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I am submitting herewith a thesis written by James Christopher Sherrell entitled "Effect of Dietary n-3 Fatty Acids and γ-Linolenic Acid on Protein Metabolism in the Thermally Injured Rat." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Comparative and Experimental Medicine.

Michael D. Karlstad, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

EFFECT OF DIETARY n-3 FATTY ACIDS AND γ-LINOLENIC ACID ON PROTEIN METABOLISM IN THE THERMALLY INJURED RAT

A Thesis
Presented for the
Master of Science
Degree
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James Christopher Sherrell
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DEDICATION

This thesis is dedicated to my parents

Mr. James Michael Sherrell and Mrs. Myra Gage Sherrell

who have sacrificed so much in order to give me every available educational opportunity

and

to my beautiful wife who has given me so much support throughout
the preparation of this manuscript

Regina King Sherrell

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ABSTRACT

In this study, it is determined what the effect of dietary n-3 fatty acids found in fish oil and γ-linolenic acid found in borage oil is on protein metabolism in burned rats given endotoxin versus feeding a more traditional corn oil-based diet. Animals were given a non-lethal, anesthetizing scald burn encompassing 30% of total body surface area and immediately injected IP with 1 mg/kg *S. enteritidis* endotoxin. Rats were then fed via gastrostomy isocaloric, isovolemic diets containing high amounts of either fish and borage oil or corn oil for 3 days. Tolerance to each diet formula was monitored throughout the 72 hour feeding period. On day three, whole-body leucine kinetics was measured, as well as fractional synthetic rates of protein in liver and rectus muscle. In burned rats given endotoxin, there was no significant ($p \leq 0.05$) difference between diet groups in either leucine kinetics or fractional protein synthetic rates (FSR) in liver or rectus muscle. However, burned animals had significantly better gastrointestinal tolerance to the diet containing fish and borage oil than to the corn oil-based diet. This study shows that although there may be no difference in whole-body protein kinetics and fractional protein synthetic rates in liver and muscle between burned rats enterally fed fish and borage oil or corn oil-based diets for 3 days, these animals did tolerate the fish and borage oil formula much better than the corn oil diet, which may have important implications in improving the tolerance of critically injured patients to enteral feeding.

TABLE OF CONTENTS

LIST OF FIGURES

PART I

INTRODUCTION

Increased whole-body protein catabolism is a primary complication seen in patients with systemic inflammatory response syndrome (SIRS) due to multiple trauma, severe burns, or sepsis. Over the past 25 years, improvements in supportive care have significantly improved patient survival, however the mortality rate (40-50%) remains unacceptably high. A primary goal of conventional approaches to dietary maintenance of the post trauma patient is to establish positive whole-body protein balance. It is essential that optimal protein synthesis rates be maintained in order for wound repair and host defense mechanisms to remain functional. However, the physiological mediation of protein metabolism in the stressed patient is a very complex process of which very little is as yet understood. Recent observations suggest that the catabolic response in critical injury is not only hormonally influenced, but that cytokines and certain metabolites of arachidonic acid may also play an important regulatory role (Flores, et al., 1989; Rodemann, et al., 1982). Rodemann, et al. (1982) demonstrated that arachidonic acid and prostaglandin E_2 had a net proteolytic effect on various types of striated rat muscle, while prostaglandin F_2 stimulated protein synthesis. Others (Flores, et al., 1989) have shown that the cytokines tumor necrosis factor-α (TNF) and interleukin-1 (IL-1) are also involved in net loss of lean-body protein. These studies indicate that TNF enhances muscle proteolysis and that the addition of IL-1 synergistically augments this process. TNF and IL-1, along with arachidonic acid and the eicosanoids PGE_2 and PGF_2, typically increase in the circulation during systemic inflammation. Each of these mediators not only contribute to the generalized inflammatory response which eventually leads to an increase in tissue protein turnover, but also regulate the synthesis of additional mediators involved in the pathogenesis of SIRS and other related conditions, such as acute

respiratory distress syndrome (ARDS) (Bone, 1991). Synthesis of TNF and IL-1 by tissue macrophages is critical in the development and progression of inflammatory multi-system organ dysfunction and may be central to increasing protein catabolism.

Critically injured patients are normally fed a high fat, low carbohydrate diet to reduce carbon dioxide production and respiratory quotient. However, the normal source of fat for this diet is corn oil, a rich source of n-6 fatty acids, which may exacerbate the severity of protein catabolism by enhancing the synthesis of cytokines and proinflammatory arachidonic acid metabolites (Karlstad et al., 1996). Diets enriched with n-3 fatty acids, derived from fish oil, and with γ-linolenic acid, derived from borage oil, reduce the synthesis of proinflammatory eicosanoids by reducing arachidonic acid pools in the cell membrane, and decreasing the activity of many enzymes responsible for arachidonic acid metabolism (Whelan et al., 1991). Moreover, the synthesis of antiinflammatory eicosanoids, derived from n-3 fatty acids and γ-linolenic acid, is increased after ingestion of diets enriched with fish oil and borage oil (Karlstad et al., 1996). However, there is conflicting information about cytokine synthesis after ingestion of antiinflammatory diets. Cytokine synthesis by human blood mononuclear cells, rat Kupffer cells, and equine peritoneal macrophages is reduced by ingestion of diets enriched with n-3 fatty acids. In contrast, cytokine synthesis by rat peritoneal macrophages is increased by ingestion of similar diets (Carrick, et al., 1994; Endres, et al., 1989; Hardard'ottir, et al., 1992; Meydani, et al., 1991). Although we have recently shown that ingestion of diets enriched with n-3 fatty acids and γ-linolenic acid reduced the severity of sepsis-induced lung injury (Mancuso, et al., 1994), the effect of this diet on post-trauma whole-body protein metabolism is not known. Because of the apparent

involvement of specific cytokines and eicosanoids in SIRS, it is important to determine whether dietary intervention alters cytokine synthesis and prevents the deleterious consequences of thermal injury-induced protein hypercatabolism. This study investigated the role of dietary intervention on cytokine/eicosanoid-mediated protein breakdown during the pathogenesis of systemic inflammatory response syndrome resulting from a severe burn injury.

The Pathophysiology of Burn Injury and Sepsis

The metabolic response to severe injury was first characterized by Cuthbertson in 1930 where he described an initial resuscitative *ebb* phase and a much later anabolic recovery *flow* phase. The ebb phase consisted of decreased intravascular volume, poor tissue perfusion, and low cardiac output accompanied by a hypometabolic state during which whole-body oxygen requirements are below normal (Cuthbertson, 1930; Tredget et al., 1992). Cunningham and colleagues elaborated on the flow phase originally described by Cuthbertson, stating that this stage occurs only after complete resuscitation and is characterized by a physiologic state of increased cardiac output, elevated energy expenditure, loss of lean body mass, and abnormal substrate production and utilization (Cuthbertson, 1930; Tredget et al., 1992; Cunningham et al., 1989). The flow phase does not occur until well after wound closure and persists until the remodeling of healing wounds is completed (Tredget et al., 1992; Cunningham et al., 1989).

The increase in metabolic rate due to thermal injury is reflected by oxygen consumption which can be measured using indirect calorimetry methods. The metabolism of severe burn patients has been shown to rise linearly and increase with the percent of the

total body surface area burned (TBSA). This linear trend continues until an increase of 100% above the resting energy expenditure (REE) is achieved with burns of greater than 60% of TBSA (Tredget et al., 1992; Gump et al., 1971; Saffle et al., 1985; Phang et al., 1990; Cunningham et al., 1989; Ireton et al., 1986). Both body core temperature and skin temperature are normally elevated during week one following burn injury. Cold stress or painful stimuli generally produce a significant increase in oxygen consumption which necessitates the establishment of a thermoneutral (patients usually request 31° to 33°C) patient care environment which is designed to control heat and evaporative water loss while at the same time providing adequate analgesic relief (Ireton et al., 1986; Tredget et al., 1992; Wilmore et al., 1974). Urinary nitrogen excretion also typically increases in severe burn injury and patients losing greater than 40 grams per day, 80% to 90% of which is urea, are characteristically most hypermetabolic (Goodwin, 1985). Appearance of nitrogen in the urine is linked directly to increased protein breakdown which occurs primarily in skeletal muscle. The amino acids resulting from this peripheral proteolysis are released into the plasma primarily as alanine and glutamine (Aulick et al., 1978) and subsequently taken up by the liver where they are used in the synthesis of acute phase proteins and other proteins of greater biological importance (Perlmutter et al., 1986).

Accelerated whole-body protein turnover and negative nitrogen balance in severe burn injury is thought to be mediated by elevations in circulating catecholamines, cortisol, glucagon, and other cytokine and eicosanoid mediators of the stress response (Karlstad et al., 1992; Gelfand et al., 1984; Bessey et al., 1984). Protein loss occurring from severe burn or sepsis results from both a decrease in protein synthesis and an increase in protein degradation

(Cooney et al., 1996). The inhibition of protein synthesis in fast-twitch muscle is thought to be achieved by a reduction in the efficiency of mRNA translation into protein. This loss of translation efficiency appears to be the result of a decrease in the rate of peptide-chain initiation which may be mediated in part by the diminished activity of an important guanine nucleotide exchange factor termed eIF-2B. It is not well understood how the activity of eIF-2B is decreased, however it has been postulated that the expression of this eukaryotic initiation factor is regulated by decreased transcription or mRNA stability (Cooney et al., 1996).

Protein breakdown in trauma and sepsis occurs more readily in white, fast-twitch muscles than in those containing red, slow-twitch fibers and is more pronounced in myofibrillar proteins (Cooney et al., 1996). There are many proteolytic systems by which cellular proteins are degraded, the best characterized of which is the lysosomal pathway. Lysosomal protein degradation utilizes the cathepsin family of proteases, which are believed to be non-selective in respect to specific protein types (Cooney et al., 1996). Other mechanisms by which muscle protein degradation occurs include the Ca^{2+}-dependent cysteine proteinases μ- and m-calpain and ATP-dependent ubiquitin-conjugated proteolysis (Cooney et al., 1996).

The metabolic response to sepsis is an immune-mediated reaction that is often a primary additive complication of severe burn trauma. The resting energy expenditure of septic patients can rise to as much as 60% above normal values when tissue breakdown and subsequent mobilization of protein, carbohydrates, and fat occur (Elwyn et al., 1992; Wojnar et al., 1995; Jeejeebhoy, 1995). In addition to increased energy requirements, septic patients

have elevated levels of glucagon, cortisol, insulin, and catecholamines which are all believed to play a central role in propagating the body's metabolic response to sepsis (Elwyn et al., 1992; Wojnar et al., 1995; Jeejeebhoy, 1995). Glucagon, the hormone of pancreatic origin, is a strong promotor of gluconeogenesis from both lactate (Cori cycle) and alanine in the liver. Glucose is synthesized primarily from lactate and alanine in septic patients because of limited glycogen reserves (Jeejeebhoy, 1995), however glycogen breakdown is actually lower in septic patients when compared to otherwise healthy patients who are fed the same diet (Elwyn et al., 1992). Wojnar and colleagues (1995) demonstrated that septic patients are both hyperglycemic and hyperinsulinemic and can develop resistance to insulin. Upon increased demand, the high level of glucose in the blood of septic and insulin-resistant patients can provide the brain and other non-insulin dependent tissues with needed energy (Elwyn et al., 1992).

In addition to elevations in metabolic hormone activity, septic patients also undergo an increase in fatty acid and glycerol turnover which corresponds to a rise in lipid oxidation (Elwyn et al., 1992; Wojnar et al., 1995; Jeejeebhoy, 1995; Kinney, 1995). It is believed that catecholamines up-regulate lipid breakdown by increasing the activity of the lipase enzyme, resulting in the release of free fatty acids which can then provide much needed energy to skeletal and cardiac muscle (Wojnar et al., 1995). The energy supplied to muscles by lipolysis subsequently allows glucose to function as the main fuel source for the central nervous system (Wojnar et al., 1995).

Protein breakdown and muscle loss are also common characteristics of the metabolic response to sepsis (Elwyn et al., 1992; Wojnar et al., 1995; Jeejeebhoy, 1995; Kinney,

1995). Stress hormones, including cortisol, mediate the release of amino acids from skeletal muscle and other peripheral tissues for use in gluconeogenesis (Jeejeebhoy, 1995; Kinney, 1995). Without intervention, protein breakdown can result in a 24 hour net loss of up to 15 g of nitrogen which corresponds to an approximately 0.5 kg decrease in lean body mass per day (Wojnar et al., 1995).

Modulation of the Inflammatory Response with Antiinflammatory Fatty Acids

Linoleic acid (18:2n-6) makes up a significant portion (7%) of the total calories in the average American diet and is the primary polyunsaturated fatty acid (PUFA) constituent in plant seed oils (Kinsella et al., 1990; Leaf et al., 1988). Linoleic acid is termed an n-6 or ω-6 fatty acid because its first double bond is six carbon atoms from the methyl end of the fatty acid chain (Kinsella et al., 1990; Leaf et al., 1988). Humans lack the ability to synthesize fatty acids with a double bond more than nine carbon atoms from the carboxyl end of the fatty acid chain, therefore linoleic acid is considered an essential fat (Leaf et al., 1988). Linoleic acid can be oxidized to provide energy, stored in cell membranes, or metabolized to arachidonic acid by the enzymes Δ-6 desaturase, Δ-5 desaturase, or elongase (Kinsella et al., 1990; Leaf et al., 1988). Arachidonic acid (20:4n-6) is necessary for adequate development of the visual and nervous systems of human infants (Salem et al., 1996) and is also the metabolic precursor to proinflammatory eicosanoids which are involved in the development and progression of many systemic inflammatory responses (Karlstad et al., 1996).

α-Linolenic acid (18:3n-3) is an essential fatty acid synthesized only in the chloroplasts of plants (Leaf et al., 1988) which in humans can be elongated and desaturated to form

eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). However in humans, arachidonic acid makes up a much larger percentage of the total amount of phospholipid found in tissues than does either eicosapentaenoic (EPA) or docosahexaenoic acid (DHA) (Endres et al., 1989; Mori et al., 1992). This observation may be because of the large amount of n-6 fatty acids in Western diets, where these and the n-3 fatty acids compete for the same enzymes responsible for forming EPA and DHA from α-linolenic acid (Leaf et al., 1988; Holman, 1964).

Many researchers have shown that dietary intake of fatty acids can modify the composition of those found in cell membrane phospholipids (Mori et al., 1992; Calder et al., 1990; Chapkin et al., 1990; Lee et al., 1985; Pullman-Mooar et al., 1990). Oral administration of fish oil containing EPA and DHA has been shown to decrease the amount of arachidonic acid while increasing the amounts of EPA and DHA in tissue phospholipids (Mori et al., 1992; Leaf et al., 1988; Nassar et al., 1986; Lands, 1992; von Schacky et al., 1985). EPA and DHA decrease Δ-6 desaturase activity (which slows the conversion of linoleic acid to arachidonic acid) and compete for binding sites on those acyltransferase enzymes which promote the incorporation of n-3 fatty acids into cell membranes over that of n-6 fatty acids (Kinsella et al., 1990; Stubbs et al., 1984).

