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NAFTIDROFURYL, EXOGENOUS ENERGY SUBSTRATES AND THE METABOLIC RESPONSE TO SURGERY

By

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Entre le poignard de l'assasin et le bistouri du chirurgien, il y a une différence d'intention, non de moyens.

R. Leriche

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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A major metabolic effect of injury is to increase the net degradation of muscle proteins to provide precursors for alternative energy substrates and maintain synthesis of visceral and acute phase proteins. Loss of a small proportion of total body protein is probably inevitable and in the majority of cases causes little harm. However, if the injury is severe, the response to it prolonged and the nutritional intake inadequate, then wasting of muscle proteins is marked and the immune system and healing process are compromised resulting in increased morbidity.

Recently, there has been much interest in the possibility of pharmacological manipulation of the metabolic response to injury and previous work had suggested that naftidrofuryl oxalate may improve nitrogen balance following elective abdominal surgery.

Studies in this thesis investigated the effect of naftidrofuryl oxalate on the metabolic response to elective abdominal surgery in patients who received a peripheral intravenous infusion of dextrose-saline (25 kJ kg^{-1} day^{-1}) or isotonic amino acids only (0.15 g nitrogen kg^{-1} day^{-1}) or a central venous infusion of glucose (50 kJ), amino acids (0.15 g nitrogen) and fat (50 kJ) kg^{-1} day^{-1}.

The infusion of 200 mg naftidrofuryl oxalate twice daily resulted in attenuation of the expected post-operative rise in venous blood lactate and pyruvate concentrations and also in reduction of the lactate to pyruvate ratio in the glucose-containing infusion regimens. However, these potentially beneficial changes were not associated with any effects on other intermediary metabolites or the daily or cumulative 3 and 6-day nitrogen balance.

Comparison of the three infusion regimens showed that the provision of isotonic amino acids only resulted in increased ureagenesis with no improvement in nitrogen balance compared to the dextrose-saline infusion. The provision of glucose, amino acids and fat resulted in great improvement in nitrogen balance compared to the other two infusion regimens, thereby confirming the beneficial use of such an infusion.

DEDICATION

This thesis is dedicated to my wife, Marie-Rose and my children, Anthony and Nadine who, most unknowingly, have made great contributions to it and to the memory of my father who taught me that success requires honesty, and perseverence.

STATEMENT OF ORIGINALITY.

The work done in this thesis is the original work of the author with help from others as stated in 'acknowledgements'.

This work has not been submitted to any other university.

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GLOSSARY OF ABBREVIATIONS NOT DEFINED IN THE TEXT.

Co A	: coenzyme A
^{o}C	: degree(s) centigrade
e.g.	: for example (exempli gratia)
et al.	: and others (et alii)
FG	: French gauge
d	: deci- (10^{-1})
g	: gram(s)
hr	: hour(s)
i.e.	: that is (id est)
k	: kilo- (10^{3})
l	: litre
l/l	: litre per litre whole blood
M	: molar (mole(s) per litre)
M	: mega- (10^{6})
m	: meter(s)
m	: milli- (10^{-3})
min	: minute(s)
mol	: mole(s)
n	: nano- (10^{-9})
pH	: negative logarithm of the hydrogen ion concentration.
(R)	: registered trademark
RPM	: revolution(s) per minute
U	: unit(s)
µ	: micro- (10^{-6})
vs	: versus
w/v	: weight for volume
<	: less than
>	: greater than
$\pm$	: plus or minus
%	: per cent

SECTION I

INTRODUCTION

CHAPTER 1 BACKGROUND TO THE STUDIES

CHAPTER 2 REVIEW OF NORMAL METABOLISM
A simplified description of the normal metabolism in the fed and fasting states

CHAPTER 3 METABOLIC RESPONSE TO INJURY
A description of the phases of the response and the endocrine and metabolic changes and their relationship

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CHAPTER 1

BACKGROUND TO THE STUDIES

Increased urinary nitrogen excretion and reduced synthesis of tissue protein are accepted features of the metabolic response to injury (Cuthbertson 1930; O'Keefe et al., 1974). Garrow et al. (1965) have shown that man can only tolerate the loss of about one third of his body nitrogen, and Lawson (1965) noted the poor chances of survival in patients losing more than 30% of their body weight in a short time. However, this 'autocannibalism' is found in the majority of patients suffering accidental injury or undergoing elective or emergency surgery with a complete recovery without complications. In these cases, loss of a small proportion of the total body proteins is probably inevitable and causes little harm. However, if the injury is severe, the response to it prolonged and the nutritional intake inadequate, then wasting of muscle proteins is marked and the healing process and immune system are compromised resulting in increased morbidity and mortality. Therefore, there may be an unduly high morbidity resulting not directly from the injury but from the subsequent metabolic response.

Attempts to alter what might otherwise be termed as the normal response to injury has attracted the attention of clinical investigators for years. Recently, there has been much interest in the possibility of pharmacological manipulation of this response. Several workers have suggested that inhibition of intracellular metabolic pathways may be the basis of abnormal protein metabolism after injury (Border at al., 1970; Burns et al., 1978; Ryan, 1976). Pharmacological stimulation of cellular metabolism may therefore be a means of improving nitrogen balance.

Naftidrofuryl has been shown to have several metabolic effects and could conceivably stimulate cellular metabolism after injury. It reduces lactate accumulation in ischaemic brain tissue (Maynaud et al., 1973). It increases ATP content of ischaemic muscle (Elert et al., 1976) and improves aerobic metabolism in normal exercising man (Shaw and Johnson 1975).

Burns et al. (1981) claimed that naftidrofuryl infusion improved nitrogen balance in patients undergoing elective surgery of moderate severity. The aim of the work presented in this thesis was to investigate this claim in surgical patients receiving not only dextrose-saline (as in the study of Burns et al., 1981) but also exogenous energy substrates (amino acids alone and amino acids + fat + glucose) to study their possible interaction with such a nitrogen sparing effect of naftidrofuryl. The study of Burns et al. (1981) did not include data on substrate metabolism and was limited to the nitrogen losses on the operative and first two post-operative days. Maximum nitrogen excretion may, however, occur beyond this period (Swaminathan et al. 1979). Therefore, the studies in this thesis were carried out over the operative and first five post-operative days and included detailed biochemical investigations so that the mode of action of naftidrofuryl could be more clearly examined. In addition, a separate group of patients was studied to determine whether the nitrogen loss was also reduced when naftidrofuryl was started post-operatively as would be the case in traumatic injury.

To determine the metabolic response to injury and/or the therapeutic effect of an infusion regimen or a drug, it is necessary to ensure that the study period is of sufficient duration for the results to reliably represent the overall response. Additionally, it is also important to study the daily pattern as it gives information on the possible variations and time course of the response. In practice, clinical studies are limited by ethical considerations, patients' compliance and

investigators work loads. Thus, some investigators have studied the metabolic response to injury for 2-3 days (O'keefe et al., 1974; Burns et al., 1981; Inglis et al., 1983) whereas others have studied it for a longer period (Cuthbertson et al., 1972; Harris et al., 1982; Leander et al., 1985).

In this study, the assumption is made that a 6-day period is a valid indicator of the metabolic response to injury and/or treatment although no information is available on the extent or duration of the catabolic phase. Therefore, the studies presented in this thesis were carried out over 6 days.

CHAPTER 2

REVIEW OF NORMAL METABOLISM

The energy required for body function is provided by metabolic substrates which are derived from diet and storage depots (e.g. fat and glycogen) and are transported to tissues by the blood stream. The main substrates which can be directly utilised as a source of energy are: glucose, lactate, fatty acids, ketone bodies and amino acids.

Glucose

The major carbohydrate metabolite is glucose which is utilised by all tissues. It is released into the blood stream by the liver and to a small extent by the kidneys. It is the main source of energy for brain, erythrocytes, leucocytes, renal medulla, retina, intestinal mucosa and skeletal muscle (during resting and short-term exercise).

Lactate

Lactate is derived from glucose by glycolysis, particularly in muscle (figure:2-1). The role of lactate as a fuel is of importance in severe exercise where lactate is readily formed due to anaerobic glycolysis and in fasting where lactate is also an important glucose precursor (Cahill, 1970). Lactate is mainly removed from the blood by the liver and kidneys (Alberti and Nattrass, 1977).

Fatty acids

Fatty acids are formed by the hydrolysis of triglycerides and are a major source of energy for liver, kidney cortex, cardiac muscle and skeletal muscle (during severe exercise).

Ketone bodies

The ketone bodies, acetoacetate and 3-hydroxybutyrate, are derived from the beta-oxidation of fatty acids in the liver. They can be oxidised by extra-hepatic tissues such as brain, cardiac muscle, renal cortex and skeletal muscle.

Amino acids

The majority of amino acids are degraded in the liver and kidney. However, the branched-chain amino acids (BCAA: valine, leucine and isoleucine) differ from the other amino acids in that the enzymes responsible for initiating their catabolism are found predominately in muscle; they are utilised to only a slight extent by the liver (Miller, 1962). During starvation and exercise, BCAA serve as important fuels for energy production in muscle.

Glucose, lactate, fatty acids, ketone bodies and amino acids are all oxidised via a common terminal metabolic pathway, the tricarboxylic acid cycle (figure:2-3). This yields carbon dioxide, water and energy. The resulting energy is stored as high energy bonds in adenosine triphosphate (ATP) which is formed in the respiratory chain. ATP can be used to perform work (e.g. muscular, secretory) or to liberate heat in order to maintain body temperature.

Alterations in intermediary metabolism during the fed and fasting states

During the fed state, surplus carbohydrate is converted to fat which is laid in the fat depots and to glycogen which is stored in the liver and muscle. However, the energy that can be stored as glycogen is comparatively low and represents around one day's need. Glucose requirements are usually provided by the average dietary intake but following a 24-36 hours fast, when glycogen stores are depleted, glucose is synthesised in

the liver and to a lesser extent in the kidneys from non-carbohydrate sources. Quantitatively, the most important precursors for gluconeogenesis are lactate, amino acids and glycerol. Lactate is produced by glycolysis mainly in muscle (figure:2-1) and is transported to the liver via the blood for conversion back to glucose. The majority of amino acids are gluconeogenic, though some, notably leucine, are ketogenic. Alanine is quantitatively the most important gluconeogenic amino acid (Felig, 1973). When required, triglyceride stores in adipose tissue are mobilised and after hydrolysis yield fatty acids and glycerol. The fatty acids are re-esterified to triglyceride or oxidised to acetyl CoA and either join the tricarboxylic acid cycle (TCA) to be completely oxidised, or else form ketone bodies. For acetyl CoA to be utilised in the TCA cycle, it must combine with oxaloacetate, which is an end-product of carbohydrate catabolism, arising from the conversion of pyruvate to oxaloacetate (figures:2-3 & 2-5). However, with impaired or limited glucose availibility (i.e. prolonged fasting), the citric acid cycle is rate-limited by the diminished amount of oxaloacetate present and thus fatty acid breakdown is shunted by way of acetyl CoA into ketone formation (figure:2-5). Ketones are the main source of body fuel during prolonged fasting, and appear to minimise amino acid release from muscle, thus aiding protein conservation (Marliss and Nakhooda, 1974; Landaas, 1977; Rich and Wright, 1979).

If more protein is ingested than is needed for growth and replacement of tissues, degradation and not storage of the excess amino acids occurs. The excess amino acids are partly oxidised as a fuel and partly converted to carbohydrate and fat for storage, their amino groups being excreted as urea in the urine. Thus, adipose tissue is the main energy store in man, and triglycerides may be synthesised from dietary fats or be formed from carbohydrate or protein breakdown.

Carbohydrate, fat and protein are digested in the alimentary canal before absorption. With the exception of long-chain fats (chain length greater than 11 carbon atoms), transported as chylomicrons by the lymphatic ducts to the systemic circulation, the liver receives all products of digestion directly via the portal circulation. The liver has a fundamental role in the control of metabolism. It has enzymes responsible for the synthesis and degradation of carbohydrate, fat and protein. Only the liver, and to a lesser extent the kidneys, possess the enzyme glucose-6-phosphatase (figure:2-4) which is necessary for the release of glucose into the blood circulation.

The liver is particularly responsible for maintaining the blood glucose concentration by the following regulatory mechanisms (figure:2-4):

a)- Interconversion of glycogen and glucose.

b)- Conversion of other hexoses (e.g. fructose, galactose) to glucose.

c)- Regulation of the removal of glucose from the blood and its conversion to triglyceride.

d)- Regulation of glucose formation from gluconeogenic amino acids (e.g. alanine).

Skeletal muscle also plays an important role in the metabolism of glucose and amino acids. Alanine is the major gluconeogenic amino acid in man (Felig, 1973), being released from muscle and forming glucose in the liver. This glucose may be taken up again by muscle and and the pyruvate thus formed be transaminated with NH_2 groups, obtained from amino acids (mainly branched-chain amino acids), to release alanine again. This is known as the glucose-alanine cycle (figure:2-2). The NH_2 formed from alanine degradation in the liver are excreted as urea in the urine.

The standard unit of heat energy is the calorie (cal) which is defined as the amount of heat energy necessary to raise the temperature of 1 g of water 1 centigrade degree, from 15^{o} to 16^{o} C. The unit commonly used in physiology and medicine is the kilocalorie (kcal) which equals 1000 calories. However, the calorie is not included in the Système International d'Unités and the joule (J) is used for both energy and heat. 1 kilocalorie (kcal) equals 4.186 kilojoules (kJ).

The catabolism of carbohydrate and proteins yields 4.1 kcal (17.2 kJ) per gram while fat yields 9.3 kcal (38.9 kJ) per gram.

