This is to certify that the

dissertation entitled

Studies of Blood Flow, Amino Acid and Branched-Chain Keto Acid Metabolism in the Hindhalf of Steers

presented by

Scott Michael Barao

has been accepted towards fulfillment
of the requirements for

Doctor of Philosophy degree in Animal Science

Werner G. Bergen
Major professor

Date March 3, 1986

STUDIES OF BLOOD FLOW, AMINO ACID AND BRANCHED-CHAIN KETO ACID METABOLISM IN THE HINDHALF OF STEERS

By

Scott Michael Barao

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1986

ABSTRACT

STUDIES OF BLOOD FLOW, AMINO ACID AND BRANCHED-CHAIN KETO ACID METABOLISM IN THE HINDHALF OF STEERS

by

Scott Michael Barao

Two groups of experimental animals consisting of 4 steers each were used in a split plot design to study both the effects of insulin (IN) and hydrocortisone (HC) and duration of fasting (FST) on amino acid (AA) and branched-chain ketoacid (BCKA) metabolism in ruminant skeletal muscle. The hindhalf of the steer was used as the model. Localized blood flow (BF) was measured and net nutrient flux was calculated. Steers were allotted into 2 main effect groups representing fat and lean body composition. The fat group (GR1) had an average weight of 565 kg and external fat cover between 1.1 and 1.6 cm. The lean group (GR2) had an average weight of 287 kg and less than .25 cm of external fat. IN level was 0.5 IU/kg bw (IN1) and 1.0 IU/kg bw (IN2), HC level was 5.0 mg/kg bw and length of FST was 24, 48,72 and 96 hr.
GR1 tended to release all AA in the fed control state while GR2 was near AA balance. IN1 produced few changes in AA or BCKA metabolism while IN2 tended to stimulate AA uptake. HC had no effect on AA or BCKA metabolism. Net flux of AA was

unchanged at 24hr of FST but as duration of FST increased net AA flux favored release. GR2 tended to respond more quickly to FST in terms of AA release. FST of 24hr resulted in BCKA release with a change to uptake of BCKA at 48hr in the group 1 steers.

BF was affected by both IN and FST. IN1 increased BF in GR1 ($P<.01$) and IN2 increased BF in both groups. FST for 24hr reduced BF by approximately 50% ($P<.01$) in both groups and the reduced BF was observed for the duration of the FST. FST increased N-t-methylhistidine (NMH) excretion in GR1 at 24 and 48hr ($P<.05$) and in GR2 at 48hr ($P<.01$). Daily NMH release from the hindhalf represented 2.99% and 3.05% of total pool NMH for GR1 and GR2 respectively.

ACKNOWLEDGMENTS

I wish to extend sincere thanks to Dr. Werner G. Bergen for his guidence, encouragement and friendship throughout my graduate training. Dr. Bergen allowed me the freedom and flexibility to gain experience in a number of academic pursuits and I thank him for that.

I also wish to thank Dr. Hawkins and Dr. Emery for their help and encouragement throughout the preparation of this dissertation. Special thanks are offered to Dr. Hawkins for his support when it was most needed.

To Dr. N. Kent Ames, it is hard to express in a few words how much I appreciate your help and encouragement. Your expertise as a surgeon and dedication as a teacher will never be forgotten. I feel fortunate to have been associated with you and thank you again for all you have done.

To my parents, Frank and Deanna, and my grandmother, Clara, I thank you for a lifetime of love and support.

To my wife, Janet, I thank you for your confidence and your love. I know the best is yet to be.

TABLE OF CONTENTS

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

LIST OF FIGURES

INTRODUCTION

Dietary proteins are digested and the resulting amino acids are utilized to meet body needs for maintenance and growth or production. The metabolic processes within the animal will use these amino acids primarily but not exclusively for synthesis of body protein. In cattle the amino acids are used for the synthesis of protein partitioned first towards the maintenance of body tissues and second, for growth above maintenance of muscle tissue and for functions such as milk production. The coordination and execution of these processes requires both a supply of preformed amino acids, the indispensible amino acids, and suitable other nitrogen sources to synthesize the dispensible amino acids.

The synthesis of body protein above that needed for maintenance results in the accumulation of tissue protein referred to as protein accretion. Accretion is the net result of the processes of protein synthesis and protein degradation or turnover. When synthesis exceeds turnover, accretion is the result. It is clear then, to enhance protein accretion, a desirable endpoint in the production of red meat, one could attempt to stimulate the process of

protein synthesis and or inhibit the process of protein degradation to an extent such that net protein accretion is enhanced.

A number of endogenous and exogenous factors play a role in the regulation of protein growth. In muscle, protein synthesis is the primary site of regulation since its rate is very sensitive to dietary changes (Waterlow and Stephen, 1968; Millward and Garlick, 1972). Protein breakdown is also a regulated process with rates of proteolysis being supressed by insulin (Mortimore and Mondon, 1970), energy supply (Parrilla and Goodman, 1974) and certain amino acids (Woodside and Mortimore, 1972). In particular, the role of the branched chain amino acid leucine and its keto analog, ketoisocaproate, as possible regulators of protein turnover in skeletal muscle is of much interest.

The following pages will review the current knowledge related to whole-body protein metabolism with emphasis on branched chain amino and keto acid metabolism of the ruminant. The development of a suitable in vivo system for intensive study of this area will be explained in detail and results from several experiments utilizing this system will be presented.

Review of Literature

Growth Concepts - Protein Synthesis and Degradation

In general terms, growth occurs only when the needs of body maintenance have been met. Inescapable losses of protein from the body occur during normal metabolic processes, appear both in urine and feces and include lossses such as skin and hair. Degradation of tissue protein is a dynamic process and a new supply of amino acids must be provided to complement an incomplete reuse of amino acids released during protein catabolism (Waldo and Glenn, 1984). The primary components of protein needed for maintenance are urinary nitrogen of endogenous origin, a measure of tissue requirements, and metabolic fecal nitrogen, a measure of endogenous protein secreted into the gastrointestinal tract which is not digested or absorbed. Sloughed epithelial cells, enzymes, bile and mucus contribute to this nitrogenous component (Swanson, 1982). Metabolic fecal nitrogen in the ruminant also represents nitrogenous residues of microorganisms from the rumen and large intestine. Estimates as high as 75% of total metabolic fecal nitrogen being of microorganism origin have been reported (Hogan, 1975). Regardless of origin, metabolic fecal nitrogen is a loss which must be replaced and contributes to the needs for maintenance.

Once the protein needs for maintenance have been satisfied, excess protein can be diverted towards processes of production such as lactation or tissue gain. Protein deposition in the form of tissue gain is affected by factors such as physiological maturity, previous and current nutrition, breed, sex and exogenous inputs such as anabolic agents. The difference between protein synthesis and protein degradation is represented by deposition. Protein turnover, the continual synthesis and degradation of body proteins, is quantitatively large in relation to total protein accretion and may exceed the daily requirement for protein by five fold (Millward et al., 1975). Reutilization of amino acids is an essential component during the process of protein turnover and values of 80% reutilization have been observed (Bergen, 1979). As demonstrated by McCarthy et al. (1983) in cattle gaining 1.1 kg/day muscle protein breakdown was 85 to 90% of muscle protein synthesis. Millward et al. (1975) demonstrated that the breakdown rate was directly proportional to the growth rate, so that rapid growth on a diet adequate in energy and protein was accompanied by high rates of protein breakdown, necessitating even higher rates of protein synthesis. The extent to which rapid protein turnover would limit growth depends on the extent to which increased demands for amino acids and energy imposed by the elevated rates of protein synthesis can be met. Amino acid supply is probably not rate-limiting since the demands for amino acids are largely

met by protein breakdown within the tissue. Only a small fraction of amino acids can not be recycled because of post-translational modification.

Protein turnover is an energetically expensive process based on an efficiency of metabolizable energy use for synthesis of tissue protein in the range of 40 to 60% (VanEs, 1980). Webster (1981) estimated, on a between species basis, that 20 to 25% of total heat production may be accounted for by body protein deposition.

As stated earlier, rates of protein synthesis and degradation are influenced by a host of factors including the endocrine system, possibly the most important and complex of all inputs. Insulin, growth hormone, thyroid hormone, adrenal steroids and sex steroids are known to influence muscle turnover. Insulin is anabolic resulting in stimulation of protein synthesis and inhibition of protein degradation (Young, 1980). Uptake and release of amino acids from muscle parallel the rise and fall of circulating insulin concentrations (Felig, 1975). Several investigators have reported reduced rates of protein synthesis in diabetic subjects (Hay and Waterlow, 1967; Senden and Garlick, 1973; Harmon et al., 1984). Harmon et al. (1984) observed decreased rates of protein synthesis in response to both diabetes and starvation. These workers (Harmon et al., 1984) demonstrated acute regulation of protein synthesis in skeletal muscle by insulin at the level of polypeptide-chain initiation. The control of initiation was apparently

mediated by the activity of initiation factor eIF-2. In contrast, Goldstein and Reddy (1967) were unable to demonstrate insulin stimulated protein synthesis often apparent in insulin-induced amino acid uptake. Young (1980) concluded that growth hormone affects amino acid transport, RNA metabolism and general ribosomal aspects of protein synthesis. Bergen (1975) suggested a stimulation of protein synthesis by growth hormone.

Glucocorticoid effects on muscle are generally catabolic as these hormones have important action in facilitating the mobilization of amino acids from muscle. A number of investigators have observed reduced muscle protein synthesis after glucocorticoid administration (Kostyo and Redmond, 1965; Goldberg, 1969; Tomas et al., 1979). In addition, Tomas et al. (1979) observed increased protein degradation as measured by 3-methylhistidine excretion.

Millward and Waterlow (1975) have reviewed the effect of nutrition on skeletal muscle turnover. Rates of protein synthesis and degradation appear sensitive to diet quality and feeding frequency. Garlick et al. (1973) reported increased muscle protein synthesis following refeeding with a concomitant decrease to approximately 50% as the fasting period lengthened. Fractional degradation rate was lowest immediately following a meal and rose to its highest levels 12 to 16 hours after feed removal. Millward and Waterlow (1978) have proposed that fractional synthesis is related to the ratio of protein to DNA, a descriptive measure of the

size of the DNA unit. A decrease in fractional synthesis rate would be expected to follow an increase in DNA unit size. Similarly, RNA activity , in terms of grams of protein synthesized per gram of RNA, has been used in nutritional comparisons related to fractional synthesis rate (Millward et al., 1975; Millward and Waterlow, 1978).

Hormonal Effects on Nutrient Metabolism

Hormonal mechanisms are involved in regulating nutrient supply and utilization at the tissue level. The endocrine system maintains homeostasis within the body through regulation of energy, mineral and water balance and protein, lipid and carbohydrate metabolism. Whole-body protein metabolism is regulated directly by the action of hormones on muscle tissue and indirectly through hormonal regulation of feed intake and nutrient partition within the body tissues. The interaction of hormones at various points during protein synthesis was identified by Bergen (1975).

Insulin

In nonruminant species, insulin lowers plasma amino acid levels by promoting uptake and/or depressing the release of amino acids by tissues (Manchester, 1970). Insulin has immediate effects on muscle protein synthesis by increasing amino acid incorporation into a large number of different proteins with no evidence of selective stimulation

(Manchester, 1970). Cahill et al. (1972) and Goldberg et al. (1980) indicated that the level of insulin is probably the most important factor regulating protein balance in skeletal muscle. Insulin increases the accumulation of labeled amino acids by muscle although this accumulation did appear to be somewhat selective.

A similar response was oserved in sheep where plasma levels of valine, leucine, isoleucine, lysine and tyrosine were decreased by insulin infusions (Brockman, 1978; Prior and Christenson, 1978). Brockman (1978) concluded that insulin had no effect on hepatic removal of amino acids, leaving muscle tissue as the logical source for increased uptake of these selective amino acids. Prior and Smith (1983) demonstrated increased plasma levels of the branched-chain amino acids in alloxan-diabetic steers. Subsequent insulin therapy normalized and maintained concentrations of these amino acids. Infusion of glucose plus injection of insulin also decreased plasma concentrations of branched-chain amino acids (Prior and Smith, 1983). Insulin injection without glucose infusion tended to decrease plasma branched-chain amino acid levels initially (day 1) with a return to normal plasma values on subsequent days (days 2-7). Prior and Smith (1983) concluded that insulin or insulin plus glucose appeared to stimulate the selective removal of branched-chain amino acids from plasma. Kanter (1976) reported similar results with human forearm muscle. Insulin infusion produced an acute decrease in the rate of

total amino acid release with the most marked decrease being observed with the branched-chain amino acids. Call et al. (1972) observed a 17% reduction in plasma non-essential amino acids and a 34% reduction in plasma essential amino acids following insulin injection in sheep. Of the branched-chain amino acids, only leucine and isloeucine were significantly depressed. Brockman and Bergman (1975) and Brockman et al. (1975) also studied the effects of insulin on amino acid metabolism in sheep. Insulin was shown to decrease the plasma concentrations of branched-chain amino acids suggesting a stimulation in muscle protein synthesis. Hepatic amino acid removal was unaffected. Although it is apparent, both in the ruminant and non-ruminant, that insulin has a stimulatory effect on muscle protein synthesis, other reports indicate that in the absence of insulin, all muscle proteins still continue to be synthesized, but in reduced amounts (Trenkle, 1974).

The primary mode of action of insulin in the stimulation of muscle protein synthesis is through the increased transport of amino acids in skeletal muscle cells (Snipes, 1967). The theory of stimulation of amino acid transport by insulin was supported by findings of Goldstein and Reddy (1970) who were unable to demonstrate a stimulatory effect of insulin on muscle tissue incubated with high concentrations of amino acids. Also, Fehlman et al (1979) and Horowitz and Pearson (1981) reported insulin stimulation of amino acid transport into isolated rat

hepatocytes and active membrane transport stimulated by insulin, respectively.

The relationship between increased plasma insulin and decreased plasma amino acids is of particular intrest with respect to the branched-chain amino acids, leucine, isoleucine and valine. Pozefsky et al. (1969) reported that the uptake of the branched-chain amino acids was under insulin control in the peripheral tissues of man. These workers (Pozefsky et al., 1969) infused insulin to attain postprandial levels across the human forearm and observed decreased net output of amino acids from the muscle. While most amino acids appeared to contribute to the decline in total amino acid nitrogen output from the muscle, consistent declines were only observed for threonine, isoleucine, leucine, tyrosine, phenyalanine and glycine.

Schander et al. (1983) studied the effect of insulin on both the branched-chain amino acids and their corresponding branched-chain keto acids in healthy human subjects. Intravenous administration of insulin was associated with a fall in blood levels of the branched-chain amino acids leucine, isoleucine and valine. The corresponding branched-chain keto acids, ketoisocaproate, ketomethylvalerate and ketoisovalerate also declined with insulin administration. The time course of response to infused insulin differed between the branched-chain amino and keto acids. Branched-chain amino acid levels had declined within twenty minutes after the start of insulin infusion. Branched-chain keto

acid levels were affected only after sixty minutes of infusion. Also, the amino acids tended to return to normal levels sooner than the corresponding keto acids. The effect of insulin on mechanisms regulating metabolism of the branched-chain keto acids both in skeletal muscle and adipose tissue have been recently studied (Frick and Goodman, 1980; Hutson et al., 1980). Hutson et al. (1980), using perfused hindquarter muscle from fed rats, observed that insulin addition decreased leucine transamination. In starved rat hindquarter muscle, intracellular leucine and alpha-ketoisocaproate concentrations were elevated and net release of leucine carbon was observed during the 90 minute perfusion, in the absence of insulin. When insulin was added, changes in net leucine uptake and intracellular leucine concentrations were similar to those observed in fed rat hindquarters with insulin. Muscle and blood branched-chain alph-keto acid concentrations were increased significantly during starvation (Hutson et al., 1980).

Working with rat adipose tissue, Frick and Goodman (1980) observed a substantial increase in rate of oxidation of alpha-ketoisocaproate when insulin was added to the medium. The findings under these conditions (Frick and Goodman, 1980) suggest that insulin decreased the km of the branched-chain alph-keto acid dehydrogenase for its substrate. It is not clear if this is the only mechanism by which insulin accelerates the decarboxylation of alpha-ketoisocaproate.

Glucocorticoids

Adrenal corticosteroids that possess glucocorticoid activity, represented by cortisol and corticosterone, influence the metabolic processes of nearly every cell in the body. Glucocorticoids possess an unparallelled range of effects because cell type affected determines the nature of the response. The structural differences among the glucocorticoids influence the intensity, duration and subsequent metabolic significance of these effects which also vary over a wide range of tissue concentrations (Wilcke and Davis, 1982).

Glucocorticoids exert an anti-insulin effect on protein metabolism resulting in decreased peripheral synthesis of protein and a relative increase in catabolism versus anabolism (Munro, 1964). These inverse changes associated with corticosterone and insulin constitute a very sensitive regulatory mechanism for muscle protein synthesis (Millward et al., 1983).

Russell et al. (1982) reported an alteration in amino acid transport by hydrocortisone treated human fibroblasts. The sodium-dependent A system for amino acid transport, one of three overlapping systems in human fibroblasts, was subject to alterations in activity by the presence of hydrocortisone. Cortisol, as well as other glucocorticoids, increased the rate of amino acid uptake in diploid human fibroblasts (Russell et al., 1982). This transport system

was the most responsive to hormonal regulation in a number of cell types. Corticosteroids have been shown to directly inhibit DNA and protein synthesis and cell replication as well as growth hormone and possibly somatomedin production (Loeb, 1976; Baxter, 1978). Munck et al. (1971) speculated that corticosteroids may induce the synthesis of inhibitory proteins that block the synthesis of RNA. Young et al. (1968) showed that following hydrocortisone administration, ribosomes isolated from rat skeletal muscle were less aggregated than those from control rats. Glucocorticoids result in reduction of muscle protein although the exact mechanism of this action is not known.

Nutritional Effects - Starvation

Felig (1975) described a biphasic metabolic response to fasting. This response includes a shift in energy metabolism from body fuel sources as the fasting period lengthens. Initially, gluconeogenesis in the liver is accelerated to maintain hepatic glucose output. This includes an increased hepatic uptake of gluconeogenic substrate, primarily alanine, to enable the maintenance of glucose supply. During the initial stage of starvation, increased alanine release from muscle is observed (Odessey and Goldberg, 1972; Ballard et al., 1976; Bergman and Heitman, 1978). Total splanchnic extraction of alanine is also enhanced. In compensation for the increased alanine

removal by the liver, alanine removal by the kidney declined, and hindquarter release of alanine and glutamine was increased (Heitman and Bergman, 1980). This is in contrast to arterio-venous concentration data reported by Ballard et al. (1976) which failed to demonstrate alanine release from the muscle of fasted sheep. Bergman and Pell (1982) reported increased plasma leucine concentrations in starved sheep and attributed this to an overcompensation in net leucine production by the peripheral tissues in the face of negligable leucine absorption by the portal-drained viscera. Although plasma leucine rose, leucine utilization by other tissues did not increase. Oddessey et al. (1974) found that alanine production was linked to branched-chain amino acid metabolism and that addition of these amino acids, but no other plasma amino acids, stimulated alanine production. In addition, the catabolism of branched-chain amino acids increased in muscles from fasting rats and in such tissue, stimulated alanine production more than in muscle from fed rats.

An increase in plasma concentrations of branched-chain amino acids (BCAA) during fasting has been observed in rats (Goldberg and Odessey, 1972; Adibi, 1976; Goldberg and Tischler, 1981), humans (Felig et al., 1969; Felig, 1975; Adibi, 1976; Williamson, 1980), dogs (Nissen and Haymond, 1981) and sheep (Ballard et al., 1976; Bergen, 1979; Heitman and Bergman, 1980) however, the length of fast required to initiate this response has varied. Goldberg and Odessey

(1972) reported that a 24 hour fast increased plasma valine and isoleucine by 25% while leucine concentration was unchanged. A decrease in all three BCAA was observed after two and three days of fasting in 60 to 90 gram rats. In contrast, Hutson and Harper (1981) reported increased plasma BCAA concentrations in 160 to 190 gram rats following a three day fast. In humans, plasma BCAA increased for the first one to two weeks of fast and then returned to pre-fast levels (Felig, 1969). Adibi (1976) reported that a six day fast was required to raise plasma BCAA concentrations in rats while only one day of fast produced similar results in human subjects.

In sheep, plasma concentrations of BCAA are increased by approximately 50% following a three day fast (Bergman and Pell, 1983). Hepatic removal of BCAA was continuous and relatively constant even after the three day fast but extra-hepatic tissue switched from the net uptake of BCAA observed in the fed state to net release during fasting (Heitman and Bergman, 1980; Bergman and Pell, 1983).

Millward et al. (1976) presented data indicating that during starvation in young and adult rats, protein synthesis rates decreased initially with a subsequent decline in breakdown rate. As muscle becomes the primary source of gluconeogenic energy precursors, breakdown rate shows a progressive increase. Millward and Waterlow (1978) observed nearly a two-fold increase in fractional breakdown rate of muscle in four day starved rats. As length of fast

increases, body metabolism is shifted towards maintenance of body protein by minimizing protein degradation. Vital tissue such as the brain switch from glucose to ketones as the primary energy source (Felig, 1975).

Assessment of Muscle Degradation

The urinary excretion of 3-methylhistidine (N-t-methylhistidine) is used as an index of muscle protein degradation in vivo (Asatoor and Armstrong, 1966; Young and Munro, 1978). Asatoor and Armstrong (1966) were first to suggest that measurement of the rate of excretion of 3-methylhistidine could provide an estimate of myofibrillar protein degradation. 3-methylhistidine, a post-translationally formed constituent of actin and myosin, is not reutilized for protein synthesis and is quantitatively excreted into the urine. Actin and myosin are present generally in eucaryotic cells and the quantitative contribution of skeletal muscle 3-methylhistidine to total 3-methylhistidine output must be assessed if this amino acid is to be used as an index of myofibrillar protein degradation. Nishizawa et al. (1977a) using rats, and Nishizawa et al. (1979) and Harris and Miline (1981a) using cattle, reported that 93 to 84% of the protein bound 3-methylhistidine pool was associated with skeletal muscle. Millward and Bates (1983) investigated the contribution of non-skeletal muscle tissue to the excreted 3-methylhistidine

pool and found that the gastrointestinal tract and skin contributed amounts ranging from 48 to 62% of total urinary 3-methylhistidine output. Nishizawa et al. (1976b) reported from studies on the comparitive turnover of methylhistidine-containing proteins in intestine, skin and muscle that intestine and skin may contribute approximately 17% of the total urinary output of this amino acid. Dietary contribution of 3-methylhistidine was also suggested as a source of error when measuring the excretion of this amino acid to estimate skeletal muscle turnover (Nishizawa et al., 1979). In some species, pigs and sheep in particular, 3-methylhistidine excretion is not a suitable indicator of muscle protein degradation as the majority of this amino acid is tied up into a large, expandable dipeptide pool (Harris and Miline, 1981b).