In addition to n-3 PUFAs such as EPA and DHA, γ-linolenic acid, a fatty acid found in large amounts in the oil of the borage plant, has also shown the ability to lower concentrations of arachidonic acid in cell membranes (Das et al., 1988; Carter, 1988; Karlstad et al., 1993). γ-Linolenic acid has not been shown to accumulate in tissue phospholipids, however it is rapidly elongated to dihomo-γ-linolenic acid which *is* readily

incorporated into cell membranes (Carter, 1988; Karlstad et al., 1993; Manku et al., 1988; Ziboh et al., 1992).

Many studies have shown that oral supplementation with n-3 PUFAs such as those found in fish oil and γ-linolenic acid from borage oil can have an important effect on the arachidonic acid cascade by significantly reducing the synthesis of proinflammatory eicosanoids (Mori et al., 1992; von Schacky et al., 1985; Karlstad et al., 1993; Ziboh et al., 1992; Sperling et al., 1993; Barre et al., 1993). Feeding diets enriched with eicosapentaenoic acid, docosahexaenoic acid, and γ-linolenic acid increases the levels of these fatty acids in cell membranes (γ-linolenic acid in its dihomo form) which has been shown to decrease proinflammatory eicosanoid synthesis by both competing for binding sites on the enzymes cyclooxygenase and 5-lipoxygenase and changing the substrate for eicosanoid formation (Kinsella et al., 1990; Leaf et al., 1988; Karlstad et al., 1993). Increasing the incorporation of EPA, DHA, and dihomo-γ-linolenic acid into tissue phospholipids correspondingly decreases the amount of arachidonic acid found in these cell membranes (Kinsella et al., 1990; Diboune et al., 1992). This in turn reduces the substrate quantity for synthesis of proinflammatory 2- series prostaglandins and 4- series leukotrienes which are key mediators of protein catabolism and other systemic inflammatory responses such as acute respiratory distress syndrome (ARDS) (Kinsella et al., 1990; Loyd et al., 1984; Rodemann et al., 1982). Displacement of arachidonic acid (AA) by eicosapentaenoic acid therefore shifts cyclooxygenase-mediated eicosanoid production in favor of both the 3- series prostaglandins and 3- series thromboxanes which are less biologically active than their 2- series counterparts derived from AA (Karlstad et al., 1996; Leaf et al., 1988; Fischer et al., 1984; Needleman

et al., 1979). In addition, leukotriene B_5 which is formed via 5-lipoxygenase from eicosapentaenoic acid, has been shown to be less actively involved in neutrophil recruitment than leukotriene B_4, which is synthesized from arachidonic acid (Lee et al., 1985; Lee et al., 1984). Dihomo-γ-linolenic acid can not be metabolized by 5-lipoxygenase, but it can be oxidized by cyclooxygenase to form the antiinflammatory prostaglandin PGE_1 (Chilton-Lopez et al., 1996; Fan et al., 1992).

In addition to the effects on eicosanoid production, dietary supplementation with n-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid has been shown to reduce synthesis of the inflammatory and pro-catabolic cytokines TNF-α and IL-1β by mononuclear cells (Biliar et al., 1988; Endres et al., 1989). Feeding diets enriched with n-3 fatty acids has also been demonstrated to reduce pulmonary leukosequestration and improve arterial oxygen pressure (PaO_2), lung morphology, and survival in animals given either endotoxin (Pomposelli et al., 1991; Murray et al., 1993) or live *Escherichia coli* (Murray et al., 1991) when compared to animals which were pre-fed diets containing linoleic acid.

Diets enriched with n-3 fatty acids have been shown to have positive therapeutic effects in the treatment of many inflammatory diseases such as rheumatoid arthritis, lupus, and psoriasis (Bittiner et al., 1988; Kremer et al., 1987; Kelley et al., 1985). Oral supplementation of n-3 fatty acids has also been demonstrated to be beneficial in experimental models of acute lung injury and sepsis (Miura et al., 1993; Murray et al., 1991; Murray et al., 1993; Murray et al., 1995). Feeding diets enriched with γ-linolenic acid from either borage oil or black currant seed oil has been shown to have a positive effect in the treatment of rheumatoid arthritis and atopic eczema (Leventhal et al., 1994; Wright et al., 1982). In

addition to having demonstrated beneficial effects in the treatment of many different inflammatory or immune-related diseases, researchers have also shown that early enteral feeding with structured lipids containing n-3 fatty acids can reduce the amount of net protein catabolism in burned rats (Selleck et al., 1994; Teo et al., 1988). However, the effect of a diet containing n-3 fatty acids *as well as* therapeutically-promising γ-linolenic acid on protein metabolism in thermal injury has yet to be addressed in the literature.

Nutritional Support in Critical Illness

The primary objectives of nutritional support for the hypermetabolic critically injured patient are to provide the proper amount of calories, prevent the loss of lean body mass, and to avoid development of respiratory complications by keeping CO_2 production at or near normal levels (Pinard et al., 1995). The criteria generally accepted for placing a patient on nutritional support are: 1) loss of at least one tenth of normal body weight, 2) zero nutrient intake for 5 to 7 consecutive 24 hour periods, or 3) likelihood of no oral nutrient intake for 10 consecutive days or longer (Bower, 1993). In patients unable to be orally fed, enteral feeding has been demonstrated to be the most effective means of nutrient delivery (Grote et al., 1987). Previous studies have indicated that patients fed parenterally, when compared to those fed enterally, are more susceptible to bacterial translocation across the gut barrier and those who are severely burned are at greater risk of becoming septic due to contamination at the feeding site (Bower, 1993; Alverdy et al., 1988; Mochizuki et al., 1984). These observations indicate that parenteral nutrition should be reserved only for those patients who are intolerant of enteral feeding, whose injuries prevent enteral feeding, or who are in need

only of short-term supplementation to otherwise deficient oral or enteral nutrient delivery (Bower, 1993).

Patients with severe burn injury or sepsis are at significant risk for muscle wasting and loss of lean body mass due to increased protein catabolism. This catabolic state can result in impaired respiratory muscle function and reduction in vital capacity and minute ventilation (Pinard et al., 1995). Traditional high carbohydrate and high protein enteral formulas have been shown to decrease the amount of muscle loss in critically injured patients, however these diets have also been demonstrated to increase ventilatory demand, placing these patients at greater risk for developing acute respiratory failure (Pinard et al., 1995; Burzstein et al., 1992; Askanazi et al., 1981). Recent studies have shown that enteral nutrition formulas containing high amounts of fat and low quantities of carbohydrate can satisfactorily meet the increased metabolic needs of critically injured patients on the ventilator without substantially increasing ventilatory demand (Burzstein et al., 1992; Al-Saady et al., 1989).

In conclusion, severe burn injury and sepsis initiates a series of inflammatory events that leads to the establishment of a hypermetabolic state characterized by increased resting energy expenditure, increased rate of glucose production and utilization, decreased lipid metabolism, and an increased rate of both protein catabolism and anabolism. The systemic inflammatory response to thermal injury and sepsis leads to the increased production of proinflammatory eicosanoids and cytokines which up-regulate the hypermetabolic/catabolic cascade. Dietary supplementation with antiinflammatory fatty acids such as eicosapentaenoic acid, docosahexaenoic acid, and γ-linolenic acid can alter the phospholipid composition of cell membranes, reduce the formation of proinflammatory arachidonic acid metabolites, and

attenuate the severity of inflammatory and immune-related diseases in both humans and laboratory animals. It may therefore be possible to modulate increased protein catabolism and muscle wasting in an animal model of severe burn injury and sepsis by early enteral feeding of a high fat, low carbohydrate nutrition formula containing antiinflammatory fatty acids derived from both fish and borage oil when compared to feeding a standard corn oil-based diet.

PART II

MATERIALS AND METHODS

Male Long-Evans rats weighing 200-220g were obtained from Charles River Laboratories (Wilmington, MA) and housed in pairs with *ad libitum* access to chow and water for one week prior to being used in this study. At the conclusion of this acclimation period, the average weight of the animals used in the experiment was approximately 250g.

Preparation of Infusion Swivel

Dual-channeled brass infusion swivels were obtained from Instech Laboratories (Plymouth Meeting, PA) and fitted with a polyethylene gastric catheter and a silastic venous catheter. The gastric catheter was prepared using a length of Intramedic PE-50 (Clay Adams Division of Becton Dickinson and Co., Parsippany, NJ) sufficient to loosely extend from one effusion port to the duodenum of the rat. Using a heating element, a non-occluding bubble was placed in the line approximately two centimeters from the end of the catheter so as to provide an obstacle to the accidental removal of the feeding tube once it had been sutured in place. The venous catheter (0.025" ID, 0.047" OD, Baxter Healthcare Corp., Deerfield, IL) was made from medical - grade silastic tubing and measured to extend from the second swivel effusion port to the left jugular vein of the rat. A 3mm 20 gauge steel bridge (made from scoring and breaking 20 gauge hypodermic needles) was inserted into each venous line approximately one inch from the end of the catheter by swelling the tubing via immersion in ether. The gastric and venous catheters were threaded simultaneously through the swivel spring using thin copper wire. In addition, both infusion port lines attached to each swivel were made from silastic tubing. Once assembled, the swivels were wrapped and sterilized using ethylene oxide and stored ready for use.

Surgical Procedure

Twelve hours prior to surgery, rats were prevented access to chow but were allowed water *ad libitum*. On the morning of surgery, the animals were first weighed and then anesthetized with Isoflurane inhalation anesthesia (Abbott Laboratories, North Chicago, IL) using a Heidbrink Kinet-O-Meter Anesthesia Machine (Ohio Medical Products, Madison, WI) at an initial induction dosage of 5%. Anesthetic was mixed with pure oxygen and the flow rate (and vacuum) was established at 1 liter per minute. While anesthetized, fur was clipped from the dorsum, midscapular region, abdomen, and the left anterior cervical triangle of the rat. After being clipped, the rat was promptly replaced under anesthesia and maintained at a dosage of 3%.

Subcutaneous Tunneling of Catheters

With the animal in the prone position, the midscapular region was marked with an indelible pen. The rat was then placed in the supine position and the abdominal and cervical incision sites were prepped with betadine solution. Visualizing the pulsating left jugular vein, a longitudinal incision was made in the dermis of the left anterior cervical triangle. A second longitudinal dermal incision was made along the abdominal midline. A dulled trochar was then tunneled subcutaneously from the cervical incision site to the marked midscapular region where the tip was exteriorized. The venous catheter was then threaded from the exit point at the midscapular region through the cervical incision using the trochar as a guide. The gastric feeding tube was tunneled under the skin to the midline incision in the same manner and exteriorized commonly with the venous catheter. At this point, the venous catheter was

filled with heparinized saline (10 units/ml, stock: Heparin Sodium for Injection, Upjohn, Kalamazoo, MI) and the gastric catheter filled with normal saline (0.9%). The abdominal wound was then lightly irrigated with saline and covered with cellophane. Isoflurane dosage was reduced at this time to 2% for the remainder of the surgical procedure.

Insertion of Venous Catheter

The venous catheter was inserted into the external jugular vein just superior to the clavicle. From the cervical incision site, a segment of the left jugular vein was isolated using blunt dissection techniques. Once isolated, the vein was ligated approximately 3-5mm from the catheter insertion site using 3-0 surgical silk (Ethicon of Johnson and Johnson, Somerville, NJ). An additional segment of 3-0 silk was tied loosely around the jugular vein just inferior to the insertion point. The catheter was trimmed (so as to produce a sharp bezeled tip) to approximate (from stent to tip) the distance within the vessel from the catheter insertion site to just superior to the heart. While holding both the superior ligature to stabilize the vessel and the inferior tie to control bleeding, an incision was made in the left jugular vein. The catheter was then inserted into the vein and advanced until the proximal end of the stent reached the venous incision. After confirming patency of the catheter by observing free back flow of blood into the line following drawback of heparinized saline, the inferior 3-0 silk tie was secured simultaneously around both the vessel wall and the stent, thereby affixing the catheter firmly within the lumen of the vessel. After trimming sutures and correctly positioning the catheter within the wound, line patency was again confirmed and the incision

was closed with 3-0 silk (cutting ps-1: Ethicon of Johnson and Johnson) in an uninterrupted pattern.

Insertion of Gastric Catheter

After removing the protective cellophane from the abdomen, the muscle wall was incised along the midline in a careful manner, ensuring that no internal structures were compromised. The stomach was carefully exteriorized using paddle forceps and the gastric-duodenal interface was identified. A catheter insertion site was selected on the gastric wall approximately 5mm from the estimated location of the pylorus. The gastric catheter was then trimmed so as to provide a length allowing the line to advance 1-2 cm into the small bowel (with the bubble resting just inside the gastric wall). The catheter was cut in such a manner that would leave no sharp edges or bezel at its end. Once the insertion site was selected, an open purse string suture (5mm diameter, 3-0 silk from Ethicon) was centered around that point in the superficial layer of the stomach wall. The gastric wall was then punctured at the insertion site with a sharp set of micro pick-ups. The catheter was inserted into the stomach through the puncture wound and carefully threaded through the pylorus into the duodenum. The bubble was advanced just past the gastric puncture and the purse string was closed behind it, thereby securing the catheter in place. Patency of the feeding line was confirmed by minimal free - infusion of saline. The midline laparotomy was closed using 3-0 silk (Ethicon) in an uninterrupted pattern and the catheter was exteriorized from the peritoneum between the first and second of these sutures. Any excess length of feeding tube was looped

under the skin of the rat and the dermal incision was also closed in an uninterrupted pattern with 3-0 silk (Ethicon).

Securing the Swivel Button

Once both catheters were installed, the animal was returned to the prone position and the swivel button was secured centrally to the midscapular exit site with four evenly-spaced interrupted 2-0 silk (Ethicon) sutures. The trochar was used as a spacer to ensure that the button was attached loosely to the midscapular dermis. Once attachment was completed, surgical tape was wrapped around the button so as to prevent the animal from clawing the lines and sutures.

Experimental Groups

Animals were randomized to receive either injury or sham treatment and with regard to diet therapy. There were four experimental groups consisting of eight animals in each group: Group 1- burn/endotoxin treatment + diet 1; Group 2- sham treatment + diet 1; Group 3- burn/endotoxin treatment + diet 2; Group 4- sham treatment + diet 2.

Injury Model

Approval to conduct this study using the following injury model was granted by the Animal Care and Use Committee of The University of Tennessee Graduate School of Medicine in accordance with guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Burn Injury

While still maintained under anesthesia from the surgical portion of the experiment, the shaven dorsal surfaces of those rats assigned to the injury group were immersed through a pre-formed mold in a 90°C water bath (Precision, Chicago, IL) for 15 seconds so as to produce a well-demarcated, fully-anesthetizing third-degree scald burn. The mold was designed so as to produce an average burn size of 30% relative to total body surface area. Immediately following the 15 second burn period, the dorsum of the rat was immersed in room temperature water for an additional 15 seconds. Upon being removed from the water baths, the rat was blotted dry and the wound was examined for poor demarcation or blistering. If any such unsatisfactory characteristic of the burn injury was observed, the animal was promptly euthanized. Each animal was also carefully observed for respiratory complications resulting from the injury and resuscitation efforts were begun if necessary. Control animals were immersed dorsally for a period of 15 seconds in room temperature water only.