Figure:2-1 GLUCOSE-LACTATE CYCLE

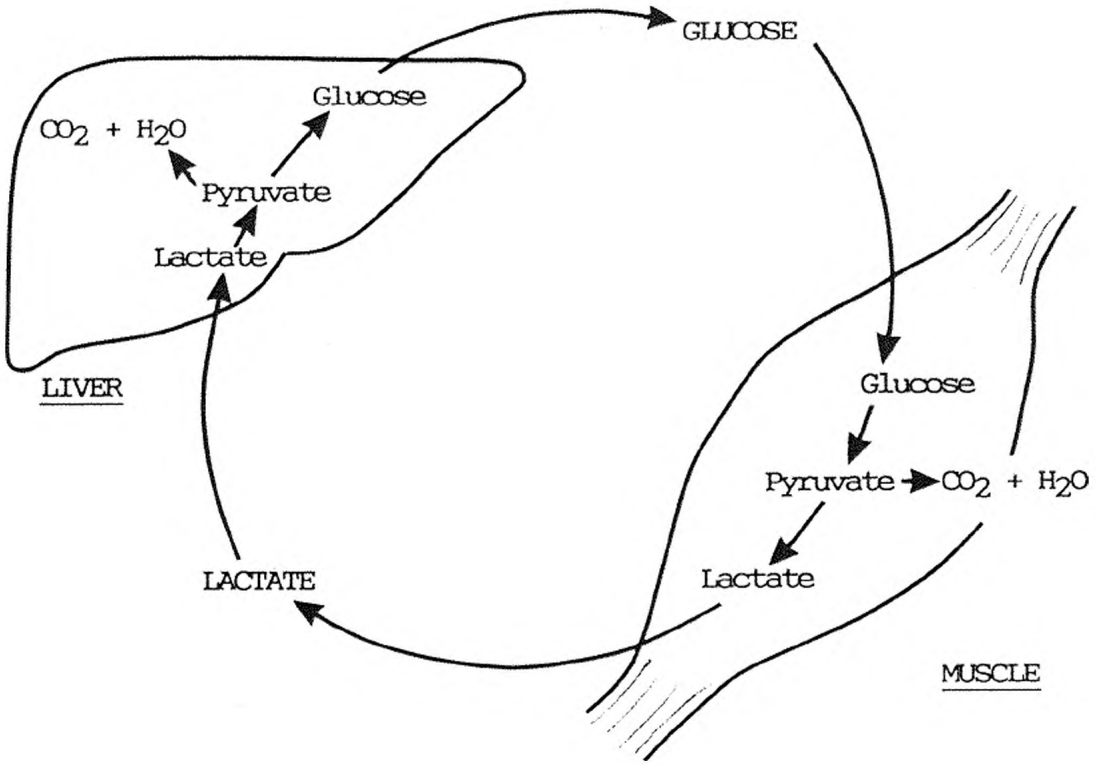


Figure:2-2 GLUCOSE-ALANINE CYCLE

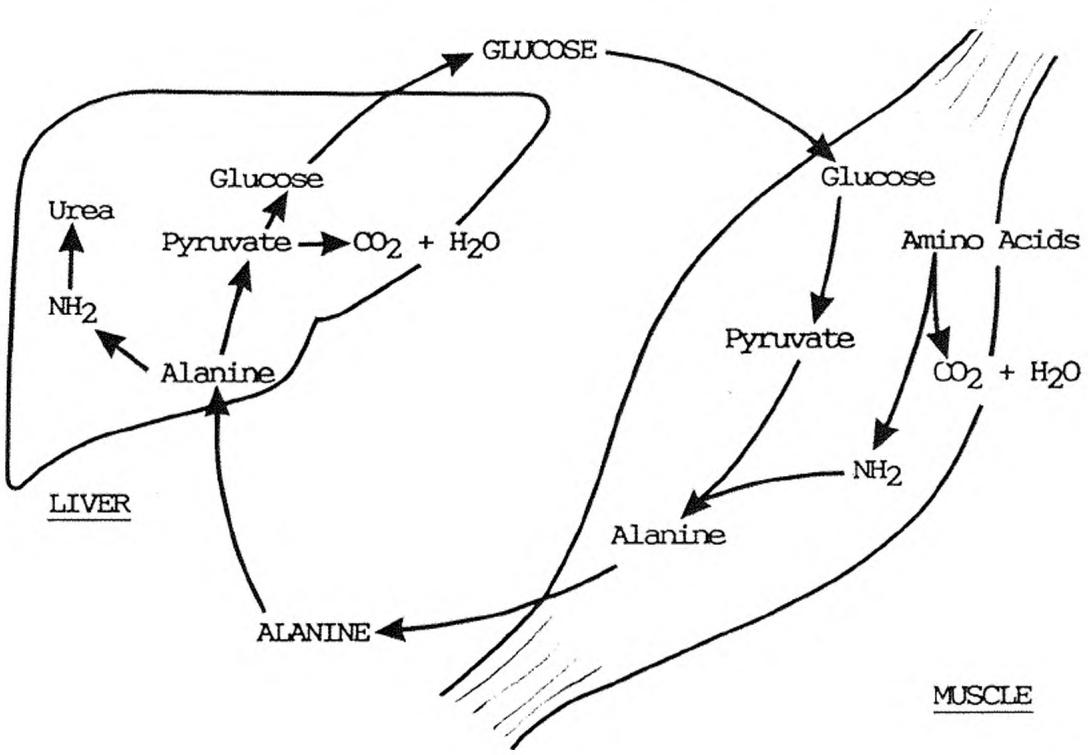
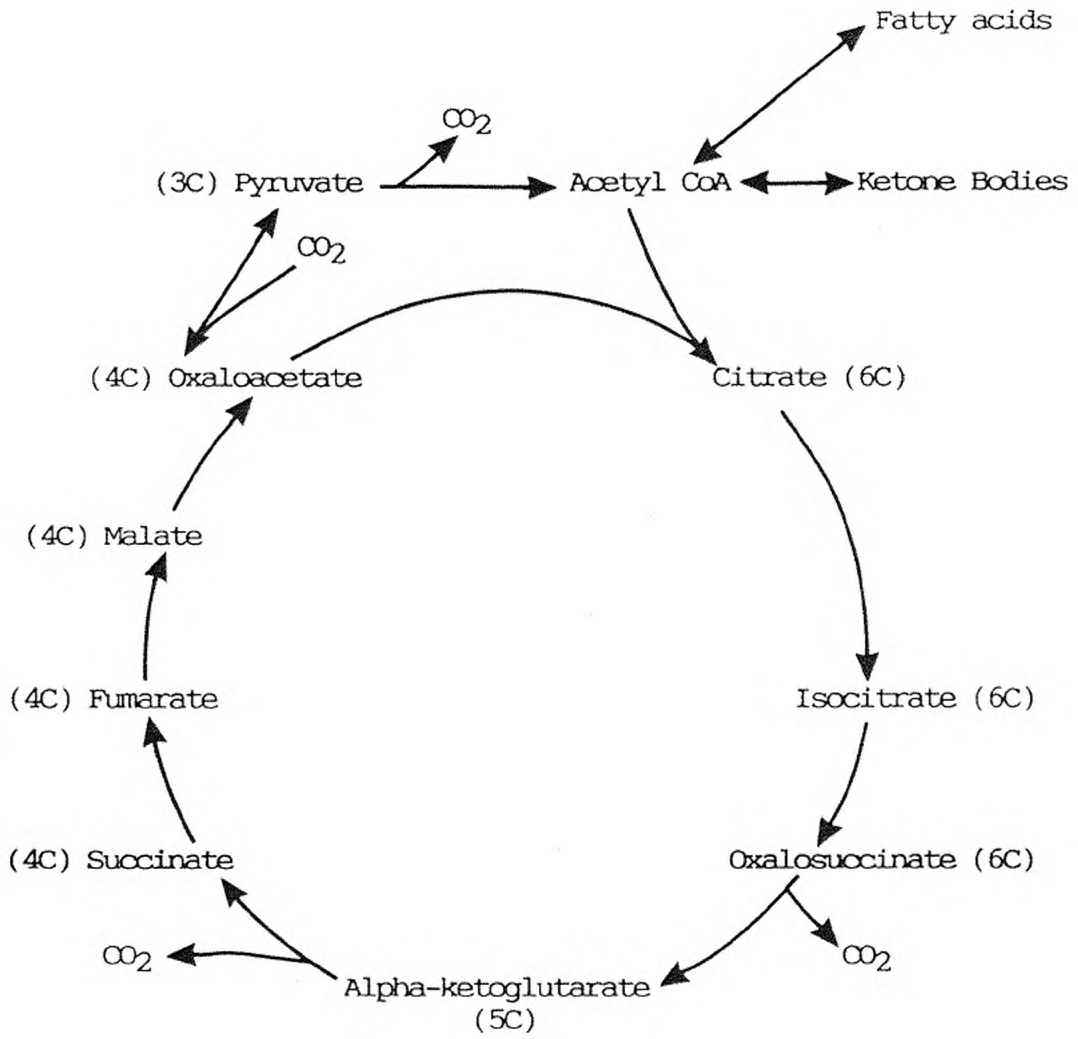
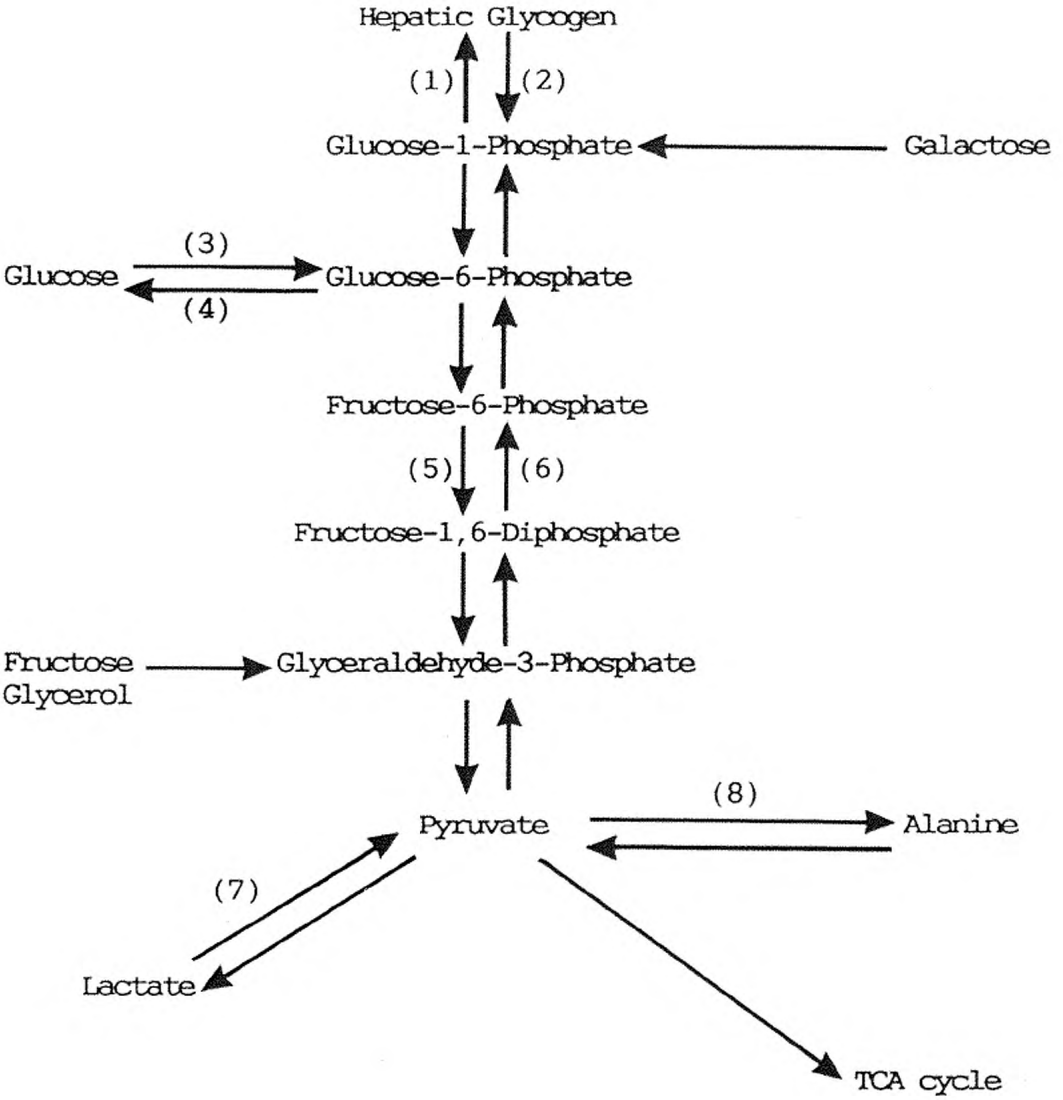


Figure:2-3 MAJOR STEPS IN TRICARBOXYLIC ACID CYCLE



The figures between brackets (6C, 5C, 4C, 3C) indicate the number of carbon atoms in each of the cycle intermediates. The oxidation of 1 mole of acetyl CoA yields carbon dioxide and water and generates by oxidative phosphorylation a total of 12 moles of ATP (adenosine triphosphate).

Figure:2-4 MAJOR STEPS IN HEPATIC GLYCOLYSIS AND GLUCONEOGENESIS



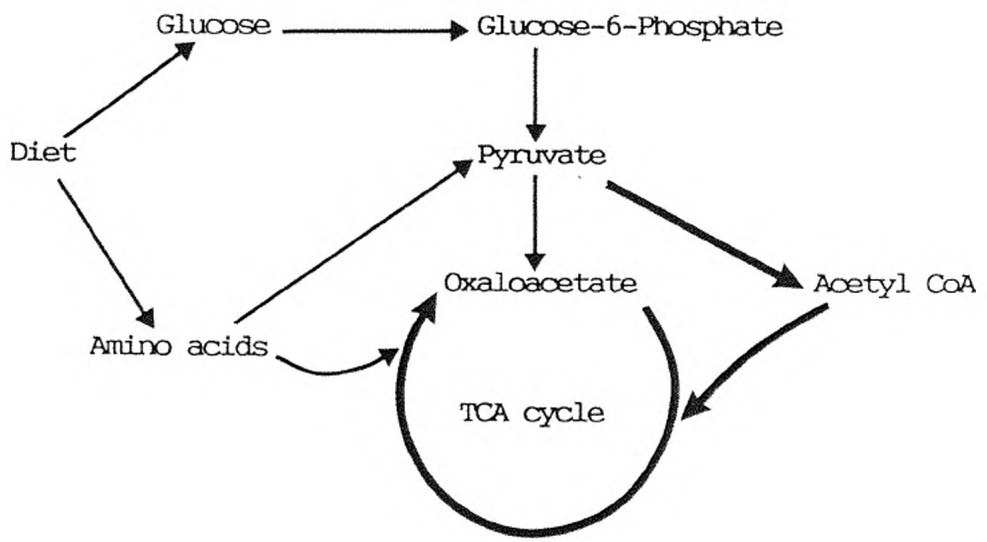
Enzyme(s) for reaction:

(1) Glucose-1-phosphate uridyl transferase and glycogen synthetase
(2) Phosphorylase
(3) Hexokinase
(4) Glucose-6-phosphatase
(5) Phosphofructokinase
(6) Fructose-1,6-diphosphatase
(7) Lactate dehydrogenase
(8) Alanine aminotransferase

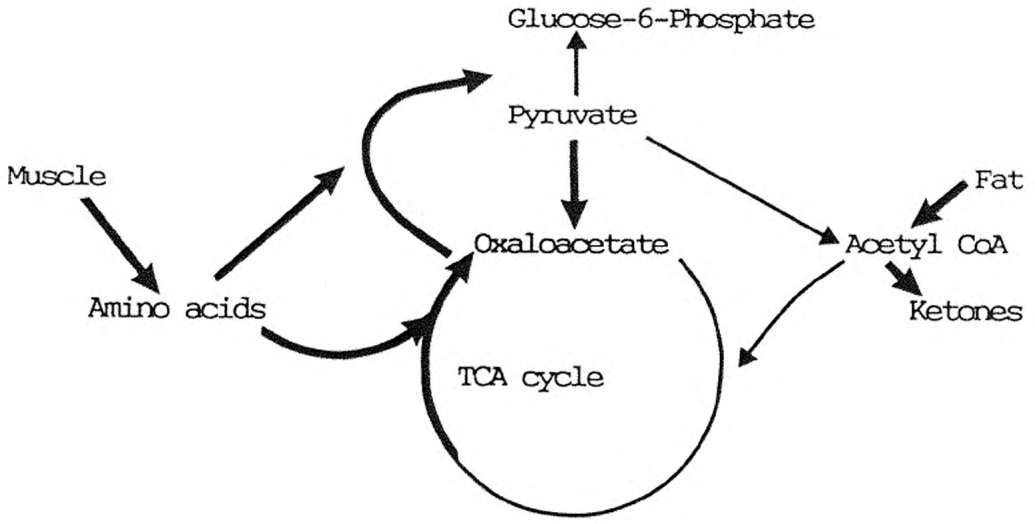
Figure:2-5 THE FLOW OF SUBSTRATE DURING THE FED AND FASTING STATES

(After Wilmore, 1977c)

FED STATE



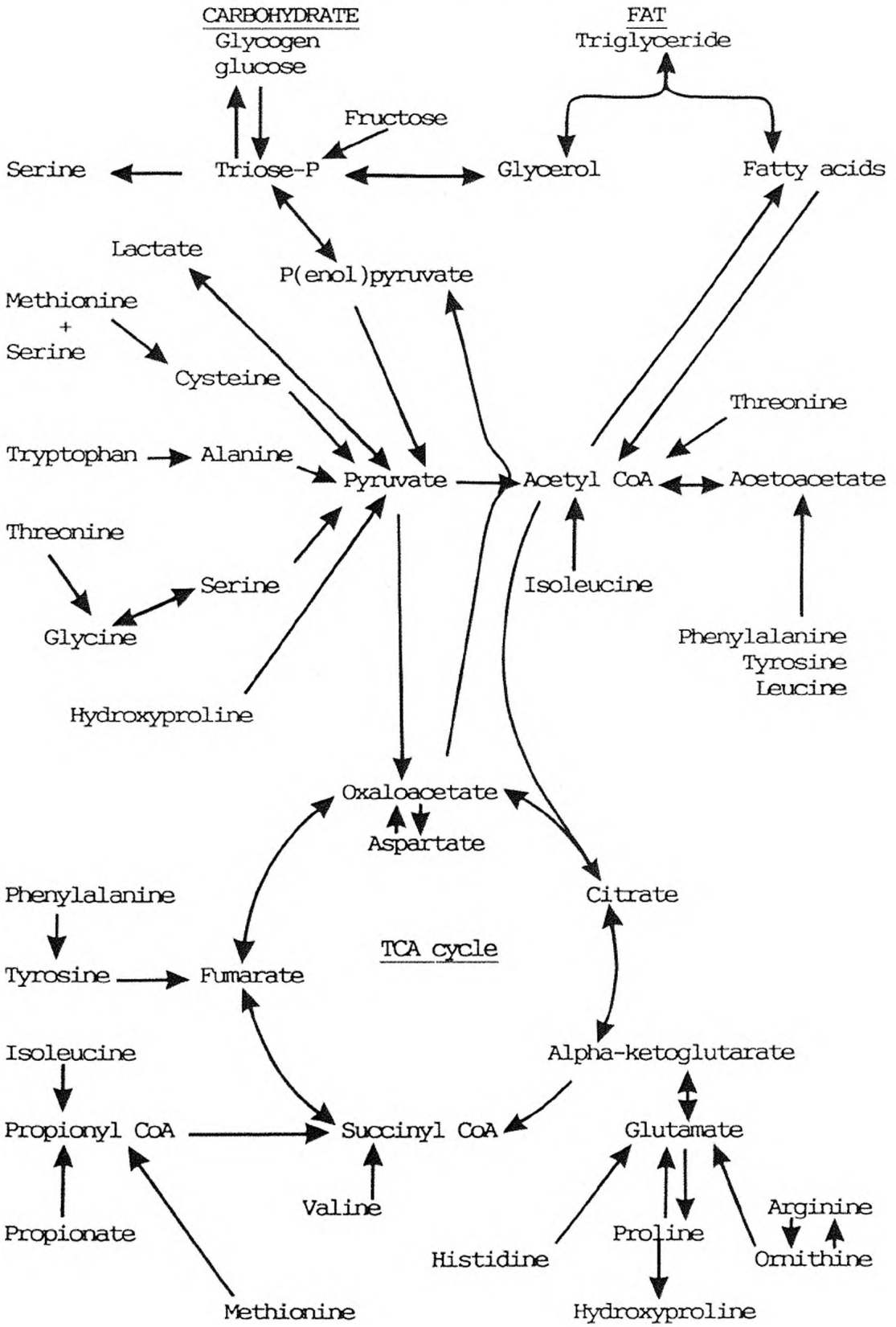
FASTING STATE



The bold arrows indicate the preferential route for the flow of substrate.

Figure:2-6 INTERCONVERSION OF METABOLIC FUELS

After Mayes, 1983



CHAPTER 3

THE METABOLIC RESPONSE TO INJURY

The elements of successful treatment of injury were slowly established through careful observations and clinical trials. Over half a century ago, the high incidence of non-union in fractures of the lower third of the tibia stimulated Sir David Cuthbertson (1930, 1932) to undertake his pioneering metabolic studies. His initial findings stimulated a great interest in the metabolic response to injury which led to a better understanding of the physiology and to considerable improvement in the management of the injured patient.

The body's response to injury may be divided into local and systemic effects. The local effect depends upon the type of injury, the anatomy involved and the integrity of the local microcirculation. It is usually resolved by specific local therapy but may result in loss of function depending on the injured tissue. The severity of the systemic response is highly dependent upon the nature and intensity of the local injury.

Phases of the metabolic response

Cuthbertson (1942) introduced the terms of "ebb" and "flow" to describe the rising and falling phases of metabolic activity in response to injury. These phases are easily defined in major injury and complicated surgery, but during elective surgery of minor or moderate severity they may pass unnoticed with little metabolic changes.

The ebb phase represents the immediate period after injury and lasts from a few hours after elective surgery of moderate severity to 24-72 hours after a more severe trauma. It is characterised by "depressed

vitality" and decreases in oxygen consumption and energy production. In patients who proceed to recovery, the ebb phase is followed by the flow phase. The duration of this phase is often less than two weeks but may last up to several months after severe injury (Cuthbertson, 1980a, 1980b). It is associated with "resurgence of vitality", increased oxygen consumption, rise in energy production and negative nitrogen balance. In clinical practice the changes are not always straightforward. Where complications or new trauma occur, there may be a change from the flow phase back to the ebb phase.

Moore (1953) divided the response to injury into 4 phases based on hormonal and metabolic relationships:

1- The adrenergic corticoid phase is characterised by increased secretion of catecholamines and corticosteroids. It lasts 1-4 days depending on the severity of the injury.

2- The corticoid withdrawal phase which occurs when the secretion of adrenal hormones returns to normal. It is associated with increased gut peristalsis, appetite and return to physical activity and usually represents the period between the 4th and 7th post-operative days.

3- The spontaneous anabolic phase is associated with a positive nitrogen balance and increased muscular strength. It usually lasts 7-10 days after major abdominal surgery.

4- The fat gain phase is accompanied by body weight gain due to the accumulation of fat and other energy stores (e.g. glycogen).

Other important endocrine changes were recognised as methods for hormone measurements were developed. Allison (1970) suggested that the metabolic changes observed might be explained by hypersecretion of the catabolic hormones (e.g. cortisol, catecholamines and glucagon) and relative deficiency of anabolic hormones (e.g. insulin).

The early work of Cannon (1939), Edghal et al.

(1958) and Hume and Edghal (1959) demonstrated the importance of the central and autonomic nervous systems in controlling the endocrine secretions. This neuroendocrine response to injury has the delicate task of preserving the body's "milieu intérieur", the integrity of which is essential for the preservation of animal life.

THE NEUROENDOCRINE RESPONSE TO INJURY

All the organs of the body are under neurological control either directly or indirectly by regulation of blood flow. The neuroendocrine response to injury is a basic neurophysiological reflex with afferent and efferent pathways integrating at the central nervous system.

The afferent impulses are mediated via somatic sensory (Hume and Edghal, 1959; Hume et al., 1962; Newsome and Rose, 1971) and autonomic (Cannon, 1939; Koizumi and McBrooks, 1974) nervous pathways. Bacterial toxins and chemical mediators released by injured tissues produce similar responses to the physiological changes such as blood and fluid loss and hypotension (Monnier, 1975). The emotional stimuli arise from the cerebral cortex (Wilmore et al., 1976a).

The spinal cord (Sato and Schmidt, 1971), hypothalamus (Crosby et al., 1962), medulla oblongata (Guyton et al., 1971) and cerebral cortex (Edghal, 1961) have been shown to be involved in the integration of the response to injury. The hypothalamus has the most important co-ordinating effect on the neuroendocrine response (Stoner, 1977). It is in direct contact with the autonomic nervous system and mediates both the adrenergic and pituitary responses.

The neuroendocrine response, through the modulating effects of hormones on fluid and electrolyte balance (Gann, 1976), cellular metabolism (Wilmore, 1976a) and the immune response (Ahlqvist, 1976), has an important and vital role in maintaining homeostasis following injury whether accidental or surgical.

CHANGES IN HORMONAL SECRETIONS FOLLOWING INJURY

Catecholamines

Adrenaline and noradrenaline have been shown to increase in response to trauma and surgical procedures

(Franksson et al., 1954), burns (Goodall et al., 1957; Harrison et al., 1967; Wilmore et al., 1974a), infection (Groves et al., 1973) and myocardial infarction (Christensen and Jorgeon, 1974). The magnitude of the response and its persistence depend on the severity and the duration of the injury. In major burns the increase in catecholamine secretion may persist for some weeks (Birke et al., 1957; Wilmore et al., 1976b).

The metabolic effect of catecholamines is characterised by an increase in metabolic rate with a rise in blood glucose and plasma free fatty acid concentrations and a suppression of the expected rise in insulin levels (Porte et al., 1966). Mobilisation of free fatty acids is the result of the direct action of catecholamines on fat cells. This increase in lipolytic activity is potentiated by the catecholamine-mediated suppression of insulin release.