In light of these varied estimates of non-skeletal muscle 3-methylhistidine output, it would seem beneficial to employ a system for studying skeletal muscle turnover which could minimize the contribution of non-skeletal muscle tissue 3-methylhistidine sources. The hind half veno-arterial sampling technique may be of great advantage. This system would circumvent the gastrointestinal tract contribution and although skin would still be included, its contribution to total myofibrillar protein degradation is not significant (Wassner and Li, 1982).

Branched-Chain Amino and Keto Acid Metabolism

The branched-chain amino acids (BCAA) leucine, isoleucine and valine cannot be synthesized and are therefore essential nutrients for all animals. These amino acids are required specifically for protein synthesis and comprise approximately 35% of the indispensable amino acids in muscle protein (Garper et al., 1984). The BCAA make up almost 50% of the indispensable amino acids in feedstuffs and as such, nutritional deficiencies do not usually occur.

Early reports of regulation of muscle protein turnover by the BCAA (Fulks et al., 1975; Buse and Reid, 1975) has stimulated much activity towards a better understanding of BCAA metabolism. The pathways of BCAA metabolism include an initial reversible transamination which yields the corresponding branched-chain keto acid (BCKA). Each BCKA then undergoes an irreversible oxidative decarboxylation yielding an acetyl-CoA derivative with one less carbon (Harper et al., 1984). The endproducts of BCAA metabolism can then enter the tricarboxylic acid cycle. Acetyl CoA and propionyl CoA, both glucogenic and ketogenic endproducts, are produced upon isoleucine catabolism. Leucine yields acetoacetate and acetyl-CoA and is therefore ketogenic and valine yields the glucogenic precursor, succinyl-CoA.

The initial transamination of the BCAA is carried out by the enzyme BCAA aminotransferase, (BCAAT), an enzyme with

extensive tissue distribution (Mathews et al., 1981). BCAAT is a pyridoxal phosphate-dependent enzyme and accepts all three BCAA as substrates. Among the tissues assayed for BCAAT enzyme, acivity per gram of wet tissue was highest in heart and kidney, intermediate in skeletal muscle and lowest in liver (Ichihara and Koyama, 1966). In contrast, Busboom (1984) found leucine aminotransferase (LAT) activity to be highest in adipose followed by skeletal muscle, kidney and finally liver in both fed and fasted lambs. These results were in agreement with earlier work by Rosenthal et al (1974) in which LAT activity in the rat, a relatively lean animal, was found to be high in adipose, second only to skeletal muscle. It has since been postulated that as the content of adipose tissue increases as a proportion of total body composition, adipose may play a more extensive role than muscle in BCAA metabolism (Goldberg and Tischler, 1981). Further degradation of the branched-chain keto acids (BCKA) occurs as the result of an irreversible decarboxylation of the BCKA to a branched-chain acyl Coa. This reaction is catalyzed the BCKA dehydrogenase enzyme complex (BCKA-DH). The BCKA-DH complex is subject to regulation by covalent modification and is activated by dephosphorylation and deactivated by phosphorylation (Harris et al., 1985). BCKA-DH kinase is inhibited by BCKA, especially alpha ketoisocaproate (Harris and Paxton, 1985) such that accumulation of BCKA would enhance BCKA-DH activity. Phosphatase responsible for the dephosphorylation

is reportedly inhibited by sodium flouride in vitro (Harris et al., 1985).

Branched-chain amino acid catabolism produces nitrogen which must be transported to the liver for conversion to urea. Interorgan cooperativity is essential for the disposal of both the nitrogen and the carbon of the BCAA. In studies of arterio-venous differences in postabsorptive man, Felig et al. (1970) observed that BCAA were taken up by muscle and that alanine was released in an amount in excess of its relative abundance in muscle proteins. The splanchnic bed was the major site of removal of the alanine (Pozefsky et al., 1969). Felig et al. (1970) proposed the existence of a glucose-alanine cycle for the shuttling of nitrogen and glucogenic substrate from muscle to liver. Marliss et al. (1971) observed that muscle released glutamine as well as alanine. Raderman and Berger (1974) used perfused rat hindquarter and Odessey et al. (1974) used rat hemidraphram to demonstrate that after the addition of BCAA to the media, alanine and glutamine were released in amounts well in excess of their relative abundance in muscle proteins. These observations indicated that amino groups from BCAA were being used for synthesis of alanine and glutamine. Haymond and Miles (1982) infused [^{15}N]-leucine into postabsorptive man and found 28% of the leucine nitrogen in alanine. Galion et al. (1980) found 30-53% of [^{15}N]-leucine nitrogen in alanine in dogs.

The de novo synthesis of alanine requires a source of carbon. Reports from several groups (Felig et al., 1970; Felig, 1975) indicate that pyruvate from glycolysis is the source of alanine carbon. Chang and Goldberg (1978a) reported that both in vitro and in vivo, exogenous glucose appears to be the major carbon source for alanine synthesis. Earlier, other investigators (Garber et al., 1976a,1976b; Goldstein and Newsholme, 1976) had proposed that amino acids derived from net protein breakdown provide carbon skeletons for alanine synthesis. This conclusion was not supported by Chang and Goldberg (1978a) who concluded that among the amino acids released by the net breakdown of muscle protein, only aspartate, asparagine, glutamate, isoleucine and valine could possibly provide carbon skeletons for the de novo synthesis of alanine or glutamine since they are the only ones that can enter the tricarboxylic acid cycle. The major fate of these amino acids was shown to be conversion to glutamine (Chang and Goldberg, 1978b). Glutamine serves as an energy source for the small intestine and as a source of ammonia and a substrate for glucose formation in the kidney (Hems, 1972).

Interorgan Cooperativity

Adibi (1976) reported that transamination and to some extent, decarboxylation, of the branched-chain amino acids occurs in extrahepatic tissues, primarily skeletal muscle. The resultant branched-chain keto acids (BCKA) are then transported in the blood to the liver where further metabolism of the BCKA yields ketones, an important source of energy provided to the peripheral tissues. This interorgan transfer and subsequent metabolism of the BCAA and BCKA is confounded by species differences. In dogs (McMenamy et al., 1962) and humans (Wagren et al., 1976; Elia and Levesey, 1981) the BCAA are metabolized primarily by the extrahepatic tissues. BCKA metabolism occurred primarily in the liver in dogs, a finding similar to that of the rat (McMenamy et al., 1965). Findings reported by Khatra (1977) regarding the distribution of BCKA dehydrogenese activity in human and rat muscle, indicated that human muscle contained a six-fold higher concentration of this enzyme. These findings (Khatra, 1977) were used to substantiate later observations by Elia and Levesey (1981) in which BCKA release from the human forearm was significantly less than the corresponding release across the rat hindlimb.

Studies with sheep have demonstrated relatively higher concentrations of BCAA in free amino acid pools than those reported for rats or humans (Bergen, 1979). Ruminants

release an amount of BCAA equivalent to their muscle amino acid concentration (Lindsay, 1980) with a combined BCAA output of 145% of the alanine output in the same tissue (Ballard et al., 1976). Reports of Ballard et al. (1976), Buttery (1979) and Busboom (1984) indicate that ruminant skeletal muscle has only limited ability to oxidize BCAA. Heitman and Bergman (1980) concluded that unlike nonruminant liver, sheep liver removed BCAA. Thompson et al. (1978) reported a net hepatic removal of leucine and a net hepatic release of valine and isoleucine. Heitman and Bergman (1980) also reported that the hindquarter of both fed and fasted sheep released BCAA. However, a small net uptake of BCAA by the hindleg of the fed sheep (Ballard et al., 1976) and cattle (Bell et al., 1975) has also been reported. More recently, Lindsay (1982) has estimated that approximately 40% of the total leucine oxidized in sheep is oxidized in muscle. Early et al. (1984) suggested that ruminants increase the release of BCAA from muscle tissue during fasting rather that use them as energy sources or transport them as BCKA to other tissue for further catabolism. These workers (Early et al., 1984) observed a decreased BCKA release by the steer hindlimb during fasting. This is in direct contradiction with the finding of Bell et al. (1983) in which a large net release of ketoisocaproate was observed from sheep hindquarter during starvation. Teleni et al. (1983) had reported data from hindlimb perfusions of [1-C^{14}]-valine in sheep that suggested transaminase activity

greatly increased during fasting but dehydrogenose activity did not. This situation would favor accumulation of BCKA in tissue and perhaps promote a greater release of the BCKA from the tissue, possibly supporting the findings of Bell et al. (1983). This is in contrast both with a report by Busboom et al. (1983) that the enzymes necessary for the catabolism of leucine, leucine transaminase and alpha-ketoisocaproate dehydrogenase, decrease during fasting in the ruminant skeletal muscle. Also, Wijayasinghe et al. (1983) concluded that in sheep, the rate of oxidation of the carbon skeleton of the leucine being metabolized was not as great in muscle as in liver and kidney and the completeness of the oxidation in the skeletal muscle was reduced by starvation.

Other tissues such as adipose tissue may have the capacity to catabolize BCAA. The oxidation of leucine to carbon dioxide has been demonstrated in adipose tissue of rats (Goodman, 1964), humans (Rosenthal et al., 1974) and cattle (Vasilatos-Younken et al., 1984). In addition to incorporation of leucine into protein by muscle, adipose tissues of rats, pigs and cattle have been shown to incorporate leucine into cell protein and lipid components (Goodman, 1964; Rosenthal et al., 1974; Vasilatos-Younken et al., 1984). Rosenthal et al. (1974) suggested that adipose tissue is a major site of leucine degradation second only to skeletal muscle since on a tissue basis, in 200 gram rats, adipose tissue utilized three times more leucine than liver

and 50% as much as skeletal muscle. Caster et al. (1956) reported that a 200 gram rat would have only 7% dissectable body fat, a very small amount in proportion to total skeletal muscle. In a more obese rat or a species that contains a higher percentage of total body fat such as humans (Goldberg and Tischler, 1981) or sheep (Wijayasinghe et at., 1983; Busboom, 1984), BCAA catabolism by adipose may be of greater physiological significance.

Regulation of Protein Turnover - Role of BCAA and BCKA

Possibly the most important aspect of BCAA and BCKA metabolism is related to reports of their control of muscle protein turnover. Several studies have indicated that the branched-chain amino acids, particularly leucine, are involved in the regulation of protein synthesis. Direct stimulation of protein synthesis in muscle tissue in vitro has been demonstrated by Buse and Reid (1975) and Fulks et al. (1975). Buse and Reid (1975) studied the incorporation of radiolabeled precursors of muscle protein into isolated rat hemidiaphrams. A mixture of the three branched-chain amino acids in equimolar proportions was added to media containing glucose. Incorporation of [^{14}C]-lysine into muscle protein was stimulated. When tested separately, valine was ineffective, isoleucine was inhibitory and leucine was stimulatory to protein incorporation of labeled precursor. At nearly the same time, Fulks et al. (1975)

reported that five times the normal plasma concentration of the branched-chain amino acids promoted protein synthesis and inhibited protein degradation in isolated quarter diaphrams of young rats. A mixture of the remaining plasma amino acids proved ineffective. These workers (Fulks et al., 1975) also reported that leucine by itself or a mixture of isoleucine and valine together were able to promote the incorporation of labeled tyrosine into protein and inhibit tyrosine release. Together these studies have provided evidence for a role of leucine alone in regulating both the synthesis and degradation of muscle protein in vitro. This regulation was not shared with the other branched-chain amino acids and was not related to the provision of energy for the tissue through leucine oxidation. These findings are of particular interest because they are unique to muscle and can be demonstrated with leucine concentrations ranging between 0.1 and 0.5 millimolar, which is in the range over which blood concentrations of leucine change in fed and fasted states (Adibi, 1976). Similar direct and rapid changes in protein synthesis in muscle tissue in response to leucine have been shown in perfused hemicorpus (Li and Jefferson, 1978) and perfused heart (Chua et al., 1979). Atwell et al. (1977) and Hedden and Buse (1979) showed that in incubations of rat diaphram, BCAA stimulated the incorporation of labeled tyrosine into myofibrillar, soluble and total protein by 50 to 60%. Tischler et al. (1982) used cycloserine to inhibit leucine transamination and determine

if leucine itself or the product of leucine transamination, alpha-ketoisocaproate, was responsible for the observed effects on protein turnover in skeletal muscle. Results from this work (Tischler, 1982) indicate that ketoisocaproate or some product of its further metabolism inhibits protein degradation while leucine itself is probably responsible for the stimulation of protein synthesis. This was in good agreement with work by Goldberg and Tischler (1981) using rat diaphram incubated with cycloserine in which the addition of leucine stimulated protein synthesis just as it did in the absence of the inhibitor. Addition of the inhibitor however, completely prevented the reduction in protein breakdown in skeletal or atrial muscle by leucine. The inhibition of protein breakdown seemed to require leucine catabolism while the enhancement of synthesis did not (Goldberg and Tischler, 1982). Further support for this conclusion came when these same workers (Goldberg and Tischler, 1982) incubated muscles with the ketoacid derivative of leucine. Results demonstrated that leucine's inhibitory effects on protein breakdown could be mimicked with alpha-ketoisocaproate, but unlike leucine, the ketoacid derivitive did not increase protein synthesis. Buse and Weigand (1977) had reported earlier that leucine, but not ketoisocaproate, inhibited protein degradation.

The role of branched-chain amino acids in regulating protein metabolism has also been investigated in intact

animals and man. Although evidence is less direct, an ability of BCAA to enhance protein synthesis has been inferred. Buse et al. (1979) suggested that an increase in the proportion of aggregated polyribosomes isolated from muscle tissue of starved rats (glucose and insulin were provided) indicated a stimulation of protein synthesis. Sherwin (1978) obtained a 25 to 30% decrease in negative nitrogen balance when leucine was infused into either three day or four week fasted humans. 3-methylhistidine excretion, a measure of muscle protein turnover, was unchanged despite the improved nitrogen balance and this finding led to the suggestion that leucine affected nitrogen balance by promoting protein synthesis. Mitch et al. (1981) measured the effect of seven consecutive daily infusions of alpha-ketoisocaproate on leucine or urinary urea and total nitrogen excretion during fasting in obese patients. The results indicated that the effect of administration of alpha-ketoisocaproate in fasting subjects is different from the effects of administering leucine even though leucine must have been metabolized to its keto analog. Intravenous infusion of ketoisocaproate reduced urea nitrogen and calculated total nitrogen excretion in the obese patients during starvation. This was not observed when leucine was administered. These findings (Mitch et al., 1981) contrast with those of Sherwin (1978) who found that total urinary nitrogen excretion of fasting subjects was reduced by infusion of leucine. In a series of experimental studies

with rats that were subject to operative injury consisting of laparotomy and jugular vein cannulation, increasing amounts of BCAA resulted in improved nitrogen retention (Freund et al., 1978). The infusion of a solution consisting only of the three BCAA resulted in nitrogen equilibrium and near normal plasma and muscle amino acid patterns. In the same rat-injury model, separate infusions of all three BCAA increased the fractional synthesis rate in liver and decreased the total body protein breakdown rate as measured by [^{14}C]-tyrosine (Freund et al., 1981). In similar metabolic studies in patients undergoing moderate operative stress, Freund et al. (1979) were able to demonstrate that an infusion of a 100% BCAA solution with 5% dextrose resulted in nitrogen equilibrium and near normal plasma amino acid patterns. Recently, Bower et al. (1985) observed an improvement in nitrogen balance with no increase in 3-methylhistidine excretion when patients undergoing severe postoperative metabolic stress were given infusions of a BCAA enriched solution.

The conditions that favor the sparing of body nitrogen by leucine and whether or not this effect is mediated by a direct action of leucine in stimulating muscle protein synthesis in vivo remains unclear. McNurlan et al. (1982) measured the effect of leucine in vivo on protein synthesis in a number if tissues of rats that were either fed or were losing body nitrogen as a result of starvation or lack of dietary protein. These workers (McNurlan et al., 1982) were

unable to detect any effect of leucine (100 micromoles) on the rate of protein synthesis in vivo, in muscle or in visceral tissues of fed, two-day starved or protein-deprived rats. The results in fed rats are not unexpected in light of the fact that positive reports of an effect of leucine on protein synthesis are generally from tissues in catabolic states. The results from starved and protein-restricted rats were somewhat unexpected. In both starvation and protein deprivation depressed rates of protein synthesis were accompanied by low concentratins of plasma insulin. It was under these conditions (low insulin) that Li and Jefferson (1978) were able to demonstrate leucine stimulation of protein synthesis in perfused muscle. Studies that have demonstrated an effect of leucine on nitrogen balance in vivo have involved exposure to leucine for 12 hours (Sherwin, 1978) or longer (Freund et al., 1979) whereas these investigators (McNurlan et al., 1982) used a short exposure (10 minutes) to the leucine. It may be possible that this was not long enough to manifest an observable response. The investigators (McNurlan et al., 1982) concluded that the ability of leucine to stimulate protein synthesis does not apear to be a general phenomenon and may only occur under rather special conditions such as catabolic states associated with severe metabolic stress. Incubated or perfused muscle tissue are generally more catabolic than tissues in vivo and hence in vitro conditions may favor the leucine stimulation observed. Starvation for

two days and or protein deprivaton for nine days may not have been severe enough to induce conditions which were sufficiently catabolic in any of the tissues studied (McNurlan et al., 1982).

While results in isolated muscles indicate that leucine or its metabolites have a positive regulatory effect on protein balance, the in vivo data is unclear. Reports of enhancement of protein synthesis by the BCAA or BCKA in normal healthy subjects (animal or human) are not available.

Long-Term Catheterization

Quantitative determination of nutrient or metabolite flux across a given system requires long-term functional catheterization of specific blood vessels. Measurement of arteriovenous differences in nutrient or metabolite concentrations and regional blood flow is essential.

The cannulation or catheterization of individual blood vessels is difficult in terms of the maintenance of catheter patency for extended periods of time (14-60 days) such as would be required for nutritional adaptation of animals prior to a sampling period. In general, the period of catheter patency is a function of: blood vessel pharmacology (Kalsner, 1984), catheter material used (Heckler and Scandrett, 1985), both size and composition, size of the blood vessel to be catheterized, blood flow past the catheter tips, surgical procedures used to insert the catheters and the species of animal being catheterized (Bergman, 1985). Heckler and Scandrett (1985) studied 37 types of catheters of similar diameter made from plastics including silicon rubber, polyvinyl chloride, polyethylene and polyurethane for thrombogenisis in the cephalic vein of sheep. Thrombus mass was measured on each catheter nine days after insertion. Further study using scanning electron microscopy compared surface roughness between individual catheter materials. Results from this work indicated that polyethylene catheters tended to be more thrombogenic than

all other types studied. Catheters with rougher surfaces were usually more thrombogenic than those with smooth surfaces. The least thrombogenic of all catheter materials studied was silicon rubber and this was attributed primarily to the smooth surface of this material. These findings are in practical agreement with results published by Kaufman and Bergman (1971). These workers (Kaufman and Bergman, 1971) were able to extend renal catheter patency in sheep from 7 to 28 days out to 28 to 45 days when the original polyvinyl catheters used were fitted with short silicon rubber tips. Tissue reaction was observed as severe in sheep with polyvinyl catheters but was less severe when silicon tips were used. Heckler et al. (1979) reported on different types of catheters inserted into the aorta and inferior vena cava of newborn lambs, vessels larger than either the cephalic or renal vessels discussed earlier. There was considerable variation in the amounts of thrombosis that formed although silicon rubber was significantly less thrombogenic. In addition to these findings, Heckler et al. (1979) observed that treating each type of catheter with a polymerized heparin complex (TDMAC process) was of limited value in preventing thrombogenisis. These findings were in contrast to reports by Bruck (1974) in which in vitro tests indicated the TDMAC process increased thromboresistance.

The catheter to vessel size ratio is also an important factor in extending catheter patency. Problems associated with thrombogenesis of certain catheter materials (polyvinyl

and teflon) appear to be somewhat overcome when these catheters are placed in larger blood vessels (Katz and Bergman, 1969; Hoey and Hopkins, 1983). Higher blood flow past the tips of these catheters may in part account for the lengthened patency observed.

In many instances loss of catheter patency is related to a cap of fibrous material which extends over the tip of the catheter, especially within the venous system (Webb, 1985; Huntington, 1985). An early indication of this type of problem is the ability to infuse into a catheter placed in a vessel but the inability to withdraw a sample. It is in this instance that the beginning of this fibrous type cap is noted as, speculatively, it swings away from the catheter tip upon infusion but upon sampling, is withdrawn to occlude the catheter. Venous catheters removed from two steers 12 weeks after insertion were found to be completely covered by a fibrous bulb while arterial catheters from the same animals were free of adhering material (Barao, personal observation).

Fibrin formation is a response to vessel injury and is stimulated upon insertin of the catheter into the vessel (Doolittle, 1984). Fibrin formation results from the conversion of fibrinogen to fibrin triggered by the thrombin-catalyzed release of small peptides from the amino-terminal segments of the fibrinogen. As long as the catheter remains in the vessel the signal for this fibrinogen to fibrin transformation remains (Doolittle,

1984). Species differences in blood vessel pharmacology may affect the rate and extent of tissue reaction to the inserted catheter as well (Kalsner, 1984). Further understanding of the specific tissue reaction, possibly in terms of prostaglandin release, within and across species is essential.

Techniques to Measure Blood Flow

A number of both indirect and direct techniques for blood flow measurement are available. Examples of indirect methods include indicator dilution measurements first introduced by Fick (1870) and first used by Stewart (1897) to measure blood flow, radioactive microspheres (Hales, 1974) and the use of freely diffusible tracers (Kety and Schmidt, 1945). Direct in-vessel measures include drop recording (Folkow, 1953), thermodilution (Haggmark et al., 1982) and electromagnetic flow metering (Denison et al., 1965).

Indicator dilution is the most common technique used to quantitate blood flow. To estimate flow from dilution of an indicator, several characteristics of the vascular bed and indicator must be assumed. The vascular bed in which flow is measured must be a closed system such that injection and sampling sites are located in a way that all of the blood flow mixes with the indicator at some point in the circuit. Flow and volume in the system must be constant while the indicator concentration is measured. Neither the volume of

indicator injected nor the nature of the indicator should alter flow through the system and indicator cannot be lost or gained from the system between the points of injection and measurement (Hamilton and Remington, 1949). In addition, any indicator which recirculates past the sampling point must be subtracted from the measured concentration in order to calculate true blood flow by simple indicator dilution. Some indicators which have been used include Evans blue and indocyanine green dyes (Fox et al., 1957), para amino hippuric acid (Roe et at., 1966) and bromosulpythalein (Clarkson et al., 1976). Indicator choice depends on the anatomical area to be studied as each marker is cleared from the circulation by different organs and at different rates.