Endotoxin Administration

Immediately following the burn injury and while continuously maintained under anesthesia, rats in the injury group were given an intraperitoneal injection of *Salmonella enteritidis* endotoxin (Difco Laboratories, Detroit, MI) at a dosage of 1 mg/kg of body weight which was suspended in 1 ml of sterile saline. Control animals were given an intraperitoneal injection of 1 ml of sterile saline only. Rats were then weighed to determine post-operative mass (animal + infusion swivel).

Fluid Maintenance

All rats were fluid rescusitated with 2.5 ml of intravenous saline per hour (via syringe pump, Harvard Apparatus, South Natick, MA) for a period of 4 hours following surgery. At the conclusion of this period, the flow rate was reduced to 0.15 ml/hr in order to keep the vein open until the isotope infusion portion of the study. Venous lines were flushed twice daily with 0.5 ml heparinized saline (10 units/ml).

Housing

Following surgery and injury or sham treatment, rats were placed individually in metabolic cages and housed in a controlled temperature (21-23°C) and light-cycle (12 hours light, 12 hours dark) environment for the duration of the study.

Diet Therapy

Rats were fed one of two commercially prepared enteral nutrition formulas (Ross Products Division of Abbott Laboratories, Columbus, OH) for a therapeutic period of 72 hours. Each diet was nutritionally complete, fortified with vitamins and minerals, and contained 55.2% of the total calories from fat (16.7% protein, 28.1% carbohydrate). The fat source in diet 1 was 97% corn oil; diet 2 contained 20% fish oil and 20% borage oil. The fatty acid content of each diet is given in Table 1. Each animal was fed an isocaloric regimen of 300 kcal/kg/day through the surgically implanted gastric catheter via syringe pump (Harvard Apparatus). Gastric infusion syringes were changed daily to prevent bacterial contamination and all feeding lines were carefully observed for any occlusions or ruptures.

	% of Total, by Weight	
Fatty Acid	Corn Oil	20% Fish Oil and 20% Borage Oil
Caproic (6:0)	ND	0.09
Caprylic (8:0)	ND	13.05
Capric (10:0)	ND	9.93
Lauric (12:0)	0.09	0.20
Myristic (14:0)	0.15	1.24
Palmitic (16:0)	11.3	5.95
Palmitoleic (16:1n-6)	0.04	1.69
Stearic (18:0)	2.27	1.86
Oleic (18:1n-9)	24.59	25.96
Linoleic (18:2n-6)	59.35	16.68
γ-linolenic (18:3n-6)	ND	4.63
α-Linolenic (18:3n-3)	1.01	3.92
Stearidonic (18:4n-3)	ND	0.71
Aracidic (20:0)	0.41	0.27
Eicosenoic (20:1n-9)	0.30	1.92
Arachidonic (20:4n-6)	ND	0.38
Eicosapentaenoic (20:5n3)	ND	5.31
Erucic (22:1n-9)	0.04	1.12
Docosapentaenoic (22:5n3)	ND	0.54
Docosahexaenoic (22:6n3)	ND	2.52
Others	0.45	2.03
Total n-6/total n-3	58.76	1.67

Table 1. Fatty acid content of enteral diets.

ND, Not detected; total n-6/total n-3, total n-6 to n-3 fatty acids.

In addition, all animals were provided *ad libitum* access to water throughout the diet therapy portion of the study. Rats were also observed twice daily during the feeding period for signs of distress (lethargy, wound licking, piloerection, etc.) related to surgery or the injury treatment; those animals exhibiting such characteristics were promptly euthanized.

Diet Tolerance

Animals were observed during the 72 hour feeding period for signs of intolerance to the enteral diet formulas. Intolerance was defined as any animal which developed loose or running stools (diarrhea) compared to normal well - formed fecal pellets at any time during the three day diet therapy period.

Kinetics Experiment

At the conclusion of the 72 hour feeding period, the diet pumps were stopped and the venous lines were flushed with 0.5 ml heparinized saline (10 units/ml) to confirm patency. The animals were then weighed and allowed to acclimate to new metabolic cages for a period of 1-2 hours before beginning the isotope infusion portion of the study. Before the animals were transferred to the second metabolic cage, a Masterflex Pump (Cole-Parmer, Chicago, IL) was affixed to the effusion port of each cage and adjusted to draw air through the enclosure at a rate of 1600 ml/min. Flow rates were measured throughout this segment of the experiment using a Matheson Gas Products Flow Meter (Secaucus, NJ). During the acclimation period, the Carbon Dioxide Analyzer (Ametek Thermox Instruments Division, Pittsburgh, PA) used for measuring CO_2 concentration in expired breath was calibrated with

O_2/CO_2 standard calibration gas (Ciba-Corning, Norwood, MA) to settings of 0.3% background ($\%CO_2$ in the room) to a maximum of 5.0% ($\%CO_2$ in the standard).

After diet infusion had been stopped for 1-2 hours, the animals were infused with a 0.8 μCi/ml solution of [1-^{14}C]L-leucine (100μCi/ml, ICN Pharmaceuticals, Costa Mesa, CA) in normal saline at a rate of 2 ml/hr (syringe pump, Harvard Apparatus) for a period of 4 hours. At 30 minute intervals during the 4 hour period, samples of expired breath were taken from the animals by collecting effluent gas from each respective Masterflex Pump (flow rate = 1600 ml/min) for 1 minute. This gas sample was then passed through Drierite (W.A. Hammond Co., Xenia, OH) to the gas analyzer to determine $\%CO_2$. Immediately following each gas collection, expired breath (air pumped from the cage at 1600 ml/min) was bubbled through 5 ml hyamine hydroxide solution [10 ml hyamine hydroxide (National Diagnostics, Manville NJ), 10 ml 0.1% phenolphthalein (Mallinckrodt, Paris, KY) in absolute ethanol, 230 ml 200 proof ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY)] until saturation was indicated by complete loss of color. The gas was allowed to bubble for an additional 5 seconds to ensure complete saturation with CO_2 and the total time was recorded. The hyamine hydroxide solution was then mixed with 10 ml Betafluor (National Diagnostics, Manville, NJ) and counted (dpm, 5 minute count time) using the Tri-Carb Liquid Scintillation Analyzer (Packard Instrument Co., Downers Grove, IL).

At the conclusion of the 4 hour isotope infusion period, each rat was transferred to a separate chamber and anesthetized with Halothane inhalation anesthesia (Ayerst Laboratories, Philadelphia, PA). Isotope infusion was maintained until the animal was anesthetized. Each rat was then removed from the anesthesia chamber and promptly

euthanized via exsanguination from the abdominal aorta. Blood was drawn from the aorta into pre-labeled Monovette blood collection tubes (Sarstedt, Newton, NC) containing 300 µl of 5% ethylenediamine tetraacetate (EDTA) (Sigma Chemical Co., St. Louis, MO). Immediately following exsanguination, the liver was removed, weighed, and a portion was wrapped in labeled foil and snap-frozen in liquid nitrogen. A portion of rectus muscle was then collected and frozen in the same manner.

At the conclusion of the kinetics experiment, the average time per animal (in seconds) required to saturate 5 ml of hyamine hydroxide solution at each 30 minute sample interval during the 4 hour infusion period was calculated. Each metabolic cage was then assayed for background CO_2 by bubbling cage air (no animal present) through 3 additional 5 ml aliquots of hyamine hydroxide (via the Masterflex pump at 1600 ml/min) for the average time of saturation calculated for each respective cage (with the animal present). The three aliquots of hyamine hydroxide corresponding to each cage were then titrated individually (Digital Buret: Brinkmann Instruments, Westbury, NY) with 0.1 N HCl (stock: Mallinckrodt, Paris, KY) until colorless and the average volume required to produce the color change was calculated.

Upon completion of the experiment, 100 µl of the ^{14}C-leucine infusate given to each animal was counted (in triplicate) in 10 ml of Betafluor (5 minute counts, Packard Tri-Carb Liquid Scintillation Analyzer). Blood was centrifuged to separate plasma, which was collected in two 1 ml aliquots and frozen for storage at -80°C. Tissue samples were removed from the liquid nitrogen and also frozen at -80°C.

Analysis of Plasma and Tissues

Plasma

Plasma samples were removed from the -80°C freezer and allowed to thaw at room temperature. 250 µl of plasma was then diluted in a 1:1 ratio with 250 µl of 10% sulfosalicylic acid (SSA) (stock: Mallinckrodt, Paris, KY). Precipitated protein was then separated by centrifuging the solution at high speed (Eppendorf Centrifuge 5415: Brinkmann Instruments, Westbury, NY) for 10 minutes. After centrifugation, the supernatant was decanted and reserved while the tube containing the protein pellet was discarded.

Following deproteinization, the leucine analog ketoisocaproate was removed from each of the plasma samples using cation-exchange resin columns (AG 50W-X8 200-400 mesh hydrogen form resin: Bio Rad Laboratories, Hercules, CA). Each column was equilibrated with 5 ml of 10% SSA prior to loading 250 µl of each sample. The columns were then washed with 5 ml of 0.1 M HCl (stock: Mallinckrodt, Paris, KY) followed by two 5 ml aliquots of distilled water. The amino acids (minus ketoisocaproate) were collected from each column in 6 ml of 4 N ammonium hydroxide (stock: Sigma Chemical Co., St. Louis, MO).

After removal of ketoisocaproate, the amino acid / ammonium hydroxide solution was dried using the Savant Speed Vac vacuum dryer (Savant Instruments, Farmingdale, NY). Adequate drying of all samples in this experiment was achieved at a reading below 300 on the Speed Vac control box. After drying was complete, the amino acids in each plasma sample were reconstituted in 200 µl of distilled water. 50 µl of the reconstituted sample was then added to 10 ml Ecolite aqueous scintillation fluor (ICN Pharmaceuticals, Costa Mesa, CA)

and counted in duplicate (15 min/sample) using the Packard Tri-Carb Liquid Scintillation Analyzer (Packard Instrument Co, Downers Grove, IL). Background radiation was automatically subtracted from each sample by first counting only 10 ml of Ecolite.

In order to determine plasma leucine concentration, a 40 µl aliquot of each reconstituted sample was diluted in a 1:1 ratio with 40 µl of 1 mM norleucine (stock: Sigma Chemical Co., St. Louis, MO). L-norleucine was diluted in 0.1 M HCl (stock: Mallinckrodt, Paris, KY). 25 µl of the sample / norleucine solution was then transferred to a 6 mm glass culture tube and dried concurrently in the Savant Speed Vac with 25 µl of 1 mM norleucine and 25 µl of amino acid standard solution. The amino acid solution consisted of 200 µl acid - neutral amino acid standard solution (Sigma Chemical Co., St. Louis, MO), 200 µl basic amino acid standard solution (Sigma Chemical Co., St. Louis, MO), 850 µl distilled water, and 1250 µl 1 mM norleucine. After drying was complete, any residual acids were neutralized by adding 20 µl of redry solution [200 µl B&J brand high purity methanol (Baxter Healthcare Corp., Muskegon, MI), 200 µl 1 M sodium acetate (stock: Sigma Chemical Co., St. Louis, MO), 100 µl triethylamine (Sigma Chemical Co., St. Louis, MO)] to each sample / standard vial. The samples and standards were then again dried using the Savant Speed Vac. 20 µl of coupling solution [420 µl high purity methanol, 60 µl HPLC grade water (Fisher Chemical Co., Fair Lawn, NJ), 60 µl triethylamine, 60 µl phenylisothiocyanate (Pierce, Rockford, IL)] was then added to each redried sample / standard and incubated at room temperature for 10 minutes. Following this derivitization step, the samples and standards were once more dried (carefully to prevent encapsulation) using the Savant Speed Vac.

When completely dried, each standard and sample was reconstituted in 100 µl Pico-

Tag diluent (Waters Division of Millipore, Milford, MA) and transferred to an ultra low volume injection vial. Concentrations of amino acids in the samples and standards were then determined by high performance liquid chromatography (Pico-Tag Method of Physiologic Amino Acid Analysis, Waters 510 pumps, 715 Ultra Wisp Sample Processor, Waters 484 Tunable Absorbance Detector: Waters Division of Millipore Corp., Milford, MA). 20 µl of each sample reconstituted in diluent was injected onto the column and run for a total of 82 minutes; absorbance was detected at 254 nm. The resulting chromatograms were analyzed using Maxima 820 Chromatography Software (Millipore Corp., Milford, MA) and the concentration of leucine in each plasma sample was calculated.

Tissue

Tissues were removed from the -80°C freezer and a 0.5 g sample was quickly weighed out and placed in 5 ml of cold 10% SSA. Any remaining tissue was promptly replaced into the freezer. Each tissue sample was homogenized on ice in SSA (tissue homogenizer: Brinkmann Instruments, Westbury, NY) and then cold - centrifuged at 4000 g for 15 minutes (Sorvall RT6000B Refrigerated Centrifuge: Sorvall, Inc., Newtown, CT). The supernatant was decanted and stored under refrigeration. The protein pellet was then dissolved under 80-90°C heat (Precision Water Bath: Precision Scientific, Chicago, IL) with 2 ml of 2 N NaOH (stock: Mallinckrodt, Paris, KY) and 0.5 ml of 20% deoxycholic acid (DOC) (stock: Mallinckrodt, Paris, KY).

Protein concentration in the dissolved pellet was determined by the Biuret assay for protein. The standard curve was constructed with a solution of bovine albumin (10 mg/ml,

stock: Sigma Chemical Co., St. Louis, MO) with 100 µl of 10% DOC added to each of the standard samples (total volume equaled 250 µl). To assay protein concentration in the tissue samples, 100 µl of each solubilized pellet was diluted with 1150 µl of distilled water and 250 µl of this dilution was aliquoted to a separate vial. 1 ml of Biuret solution [60 g NaOH (Mallinckrodt, Paris, KY) + 600 ml dH_2O added to 3 g cupric sulfate (Sigma Chemical Co., St. Louis, MO) + 12 g sodium potassium tartrate (Sigma Chemical Co., St. Louis, MO) + 1 L dH_2O and brought up to 2 L total volume] was then added to both the standard curve samples and the tissue samples and incubated at room temperature for 30 minutes. Samples were then analyzed using the UV-Visible Recording Spectrophotometer (Shimadzu Corp., Columbia, MD) at 540 nm and the concentration of protein in each tissue was calculated. 200 µl of each solubilized protein pellet was also counted (in duplicate, 15 min count) in 10 ml Ecolite (ICN Pharmaceuticals, Costa Mesa, CA) using the Packard Tri-Carb Liquid Scintillation Analyzer (Packard Instruments, Downers Grove, IL).