Pituitary-adrenal axis

a-Adrenocorticotrophic hormone (ACTH)

This polypeptide hormone, released from the anterior pituitary, increases during surgery under general anaesthesia (Oyama, 1973; Oyama et al., 1979a, 1979b) but not under spinal anaesthesia (Newsome and Rose, 1971). There is no correlation between the concentrations of plasma ACTH and plasma corticosteroids during surgery (Ichikawa et al., 1971; Newsome and Rose, 1971).

b-Cortisol

Cope et al. (1943) first described increased corticosteroid secretion after injury. Albright (1943) noted the similarity between post-operative metabolic changes and those seen in Cushing's syndrome. Raised glucocorticoid levels have been shown in patients after severe injury (Hume et al., 1962; Meguid, 1974), burns (Wise et al., 1972; Popp et al., 1977), surgery (Steenburg et al., 1956; Clarke et al., 1970) and infection (Beisel,

1977). However, moderate haemorrhage in healthy man does not produce an increase in serum cortisol (Skillman 1967). In surgery of moderate severity (e.g. cholecystectomy) the degree of corticosteroid response can be markedly altered by the degree of pre- and peri-operative hydration (Gibbs et al., 1972).

Selye (1946) suggested a relationship between the increase in secretion of corticosteroids and the negative nitrogen balance. However, Ingle et al. (1947) failed to find this correlation in rats. Campbell et al. (1954) demonstrated that the metabolic response to trauma, although abolished in adrenalectomised animals, was normal when constant cortisone replacement therapy was given. Jepson et al. (1957) showed similar findings in humans. It has since been believed that the role of corticosteroids is "permissive" in that they are necessary for the metabolic response to injury but do not mediate it (Ingle, 1954). There is little doubt that the secretion is increased following injury and that the biochemical effects are rather too great to be dismissed.

Cortisol enhances hepatic glucogenesis (Wilmore, 1977b). It decreases glucose phosphorylation in the muscle cell (Morgan et al., 1961) with reduction of glucose uptake by peripheral tissues. This will, overall, have a glucose-sparing effect. In addition cortisol is an important proteolytic hormone and increases the flow of glucogenic amino acids to the liver. It also potentiates the lipolytic effect of catecholamines with a further increase in fatty acid utilisation and inhibition of glucose oxidation (Randle et al., 1963).

Pituitary-thyroid axis

a-Thyroid-stimulating hormone (TSH)

The TSH plasma concentrations do not change during moderate and major surgery (Chan et al., 1978; Hagenfeldt et al., 1979) or in response to acute illness (Burger et al., 1976) and burns (Popp et al., 1977) but may decrease

in the immediate post-operative period (Sowers et al., 1977; Adami et al., 1978).

b-Thyroid hormones

The known influence of these hormones on the metabolic rate and oxygen consumption encouraged much investigation which resulted in conflicting reports regarding the activity of the thyroid gland following injury. Cuthbertson and Tilstone (1969) found no consistent changes to implicate the thyroid hormones in the metabolic response to injury. Conversely, Richards et al. (1973) showed a good correlation between post trauma nitrogen loss and turnover of these hormones. Measurement of thyroxine (T4), Triiodothyronine (T3) and reverse T3 (rT3) following surgery (Burr et al., 1975) and after acute illness (Burger et al., 1976) has shown normal T4, decreased T3 and increased rT3. This increase in rT3 may not be due to increased conversion rate of T4 to rT3 but to reduced disappearance of rT3 from blood (Eisenstein et al., 1978). The decrease in T3 has also been demonstrated in burn patients (Popp et al., 1977) with an inverse correlation between the circulating levels of T3 and those of adrenaline and noradrenaline (Becker et al., 1980). These findings are not explained by changes either in prealbumin or thyroid-binding globulin (Schimmel and Utiger, 1977).

The decrease in circulating T3 in acute stress states does not cause hypothyroid symptoms (Spector et al., 1976), in spite of T3 being the active hormone in the periphery. Treatment of burn patients with metabolically active T3 causes a rise in circulating T3 levels but has no effect on metabolic rate (Becker et al., 1980). Thus, although decreased T3 concentration is common after stress and injury, its clinical significance remains unknown and further investigations are warranted.

Pituitary-gonadal axis

a-Luteinising hormone (LH)

LH is theoretically the most important of the gonadotrophic hormones as it controls the circulating levels of oestrogen and testosterone. There is an increase in serum LH concentration during surgery. It occurs only in men, not in women (Aono et al., 1976; Oyama et al., 1977). This increase returns to basal levels during the operation and decreases at the end of surgery both in men and women to below basal levels for up to 7 days afterwards (Aono et al., 1976).

b-Follicle-stimulating hormone (FSH)

FSH is of interest as a controller of the amenorrhoea often occurring following injury. Serum FSH concentrations are decreased following burns (Popp et al. 1977) but are not affected by surgery (Aono et al., 1976; Charters et al., 1969).

c-Prolactin

Plasma prolactin concentration increases markedly during surgery regardless of the type of anaesthetic used (Brandt et al., 1976). The concentration returns to normal by 3 days (Noel et al., 1972; Chan et al., 1978). However, in some mastectomy patients, the prolactin concentration remains high for months, although not as a stress response (Herman et al., 1981).

d-Oestrogens, progesterone and testosterone

No change has been observed in the plasma oestrogen concentration after surgery. By contrast, the plasma progesterone concentration decreases post-operatively in young women (Soules et al., 1980).

Testosterone is of particular interest because of its anabolic effect on skeletal muscle (Young, 1969). Its plasma concentration decreases for periods of one to two weeks following moderate or major surgery but not after minor operations (Aono et al., 1976; Carstensen et al., 1972; Matsumoto et al., 1970).

Growth hormone (GH)

The anterior pituitary growth hormone has been extensively studied as a possible mediator of the metabolic response to injury. It promotes protein synthesis, inhibits utilisation of glucose and stimulates lipolysis and fat oxidation (Daughaday and Kipnis, 1966).

The circulating levels of growth hormone are increased in response to severe injury and shock (Carey et al., 1971), surgery (Charters et al., 1969; Wright and Johnston, 1975; Newsome and Rose, 1971; Noel et al., 1972) and exercise (Noel et al., 1972). Wright and Johnston (1975) showed a relationship between the severity of injury and the subsequent increase in GH concentration which returned to normal in about a week after major surgery. This increase is less during surgery under epidural and spinal anaesthesia than under general anaesthesia (Newsome and Rose, 1971; Brandt et al., 1976).

Wilmore et al. (1974b) reported that administration of GH in the presence of sufficient energy intake can enhance positive nitrogen balance in burn patients by elevating circulating insulin levels. Recently Ward et al. (1984) reported a 44% increase in fat oxidation with increased energy expenditure but decreased nitrogen excretion in post-operative patients given synthetic human growth hormone.

Antidiuretic Hormone (ADH)

The main functions of ADH are the regulation of osmotic pressure in the extracellular fluid and the regulation of blood volume by stimulating the tubular reabsorption of water. At high plasma levels it additionally has a vasoconstrictor effect by its direct action on the smooth musculature of the vascular bed. It has also been reported to have a rapid potent stimulatory effect on hepatic glycogenolysis (Hems, 1978; Hems and Whitton, 1973; Hems et al., 1976 and 1978) and gluconeogenesis (Whitton et al., 1978).

During surgery there is a rapid increase in the plasma ADH concentration (Haas and Glick, 1978 ; Moran et al., 1964). This response is blocked by epidural anaesthesia (Harari et al., 1980). ADH is also released by a variety of non-specific stress stimuli including injury (Becker and Daniel, 1973) and hypoglycaemia (Bayliss and Heath, 1977). The practical significance of this system is that it prevents injured patients from excreting a free water load.

Renin-angiotensin-aldosterone system:

This system is jointly controlled by the sympathetic nervous system, the arteriolar perfusion pressure of the renal juxtaglomerular apparatus and by the sodium flux at the macula densa of the kidney (Oparil and Haber, 1974). The plasma renin activity increases during surgery (Robertson and Michelakis, 1972) and in burns (Popp et al., 1981). Aldosterone secretion also increases during surgery (Oyama et al., 1979a, 1979b) and in the early post-operative period (Zimmerman et al., 1956). Brandt et al. (1979) found no change in plasma aldosterone under epidural anaesthesia.

The other main factors affecting this response are the length of overnight dehydration and the nature of fluids administered during surgery. After cholecystectomy the magnitude of rise in plasma aldosterone was less after a high sodium intake compared with a low sodium intake (Cochrane, 1978) and when saline instead of glucose was infused during surgery (Engquist et al., 1978).

Insulin

Insulin is an anabolic hormone that promotes the storage of metabolic fuels within the cells. Studies on the secretion of this hormone following burns have shown a suppression of insulin secretion in the acute phase of injury followed by a period of insulin resistance (Allison et al., 1968). This is characterised by an inappropriately large production of insulin that does not have its

expected effect. Catecholamines may suppress the release of insulin during the ebb phase following surgery, infection or burn shock (Porte and Robertson, 1973). This action is mediated by the alpha receptors, while the adrenergic beta receptors which stimulate insulin production (Iversen, 1973) come into action during the flow phase (Wilmore et al. 1976c).

Insulin activates and facilitates glucose transport into muscle and adipose tissue. Glycogen synthesis and glycolysis are stimulated whereas gluconeogenesis and glycogenolysis are decreased. Insulin is also a major hormone in the metabolism of fat and protein. Low insulin concentrations stimulate lipolysis. Conversely insulin with dietary carbohydrate stimulates lipogenesis by facilitating the formation of acetyl CoA from carbohydrates, by providing alpha-glycerol phosphate for esterification of fatty acids to form triglycerides and by generating NADPH (dihydronicotinamide adenine dinucleotide phosphate) which is a necessary factor for fat synthesis (McGilvery, 1970). Thus insulin acts to convert energy ingested as carbohydrate to a storage form as lipid.

Low insulin concentration increases amino acid flux from muscle and this effect is reversed by provision of insulin (Pozefsky et al., 1969). Brennan et al. (1972) also showed that insulin is central to the regulation of protein metabolism and changes in its plasma concentration are associated with the uptake or release of amino acids.

Glucagon

Glucagon is suppressed following ingestion of carbohydrates and rises in response to starvation (Aguilar-Parada et al., 1969). Its concentration is raised in response to surgery (Russel et al., 1975), injury (Lindsey et al., 1974), burns (Wilmore et al., 1974c) and sepsis (Rocha et al., 1973) and returns to normal with recovery (Wilmore et al., 1974c).

Glucagon release is stimulated by hypoglycaemia, the autonomic nervous system (Iversen, 1973) and specific amino acids, particularly the glucogenic amino acids (Rocha et al., 1972). Glucagon increases hepatic glucose output by stimulating hepatic glycogenolysis and gluconeogenesis through increased gluconeogenic 3-carbon precursor uptake (Exton, 1972). Additionally, it may increase lipolysis and ketone production but this is significant only in the presence of insulin deficiency (Alberti and Nattrass, 1977; Liljenquist et al., 1974). In general, the role of glucagon is to act in conjunction with insulin to ensure a steady supply of substrate from liver under a wide range of physiological conditions (Unger, 1971a). While insulin is referred to as a storage hormone, glucagon can be viewed as a hormone of energy release (Unger, 1971a). However, glucagon does not appear to be a primary stress hormone but is permissive in its actions (Wilmore et al., 1983). Unger (1971b) has proposed the use of the molar insulin/glucagon (I/G) ratio to assess the overall effects of these hormones. A relative insulin increase (I/G>5) creates a hormonal milieu that favours anabolism and protein conservation whereas a relative glucagon increase (I/G<3) favours increased glycogenolysis, gluconeogenesis and ureagenesis at the expense of protein synthesis (Wilmore et al., 1983).

The response of different hormones to injury has been described. The overall metabolic response should be viewed as the result of the hormonal environment created by the interaction of all the above described hormones.

THE METABOLIC CHANGES FOLLOWING INJURY

Changes in metabolism following injury will be described for the individual components of body composition.

Changes in total body weight

It is accepted that tissue catabolism is accompanied by weight loss, the extent and rate of which are related to the severity and duration of starvation and/or injury. A comparison of the balance studies by Kinney (1978) on patients maintaining oral intake following major injury with results of total starvation reported by Benedict (1915) shows that over a 21-day period the contribution of protein to the total weight loss after injury (12%) is similar to that in total starvation (12.6%). The major difference in weight loss is that fat contributed 13.8% of the total in the major injury group and 24.6% in the total starvation group.

As fluctuations in body fluid response mask changes in lean and fat body mass, investigators focused their attention on the individual components of body composition: protein, fat, carbohydrate and water and electrolytes.

Changes in protein and amino acid metabolism

A major metabolic effect of injury is to increase the net degradation of muscle proteins (Cuthbertson, 1930; O'Keefe et al., 1974) to provide alternative energy substrates (Cairnie et al., 1957; Kinney et al., 1970) and to maintain synthesis of visceral and acute phase proteins (O'Keefe et al., 1974; Powada, 1977; Traynor and Hall, 1981). Duke et al. (1970) showed that the energy contribution of protein breakdown was only about 20% of the total expenditure in surgical patients, but the importance of released amino acids for synthesis of new proteins was vital. This metabolic response is usually

associated with a negative nitrogen balance the extent of which reflects the severity of the injury (Cuthbertson, 1932; Kinney, 1967). The nitrogen loss is greater after surgery with consequent fasting than after fasting alone for the same time (Foster et al., 1979) and its magnitude is reduced by epidural anaesthesia (Brandt et al., 1978).

Attempts to define whether the net protein catabolism results from changes in protein breakdown, synthesis or a combination of both have attracted considerable attention. Skeletal trauma (Birkhahn et al., 1980), infection (Long et al., 1977), and severe burns (Kien et al., 1978) cause marked increases in both synthesis and catabolism. Elective surgery (Crane et al., 1977; O'Keefe et al., 1974) and mild burns (Kien et al., 1978) cause only minimal changes and may result in decreased protein synthesis and unchanged catabolism. Unfortunately, these studies have not taken into consideration the effects of immobilisation nor corrected for nutrient intake which is a major factor influencing protein synthesis (Golden et al., 1977). Recently, Clague et al. (1983) studied whole body protein metabolism in surgical patients. The nutritional intake was controlled and the degree of surgical trauma assessed. They found that, pre-operatively, whole body protein flux and synthesis and amino acid oxidation increased with nutritional intake whereas protein breakdown remained unchanged. Post-operatively, whole body protein metabolism was determined on fasted or fed patients and the change in metabolism in each individual patient, from a pre-operative fed state, was calculated. Whole body protein breakdown increased in both groups but was significant only in the group fasted post-operatively. However, protein synthesis increased in the fed patients but fell in the fasted group. Clague et al. (1983) concluded that protein breakdown in response to injury is largely obligatory whereas synthesis responds to substrate availability.

The availability of improved analytical techniques has permitted the study of plasma and muscle amino acids. Most plasma amino acids decrease promptly following abdominal surgery. The non-essential amino acids continue to fall for the first two days or more, while the essential amino acids return to higher concentrations within two days (Dale et al., 1977). Hoover-Plow et al. (1980) also reported a fall of the plasma amino acids after surgical procedures, with the exception of the immediate increase in tyrosine, phenylalanine and tryptophan. Major fractures are associated with a decrease in ornithine, taurine and aspartic acid (Woolf et al., 1976). Severe injury produces a twofold rise in the branched-chain amino acids (BCAA) and a slight decrease in the values of circulating alanine, glycine and glutamate (Wedge et al., 1976). These authors also showed that the magnitude of rise of BCAA was less in the patients who showed an initial hyperketonaemia (> 2 mmol/l) and lower nitrogen excretion than those with normal ketone concentrations and higher nitrogen excretion. Severe infection causes a greater elevation of phenylalanine and methionine plasma concentrations than in injury (Freund et al., 1978; Woolf et al., 1976). These changes were interpreted as representing impaired liver function.

The current evidence suggests that the decrease in plasma amino acids is due to surgery and not to fasting or anaesthesia (Dale et al., 1977; Woolf et al., 1976).

About 80% of free amino acids in the body are found in skeletal muscle. It is, therefore, reasonable to expect that changes in the muscle free amino acid pattern might also reflect changes in protein metabolism. Vinnars et al. (1976) and Fürst et al. (1977), using the Bergström needle biopsy technique (Bergström, 1962 and 1974), showed that, following uncomplicated abdominal surgery and total hip replacement, there are increased muscle concentrations of BCAA, phenylalanine, tyrosine and methionine, glycine and alanine and decreased concentrations of lysine and glutamine. These changes were not always reflected in the

plasma and appeared not to be due to differences in membrane potentials. More recently, Askanazi et al. (1980a) also reported, following total hip replacement, increases in intracellular muscle BCAA, phenylalanine, tyrosine and methionine. Plasma amino acids displayed similar changes. There was a large decrease in intracellular glutamine which was not accompanied by a similar plasma change. These changes were not affected by infusions of amino acids, dextrose or a combination of both. Popp and Brennan (1983) postulated that the increases in plasma and muscle BCAA would be expected to stimulate protein synthesis and actively limit rather than passively reflect the post-injury protein catabolism.

Surgery is associated with an amino acid disturbance which is relatively unaffected by hypocaloric intravenous nutrition (Askanazi et al., 1980a). The decrease in glutamine observed in injured patients seems to be unique to surgical catabolism and may offer a way to separate the influence of surgery from that of starvation (Kinney, 1977).

Individual plasma proteins have been intensively studied. Investigators recognised long ago that the concentrations of plasma proteins are altered after injury (Cuthbertson and Tompsett, 1935). Electrophoresis of serum after trauma shows a decrease in the albumin band and increases in the alpha-1 and alpha-2 globulin bands (Owen, 1967; Fleck, 1976).

The concentrations of many plasma proteins increase in response to injury (Gordon, 1976). These are the so called acute-phase proteins which include the C-reactive protein, alpha-1-acid glycoprotein, alpha-1-antitrypsin, haptoglobin and fibrinogen (Kindmark et al., 1976; Colley et al., 1983; Myers et al., 1984). Recent research has led to better understanding of the mediators and mechanisms involved in the acute phase response (Dinarello, 1984; Lomedico et al., 1984). The products of tissue damage stimulate the macrophages to

produce interleukin-1 which causes the liver to synthesise the acute phase proteins. Some investigators have suggested that muscle breakdown and negative nitrogen balance are related to this response (Clowes et al., 1983; Loda et al., 1984).

In contrast to the acute phase proteins, albumin, transferrin, prealbumin and retinol-binding protein concentrations fall after injury and remain low for several days (Aronsen et al., 1972; Johansson et al., 1972; Moody, 1982). The concentration of albumin reaches a minimum 4-5 days after injury (Ballantyne and Fleck, 1973a). There is evidence that catabolism of albumin increases after injury (Ballantyne and Fleck, 1973b; Davies et al., 1962; Davies, 1982) but not enough to account for the decrease in concentration (Owen, 1967; Fleck, 1985). Other factors are involved, mainly an increase in vascular permeability which leads to loss of proteins to the extravascular space (Hurley, 1972; Arturson and Jonsson, 1979). The transcapillary escape rate of albumin has been shown to increase by more than 300% in septic shock and by up to 200% after cardiac surgery (Fleck et al., 1985). These changes would contribute to the measured decrease in plasma albumin (Ballantyne and Fleck, 1973a) and increase in extravascular albumin after injury (Ballantyne and Fleck, 1973b; Davies, 1982).

Changes in carbohydrate metabolism

The early studies of Claude Bernard (1877), demonstrating that hyperglycaemia and glycosuria are responses common to many types of injury, have been confirmed repeatedly. Hyperglycaemia is the most prominent derangement of carbohydrate metabolism following uncomplicated elective surgery (Stoner and Heath, 1973), infection and trauma (Gump et al., 1974; Wilmore, 1976b). This response is similar in young and old people (Blichert-toft et al., 1979) and increases with the severity of the operative procedure (Clarke, 1970; Wright,

1979). It occurs irrespective of the general anaesthetic used (Clarke et al., 1974) but is inhibited by high-dose fentanyl (Hall et al., 1974) and epidural anaesthesia (Kehlet et al., 1979). This response is regulated largely, as previously discussed, by the neuroendocrine response to injury.

