased flux through the citric acid cycle.

Enzyme activities are not the only indicators of energy-producing potential. The alanine-glucose cycle unites activities of skeletal muscle with those of the liver. This cycle is important in removing nitrogenous waste from exercised muscle, in routing pyruvate produced from glycolysis away from lactate production into alanine in muscle, and in forming glucose within the liver from alanine released from skeletal muscle. Increased activity of the alanine-glucose cycle may thus be beneficial to an exercising dog that is producing nitrogenous waste and has a need for maintaining circulating glucose

concentrations. There were no significant differences in any hindleg utilization or release of substrates determined in the study conducted. Specifically measured were alanine, glutamate, leucine, isoleucine, valine, glycine, glucose, and lactate. Glycine concentrations, however, were significantly increased in arterial, venous and muscle samples when dogs were fed the corn-soy diet. The following trends in arterial and venous concentrations were notable, but, not statistically significant ($P > 0.05$). More alanine was released from dogs hindlegs when they were fed the corn-soy diet and trained than when they were fed the meat-mixed diet and trained. When dogs were fed the corn-soy diet regardless of whether or not they were exercised, they had less lactate accumulation in both arterial ($P > 0.05$) and venous ($P \leq 0.05$) plasma samples. Training 3 times /week caused an increase in resting glutamate release from the hindleg when dogs were fed the corn-soy diet. Training caused a significant increase in resting circulating glucose concentration regardless of diet consumed. These trends in alanine and glutamate utilization and in lactate, glucose, and glutamate concentrations in arterial and venous plasma may indicate that the corn-soy diet increases glycolytic ability in trained greyhounds by increasing potential substrate flux through the alanine-glucose cycle.

In summary, the increase in citrate synthase activity in those dogs fed the corn-soy diet may indicate that the corn-soy diet increases oxidative capacity of greyhound skeletal muscle. Although alanine-glucose cycle substrate utilization was not different between diet or exercise groups examined, trends in substrate concentration were notable and may indicate that the corn-soy also diet increases glycolytic potential of greyhound skeletal muscle by increasing potential substrate flux through the alanine-glucose cycle. Finally, the thermodilution technique was shown to be a reliable method of determining resting blood flow in the external iliac vein of conscious greyhounds. The effect of the corn-soy diet on racing performance needs to be addressed, however, before it can be recommended

for performance enhancement. It is possible that dogs prefer the raw-meat diet, therefore, a psychological factor may affect their performance when fed the meat-mixed diet.

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APPENDIX

Anova Table Format used in Paper 3

Dependent Variable: AGLY

Source	DF	Sum of Squares	F Value	Pr > F
Model	12	32738.2089947	2.26	0.0452
Error	23	27805.4298942		
Corrected Total	35	60543.6388889		

R-Square	C.V.	AGLY Mean
0.540737	20.84445	166.805556

Source	DF	Type I SS	F Value	Pr > F
Dog	9	13758.6388889	1.26	0.3073
Exercise	1	1200.0000000	0.99	0.3295
Diet	1	16620.0455581	13.75	0.0012
Exercise*Diet	1	1159.5245477	0.96	0.3376

Source	DF	Type III SS	F Value	Pr > F
Dog	9	12356.2066138	1.14	0.3788
Exercise	1	1709.4722797	1.41	0.2465
Diet	1	15113.3969851	12.50	0.0018
Exercise*Diet	1	1159.5245477	0.96	0.3376

Least-squares means

Exercise	Diet	AGLY LSMeans
1	2	145.800000
1	2	199.300000
2	3	142.981217
2	3	173.272222

Exercise level 1=not trained
Exercise level 2=trained

Diet level 2=meat-mix diet
Diet level 3=corn-soy diet