Leucine concentration in the tissues was determined in a manner very similar to that used for plasma. Ketoisocaproate was removed from the acid - soluble fraction of the tissues (homogenate supernatant) using cation - exchange resin columns (AG 50W-X8 200-400 mesh hydrogen form resin: Following thorough drying, each sample was reconstituted in 400 µl of distilled water. Bio Rad Laboratories, Hercules, CA). After equilibrating the columns with 5 ml of 10% SSA, 2.0 ml of each sample supernatant was passed through the resin. The columns were then washed with 5 ml of 0.1 M HCl (stock: Mallinckrodt, Paris, KY) and two 5 ml aliquots of distilled water. The amino acids in each acid - soluble fraction of tissue were collected in 6 ml of 4 N ammonium hydroxide (stock: Sigma Chemical Co., St. Louis, MO)

and dried overnight in the Savant Speed Vac. 200 µl of each reconstituted sample was counted (15 minute counts) in 10 ml of Ecolite (ICN Pharmaceuticals, Costa Mesa, CA) using the Tri-Carb Analyzer (Packard Instruments, Downer's Grove, IL). 40 µl of reconstituted amino acid solution was then removed from each original vial and diluted 1:1 with 1 mM norleucine (stock: Sigma Chemical Co., St. Louis, MO). 25 µl of this dilution was then transferred to a 6 mm glass culture tube, as was 25 µl of 1 mM norleucine and 25 µl of amino acid standard solution (previously described). The tissue samples and standards were then processed and analyzed via high performance liquid chromatography as discussed previously in the above section entitled "Plasma."

Calculations

Leucine flux into plasma (Q) was determined from the following equation:

$$Q = F/Sp\ max$$

where Q (flux) is the rate of appearance (or disappearance) of leucine in the plasma pool expressed as µmoles/hour, F is the infusion rate of tracer isotope in dpm/hour, and Sp max is the specific activity of leucine at steady state.

Protein synthesis and breakdown were calculated using the following mass balance equation:

$$Q = I + B = O + S$$

where Q equals flux, I is the dietary intake of leucine (zero in the post-absorptive animal), B is the leucine derived from protein breakdown and appearing in plasma, O is the amount of leucine oxidized, and S is the leucine used in protein synthesis.

The rate of leucine oxidation was estimated from the appearance of $^{14}CO_2$ in expired breath:

$$O = E/(0.8 \cdot S_p)$$

where O is the oxidation rate of leucine expressed in μmol/min, E is the rate of $^{14}CO_2$ expired in breath in dpm/min, S_p is the specific activity of leucine in plasma at isotopic steady state in dpm/μmol, and 0.8 represents the fractional recovery of $^{14}CO_2$ in expired breath during an infusion of [^{14}C]bicarbonate, which corrects for CO_2 fixation and other losses (Ling et al., 1987).

The equation of Garlick et al (1973) was used to calculate the fractional synthetic rates (K_s) of muscle and liver protein:

$$S_b / S_i = \lambda_i / (\lambda_i - K_s) \times (1 - e^{-Kst}) / (1 - e^{\lambda it}) - K_s / (\lambda_i - K_s)$$

where S_b is the specific activity of leucine bound in protein, S_i is the specific radioactivity in the free acid - soluble pool expressed in dpm/μmol, λ_i is the rate constant for the increase in specific radioactivity in the acid - soluble free amino acid pool in days^{-1}, t is the length of [1-

^{14}C]leucine infusion in days, and K_s is the fraction of protein mass renewed each day in %/d. In this experiment, $\lambda_i = 38\ d^{-1}$, as was reported in a previous study (Laurent et al., 1984).

Statistical Analysis

Data are reported as means ± SEM (standard error of the mean). Comparisons among study groups were made by one - way analysis of variance (ANOVA) followed by pairwise multiple comparison procedures using the Student-Newman-Keuls test. A $p \leq 0.05$ was considered significant (Stat Most 2.5 for Windows / Statistical Analysis and Graphics, Data Most Corp., Salt Lake City, UT). Figures were produced using Sigma Plot Scientific Graphing Software (Jandel Scientific, San Rafael, CA).

PART III

RESULTS

Percent Protein in Liver and Rectus Muscle

The percent protein in liver for sham and injured rats is shown in Figure 1. The percent protein in liver for control animals was higher in comparison to those which received the burn/endotoxin treatment. There was no significant difference ($p \geq .05$) between groups which received the same sham/injury treatment, however there was a difference between control and burn/endotoxin animals in both diet groups.

The percent protein in rectus muscle for sham and injured rats is shown in Figure 2. The percent protein in rectus muscle for animals which received the sham/saline treatment was higher than the percentage found in animals which were burned and given endotoxin. There was a significant difference ($p \leq .05$) between control and injured animals in both diet groups but no difference between those in either diet group which were given the same sham/saline or burn/endotoxin treatment.

Percent of Plasma Leucine Flux Oxidized

The percent of plasma leucine flux oxidized for sham and injured rats is shown in Figure 3. There was no significant difference ($p \geq .05$) in percent flux oxidized between control and injured animals fed either diet formula. The percent of plasma leucine flux oxidized was modestly greater in burned animals fed the corn oil - based diet (compared to control) than in burned animals fed the fish and borage oil diet.

Plasma Leucine Oxidation

The amount of plasma leucine oxidized for sham and injured animals is shown in

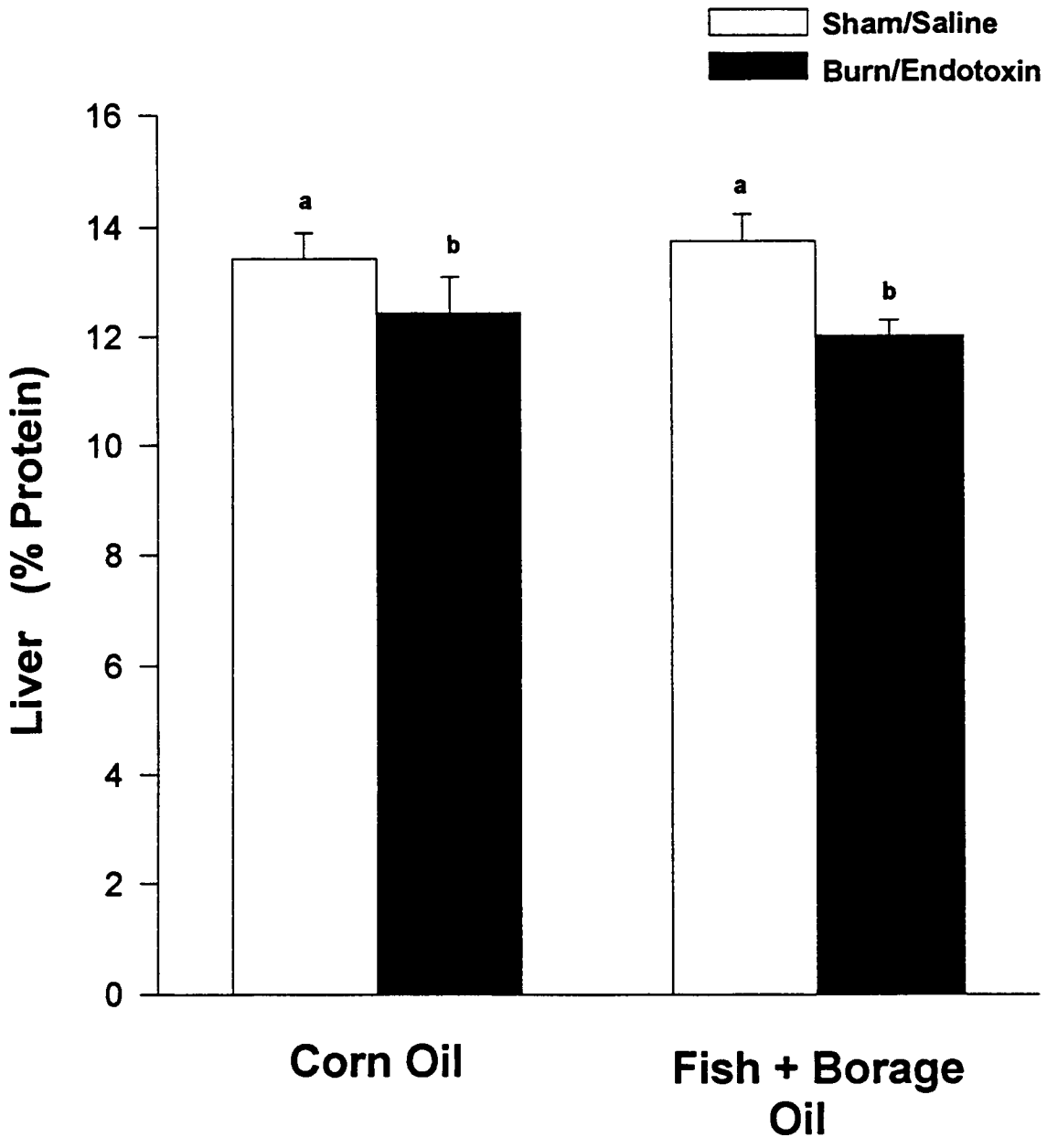

Figure 1. Percent protein in liver for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. Values with different-letter footnotes are significantly different ($p \leq .05$).

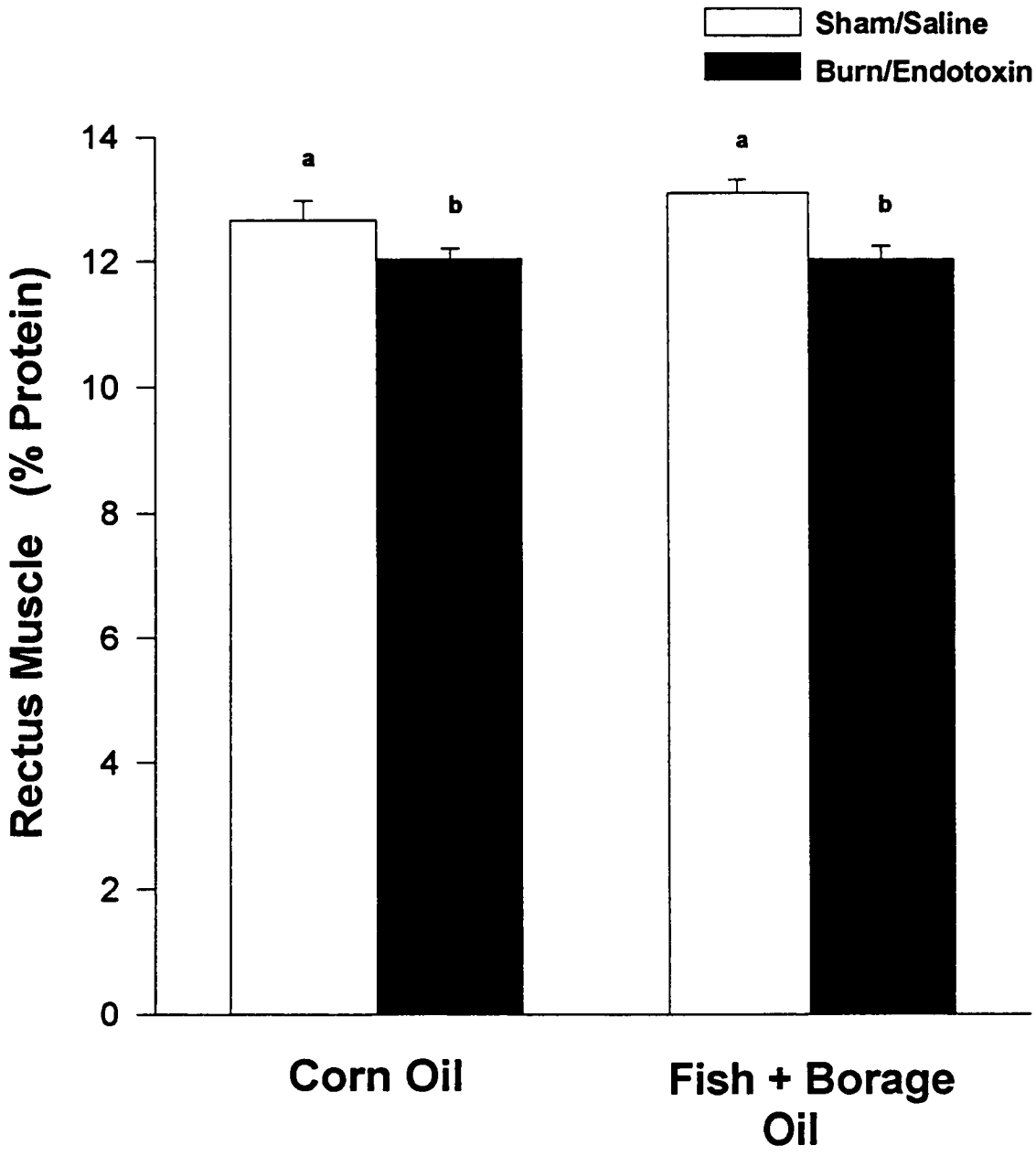

Figure 2. Percent protein in rectus muscle for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. Values with different-letter footnotes are significantly different ($p \leq .05$).

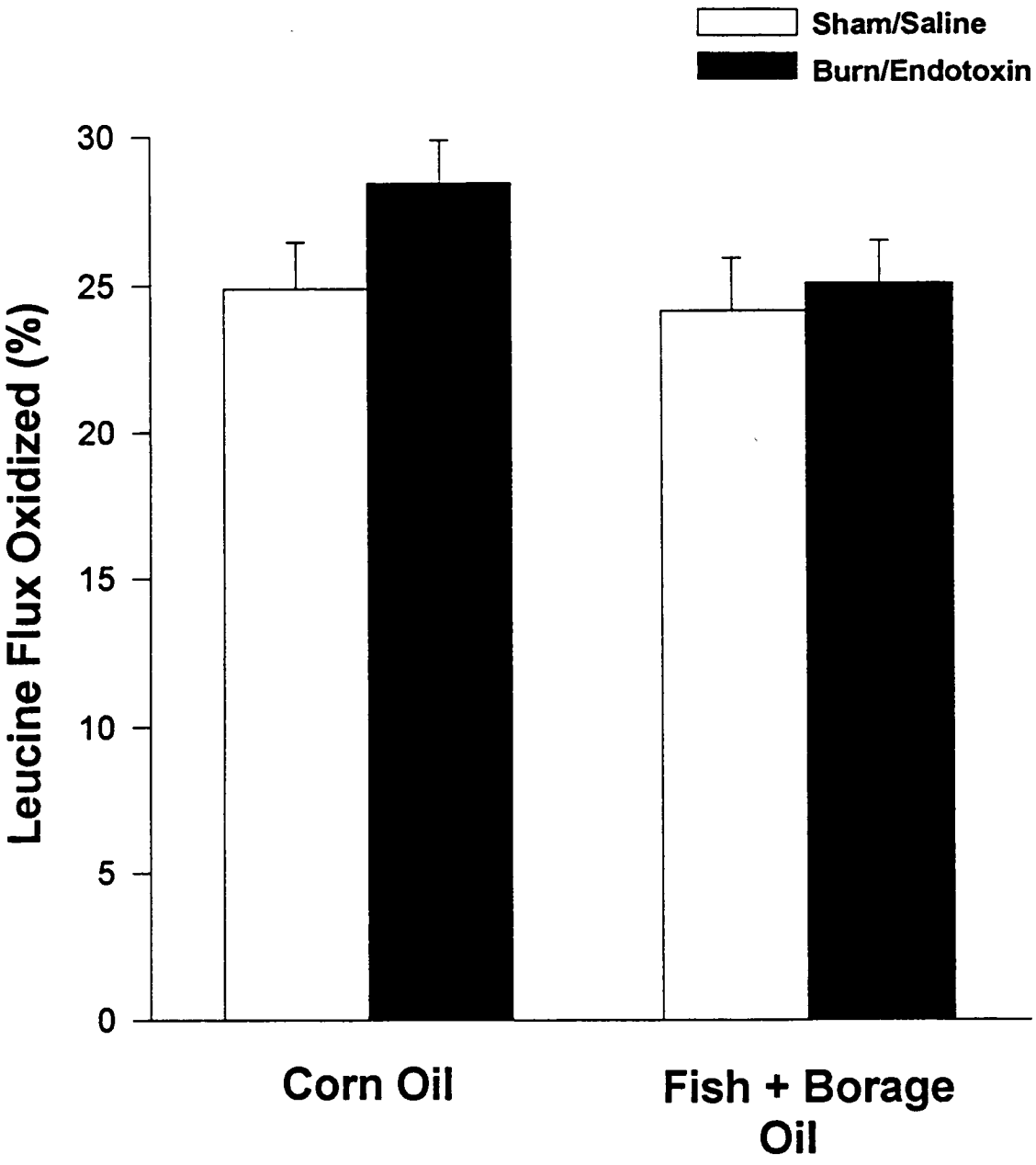

Figure 3. Percent of plasma leucine flux oxidized for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. There was no significant difference ($p \geq .05$) between groups.