Metabolizable radioactive microspheres made from serum albumin follow the indicator dilution principles and are presently receiving wide application in clinical studies. Similarly, microspheres made of inert plastic material have found important applications in experimental research (Hales, 1974). As generally applied, radioactive indicators such as rubidium allow only one measurement to be made because the animal must be killed and the circulation stopped within a few seconds of indicator injection. The radioactive microsphere technique is not subject to this limitaion because the indicator remains in the tissue and different doses may be characterized by a particular isotopic label. The number of measurements possible then

depends on the physiological limits of tolerance within the microvasculature and on radioassay instrumentation available. Up to five measurements have been made in one animal (Forsyth, 1970). Diffusible indicators and particles both offer the advantage, compared with electromagnetic on ultrasonic techniques, of enabling the measurement of blood flow in tissues to which access is very difficult.

Indirect blood flow measurement with a freely diffusible tracer such as Xenon-133 is used to assess individual organ flows. The radionuclide Xenon-133 is the most commonly used inert gas tracer for this purpose. Usually the radioactive xenon is administered either directly into the tissue or as a bolus injection via the arterial supply. Several methods have been developed to calculate the organ blood flows from the measured washout of the isotope, with exponential analysis being the most common (Zierler, 1965). Exponential analysis is based on the assumption that diffusion equilibrium between the xenon in blood phase and the xenon in tissue phase is established within the order of a second or less. The concentration of xenon in venous blood leaving the organ is therefore in continuous equilibrium with the tissue concentration. The label is thus transported from the tissue in question at a rate which is proportional to the blood flow through that tissue (Zierler, 1965).

Organ blood flows have also been measured with tritiated water, a method which produces results highly

correlated with the radioactive microsphere technique (Oddy et al., 1981). This technique involves prolonged infusion of the tritiated water with equilibrium being established between 30 and 60 minutes after the start of the infusion. Venous blood is sampled continuously during the entire infusion period and the resultant flow values correlate well with those determined using the microsphere method. Oddy et al. (1981) and Brown et al. (1982) have obtained satisfactory estimates of blood flow in both the sheep hindlimb muscle and sheep uterus respectively, using the tritiated water technique.

Direct measures of blood flow such as electromagnetic flow metering (Denison et al., 1965) are less common and require specialized and expensive apparatus. This technique involves the installation of electromagnetic flowimeters on the blood vessel of interest and as such find limited application when vessel isolation is extremely invasive. When installed, this apparatus can provide instantaneous measures of blood flow through a vessel over an extended period of time. An important assumption with this technique is that the flowimeter itself does not interfere with and alter flow through the vessel.

Thermodilution is another direct method for measuring blood flow based on indicator temperature gradients prior to and after being mixed with blood (Haggmark et al., 1982). The accuracy of this technique has been established in the flow range of 300-1500 ml/minute. Prerequisites for the

continuous thermodilution method include: constant intravascular temperature, stable catheter position, absence of blood flow reflux and adequate indicator-blood mixing. A particular advantage to the thermal dilution technique is that the injection of the indicator and the detection of the indicator thermal dilution curve can be accomplished with a single thermistor-tipped catheter. Thermal dilution measurements give a reliable estimate of flow and may be repeated frequently without harm or buildup of background.

MATERIALS AND METHODS

General Design

Two groups of experimental animals consisting of four steers each were used to study both the effects of various hormones and the duration of starvation on amino and ketoacid metabolism in skeletal muscle. Localized blood flow and a number of other blood consitiuents were studied as well.

Steers were allotted into two main effect groups representing fat and lean body composition. Group one, those animals with a high degree of body fat accumulation, were 15 to 18 months of age and were either small framed Hereford or Hereford crossbred steers. The average weight of this group at the time they were used in the experiment was 565 kg and external body fat was estimated at between 1.1 and 1.6 cm. Carcass data from this group collected at slaughter approximately 2-6 weeks post experiment is presented in table (4).

Cattle in group two, the lean group, were 8 to 9 months of age when used in the experiment. They had an average weight of 287 kg and were estimated to have less than .25 cm of external body fat. These cattle were larger framed

crossbred cattle representing Shorthorn, Angus, Gelbevih, Holstein and Simmental crosses.

The experimental procedures for treatment and sampling proceded using pairs of animals within each group during each of four experimental periods. This regime was necessary due to procedural restrictions in the surgical preparation of the animals. This will be explained in great detail later.

Experimental Diets and Adaptations

Animals chosen for group one, the fat group, were gradually adapted to and maintained on a diet consisting of approximately 86.5% concentrate (Table 1). The energy content was 1.4 mcals/kg of diet DM and the crude protein level was 12.0% (calculated).

TABLE 1. FINISHING DIET GROUP 1

Item	International Reference No.	% of Diet DM
Corn, Dent Yellow Grain, High Moisture	40-20-720	81.6
Corn, Silage, Well Eared	3-28-250	10.0
Supplement[1]	---------	8.4

1. Supplement contained 71.4% soybean meal (5-20-637) 10.0% limestone, 15.9% potassium chloride and 3.0% trace mineral salt.

This finishing diet was fed ad lib for approximately 210 days to ensure adequate fat accumulation in these animals. Following the fattening period, these animals were switched to the experimental diet and fed ad lib quantities of this diet for 6 weeks (Table 3).

Animals designated for use on the lean group were maintained for approximately 65 days on a diet consisting of corn silage and protein supplement (Table 2) fed ad lib. The energy content of this diet was 1.1 mcals/kg of diet DM and crude protein was a calculated 12.0%.

TABLE 2. Growing Diet Group 2

Item	International Reference No.	% of Diet DM
Corn, Silage, Well Eared	3-28-250	89.8
Supplement[1]	---------	10.2

1. Supplement contained 92.7% soybean meal (5-20-637) 4.9% dicalcium phosphate and 2.4% trace mineral salt.

These animals were then switched to the experimental diet (Table 3) and fed for 6 additional weeks before being used in the experiment. During the adaption periods all animals (both groups) were housed outside in partially covered, bedded pens with free access to water. Feed was provided once daily.

TABLE 3. COMPOSITION OF EXPERIMENTAL DIET

Item	International Reference No.	% of Diet DM
Corn, Silage, Well Eared	3-28-250	74.70
Corn, Dent Yellow, Grain, High Moisture	40-20-720	16.30
Soybean Seed, Meal, Solvent Extracted, 44.0% Protein	5-20-637	6.70
Potassium Chloride	6-03-755	1.32
Limestone, Ground[a]	6-02-632	0.34
Dicalcium Phosphate[b]	6-01-080	0.29
Trace Mineral Salt[c]	---------	0.25
Selenium-90 Premix[d]	---------	0.10

a. Calcium carbonate 38% calcium
b. 23% calcium, 18% phosphorus
c. Contained in %, Zn, mn 0.35, Mn, mn 0.2, Fe, mn 0.2, Mg,mn 0.15, Cu, mn 0.03, Co, mn 0.05, I, mn 0.007, NaCl, mx 98.5
d. 90 mg Se/kg

TABLE 4. CARCASS DATA - FAT GROUP

Animal #	Live Wt.(kg)	HCW[1](kg)	Ext. Fat(cm)	YG[2]	QG[3]
180	604	365	1.02	3.8	CH-
8448	617	372	1.02	3.3	GD+
175	572	348	1.52	3.8	CH
118	570	349	1.47	3.9	CH+

1. Hot carcass weight.
2. Yield grade.
3. Quality grade.

One week prior to the start of an experimental period a pair of animals was moved into the metabolism room and housed in individual pens. Fresh feed (experimental diet) was offered twice daily and water was always present. Surgical preparation of the experimental animals occurred during this week prior to the start of the experiment. Surgical preparation and technique will be explained in great detail later in this section.

Experimental System Development - Single Hind Limb

The initial experimental system chosen to study the amino and branched-chain keto acid metabolism in skeletal muscle was the single hindlimb of the steer. This system was preferred because of the high proportion of total skeletal muscle to non-muscle tissue (skin, bone, etc.) present in this area. Catheters would need to be placed in the primary large blood vessels supplying and draining this area to provide the represntative blood samples required to study blood flow nutrient flux. The choice of blood vessels for this purpose, in order of preference as to total muscle drainage, include the deep femoral artery and vein, accessed directly, the femoral artery and vein accessed via the saphenous artery and vein and a short section of the iliac artery and vein accessed either directly or via the circumflex artery and vein (Figure 1). The diameter of these vessels appeared adequate to support catheters such

that sufficient blood could be withdrawn in a short period of time (less than 5 seconds).The point of access to each of these vessels would be different and subsequent distance of catheter insertion would vary with the vessel chosen and location of access. There is little published literature available to document the length of the various vessels of the hind limb which could serve as a reference for correct catheter insertion. This problem was especially compounded since animals of varying weights and frame sizes were used. It was for this reason that a separate preliminary study was undertaken to attempt to characterize vessel lengths and locations while using external landmarks on various size and weight cattle.

Carcass Dissection

The hindhalf sections of five animals varying in weights and hip height between 370 kg and 475 kg and 126 cm and 134 cm respectively, were disected to expose the major blood vessels. Hindhalf sections were obtained either at time of slaughter at both the Michigan State University meat science laboratory and a local packing company, or during necropsy of a particular animal at the Michigan State University College of Veterinary Medicine. Following careful dissection and exposure of the blood vessels, measurements were recorded starting at the level of the hock, the external landmark, working proximally to the level

of the dorsal aorta and caudal vena cava. Bifurcations were noted and approximate vessel diameter was observed. Table (5) presents a summary of the results obtained from this work. Using these data it was then possible to surgically implant catheters in the hindlimb with a greater degree of confidence as to the final location of the catheter tips within a given animal. X-ray validation of catheter tip location was not available during these experiments.

Regardless of which vessel was chosen for catheter placement, the surgical procedure would be moderately to highly invasive and require general anesthesia of the animal. A total of 11 practice surgeries were performed using different vessels at different access sites and implanting an assortment of types and diameter catheters.

Access to and insertion of catheters in the deep femoral vessels was the most difficult and least successful. A number of muscle layers had to be dissected through to expose only a small portion of these deep vessels. Catheter insertion was difficult and tip location was uncertain due to the lack of a sufficient external landmark as a guide.

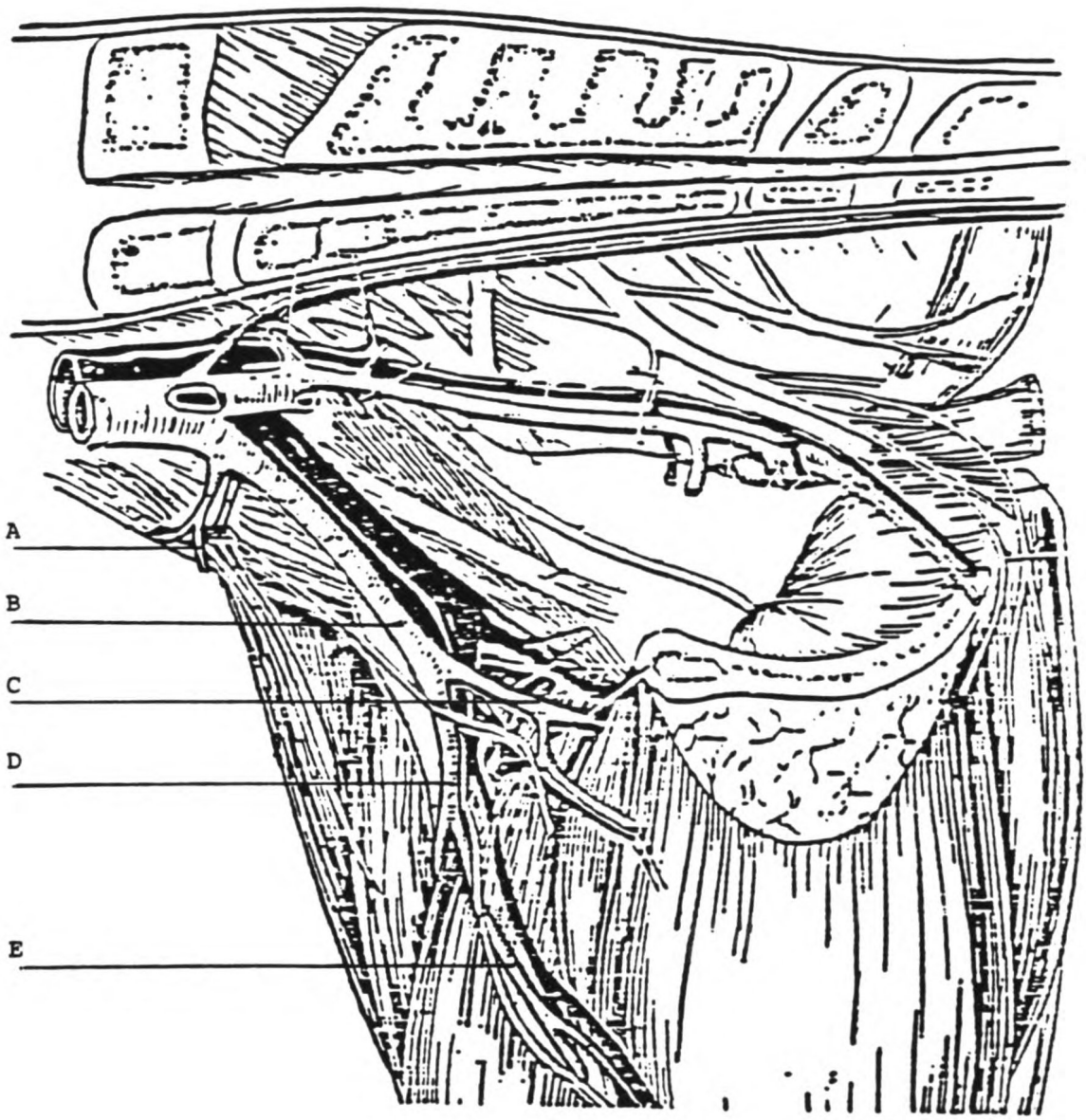
Direct access to and success with the external iliac vessels was surrounded by problems similar to those encountered with the deep femoral surgeries. Catheter placement in the femoral vessels via insertion through the saphenous vessels was the most promising of all the initial procedures attempted. The saphenous artery and vein are readily accessed at the level of the hock in the inside of

TABLE 5. SUMMARY OF MEASURED VESSEL LENGTHS[1]

Animal Wt. (kg)	320	340	398	410	475
Hip Height (cm)	126	128	129	132	133
Vessel Segment (Figure 1)					
	---------- cm ----------				
Bifurcation of External Iliac at Aorta to Circumflex Iliac	5.5	6	6	6	6
Bifurcation of External Iliac at Aorta to Deep Femoral	15	14	12	11	15
Deep Femoral to Femoral/Saphenous Junction	13	13	13	15	13
Saphenous at Hock to Bifurcation of External Iliac at Aorta	75	75	72	76	75
Bifurcation of External Iliac at Aorta to:					
- Post. Mesenteric	14	13	13	14	14
- Renal Vessels	15.5	16	16	16	--
Circumflex Iliac at Lymph Node to Aorta - External Iliac Bifurcation (Figure 2)	---	--	26	28	26

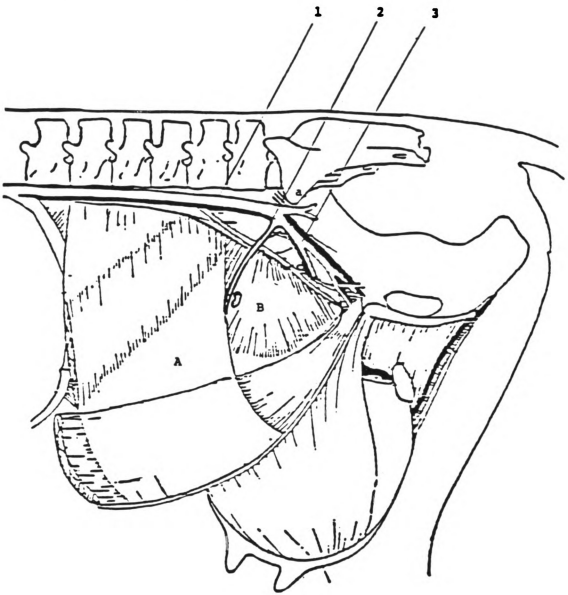
1. Leg positioned in "standing" fashion.

Figure 1. Blood Vessels of the Hindlimb



A. Circumflex Iliac Artery
B. External Iliac Artery
C. Deep Femoral Artery
D. Femoral Artery
E. Saphenous Artery

Figure 2. Blood Vessels and Muscles of the Hindlimb



A. Internal Abdominal Oblique
B. External Abdominal Oblique

a. Tuber coxae

1. Aorta
2. Circumflex Iliac Artery
3. External Iliac Artery

the leg and are found rather superficially. The size of these vessels was somewhat but not entirely restrictive as to size of catheter that could be used. Following insertion of the catheters into these vessels for a suitable distance, the catheter material remaining outside the vessel was then directed under the hide of the animal such that the catheters exited on top of the rump to allow easy access and blood sampling. Although problems with catheter patency had been encountered, this procedure was chosen as the best candidate for use in the experimental animals.

Eight more attempts at this procedure using various catheter materials were performed in an attempt to overcome problems of loss of catheter patency related primarily to fibrous cap formation over the venous catheter tips. Kinking of some catheters due to the lack of flexability of certain catheter materials (teflon and polyethylene) following insertion under the hide was also a problem.

Catheter materials used included: polyvinyl, polyethylene, teflon, silicon rubber and combinatins of teflon inside the vessel with silicon rubber or polyvinyl outside the vessel. The catheters inside diameter ranged from .55 mm to 2.2 mm (Table 8). All catheters were coated inside and out with TDMAC heparin (Polyscience Co.), a heparin-polymer compound to prevent blood clots. Catheters were cut from bulk material and the tips were polished prior to insertion to further inhibit clot formation. All catheters were gas-sterilized prior to use. After several

months of work, the venous catheter patency problem had not been overcome. It was concluded that the rate of blood flow past the catheter tips, especially on the venous side, played a critical role in maintaining catheter patency. In each of the attempts, venous catheter patency was lost within 7 to 10 days while the arterial catheter worked indefinitely. All catheters were removed from the animal upon loss of patency and examined for abnormalities. Venous catheters were covered with a fibrous coat extending over the tip in some cases which allowed infusion into the catheter but prevented a sample from being drawn out. Some venous catheters appeared free of this fibrous material although it may have been stripped off upon removal of the catheter. All arterial catheters were free of any fibrous material or clots. In general, it was recognized that silicon rubber was the material of choice inside the vessel and polyvinyl was the choice outside the vessel.

Additional problems encountered with the single hindlimb procedure included loss of feed intake with slow recovery of pre-surgery feed intake, a problem related to the general anesthesia necessary for the procedure. Infection at both the surgery site and along the route of the catheter under the hide was a serious problem as well. It became clear that an alternative procedure would be required to achieve the initial experimental goals. Easier access to larger vessels with less invasive methods would be required.

Total Hind Half System

A new system involving the catheterization of the blood vessels supplying and draining the total hindhalf of the animal was developed. This procedure allowed catheter placement in the dorsal aorta and caudal vena cava which in turn prolonged venous catheter patency in large part due to the higher rate of blood flow and a 2 to 4 fold greater vessel diameter. In this case catheter insertion was via the circumflex iliac vessels which run superficially approximately half-way between the tuber coxae and the flank. A lymph node located at this exact location serves as a reliable landmark (Figure 2).

The surgical procedure involved a 15 cm skin incision at the level of and immediately anterior to the lymph gland. The animals were standing and local anesthesia was used which allowed the animals to maintain feed intake. Superficial fascia was incised and the circumflex iliac vessels were exposed. These vessels transverse the internal abdominal oblique muscle and are easily accessable in this area.

Following excision of fascia surrounding the individual vessels a small cut was made in the vessel with a scapel blade and the catheter was inserted into the vessel for a predetermined distance. A silk suture was placed around the vessel and catheter to prevent leakage at the insertion

site. The catheters were then secured in two additional places inside the incision to prevent slippage of the catheter from the vessel. Catheters were exited from the animal through a small hole in the hide anterior to the incision. The catheters used in this procedure were 65 cm long and made of polyvinyl with a 5 cm silicon rubber tip secured to the end of the venous catheters. Both catheters were inserted 32 cm into each vessel which left the catheter tips in the dorsal aorta and caudal vena cava 4 to 6 cm anterior to the bifurcation of the iliac vessels. A 7 cm silicon rubber collar was installed over all catheters at the point which the catheters were secured inside the animal. This procedure prevented constriction of the sampling catheter by the sutures used to secure it in place.

This procedure resulted in venous catheter patency of an average 21 to 28 days and indefinite arterial catheter patency. Thus the procedure was employed on all subsequent experimental animals. This surgical procedures resulted in little post-operative infection and no serious complications. An important advantage of this procedure is that there are no bends in the original catheter as it exits from the vessel and the animal and this facilitates the insertion of a smaller catheter or a cleaning wire to overcome occasional clotting problems.

Once inserted, catheters were flushed and filled with heparinized saline (20 units heparin/ml). Catheters were flushed once daily throughout the experimental period.

Experimental Protocol

Each experimental period began two days post surgery and continued for seven days. Each animal served as its own fed control in an untreated state immediately prior to the treatment and sampling regime. Table (6) outlines the treatment and sampling procedure. Sampling began each day at 9:00 AM, two hours after fresh feed was provided.

All treatment injections were via a jugular catheter inserted into the animal on the evening prior to the start of the experimental period. At each sampling time 20 ml of blood was withdrawn from each animal's arterial and venous catheters simultaneously. Blood samples were placed in heparinized tubes and stored on ice. Plasma was separated by a brief centrifugation (10 minutes at 6000 rpms) within 15 minutes of sampling. Plasma was extracted and half the plasma was immediately frozen for later analysis of glucose, creatinine, insulin and branched chain keto acids. The remaining plasma was prepared immediately for analysis. An additional 50 ml of blood was taken for blood flow analysis at the times indicated in Table (6).