Hyperglycaemia may result from either increased production or decreased utilisation of glucose or both. Early studies of glucose tolerance curves gave rise to the concept of stress diabetes and suggested insulin deficiency and reduced glucose utilisation. However, recent work suggests that glucose production and utilisation are increased in injured patients. Wilmore et al. (1976c, 1980) have demonstrated increased glucose flow in burned and in injured patients with sepsis. Long et al. (1971), using tracer studies, reported little change in glucose turnover following surgery, but both the glucose turnover and pool size doubled in injured patients. Wilmore et al. (1977) studied patients with 40% burns with and without leg involvement. All patients had similar increases in cardiac output and oxygen consumption. Patients with burned legs had increased blood flow, glucose utilisation and lactate production but no rise in oxygen consumption in that leg. This study suggests that the wound depends highly on anaerobic energy production from glucose.

Attempts to define the substrates utilised for this increased glucogenesis attracted the attention of many investigators. It is generally accepted that fat is not converted to glucose, although glycerol, released by lipolysis, could be readily converted to glucose (White et al., 1978). Carpentier et al. (1979) have shown increased glycerol turnover rate after injury but further studies are required to estimate the utilisation of this substrate for gluconeogenesis. Data from studies by Wilmore et al. (1977, 1980) suggest that lactate can provide up to 45% of the gluconeogenic substrates for the liver in severely injured patients with complications. Felig et al. (1970),

Felig and Wharen (1971) and Felig (1973) have reported that alanine is synthesised de novo in muscle and accounts for as much as 33% of the substrates utilised by the liver for gluconeogenesis in the post-absorptive state, during exercise and also after prolonged starvation. Similar changes were also demonstrated in patients with major burns (Aulick and Wilmore, 1979). Wilmore et al. (1980) measured hepatic glucose production and splanchnic amino acid uptake in burned patients and concluded that amino acids provided 22% to 34% of the substrates for gluconeogenesis. This data suggests that, following injury, there is enhanced activity of the alanine shuttle which moves three carbon fragments and ammonia from skeletal muscle to liver for production of glucose and urea. The production of radioactive glucose from infusion of labelled alanine, in injured patients, is further evidence of this alanine shuttle (Felig, 1973; Long et al., 1976).

Changes in fat metabolism

The utilisation of fat stores to meet energy requirements after injury is of great importance, especially when intake is restricted. Moore (1959) reported the large loss of adipose tissue following injury and indirect calorimetric studies on surgical patients have shown that 75% to 90% of the negative energy expenditure is met by fat oxidation (Duke et al., 1970).

Lipolysis increases in response to injury as shown by increased concentrations of free fatty acids and glycerol (Allison et al., 1969; Wiklund and Jorfeldt, 1975; Äärimaa et al., 1978). This response is inhibited by epidural anaesthesia (Cooper et al., 1979; Kehlet et al., 1979). The turnover rate of glycerol and the net oxidation of fat as well as the turnover of exogenously administered lipids are increased after injury (Carpentier et al., 1979). Catecholamines stimulate lipolysis by cyclic AMP-mediated increases in hormone-sensitive lipase (Steinberg et al., 1975) which hydrolyses triglyceride

into free fatty acids and glycerol. Insulin is the chief storage hormone and low concentrations favour free fatty acids release (Cahill, 1971).

The reported changes in the plasma ketone bodies in response to injury are variable but there appears to be an inverse relationship between the degree of nitrogen loss and the early concentration of ketones in the blood (Smith et al., 1975; Wedge et al., 1976).

Changes in water and electrolytes

Injury is associated with increased secretion of ADH resulting in intra- and extracellular water retention. Wynn (1960) described, following surgery, the fall in plasma osmolality, sodium retention and potassium loss. He concluded that the immediate post-operative oliguria is probably secondary to renal haemodynamic changes and that its persistence is due to ADH.

There is an increase in cell membrane permeability causing net gains in sodium, chloride and water and net loss of potassium (Flear, 1970). Cuthbertson (1972) described increased serum potassium concentration and urinary excretion in the first 24 to 48 hours after moderate injury, followed by decreased excretion for the next week. After severe injury such as large burns, the increased potassium excretion may continue for much longer (Hinton et al., 1971; Hinton et al., 1973).

Energy metabolism following injury

Adequate energy supply is required, following injury, to fuel the increasing demands of the reparative process. Skeletal trauma and infection can cause increases up to 140% of the normal resting needs, whereas major burns can lead to increases of 180% to 200% (Kinney, 1974; Wilmore et al., 1974a). The magnitude of rise in metabolic rate, in burned patients, is proportional to the size of the burn (Wilmore et al., 1974a). There is, however, minimal or no increase in the energy requirements in uncomplicated post-operative patients (Kinney, 1975).

This response indicates the need of the injured for additional energy sources. Kinney (1975) has estimated that an average excess of 50% over resting metabolic expenditure is necessary for anabolism.

CHAPTER 4

FACTORS MODIFYING THE METABOLIC RESPONSE TO INJURY

The magnitude of the metabolic changes following injury may be altered by various factors. Since this thesis is concerned with the possibility of beneficial modification of this response, mainly in terms of nitrogen sparing, these factors will be discussed.

PHYSICAL FACTORS

Environmental temperature

Caldwell (1962) housed rats after thermal injury at 30° C and 20° C. The animals at the higher temperature lost less weight and less nitrogen than the others. This observation led to the hypothesis that ambient temperature has profound effects on the magnitude of the metabolic response to injury, but subsequent investigations of the effect have produced conflicting results.

Spivey and Johnston (1972) showed no effect of the ambient temperature on post-operative nitrogen balance after surgery, while Carli et al. (1982) were able to reduce post-operative nitrogen losses in elderly patients by the use of metallised blankets, warmed intravenous fluids and a hot water mattress. Ryan (1983), using the Clinitron fluidised bead bed where environmental temperature can be closely regulated, demonstrated that those patients nursed at 32° C had a significantly lower nitrogen loss than those patients nursed at 22° C. Further studies by Harris et al. (1983), investigating the post-operative response to injury of moderate severity at either 20° C or 28° C failed to show any differences in energy expenditure or nitrogen excretion over the first 4

post-operative days. However, it is unlikely that their patients were in practice exposed to such a wide variation in ambient temperature, since they wore bed gowns and were covered by a sheet and duvet. More recently, Jones at al. (1984), using the Clinitron bed, demonstrated that an elevated ambient temperature of 32^{o} C produced nitrogen sparing after surgery by reducing protein breakdown.

Studies on burned patients have shown that the metabolic rate (Barr et al., 1968) and nitrogen excretion (Davies and Liljedhal, 1970) are reduced when these patients are nursed in a warm dry environment. These findings are probably due to reduced evaporative heat loss. Nitrogen loss was similarly reduced after fractures in man by raising the ambient temperature to 30^{o} C (Cuthbertson et al., 1968; Cuthbertson et al., 1972). This change in the metabolic response was greater in those who had a more severe injury.

These studies demonstrate that environmental temperature must be carefully controlled to obtain a measurable effect and that a relatively large increase in ambient temperature is required.

Mobility

Cuthbertson (1929) and Deitrick et al. (1948) showed that immobilisation in plaster was associated with increased urinary nitrogen excretion. Schønheyder et al. (1954) carried out isotope studies in three immobilised healthy subjects and suggested reduced protein synthesis with unchanged breakdown. The immobilisation in plaster may have been the main contributing factor to the nitrogen loss since, in normal subjects on bed rest without plaster immobilisation for 2 weeks, there was no significant change in urinary urea excretion (Greenleaf et al., 1977). Leonard et al., (1983) studied the loss of weight and lean body mass and the negative balance of nitrogen and potassium observed during prolonged space flight and suggested that exercise may ameliorate these losses.

Covering of Burns

The use of occlusive dressings to reduce evaporative heat loss in burned patients gave conflicting results. Zawacki et al. (1970) were unable to show any effect upon metabolic rate, while Caldwell et al. (1981) succeeded in reducing the metabolic rate in response to burns. Early skin grafting of burned areas has been advocated (Caldwell and Levitsky, 1963; Demling, 1983) as it would reduce the source of evaporative heat loss and that of afferent mediators to the metabolic response to injury.

NUTRITIONAL FACTORS

Nutritional status

Munro and Cuthbertson (1943) reported that the nitrogen loss after a standard injury was smaller when animals had been starved prior to injury. Munro and Chalmers (1945) postulated that the cause was a reduction in "dispensible protein reserves" mobilisable after injury. Abbott and Albertson (1963) showed that in man also, the metabolic response was reduced by pre-injury starvation.

This reduction in nitrogen loss in starved patients would seem advantageous. However, it is well established that malnutrition may lead to impaired wound healing (Lawson, 1965) and immunological incompetence (Law et al., 1974), which are likely to be more deleterious in terms of morbidity and mortality than the loss of a certain amount of body protein. Involuntary weight loss greater than 15-20% before duodenal ulcer surgery (Studley, 1936), oesophageal surgery (Conti, 1977) and colorectal surgery (Hickman, 1980) was associated with a large increase in the mortality rate. Recently Bastow et al. (1983), investigating patients with fractured neck of femur, reported that patients who were "well nourished" had a mortality of 4.4%, those that were "thin" 8% and

those that were "very thin" 18%.

Cuthbertson et al. (1937) suggested that the metabolic response to injury might be amenable to nutritional manipulation, and Cuthbertson and Munro (1937) showed that the provision of excess calories was protein sparing.

Nutritional manipulation

The non-nitrogen energy sources available are the fat emulsions (Triglycerides of long chain, and medium and short chain fatty acids), the carbohydrates (glucose, fructose and maltose), the polyols (sorbitol, xylitol,and glycerol), the diols (1,3-propanediol and 1,2-butanediol), ethanol and keto-compounds (monoacetoacetin). Of these only the most commonly used substrates will be discussed: glucose and fat emulsions.

a- Glucose

Over 70 years ago, Woodyatt et al. (1915) showed that glucose in the amount of 0.85 g $kg^{-1}hr^{-1}$ could be given intravenously to man without inducing glycosuria. Dudrick et al. (1969) reported the intravenous administration of up to 1.2 g $kg^{-1}hr^{-1}$ glucose without any adverse reactions.

The utilisation of glucose as an energy source is well documented. O'Connell et al. (1974) reported a decrease in nitrogen excretion with increasing doses of intravenous glucose in fasting volunteers. Radcliffe et al. (1980) reported a similar progressive reduction in nitrogen excretion with increasing carbohydrate intake after elective surgery. However, glucose is not as effective in hypermetabolic injured man as in hypometabolic starving subjects (Askanazi et al., 1980b). Other studies also suggest that glucose will suppress gluconeogenesis in the injured man, but that much higher rates of glucose infusion are necessary than in the starving man (Burke et al., 1979; Elwyn et al., 1979).

Investigators also studied the effect of glucose

infusion rates that were in excess of energy expenditure. Jacot et al. (1982) investigated the oxidation rate of glucose given intravenously using the glucose clamp technique. They showed that the capacity of the body to oxidise glucose was 2-3 mg $kg^{-1}min^{-1}$ and did not exceed 4 mg $kg^{-1}min^{-1}$. These studies were carried out on healthy young volunteers during hyperglycaemia and hyperinsulinaemia within physiological ranges. The oxidative capacity was not influenced by the total amount of administered glucose. Injury is accompanied by hyperglycaemia and insulin resistance. In spite of these effects, glucose oxidation does not decrease in response to injury (Kinney et al., 1970; Stoner, 1970). The maximal oxidation rate following injury is 5 mg $kg^{-1}min^{-1}$ (Burke et al., 1979; Elwyn et al., 1979).

Burke et al. (1979) noted definite increases in non-protein respiratory quotient above 1.0 and documented extensive fat deposits in the liver in burned patients dying after intravenous glucose infusions of 9.3 to 17 mg $Kg^{-1}min^{-1}$. Askanazi et al. (1980c, 1980d) also showed the increased ventilation necessary to increase oxygen uptake and carbon dioxide excretion at high levels of glucose infusion. These studies suggest that glucose loads in parenteral nutrition should not greatly exceed metabolic expenditures.

b- Fat emulsions

The use of fat emulsions as an energy source was clinically investigated in the early 1960s (Schuberth and Wretlind, 1961). The use of exogenous fat, in a parenteral regimen to replace part of the calories provided by glucose, has been shown to reduce markedly the carbon dioxide production and consequently the minute ventilation (Askanazi et al., 1981). This procedure has allowed the weaning off artificial ventilation in patients with respiratory failure induced by high carbohydrate intake. Jeejeebhoy et al. (1973) also showed that the use of intravenous fat could reduce the degree of hepatic

steatosis in patients previously fed with glucose as the sole calorie source. Nordenström et al. (1982) studied surgical patients receiving parenteral nutrition with either glucose alone or glucose with fat as the energy source. They showed that the fat oxidation was reduced in the glucose regimen whereas a large proportion of the administered fat was used as an immediate source of energy in the glucose-fat regimen. They also demonstrated a linear relationship between the rate of intravenous fat administration and net fat oxidation, indicating that the infused fat is oxidised in the same way as endogenous fat. These results emphasised the importance of administering fat as part of the calorie intake, but the protein sparing effect of this emulsion has been greatly debated.

Long et al. (1977) and McDougal et al. (1977) have reported that, despite optimal utilisation, the infused lipids did not reduce nitrogen loss in patients with extensive burns, in contrast to the positive balance obtained after glucose infusion. The urea nitrogen excretion was inversely related to carbohydrate intake and directly related to metabolic rate (Long et al., 1977). However, Wolfe et al. (1979), Burke et al. (1979) and Askanazi et al. (1980b) have shown that injured patients with sepsis, including those with burns, have an obligatory need for fat energy. Such patients continue to oxidise significant amounts of fat despite being provided with sufficient glucose calories to meet their needs. Others have reported that lipid and glucose are comparable in promoting nitrogen retention in a variety of critical conditions (Bark et al., 1976; Bocking et al., 1980). Roulet et al. (1980) found, in a controlled study, that acutely ill patients in respiratory failure had a significant increase in net protein synthesis (over catabolism) only when a mixed substrate of fat and carbohydrate was infused. More recently, Macfie et al. (1981) showed that the infusion of an amino acid, glucose and lipid mixture promoted nitrogen retention better than the infusion of an amino acid and glucose solution.

It seems that the injured patient needs a mixed substrate of fat and carbohydrate for meeting non-nitrogen energy needs. However, caution is needed to avoid too fast or too massive infusion of exogenous fat. Indeed this could result in side effects such as an inhibition of various functions of polymorphonuclear leucocytes and possibly macrophages (Jarstrand et al., 1978).

The sources of lipids commonly used in parenteral nutrition are the triglycerides of long-chain fatty acids derived from either soybean or safflower oil. Recently, there has been increasing concern about the utilisation of long-chain fatty acids during severe stress, with reported reduction in muscle carnitine levels which may limit fatty acid oxidation within the mitochondria (Border et al., 1970). Studies are in progress to solve this problem and investigators have adopted two different approaches. One consists of adding L-carnitine to exogenous fat in an effort to facilitate the transfer of long-chain fatty acids into the mitochondria; the other consists of introducing medium-chain triglycerides in the fat emulsion in order to take advantage of the fast metabolic handling of these shorter chain fatty acids. Their metabolism is carnitine-independent and their oxidation is not restricted by insulin (Lossow et al., 1956) which is in contrast to the oxidation of the long-chain fatty acids. Studies in this field should not only define the metabolic efficacy of these "future" fat emulsions but should also determine any possible short- or long-term side effects.

c- Amino acids

Amino acids should be supplied in order to maintain the body proteins. Blackburn et al. (1973) suggested that a peripheral infusion of isotonic amino acids, with no other energy source, would suppress insulin production and allow endogenous fat mobilisation and ketone production to meet energy requirements. This would avoid gluconeogenesis from amino acids and lead to improved nitrogen balance. Subsequently, a number of

workers have investigated this hypothesis and conflicting results have been obtained. O'Keefe et al. (1974) have shown that amino acid infusions following surgical stress increase the rate of protein flux and synthesis but have no effect on protein breakdown. Skillman et al. (1976) have demonstrated an increase in albumin synthesis rates in post-operative patients infused with amino acids alone. Tweedle et al. (1977) infused isotonic amino acids in fasting normal men and were unable to demonstrate positive nitrogen balance. Shizgall (1978) reported that amino acids alone improved the lean body mass and the total exchangeable potassium with a decrease in body fat. More recently, Harris et al. (1982) and Garden et al. (1983) failed to improve nitrogen balance following surgery by the infusion of isotonic amino acids.

Intravenous feeding with amino acids and calorie sources after vagotomy and pyloroplasty produced an improvement in mean daily nitrogen balance over 5 days in patients fed with low calorie intravenous feeding, high calorie intrajejunal feeding and high calorie intravenous feeding. The biggest improvement in nitrogen balance was seen in those patients having the high calorie intravenous feeding (Johnston et al., 1966). Wolfe et al. (1977) compared amino acids alone with glucose alone, lipid alone and combinations of amino acids, glucose and lipid in fasting normal man. These studies suggest that nitrogen balance is most dependent on calorie intake, but that the provision of amino acids and energy substrate is better than the provision of energy substrate alone (Wolfe et al., 1977). Similar, although less extensive, studies of isotonic amino acid infusions with and without energy substrate have been conducted in post-operative (Greenberg et al., 1976) and burned (McDougal et al., 1977) patients. Studies in post-operative patients demonstrated a protein sparing effect of amino acid infusions and Greenberg et al. concluded that the metabolic pattern, described by Blackburn et al. (1973), of fat mobilisation and ketosis was not necessary to utilise the infused amino acids. The

study in burned patients indicated a similar effect of isocaloric infusions of amino acids or glucose in patients with or without bacteraemia. The effects of both infusions were additive and nitrogen balance was most dependent on calorie intake (McDougal et al. 1977).

Nuwer et al. (1983) have shown that solutions containing increased amounts of branched-chain amino acids have improved nitrogen balance and increased total lymphocyte count and skin test reactivity in patients with a high level of surgical stress. Most essential amino acids can be utilised in the alpha-keto rather than the alpha-amino form. The possibility that leucine may improve protein synthesis (Buse and Reid, 1975) has led to investigations of the effect of leucine and its analogue ketoleucine on nitrogen metabolism following surgery. Sapir et al. (1983) infused glucose, leucine and ketoleucine to three separate groups of surgical patients and found that the 3-methylhistidine excretion was lower in the ketoleucine group than in the groups receiving either glucose or leucine. Nitrogen balance was less negative in the ketoleucine group than in the group receiving glucose. When glucose was given at the same time as leucine or ketoleucine no such effect on nitrogen excretion was seen but a decrease in 3-methylhistidine occurred with ketoleucine (François et al., 1984).

Controversial results have been obtained with the infusion of amino acids alone after injury. There is, however, convincing evidence that amino acids plus energy substrates improve nitrogen balance following trauma. The role of branched-chain amino acids and alpha-keto analogues needs further evaluation.

ENDOCRINE FACTORS

It is well established that hormones play an important role in mediating the metabolic response to trauma. Hormonal manipulation has been used by many

investigators to reduce the magnitude of the metabolic response to injury.

Insulin

Insulin is an anabolic hormone which increases the rate of muscle protein synthesis. Hinton et al. (1971) reported that insulin can improve nitrogen balance when administered with large quantities of glucose and potassium. Subsequently, Woolfson et al. (1979) demonstrated that those patients, in their study, who were more severely catabolic excreted less nitrogen when given insulin. However, Powell-Tuck et al. (1983), studying parenterally fed undernourished patients showed no beneficial clinical effect of the use of insulin as a means of modifying protein losses after major surgery. The reason why these studies produced conflicting results needs further evaluation.