Figure 4. There was no significant difference ($p \geq .05$) in leucine oxidation between groups. There was a moderate increase in the amount of leucine oxidized in burned animals which were fed the corn oil - based diet versus control. This slight increase was not observed in animals fed the fish and borage oil diet.

Protein Breakdown

The amount of leucine derived from protein breakdown appearing in plasma for sham and injured rats is shown in Figure 5. There was no significant difference ($p \geq .05$) between treatment groups fed either diet. There was a very slight decrease in plasma leucine appearance in burned animals given endotoxin compared to sham/saline - treated rats in both diet groups.

Protein Synthesis

The amount of plasma leucine used in protein synthesis for sham and injured animals is shown in Figure 6. There was no significant difference ($p \geq .05$) between control or burned animals in either diet group. Protein synthesis was modestly lower in burned animals versus control, however this difference was not statistically significant and was irrespective of diet.

Fractional Protein Synthetic Rate in Liver and Rectus Muscle

The fractional protein synthetic rate (FSR) in liver for sham and injured rats is shown in Figure 7. One-way analysis of variance showed no significant difference between sham/saline or burn/endotoxin - treated animals fed either diet. In animals fed corn oil, there

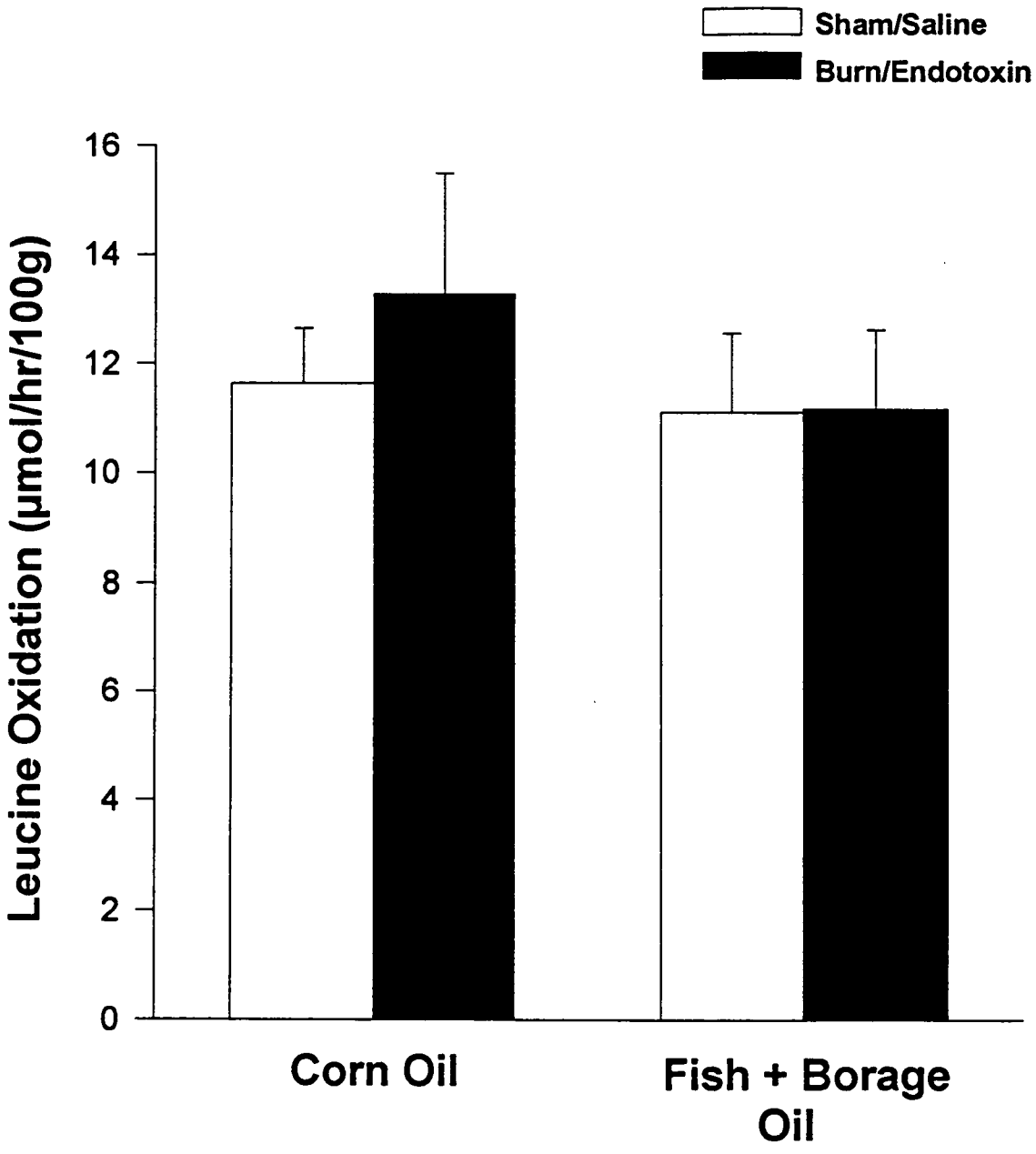

Figure 4. Total plasma leucine oxidized for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. There was no significant difference ($p \geq .05$) between groups.

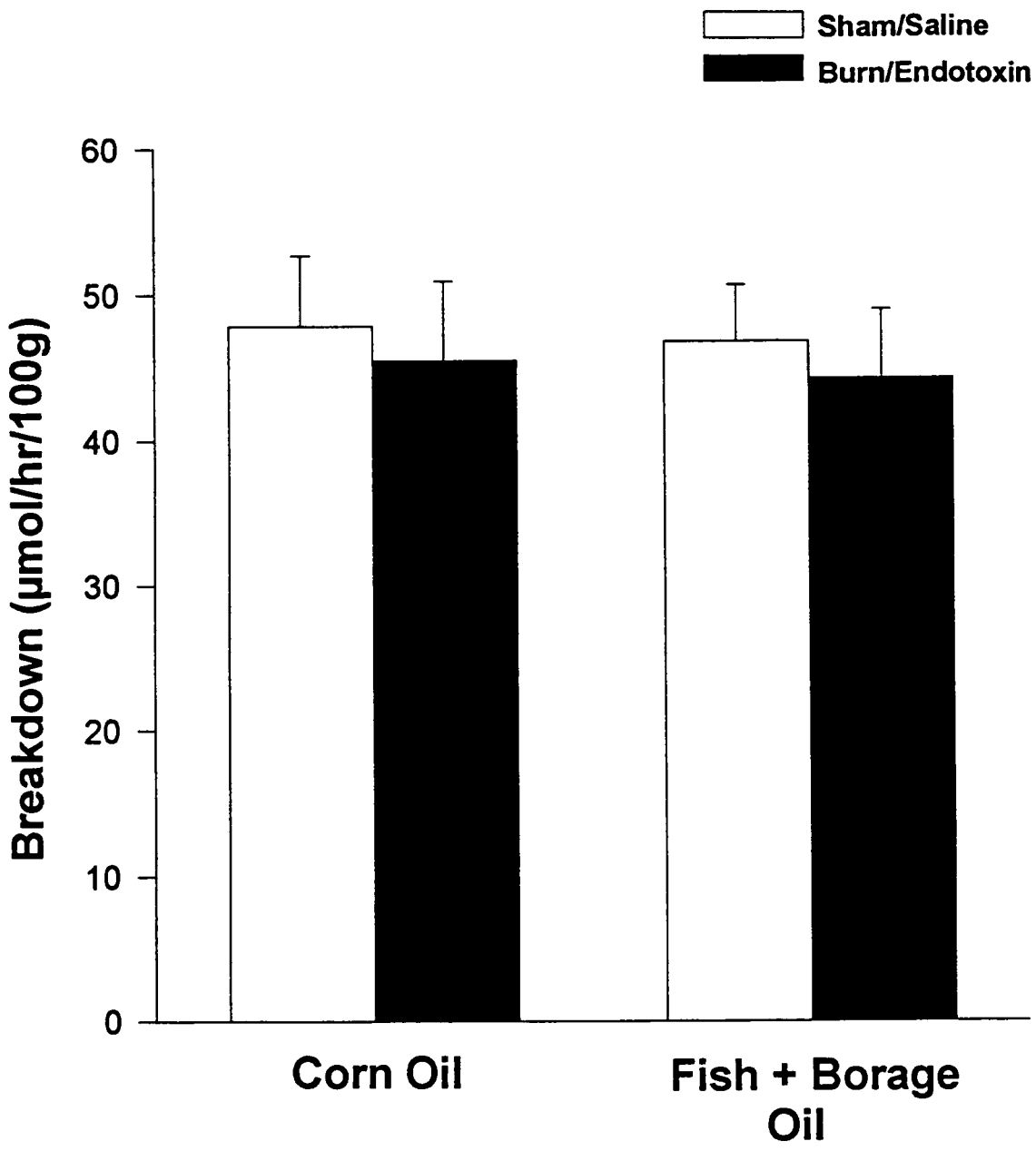

Figure 5. Amount of leucine derived from protein breakdown appearing in plasma for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. There was no significant difference ($p \geq .05$) between groups.

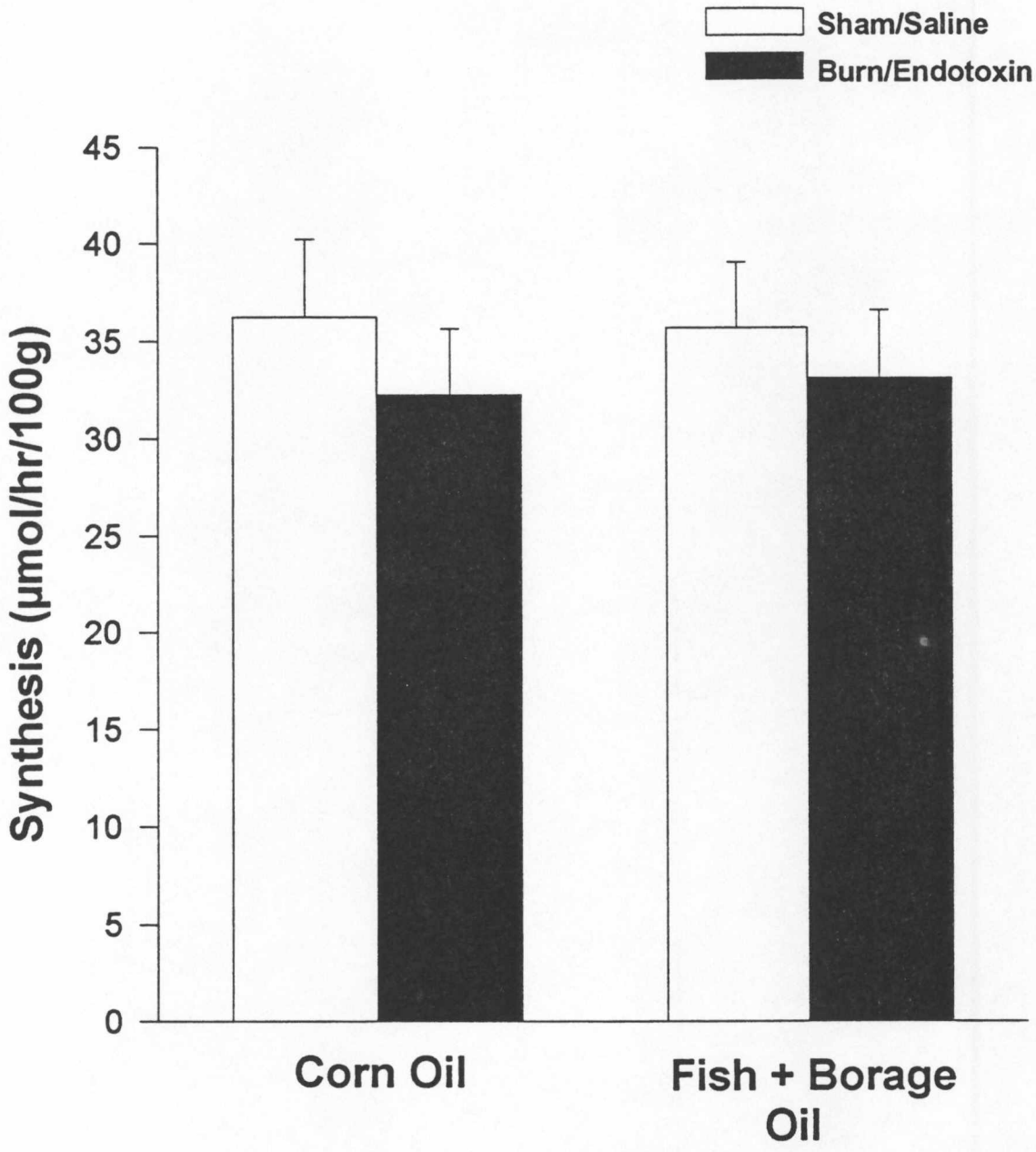

Figure 6. Amount of plasma leucine used in protein synthesis for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. There was no significant difference ($p \geq .05$) between groups.