Amino Acid Analysis

Plasma samples for amino acid analysis were deproteinized with .1 volume of sulfursalicylic acid and

TABLE 6. EXPERIMENTAL PROTOCOL

Day	Treatment	Sample (minutes post trtmt.)
1	Fed Control[d]	
	Insulin Infusion[a]	15, 30[d], 60, 90
2	Fed Control[d]	
	Insulin[b]	15, 30[d], 60, 90
3	Fed Control[d]	
	Hydrocortisone Infusion[c]	15, 30[d], 60, 90
4	24 Hour Fasted[d]	e
5	48 Hour Fasted[d]	e
6	72 Hour Fasted[d]	e
7	96 Hour Fasted[d]	e

a. 0.5 units Insulin per kg BW
b. 1.0 units Insulin per kg BW
c. 5 mg/kg BW
d. Blood flow measured
e. Point sample only

prepared according to procedures described by Bergen et al (1973). Amino acid concentrations were determined by ion-exchange chromatography using an automated Durrum amino acid analyzer. The sample volume applied to the amino acid column was .1 ml and the visible detector (570 nm) was set at .5 AUFS (absorbance units full scale). The amino acid column was 26.5 cm long with an inside diameter of 3.2 mm. Column packing was DC-4A Dionex resin. Mobile phase flow rate was 12 ml/hr. A four step running buffer gradient was used to elute the amino acids from the column as follows: .25N Li citrate (pH 2.7); .6N Li citrate (pH 3.2); 1.0N Li citrate (pH 4.2) and 1.3N Li citrate (pH 5.1). Post-column derivitization was achieved with ninhydrin (6 ml/hr).

Plasma Glucose Analysis

Glucose was determined in previously frozen plasma samples using the hexokinase catalyzed conversion of glucose to glucose-6-phosphate. This is a colorimetric procedure in kit form and was obtained from Sigma Diagnostice Co. (Sigma Kit #115).

Creatinine Analysis

Creatinine was determined in previously frozen samples by a direct colorimetric procedure developed by Heinegard and Tiderstrom (1973). A kit was obtained for this procedure from the Sigma Diagnostic Co. (Kit # 555).

Insulin Analysis

Plasma insulin was determined using a Micromedics Autopack Insulin Radio Immune Assay Kit (B-1802). This procedure was validated for quantifying insulin in cattle blood by Reimers et al (1982). Inter assay precision was between 6.7 and 20.1%.

Branched Chain Keto Acid Analysis

Measurement of the branched-chain alpha-ketoacids in the plasma was accomplished using high performance liquid chromatography following the procedure described by Nissen et al (1982). Ketoacids for use in standards (.005-.05 mM) were obtained from the Sigma Chemical Co. A modification in the mobile phase was required to achieve better peak separation and clarity. This modification entailed the use of a mixture of 80% .05 Molar sodium phosphate (pH 7.0) and 20% methanol as the running buffer in place of the sodium phosphate-acetonitrile mixture described in the reference.

Plasma Sample Preparation

Plasma samples (1 ml) were adjusted to pH 1 with 1 M hydrochloric acid (approximately 200 microliters). Twenty nanamoles of internal standard (alpha-ketocaproate) were added to each sample. Standard solutions of .05 millimolar branched chain keto acids (BCKA) were processed along with the plasma. The BCKA were initially fractionated by transferring the plasma to a 4 X 8 cm column containing 50% aqueous solution of cation exchange resin (H+ from Bio-Rad

Labs # 731-6213). The columns were washed with four 1 ml aliquots of .01 M hydrochloric acid and the effluent plus washings were collected into test tubes for BCKA analysis. This effluent was extracted once with 35 ml of methylene chloride and after centrifugation for five minutes (800 x g) the supernatant (aqueous layer) was aspirated and discarded. The methylene chloride layer was back extracted with 350 microliters of .1 M sodium phosphate buffer (pH 7.0). Following a second centrifugation for five minutes (800 x g) the aqueous layer was transferred to 300 microliter reaction vials. Centrifugation took place again for five minutes (800 x g) and 150 microliters of the aqueous solution was injected into the HPLC system. BCKA concentrations were calculated using peak area ratios of the standards and the internal standard, alpha-ketocaproate.

Blood Flow Determination

Hindhalf blood flow was determined using indocyanine green dye infusion according to the method described by Bell et al (1974). This method is a short-term continuous dye infusion first developed by Wahren (1966) and later modified by Jorfeldt and Wahren (1971). Indocyanine dye (Sigma Chemical # I2633) was infused into the dorsal aorta for seven minutes at 1.2 mg/minute in a dilution giving an infusion rate of 2.2 to 25 ml/minute. Blood was sampled from the caudal vena cava immediately before dye infusion to serve as a control, then simultaneously from the caudal vena

cava and the jugular vein at five and seven minutes during the dye infusion. An additional 10 ml sample was taken for PCV measurement prior to the start of each infusion. A 10 milliliter sample of blood was withdrawn at each time and plasma was separated. Dye concentration was measured directly in plasma spectrophotmetrically at a wavelength of 800 nanamoles. A standard curve was constructed using known amounts of dye added to blood collected immediately before dye infusion was begun. Calculations of blood flow and nutrient flux are presented in table 7. The indocyanine green dye was observed to follow Beer's law over the range of concentrations studied (1.2 - 4.8 micrograms/ml). The infused dye was cleared from the circulation between 30 and 45 minutes after the infusion was stopped.

Statistical Analysis

Two separate split plot analyses were used to statistically evaluate the data according to Gill (1978). Bonferroni contrasts of means were constructed to compare treatment effect within and between main effect groups. The Genstat statistical package was used for all analyses.

TABLE 7. CALCULATIONS

Blood Flow

$$F_B = \frac{I \times C_I}{C_v - C_A}$$

$$F_P = F_B \times \frac{100 - PCV}{100}$$

Net Nutrient Flux

$$\text{Net Flux (in mmoles/hour)} = F_P (C_{XA} - C_{Xv})$$

(+) = uptake

(-) = release

Where:

F_B = Blood flow (ml/min)

F_P = Plasma flow (l/hr)

I = Dye infusion rate (ml/min)

C_I = Dye concentration in infusate (mg/ml)

C_v = Venous dye concentration (mg/ml)

C_a = Remote artery dye concentration (mg/ml) for recirculation value

P_{CV} = Packed cell volume (%)

C_{XA}, C_{Xv} = Nutrient concentration in arterial and venous blood respectively (mmoles/l)

TABLE 8. SUMMARY OF CATHETERS USED

Composition	Inside Dia. (mm)	Source
Teflon, Medical Grade Ultra Thin Wall	.55	1
Teflon, Medical Grade Ultra Thin Wall	.90	1
Teflon, Non-Medical Standard Wall	1.50	2
Polyethylene, Formocath™	1.40	1
Polyethylene, Formocath™	2.20	1
Polyethylene, Intramedic™	1.20	1
Polyvinyl	1.10	3
Polyvinyl	1.40	3
Polyvinyl	2.20	3
Silastic™, Medical Grade	2.20	4

1. Becton, Dickerson Co., Parsippany, NJ
2. Essex Group Wire Co., Ft. Wayne, IN
3. Ico-Rally Co., Palo Alto, CA
4. Dow-Corning Co., Midland, MI

RESULTS

Experimental results will be presented first in a within-group, across-treatment fashion for amino acid (AA) and branched-chain keto acid (BCKA) net flux (mMoles/hr) across the hindhalf of the steer (tables 9-34). Results of treatment response between the two experimental groups will be presented next (tables 35-47). Blood flow data and arterial concentrations of glucose and insulin will follow (tables 48-56). Finally, N-t-methylhistidine (NMH) release and NMH/creatinine ratios for both experimental groups and all treatments will be presented (tables 57-60). Throughout the results and discussion, uptake and removal, and also release and addition, will be used interchangably.

Amino Acid Net Flux Within-Group

The effect of insulin (at two levels) on net hindhalf flux for animals in group 1 (fat group) is presented in tables 9 and 10. Insulin injection at the 0.5 Iu/kg bw level (table 9) produced results not different from fed control values, with the exception of a significant ($P<.05$) change in threonine net flux from removal in the control to release in the treated condition. In both conditions (control and insulin level 1) the AA flux favored release. Insulin injection at the 1.0 Iu/kg bw level (table 10) produced significant ($P<.05$) removal of alanine, glutamine and leucine with a number of other AA tending towards

Table 9. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate		18.98		21.48	11.91
Glycine		120.40		73.14	37.33
Serine		8.39		14.44	9.85
Alanine		209.01		180.61	42.32
Glutamine		96.83		83.08	22.62
Glutamate	79.40		53.79		28.21
Threonine	10.20[a]			57.24[b]	11.14
Methionine		13.38		14.10	5.96
Histidine		40.50		15.51	15.81
Arginine		39.98		31.66	19.90
Cystine		30.39		12.03	10.36
Phenlyala		43.63		45.55	24.62
Tyrosine		22.97		15.82	5.57
Lysine		40.09		41.67	33.43
Leucine		60.49		70.16	18.55
Isoleucine		50.68		31.25	13.01
Valine		162.51		180.62	70.90

1. Means of 4 animals. Group 1, fat animals.
2. 0.5 Iu/kg bw.
a,b Row means with different superscripts differ ($P<.05$).

Table 10. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Aspartate		17.72	23.62		11.91
Glycine		112.30	12.75		37.33
Serine	1.71		.92		9.85
Alanine		188.12[a]	48.28[b]		42.32
Glutamine		54.73[a]	68.34[b]		22.62
Glutamate	97.49			14.55	28.21
Threonine		5.67	6.45		11.14
Methionine		6.09		2.39	5.96
Histidine		26.64	3.33		15.81
Arginine		24.29		12.17	19.90
Cystine		36.21	4.40		10.36
Phenlyala		22.49		3.78	24.62
Tyrosine		20.77		3.03	5.57
Lysine		110.01		179.30	33.43
Leucine		64.72[a]	6.15[b]		18.55
Isoleucine		37.70		4.73	13.01
Valine		55.97		6.26	70.90

1. Means of 4 animals. Group 1, fat animals.
2. 1.0 Iu/kg bw.
a,b Row means with different superscripts differ (P<.05).

Table 11. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Amino Acid	Fed Control		Hydrocortisone		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate		19.17		56.98	11.91
Glycine		46.99		72.48	37.33
Serine		5.02		2.89	9.85
Alanine		93.56		92.40	42.32
Glutamine		70.36	1.11		22.62
Glutamate	99.09		51.43		28.21
Threonine		8.05		4.95	11.14
Methionine		1.34		6.66	5.96
Histidine		36.72		34.51	15.81
Arginine		48.86		12.58	19.90
Cystine		35.57		11.62	10.36
Phenlyala		12.99		47.11	24.62
Tyrosine		6.67		4.91	5.57
Lysine		101.92		47.72	33.43
Leucine		24.33		10.43	18.55
Isoleucine		11.66		13.26	13.01
Valine		29.05		39.45	70.90

1. Means of 4 animals. Group 1, fat animals.
2. 5.0 mg/kg bw.

removal as well (aspartate, glycine, threonine, histidine, cystine) compared to the control.

Hydrocortisone administration (table 11) produced no significant changes in AA net flux compared to control for animals in group 1. The vast majority of AA were released from the muscle under both conditions (control and hydrocortisone). With the exception of insulin at level 2 (table 10), glutamate was the only AA consistently removed from the blood.

The effect of fasting (24-96 hr) for animals in group 1 is presented in tables 12-15. Fasting for 24 hours (table 12) produced a significant ($P<.05$) decrease in alanine release from muscle compared to control and a significant ($P<.01$) change in glutamine metabolism from measured release in the control state to uptake in the 24 hour fasted state. With the exception of glutamate (net removal) all other AA were released from muscle in both the control and 24 hour fasted state. A 48 hour fast (table 13) produced a significant ($P<.05$) change in glycine and isoleucine net flux from release in the control state to uptake in the fasted state. A similar result ($P<.01$) was observed for cystine. Alanine release was significantly ($P<.05$) less in the fasted condition and glutamine, which was taken up after a 24 hour fast (table 12), was released from muscle after 48 hours of fasting. Fasting glutamine release was significantly ($P<.05$) less than control at 48 hours. Following a 72 hour fast (table 14) alanine and glutamine

Table 12. Net Hindhalf Metabolism[1] Effect of Fasting (24 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate		18.62		45.75	13.17
Glycine		93.29		2.12	39.58
Serine		3.90		3.80	10.60
Alanine		163.62[a]		55.36[b]	36.57
Glutamine		73.97[c]	29.43[d]		22.08
Glutamate	91.99		54.26		25.08
Threonine		1.17		11.18	11.01
Methionine		6.94		2.30	5.04
Histidine		34.62		22.46	15.79
Arginine		37.71		18.23	18.36
Cystine		34.06		15.34	9.04
Phenlyala		26.37		3.92	19.07
Tyrosine		16.80		5.58	13.62
Lysine		84.03		20.59	31.21
Leucine		49.85		13.88	16.42
Isoleucine		33.35		4.55	12.06
Valine		82.53		51.38	68.83

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 13. Net Hindhalf Metabolism[1] Effect of Fasting (48 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate		18.62	7.34		13.17
Glycine		93.29[a]	55.13[b]		39.58
Serine		3.90		2.11	10.60
Alanine		163.62[b]		74.73[b]	36.57
Glutamine		73.97[a]		13.95[b]	22.08
Glutamate	91.99		56.06		25.08
Threonine		1.17	12.05		11.01
Methionine		6.94		2.63	5.04
Histidine		34.62		16.01	15.79
Arginine		37.71		3.96	18.36
Cystine		34.06[c]	14.33[d]		9.04
Phenlyala		26.37		5.74	19.07
Tyrosine		16.80		4.80	13.62
Lysine		84.03		17.13	31.21
Leucine		49.85		11.13	16.42
Isoleucine		33.35[a]	6.95[b]		12.06
Valine		82.53		17.50	68.83

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

Table 14. Net Hindhalf Metabolism[1] Effect of Fasting (72 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate		18.62		4.56	13.17
Glycine		93.29		51.67	39.58
Serine		3.90		16.15	10.60
Alanine		163.62[a]		51.64[b]	36.57
Glutamine		73.97[a]		11.41[b]	22.08
Glutamate	91.99		37.58		25.08
Threonine		1.17	7.84		11.01
Methionine		6.94		3.82	5.04
Histidine		34.62		2.62	15.79
Arginine		37.71		2.12	18.36
Cystine		34.06[c]	1.47[d]		9.04
Phenlyala		26.37		13.43	19.07
Tyrosine		16.80	4.71		13.62
Lysine		84.03[a]	5.04[b]		31.21
Leucine		49.85[c]	18.57[d]		16.42
Isoleucine		33.35[c]	32.03[d]		12.06
Valine		82.53	19.79		68.83

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 15. Net Hindhalf Metabolism[1] Effect of Fasting (96 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate		18.62	3.77		13.17
Glycine		93.29		10.51	39.58
Serine		3.90		1.58	10.60
Alanine		163.62[a]		21.48[b]	36.57
Glutamine		73.97[a]		8.21[b]	22.08
Glutamate	91.99[a]			4.61[b]	25.08
Threonine		1.17		5.13	11.01
Methionine		6.94		2.72	5.04
Histidine		34.62		5.16	15.79
Arginine		37.71	1.06		18.36
Cystine		34.06[a]	.17[b]		9.04
Phenlyala		26.37		8.85	19.07
Tyrosine		16.80		5.23	13.62
Lysine		84.03		5.63	31.21
Leucine		49.85		15.47	16.42
Isoleucine		33.35		18.29	12.06
Valine		82.53		40.34	68.83

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ ($P<.05$).

release was significantly ($P<.05$) less than control and a significant ($P<.05$) change from release in the control state to uptake in the fasted state was observed for lysine. Net flux for branched-chain amino acids (BCAA), leucine and isoleucine, switched from release in the fed control state to uptake following the 72 hour fast ($P<.01$). A similar result was observed for cystine. Glutamate continued to be removed from the blood at all three fasting periods (24,48,72 hr). Following 96 hours of fasting, all measured AA were released into the blood with the exception of aspartate, arginine and cystine, which were removed in small quantities (table 15). Alanine and glutamine release was significantly ($P<.05$) reduced and glutamate, which had been removed at the previous three fasting times, was now released in an amount significantly ($P<.05$) different from control. Net flux favored release in both the control and fasted states with the exception of the 48 hour fast (table 13) but total AA release appeared less during fasting.

The effect of insulin (at two levels) on net hindhalf AA flux for animals in group 2 (lean group) is presented in tables 16 and 17. Insulin injection at the 0.5 Iu/kg bw level (table 16) produced no significant changes in AA net flux. The tendency in both the control and insulin treated states was towards AA uptake. Likewise, insulin injection at the 1.0 Iu/kg bw level did not significantly change net AA flux compared to control in animals from group 2 (table 17). A much stronger tendency towards net removal of AA was

Table 16. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate	.94			1.58	11.91
Glycine		129.67		34.01	37.33
Serine	27.68			.91	9.85
Alanine		58.38	24.99		42.32
Glutamine		89.19		95.87	22.62
Glutamate	84.90		2.47		28.21
Threonine	32.94		10.11		11.14
Methionine	7.56		2.29		5.96
Histidine	26.69		28.57		15.81
Arginine		58.47		14.47	19.90
Cystine		8.22	13.56		10.36
Phenlyala		14.05		29.08	24.62
Tyrosine		10.32		14.49	5.57
Lysine	94.37		86.69		33.43
Leucine	21.37			8.31	18.55
Isoleucine	34.67		5.02		13.01
Valine	80.25		179.70		70.90

1. Means of 4 animals. Group 2, lean animals.
2. 0.5 Iu/kg bw.

Table 17. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate	5.51		3.88		11.91
Glycine		53.76	59.39		37.33
Serine	46.23		39.99		9.85
Alanine		38.60	14.10		42.32
Glutamine		41.21		23.68	22.62
Glutamate	109.70		49.37		28.21
Threonine	17.78		25.41		11.14
Methionine	11.49		7.28		5.96
Histidine	1.14		59.28		15.81
Arginine		53.41	27.66		19.90
Cystine		2.14	4.14		10.36
Phenlyala		12.52	17.13		24.62
Tyrosine	.86			9.54	5.57
Lysine	27.70		98.92		33.43
Leucine	37.30		15.75		18.55
Isoleucine	21.17			5.29	13.01
Valine	139.33			96.55	70.90

1. Means of 4 animals. Group 2, lean animals.
2. 1.0 Iu/kg bw.

Table 18. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Amino Acid	Fed Control		Hydrocortisone		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate	.23			3.27	11.91
Glycine		60.58[a]	92.98[b]		37.33
Serine	45.46		39.47		9.85
Alanine		38.59[a]	72.03[b]		42.32
Glutamine		33.62		18.85	22.62
Glutamate	122.68		86.28		28.21
Threonine	33.07		22.58		11.14
Methionine	18.63		15.20		5.96
Histidine	28.23		31.15		15.81
Arginine		37.05[a]	62.52[b]		19.90
Cystine		29.16[a]	16.64[b]		10.36
Phenlyala		11.32	18.63		24.62
Tyrosine		6.30	2.12		5.57
Lysine	52.04			63.57	33.43
Leucine	37.29		46.64		18.55
Isoleucine	4.46		39.43		13.01
Valine	259.81		242.25		70.90

1. Means of 4 animals. Group 2, lean animals.
2. 5.0 mg/kg bw.
a,b Row means with different superscripts differ ($P<.05$).

observed following the administration of insulin at level 2 (1.0 Iu/kg bw). Hydrocortisone administration in group 2 resulted in a number of significant changes in net AA flux (table 18). A significant ($P<.05$) change from net addition in the control state to net removal in the treated state was observed for glycine, alanine, arginine, and cystine. All other AA with the exception of aspartate, glutamine and lysine tended to be removed from blood following hydrocortisone treatment. This is in contrast to reports by Tomas et al. (1984) of glucocorticiod-induced acceleration of myofibrillar protein turnover.

The effect of fasting (24-96 hr) on animals from group 2 is presented in tables 19-22. A 24 hour fast produced few significant changes in AA net flux in these animals (table 19). AA flux patterns were nearly indentical to control following the 24 hour fast with the exception of serine and glutamine. Serine net removal increased significantly ($P<.05$) in the 24 hour fasted state and glutamine switched from net release in the fed control to uptake following the 24 hour fast ($P<.05$). A number of additional significant changes in net AA flux were observed following a 48 hour fast in animals from group 2 (table 20). Glutamine and glycine changed significantly ($P<.05$ and $P<.01$ respectively) from release in the control to uptake in fasted (48 hr) state. A significant ($P<.05$) change in net methionine flux from removal in the control to release in the fasted state was observed. Glutamate removal and arginine addition were

Table 19. Net Hindhalf Metabolism[1] Effect of Fasting (24 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
	(mMoles/hr)				
Aspartate	2.22		1.84		13.17
Glycine		81.34	15.07		39.58
Serine	39.78[a]		75.92[b]		10.60
Alanine		45.19		1.65	36.57
Glutamine		54.67[a]	11.37[b]		22.08
Glutamate	105.76		49.63		25.08
Threonine	27.93		37.18		11.01
Methionine	12.56		2.17		5.04
Histidine	18.69			.79	15.79
Arginine		49.64		11.10	18.36
Cystine		13.17		2.73	9.04
Phenlyala		12.63		1.54	19.07
Tyrosine		5.25	9.49		13.62
Lysine	58.04			2.31	31.21
Leucine	31.96		.55		16.42
Isoleucine	20.10		.63		12.06
Valine	159.79		105.01		68.83

1. Means of 4 animals. Group 2, lean animals.
a,b Row means with different superscripts differ ($P<.05$).

Table 20. Net Hindhalf Metabolism[1] Effect of Fasting (48 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
		(mMoles/hr)			
Aspartate	2.22		7.44		13.17
Glycine		81.34[c]	112.08[d]		39.58
Serine	39.78		13.26		10.60
Alanine		45.19	7.46		36.57
Glutamine		54.67[a]	10.02[b]		22.08
Glutamate	105.76[a]		15.21[b]		25.08
Threonine	27.93		4.31		11.01
Methionine	12.56[a]			1.31[b]	5.04
Histidine	18.69			2.33	15.79
Arginine		49.64[a]		14.49[b]	18.36
Cystine		13.17		1.27	9.04
Phenlyala		12.63	5.37		19.07
Tyrosine		5.25		4.66	13.62
Lysine	58.04		5.07		31.21
Leucine	31.96		17.27		16.42
Isoleucine	20.10		6.83		12.06
Valine	159.79		36.32		68.83

1. Means of 4 animals. Group 2, lean animals.
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 21. Net Hindhalf Metabolism[1] Effect of Fasting (72 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
	(mMoles/hr)				
Aspartate	2.22		.94		13.17
Glycine		81.34		20.49	39.58
Serine	39.78[c]			5.67[d]	10.60
Alanine		45.19		14.44	36.57
Glutamine		54.67[a]	3.39[b]		22.08
Glutamate	105.76[c]		5.40[d]		25.08
Threonine	27.93[a]			9.18[b]	11.01
Methionine	12.56[a]			2.17[b]	5.04
Histidine	18.69			16.30	15.79
Arginine		49.64[c]	40.96[d]		18.36
Cystine		13.17		3.19	9.04
Phenlyala		12.63		19.63	19.07
Tyrosine		5.25	66.21		13.62
Lysine	58.04[a]			25.03[b]	31.21
Leucine	31.96[a]			24.34[b]	16.42
Isoleucine	20.10			11.56	12.06
Valine	159.79[c]			140.01[d]	68.83

1. Means of 4 animals. Group 2, lean animals.
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 22. Net Hindhalf Metabolism[1] Effect of Fasting (96 hr)

Amino Acid	Fed Control		Fasted		
	Removed	Added	Removed	Added	SEM
	(mMoles/hr)				
Aspartate	2.22			2.12	13.17
Glycine		81.34		30.48	39.58
Serine	39.78[c]			12.35[d]	10.60
Alanine		45.19		32.36	36.57
Glutamine		54.67		2.21	22.08
Glutamate	105.76[c]		3.73[d]		25.08
Threonine	27.93[a]			12.14[b]	11.01
Methionine	12.56[a]			4.71[b]	5.04
Histidine	18.69		1.22		15.79
Arginine		49.64		1.79	18.36
Cystine		13.17		3.17	9.04
Phenlyala		12.63	1.93		19.07
Tyrosine		5.25		5.37	13.62
Lysine	58.04			2.35	31.21
Leucine	31.96			8.71	16.42
Isoleucine	20.10			5.13	12.06
Valine	159.79			10.78	68.83

1. Means of 4 animals. Group 2, lean animals.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

significantly decreased ($P<.05$) following the 48 hour fast. Fasting for 72 hours (table 21) produced the greatest number of significant changes in net AA flux among animals in group 2. Threonine, methionine, lysine and leucine showed a significant ($P<.05$) change in net flux from uptake in the fed control to release in the 72 hour fasted state. Serine and valine experienced a similar change ($P<.01$). Arginine ($P<.01$) and glutamine ($P<.05$) switched from net release to net removal following the 72 hour fast. Glutamate uptake was significantly ($P<.01$) reduced following fasting. Fasting for 96 hours produced results similar to those observed at 72 hours for serine, threonine, methionine and glutamate (table 22). No other significant differences in net AA flux were detected at 96 hours of fasting. In general, early fasting (24-48 hr) favored net uptake of AA while later stages of fasting (72-96 hr) favored release. Overall, total net AA release during fasting appeared less than control.