Anabolic Hormones

Androgens are known to have strong anabolic properties. Testosterone has been observed to reduce nitrogen excretion after injury (Abbott et al., 1946), but its virilising effects precluded its use in women and children. Anabolic steroids have minimal virilising effects but retain the anabolic properties of androgens.

Johnston and Chenour (1963) and Tweedle (1973) reported improved nitrogen balance with anabolic steroids following surgery. Lewis et al. (1981) reported no beneficial clinical effect of nandralone decanoate. However, Michelsen et al. (1982), studying the effect of the same anabolic hormone in surgical patients, showed a significant decrease in nitrogen excretion. Young et al. (1983) failed to demonstrate any increase in total body nitrogen in patients given anabolic steroids. Blamey et al. (1984) showed that the potent anabolic steroid stanazolol, with an anabolic to androgen ratio of 100:1, significantly decreased the nitrogen excretion following general surgical procedures.

Growth Hormone

Johnston and Hadden (1963) failed to identify any beneficial effect of growth hormone in male patients following unilateral inguinal herniorrhaphy. Wilmore et al. (1974b), investigating burned patients, demonstrated that growth hormone improved nitrogen balance in the presence of sufficient energy intake. Recent work by Ward et al. (1984) has demonstrated decreased nitrogen excretion in post-operative patients given biosynthetic growth hormone.

Corticosteroids

Corticosteroids have been given to shocked patients after severe injury. They produced no decrease in mortality but there was a suggestion that the incidence of fat embolism might be reduced (Rokkanen 1974).

The use of high-dose steroids in the treatment of septic shock has produced conflicting results. Shumer (1976) has claimed their efficacy, whereas Shine et al. (1980) have demonstrated adverse effects. A recent prospective, controlled study has shown no improvement in mortality with the use of either methylprednisolone or dexamethasone (Sprung et al., 1984).

These studies indicate that endocrine manipulation may be an effective tool to be included in the armamentarium to combat the post-injury metabolic derangement. However, it still needs further evaluation before its clinical use is established.

ANAESTHETIC FACTORS

Anaesthesia offers two main hypothetical methods for modifying the metabolic response to injury. First, the intravenous administration of large doses of opiates to block hypothalamic pituitary function. Secondly, the

use of afferent neuronal blockade, by epidural or spinal analgesia, to inhibit the transmission of impulses from the site of injury.

The ability of morphine to inhibit ACTH secretion was described by McDonald et al. (1959). George et al. (1974) found that intravenous morphine decreased the endocrine changes found during abdominal and cardiac surgery until the start of cardiopulmonary bypass. Taylor et al. (1976) demonstrated that one systemic dose of morphine reduced nitrogen excretion in burned patients. However, the use of large doses of morphine resulted in an unacceptably prolonged recovery period and respiratory depression. The introduction of fentanyl, a potent opiate analgesic with a short duration of action, has enabled the investigators to study in detail the effects of high-dose opiate anaesthesia.

The intravenous administration of high-dose fentanyl before the onset of surgery abolished the glucose, lactate, cortisol and growth hormone response to pelvic surgery of 3 hours duration (Hall et al., 1978). The same dose of fentanyl was less effective in gastric surgery (Cooper et al., 1981). The available evidence indicates that this technique is only effective if fentanyl is administered before the onset of surgery (Bent et al., 1984).

The use of epidural anaesthesia to modify the metabolic response to injury has produced conflicting results. It inhibits the endocrine metabolic response to pelvic (Brandt et al., 1978) and upper abdominal surgery (Asoh et al., 1983). However, others have reported that epidural blockade could not attenuate oxygen uptake (Rutberg et al., 1984) and had no effect on the increased splanchnic uptake of amino acids (Lund et al., 1984) during cholecystectomy. Recently, Reinhart et al. (1985) have reported that epidural analgesia, with bupivacaine 0.5%, does not modify the metabolic response to surgery.

It is important to note that in all these studies

the epidural block was established before the onset of surgery. The use of this technique after surgery has only limited effects on circulating glucose and cortisol values (Moller et al., 1982).

Recently, Hall (1985) reviewed the effects of anaesthesia on the metabolic response to surgery and concluded that the two main anaesthetic methods available for ameliorating this response, high-dose opiate anaesthesia and epidural analgesia, have serious clinical limitations.

PHARMACOLOGICAL FACTORS

The possibility that the metabolic response to injury might be modified by direct pharmacological means has attracted much attention recently. Investigators have studied the effects of drugs alone or in association with feeding to enhance the anabolic effect.

Naftidrofuryl

Since this thesis is concerned with the possible beneficial effect of Naftidrofuryl on the metabolic response to surgery, this drug will be discussed in detail under a separate heading.

Indomethacin

Baracos et al. (1983) have demonstrated that indomethacin prevented muscular prostaglandin E_2 synthesis and abolished the increased proteolysis in rat muscles incubated with purified human leucocytic pyrogen. They concluded that cyclo-oxygenase inhibitors, such as indomethacin, may be useful in the treatment of negative nitrogen balance in fever.

Ornicetil

The salt complex of alpha-ketoglutarate and ornithine (L (+) ornithine alpha-glutarate, Ornicetil) is

able to bind six amino groups per molecule without enzymatic intervention. It has been claimed that intravenous administration of Ornicetil, together with parenteral or enteral nutrition, can improve nitrogen economy (Nicolas and Rodineau, 1982; Tremolières et al., 1972). Recently, Leander et al. (1985) have reported that Ornicetil improves nitrogen balance in patients fed intravenously after colonic resection. Eriksson et al., (1985) failed to demonstrate, in healthy volunteers, a significant effect of Ornicetil on hepatic or skeletal muscle protein metabolism.

The use of Ornicetil with exogenous energy substrates seems promising but needs further evaluation.

Adrenergic blockade

Combined alpha- and beta-adrenergic blockade has been shown to reduce oxygen consumption in burned patients, although not to the levels seen in non-burned controls, while alpha-adrenergic blockade alone had no effect (Wilmore et al., 1974a). Beta-adrenergic blockade with propranolol was shown to diminish the rise in non-esterified fatty acids, lactate and glucose and the fall in circulating phosphate levels associated with surgery (Tsuji et al., 1980; Lovén et al., 1982; Body et al., 1983). Alpha-adrenergic blockade with phentolamine had no effect upon the lipolytic response to surgery (Walsh et al., 1982), although its effects upon the hyperglycaemia associated with surgery or the impaired insulin response to glucose are disputed (Allison et al., 1969; Nakao and Miyata, 1977).

Benzodiazepines

Diazepam has been shown to inhibit the catecholamine response to dental extraction (Goldstein et al., 1982) but its use after injury or major surgery has not been evaluated.

Pain is a powerful stimulus for catecholamine secretion and causes a rise in the metabolic rate

(Arturson et al. 1977). Sleep is important in promoting the healing process (Adam and Oswald, 1984). Perhaps the combination of sedative or hypnotic agents with analgesia plays a significant part in allowing regular sleep to occur.

Fructose 1,6-diphosphate

Iapichino et al. (1983) have reported that fructose 1,6-diphosphate improved nitrogen balance in critically ill parenterally fed patients. They suggested that this improvement depended on the increase of body protein synthesis since muscular catabolism (evaluated by urinary 3-methylhistidine) was not significantly different in the control group. They also postulated that this substance exerted its anabolic activity by improving metabolic substrate utilisation by means of ATP production.

Bradykinin

There has been one study in which intravenous infusion of bradykinin improved nitrogen balance and glucose tolerance after abdominal surgery (Dietze et al., 1982). Its mechanism of action is not fully understood and further studies are warranted.

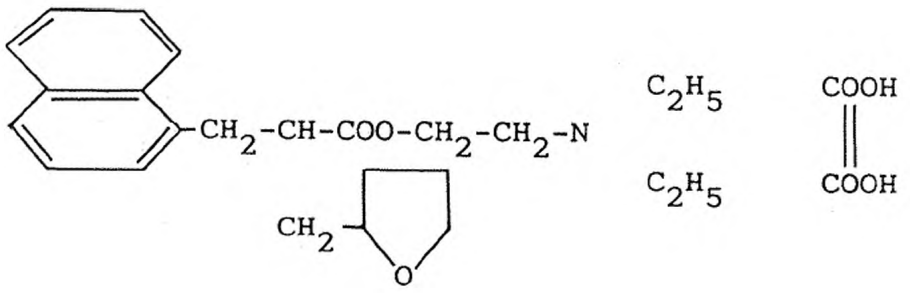
Despite the intense interest generated by these initial reports, there is not yet unambiguous evidence to support the use of any of these drugs in the routine clinical management of the injured patient.

CHAPTER 5

NAFTIDROFURYL

Structure

Naftidrofuryl was synthesised by E. Szarvasi and his team (Szarvasi et al. 1965, 1966, 1967a, 1967b). It is the acid oxalate of ethyl-N-diethylamino-2'''-(naftyl-1')- 3-(tetrahydrofurfuryl-2'')-2-propionate. Its structure is as follows:



It is colourless, odourless (melting point 110 to 111°C, heated plate) and is readily soluble in water.

Presentation

Naftidrofuryl is marketed in the United Kingdom by Lipha Pharmaceuticals Limited.

- Oral form: PRAXILENE, pale pink capsules overprinted 'PRAXILENE-Lipha', each containing 100 mg of naftidrofuryl oxalate (Product licence number: 3759/0002).

- Intravenous form: PRAXILENE FORTE, clear and colourless, 10 ml ampoules imprinted 'PRAXILENE FORTE', each containing 200 mg of naftidrofuryl oxalate (20 mg/ml). (Product licence number: 3759/0004).

Pharmacology

Naftidrofuryl is a direct acting, papaverine-like vasodilator that also has antinicotinic and antibradykinin effects (Fontaine et al., 1968, Cook and James, 1981).

The drug is a potent vasodilator when given by direct intra-arterial injection in animals (Fontaine et al., 1968), however it does not cause hypotension or tachycardia when given orally in therapeutic doses to humans and it appears to be a relatively weak vasodilator under these conditions (Cox, 1975). In animals, it inhibits platelet aggregation in the same way that dipyridamole does and has no effect on clotting or thrombin times. It also increases biliary output by 40%, and has no effect on diuresis (Fontaine et al., 1968). The LD 50 (Lethal dose 50 = dose which kills 50% of the population studied) in rats is 1736 mg kg^{-1} for oral route and 16.5 mg kg^{-1} for intravenous administration, whereas the LD 100 (lethal dose 100 = dose which kills all studied population), following intravenous administration, is 30 mg kg^{-1} in the rat, 24 mg kg^{-1} in the rabbit and 71 mg kg^{-1} in the dog (Fontaine et al., 1968).

Naftidrofuryl has been shown, in animals, to have a direct effect on intracellular metabolism (Maynaud et al., 1973), as a result of which there is a reduction in lactate concentrations and an increase in ATP (adenosine triphosphate) concentrations under ischaemic conditions (Maynaud et al., 1975). These findings have been confirmed both in healthy volunteers (Shaw and Johnson, 1975) and in patients suffering from peripheral vascular disease (Elert et al., 1976). Shaw and Johnson (1975) have demonstrated that naftidrofuryl is able to enhance cellular oxidative capacity; a single dose of 300 mg naftidrofuryl increases the rate of decline of the blood lactate/pyruvate ratio following exercise in healthy volunteers. In patients with severe lower limb ischaemia, assays on muscle biopsies performed before and soon after several daily intravenous infusions of naftidrofuryl showed a decline in lactate concentration and an increase in ADP (adenosine diphosphate) and ATP (adenosine triphosphate) formation (Elert et al., 1976). How naftidrofuryl produces these effects remains unclear. Maynaud et al. (1973) suggested that it has an

insulin-like action, whereas Shaw and Johnson (1975) postulated that it stimulates the activity of the tricarboxylic acid cycle.

Pharmacokinetics

Animal studies by Rico et al. (1974) have shown that after a single intramuscular injection (40 mg Kg^{-1}), ^{14}C-naftidrofuryl was rapidly and totally absorbed. Peak blood concentrations occurred between 1 and 4 hours and fell thereafter with virtually all trace of radioactivity disappearing after 48 hours. There was no tissue storage but high concentrations were recorded in lungs, kidneys and liver, and lower concentrations were detected in brain and muscle tissue. There was substantial biliary excretion. However, the presence of an enterohepatic cycle, previously demonstrated by Fontaine et al. (1969), explained the very low faecal excretion (12% over 3 days) and high urinary excretion in the form of metabolites (60% in the first 24 hours, 80% in the first 72 hours). Radioactivity in the expired gases was very low for the first 8 hours and then absent, demonstrating minimal respiratory clearance of the drug. Tatematsu et al. (1975) have reported, in animal studies following oral administration of ^{14}C-naftidrofuryl, a high biliary excretion of radioactivity (72% of the total administered dose). However, unlike Rico et al. (1974) who also used the rat as an experimental model, they reported very little reabsorption with 30% of the radioactivity being recovered in the urine and the rest being excreted in faeces. Autoradiography indicated no accumulation of the radioactivity in organs other than digestive tract 24 hours after administration (Tatematsu et al., 1975).

Few investigators have studied the pharmacokinetics of naftidrofuryl in humans. Lartigue-Mattei et al. (1978) reported a good absorption of the drug following oral administration (50 mg) in five healthy volunteers. The peak plasma concentration occurred one hour after dosing, the elimination half-life

was 40.5 minutes (range: 32.5 - 46.6 minutes) and the bio-availibilty was 78.6% (range: 45.4 - 100.6%) indicating a large variation between individuals.

Unpublished data on humans (Lipha Pharmaceuticals Limited, 1986), following oral and intravenous administration of 200 mg of naftidrofuryl oxalate, indicated that the pharmacokinetics of this drug are best described by a two-compartment model. The bio-availability varied considerably (14 - 60%). In the dog, bio-availabilty of this drug after an oral administration of 250 mg was found to be only 0.3 - 2.7% (Garret, 1984). Drugs which undergo substantial first pass metabolism (The drug is absorbed and metabolised by the liver to a great extent before it reaches the systemic circulation) usually show considerable inter-individual variation in bio-availabilty of the drug. Therefore, the low and varied values suggest that there is pre-systemic first pass metabolism of naftidrofuryl.

The mean half-life is 1.92 hours (range: 1.01 - 2.89 hours) and 0.99 hour (range:0.63 - 1.5 hours) respectively following oral or intravenous administration. The mean clearance value following intravenous infusion is 773 mls min^{-1} (range: 438 - 1528 mls min^{-1}) indicating a very high clearance rate of the drug. Naftidrofuryl has been found to have a mean volume of distribution of 92 litres (range: 52 - 136 litres) (Lipha Pharmaceuticals Limited, 1986). This exceeds the value commonly taken to represent total body water (55 litres). A large volume of distribution indicates that the drug is not highly bound to plasma protein and therefore is contained within the vascular compartment, but is free to distribute into tissues (two-compartment model) where binding produces an enormously high volume of distribution. This is in agreement with the large total body volume determined by Garret (1984) in dogs.

In the design of a dosage regimen to maintain therapeutic levels of a drug, the half-life, bio-availability, minimum therapeutic dose and therapeutic

index (ratio of toxic dose to therapeutic dose) must all be considered. In theory, dosage interval should be equal to the half-life of the drug to maintain therapeutic serum levels and therefore, the manufacturers recommended dosage interval of 8 and 12 hours respectively for oral and intravenous administration is questionable. However, naftidrofuryl does not have a recognised therapeutic serum level to aim for and there is no published data to indicate that there is any relation between the serum concentration of this drug and the therapeutic effect in humans.

The mode of action of naftidrofuryl remains poorly defined and there is no known correlation between its serum concentration and therapeutic effects. Further studies are needed to clarify this particular point.

Adverse effects

The drug appears to be well tolerated in humans and the incidence of adverse effects is low. Nausea, epigastric pain, headache, insomnia and rashes have all been reported (Drug and Therapeutic Bulletin, 1979; Martindale Pharmacopia, 1982). Thrombophlebitis may occur at peripheral intravenous infusion sites (Woodhouse and Eadie, 1977). Thrombophlebitis is common when naftidrofuryl is given as a continuous drip but no trouble has been experienced when it is given as an infusion (200 mg in 500 mls dextrose-saline) over 2 hours, the line being kept open with normal saline until the next infusion is due (Gann 1977).

Clinical use

Naftidrofuryl is used in cerebrovascular disease (Leading article, British Medical Journal, 1979; Admani, 1978; Cook and James, 1981), peripheral vascular disorders (Clyne and Lask, 1978; Nilsen, 1979; Drug and Therapeutic Bulletin, 1979) and Ménière's disease (Gibson et al., 1977). Its use in the above clinical contexts is outside the scope of this thesis and will not be discussed.

However, the reported effects of naftidrofuryl on the metabolic response to injury will be discussed.

Naftidrofuryl and the metabolic response to injury

Burns et al. (1981) reported that a twice daily naftidrofuryl infusion significantly improved post-operative nitrogen balance in patients undergoing elective surgery of moderate severity. They postulated that the drug may stimulate catabolism of carbohydrate and fat in the post-operative period, thus sparing tissue protein. In an attempt to explain this finding, Burns (1982) studied the effects of the drug on the concentrations of pyruvate, citrate and alpha-oxoglutarate in post-absorptive normal mouse liver. Four hours after naftidrofuryl injection, hepatic pyruvate and alpha-oxoglutarate contents were significantly elevated and hepatic citrate content was significantly decreased when compared to controls. By eight hours citrate and alpha-oxoglutarate had returned to normal but pyruvate content remained elevated. Burns concluded that the drug may stimulate glucose oxidation and increase the rate of cycling of the TCA cycle. These findings seem to support the hypothesis that naftidrofuryl improves nitrogen balance after surgery by making more carbohydrate available for oxidation and by improving the efficiency of energy production by the tricarboxylic acid cycle.

SECTION II

PATIENTS, MATERIALS AND METHODS

CHAPTER 6 ETHICAL CONSIDERATIONS

CHAPTER 7 METHODS AND MATERIALS

CHAPTER 8 PATIENTS STUDIED

CHAPTER 6

ETHICAL CONSIDERATIONS

ETHICAL COMMITTEE APPROVAL

The study was approved by the Ethical Committee of the Portsmouth and South East Hampshire Health Authority.

HAMPSHIRE AREA HEALTH AUTHORITY (TEACHING)

PORTSMOUTH AND SOUTH EAST HAMPSHIRE HEALTH DISTRICT

DISTRICT OFFICE SAINT MARY'S HOSPITAL PORTSMOUTH PO3 6AD

Portsmouth (0705) 27641 Ext. 227 Please ask for Mr Marshall

Our Ref. BM/RAP/32/1 Your Ref.

10th May 1982

Dear Mr Khawaja

The Effect of Praxilene (Naftidrofuryl) on the Metabolic Response To Surgery

Thank you for attending the meeting of the Ethical Committee held on Friday 7th May 1982. The members unanimously agreed that approval be given for the above project to proceed.

John Wiley and Sons have asked for co-operation in compiling a medical research directory, and if you are prepared to assist them by submitting details of this study, I enclose the relevant form which you should complete and return to them direct.

Yours sincerely

[redacted]

Chairman
Ethical Committee

The Ethical Committee consists of four members of the medical staff of the hospitals and a lay member nominated by the District Health Authority. It is guided by the code of ethics of the World Medical Association (Declaration of Helsinki). Its general terms of reference are "to ensure that high ethical standards are upheld throughout the course of any procedure undertaken in any hospital in the district in the course of investigations on human subjects".

Research projects submitted require the following information: responsible investigator, title of project, hypothesis to be tested, design of study, whether consent is to be obtained, details of patients selection, details of substances to be used and routes of administration, samples to be taken and discomfort and possible side effects to patients.