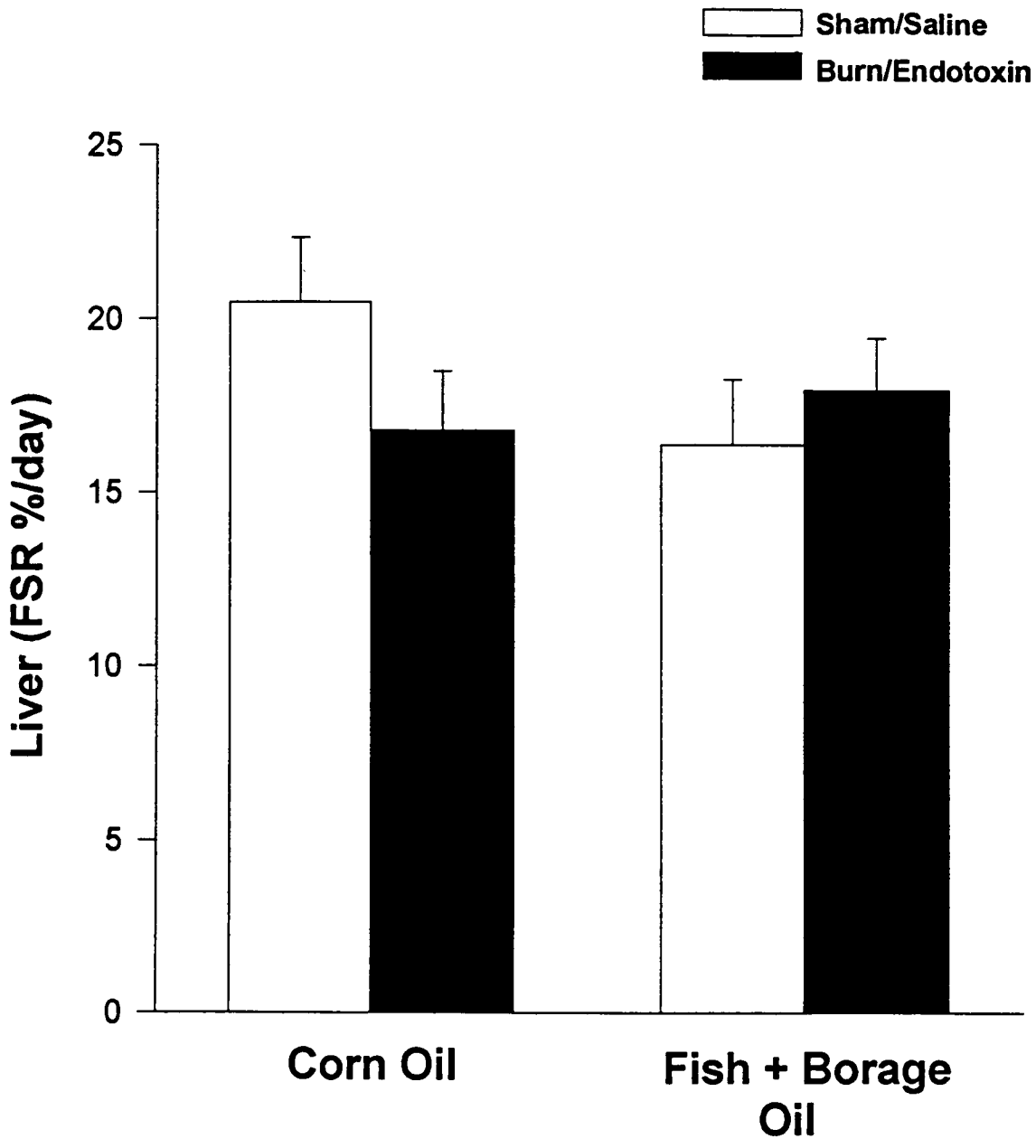

Figure 7. Fractional protein synthetic rate in liver for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. There was no significant difference ($p \geq .05$) between groups.

was a slight decrease in liver FSR in burned animals compared to control. This effect was not seen in animals fed the fish and borage oil diet, however liver FSR was lower in these control animals compared to those fed corn oil.

The fractional protein synthetic rate in rectus muscle for sham and injured animals is shown in Figure 8. The rectus muscle FSR was significantly ($p \leq .05$) lower in sham/saline animals fed fish and borage oil than those fed corn oil, however there was no significant difference between diet groups in burned animals. In animals fed the fish and borage oil diet, the rectus muscle FSR was significantly higher in burned animals than in sham/saline - treated rats.

Total Liver Weight

Total liver weight per 100 grams of body weight for sham and injured rats is given in Figure 9. In both diet groups, total liver weight was significantly higher ($p \leq .05$) in burned animals than in sham - treated rats. Total liver weight was also significantly higher in burned rats fed fish and borage oil than in injured animals fed the corn oil diet.

Total Protein Content in Liver

Total protein content in liver per 100 grams of body weight for sham and injured animals is shown in Figure 10. In animals fed both corn oil and fish and borage oil - based diets, there was a significant increase ($p \leq .05$) in total liver protein content in burned animals versus control. In addition, total protein content in liver was significantly higher in sham - treated animals fed fish and borage oil than in those fed corn oil - based diets.

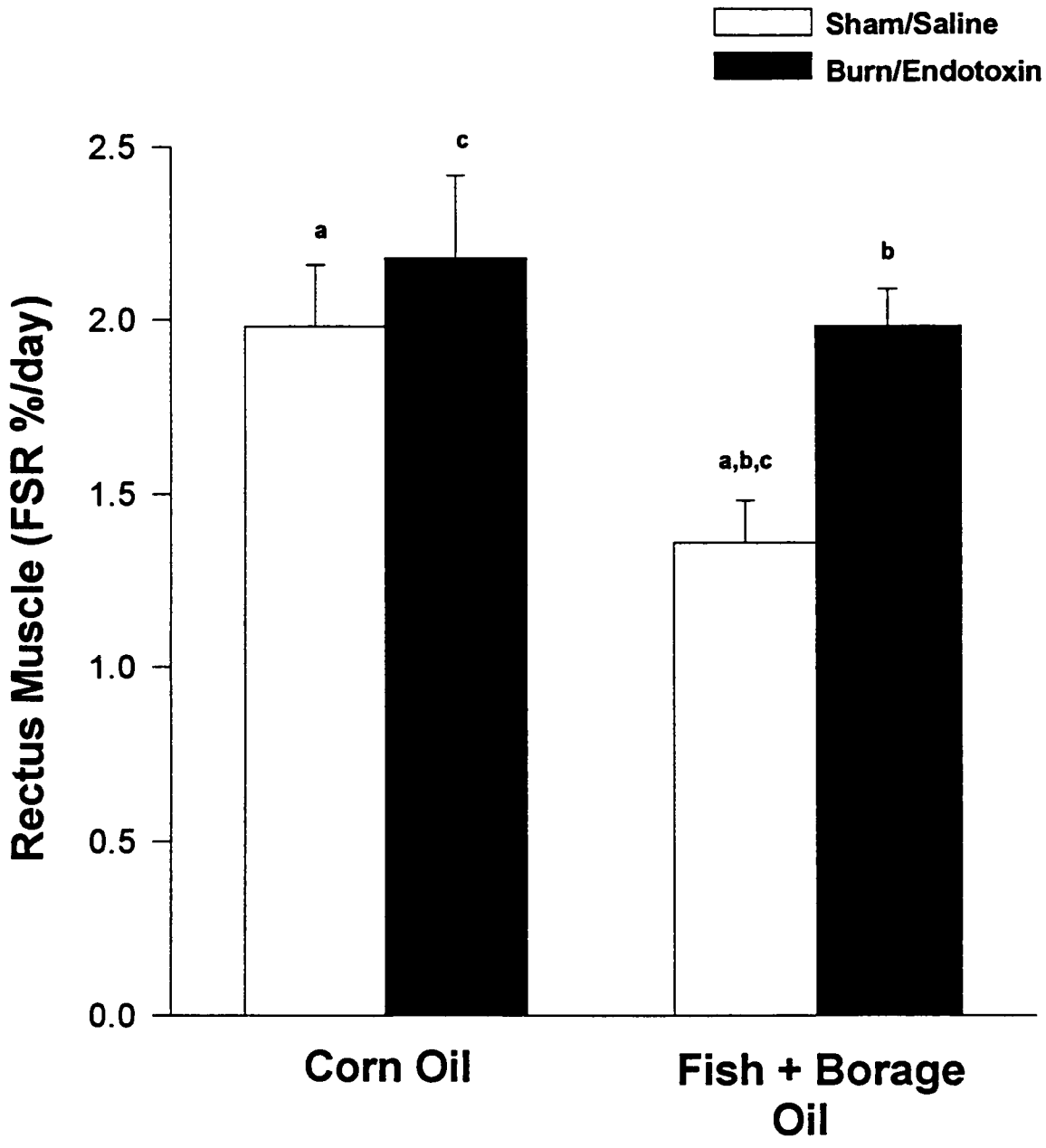

Figure 8. Fractional protein synthetic rate in rectus muscle for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. Values with the same-letter footnote are significantly different ($p \leq .05$).

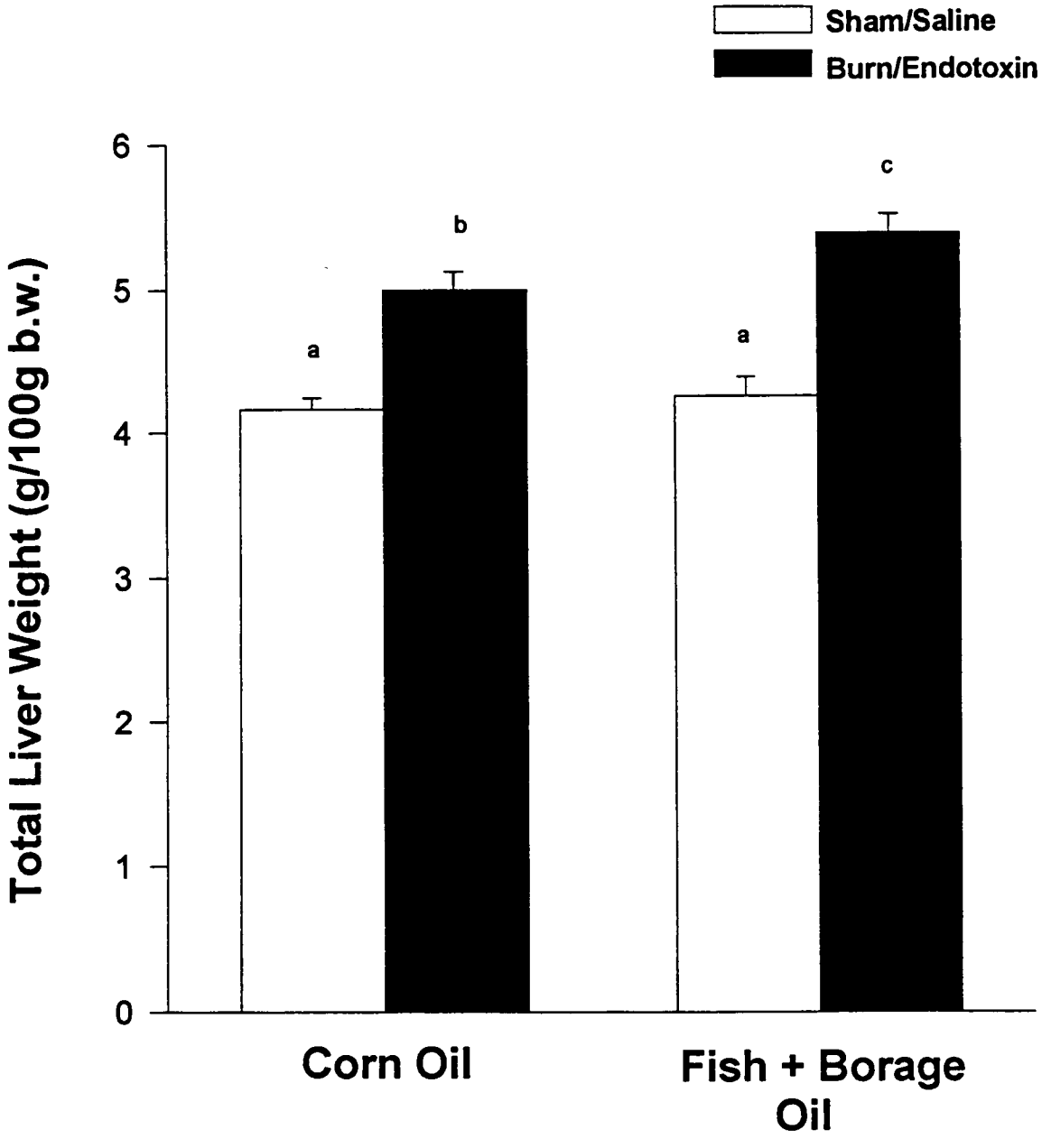

Figure 9. Total liver weight per 100 g of body weight for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. Values with different-letter footnotes are significantly different ($p \leq .05$).

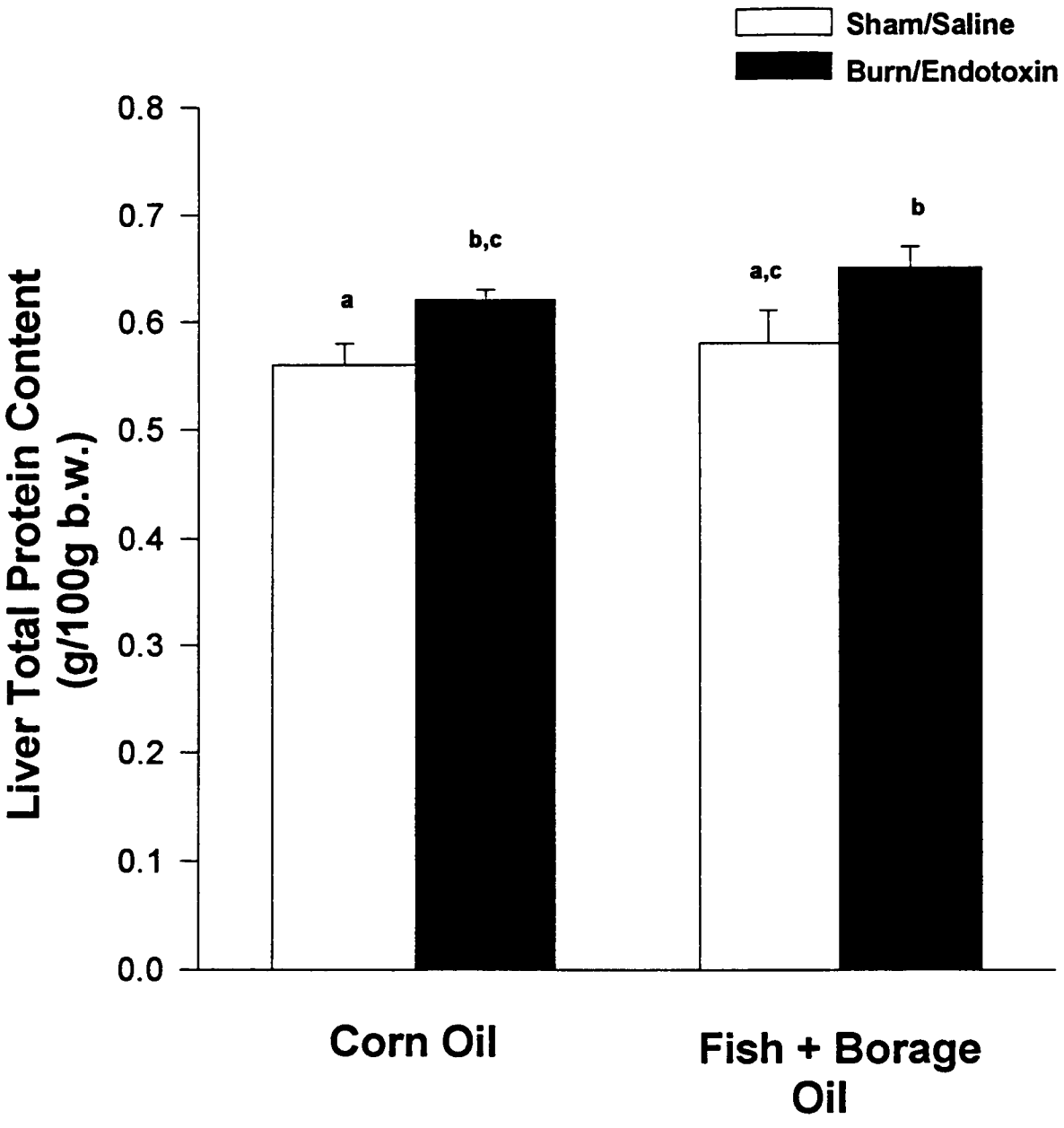

Figure 10. Total protein content in liver for sham and injured rats fed diets containing corn oil or fish and borage oil.