Branched-Chain Alpha Ketoacid Net Flux Within-Group

The effect of insulin (two levels) on branched-chain ketoacid (BCKA) net flux for animals in group 1 (fat group) is presented in tables 23 and 24. Insulin at the 0.5 Iu/kg bw level produced a significant ($P<.05$) change in ketoisovalerate (KIV) net flux from release in the control state to uptake in the treated state (table 23). Keto-methylvalerate (KMV) and ketoisocaproate (KIC) net flux was not changed. Both KMV and KIC were released into blood in

Table 23. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.21[a]		2.06[b]		.33
Methylvaleric	1.20			1.79	.37
Isocaproic	1.09			.83	.38

1. Means of 4 animals. Group 1, fat animals.
2. 0.5 Iu/kg bw.
a,b Row means with different superscripts differ ($P<.05$).

Table 24. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.23		.31		.33
Methylvaleric		1.05	1.10		.37
Isocaproic		.60	1.54		.38

1. Means of 4 animals. Group 1, fat animals.
2. 1.0 Iu/kg bw.

Table 25. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Alpha Keto Acid	Fed Control		Hydrocortisone		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.20[a]		1.50[b]		.33
Methylvaleric	1.21[c]			2.62[d]	.37
Isocaproic	1.15			.48	.38

1. Means of 4 animals. Group 1, fat animals.
2. 5.0 mg/kg bw.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

both the control and insulin (level 1) treated state. Administration of the higher level of insulin (1.0 Iu/kg bw) to animals in group 1 failed to produce any significant changes in BCKA net flux (table 24). All BCKA were released into the blood during insulin administration (level 2).

Hydrocortisone injection produced a significant ($P<.05$) change from net addition of KIV in the control to net removal in the treated state (table 25). KMV was added to the blood in both control and treated states although net addition was greater ($P<.01$) following hydrocortisone injection. KIC flux was unchanged.

Results of the effect of fasting for 24, 48 and 96 hours on BCKA flux are presented in tables 26-28. Fasting for 24 hours produced a significant ($P<.01$) increase in KIV release and a concomitant significant ($P<.05$) decrease in KIC release (table 26). Although not significant, KMC net flux tended to increase following the 24 hour fast. KIV release remained greater than control ($P<.05$) after 48 hours of fasting (table 27). This fast (48 hr) produced a significant ($P<.01$) shift in both KMV and KIC net flux from release in the fed control state to net removal in the fasted state. By the end of the fasting period (96 hr) no significant differences could be detected in BCKA net flux (table 28). In general, net flux tended to favor addition of BCKA at 24 and 96 hours of fasting and removal at 72 hours. Total net flux appeared greatest during fasting

Table 26. Net Hindhalf Metabolism[1] Effect of Fasting(24 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.06[c]		1.42[d]		.29
Methylvaleric	1.15		2.73		.35
Isocaproic	.95[a]		.13[b]		.33

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

Table 27. Net Hindhalf Metabolism[1] Effect of Fasting(48 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.06[a]			.94[b]	.29
Methylvaleric	1.15[c]		.72[d]		.35
Isocaproic	.95[c]		2.61[d]		.33

1. Means of 4 animals. Group 1, fat animals.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

Table 28. Net Hindhalf Metabolism[1] Effect of Fasting(96 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.06		.08		.29
Methylvaleric	1.15		1.03		.35
Isocaproic	.95		.32		.33

1. Means of 4 animals. Group 1, fat animals.

compared to control at both 24 and 48 hours of fast (table 26,27).

The effect of insulin (two levels) on BCKA net flux for animals in group 2 (lean animals) is presented in tables 29 and 30. Insulin at the 0.5 Iu/kg bw level (table 29) and at the 1.0 Iu/kg bw level (table 30) failed to produce any significant change in net BCKA flux among animals in group 2. Although not significant, insulin injection at the lower level (table 29) tended to result in overall increased net release of the BCKA compared to control. No such tendency was observed for the higher insulin level.

Hydrocortisone injection produced a significant ($P<.01$) change in KIV net flux from release in the control state to uptake in the treated state (table 31). A tendency towards increased removal of KMV and KIC was observed although the differences were not statistically significant.

Results of the effect of fasting for 24, 48 and 96 hours on BCKA flux in animals from group 2 are presented in tables 32-34. Starvation for 24 hours (table 32) produced no observable changes in net BCKA flux. In contrast, the 48 hour fast did produce a significant change in both KIV and KMV net flux (table 33). KIV net flux changed from release in the fed control state to uptake in the fasted (48 hr) state ($P<.01$). Likewise, KMV which was removed in the fed state was added in significant amounts ($P<.05$) in the fasted state. KIC was unchanged. Fasting for 96 hours resulted in no significant differences in net BCKA flux compared to the

Table 29. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.80		2.04	.33
Methylvaleric	.14			.98	.37
Isocaproic		.37		1.23	.38

1. Means of 4 animals. Group 2, lean animals.
2. 0.5 Iu/kg bw.

Table 30. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Fed Control		Insulin		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.91		.28	.33
Methylvaleric	.45		.72		.37
Isocaproic		.24		.42	.38

1. Means of 4 animals. Group 2, lean animals.
2. 1.0 Iu/kg bw.

Table 31. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Alpha Keto Acid	Fed Control		Hydrocortisone		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.55[a]	1.99[b]		.33
Methylvaleric	.60		1.92		.37
Isocaproic		.46	.22		.38

1. Means of 4 animals. Group 2, lean animals.
2. 5.0 mg/kg bw.
a,b Row means with different superscripts differ (P<.01).

Table 32. Net Hindhalf Metabolism[1] Effect of Fasting(24 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Isovaleric		.75		.14	.29
Methylvaleric	.40		.11		.35
Isocaproic		.36	.05		.33

1. Means of 4 animals. Group 2, lean animals.

Table 33. Net Hindhalf Metabolism[1] Effect of Fasting(48 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.75[c]	1.12[d]		.29
Methylvaleric	.40[a]			.97[b]	.35
Isocaproic		.36		.17	.33

1. Means of 4 animals. Group 2, Lean animals.
a,b Row means with different superscripts differ (P<.05).
c,d Row means with different superscripts differ (P<.01).

Table 34. Net Hindhalf Metabolism[1] Effect of Fasting(96 hr)

Alpha Keto Acid	Fed Control		Fasted		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.75		.03	.29
Methylvaleric	.40		.46		.35
Isocaproic		.36		.21	.33

1. Means of 4 animals. Group 2, lean animals.

control (table 34). Fasting for 48 hours (table 33) produced the only significant changes in the BCKA flux in this group of animals. Total net BCKA flux did not appear different at any of the fasting periods compared to the controls.

Amino and Keto Acid Response Across-Groups

Tables 35-47 contain AA and BCKA net flux data represented as an across-group response to treatment. The statistical comparison of means is based upon the control versus treated response of the two experimental groups.

The effect of insulin (two levels) on AA net flux is presented in tables 35 and 36. Response to insulin treatment at the 0.5 Iu/kg bw level was not different between groups (group 1, fat; group 2, lean) as indicated in table 35. Likewise, no significant differences in across-group response was noted for insulin at the higher (1.0 Iu/kg bw) level (table 36).

As with the two previous treatments (insulin level 1 and 2), hydrocortisone response was not different between experimental groups (table 37).

Group response to fasting in terms of AA net flux did exhibit some differences as indicated in tables 38-41. Group response to the 24 hour fast was significantly ($P<.05$) different in terms of lysine, leucine, and isoleucine net flux (table 38). Animals in group 1 released lysine into blood in both the control and fasted (24 hr) state (table 12) while animals in group 2 switched from lysine uptake to

Table 35. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate		21.48		1.58	8.42
Glycine		73.14		34.01	26.40
Serine		14.44		.91	6.97
Alanine		180.61	24.99		29.92
Glutamine		83.08		95.87	15.99
Glutamate	53.79		2.47		19.95
Threonine		57.24	10.11		7.88
Methionine		14.10	2.29		4.21
Histidine		15.51	28.57		11.18
Arginine		31.66		14.47	14.07
Cystine		12.03	13.56		7.33
Phenlyala		45.55		29.08	17.41
Tyrosine		15.82		14.49	3.94
Lysine		41.67	86.69		23.64
Leucine		70.16		8.31	13.12
Isoleucine		31.25	5.02		9.20
Valine		180.62	179.70		50.13

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.

Table 36. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate	23.62		3.88		8.42
Glycine	12.75		59.39		26.40
Serine	.92		39.99		6.97
Alanine	48.28		14.10		29.92
Glutamine	68.34			23.68	15.99
Glutamate		14.55	49.37		19.95
Threonine	6.45		25.41		7.88
Methionine		2.39	7.28		4.21
Histidine	3.33		59.28		11.18
Arginine		12.17	27.66		14.07
Cystine	4.40		4.14		7.33
Phenlyala		3.78	17.13		17.41
Tyrosine		3.03		9.54	3.94
Lysine		179.30	98.92		23.64
Leucine	6.15		15.75		13.12
Isoleucine		4.73		5.29	9.20
Valine		6.26		96.55	50.13

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 1.0 Iu/kg bw.

Table 37. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Aspartate		56.98		3.27	8.42
Glycine		72.48	92.98		26.40
Serine		2.89	39.47		6.97
Alanine		92.40	72.03		29.92
Glutamine	1.11			18.85	15.99
Glutamate	51.43		86.28		19.95
Threonine		4.95	22.58		7.88
Methionine		6.66	15.20		4.21
Histidine		34.51	31.15		11.18
Arginine		12.58	62.52		14.07
Cystine		11.62	16.64		7.33
Phenlyala		47.11	18.63		17.41
Tyrosine		4.91	2.12		3.94
Lysine		47.72		63.57	23.64
Leucine		10.43	46.64		13.12
Isoleucine		13.26	39.43		9.20
Valine		39.45	242.25		50.13

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 5.0 mg/kg bw.

lysine release upon fasting (table 19). The branched-chain amino acids (BCAA), leucine and isoleucine, were released in both the fed and 24 hour fasted condition by group 1 (table 12) while group 2 removed these BCAA during similar conditions (table 19). Fasting for 48 hours produced significant ($P<.05$) differences in group response for cystine, lysine and isoleucine net flux (table 39). Lysine was added to blood both during the control period and following a 48 hour fast in group 1 animals (table 13). At the same times, group 2 animals removed lysine (table 20). Isoleucine was added during the control period and removed during fasting (48 hr) by group 1 animals (table 13) while this BCAA was removed under both control and 48 hour fast conditions in group 2 animals (table 20). Similarly, cystine was added during control and removed during fast in group 1 (table 13) while this AA was added under both conditions (control and 48 hour fast) by group 2 (table 20).

A 72 hour fast resulted in differing group response for net flux of threonine, histidine and lysine ($P<.05$) as well as the three BCAA, leucine, isoleucine and valine ($P<.01$) as indicated in table 40. Lysine and threonine were both added to blood in the control state by group 1 and then removed following a 72 hour fast (table 14). In contrast, lysine and threonine were removed in the control state and later released during fasting by group 2 (table 21). Histidine was always added (control and 72 hour fast) by group 1 animals (table 14) while this AA was initially removed

Table 38. Net Hindhalf Metabolism[1] Effect of Fasting (24 hr)

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Aspartate		45.75	1.84		9.31
Glycine		2.12	15.07		28.18
Serine		3.80	75.92		7.49
Alanine		55.36		1.65	25.86
Glutamine	29.43		11.37		15.61
Glutamate	54.26		49.63		17.73
Threonine		11.18	37.18		7.79
Methionine		2.30	2.17		3.56
Histidine		22.46		.79	11.17
Arginine		18.23		11.10	12.98
Cystine		15.34		2.73	6.39
Phenlyala		3.92		1.54	13.48
Tyrosine		5.58	9.49		9.63
Lysine		20.59[a]		2.31[b]	22.07
Leucine		13.88[a]	.55[b]		11.61
Isoleucine		4.55[a]	.63[b]		8.53
Valine		51.38	105.01		48.67

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.05$).

Table 39. Net Hindhalf Metabolism[1] Effect of Fasting (48 hr)

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate	7.34		7.44		9.31
Glycine	55.13		112.08		28.18
Serine		2.11	13.26		7.49
Alanine		74.73	7.46		25.86
Glutamine		13.95	10.02		15.61
Glutamate	56.06		15.21		17.73
Threonine	12.05		4.31		7.79
Methionine		2.63		1.31	3.56
Histidine		16.01		2.33	11.17
Arginine		3.96		14.49	12.98
Cystine	14.33[a]			1.27[b]	6.39
Phenlyala		5.74	5.37		13.48
Tyrosine		4.80		4.66	9.63
Lysine		17.13[a]	5.07[b]		22.07
Leucine		11.13	17.27		11.61
Isoleucine	6.95[a]		6.83[b]		8.53
Valine		17.50	36.32		48.67

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.05$).

Table 40. Net Hindhalf Metabolism[1] Effect of Fasting (72 hr)

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Aspartate		4.56	.94		9.31
Glycine		51.67		20.49	28.18
Serine		16.15		5.67	7.49
Alanine		51.64		14.44	25.86
Glutamine		11.41	3.39		15.61
Glutamate	37.58		5.40		17.73
Threonine	7.84[a]			9.18[b]	7.79
Methionine		3.82		2.17	3.56
Histidine		2.62[a]		16.30[b]	11.17
Arginine		2.12	40.96		12.98
Cystine	1.47			3.19	6.39
Phenlyala		13.43		19.63	13.48
Tyrosine	4.71		66.21		9.63
Lysine	5.04[a]			25.03[b]	22.07
Leucine	18.57[c]			24.34[d]	11.61
Isoleucine	32.03[c]			11.56[d]	8.53
Valine	19.79[c]			140.01[d]	48.67

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 41. Net Hindhalf Metabolism[1] Effect of Fasting (96 hr)

Amino Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Aspartate	3.77			2.12	9.31
Glycine		10.51		30.48	28.18
Serine		1.58[a]		12.35[b]	7.49
Alanine		21.48		32.36	25.86
Glutamine		8.21		2.21	15.61
Glutamate		4.61	3.73		17.73
Threonine		5.13		12.14	7.79
Methionine		2.72[a]		4.71[b]	3.56
Histidine		5.16	1.22		11.17
Arginine	1.06			1.79	12.98
Cystine	.17			3.17	6.39
Phenlyala		8.85	1.93		13.48
Tyrosine		5.23		5.37	9.63
Lysine		5.63[a]		2.35[b]	22.07
Leucine		15.47[a]		8.71[b]	11.61
Isoleucine		18.29		5.13	8.53
Valine		40.34		10.78	48.67

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.05$).

(control) and then later released (table 21) by group 2 animals. Net BCAA flux between the fed control and 72 hour fasted states was opposite across the two experimental groups. Group 1 animals showed BCAA release during control and uptake following the 72 hour fast (table 14). In contrast, group 2 animals removed the BCAA during the fed control period and released these AA during the fast (table 21).

Fasting for 96 hours produced significantly ($P<.05$) different across-group response in serine, methionine, lysine and leucine net flux (table 41). Each of these AA were released into the blood during the control period and removed at 96 hours of fasting by group 1 animals (table 15). The opposite was observed in group 2 animals. These animals removed the above AA during control and released them upon 96 hours of fast (table 22).

Tables 42-44 present BCKA group response data for insulin (two levels) and hydrocortisone treatment. Response differences were not observed in BCKA net flux across groups for any of the treatments; insulin .05 Iu/kg bw (table 42), insulin 1.0 Iu/kg bw (table 43), hydrocortisone (table 44). The effect of fasting (24,48,96 hr) on BCKA response is presented in tables 45-57. Fasting for 24 hours produced significantly ($P<.01$) different group response for both KIV and KMV net flux. KIV was added to the blood by both experimental groups (tables 26,32) in the fed control and 24 hour fasted states although the amount added was greater

Table 42. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	2.06		2.04		.21
Methylvaleric		1.79	.98		.26
Isocaproic		.83	1.23		.27

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.

Table 43. Net Hindhalf Metabolism[1] Effect of Insulin[2]

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric		.31		.28	.21
Methylvaleric		1.10	.72		.26
Isocaproic		1.54		.42	.27

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 1.0 Iu/kg bw.

Table 44. Net Hindhalf Metabolism[1] Effect of Hydrocortisone[2]

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	1.50		1.99		.21
Methylvaleric		2.62	1.92		.26
Isocaproic		.48	.22		.27

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 5.0 mg/kg bw.

Table 45. Net Hindhalf Metabolism[1] Effect of Fasting(24 hr)

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	1.42[a]		.14[b]		.21
Methylvaleric	2.73[a]	.11[b]			.25
Isocaproic	.13	.05			.23

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ (P<.01).

Table 46. Net Hindhalf Metabolism[1] Effect of Fasting(48 hr)

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
		(mMoles/hr)			
Isovaleric		.94[a]	1.12[b]		.21
Methylvaleric	.72[a]			.97[b]	.25
Isocaproic	2.61[a]			.17[b]	.23

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.01$).

Table 47. Net Hindhalf Metabolism[1] Effect of Fasting(96 hr)

Alpha Keto Acid	Group 1		Group 2		SEM
	Removed	Added	Removed	Added	
	(mMoles/hr)				
Isovaleric	.08			.03	.21
Methylvaleric		1.03	.46		.25
Isocaproic		.32		.21	.23

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).

during fasting compared to control for group 1 (table 6) and less (same comparison) for group 2 (table 32). KIV release by group 1 was 23 times greater at 24 hours of fast compared to the control ($P<.01$). KMV was added to the blood at both control and 24 hours fast by group 1 while this BCKA was removed at the same times by group 2. The 48 hour fast produced a different group response for KIV net flux ($P<.01$). KIV was released by group 1 animals at both the control and 48 hour fast periods (table 27). Group 2 animals released KIV during the fed control period but removed this BCKA following a 48 hour fast (table 33). There were no longer any observable group response differences in BCKA net flux by 96 hours of fasting (table 47).

Hindhalf Blood Flow

The effect of treatment on blood flow for each experimental group is presented in table 48. Insulin injection at the 0.5 Iu/kg bw level significantly ($P<.01$) increased blood flow in group 1 (fat animals) but not in group 2 (lean animals). A 15% increase was observed in group 1 hindhalf blood flow upon insulin (level 1) injection. The higher level of insulin (1.0 Iu/kg bw) produced a significant ($P<.01$) rise in blood flow in both groups. Group 1 responded with a 25% increase while group 2 had an 18% increase in hindhalf blood flow. Hydrocortisone treatment had no significant effect on hindhalf blood flow.

Table 48. Hindhalf Blood Flows (l/hr)[1] Effect of Treatment

Group	Treatment		SEM
	Control	Insulin[2]	
1	434.25[a]	500.25[b]	10.58
2	532.25	541.25	
	Control	Insulin[3]	
1	418.25[a]	524.50[b]	10.58
2	468.25[a]	554.75[b]	
	Control	Hydrocortisone[4]	
1	444.50	435.00	10.58
2	524.25	541.25	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

a,b Row means with different superscripts differ ($P<.01$).

Table 49. Hindhalf Blood Flows (l/hr)[1] Effect of Fasting

Group	Fasted					SEM
	Control	24hr	48hr	72hr	96hr	
1	432.25[a]	256.50[b]	231.25[b]	211.25	98.75[b]	8.75
2	508.25[a]	251.25[b]	258.50[b]	215.75[b]	160.50[b]	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.01$).

Table 50. Hindhalf Blood Flows (l/hr)[1] Effect of Treatment

Treatment	Group 1	Group 2	SEM
Fed control	432.25	508.25	6.19
Insulin[2]	500.25[a]	541.25[b]	
Insulin[3]	524.50	554.75	
Hydrocortisone[4]	435.00	541.25	
Fasted (24hr)	256.50[a]	251.25[b]	7.48
Fasted (48hr)	231.25	258.50	
Fasted (72hr)	211.25[a]	215.75[b]	
Fasted (96hr)	98.74[a]	160.50[b]	

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

a,b Row means with different superscripts differ ($P<.05$).

The effect of fasting on blood flow is presented in table 49. Blood flow was significantly ($P<.01$) decreased (40-50%) following a 24 hour fast in both groups of animals. Blood flow was significantly lower than control at each fasting period. By 96 hours of fast, group 1 blood flow was only 23% of control and group 2 blood flow was 32% of control. Across-group treatment response of blood flow is presented in table 50. The group 1 response to treatment in terms of decreased blood flow was significantly ($P<.05$) different than group 2 for insulin at level 2 as well as at 24, 72 and 96 hours of fasting. At these times, the drop in blood flow was greater in group 2 animals.