PATIENTS' CONSENT

The Wessex Regional Research Fund Committee found it advisable to include the patients' written consent form in the protocol of the study before approving a grant for the project. The research study was explained to the patients, in all cases in the presence of a trained nurse, and whenever possible in the presence of the next of kin, before they signed the consent form (see below).

CONSENT FOR PARTICIPATION IN A CLINICAL STUDY

Background

Following surgical operations it is known that there is a breakdown of body proteins. This is clearly undesirable but it can be lessened by giving intravenous solutions of various sugars, protein and fat. In addition, there is some evidence to indicate that the drug known as Praxilene may carry additional benefits. We wish to study whether this is the case and if so, how this drug

produces its benefits. In order to achieve these aims we need to compare the various solutions and the drug (Praxilene) for effects on protein breakdown and the concentrations of various substances present in the blood.

What the study will involve

If you decide to take part in this study, you will receive an infusion of fluids via a vein for 5 days after your operation. You will be allowed clear water to drink but no food or other drinks. It will be necessary to take a blood sample before your operation and on the first, third and fifth post-operative days. You will also be weighed and have the thickness of your skinfolds measured in a simple, non-painful way. Your urine will be collected throughout the study period.

The solution which you receive will be chosen independently and it may be given either into a vein in your arm or via another vein in your neck or chest. In addition, about half of the patients involved in this study will receive the drug, Praxilene, via an arm vein twice a day. Apart from the fact that it may reduce the breakdown of protein after your operation, this drug has no major side-effects. It has been safely used for a number of years in the treatment of other conditions and also in a pilot study in this hospital on 19 patients.

Apart from not being allowed to eat or drink fluids other than water, your treatment after the operation will otherwise be as normal and it should not in any way influence your recovery. However, you are at liberty to withdraw from the study at any time if you are unhappy about it.

I,.....hereby consent to take part in the above clinical study. Its

nature and purpose have been explained to me by:

.....

Patient's signature:.....

Doctor's signature:.....

Witness's signature:.....

Date: .....

CHAPTER 7

METHODS AND MATERIALS

ANTHROPOMETRIC MEASUREMENTS

Standing height, body weight and mid-arm circumference (MAC) were recorded. Skinfold thickness was measured with Holtain skin calipers (Holtain Ltd., U.K.) which exert a constant pressure at varying openings of the instrument-jaws. The triceps skinfold thickness (TSF) and MAC were measured, on the non dominant arm, mid-way between the olecranon and the tip of the acromion, with the upper arm hanging vertically (Edwards et al, 1955). Mid-arm muscle circumference (MAMC) was calculated from the formula: MAMC = MAC - π x (TSF). Lean body mass (LBM) was calculated according to the equations, appropriate for age and sex, as derived by Durnin and Womersley (1974).

PHYSIOLOGICAL AND OTHER MEASUREMENTS

Body temperature, fluid intake and urine output were recorded using standard ward equipments and methods. Particular care was taken to ensure accuracy of the urine collections. The ward ambient temperature was measured using a button resetting maxima and minima thermometer (Diplex, West Germany).

GENERAL ANAESTHETIC

All patients received a standard general anaesthetic in order to exclude the possibility of additional variation in the metabolic response due to different anaesthetic techniques. The premedication consisted of

1 - 2 mg Lorazepam (Ativan(R), Wyeth Laboratories) orally 2 - 3 hours before surgery.

The general anaesthetic was induced with 100 - 200 μg of fentanyl citrate (Sublimaze(R), Janssen Pharmaceuticals Ltd.), 3 - 4 mg kg^{-1} of thiopentone (Intraval(R), May and Baker Ltd.) and 200 - 250 μg kg^{-1} of alcuronium chloride (Alloferin(R), Roche Products Ltd.). An oral cuffed endotracheal tube was then sited (or an oral cuffed double lumen tube when thoracotomy was envisaged). The anaesthetic was then maintained with nitrous oxide.oxygen (67%.33%) and 0.2 - 1% enflurane (Ethrane(R), Abbott Laboratories Ltd.) using isocapnic intermittent positive pressure ventilation. Increments of fentanyl and alcuronium were used as required. The muscle relaxant was reversed with 0.5 mg of glycopyrronium bromide (Robinul(R), A.H. Robbins Co. Ltd.) and 2.5 mg of neostigmine methylsulfate (Prostigmin (R), Roche Products Ltd.).

Patients having a hiatus hernia repair underwent a 'crash induction' using pre-oxygenation, thiopentone (in the dose described above), suxamethonium chloride (1 - 1.5 mg kg^{-1}; Scoline(R), Duncan, Flockhart & Co. Ltd.) and intubation sequence. Once the effect of suxomethonium wore off, the patients were given alcuronium and fentanyl and the anaesthesia was maintained as above.

Post-operative analgesia was maintained with a peripheral vein infusion of 0.1% pethidine (500 mg in 500 mls of normal saline; Roche Products Ltd.) given at the rate of 0.2 - 0.3 mg kg^{-1} hr^{-1} for the first 36 - 48 hrs. Thereafter, the patients received, when needed, sublingual buprenorphine (Temgesic(R), Reckitt and Colman Pharmaceutical Division) in the dose of 200 -400 μg and intra-muscular injections of 2.5 - 5 mg perphenazine (Fentazin(R), Allen and Hanburys Ltd.) every 6 hours as required. Patients who had a thoracotomy received at time of surgery an intercostal nerve block using 0.5% plain bupivacaine hydrochloride (Marcain Plain(R), Astra Pharmaceuticals Ltd.).

Convulsions have been reported following enflurane anaesthesia (Kruczeck et al., 1980; Allan, 1984; Jenkins and Milne, 1984; Grant, 1986). For this reason patients with a known history of seizures were not considered for the study.

NUTRIENTS USED

A - NITROGEN SOURCES

The commercial sources of nitrogen used throughout this study were Perifusin(R) (E. Merck Ltd.) for the isotonic amino acid (IAA) group and Aminoplex 12(R) (Geistlich Sons Ltd.) for the total parenteral nutrition (TPN) group.

Table 7-1 shows the comparative nitrogen, energy and electrolyte content of each solution. The amino acid composition of these two mixtures is different (table:7-3) with much higher glycine and glutamic acid concentrations in Perifusin(R) than Aminoplex 12(R) (for a similar weight of nitrogen).

B - ENERGY SOURCES

1 - Carbohydrate

a- Glucoplex 1000(R) (400g/l anhydrous glucose; Geistlich Sons Ltd.) used for the TPN group.

b- 4.0% w/v dextrose + 0.18% w/v sodium chloride (Travenol Laboratories Ltd.) used for the dextrose-saline (D/S) group.

Table 7-2 shows the comparative energy and electrolyte content of the Glucoplex 1000(R) and Dextrose-Saline solutions.

2 - Fat

The fat source used was Intralipid 10%(R) (KabiVitrum Ltd.). This is a 10% emulsion of Soya bean

oil in water, stabilised with egg yolk phosphatides, lecithin and glycerol. It provides 1100 Kcal (4600 kJ) (100g fat) and 22.5 g of glycerol per litre.

C - ELECTROLYTES SOURCES

The infusion solutions were the major source of electrolytes. Table 7-4 shows the comparison, including the electrolyte concentrations, between the three infusion regimens. All patients received 1 litre of Hartmann's solution (compound sodium lactate; Travenol Laboratories Ltd. Electrolytes content in mmol/l: Na^+ = 131, K^+ = 5, Ca^{++} = 2, Cl^- = 111 and lactate = 29) during the peri-operative period.

Water and electrolytes were also provided daily between 01.00 and 09.00 hrs by isotonic sodium chloride solution [0.9% w/v;(154 mmol/l)].

ADMINISTRATION OF NUTRIENTS

A - Methods of administration

The 4% dextrose-saline and isotonic amino acids solutions were infused into the upper limb peripheral veins. The TPN solution was administered into the central venous system by catheters lying in large central veins. This is because such solutions are in general extremely hypertonic and rapidly cause thrombosis of small veins.

The 4% dextrose-saline and isotonic amino acid solutions were administered at a constant drip-rate regulated by the nursing staff. The TPN administration was regulated by an infusion pump (IVAC, IVAC corporation, USA).

B - Catheters used

1 - Peripheral venous catheters: Only teflon catheters were used (Venflon , Viggo AB., Sweden; size 14-16 FG).

2 - Central venous catheters: Size 14 FG were used.

- The 'Nutricath "S"' (Vygon, France) silicone catheter.

- The 'Drum-cartridge' catheter (Abbott Laboratories, Kent). This was used in five patients only.

Both catheters are radio-opaque and thus their tips may be easily ascertained.

C - Catheters siting

1 - Peripheral venous catheters

The catheters were preferably sited in the forearm veins (Cephalic or basilic veins or their branches). The dorsum of the hand and the anticubital fossa were avoided whenever possible to minimise the patients discomfort. The cannula was resited on clinical evidence of superficial thrombophlebitis (Maddox grade 2 or more; Maddox et al., 1977; table:8-2).

2 - Central venous catheters

To avoid the venous thrombosis caused by administration of hyperosmolar solutions into small veins, the catheters were manoeuvered so that their tips lay in the superior vena cava or innominate veins. This position was always checked by a chest Xray before starting the infusion (this was also checked to exclude a pneumothorax).

All central catheters were inserted by a single operator whilst the patient was still under general anaesthetic and using strict aseptic techniques. The 'Drum-cartridge' catheters were inserted via one or other anti-cubital fossa veins. The preferred access for the 'Nutricath"S"' catheters was via one of the internal jugular or subclavian veins. A skin tunnel was created, in all cases, by means of passing the intravenous cannula subcutaneously to meet the initial puncture site. Because of the danger of damaging the thoracic duct, the right

side was used whenever possible.

COLLECTION OF SPECIMENS

All specimens were collected by the same investigator.

1- BLOOD

Care was taken to ensure that the sampling was undertaken before the patient did any physical activity such as washing or walking. All blood specimens were taken, with the patient in the supine position, between 07.00 and 08.00 hrs to ensure that no errors in comparison arose from differences due to posture and circadian variations in concentrations. Peripheral venepuncture was performed with a cannula (Butterfly, size 21, Abbott Laboratories Ltd.) and care was taken to ensure that samples could be obtained without stasis.

Fasting blood samples were taken pre-operatively (Day 0) and on Days 1, 3 and 5 after operation, and following a 6 to 7 hours infusion-free time (01.00 hrs to 07.00-08.00 hrs) during which the patients received a 0.9% w/v sodium chloride infusion to maintain venous access.

Blood for estimation of serum urea, creatinine, sodium, potassium, bicarbonate, albumin and total protein concentrations was taken into plain 10mls glass tubes. For blood glucose measurements, 2 mls of blood were collected into fluoride oxalate tubes. For full blood count estimations 4 mls of blood were collected into EDTA (Ethylenediaminetetra-acetic acid) tubes. The 'glucose' and 'full blood count' tubes were agitated gently for 15 seconds to ensure thorough mixing. These specimens were sent without delay to the main hospital laboratory for routine analysis.

Samples (5 ml blood) for whole blood intermediary metabolites (lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and glycerol) were collected in

pre-weighed (Oertling R20 microbalance; L. Oertling Ltd., U.K.) cooled plastic tubes containing 15 ml of 8% w/v perchloric acid. The sample tubes were mixed thoroughly, put in ice immediately, transported to the laboratory as soon as possible, weighed (Oertling microbalance; thus calculating the weight of the added blood) and centrifuged at 4^{o} C (Mistral 6L; Measuring and Scientific Equipments, U.K.; 2400 RPM) for 15 minutes. A dilution factor was calculated from the volumes of perchloric acid and added blood. Blood volume was derived from weight using a standard specific gravity of 1.06 (Documenta Geigy, 1975). Acid volume was calculated from weight of acid and a specific gravity of 1.043 for 8% w/v perchloric acid. The pyruvate and acetoacetate were analysed immediately (within 90 minutes from the time of blood sampling). The remainder of the acid extract (the supernatant) was stored at -20^{o} C for later analysis.

Samples (15 ml blood) for estimation of amino acids, insulin and free fatty acids (FFA) were collected in lithium heparin tubes and transported on ice to the laboratory where they were centrifuged at 4^{o} C (Mistral 6L; 2400 RPM) for 15 minutes. For insulin and FFA the plasma was stored at -20^{o} C until analysis. For the amino acids the plasma was precipitated (within 90 minutes from blood sampling) in an equal volume of 6% sulphosalicylic acid in a 0.4 M lithium buffer and containing norleucine at 200 µmol/l as an internal standard. The precipitated proteins were separated by centrifugation at 4^{o} C (Mistral 6L; 2400 RPM) for 25 minutes and the supernatant stored at -20^{o} C until analysis.

2- URINE

Sequential 24 hour urine collections were started on the morning of the operation and were continued for 5 days post-operatively. The urine was collected in plastic bottles each containing 10 ml of 10% w/v thymol in isopropanol to inhibit bacterial growth. In order to

minimise possible errors, urine collection was checked by two trained nurses and the time and measured volume entered in the patient's fluid chart and on the urine bottle. At the end of each 24-hour period the total volume was measured, checked against the volume recorded on the fluid chart and the urine thoroughly mixed. 10 ml aliquots were collected into plain glass tubes and stored at -20^{o} C until analysis.

ANALYTICAL METHODS

Serum urea, creatinine, electrolytes, total protein, albumin, blood glucose, haemoglobin and packed cell volume were measured in the main hospital laboratory. The remainder of the blood and urine analyses were carried out in the metabolic research laboratory.

A- BLOOD

1 - The following estimations were done on the Technicon SMA 12/60 Auto-Analyser:

- Serum sodium and potassium estimation was by flame photometry with lithium as a reference. Aspiration was into a propane/air flame.

- Serum bicarbonate: The method used measures bicarbonate and physically dissolved carbon dioxide concentrations in the sample. It relies on the colour change of cresol red with varying hydrogen ion concentrations.

- Serum urea was determined by the diacetyl monoxime method.

- Serum creatinine was measured by the Jaffe method using alkaline picrate.

- Total protein were measured by the Biuret reaction and albumin by the bromo-cresol green dye binding method.

2 - Haematology

Haemoglobin and packed cell volume ratio (PCV) were measured on Coulter Counter S+1 (Coulter Electronics Ltd., UK)

3 - Glucose

Whole blood glucose was measured on a Technicon single channel Series 1 Auto-Analyser using a glucose oxidase reaction.

4 - Insulin

Plasma insulin concentrations were measured using a pre-precipitated double antibody radio-immunoassay (Immuno Nuclear Corporation - RIA, U.K. Ltd.). In this assay unlabelled insulin from the sample (antigen) competes with labelled insulin in the assay system for a limited number of available antibody binding sites. Antigen bound to the insulin antibody is precipitated with a pre-precipitated second antibody to the insulin antibody. Thus, the level of bound radio-activity in the precipitate is inversely related to the concentration of antigen but in a non-linear manner and may be quantified after counting in a gamma counter (Auto-Gamma 500C Radiation Measurement System, Packard instrument Company, INC., USA)) and comparison with a series of standards. For each sample, standard or unknown, the binding as a percentage of the binding in a zero standard is calculated. A semi-logarithmic plot of the percentage binding against concentration for the standard samples allows concentrations of the unknown samples to be determined.

5 - Pyruvate and acetoacetate

5 ml of acid extract (see 'Blood

sampling') were neutralised with 10% w/v potassium hydroxide and 10 µl Universal Indicator (BDH Chemicals Ltd., UK) and this dilution factor was calculated from the volumes of the acid extract and diluent. Pyruvate and acetoacetate were then analysed by a manual spectrophotometric method (Czok and Lamprecht, 1974; Williamson et al, 1962) within 90 minutes of blood sampling. This measured the reduction of absorbence of dihydronicotinamide adenine dinucleotide (NADH) at a wavelength of 340 nm (SP6 - 500 UV spectrophotometer, Pye Unicam) in a phosphate buffer (pH 7.0) by the addition of lactate dehydrogenase (LDH) or Beta-hydroxybutyrate dehydrogenase (BHBDH) respectively. For pyruvate the final absorption was read at 5 minutes. For acetoacetate this absorption was monitored at 5 minutes intervals. The final reading was taken when the absorption change was less than 0.005. The measured concentrations were corrected for dilution of blood in perchloric acid (see above: collection of specimens) and dilution of the acid extract during neutralisation.

6 - Lactate, 3-hydroxybutyrate and glycerol

The concentrations of these substances in the acid extract of whole blood (prepared as above) were measured by the semi-automated fluorometric method of Lloyd et al. (1978). The increases in NADH, following the addition of LDH, BHBDH and glycerol kinase (GK) respectively were measured by fluorometry (excitation at 355 nm and emission at 485 nm, Technicon Fluorometer 2). All samples were corrected for blank fluorescence by omitting the enzyme from a second run.

7 - Triglycerides

Total triglyceride concentration was measured by an enzymatic method (Wahlefeld, 1974; Boehringer Mannheim GmbH, Boehringer Corporation (London) ltd.). In this assay, enzymatic hydrolysis of

triglyceride leads to a series of stoichiometrically linked reactions:

$$\text{triglycerides} \xrightarrow{\text{lipase/esterase}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate + ADP}$$

$$\text{ADP + phospho(enol)pyruvate} \xrightarrow{\text{phosphokinase}} \text{pyruvate + ATP}$$

$$\text{pyruvate + NADH + } H^{+} \xrightarrow{\text{Lactate dehydrogenase}} \text{lactate + } NAD^{+}$$

Total triglycerides were quantified by measuring changes in NADH absorbence at a wavelength of 340 nm (Technicon Fluorometer 2).

8 - Free fatty acids

FFA were measured by a method based on the modification by Turnell et al. (1980) of the radiometric assay of Ho and Meng (1969). FFA were extracted into an organic phase. It was found necessary to use 0.5 M sulphuric acid for the extraction mixture, as in a standard Dole extraction (Dole, 1956), otherwise the extraction efficiency of the Turnell et al. method was very low.

On shaking with an aqueous solution containing ^{57}cobalt, stoichiometric formation of cobalt soaps of FFA caused the extraction of cobalt into the organic phase. Centrifugation was then performed at 4^{o} C (Mistral 6L; Measuring and Scientific Equipments, U.K.) to decrease isopropanol solubility in chloroform and reduce the blank effect. An aliquot of the organic phase was counted in a gamma counter (Auto-Gamma 500C Radiation Measurement System, Packard Instrument Company, INC., USA) and samples were quantified by comparison with a series of

standards.

9 - Amino acids

After pre-treatment of the plasma (see above) to remove the plasma proteins and add an internal standard, an aliquot was filtered using a 0.2μm cellulose nitrate membrane (Whatman membrane filters, Whatman Ltd., U.K.), prior to analysis on the 'Chromaspek J180' (Hilger Analytical, U.K.).

This analyser uses ion-exchange chromatography with a single column, gradient elution using two lithium citrate buffers (Thomas, 1970), ninhydrin reaction (Spackman et al., 1958) and potassium cyanide as a reducing agent (Thomas et al., 1966).

Ninhydrin is a powerful oxidising agent which causes oxidative decarboxylation of the alpha-amino acids and produces carbon dioxide, ammonia and an aldehyde with one carbon less than the parent amino acid. Reduced ninhydrin (hydrindantin) then reacts with the liberated ammonia to form a blue complex (Ruhemann's Purple) which maximally absorbs light of 570 nm wavelength. The intensity of this blue complex is a linear function of the concentration of alpha-amino groups present. The imino acid, proline, reacts with ninhydrin to form a yellow colour which is measured at 440 nm.

The amino acids were quantified automatically using the 'Supergrator-3' computing integrator (Columbia Scientific Industries, USA).

B - URINE

1 - Total nitrogen

Nitrogen compounds in 1-ml urine samples were oxidised to ammonium sulphate by acid digestion (Kjeldahl, 1883). This method uses concentrated sulphuric acid (96 -98%) heated to 420^{o} C, 0.5 g of copper sulphate as a catalyst, 5 g potassium sulphate to raise the boiling point of the mixture and hydrogen peroxide to aid in the

oxidation of the carbon compounds (Akesson, 1978; Mossberg, 1978; Technicon BD20 Method No. 369-75A/A, 1975).