Values are means ± SE; n = 8-9. Values with different-letter footnotes are significantly different ($p \leq .05$).

Diet Tolerance

Intolerance to diet formulas observed as incidence of diarrhea during the 72 hour feeding period for sham and injured rats is shown in Figure 11. Incidence of diarrhea was significantly higher ($p \leq .05$) for burned animals fed the corn oil - based diet compared to all other experimental groups. Sham - treated animals fed the corn oil diet had a relatively low incidence of diarrhea on days one and two of the three day feeding period and no diarrhea on day three. Burned animals fed the corn oil diet had a moderate incidence of diarrhea (peaking at 60% on day 2) during the entire 72 hour period. Sham - treated animals fed the fish and borage oil diet had no diarrhea on days one and three and a relatively minor incidence (25%) on day two. Burned animals fed the fish and borage oil diet had a relatively minor incidence of diarrhea on days one and two and none on day three, an identical result to that seen in the sham - treated group fed corn oil.

Sham - treated animals fed the fish and borage oil diet which developed diarrhea (2 of 8) did so on day two only. Among the two sham - treated animals fed the corn oil diet which developed diarrhea, one did so only on day two and the other was symptomatic for days one and two of the three day feeding period. Of the two burned animals fed the fish and borage oil diet which developed diarrhea, one showed symptoms for days one and two and the other did so only on day two. Those burned animals fed the corn oil - based diet which developed diarrhea (6 of 9) showed symptoms for an average of all three days of the 72 hour feeding period. One of the six animals in this group with diarrhea was symptomatic for two days (day 2 and day 3).

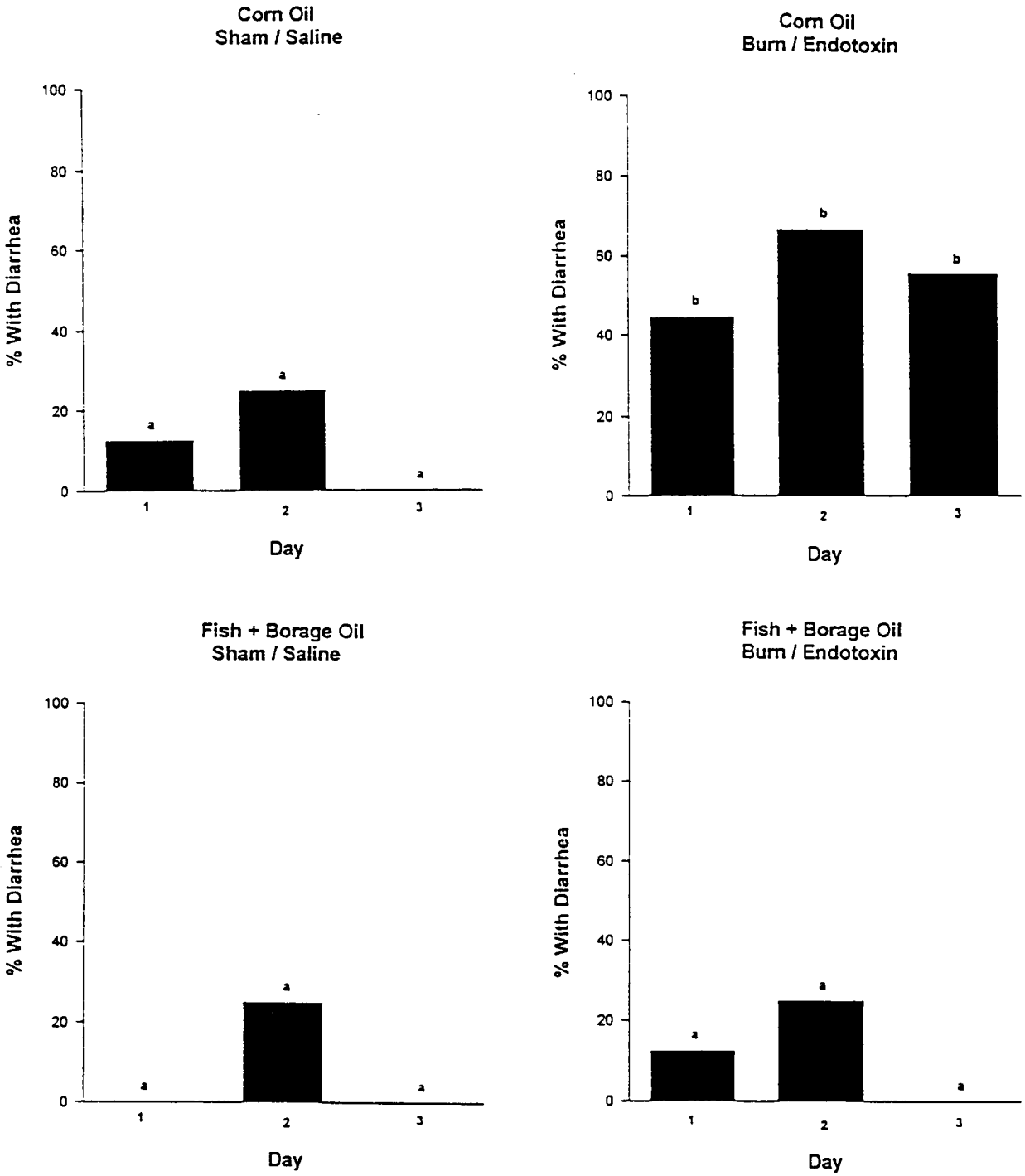

Figure 11. Incidence of diarrhea during 72 hour enteral feeding of sham and injured rats.

Values are means ± SE; n = 8-9. Values within the same day with different-letter footnotes are significantly different ($p \leq .05$).

PART IV

DISCUSSION

Recent observations have shown that the catabolic response in critical injury is not only hormonally influenced, but that cytokines and certain eicosanoid metabolites of arachidonic acid likely play a significant role in the pathogenesis of sepsis and trauma-related whole-body protein breakdown (Flores et al., 1989; Rodemann, et al., 1982). It has also been demonstrated that early enteral feeding can lessen the hypermetabolic response and support intestinal mucosal growth in burn injury (Mochizuki et al., 1984). In addition, enteral diets enriched with n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) commonly found in fish oil can modulate the synthesis of 1-, 2-, and 3- series prostaglandins (PG's) and 4- and 5- series leukotrienes (LT's) (Kinsella et al., 1990) and reduce the formation of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) by mononuclear cells (Billiar et al., 1988; Endres et al., 1989). Diets supplemented with γ-linolenic acid (GLA), which is found in high amounts in the oil of the borage plant, have been shown to suppress the production of pro-inflammatory 2- series prostaglandins and thromboxanes and 4- series leukotrienes by competing with arachidonic acid for binding sites on cyclooxygenase and lipoxygenase (Tate et al., 1989; Kunkel et al., 1981). In this study, we examined the effects of feeding enteral diets containing corn oil or a mixture of fish and borage oil for 3 days on protein metabolism in rats which were burned and then given endotoxin. We demonstrated that although there was no over-all significant difference in whole-body protein kinetics among burned animals fed either diet, there was a marked increase in animal tolerance to the formula containing fish and borage oil when compared to the conventional corn oil-based diet. These results, in conjunction with previous studies conducted in our laboratory involving lung injury and enteral nutrition with fish and borage

oil (Mancuso et al., 1997), suggest that although 3-day post-burn feeding with fish and borage oil may have no measurably different effect upon protein metabolism in burned and endotoxic rats than conventional therapies with corn oil-based diets, enteral nutrition formulas enriched with fish and borage oil *have* demonstrated substantial antiinflammatory and respiratory-protective benefits as well as important improvements in gastrointestinal tolerance when compared to similar diets containing corn oil as the fat source.

In this experiment, total liver weight and total protein content in liver was elevated in burned animals compared to control and total liver weight of those rats fed the fish and borage oil diet was slightly greater than those fed corn oil. This observation is supported by the findings of Wilmore (1990) that following burn injury, there is an increased release of amino acids from the peripheral tissues which is then accompanied by an increased uptake by the central organs, including the liver, which then uses the amino acids for such purposes as gluconeogenesis and the synthesis of inflammatory hormones, cytokines, and eicosanoids. The increase in liver size and protein content in response to burn injury can also be attributed to the increase in both splanchnic blood flow and capillary wall permeability which is a characteristic physiological event in response to a major burn (Wilmore et al., 1990).

In the leucine kinetics portion of the study, there was no significant difference between groups of injured or control animals fed either diet for nearly all of the parameters measured. These results may be explained by the duration and intensity of the metabolic response after burn injury. There are three phases in the post-burn period: early hypometabolism, the hypermetabolic state, and the recovery or anabolic phase (Cynober, 1989). At about the middle of the hypermetabolic state, the rate of net protein catabolism begins to decrease

(Cynober, 1989). It is possible that these animals had reached the anabolic recovery phase by day 3 when the kinetics experiment was performed. It is also possible that the injury model consisting of a 30% of total body surface area burn accompanied by a *one time* 1 mg/kg IP dose of endotoxin did not induce a catabolic state of the severity required to adequately compare the amino acid kinetics of an injured animal to that of a surgically stressed control animal. Yet, since all reported kinetics values were well within the accepted physiological ranges, and there were measurable and logical differences between control and burned animals, it is perhaps more likely that there was a nearly equal protein-sparing benefit to burned animals fed either diet. The aid of early enteral feeding in severe thermal injury is well documented (Mochizuki et al., 1984) and it can therefore be assumed that therapy with either of these enteral diets (in patients suitable for enteral feeding) would be more desirable than none at all. In addition, Mochizuki and colleagues (1984 and 1985) have reported that the utilization of continuous enteral tube feeding beginning immediately following burn injury can prevent the postburn hypermetabolic response. Since there were no control groups in this experiment which were fed either parenterally, not at all, or not until days after the burn injury, it is possible that the animals in these current study groups never achieved the hypermetabolic state due to having been fed immediately postburn.

The high energy and nutrient requirements of severely burned patients has created the difficulty of developing sufficient nutrition formulas which can be easily tolerated by the patient. Most often, the higher amounts of nutrients needed because of increased energy needs are a direct cause of gastrointestinal (GI) intolerance (Mochizuki, 1984). Fats containing primarily long-chain triglycerides (LCTs) such a corn oil and other vegetable oils

generally are less easily absorbed across the gut mucosa because of their high molecular weight and low solubility in water (Selleck et al., 1994). Structured lipids which contain shorter triglycerides including those found in fish oil and borage oil are rapidly hydrolyzed and absorbed because medium-chain triglycerides (MCTs) are much smaller and more water soluble than LCTs (Babayan, 1987; Jandacek et al., 1987).

In this experiment, diet intolerance was determined as incidence of diarrhea during the 72 hour feeding period immediately following the burn injury and endotoxin administration. Among control animals which received the sham/saline treatment, the occurrence of diarrhea was relatively low. On day one, none of the control animals which were fed the fish and borage oil diet developed diarrhea, while approximately 15% of those which were fed corn oil did have loose or running stools. This observation could indicate that early intolerance may be attributable to possible GI irritation resulting from post-surgical stress which appears to be avoidable by feeding a more readily absorbable MCT diet containing such fats as fish and borage oil. On day two, sham/saline animals fed both diets had an equal incidence of diarrhea which might be explained by continuing surgical stress or by simply the need of a short acclimation period to an entirely liquid diet. This proposed period of GI adjustment appeared to be over by the end of day two, as all symptoms of diarrhea in these animals were gone by the third day.

Among the animals which were burned and given endotoxin, there was a dramatically higher incidence of diarrhea seen in those fed the corn oil diet compared to the group fed fish and borage oil. On day two, the number of animals with diarrhea in the corn oil group was three times that of the fish and borage oil group. In addition, approximately half of the

burned animals fed corn oil had diarrhea also on days one and three, with the incidence on day three being greater than 50% while at the same time animals in all other groups of either treatment fed either diet had no diarrhea whatsoever. Perhaps most interesting is that the incidence of diarrhea during the 3 day feeding period among the sham/saline animals fed corn oil exactly paralleled the occurrences of GI intolerance among the *burned* animals fed the diet containing fish and borage oil. These observations indicate that although there may be some initial gastrointestinal intolerance to high-fat enteral nutrition formulas most likely due to post-surgical stress, the animals in this study tolerated the fish and borage oil diet much more readily than the corn oil-based formula, especially when challenged with both a severe burn and endotoxin.

In summary, this experiment has shown that in burned rats given endotoxin and fed enterally for 72 hours, total liver weight and total protein content in liver was increased. These observations are in accordance with previously published thermal injury data (Wilmore, 1990) which suggests that amino acid release is increased in the peripheral tissues while uptake is increased in the liver. In the liver, these amino acids are used to assemble the proteins involved in gluconeogenesis, the synthesis of various inflammatory hormones, and the production of cytokines and eicosanoids. In addition, both increased splanchnic blood flow and greater capillary wall permeability can attribute to increased liver size in response to burn injury (Wilmore et al., 1990).

In the leucine kinetics portion of this experiment, there was no significant difference between treatment groups fed either diet for most of the parameters measured. The lack of difference between any of these groups is most likely because all burned animals in this study

were fed enteral diet formulas beginning a very short time after sustaining injury. Mochizuki and colleagues (1984) demonstrated that immediate enteral feeding could reduce postburn hypercatabolism and reduce the hypermetabolic state. It is conceivable that this early diet therapy reduced the catabolic response in both diet groups to the extent that a detailed comparison between the two was not possible. It is also possible that a small one time intraperitoneal dose of endotoxin was not sufficient to maintain sepsis over the entire 72 hour period and that animals were entering the anabolic recovery phase prematurely. Perhaps in future experiments, multiple doses could be administered over the three day study to ensure that animals remain catabolic. Conversely, it is also possible that these animals never surpassed the initial hypometabolic phase of the burn injury and thus never became hypermetabolic. In order to test these theories, future studies would need to be conducted in which the feeding period is extended to greater than 72 hours, perhaps to five, seven, ten, or even fourteen days. Additional experiments involving both greater or more frequent doses of endotoxin and increased length of diet therapy would undoubtedly answer many of the remaining questions concerning protein kinetics in this study.