Hindhalf Arterial Glucose and Insulin

The effect of treatment on arterial glucose is presented in table 51. Insulin treatment at both levels significantly ($P<.01$) decreased and hydrocortisone significantly ($P<.01$) increased plasma glucose level in both experimental groups. Insulin at the .05 Iu/kg bw level decreased plasma glucose an average of 35% while level 2 insulin (1.0 Iu/kg bw) decreased plasma glucose an average of 45%. Hydrocortisone increased plasma glucose by an average of 23% in group 1 animals only.

Fasting significantly ($P<.01$) decreased plasma glucose in group 1 animals after 72 and 96 hours as indicated in table 52. Plasma glucose was also significantly ($P<.01$) less than control for group 2 animals at 48, 72, and 96 hours of fasting. A 96 hour fast decreased plasma glucose

Table 51. Hindhalf Arterial Glucose (mg/dl)[1] Effect of Treatment

Group	Treatment		SEM
	Control	Insulin[2]	
1	78.91[a]	51.73[b]	1.53
2	88.05[a]	58.53[b]	
	Control	Insulin[3]	
1	77.15[a]	43.76[b]	1.53
2	83.96[a]	47.53[b]	
	Control	Hydrocortisone[4]	
1	77.90[a]	98.97[b]	1.53
2	85.06[a]	72.29[b]	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

a,b Row means with different superscripts differ ($P<.01$).

Table 52. Hindhalf Arterial Glucose (mg/dl)[1] Effect of Fasting

Group	Fasted					SEM
	Control	24hr	48hr	72hr	96hr	
1	78.02[a]	77.61	75.05	69.72[b]	66.57[b]	1.55
2	85.69[a]	85.08	70.36[b]	68.69[b]	33.40[b]	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.01$).

Table 53. Hindhalf Arterial Glucose (mg/dl)[1] Effect of Treatment

Treatment	Group 1	Group 2	SEM
Fed control	78.02	85.69	1.08
Insulin[2]	51.73	58.53	
Insulin[3]	43.76	47.53	
Hydrocortisone[4]	98.97[a]	72.29[b]	
Fasted (24hr)	77.61	85.08	1.10
Fasted (48hr)	75.05[a]	70.36[b]	
Fasted (72hr)	69.72[a]	68.69[b]	
Fasted (96hr)	66.57[a]	33.40[b]	

1. Values are means of 4 animals/group and represent across-group treatment response (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

a,b Row means with different superscripts differ ($P<.01$).

by 15% in group 1 animals while a 62% decrease was observed in group 2 animals after the same length of fast (table 52). Across-group response to treatment for arterial glucose is outlined in table 53. Group 2 animals had a significantly ($P<.01$) smaller rise in plasma glucose in response to hydrocortisone treatment as well as a greater decrease in plasma glucose during the 48, 72 and 96 hour fasting periods ($P<.01$).

Arterial plasma insulin levels were significantly ($P<.01$) increased by exogenous insulin administration regardless of level of insulin administered or experimental group (table 54). A ten-fold rise in plasma insulin was observed in group 1 and a seven-fold rise was observed in group 2 in response to the 0.5 Iu/kg bw treatment level of insulin. Insulin at level 2 (1.0 Iu/kg bw) increased group 1 and group 2 plasma insulin an average of fifteen-fold.

Fasting tended to decrease plasma insulin across groups although this trend was not significant (table 55). As well, across-group treatment response of plasma insulin was not different (table 56).

N-t-Methylhistidine Release and NMH/Creatine Ratios

As outlined in table 57, no significant differences in N-t-methylhistidine (NMH) release from the hindhalf were observed. Although not significant, there was a tendency towards decreased NMH excretion following insulin administration and an increase in NMH excretion following hydrocortisone administration. Fasting did produce a

Table 54. Hindhalf Arterial Insulin (uIU/ml)[1] Effect of Treatment

Group	Treatment		SEM
	Control	Insulin[2]	
1	15.24[a]	147.46[b]	11.81
2	17.22[a]	119.93[b]	
	Control	Insulin[3]	
1	16.23[a]	231.21[b]	11.81
2	17.69[a]	247.46[b]	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
a,b Row means with different superscripts differ ($P<.01$).

Table 55. Hindhalf Arterial Insulin (uIU/ml)[1] Effect of Fasting

Group	Fasted					SEM
	Control	24hr	48hr	72hr	96hr	
1	15.74	18.34	10.70	8.10	6.91	8.15
2	17.46	17.44	12.54	7.84	5.50	

1. Means of 4 animals/group (group 1, fat; group 2, lean).

Table 56. Hindhalf Arterial Insulin (uIU/ml)[1] Effect of Treatment

Treatment	Group 1	Group 2	SEM
Fed control	15.74	17.46	8.35
Insulin[2]	147.46	119.93	
Insulin[3]	231.21	247.46	
Fasted (24hr)	18.34	17.44	5.76
Fasted (48hr)	10.70	12.54	
Fasted (72hr)	8.10	7.84	
Fasted (96hr)	6.91	5.50	

1. Values are means of 4 animals/group and represent across-group treatment response. (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.

Table 57. Hindhalf 3-M-Histidine Release (mMoles/day)[1]

Group	Treatment		SEM
	Control	Insulin[2]	
1	1.06	1.71	.29
2	1.22	.98	
	Control	Insulin[3]	
1	1.68	.84	.29
2	1.28	.85	
	Control	Hydrocortisone[4]	
1	1.65	2.53	.29
2	1.36	1.88	

1. Means of 4 animals/group, (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

Table 58. Hindhalf 3-M-Histidine Release (mMoles/day)[1]

Group	Fasted					SEM
	Control	24hr	48hr	72hr	96hr	
1	1.46[a]	3.26[b]	3.34[b]	1.86	1.70	.21
2	1.29[a,c]	1.01	2.41[d]	1.29	.46[b]	

1. Means of 4 animals/group, (group 1, fat; group 2, lean).
a,b Row means with different superscripts differ ($P<.05$).
c,d Row means with different superscripts differ ($P<.01$).

Table 59. Hindhalf 3-M-Histidine/Creatinine Ratios[1]

Group	Treatment		SEM
	Control	Insulin[2]	
1	.274	.263	.02
2	.454	.440	
	Control	Insulin[3]	
1	.312	.272	.02
2	.437	.403	
	Control	Hydrocortisone[4]	
1	.284	.269	.02
2	.397	.308	

1. Means of 4 animals/group (group 1, fat; group 2, lean).
2. 0.5 Iu/kg bw.
3. 1.0 Iu/kg bw.
4. 5.0 mg/kg bw.

Table 60. Hindhalf 3-M-Histidine/Creatinine Ratios[1]

Group	Fasted					SEM
	Control	24hr	48hr	72hr	96hr	
1	.290	.334	.319	.314	.242	.03
2	.429	.386	.409	.354	.231	

1. Means of 4 animals/group, (group 1, fat; group 2, lean).

significant ($P<.05$) increase in NMH excretion in group 1 animals at 24 and 48 hours compared to the control (table 58). Also, fasting significantly increased NMH excretion in group 2 animals at 48 hours ($P<.01$) and 96 hours ($P<.05$).

Finally, tables 59 and 60 present the NMH/creatine ratios for both experimental groups and all treatments. No significant changes were observed in this ratio in response to either treatment (table 59) or fasting (table 60).

DISCUSSION

Amino Acid Metabolism

Reports of blood or plasma concentrations of amino acids or arterio-venous differences without consideration of blood flow may lack physiological significance from a tissue or system metabolism standpoint. Measurement of net nutrient flux (arterio-venous difference x blood flow) should provide a more precise view of overall metabolism. The results presented and discussed here do reflect actual net flux of the amino and branched-chain keto acids (AA, BCKA) studied. A precise description of the anatomical domain represented by the arterial and venous sampling sites is a critical factor in interpreting physiological data. Much preliminary work (see methods) was involved in the deliniation of the circulatory structure in the hindhalf system reported here. The hindhalf system represents a large anatomical region and although unlikely, it is not impossible that extraneous arterial supply to the region was unaccounted for. In the event of this unlikely occurrance, net flux results could be confounded.

The effect of insulin at two levels on AA net flux in steers with a high degree of body fat accumulation is presented in tables 9 and 10. Upon examination of the fed control AA flux data which are means of the 4 animals in

this group (fat group), it appears that these animals had a tendency to release AA during this period (fed control). This result was somewhat unexpected in light of reports of approximate AA balance across the hindlimb of fed steers and sheep (Ballard et al., 1976; Jarrett et al., 1976 and Bird et al., 1981). Closer examination of the fed control values for the individual animals in group 1 produced a plausible explanation for the observed release of AA in the fed state. Arterio-venous (A-V) concentration diferences for animal 4 were greatly skewed in favor of release of AA from the hindhalf as compared to animals 1-3 (figure 3). Combining the values for all 4 animals into the mean values presented in the corresponding tables produced the somewhat unexpected fed control results. Animal 4 was the only animal with apparently deviant control values and these values were most deviant during the control periods prior to the insulin treatments (figure 4). Statistical comparison of the control periods using the means of the 4 animals produced no significant differences and as a result, all values from all animals are included in the results. Speculation that animal 4 had not completely recovered following surgery or was harboring a subclinical infection may be appropriate although daily monitoring of body temperature provided no such evidence. The short recovery period allowed following surgery is a trade-off for insured catheter patency and produced no apparent complications in 7 of the 8 animals.

Figure 3. Control Means A–V Difference

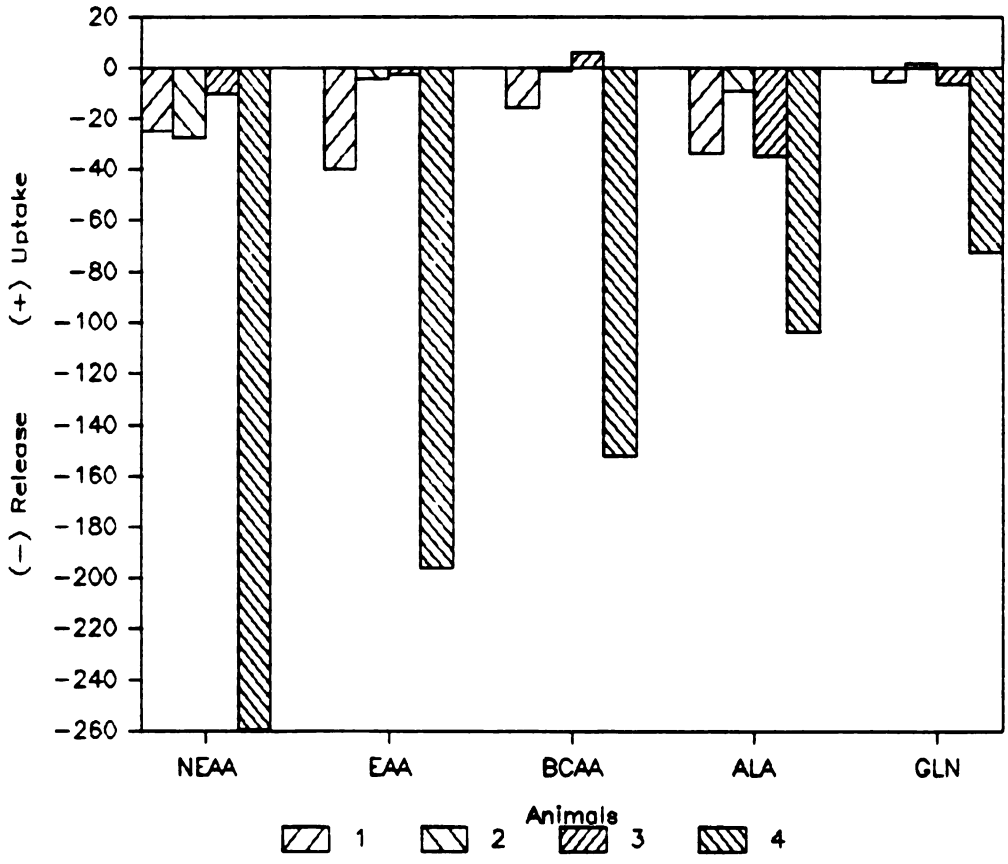
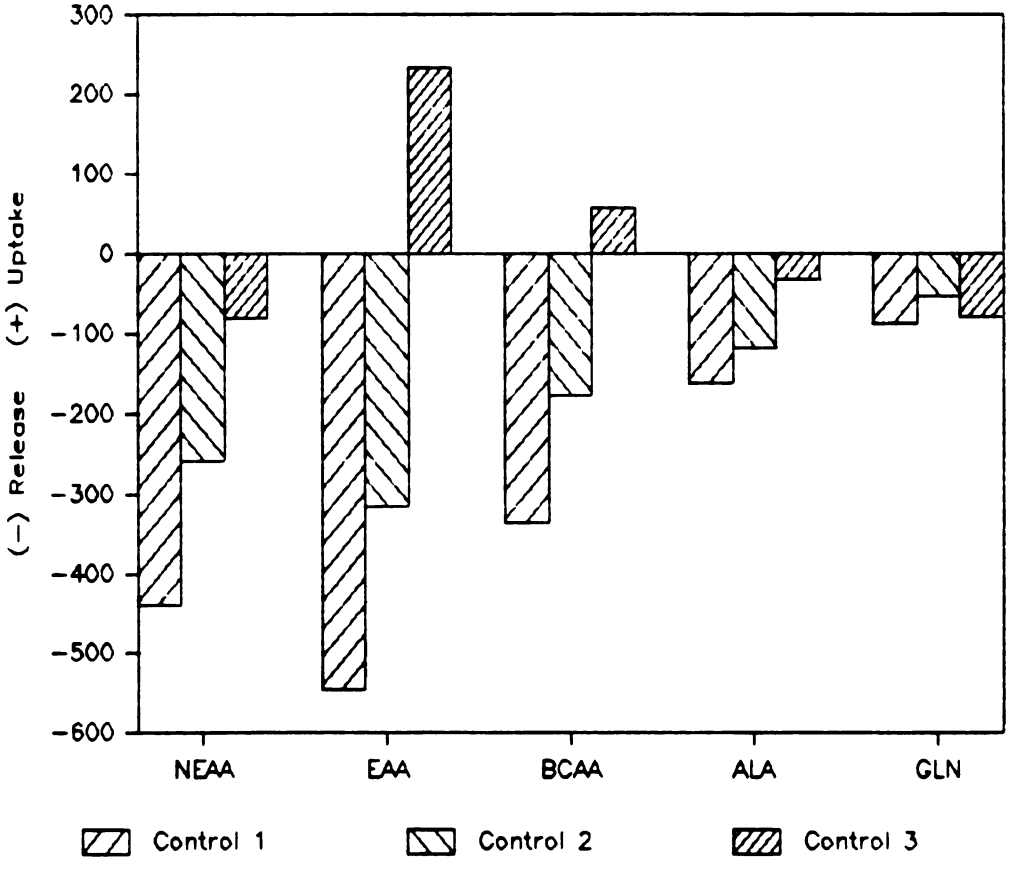


Figure 4. Control Values A–V Difference



Following insulin administration at the 0.5 IU/kg bw level (table 9) threonine net flux changed from uptake during the control period to release in the treated state. All other AA with the exception of glutamate were released from the hindhalf following insulin injection. Arterial insulin levels confirmed the fact that insulin was increased significantly ($P<.01$) following treatment (table 54). The lack of significant changes in AA net flux after administration of insulin (level 1) especially with reference to branched-chain amino acid (BCAA) net flux, is different than reports by Prior and Smith (1983). These workers (Prior and Smith, 1983) observed a decline in plasma BCAA following injection of insulin at 6 IU/kg bw, a level 12 times that reported in table 9. Call et al. (1972) also observed decreased BCAA concentration at high insulin levels (10-12 IU/kg bw).

Insulin at level 2 (1.0 IU/kg bw) produced a number of changes in AA flux (table 10). Alanine, glutamine and leucine changed significantly ($P<.05$) from net release to net uptake, results more like those observed by Prior and Smith (9183) and Call et al. (1972). Several other AA were apparently removed following insulin at level 2 although due to high standard errors, significance was difficult to detect. Many workers (Prior and Smith, 1983; Lindsay, 1982; Bird et al., 1981; Bergman and Heitman, 1978; Ballard et al., 1976 and Jarrett et al., 1976) have reported large standard errors (10-30%) when measuring A-V concentration

differences and net nutrient flux in different animals. Arterial insulin was elevated upon insulin treatment (level 2) by approximately 7 fold (table 54).

Blood flow was significantly ($P<.01$) increased by insulin treatment regardless of level (table 48) in steers from group 1. In the insulin treated state A-V differences alone do not provide a complete metabolic picture as evidenced by the 10-25% increase in blood flow. A small change in A-V difference may be less appreciated physiologically when blood flow data is absent.

Hydrocortisone administration to group 1 produced no significant changes in AA net flux (table 11). With the exception of glutamate, all AA were apparently released in similar amounts under both control and treated conditions. Corticosterone-stimulated proteolysis has been observed in rats (Odedra et al., 1983; Tomas et al., 1978) but similar data from the ruminant is lacking. Any increase in proteolysis is most likely transient (Odedra et al., 1983) and was not observed, as reflected in AA net flux data, here. Garlick et al. (1981) reported diurnal variations in the patterns of protein metabolism in man. If similar patterns occur in the ruminant, the use of a single point sample following treatment may be inappropriate. A greater number of samples over a longer period of time combined with prolonged elevation in the levels of exogenously administered hormone, may provide a more accurate estimation

of the treatment effects on muscle metabolism. Hydrocortisone treatment did not alter blood flow.

Early starvation (24 hr) in group 1 animals produced few changes in AA net flux from control (table 12). Measured release of alanine decreased ($P<.05$) in the 24 hr fasted state. Glutamine, released in the control, was removed following the 24 hr fast ($P<.01$). All other AA net flux data was unchanged. Initially these results appear to be in contradiction with reports of elevated venous AA concentrations (accelerated release from muscle) (Heitman and Bergman. 1980; Bergen, 1979 and Ballard et al., 1976). When blood flow is considered, an explanation is apparent. Fasting for 24 hr produced a near 50% decline in hindhalf blood flow ($P<.01$) in steers from group 1 (table 49). This decreased blood flow rate was maintained and even tended to drop further as length of fast increased. It is for this reason (decreased blood flow) that a net AA flux change was not observed for the largest percent of AA measured. It would be correct to assume that to produce no significant change in AA net flux in the face of decreased blood flow, A-V AA concentration differences must be substantially increased as noted by others (Heitman and Bergman, 1980; Bergen, 1979).

As length of fast increased (48-96 hr) additional net flux differences were observed (tables 13-15). Isoleucine was removed from blood at both 48 and 72 hr of fasting and leucine was removed at 72 hr. A number of other AA including

glycine, cystine and lysine switched from net release to net uptake during the 48 and 72 hr fast. A net release of alanine and glutamine was not observed in the group 1 animals even at the 72 hr fasted observation when all three BCAA were apparently being removed (table 14). This observation may indicate that although these BCAA were removed from blood, they were not degraded in the hindhalf tissue. Chang and Goldberg (1978a,b) reported that alanine and glutamine production was linked to BCAA catabolism as these AA yield alpha amino nitrogen upon catabolism which in turn contributes to the de novo production of alanine and glutamine in skeletal muscle. A increased release of the corresponding branched-chain ketoacids (BCKA) was observed at 24 and 48 hr of fasting only for the keto-analog of valine (ketoisovaleric, KIV) (table 26 and 27). Valine was apparently being released from the hindhalf at these times in the fast. Heitman and Bergman (1980) observed an increased release of BCAA from hindquarter of 72 hr fasted sheep as well as increased release of alanine and glutamine. These results (Heitman and Bergman, 1980) may be explained in part due to the lack of change in hindquarter blood flow during the period of starvation. Ballard et al. (1976) observed increased A-V differences for alanine and glutamine and the BCAA (higher venous concentrations) in sheep, but reported no net flux data.

AA net flux data for group 2 (lean animals) is presented in tables 16-22. Fed control values for all 4

animals in group 2 are similar to those reported by others (Bergen, 1979; Ballard et al., 1976 and Jarrett et al., 1976). There was an approximate net balance of AA across the hindhalf with respect to total AA flux. Insulin injection at level 1 (0.5 IU/kg bw) resulted in no significant changes in AA net flux (table 16) although arterial insulin levels were significantly ($P<.01$) elevated (table 54). Also, hindhalf blood flow did not change with this insulin treatment (table 48). This is a similar observation (for AA net flux) to that of group 1. In contrast to cattle from group 1, level 2 insulin (1.0 IU/kg bw) did not produce any significant changes in AA net flux (table 17). There was a tendency towards removal of a greater number of AA although this was not detected as significant due to large standard errors. This tendency may be accounted for by the increase in blood flow observed at this higher level of insulin (table 48). As mentioned earlier, both Prior and Smith (1983) and Call et al. (1972) observed decreased A-V concentration differences at high insulin levels, 6-10 times higher than the level 2 insulin reported here. The cattle used by Prior and Smith (1983) were similar in age and type to those used in this study in group 2 possibly indicating that an even higher dose of insulin would be required to produce similar results.