Following this Kjeldahl digestion, ammonium ions in the diluted digests were analysed on a Technicon AA II continuous flow analyser fitted with an ammoniacal nitrogen manifold. This analyser uses a colourimetric reaction in which an emerald-green colour is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at a pH of 12.8 - 13. The ammonia-salicylate complex is read at 660 nm, the intensity of the colour being directly proportional to the concentration of ammonia (Reardon et al., 1966; Searcy et al., 1967; Crooke and Simpson, 1971; Technicon Industrial Method No. 334-74A/A, 1976).

2 - Urea and creatinine

The urine samples were prediluted 1/20 in 0.9% w/v sodium chloride. The urea concentrations were determined on a 'BUN Analyzer 2' (Beckman, USA) using an enzymatic conductimetric method.

The urinary creatinine concentrations were determined on a 'Creatinine Analyzer 2' (Beckman, USA) using a kinetic Jaffe reaction with alkaline picrate.

3 - Potassium

Urinary potassium was measured by flame photometry using the emission mode of an Unicam SP90A Series 2 Atomic Absorption Spectrophotometer (Pye Unicam, UK) with an air/acetylene flame. Measurements were made at 755 nm wavelength.

REPRODUCIBILITY OF RESULTS

Accuracy and precision of the determinations were monitored by the inclusion of quality control samples in each assay batch. Where long term stability of the quality control was doubtful some samples

from the previous assay were, in addition, re-analysed. If control samples failed to meet the accuracy or the precision limits of the laboratory the assay was repeated.

CALCULATION AND PRESENTATION OF RESULTS

UNITS

All results in the text, tables and figures are expressed in S.I. units (Le Système International d'Unités) as mean ± standard error of the mean (sem). The energy was expressed in equivalent kcal as well.

NITROGEN BALANCE

The nitrogen balance was calculated as the difference between the intake of nitrogen and the urinary losses of total nitrogen for each day. The nitrogen intake was calculated from the exact amount of fluid infused (i.e. total volume less measured unused volume). Faecal, drain and perspiration losses and other losses via the skin were considered minimal and were not taken into account. The nitrogen balance was corrected for changes in total urea pool, assuming the total body water to be 60% of the body weight in males and 55% in females and the daily changes in body weight negligible. The values of total body water determined t. means of tritiated water are in good agreement with those calculated from the body weight as above (Bergström et al, 1972).

The correction was done according to the following equations:

$NB_C = NB_m$ - (increase in TUP)

TUP = SU x 0.028 x Wt x F

where F = 0.6 for males and 0.55 for females

NB_C = Corrected Nitrogen balance (g)

NB_m = Measured nitrogen balance (g)

TUP = Total urea pool (g nitrogen)

SU = Serum urea (mmol/l)

Wt = Body weight (kg)

UREA PRODUCTION

For a given time, the ureagenesis was calculated according to the following equation:

$U_p = U_e$ + (increase in TUP)

TUP = SU x Wt x F

where F = 0.6 for males and 0.55 for females

U_p = Urea production (mmol)

U_e = Urinary urea excretion (mmol)

TUP = Total urea pool (mmol)

SU = Serum urea (mmol/l)

Wt = Body weight (kg)

POTASSIUM BALANCE

This balance was calculated as the difference between the potassium infused and the urinary losses of potassium for each day. Drain and faecal losses were considered as negligible.

TOTAL KETONES

Total ketones were expressed as the sum of 3-hydroxybutyrate and acetoacetate.

ALBUMIN AND TOTAL PROTEIN

Packed cell volume ratio (PCV) reflects changes in haemodilution which affects the concentration of albumin and total protein. To account for this effect, which is marked after surgery, the post-operative concentrations of albumin and total protein were corrected to the pre-operative PCV using the equation:

$$C_C = C_m \frac{1 - PCV_m}{1 - PCV_o}$$

C_C = corrected concentration (g/l)

C_m = measured concentration (g/l)
PCV_o = PCV at Day 0
PCV_m = measured PCV

CREATININE CLEARANCE

Creatinine clearance was calculated from the formula:

$$\text{Creatinine clearance (ml/min)} = \frac{U \times 1000}{t \times P}$$

U = urinary creatinine excretion (mmol/l) during time t
t = time during which urine was collected (minutes)
P = serum creatinine (μmol/l)

TERMINOLOGY

For analytical purposes 'between group' differences compare naftidrofuryl with the control group and 'between infusion' differences compare the effects of different intravenous regimens without reference to naftidrofuryl. '3-day' and '6-day' urinary results represent the cumulative first 3 days and 6 days measurements. 'D/S', 'IAA', and 'TPN' refer to the dextrose-saline, isotonic amino acid and total parenteral nutrition groups respectively. 'Naf-pre' and 'Naf-post' represent the subgroups in the dextrose-saline infusion group which received Naftidrofuryl with the induction of anaesthesia (Naf-pre) or on the first post-operative day (Naf-post). All times refer to the 24-hour clock and 'w/v' indicates concentrations expressed on a weight to volume basis.

PRESENTATION OF RESULTS

The data is presented in tabular and graphic form at the end of each chapter.

For the total ketones which required logarithmic transformation for statistical analysis (see

below), the tables present the geometric mean but the figures (graphs) describe the untransformed data.

The figures for nitrogen balance are laid out according to Reifenstein et al. (1945). The error bars in all figures represent 1 standard error of the mean (sem).

STATISTICAL ANALYSIS

The statistical calculations were done on a Hewlett-Packard (HP-85) computer. The data was stored on tape, retrieved and analysed using programs specifically written for this study. The Student t-test was used for data which were continuous and normally distributed, whereas the Wilcoxon Rank Sum test was used for data with skewed distribution which could not be normalised. Statistical significance was attributed to probabilities of $p<0.05$ by both paired and unpaired tests. Unpaired tests were used to compare the values between two groups . Changes within the respective groups (between day changes; Days 1, 3 & 5 vs Day 0) were evaluated by paired tests. A two-tailed probability distribution was used to test for changes in either direction.

Where multiple comparisons were made as in chapter 13 (i.e. D/S group vs IAA group, D/S group vs TPN group, and IAA group vs TPN group), the use of analysis of variance was initially used. However, it revealed in many instances large differences in variance between the groups when evaluated by Bartlett's test. A prime requirement for this analysis is the assumption that "all groups are subject to the same within groups variance and if there is doubt about the near-validity of this assumption it will be safer to rely on the data from the two groups alone" (Armitage, 1971). Therefore, in view of different between group variances and the small number of comparisons (3 groups) t- or Wilcoxon Rank Sum tests were used for statistical analysis. The limitations of this technique were borne in mind when interpreting the statistical results in chapter 13 and the level of significance was

taken as $p<0.02$. This figure was calculated using the Bonferroni correction where the accepted level of significance for one comparison ($p<0.05$) is divided by the number of comparisons ($0.05/3 = 0.0166$). The 0.02 figure is the nearest generally available in standard statistical tables (Documenta Geigy, 1975).

When the measured variables were positively skewed with some very high values (e.g. total ketones), the data was logarithmically transformed. This resulted in a normal distribution which made it appropriate for parametric analysis. Afterwards, the results were converted back into the original scale for reporting. In this case, the antilog of the mean of the log data (known as the geometric mean) was used and a range of 1 sem (standard error of the mean) about the mean was quoted (Altman et al., 1983).

Where the between group pre-operative concentrations were different, the magnitude of change (paired data) between days was also compared. If this revealed a significant difference than it was tested for a correlation with Day 0 concentrations. If this were to be significant it could indicate that the change might not be due to the treatment but only to a relationship with the starting pre-operative concentrations.

LIMITATIONS OF THE STUDY DESIGN

Nitrogen balance is a measure of whole body protein metabolism and fails to identify changes in protein synthesis and breakdown. An improvement in nitrogen balance may be due to a relatively greater increase in protein synthesis over breakdown or to a greater decrease in protein breakdown than in synthesis. Isotopic labelling studies could have identified such variations but these methods have their own limitations (Waterlow, 1984; Garlick and Fern, 1985; Bier et al., 1985) and depend on the availibility of necessary equipments. 3-methylhistidine excretion was not measured since its use as an indicator of muscle protein breakdown

protein is controversial (Williamson et al., 1977; Bilmazes et al., 1978; Young and Munro, 1978, Elia et al., 1981; Ballard and Thomas, 1983; Rennie and Millward, 1983 and Rennie et al., 1984).

The kinetics of energy substrates have been investigated by ^{13}C- and ^{14}C-labelled precursors with determination of the exhaled labelled carbon dioxide. Direct and indirect calorimetry have also been used to study the utilisation of energy substrates. The net 'flux' of a substrate across defined tissues (e.g. limb) has been calculated from determination of its arterio-venous difference and the blood flow. However, these techniques have got their own limitations and require skills and equipments which are not available locally. Venous concentrations of intermediary metabolites and amino acids do not accurately reflect changes at cellular level and do not necessarily correlate with substrate or amino acid turnover. However, serial measurements give information on the metabolic changes but offer no information on fluxes.

Naftidrofuryl oxalate was used in the dosage recommended by the manufacturers (200 mg 12 hourly). In theory, dosage interval should be equal to the half-life of the drug to maintain therapeutic serum levels. Thus, the manufacturer's recommended dosage interval is questionable since the mean half-life for intravenous administration in humans is 0.99 hour (range:0.63 - 1.5 hours). Therefore, the use of naftidrofuryl oxalate in a continous infusion (Inglis et al., 1983) is more plausible. Furthermore, naftidrofuryl oxalate does not have a recognised serum therapeutic level to aim for and there is no published data to indicate that there is a relation between the serum concentration of this drug and its therapeutic effect in humans.

TABLE:7-1 **COMPOSITION OF PERIFUSIN(R) AND AMINOPLEX 12(R) (per litre)**

	Perifusin	Aminoplex 12
Nitrogen (g)	5	12.4
Energy (kcal(kJ))	132(550)	325(1300)
K^+ (mmol)	30	30
Na^+ (mmol)	40	35
Mg^{++} (mmol)	5	2.5
Cl^- (mmol)	9	67.2
Acetate (mmol)	10	5
Malate (mmol)	22.5	33.1

TABLE:7-2 **COMPOSITION OF GLUCOPLEX 1000(R) AND 4% DEXTROSE 0.18% w/v SALINE (per litre)**

	Glucoplex 1000	Dextrose-saline
Energy (kcal(kJ))	1000 (4200)	160 (670)
K^+	30	0
Na^+	50	30
Cl^-	67	30
Mg^{++}	2.5	0
H_2PO4^-	18	0
Zn^{++}	0.46	0

TABLE:7-3 AMINO ACID COMPOSITION OF AMINOPLEX 12(R) AND PERIFUSIN(R) (g AMINO ACID/10 g NITROGEN)

	Aminoplex 12	Perifusin
L-Isoleucine	3.05	2.12
L-Leucine	4.66	2.9
L-Lysine HCL	5.47	3.3
L-Methionine	3.86	2.78
L-Phenylalanine	5.53	2.9
L-Threonine	2.57	1.32
L-Tryptophan	1.13	0.66
L-Valine	3.6	1.98
L-Arginine	7.4	5.28
L-Histidine	1.77	1.32
L-Alanine	8.04	7.92
L-Glutamic acid	1.61	11.88
Glycine	3.54	13.2
L-Proline	9.65	9.24
L-Ornithine-L Aspartate	1.61	-
L-Serine	1.93	-
EAA/NEAA	0.84	0.37

EAA/NEAA = Essential to non-essential amino acids ratio

TABLE:7-4 COMPARISON BETWEEN THE INFUSION REGIMENS. (COMPOSITION per litre)

	D/S solution	IAA solution	TPN solution
Nitrogen g	0	5	4.3
Energy kcal(kJ)	160 (670)	132 (550)	685 (2880)
K^+ mmol	0	30	20.6
Na^+ mmol	30	40	29.2
Mg^{++} mmol	0	5	1.7
Cl^- mmol	30	9	46
H_2PO4^- mmol	0	0	10.8
Zn^{++} mmol	0	0	0.016
Malate mmol	0	22.5	11.4
Glycerol mmol	0	0	76.5
Acetate mmol	0	10	1.7
Fat g	0	0	31.17

D/S = Dextrose-Saline

IAA = Isotonic amino acid

TPN = Total parenteral nutrition

CHAPTER 8

PATIENTS STUDIED

SELECTION AND RANDOMISATION OF PATIENTS

Patients undergoing elective intra-abdominal surgery were studied. Only patients aged between 18 and 75 years who had lost less than 2 Kg in weight in the previous 2 months were considered. Patients with known epilepsy (see: general anaesthetic - chapter 7), metastatic cancer, localised or widespread sepsis, endocrine disorders, cardiac or respiratory insufficiency, atrioventricular block and those receiving hormonal replacement including steroids and/or the contraceptive pill were excluded. The study was terminated in patients who lost 3 or more units of blood in the peri-operative period.

The patients were allocated to one of 3 infusion groups. A dextrose-saline group (D/S) received a peripheral intravenous infusion of 4% dextrose-saline delivering 6 kcal(25 kJ)of carbohydrate kg^{-1} day^{-1}. An isotonic amino acid group (IAA) received a peripheral infusion of isotonic amino acids delivering 0.15 g of nitrogen kg^{-1} day^{-1} over the same period. A total parenteral nutrition group (TPN) received, in a 3-litre bag, a central venous infusion of carbohydrate, amino acids and fat. This regimen delivered the same amount of nitrogen daily as the IAA group and 24 non-protein kcal (100 kJ) kg^{-1} day^{-1}. The non-nitrogen energy to nitrogen ratio was 160:1[kcal:g ; 670:1 (kJ/g)] with the non-nitrogen energy given as 50% fat and 50% carbohydrate. These daily infusions were delivered over 16 hours (from 09.00 hrs till 01.00 hrs the following day). Thereafter (01.00 hrs till 09.00hrs), the patients received an infusion of normal saline to maintain venous access. On

the operative day and for each infusion group, all patients received a standard regimen regardless of body weight [D/S group = 240 kcal (1004 kJ); IAA group = 5 g nitrogen; TPN group = 5 g nitrogen and 800 kcal (3349 kJ)]. The patients were allowed clear water only by mouth throughout the study period which lasted 6 days (the operative and 5 post-operative days).

An "ideal" study would have examined all three infusion groups simultaneously to remove a possible time related effect. However, it was felt that this would have introduced even greater potential variations due to the difficulty of nursing large number of different infusion regimens simultaneously on non metabolic wards. Consequently, the D/S group was studied first followed by the IAA then the TPN groups.

Each infusion group was further divided into control and naftidrofuryl (Naf) subgroups by randomly allocating to each patient a plain coded box containing either placebo or 200 mg of naftidrofuryl oxalate per phial. The trial material was supplied by Lipha Pharmaceuticals Ltd. and the key to randomisation held by the Principal Pharmacist and made available when the data was ready for analysis.

To exclude a possible error of randomisation by the manufacturers, the contents of a small number of trial material boxes were examined for ultra-violet (U.V.) light absorption. In all cases, the U.V.absorption correlated with the coding supplied.

The patients received a twice daily infusion (09.00 hrs and 21.00 hrs) of the trial material in 250 ml of normal saline (0.9% w/v sodium chloride) given over 2 hours and starting with the induction of anaesthesia.

To determine whether the nitrogen sparing effect of naftidrofuryl would also be obtained when the drug is given post-operatively as would be the case in non-surgical trauma, a third D/S subgroup [naftidrofuryl-post (Naf-post)] was studied. These patients received the first dose of naftidrofuryl, not

with the induction of anaesthesia but on the first post-operative morning, after blood sampling. This group was not blind. Naftidrofuryl-pre patients (Naf-pre) represent the D/S patients who received naftidrofuryl with the induction of anaethesia.

PATIENTS STUDIED

149 patients were selected to enter the study but only 106 (71.1%) satisfied the criteria for inclusion in the final data analysis. 43 patients were excluded because of failure to complete the first half of the study (first 3 days), post-operative sepsis, incomplete urine collection or discovery of latent diabetes mellitus (table:8-1).

Post-operative sepsis was defined as a clinical, radiological, bacteriological and/or haematological evidence of localised or widespread infection with a single axillary temperature of 38.5^{o} C or more. Deep venous thrombosis (DVT) was diagnosed on well established clinical criteria. The only death, unrelated to the study, occured in an otherwise fit 57 year old man on the third post-operative day following an uncomplicated cholecystectomy. Post mortem examination revealed bilateral femoral and iliac vein thrombosis with a massive pulmonary embolus. It is important to note that all patients were encouraged to mobilise as early as possible and the intravenous infusion did not hinder their mobility (the patients were regularly visited by the physiotherapists and tripods-on-wheels drip stands were readily available).

Patients with pre-operative fasting blood glucose concentration greater than 6.0 mmol/l were excluded from the study and further investigated routinely to exclude the possibility of latent diabetes mellitus.

The incidence and severity of infusion phlebitis were estimated at 12-hour intervals using the method described by Maddox et al. (1977; table:8-2). Patients

with Maddox grade 3 or more phlebitis were excluded from the study. One of the five patients who had a `Drum-cartridge' central venous catheter developed a severe thrombophlebitis (Maddox grade 4), on the third post-operative day, along the course of the brachio-cephalic vein. The catheter was removed and the patient excluded from the study.

Voluntary withdrawal from the study, (either in the early period or after successfully completing the first three days of the study), was due to the patients feeling hungry and insisting on oral calorie intake.

Incomplete urine collection was due, in all cases, to the patients voiding their bladder in the toilet or the collected specimen being accidentally discarded in the sluice.

It is evident from the above (table:8-1) that the exclusions were not biased towards any particular group and that none of the conditions leading to the exclusion of patients was due to naftidrofuryl.

Tables:8-3, 8-4 and 8-5 show the operations carried out and the anthropometric data in the D/S, IAA and TPN groups respectively. The mean weight and lean body mass (LBM), in the TPN group, were heavier in the control than the naftidrofuryl patients. This was, almost certainly, due to a bias within the random selection and should not affect the interpretation of the data as the results are expressed per Kg body weight. Otherwise, the control and naftidrofuryl patients, in all infusion groups, were matched for age, sex, anthropometric measurements and the type of surgical procedure.

TABLE: 8-1 DETAILS OF PATIENTS EXCLUDED FROM AND NOT COMPLETING THE STUDY

		Dextrose-saline group			Isotonic amino acid group		Total parenteral nutrition group	
		Control	Naf-Post	Naf-pre	Control	Naf	Control	Naf
FAILURE TO COMPLETE THE FIRST HALF OF THE STUDY	Voluntary withdrawal	2	1	1	2	2	3	2
	Deep venous thrombosis	-	-	1	-	-	1	-
	Difficult venous access	1	1	1	1	2	-	1
	Infusion phlebitis	-	-	-	-	-	-	1
	Death	-	-	-	1	-	-	-
POST-OPERATIVE SEPSIS		1	-	1	1	-	1	1
INCOMPLETE URINE COLLECTION		2	-	2	2	1	2	2
FASTING BLOOD GLUCOSE > 6.0 mmol/l		-	-	1	-	1	1	-
TOTAL PATIENTS EXCLUDED		6	2	7	7	6	8	7
VOLUNTARY WITHDRAWAL AFTER SUCCESSFULLY COMPLETING THE FIRST 3 DAYS OF THE STUDY		-	-	-	3	3	2	3

TABLE:8-2 CRITERIA FOR SEVERITY OF INFUSION PHLEBITIS

(After Maddox et al., 1977)

0	No pain at intravenous site, no erythema, no swelling, no induration, no palpable venous cord.
1	Painful intravenous site, no erythema, no swelling, no induration, no palpable venous cord.
2	Painful intravenous site with erythema or some degree of swelling, or both, no induration, no palpable venous cord.
3	Painful intravenous site with erythema and swelling and with induration or a palpable venous cord less than three inches above intravenous site.
4	Painful intravenous site, erythema, swelling, induration and a palpable venous cord greater than three inches above intravenous site.
5	Frank vein thrombosis along with all signs of 4; intravenous may have stopped running due to thrombosis.