Perhaps the most valuable information learned from this experiment is the ability of the study animals to better tolerate the fish and borage oil diet compared to the conventional corn oil-based formula. A primary complication of high-fat enteral diets is GI intolerance, which is not only harmful to the patient because of decreased nutrient absorption and fluid loss, but it is also disconcerting to the nursing staff which is wholly responsible for patient hygiene. A better tolerated nutrition formula such as the fish and borage oil diet would not only make the job of those who care for the patient easier, but would also likely reduce

patient time spent under critical care because of the many well-documented antiinflammatory and respiratory-protective properties of these constituent fatty acids (Mancuso et al., 1997; Karlstad et al., 1996).

Enteral nutrition with high-fat, low-carbohydrate formulas containing antiinflammatory fatty acids such as eicosapentaenoic acid, docosahexaenoic acid, and γ-linolenic acid found in fish and borage plant oils may provide increased gastrointestinal tolerance and important immuno-suppressive and respiratory protective qualities (Karlstad et al., 1996) of which traditional vegetable oil-based enteral diets are incapable.

REFERENCES

Al-Saady NM, Blackmore CM, Bennett ED. (1989) High fat, low carbohydrate enteral feeding lowers $PaCO_2$ and reduces the period of ventilation in artificially ventilated patients. *Intensive Care Med* 15:290.

Alverdy JC, Aoys E, Moss GS. (1988) Total parenteral nutrition promotes bacterial translocation from the gut. *Surgery* 104:185.

Askanazi J, Nordenstrom J, Rosenbaum SH, et al. (1981) Nutrition for the patient with respiratory failure. *Anesthesiology* 54:373.

Aulick LH, Wilmore DW. (1978) Increased peripheral amino acid release following thermal injury. *Ann Surg* 188:778.

Babayan VK. (1987) Medium chain triglycerides and structured lipids. *Lipids* 22:417.

Barre DE, Holub BJ, Chapkin RS, et al. (1993) The effect of borage oil supplementation on human platelet aggregation, thromboxane B_2, prostaglandin E_1, and E_2 formation. *Nutr Res* 13:739.

Billiar TR, Bankey PE, Svingen BA, et al. (1988) Fatty acid intake and kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* 104:343.

Bittiner SB, Cartwright I, Tucker WFG, et al. (1988) A double-blind, randomized, placebo-controlled trial of fish oil in psoriasis. *Lancet* 1:378.

Bone RC. (1991) The pathogenesis of sepsis. *Ann Intern Med* 115:457.

Burzstein S, D'Attellis NP, Askanazi J. (1992) Nutrition and the respiratory system. In: Hall JB, Schmidt GA, Wood LDH, eds. *Principles of critical care*. New York: McGraw-Hill; p.1093.

Bower RH. (1993) Nutrition during critical illness and sepsis. *Crit Care Med* 1:348.

Calder PC, Bond JA, Harvey DJ, et al. (1990) Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* 269:807.

Carrick JB, Schnellmann RG, Moore JN. (1994) Dietary source of omega-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. *Shock* 2:421.

Carter JP. (1988) Gamma-linolenic acid as a nutrient. *Food Technol* June:72.

Chapkin RS, Carmichael SL. (1990) Effects of dietary n-3 and n-6 fatty acids on macrophage phospholipid classes and subclasses. *Lipids* 25:827.

Chilton-Lopez T, Surette ME, Swan DD, et al. (1996) Metabolism of gamma linolenic acid in human neutrophils. *J Immunol* 156:2941.

Cooney RN, Kimball SR, Vary TC. (1996) Regulation of skeletal muscle protein turnover during sepsis: mechanisms and mediators. *Submitted.*

Cunningham JJ, Hegarty MT, Meara PA, et al. (1989) Measured and predicted caloric requirements of adults during recovery from severe burn trauma. *Am J Clin Nutr* 49:404.

Cuthbertson DP. (1930) The disturbance of metabolism produced by bony and non-bony injury, with notes on certain abnormal conditions of bone. *Biochem J* 24:1244.

Cynober L. (1989) Amino acid metabolism in thermal burns. *JPEN* 13:196.

Das UN, Horrobin DF, Begin ME, et al. (1988) Clinical significance of essential fatty acids. *Nutrition* 4:337.

Diboune M, Ferard G, Ingenbleek Y, et al. (1992) Composition of phospholipid fatty acids in red blood cell membranes of patients in intensive care units: effects of different intakes of soybean oil, medium chain triglycerides, and black currant seed oil. *JPEN* 16:136.

Elwyn DH, Askanazi J. (1992) Fuel utilization in critical illness. In: Hall JB, Schmidt GA, Wood LDH, eds. *Principles of critical care*. New York: McGraw-Hill; p.1057.

Endres S, Ghorbani K, Kelley VE, et al. (1989) The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 320:265.

Fan YY, Chapkin RS. (1992) Mouse peritoneal macrophage prostaglandin E_1 synthesis is altered by dietary gamma-linolenic acid. *J Nutr* 122:1600.

Flores EA, Bistrian BR, Pomposelli CA, et al. (1989) Infusion of tumor necrosis factor/cachetin promotes muscle catabolism in the rat. A synergistic effect with interleukin 1. *J Clin Invest* 83:1614.

Garlick PJ, Millward DJ, James WPT. (1973) The diurnal response of muscle and liver protein synthesis, in vivo, in meal-fed rats. *Biochem J* 136:935.

Goodwin CW. (1985) Metabolism and nutrition in the thermally injured patient. *Crit Care Clin* 1:97.

Grote AE, Elwyn DH, Takala F, et al. (1987) Nutritional and metabolic effects of enteral and parenteral feeding in severely injured patients. *Clin Nutr* 6:161.

Hardardottir I, Whelan J, Kinsella. (1992) Kinetics of tumor necrosis factor and prostaglandin production by murine resident peritoneal macrophages as affected by dietary n-3 polyunsaturated fatty acids. *Immunology* 76:572.

Holman RT. (1964) Nutritional and metabolic interrelationships between fatty acids. *Fed Proc* 23:1062.

Ireton CS, Turner WW, Hunt JL, et al. (1986) Evaluation of energy expenditures in burn patients. *J Am Diet Assoc* 86:331.

Jandacek RJ, Whiteside JA, Holcombe BW, et al. (1987) The rapid hydrolysis and efficient absorption of triglycerides with octenoic acid in 1 and 3 positions and long chain fatty acid in the 2 position. *Am J Clin Nutr* 45:940.

Jeejeebhoy KN. (1995) Nutrition in critical illness. In: Ayres SM, Grenvik A, Holbrook PR, et al., eds. *Textbook of critical care*. Philadelphia: WB Saunders; p.1106.

Karlstad MD, DeMichele SJ, Leathem WD, et al. (1993) Effect of intravenous lipid emulsions enriched with gamma-linolenic acid on plasma n-6 fatty acids and prostaglandin biosynthesis after burn and endotoxin in rats. *Crit Care Med* 21:1740.

Karlstad MD, Palombo JD, Murray MJ, et al. (1996) The anti-inflammatory role of γ-linolenic and eicosapentaenoic acids in acute lung injury. In: Huang YS, Mills DE, eds. *γ-Linolenic acid: metabolism and its roles in nutrition and medicine*. Champaign: AOCS Press; p. 137.

Kelley VE, Ferretti A, Izui S, et al. (1985) A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and supresses lupus in MRL-lpr mice. *J Immunol* 134:1914.

Kinney JM. (1995) Metabolic responses of the critically ill patient. *Crit Care Clin* 11:569.

Kinsella JE, Broughton KS, Whelan J. (1990) Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis. *J Nutr Biochem* 1:123.

Kinsella JE, Lokesh B, Broughton S, et al. (1990) Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* 6:24.

Kremer JM, Jubiz W, Michalek A, et al. (1987) Fish oil fatty acid supplementation in active rheumatoid arthritis: a double-blind, controlled, crossover study. *Ann Intern Med* 106:497.

Kunkel SL, Ogawa H, Conran PB, et al. (1981) Suppression of chronic inflammation by evening primrose oil. *Prog Lipid Res* 20:886.

Lands WEM. (1992) Biochemistry and physiology of n-3 fatty acids. *FASEB J* 6:2530.

Laurent BC, Moldawer LL, Young VR, et al. (1984) Whole-body leucine and muscle protein kinetics in rats fed varying protein intakes. *Am J Physiol* 246:E444.

Leaf A, Weber PC. (1988) Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 318:549.

Lee TH, Hoover RL, Williams JD, et al. (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 312:1217.

Lee TH, Mencia-Huerta J, Shih C, et al. (1984) Characterization and biologic properties of 5, 12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J Biol Chem* 259:2383.

Leventhal LJ, Boyce EG, Zurier RB. (1994) Treatment of rheumatoid arthritis with black currant seed oil. *Br J Rheumatol* 33:847.

Ling PR, Bistrian BR, Blackburn GL, et al. (1987) Effect of fetal growth on maternal protein metabolism in postabsorptive rat. *Am J Physiol* 252:E380.

Long CL, Lowry SF. (1990) Hormonal regulation of protein metabolism. *JPEN* 14:555.

Loyd JE, Tagari P, Chapel H, et al. (1984) Persistent generation of peptido leukotrienes in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 29:A118.

Mancuso P, et al. (1994) *ALA/ATS International Conference* p.309.

Mancuso P, Whelan J, DeMichele SJ, et al. (1997) Effects of eicosapentaenoic acid and γ-linolenic acid on lung permeability and alveolar macrophage eicosanoid synthesis in endotoxic rats. *Crit Care Med* 25:523.

Manku MS, Morse-Fisher N, Horrobin DF. (1988) Changes in human plasma essential fatty acid levels as a result of administration of linoleic acid and gamma-linolenic acid. *Euro J Clin Nutr* 42:55.

Meydani SN, Endres S, Woods MM, et al. (1991) Oral (n-3) fatty acid supplementation suppresses cytokine production an lymphocyte proliferation: comparison between young and older women. *J Nutr* 121:547.

Miura S, Imaeda H, Shiozaki H, et al. (1993) Attenuation of endotoxin-induced intestinal micro-circulatory damage by eicosapentaenoic acid. *Am J Physiol* 264:G828.

Mochizuki H, Trocki O, Dominioni L, et al. (1984) Mechanism of prevention of postburn hypermetabolism and catabolism by early enteral feeding. *Ann Surg* 200:297.

Mochizuki H, Trocki O, Dominioni L, et al. (1985) Reduction of postburn hypermetabolism by early enteral feeding. *Curr Surg* 42:121.

Mori TA, Vandongen R, Macanian F, et al. (1992) Plasma lipid levels and platelet and neutrophil function in patients with vascular disease following fish oil and olive oil supplementation. *Metabol* 41:1059.

Murray MJ, Kumar M, Gregory TJ, et al. (1995) Enteral diets enriched in eicosapentaenoic acid and gamma linolenic acid attenuate cardiopulmonary dysfunction in a porcine model of acute lung injury. *Am J Physiol* 269:H2090.

Murray MJ, Svingen BA, Holman RT, et al. (1991) Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *JPEN* 15:152.

Murray MJ, Svingen BA, Yakesh TL, et al. (1993) Effects of endotoxin on pigs prefed n-3 vs. n-6 fatty acid-enriched diets. *Am J Physiol* 265:E920.

Nassar BA, Huang YS, Horrobin DF. (1986) Response of tissue phospholipid fatty acid composition to dietary (n-6) and replacement with marine (n-3) and saturated fatty acids in the rat. *Nutr Res* 6:1397.

Perlmutter D, Goldberger G, Dinarello CA, et al. (1986) Cachetin/tumor necrosis factor regulates hepatic acute phase gene expression. *J Clin Invest* 78:1349.

Pinard B, Geller E. (1995) Nutritional support during pulmonary failure. *Crit Care Clin* 11:569.

Pomposelli JJ, Flores EA, Blackburn GL, et al. (1991) Diets enriched with n-3 fatty acids ameliorate lactic acidosis by improving endotoxin-induced tissue hypoperfusion in guinea pigs. *Ann Surg* 213:166.

Pullman-Mooar S, LaPosata M, Lem D, et al. (1990) Alteration of the cellular fatty acid profile and the production of eicosanoids in human monocytes by gamma-linolenic acid. *Arthritis Rheum* 33:1526.

Rodemann HP, Goldberg AL. (1982) Arachidonic acid, prostaglandin E_2 and F_2, influence rates of protein turnover in skeletal and cardiac muscle. *J Biol Chem* 257:1632.

Salem NJ, Wegher B, Mena P, et al. (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc Natl Acad Sci* 93:49.

Selleck KJ, Wan JMF, Gollaher CJ, et al. (1994) Effect of low and high amounts of a structured lipid containing fish oil on protein metabolism in enterally fed burned rats. *Am J Clin Nutr* 60:216.

Sperling RI, Benincaso A, Knoell CT, et al. (1993) Dietary Ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J Clin Invest* 91:651.

Stubbs CD, Smith AD. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim et Biophys Acta* 779:89.

Tate G, Mandell BF, Laposata M, et al. (1989) Suppression of acute and chronic inflammation by dietary γ-linolenic acid. *J Rheumatol* 16:729.

Tredget EE, Yu YM. (1992) The metabolic effects of thermal injury. *World J Surg* 16:68.

von Schacky C, Fischer S, Weber PC. (1985) Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest* 76:1626.

Whelan J, Broughton KS, Kinsella JE. (1991) The comparative effects of dietary alpha-linolenic acid and fish oil on 4- and 5- series leukotriene formation in vivo. *Lipids* 26:119.

Wilmore DW. (1990) Pathophysiology of the hypermetabolic response to burn injury. *J Trauma* 30:S2.

Wilmore DW, Goodwin CW, Aulick LH, et al. (1990) Effects of injury and infection on visceral metabolism and circulation. *Ann Surg* 192:491.

Wilmore DW, Long JM, Mason AD, et al. (1974) Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653.

Wojnar MM, Hawkins WG, Lang CH. (1995) Nutritional support of the septic patient. *Crit Care Clin* 11:717.

Wright S, Burton JL. (1982) Oral evening primrose seed oil improves atopic eczema. *Lancet* 2:1120.

Ziboh VA, Fletcher MP. (1992) Dose-response effects of dietary γ-linolenic acid-enriched oils on human polymorphonuclear - neutrophil biosynthesis of leukotriene B_4. *Am J Clin Nutr* 55:39.

VITA

James Christopher Sherrell was born in Knoxville, Tennessee on March 29, 1972. He attended schools in the public system of Knoxville, Tennessee, the Knox County and Knoxville City Public Schools System, where he graduated from Bearden High School in May, 1990. He entered Wake Forest University, Winston-Salem, North Carolina during August of 1990 where in May, 1994 he received the Bachelor of Arts degree in History with a minor in Biology. He entered The Graduate School at The University of Tennessee, Knoxville in August, 1994 first in the Master's program in Zoology and then switching to the Department of Comparative and Experimental Medicine in January, 1996. The Master of Science degree in Comparative and Experimental Medicine was awarded in August, 1997.

He is presently living in Memphis, Tennessee with his wife Regina while working toward the Doctor of Medicine degree at The University of Tennessee, Memphis College of Medicine.