Hydrocortisone administration to the lean group of steers (group 2) produced significant ($P<.05$) uptake of glycine, alanine, arginine and cystine (table 18). In

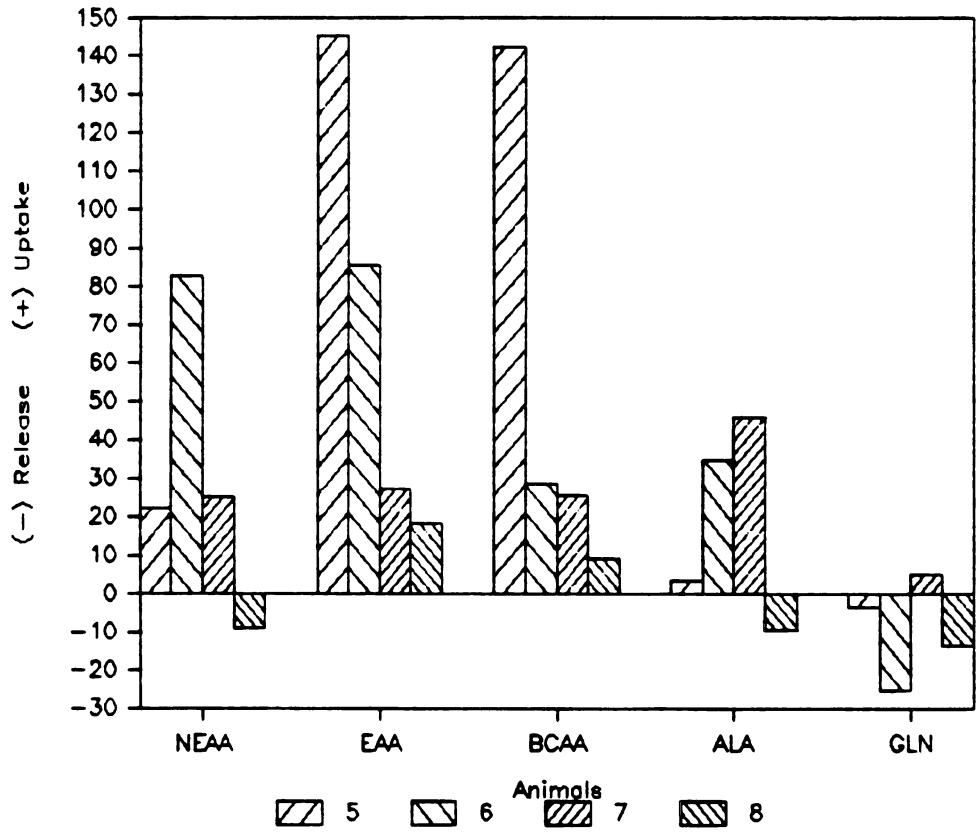
general, all AA with the exception of aspartate, glutamine and lysine, tended to be removed from the blood. This observation was unexpected and difficult to explain if in fact corticosterone is a stimulus for increased proteolysis in ruminants as has been reported in the rat (Millward and Waterlow, 1978). Possibly the 5.0 mg/kg bw level that was administered was not sufficient to produce proteolysis in these animals. The hydrocortisine level used was similar to that used in rats adjusted on the basis of metabolic body weight (Goldberg, 1969). Treatment levels were not titrated for effect. Blood flow did not change following hydrocortisone treatment.

Fasting in group 2 animals resulted in few changes in AA net flux at 24 hr (table 19). A greater number of changes occured as length of fast increased (tables 20-22). As with group 1 animals, hindhalf blood flow decreased significantly ($P<.01$) following the 24 hr fast (50%). Blood flow remained at a reduced level during all subsequent fasted measurements (table 49). It is most likely this large decrease in fasting blood flow that is responsible for the lack of AA net flux change at 24 hr. A concomitant increase in the A-V concentration differences would be expected. Fasting for 24 hr did produce increased removal of serine and glutamine ($P<.05$). Total AA flux did appear less following fasting primarily due to the decreased blood flow. Significant ($P<.05$) removal of glycine, glutamine and glutamate was observed at 48 hr of fast and it was not until 72 hr of fast

that a large number of AA were released from the hindhalf (table 21). Threonine, lysine, methionine and leucine were released in significant amounts ($P<.05$) as were serine and valine ($P<.01$). The increased release of these AA later in fasting indicates that the ruminant requires a longer length of fast to produce similar changes in AA metabolism to those of the non-ruminant (Felig et al., 1969). At 96 hr of fasting, serine threonine and methionine were still released (table 22). Total AA net flux did appear less than control as would be expected in view of the 70% fall in hindhalf blood flow observed at the 96 hr sample. BCAA net flux tended towards release as fasting progressed although high standard errors prevented detection of significance.

When comparing the between group response to treatment and fasting for AA net flux some differences are apparent (tables 35-41). Pooled means for A-V differences for groups of AA in the fed control state for both fat (figure 3) and lean (figure 5) animals do appear different. Even if animal 4 control values are discarded for comparison purposes (discussed earlier) with the lean control values, group 1 animals tend towards release of the essential, non-essential and branched-chain AA as well as alanine and glutamine. In contrast, group 2 animals appeared to be near balance or tended towards uptake relative to net flux of the same AA or groups of AA as outlined for group 1. This observation may be explained by the fact that the group 1 animals were older and more mature from a growth standpoint such that reduced

Figure 5. Control Means A–V Difference



protein and increased fat accretion would be occuring (Bergen, 1974). Group 2 animals were leaner and younger growing animals with expected higher rates of protein accretion responsible for apparent uptake of a number of AA.

AA net flux response to insulin injection at level 1 (0.5 mg/kg bw) was not different between groups (table 35). As well, no differences in response were observed at the higher insulin level (table 36). This is in good agreement with reports of Gregory et al. (1982) which indicated that insulin response was not related to the proportion of dissectible fat in the empty body of cattle. As indicated earlier, results presented in table 54 confirm the fact that high arterial insulin levels were achieved following treatment, eliminating speculation that higher exogenous doses of insulin would be required to produce a significant rise in the circulating levels of this hormone. Group 1 animals tended to favor overall release of AA with insulin at level 1 and tended towards AA net balance with insulin at level 2. Lean animal net AA flux favored balance at the lower insulin level while the higher level tended to result in uptake. There were no statistical differences in between group response, that is, the change in AA net flux following treatment, at either insulin level.

Response to hydrocortisone between groups did not differ (table 37). Group 1 animals tended to have net AA release while group 2 tended towards uptake. Hydrocortisone did not appear to be significantly proteolytic in either

group upon acute administration. Proteolytic response to corticosterone may require prolonged elevation in the levels of this hormone such as was observed in rats by Millward et al. (1983).

The between group response to fasting in terms of AA net flux was different at 24 hr for lysine, leucine and isoleucine and at 48 hr for lysine and isoleucine. Lysine was released both in the fed and fasted state (24 and 48 hr) by group 1 while lysine was removed in the control state and later released in the fasted state by animals in group 2. Isoleucine was added during the control period and removed during fasting (48 hr) by group 1 animals while this BCAA was removed under both control and fasted conditions in the group 2 animals. Fasting for 72 hr produced the greatest number of between group response differences, especially in terms of BCAA net flux (table 40). Net BCAA flux between the fed control and 72 hr fasted state was opposite across the 2 experimental groups. Group 1 showed BCAA release during control and uptake following the 72 hr fast. This was somewhat unexpected in view of reports by Prior and Smith (1983) and Heitman and Bergman (1980) of release of BCAA from hindquarters of steers and sheep (fed and fasted) respectively. However, a small net uptake of BCAA was reported in the hindleg of sheep (Ballard et al., 1976) and cattle (Bell et al., 1975). In the case of group 1, at the 72 hr fast when BCAA were apparently removed, a significant ($P<.05$) decrease in alanine and glutamine output was

observed. The decrease in magnitude of flux could be accounted for soley by the drop in hindhalf blood flow (table 49) during fasting and this observation may support the premise of limited BCAA metabolism in ruminant peripheral tissue. Animals from group 2 removed the BCAA in the fed control state and then released these AA during the fast (72 hr). This is in agreement with reports of limited catabolism of the BCAA in peripheral tissues of the hindlimb of ruminants (Heitman and Bergman, 1980; Lindsay et al., 1977; Ballard et al., 1976; Bell et al., 1975).

Group response to fasting in terms of leucine net flux was different also at 96 hr of fasting. Group 1 animals which removed leucine at 72 hr, began to release this AA following the 96 hr fast. This tended to be the case for isoleucine and valine as well. Group 2 animals continued to release the BCAA as observed at 72 hr. In general terms, it was not until 96 hr of fasting that group 1 animals apparently released the majority of AA studied while in contrast, group 2 animals required only 72 hr of fasting to produce similar observations.

Branched-Chain Ketoacid Metabolism

Reports of branched-chain ketoacid (BCKA) metabolism in ruminant muscle in vivo is limited. Pell et al. (1983) studied ketoisocaproate (KIC) metabolism by tissues of fed and starved sheep and Early et al. (1984) studied BCKA exchange across the hindlimb of fed and fasted steers. Data

is reported here for BCKA metabolism across the hindhalf of both fat (group 1) and lean (group 2) steers under a variety of treated and fasted states.

Animals in group 1 exibited net release in the fed control state for all three BCKA. As stated earlier, the corresponding BCAA were released in the fed control state as well. Insulin treatment (0.5 IU/kg bw) produced significant ($P<.05$) removal of ketoisovalerate (KIV) with no change in ketomethylvalerate (KMV) or ketoisocaproate (KIC) (table 23). Valine appeared to be released from the hindhalf upon insulin injection (level 1, table 9). Subsequent transamination of this BCAA by the liver to its corresponding BCKA (KIV) may account for the increased removal of KIV reported in table 23 if in fact, KIV released from the liver did occur. Similar speculation for the subsequent insulin or hydrocortisone treatments is not possible due to large standard errors in the AA net flux data. Bergman (1985) reported that in ruminant liver, most BCKA seemed to be oxidized to carbon dioxide and ketone bodies with some being reaminated but did not report intact BCKA release from this organ.

Higher insulin (1.0 IU/kg bw) failed to produce any changes in BCKA net flux (table 24). Hydrocortisone injection resulted in net uptake of KIV ($P<.05$) and increased release of KMV ($P<.01$) from the hindhalf of group 1 animals. These variations are difficult to interpret in light of the lack of similar studies in ruminants.

Early fasting (24 hr) in group 1 produced increased ($P<.05$) release of KIV but decreased release ($P<.01$) of KIC. At 48 hr, KIV was significantly ($P<.05$) added while KMV and KIC were removed ($P<.01$). No differences were detected in BCKA net flux at 96 hr of fast in group 1 animals. Large standard errors in AA net flux and the apparent release of the BCAA at 24 and 48 hr of fasting make explanation of the observed BCKA changes difficult. Pell et al. (1983) observed increased arterial KIC following fasting in sheep. Total peripheral tissue release of KIC was noted. Early et al. (1984) observed decreased net exchange of all three BCKA between plasma and the hindlimb of fasted steers. A 50% decrease in plasma flow through the hindlimb was also observed (Early et al., 1984) and may account, in terms of magnitude, for the BCKA response.

Insulin treatment at either level did not change BCKA net flux in steers from group 2 (tables 29 and 30). KIV and KIC release was noted at both insulin treatments while KMV was added (insulin level 1) and removed (insulin level 2). Hydrocortisone caused a shift from net KIV release to net uptake of this BCKA and KMV was removed in both the control and treated states (table 31). Removal of KMV did appear to increase following hydrocortisone treatment although this apparent increase was not statistically significant. The corresponding BCAA were removed during hydrocortisone treatment (table 18) and this observation coupled with the

observed release of KIV and KMV may support the view that BCAA catabolism in ruminant peripheral tissue is limited.

Early fasting (24 hr) produced no change in BCKA net flux in animals from group 2. The only observed changes in BCKA net flux during fasting in this group of animals occured following the 48 hr fast. At this time (48 hr), KIV was significantly ($P<.01$) removed and KMV was released ($P<.05$). BCAA net flux at 48 hr in the group 2 animals (table 20) does not appear to lend explanation to the observed changes in BCKA metabolism.

Across-group response in terms of BCKA net flux is presented in tables 42-47. Fat and lean animals responded in a similar fashion to both insulin and hydrocortisone injection. Fasting for 24 and 48 hr did produce some between group differences (tables 45 and 46). KIV was added to the blood by both groups in the fed control and 24 hr fasted states although the amount added was greater during fasting compared to control for group 1 animals and less (same comparison) for group 2. KIV release by group 1 was 23 times greater at 24 hr of fasting compared to the control ($P<.01$). Busboom et al. (1983) reported relatively high activity of the leucine aminotransferase (LAT) enzyme in sheep adipose tissue and also that this enzyme decreased during fasting in skeletal muscle. In group 1, animals with a high degree of adipose accumulation, the reports of Busboom et al. (1983) may help explain the increased KIV and KMV release from the hindhalf of these steers if in fact, the BCKA

aminotransferase enzymes responsible for metabolism of isoleucine and valine act in a similar manner to that observed for LAT. The lack of change in KIC metabolism in fed versus fasted conditions is difficult to explain. Early et al. (1984) demonstrated a pattern of BCKA release from the hindlimb of steers apparently similar in type to those from group 2 reported here.

A 48 hr fast produced a different response for KIV net flux. KIV was released by group 1 at both the control and 48 hr fasted periods. Group 2 released KIV during the control period but removed this BCKA following the 48 hr fast. Once again, large standard errors in the BCAA net flux data discussed earlier, make comparisons between BCAA and BCKA metabolism difficult.

The significantly ($P<.01$) decreased blood flows observed during fasting (table 49) further confound the interpretation of the BCKA net flux data. The approximate 50 percent drop in blood flow following the 24 hr fast may be sufficient to account for changes in net BCKA flux at this time (table 45). At 48 hr of fast, blood flow alone could no longer account for the changes in net BCKA flux.

Wijayasinghe et al. (1983) stated that due to an absence of measurements of output or uptake of the BCKA across the ruminamt hindlimb, suggestion of limited BCAA catabolism in ruminant muscle should be considered tenuous. The data reported here, although sometimes subject to high standard errors, seem to support the premise of limited BCAA

catabolism in ruminant skeletal muscle. An exception to this conclusion may be noted on two occasions of apparent BCAA uptake, (group 1, 72 hr fast; group 2, hydrocortisone treatment). Concomitant release of the BCKA was not noted at these times and this observation may suggest BCAA degradation with further catbolism of the BCKA produced within the skeletal muscle.

Arterial Glucose Concentration

Arterial glucose levels were observed for the control, treated and fasted states and are presented in tables 51-53. As expected, insulin treatment significantly ($P<.01$) decreased plasma glucose levels. Between group response of plasma glucose was similar at both insulin levels. Hydrocortisone increased ($P<.01$) plasma glucose levels in group 1 but not group 2 animals. Fasting decreased plasma glucose in both groups ($P<.01$) although to a greater extent in the lean group by 96 hr of fast. Group 1 animals appeared to be able to buffer the fall in plasma glucose as length of fast increased.

N-t-Methylhistidine Excretion and NMH/Creatinine Ratios

The use of N-t-methylhistidine (NMH) excretion as an indicator of muscle protein turnover in cattle has been documented (McCarthy et al., 1983; Harris and Miline, 1981).

NHM excretion during the control, treated and fasted states for both groups of animals is reported in tables 57-59. Insulin (level 1 or 2) or hydrocortisone treatment did not change daily NMH excretion in either group of cattle. NMH excretion was increased ($P<.05$) following 24 and 48 hr of fasting compared to the control for group 1 and at 48 hr of fasting ($P<.01$) for group 2. Also, NMH excretion was significantly ($P<.05$) decreased at 96 hr of fast in group 2 compared to control (table 58). Acceleration in muscle protein degradation following fasting would explain the observed increase in NMH excretion although the the AA net flux data discussed earlier does not bear this out.

Total NMH excretion in terms of mMoles/day measured in the venous plasma leaving the hindhalf of the steers in this study can be compared with data of McCarthy et al. (1983). These workers (McCarthy et al., 1983) used urinary NMH excretion as a measure of muscle protein turnover in cattle of differing genetic backgrounds. To make a valid comparison of muscle protein turnover in hindhalf skeletal muscle compared to total body skeletal muscle, an estimate of percent of total body skeletal muscle present in the hindhalf of steers is essential. Allen (1966) reported results from physical separation of wholesale cuts from steer carcasses. Allen (1966) used small and large framed cattle with carcass weights and fat cover varying from 230 to 340 kg and .60 to 3.20 cm respectively. Extrapolation of this data (Allen, 1966) to the animals used in the study

reported here resulted in an estimate of 47.88 percent of total body skeletal muscle present in the hindhalf of steers. Using equations outlined by McCarthy (1981), NMH excretion as a percent of the total muscle NMH pool results in an estimate of 2.99% of total NMH pool excreted per day for group 1 and 3.05% for group 2 animals. This is in good agreement with reports by McCarthy et al. (1983) of 3.95% and 3.74% of total pool NMH excreted per day in similar type cattle. This calculation is based on 47.88% of the actual values reported by McCarthy et al. (1983) to equate NMH excretion to the hindhalf muscle. Values from McCarthy et al. (1983) appear approximately 20% higher than those reported here and may be accounted for by non-skeletal muscle sources of NMH (see literature review).

NMH/creatinine ratios are reported in tables 59 and 60. This ratio was unaffected by either treatment of fasting regardless of group of animals. NMH/creatinine ratios did tend to be higher in group 2 animals indicating greater protein turnover and are in relative agreement with ratios of .235 to .297 reported by McCarthy et al. (1983).

CONCLUSIONS

1. Blood flow was significantly changed by treatment and fasting. As a result, net nutrient flux is a more precise physiological parameter when compared to arterio-venous concentration differences.

2. The effect of insulin on amino acid and branched-chain ketoacid metabolism in the ruminant may only be apparent when pharmacological doses of this hormone are administered.

3. Hydrocortisone did not appear to stimulate proteolysis in the hindhalf of the steer at the level administered here. Higher levels maintained over a longer period of time may be required to produce an effect.

4. Branched-chain amino acid catabolism appeared limited in the hindhalf of the group 2 steers.

5. Animals with a greater degree of body fat accumulation tended to release more branched-chain ketoacids from the hindhalf than lean counterparts suggesting enhanced branched-chain amino acid catabolism in the fatter animals.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adibi, S.A. 1976. Metabolism of branched-chain amino acids in altered nutrition. Metab. Clin. Exp. 25:1287.

Allen. D. 1966. The relationship of some linear and physical measurements to beef carcass composition. Ph.D. Dissertation. Michigan State University. E. Lansing, MI. 48824.

Asatoor, A.M. and M.D. Armstrong. 1967. 3-methylhistidine, a componant of actin. Biochem. Biophys. Res. Com. 26:168.

Atwell, J.R., M.P. Hedden, V.J. Mancusi and M.G. Buse. 1977. Branched-chain amino acids as regulators of protein synthesis. Diabetes. 26(suppl. 1):81 (abstr.).

Ballard, F.J., O.H. Filsell and I.G. Jarrett. 1976. Amino acid uptake and output by the sheep hind limb. Metabol. 25:415.

Baxter, J.D. 1978. Mechanisms of glucocorticoid action. Kidney Int. 14:330.

Bell, A.W., J.W. Gardner, W. Manson and G.E. Thompson. 1975. Acute cold and the metabolism of blood glucose, lactate and pyruvate, and plasma amino acids in the hind leg of fed and fasted young ox. Br. J. Nutr. 33:207.

Bell, A.W., G.E. Thompson and J.D. Findlay. 1974. The contribution of the shivering hind leg to the metabolic response to cold of the young ox. Pflugers Arch. 346:341.

Bergen, W.G. 1979. Free amino acids in blood of ruminants - physiological and nutritional regulation. J. Anim. Sci. 49:1577.

Bergen, W.G. 1975. Nutritional regulation of macromolecular synthesis in muscle. Proc. Recip. Meat Conf. 28:47.

Bergen, W.G. 1974. Protein synthesis in animal models. J. Anim. Sci. 38:1079.

Bergen, W.G., H.A. Henneman and W.T. Magee. 1973. Effect of dietary protein level and protein source on plasma and tissue free amino acids in growing sheep. J. Nutr. 103:575.

Bergman, E.N. 1985. Personal communication.

Bergman, E.N. 1985. Total splanchnic and peripheral uptake of amino acids in relation to the gut. Fed. Proc. Rum. Nutr. Symp. In Press.

Bergman, E.N. and R.N. Heitman. 1978. Metabolism of amino acids by the gut, liver, kidneys and peripheral tissues. Fed. Proc. 37:1228.

Bergman, E.N. and J.M. Pell. 1983. Metabolism in tissue of fed and starved sheep. Fed. Proc. 41:435 (abstr.).

Bergman, E.N. and J.M. Pell. 1982. Leucine metabolism in tissues of fed and starved sheep. Fed. Proc. 41:343 (abstr.).

Bird, A.R., K.D. Chandler and A.W. Bell. 1981. Effect of excercise and plane of nutrition on nutrient utilization by the hindlimb of sheep. Aust. J. Biol. Sci. 34:541.

Bower, R.H., K.A. Kern and J.E. Fischer. 1985. Use of branched-chain amino acid enriched solution in patients under metabolic stress. Am. J. Surg. 149:266.

Brockman, R.P. 1978. Effect of somatostatin on plasma glucagon and insulin and glucose turnover in excercising sheep. J. Appl. Physiol. 47:273.

Brockman, R.P. and E.N. Bergman. 1975. Effect of glucagon on plasma alanine metabolism and hepatic gluconeogenesis in sheep. Am. J. Physiol. 228:1627.

Brockman, R.P., E.N. Bergman, P.K. Joo and J.G. Manns. 1975. Effects of glucagon and insulin on net hepatic metabolism of glucose precursors in sheep. Am. J. Physiol. 229:1344.

Brown, B.W., V.H. Oddy and A.W. Jones. 1982. Measurement of organ blood flow using tritiated water. 2. Uterine blood flow in conscious pregnant ewes. Aust. J. Biol. Sci. 35:25.

Bruck, S.D. 1974. "Blood compatible synthetic polymers: An introduction." Thomas Co. Springfield, IL.

Busboom, J.R. 1984. Effects of age and nutritional state on branched chain amino acid degradation in sheep. Doctoral Dissertation. Michigan State University. E. Lansing, MI.

Busboom, J.R., A. Merkel and W.G. Bergen. 1983. The effect of dietary protein content and fasting on tissue leucine transaminase and alpha-ketoisocaproate dihydrogenase in wethers. J. Anim. Sci. 57(suppl. 1):189 (abstr.).

Buse, M.G., R. Atwell and V. Mancusi. 1979. In vitro effect of branched-chain amino acids on the ribosomal cycle in muscles of fasted rats. Horm. Metab. Res. 11:289.

Buse, M.G. and S.S. Reid. 1975. Leucine: a possible regulator of protein turnover in muscle. J. Clin. Invest. 56:1250.

Buse, M.G. and D.A. Weingard. 1977. Studies concerning the specificity of the effect of leucine on the turnover of proteins in muscles of control and diabetic rats. Biochem. Biophys. Acta. 475:81.

Buttery, P.J. 1979. Metabolism of ruminant muscle. In: Protein Metabolism in the Ruminant. (P.J. Buttery. ed.) P. 9.1. ARC, London.

Cahill, G.F., T.T. Aoki and E.B. Marliss. 1972. Insulin and muscle protein. Hndbk. Physiol. Endocrinol. 1:563.

Call, J.L., G.E. Mitchell, D.G. Ely, C.O. Little and R.E. Tucker. 1972. Amino acid, volatile fatty acids and glucose in plasma of insulin treated sheep. J. Anim. Sci. 34:767.

Chang, T.W. and A.L. Goldberg. 1978a. The origin of alanine produced in skeletal muscle. J. Biol. Chem. 253:3677.

Chang, T.W. and A.L. Goldberg. 1978b. The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. J. Biol. Chem. 253:3685.

Chua, B., D.L. Siehl and H.E. Morgan. 1979. Effect of leucine and metabolites of branched-chain amino acids on protein turnover in heart. J. Biol. Chem. 254:8358.

Clarkson, J.F., A.H. Smith and T.G. Richards. 1976. Measurement of liver blood flow by means of a single injection of bromosulphthalein, without hepatic venous catheterization. Clin. Sci. Mol. Med. 51:141.

Denison, A.B., M.P. Spencer and H.D. Green. 1965. A square wave electromagnetic flowmeter for application to intact blood vessels. Circ. Res. 3:39.

Doolittle, R.F. 1984. Fibrinogen and fibrin. Ann. Rev. Biochem. 53:195.

Early, R.J., J.R. Thompson, R.J. Christopherson and G.W. Sedgwick. 1984. Branched-chain alpha-ketoacid exchange across the portal-drained viscera and hind limb of fed and fasted steers. Can. J. Anim. Sci. 648(suppl.):276.

Elia, M. and G. Livesey. 1981. Branched-chain amino acid and oxo acid metabolism in human and rat muscle. In: Metabolism and Clinical Implications of Branched-Chain Amino and Keto Acids. (Walser, M. and J.R. Williamson. eds.) p. 257.

Fehlman, M., A. LeCam and P. Friychet. 1979. Insulin and glucagon stimulation of amino acid transport in isolated rat hepatocytes. Endocrinol. 109:253.

Felig, P. 1975. Amino acid metabolism in man. Ann. Rev. Biochem. 44:933.

Felig, P., O.E. Owen, J. Wahren and G.F. Cahill. 1969. Amino acid metabolism during prolonged starvation. J. Clin. Invest. 48:584.

Felig, P., T. Pozefsky, E.B. Marliss and G.F. Cahill. 1970. Alanine: key role in gluconeogenisis. Science. 167:1003.

Fick, A. 1870. Uber die messung des blut quantums in den herzventrikelen sitz. Physik-Med. Wurzburg:16.

Folkow, B. 1953. A critical study of some methods used in investigations on the blood circulation. Acta. Physiol. Scand. 27:118.

Forsyth, R.P. 1970. Hypothalmic control of the distribution of cardiac output in the unanesthetized rhesus monkey. Circ. Res. 26:783.

Fox, I.J., L.Q.S. Brooker, D.W. Heseltine, D.W. Essex and E.H. Wood. 1957. A tricarbocyanine dye for continuous recording of dilution curves in whole blood dependent on variations in blood oxygen saturation. Proc. Staff. Meet. Mayo Clin. 32:478.

Freund, H.R., H.C. Hoover, S. Atamain and J.E. Fischer. 1979. Infusion of the branched-chain amino acids in postoperative patients. Ann. Surg. 109:18.

Freund, H.R., J.H. James and J.E. Fischer. 1981. Nitrogen sparing mechanisms of singly administered branched-chain amino acids in the injured rat. Surgery 90:237.

Freund, H.R., N. Yoshimura, L. Lunetta and J.E. Fischer. 1978. The role of branched-chain amino acids in decreasing muscle catabolism in vivo. Surgery 83:611.

Frick, G.P. and H.M. Goodman. 1980. Insulin regulation of branched-chain alph-keto acid dehydrogenase in adipose tissue. J. Biol. Chem. 355:6186.

Fulks, R.M., J.B. Li and A.L. Goldberg. 1975. Effect of insulin, glucose and amino acids on protein turnover in rat diaphram. J. Biol. Chem. 250:290.

Galim, E.B., K. Hurska, D.M. Bier, D.E. Mathews and M.W. Haymond. 1980. Branched-chain amino acid nitrogen transfer to alanine in vivo in dogs. J. Clin. Invest. 66:1295.

Garber, A.J., I.E. Karl and D.M. Kipnis. 1976a. Alanine and glutamine synthesis and release from skeletal muscle. 1. J. Biol. Chem. 251:826.

Garber, A.J., I.E. Karl and D.M. Kipnis. 1976b. Alanine and glutamine release from skeletal muscle. 2. J. Biol. Chem. 251:836.

Garlick, P.J., G.A. Clugston, J.C. Waterlow and R.W. Swick. 1981. Diurnal pattern of protein and energy metabolism in man: a defense. Am. J. Clin. Nutr. 34:1626.

Garlick, P.J., D.J. Millward and W.T. James. 1973. The diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats. Biochem. J. 136:935.

Gill, J.L. 1978. Design and analysis of experiments in the animal and medical sciences. Vol. 1, 2, 3. Iowa State Univ. Press. Ames, Iowa.

Goldberg, A.L. 1969. Protein turnover in skeletal muscle. 2. Effects of denervation and cortisone on protein catabolism in skeletal muscle. J. Biol. Chem. 244: 3223.

Goldberg, A.L. and R. Odessey. 1972. Oxidation of amino acids by diaphrams from fed and fasted rats. Am. J. Physiol. 223:1384.

Goldberg, A.L. and M.E. Tischler. 1981. Regulatory effects of leucine on carbohydrate and protein metabolism. In: Metabolism and Clinical Implications of Branched-Chain Amino and Keto Acids. (Walser, M. and J.R. Williamson eds.). p.73. Elsevier, New York.

Goldberg, A.L., M. Tischler, G. Demartino and G. Griffin. 1980. Hormonal regulation of protein degradation and synthesis in skeletal muscle. Fed. Proc. 39:31.

Goldstein, S. and W.R. Reddy. 1970. Insulin and protein synthesis in muscle. Arch. Biochem. Biophys. 140:181.

Goldstein, S. and W.J. Reddy. 1967. Effect of insulin on the incorporation of [^{14}C]-leucine into rat caudofemoralis protein. Biochem. Biophys. Acta. 141:310.

Goodman, H.M. 1964. Stimulatory action of insulin on leucine uptake and metabolism in adipose tissue. Am. J. Physiol. 206:129.

Haggmark, S., B. Biber, J.G. Sjondin, O. Winson, B. Gustavsson and S. Reiz. 1982. The continuous thermodilation method for measuring high blood flows. Scand. J. Clin. Lab. Invest. 42:315.

Hales, J.R.S. 1974. Radioactive microsphere techniques for studies of the circulation. Clin. Exper. Pharm. Physiol. Suppl.1:31.

Hamilton, W.F. and J. W. Remington. 1949. Comparison of the time concentration curves in arterial blood of diffusible and non-diffusible substances when injected at a constant rate and when injected instantaneously. Am. J. Physiol. 148:35.

Harmon, C.S., C.G. Proud and V.M. Pain. 1984. Effects of starvation, diabetes and acute insulin treatment on the regulation of polypeptide-chain initiation in rat skeletal muscle. Biochem. J. 223:687.

Harris, C.I. and G. Miline. 1981a. The urinary excretion of N-t-methylhistidine by cattle: Validation as an index of muscle protein breakdown. Br. J. Nutr. 45:411.

Harris, C.I. and G. Miline. 1981b. The inadequacy of urinary N-t-methylhistidine excretion in the pig as a measure of muscle protein breakdown. Brit. J. Nutr. 45:423.

Harris, R.A. and R. Paxton. 1985. Regulation of branched-chain alpha ketoacid dehydrogenase by phosphorylation-dephosphorylation. Fed. Proc. 44:305.

Harris, R.A., R. Paxton and P. Jenkins. 1985. Nutritional control of branched-chain alpha ketoacid dehydrogenase in rat hepatocytes. Fed. Proc. 44:2463.

Harper, A.E., R.H. Miller and K.P. Block. 1984. Branched-chain amino acid metabolism. Ann. Rev. Nutr. 4:409.

Hay, A.M. and J.C. Waterlow. 1967. The effects of alloxan diabetes on muscle and liver protein synthesis in the rat measured by constant infusin of [^{14}C]-lysine. J. Physiol. 191:111.

Haymond, M.W. and J.M. Miles. 1982. Branched-chain amino acids as a major source of alanine nitrogen in man. Diabetes. 31:86.

Hecker, J.F., G.C. Fisk, J.M. Gupta, N. Abrahams, R.A. Cockington and B.R. Lewis. 1979. Thrombus formation on catheters in newborn lambs. Anaesth. Intens. Care. 7:239.

Hecker, J.F. and L.A. Scandrett. 1985. Roughness and thrombogenicity on the outer surface of intravascular catheters. J. Biomed. Mater. Res. 19:381.

Hedden, M.P. and M.G. Buse. 1979. General stimulation of muscle protein synthesis by branched-chain amino acids in vitro. Proc. Soc. Exp. Biol. Med. 160:410.

Heinegard, D. and G. Tiderstrom. 1973. Determination of serum creatinine by a direct colorimetric method. Clin. Chem. Acta. 43:305.

Heitman, R.N. and E.N. Bergman. 1980. Integration of amino acid metabolism in sheep: effects of fasting and acidosis. Am. J. Physiol. 239:E248.

Hems, D.A. 1972. Metabolism of glutamine and glutamic acid by isolated perfused kidneys of normal and acidotic rats. Biochem. J. 130:671.

Hoey, W.A. and P.S. Hopkins. 1983. Chronic arterial cannulation for studying the skin of sheep. Res. in Vet. Sci. 35:247.

Hogan, J.P. 1975. Influence of protein digestion on plasma amino acid levels in sheep. J. Dairy Sci. 58:1164.

Horowitz, S.B. and T.W. Pearson. 1981. Intracellular monosallaride and amino acid concentrations and activities and the mechanism of insulin action. Mol. Cell. Biol. 1:769.

Huntington, G. 1985. Personal Communication.

Hutson, S.M. and A.E. Harper. 1981. Blood and tissue branched-chain amino acid and alpha-ketoacid concentrations: effect of diet, starvation and disease. Am. J. Clin. Nutr. 34:173.

Hutson, S.M., C. Zapalowski, T.C. Cree and A.E. Harper. 1980. Regulation of leucine and alph-ketoisocaproic acid metabolism in skeletal muscle. Efffect of starvation and insulin. J. Bio. Chem. 25:2418

Ichihara, A. and E. Koyama. 1966. Transaminase of branched-chain amino acids. J. Biochem. 59:160.

Jarrett, I.G., O.H. Filsell and F.J. Ballard. 1976. Utilization of oxidizable substrates by the sheep hindlimb: Effects of starvation and excercise. Metabolism 34:523.

Jorfeldt, L., and J. Wahren. 1971. Leg blood flow during exercise in man. Clin. Sci. 41:459.

Kalsner, S. 1984. Pharmacology of human blood vessels. Fed. Proc. 44:316.

Kanter, Y. 1976. The role of insulin in regulating protein catabolism in mammalian cells. Int. J. Biochem. 7:253.

Katz, M.L. and E.N. Bergman. 1969. A method for simultaneous cannulation of the major splanchnic blood vessels of sheep. Am. J. Vet. Res. 30:655.

Kaufman, C.F. and E.N. Bergman. 1971. Cannulation of renal veins of sheep for long-term functional and metabolic studies. Am. J. Vet. Res. 32:1103.

Kety, S.S. and C.F. Schmidt. 1945. The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. Am. J. Physiol. 143:53.

Khatra, B.S., R.K. Chawla, C.W. Sewell and D. Rudman. 1977. Distribution of branched-chain alpha-keto acid. J. Clin. Invest. 59:558.

Kostyo, J.L. and A.F. Redmond. 1966. Role of protein synthesis in the inhibitory action of adrenal steroid hormones on amino acid transport by muscle. Endocrin. 79:531.

Li, J.B. and L.S. Jefferson. 1978. Influence of amino acid availablility on protein turnover in perfused skeletal muscle. Biochem. Biophys. Acta. 544:351.

Lindsay, D.B. 1982. Relationships between amino acid catabolism and protein anabolism in the ruminant. Fed. Proc. 41:2550.

Lindsay, D.B. 1980. Amino acids as energy sources. Proc. Nutr. Soc. 39:53.

Lindsay, D.B., J.W. Steel and P.J. Buttery. 1977. The net exchange of amino acids from muscle of fed and starved sheep. Proc. Nutr. Soc. 36:33A.

Loeb, J.N. 1976. Corticosteroids and growth. New Engl. J. Med. 295:547.

Manchester, K.L. 1970. Sites of hormonal regulation of protein metabolism. In: Mammalian Protein Metabolism, Vol. 4. (Munro, H.N., ed.) Academic Press. New York. p. 229.

Marliss, E.B., T.T. Akoi, T. Pozefsky, A.S. Most and G.F. Cahill. 1971. Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. J. Clin. Invest. 50:814.

Mathews, D.E., D.W. Bier, M.J. Rennie, R.H. Edwards, D. Halliday, D.J. Millward and G.A. Clugston. 1981. Regulation of leucine metabolism in man: A stable isotope study. Science 214:1129.

McCarthy, F.D. 1981. Measurement of composition of growth and muscle protein degradation in cattle. Ph.D. Dissertation. Michigan State University. E. Lansing, MI 48824.

McCarthy, F.D., W.G. Bergen and D.R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N^t-methylhistidine excretion. J. Nutr. 113:2455.

McMenamy, R.H., W.C. Shoemaker, J.E. Richmond and D. Elwyn. 1962. Uptake and metabolism of amino acids by the dog liver in situ. Am. J. Physiol. 202:407.

McMenamy, R.H., J. Vang and T. Drapanus. 1965. Amino acid and alpha-keto acid concentrations in blood and plasma of liverless dogs. Am. J. Physiol. 209:1046.

Micromedics Co. Horsham, PA 19044.

Millward, D.J. and D.C. Bates. 1983. 3-methylhistidine turnover in the whole body, and the contribution of skeletal muscle and intestine to urinary 3-methylhistidine excretion in the adult rat. Biochem. J. 214:607.

Millward, D.J. and P.J. Garlick. 1972. The pattern of protein turnover in the whole animal and the effect of dietary variations. Proc. Nutr. Soc. 31:257.

Millward, D.J., P.J. Garlick, R.J.C. Stewart, D.O. Nnanyelugo and J.C. Waterlow. 1975. Skeletal muscle growth and protein turnover. Biochem. J. 150:235.

Millward, D.J., B.R. Odedra and P.C. Bates. 1983. Role of insulin, corticosterone and other factors in the recovery of muscle protein synthesis on refeeding fasted rats. Biochem. J. 216:583.

Millward, D.J. and J.C. Waterlow. 1978. Effect of nurtition on protein turnover in skeletal muscle. Fed. Proc. 37:2283.

Mitch, W.E., M. Walser and D.G. Sapir. 1981. Nitrogen sparing induced by leucine compared with that induced by its keto analoque alph-ketoisocaproate, in fasting obese man. J. Clin. Invest. 67:553.

Mortimore, G.E. and C.E. Mondon. 1970. Inhibition by insulin of valine turnover in liver. J. Biol. Chem. 245:2375.

Munck, A. 1971. Glucocorticoid inhibition of glucose uptake by peripheral tissues: old and new evidence, molecular mechanisms and physiological significance. Perspect. Biol. Med. 14:265.

Munro, H.N. 1964. In Mammalian Protein Metabolism. (H.N. Munro and J.B. Allison eds.) Academic Press. New York.

Nishizawa, N., T. Noguchi, S. Hareyama and R. Funabiki. 1977. Fractional flux rates of N-t-methylhistidine in skin and gastrointestine: the contribution of these tissues to urinary output of N-t-methylhistidine in the rat. Brit. J. Nutr. 39:149.

Nishizawa, N.T., M. Shimbo, S. Hareyama and R. Funabiki. 1977. Fractional catabolic rates of myosin and actin estimated by urinary excretion of N-t-methylhistidine: the effect of dietary protein level on catabolic rates under conditions of restricted food intake. Brit. J. Nutr. 37:345.

Nishizawa, N., Y. Toyoda, T. Noguchi, S. Hareyama, H. Itabushi and R. Funabiki. 1979. N-t-methylhistidine content of organs and tissues of cattle in an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N-t-methylhistidine. Brit. J. Nutr. 42:247.

Nissen, S. and J.R. Haymond. 1981. Effects of fasting on flux and interconversion of leucine and alpha-ketoisocaproate in vivo. Am. J. Physiol. 241:E72.

Nissen, S.L., C. VanHuysen and M.W. Haymond. 1982. Measurement of branched-chain amino acids and branched-chain alpha ketoacids in plasma by high performance liquid chromatography. J. Chromat. 232:170.

Oddessey, R. and A.L. Goldberg. 1972. Oxidation of leucine by rat skeletal muscle. Am. J. Physiol. 223:1376.

Oddessey, R., E.A. Khairallah and A.L. Goldberg. 1974. Origin and possible significance of alanine production by skeletal muscle. J. Biol. Chem. 249:7623.

Oddy, V.H., B.W. Brown and A.W. Jones. 1981. Measurement of organ blood flow using tritiated water. 1. Hind limb muscle blood flow in conscious ewes. Aust. J. Biol. Sci. 34:419.

Odedra, B.R., P.C. Bates and D.J. Millward. 1983. Time course of the effect of corticosterone on protein turnover in rat skeletal muscle and liver. Biochem. J. 214:617.

Parrilla, R. and M.N. Goodman. 1974. Nitrogen metabolism in the isolated perfused rat liver. Biochem. J. 138:341.

Pell, J.M., E.M. Caldarone and E.M. Bergman. 1983. Alpha-keotisocaproate metabolism by tissues of fed and starved sheep. Fed. Proc. 42:815.

Pozefsky, T., P. Felig, J.D. Tobin, S. Soeldner and G.F. Cahill. 1969. Amino acid balance across the forearm in postabsorptive man. Effects of insulin at two dose levels. J. Clin. Invest. 48:2273.

Prior, R.L. and S.B. Smith. 1983. Role of insulin in regulating amino acid metabolism in normal and alloxan-diabetic cattle. J. Nutr. 113:1016.

Reimers, T.J., R.G. Cowan, J.P. McCann and M.W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine and equine insulin. Am. J. Vet. Res. 43:1274.

Roe, W.E., E.N. Bergman and K. Kon. 1960. Absorption of ketone bodies and other metabolites via the portal blood of sheep. Am. J. Vet. Res. 27:729.

Rosenthal, J., A. Angel and J. Farkas. 1974. Metabolic fate of leucine: a significant sterol precursor in adipose tissue and muscle. Am. J. Physiol. 226:411.

Ruderman, N.B. and M. Berger. 1974. The formation of glutamine and alanine in skeletal muscle. J. Biol. Chem. 249:5500.

Russell, S.B., J.D. Russell and J.S. Trupin. 1982. Alteration of amino acid transport by hydrocortisone. J. Biol. Chem. 257:9525.

Schauder, P., K. Schroder, D. Matthaei, H.V. Honning and U. Langenbeck. 1983. Influence of insulin on blood levels of branched-chain keto and amino acids in man. Metab. 32:323.

Sender, P.M. and P.J. Garlick. 1973. Synthesis rates of protein in the Langendorf-perfused heart in the presence and absence of insulin. Biochem. J. 132:603.

Sherwin, R.S. 1978. Effect of starvation on the turnover and metabolic response to leucine. J. Clin. Invest. 61:1471.

Sigma Chemical Co. St. Louis, MO 63178.

Snipes, C.A. 1967. Hormonal effects on accumulation of naturally occuring amino acids in vivo and in vitro. Am. J. Physiol. 212:279.

Stewart, G.N. 1975. Researches on the circulation time and on the influences which affect it. The output of the heart. J. Physiol. 22:159.

Swanson, E.W. 1982. Protein Requirements of Cattle. (F.N. Owens. ed.) Okla. St. Univ., Stillwater. p.1183.

TDMAC-heparin complex. Polyscience Inc. Warrington, Pa 18976.

Tischler, M.E., M. Desautels and A.L. Goldberg. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? J. Biol. Chem. 257:1613.

Thompson, G.E., W. Manson, P.L. Clark and A.W. Bell. 1978. Acute cold exposure and the metabolism of glucose and some of its precursors in the liver of fed and fasted sheep. A. J. Exp. Physiol. 63:189.

Tomas, F.M., A.J. Murray and L.M. Jones. 1984. Modification of glucocorticoid-induced changes in myofibrillar protein turnover in rats by protein and energy deficiency as assessed by urinary excretion of N-t-methylhistidine. Brit. J. Nutr. 51:323.

Tomas, F.M., H.N. Monroe and V.R. Young. 1979. Effect of glucocorticoid administration on the rate of muscle protein breakdown in vivo in rats as measured by urinary excretion of N-t-methylhistidine. Biochem. J. 178:139.

Trenkle, A. 1974. Hormonal and nutritional inter-relationships and their effects on skeletal muscle. J. Anim. Sci. 38:1142.

Van Es, J.H. 1980. Energy cost of protein deposition. In: Protein Deposition in Animals. (P.J. Buttery and D.B. Lindsay eds.) Butterworth. London.

Vasilatos-Younker, R., R.L. Prior and R.A. Britton. 1984. Metabolism of leucine by bovine adipose tissue: effects of media supplementation and insulin. Dom. Anim. Endocrinol. 1:299.

Wahren, J. 1966. Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise. Acta. Physiol. Scand. 67:269.

Wahren, J., P. Felig and L. Hagenfelt. 1976. Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. J. Clin. Invest. 57:987.

Waldo, D.R. and B.P. Glenn. 1984. Comparisons of new protein systems for lactating dairy cows. J. Dairy Sci. 67:1115.

Wassner, S.J. and J.B. Li. 1982. N-t-methylhistidine release: contributions of rat skeletal muscle, GI tract and skin. Am. J. Physiol. 243:E293.

Waterlow, J.C. and J.M.L. Stephen. 1968. The effect of low protein diets on the turnover rates of serum, liver and muscle proteins in the rat measured by continuous infusin of [^{14}C]-lysine. Clin. Sci. 35:287.

Webb, K. 1985. Personal communication.

Webster, A.J. 1981. The energetic efficiency of metabolism. Proc. Nutr. Soc. 40:121.

Wijayasinghe, M.S., L.P. Milligan and J.R. Thompson. 1983. In vitro degradaton of leucine in muscle, adipose tissue, liver and kidney of fed and starved sheep. Biosci. Reports 3:1133.

Williamson, D.H. 1980. Muscle protein degradation and amino acid metabolism in human injury. Biochem. Soc. Trans. 8:497.

Woodside, K.H. and G.E. Mortimore. 1972. Suppression of protein turnover by amino acids in perfused rat liver. J. Biol. Chem. 247:6474.

Young, V.R. 1980. Hormonal control of protein metabolism, with particular reference to body protein gain. In: Protein Deposition in Animals. (P.J. Buttery and D.B. Lindsay eds.) Butterworth. London.

Young, V.R., S.C. Chen and J. MacDonald. 1968. The sedimentation of rat skeletal-muscle ribosomes. Effect of hydrocortisone, insulin and diet. Biochem. J. 106:913.

Young, V.R. and H.N. Munro. 1978. N-t-methylhistidine and muscle protein turnover: An overview. Fed. Proc. 37:2291.

Zierler, K. 1965. Equations for measuring blood flow by external monitoring of radioisotopes. Circ. Res. 16:309.