TABLE: 8-3 PRE-OPERATIVE ANTHROPOMETRIC DATA AND SURGICAL PROCEDURES IN THE DEXTROSE-SALINE GROUP

	CONTROL			NAFTIDROFURYL						NAFTIDROFURYL ALL		
				after surgery			before surgery					
	mean	± sem	n	mean	± sem	n	mean	± sem	n	mean	± sem	n
ANTHROPOMETRIC MEASUREMENTS												
HEIGHT (cm)	167.3	± 2.35	16	168.7	± 2.59	11	170.2	± 5.0	8	169.3	± 2.50	19
WEIGHT (kg)	66.6	± 2.5	16	69.1	± 3.4	11	66.9	± 4.5	8	68.2	± 2.66	19
MID-ARM CIRCUMFERENCE (cm)	24.3	± 0.7	16	25.6	± 0.9	11	24.0	± 0.7	8	24.8	± 0.6	19
LEAN BODY MASS (kg)	45.2	± 1.8	16	50.1	± 3.4	11	46.7	± 3.2	8	48.4	± 2.2	19
AGE (years)	54.7	± 3.3	16	50.2	± 4.1	11	56	± 5.4	8	52.6	± 3.2	19
MEN:WOMEN	8:8			4:4			5:6			9:10		
SURGICAL PROCEDURES												
CHOLECYSTECTOMY	9			4			6			10		
TRANSVERSE COLECTOMY	1			-			-			-		
SIGMOID COLECTOMY	-			1			-			1		
ANTERO-POSTERIOR RESECTION	1			1			1			3		
RESTORATION OF COLONIC CONTINUITY FOLLOWING HARTMANN'S PROCEDURE	-			1			-			1		
LAPAROTOMY	1			-			-			-		
HIGHLY SELECTIVE VAGOTOMY	1			1			1			2		
PARTIAL GASTRECTOMY FOR CARCINOMA	1			-			1			1		
IVOR LEWIS OESOPHAGECTOMY	1			-			1			-		
AORTO-BIFEMORAL BYPASS GRAFT	1			-			1			1		

TABLE: 8-4 PRE-OPERATIVE ANTHROPOMETRIC DATA AND SURGICAL PROCEDURES IN THE ISOTONIC AMINO ACID GROUP

	CONTROL mean ± sem	n	NAFTIDROFURYL mean ± sem	n
ANTHROPOMETRIC MEASUREMENTS				
HEIGHT (cm)	170.7 ± 2.1	18	169.9 ± 1.6	18
WEIGHT (kg)	68 ± 3.4	18	70.3 ± 2.6	18
MID-ARM MUSCLE CIRCUMFERENCE (cm)	24.2 ± 0.7	18	24.8 ± 0.7	18
LEAN BODY MASS (kg)	47.3 ± 2.0	18	49 ± 1.7	18
AGE (years)	55 ± 3.3	18	52.4 ± 3.35	18
MEN:WOMEN	10:8		9:9	
SURGICAL PROCEDURES				
CHOLECYSTECTOMY	9		9	
CHOLECYSTECTOMY + APPENDICECTOMY	-		1	
LEFT HEMICOLECTOMY	-		1	
ANTERIOR RESECTION	2		1	
ANTERO-POSTERIOR RESECTION	1		1	
PARTIAL GASTRECTOMY FOR CARCINOMA	1		-	
HIGHLY SELECTIVE VAGOTOMY	1		1	
HIGHLY SELECTIVE VAGOTOMY AND NISSEN FUNDOPLICATION	-		1	
NISSEN FUNDOPLICATION	2		1	
IVOR LEWIS OESOPHAGECTOMY	1		1	
AORTO-BIFEMORAL BYPASS GRAFT	1		1	

TABLE: 8-5 PRE-OPERATIVE ANTHROPOMETRIC DATA AND SURGICAL PROCEDURES IN THE TOTAL PARENTERAL NUTRITION GROUP

	CONTROL mean ± sem	n	NAFTIDROFURYL mean ± sem	n
ANTHROPOMETRIC MEASUREMENTS				
HEIGHT (cm)	171.3 ± 2.3	17	167.4 ± 2.0	18
WEIGHT (kg)	72.7 ± 2.5	17	64.9 ± 2.8	18*
MID-ARM CIRCUMFERENCE (cm)	25.1 ± 0.6	17	23.3 ± 0.6	18
LEAN BODY MASS (kg)	50.2 ± 1.7	17	44.8 ± 1.8	18*
AGE (years)	58.7 ± 3.4	17	52.4 ± 2.9	18
MEN:WOMEN	9:8		9:9	
SURGICAL PROCEDURES				
CHOLECYSTECTOMY	8		7	
LEFT HEMICOLECTOMY	-		1	
RIGHT HEMICOLECTOMY	1		1	
ANTERIOR RESECTION	1		1	
ANTERO-POSTERIOR RESECTION	1		1	
RESTORATION OF COLONIC CONTINUITY FOLLOWING HARTMANN'S PROCEDURE	1		1	
PARTIAL GASTRECTOMY FOR CARCINOMA	1		1	
HIGHLY SELECTIVE VAGOTOMY	1		1	
HIGHLY SELECTIVE VAGOTOMY AND NISSEN FUNDOPLICATION	1		2	
IVOR LEWIS OESOPHAGECTOMY	1		1	
AORTO-BIFEMORAL BYPASS GRAFT	1		1	

Significance vs control group: * $p<0.05$

SECTION III

STUDIES IN THIS THESIS

CHAPTER 9	EFFECT OF NAFTIDROFURYL WITH EXOGENOUS ENERGY SUBSTRATES ON THE URINARY EXCRETION OF NITROGEN, UREA, CREATININE AND POTASSIUM
CHAPTER 10	EFFECT OF NAFTIDROFURYL WITH 4% DEXTROSE-SALINE INFUSION ON THE METABOLIC RESPONSE TO SURGERY
CHAPTER 11	EFFECT OF NAFTIDROFURYL WITH ISOTONIC AMINO ACID INFUSION ON THE METABOLIC RESPONSE TO SURGERY
CHAPTER 12	EFFECT OF NAFTIDROFURYL WITH GLUCOSE,FAT AND AMINO ACID INFUSION ON THE METABOLIC RESPONSE TO SURGERY
CHAPTER 13	EFFECT OF EXOGENOUS ENERGY SUBSTRATES ON THE METABOLIC RESPONSE TO SURGERY
CHAPTER 14	CONCLUSIONS

CHAPTER 9

EFFECT OF NAFTIDROFURYL AND EXOGENOUS ENERGY SUBSTRATES ON THE URINARY EXCRETION OF NITROGEN, UREA, CREATININE AND POTASSIUM

Injury, whether surgical or accidental is usually associated with a negative nitrogen balance, the extent of which reflects the severity of the trauma (Kinney, 1967). The methods used to moderate this nitrogen loss and improve the prognosis after surgery have included the infusion of various exogenous nutrients (Allison, 1974; Mullen et al., 1980; Williams et al. 1976).

Burns et al. (1981) suggested that the infusion of naftidrofuryl in patients undergoing elective surgery resulted in a reduction of urinary nitrogen excretion. This chapter describes the effect of naftidrofuryl oxalate, with the provision of different exogenous energy substrates on the nitrogen and potassium balances and the urinary excretion of urea and creatinine.

The study of Burns et al. (1981) was limited to the nitrogen losses on the operative and first two post-operative days. However, maximum nitrogen excretion may occur beyond this period (Swaminathan et al., 1979) and therefore the studies reported here were carried out over the operative and first five post-operative days.

PATIENTS AND METHODS

The patients and methods are those described in section II (chapters 7 & 8) and used throughout the study. The patients were assigned to one of three infusion groups. The D/S group received 4% dextrose-saline [6 kcal (25 kJ) kg^{-1} day^{-1}]. The IAA group received a peripheral intravenous infusion of isotonic amino acids [0.15 g nitrogen kg^{-1} day^{-1}]. The TPN group received a central

venous infusion of carbohydrate, amino acids and fat [0.15 g nitrogen and 24 non-nitrogen kcal (100 kJ; 50% carbohydrate and 50% fat) kg^{-1} day^{-1}]. Each infusion group was further randomised to receive, with the induction of anaesthesia, a twice daily infusion of 200 mg of naftidrofuryl oxalate (naftidrofuryl group) or placebo (control group). In the D/S infusion group a third subgroup which received the first dose of naftidrofuryl on the first post-operative day was also studied (Naf-post).

The results are reported for the full 6-day period and also for the shorter 3-day period which has been used in similar studies (Burns et al., 1981; Inglis et al. 1983).

RESULTS

Tables 9-1, 9-2 and 9-3 show the cumulative 3 and 6-day urine volume, nitrogen intake and nitrogen balance corrected for changes in total urea pool (see chapter 7), urinary excretion of urea and creatinine and potassium balance respectively in the D/S, IAA and TPN groups.

DEXTROSE-SALINE GROUP (table:9-1 & figures:9-1A & 9-1B)

There were no significant between group differences in the nitrogen intake, the nitrogen balance and the urea excretion. The 3-day urinary creatinine excretion was lower in the control group when compared to the naftidrofuryl-pre (Naf-pre; $p<0.005$) and naftidrofuryl- all (Naf-all = Naf-post + Naf-pre; $p<0.05$) groups. However, this difference was not significant for the 6-day period.

No significant between group differences were detected in any of the other measured variables.

ISOTONIC AMINO ACID GROUP (table:9-2 & figure:9-2)

There were no significant between group differences in the nitrogen intake, the nitrogen balance and the urea excretion. The cumulative 3-day urinary potassium balance was lower in the naftidrofuryl group

(Naf) but not significantly so; this difference became significant ($p<0.05$) on the cumulative 6-day measurement. There were no significant between group differences in the other measured variables.

TOTAL PARENTERAL NUTRITION GROUP (table:9-3 & figure:9-3)

No significant between group differences were detected in any of the measured variables.

In order to minimise possible interference by occult differences between subgroups, the nitrogen balance results within each infusion group were analysed for a naftidrofuryl effect in males only, females only, cholecystectomy operations only and age range (less than 40 years, between 40 and 60 years and over 60 years). No naftidrofuryl effect was observed in any of these subgroups, although it must be borne in mind that the consequent reduction in the number of patients reduces the ability to detect an effect.

Table 9-4 and figures 9-4A, 9-4B & 9-4C show no naftidrofuryl effect, in any of the infusion groups, on the daily nitrogen balance of the patients who have completed the study (6 days). There was no significant between group difference in the daily nitrogen intake (table:9-5). The results for Day 0 and Day 1 were pooled [(Day 0 + Day 1)/2] due to the known post-operative urinary bladder retention thus rendering the first 24 hr collection unrepresentative. Similar pooling of days 0 and 1 was performed for all other daily urinary measurements.

There was no between group difference, in any of the infusion groups, in the volume of the infusate (table:9-6).

During the study period, the recorded ambient temperature of the wards where the patients were studied fell between 20.9^{o} C and 25.6^{o} C with no difference between the wards.

To rule out an independent effect of naftidrofuryl on renal function, the creatinine clearance was measured

on the last day of the study in 10 control and 10 treated patients in each infusion group (table:9-7). There was no difference due to naftidrofuryl. This agrees with the study of Heidrich et al. (1975), which demonstrated no effect of Naftidrofuryl on renal function after 14 days infusion. It is also in agreement with the findings of Burns et al. (1981) who showed no difference, over 3 days, in the daily urea clearance in naftidrofuryl and control patients receiving 5% dextrose.

DISCUSSION

The above data clearly shows that a twice daily infusion of 200 mg naftidrofuryl oxalate does not have a nitrogen sparing effect, in any of the infusion groups, in patients undergoing elective abdominal surgery. These findings do not agree with the work of Burns et al. (1981) and Galloway et al.(1983) who reported that, over a 3-day period, this drug reduced nitrogen excretion following elective surgery in patients who received dextrose and saline fluid replacement only. However, they complement the findings of Inglis et al. (1983 and 1984) who showed no effect of this drug on nitrogen balance when given as a continuous infusion or as a twice daily bolus intravenous injection.

The variation in the ambient temperature over the course of the study was minimal (4.7^{o} C; 20.9^{o} C to 25.6^{o} C) and is unlikely to affect the metabolic response. Furthermore, its unlikely that the patients were exposed to such variation in ambient temperature since they wore bed gowns and were covered by a sheet or blanket.

The greater 3-day urinary creatinine excretion, in the D/S patients, by the Naf-pre and Naf-all groups when compared to the control group is not detected in the other 2 infusion groups and the difference does not reach statistical significance in the final 6-day result. It is also accepted that creatinine excretion is more closely related to lean body mass (LBM) than to body weight and

when the results are expressed per unit of LBM the difference is not significant (table:9-8). Therefore, the above finding is probably spurious and due to the 'inappropriate' use of weight as opposed to LBM as an expression unit. Consequently, this isolated finding will not be taken into account in the rest of the study.

The IAA patients showed no significant between group difference in either the cumulative 6-day potassium excreted or received. However, the difference in the potassium balance was significant with the naftidrofuryl group retaining more potassium (table:9-9). There was no significant between group difference in the pre or post-operative serum potassium concentration (see chapter 11, table:11-4) and none of the studied patients were on diuretic therapy.

Although potassium is excreted into the gut in the digestive fluids, much of this is later reabsorbed and the kidney is the principle organ for its excretion. This is markedly influenced by changes in acid-base balance and the activity of the adrenal cortex. The aldosterone response to surgery is influenced not only by the injury but also by the volume and electrolyte content of fluids administered (Engquist et al, 1978; Cochrane, 1978). None of the studied patients had diarrhoea, vomiting, drain losses greater than 250 ml over the study period or an intestinal fistula to account for any potassium losses outside the urine. The serum bicarbonate concentrations followed the same pattern in the control and treatment groups (see chapter 11, table:11-4) and therefore the difference can not be attributed to a change in acid-base balance.

The adrenal cortical activity was not measured and one possible explanation is that, in spite of the groups being matched for age, sex, anthropometric measurements, type of the surgical procedure (see chapter 8, table:8-4)and the volume and nature of the infusate (table:9-5), the naftidrofuryl group may conceivably have had a lower aldosterone response. However, given the

available data this explanation cannot be ascertained.

In conclusion, a twice daily infusion of 200 mg naftidrofuryl oxalate has no measurable effect, either deleterious or beneficial, on the nitrogen balance in patients undergoing elective abdominal surgery and receiving an infusion of 4% dextrose- saline, isotonic amino acids or a mixture of amino acids, carbohydrate and fat.

TABLE:9-1 CUMULATIVE 3 AND 6-DAY URINARY MEASUREMENTS IN THE DEXTROSE-SALINE GROUP

		Control		Naf-post		Naf-pre		Naf-all	
		mean ± sem	n	mean ± sem	n	mean ± sem	n	mean ± sem	n
Urine volume (ml)	3-day	4633 ± 314	16	4699 ± 751	8	5403 ± 597	11	5106 ± 460	19
	6-day	11660 ± 607	16	12206 ± 1076	8	12634 ± 1230	11	12454 ± 840	19
Nitrogen balance ($mg\ kg^{-1}$)	3-day	-284 ± 28	16	-359 ± 36	8	-307 ± 27	11	-329 ± 28	19
	6-day	-621 ± 55	16	-687 ± 53	8	-661 ± 41	11	-670 ± 32	19
Creatinine ($\mu mol\ kg^{-1}$)	3-day	342 ± 18	16	395 ± 39	8	418 ± 33	11**	408 ± 25	19*
	6-day	731 ± 33	16	798 ± 41	8	825 ± 55	11	814 ± 35	19
Urea ($mmol\ kg^{-1}$)	3-day	8.07 ± 0.64	16	10.25 ± 1.25	8	9.19 ± 0.78	11	9.64 ± 0.68	19
	6-day	17.16 ± 1.35	16	19.56 ± 1.35	8	17.65 ± 1.55	11	18.45 ± 1.06	19
Potassium balance ($mmol\ kg^{-1}$)	3-day	-1.84 ± 0.15	16	-2.24 ± 0.40	8	-2.02 ± 0.54	11	-2.12 ± 0.19	19
	6-day	-3.21 ± 0.31	16	-3.34 ± 0.41	8	-3.26 ± 0.38	11	-3.29 ± 0.27	19

Naf-post = group which received first dose of naftidrofuryl on first post-operative day

Naf-pre = group which received first dose of naftidrofuryl with the induction of anaesthesia

Naf-all = Naf-post + Naf-pre

Significance vs control: * $p<0.05$ ** $p<0.005$

FIGURE:9-1A

CUMULATIVE 3-DAY NITROGEN BALANCE IN THE DEXTROSE-SALINE GROUP

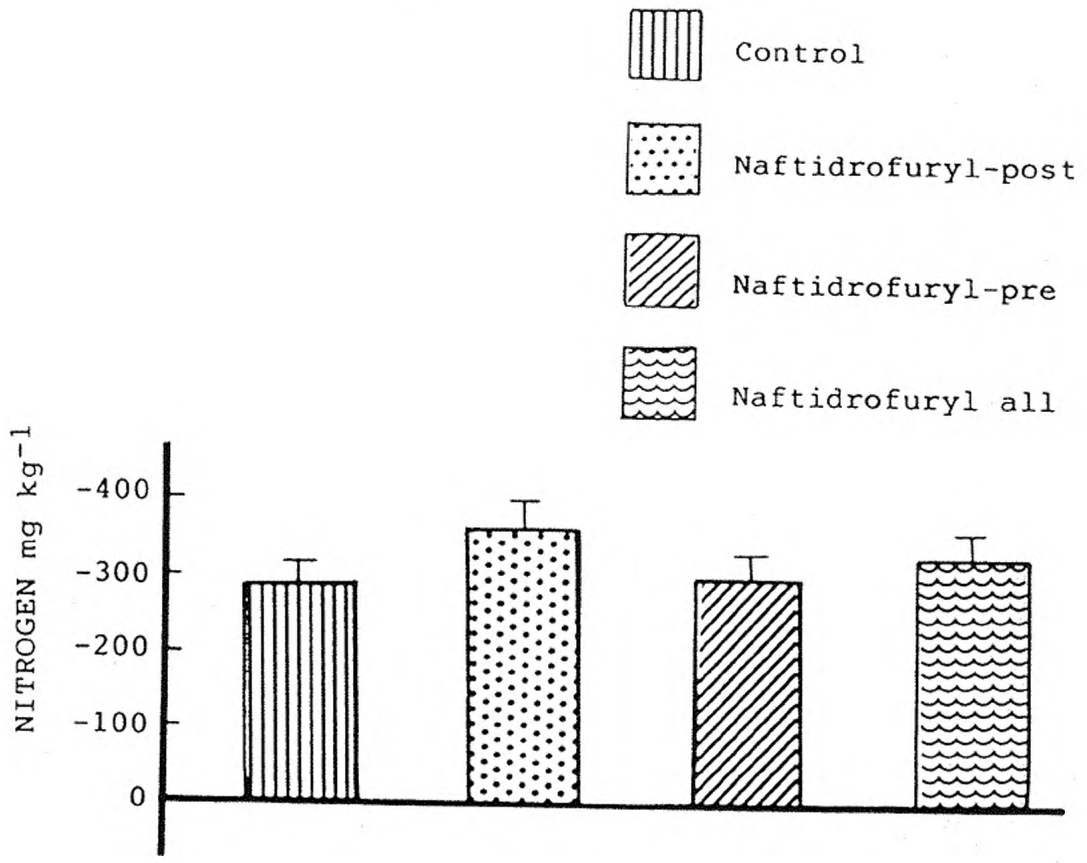


FIGURE:9-1B

CUMULATIVE 6-DAY NITROGEN BALANCE IN THE DEXTROSE-SALINE GROUP

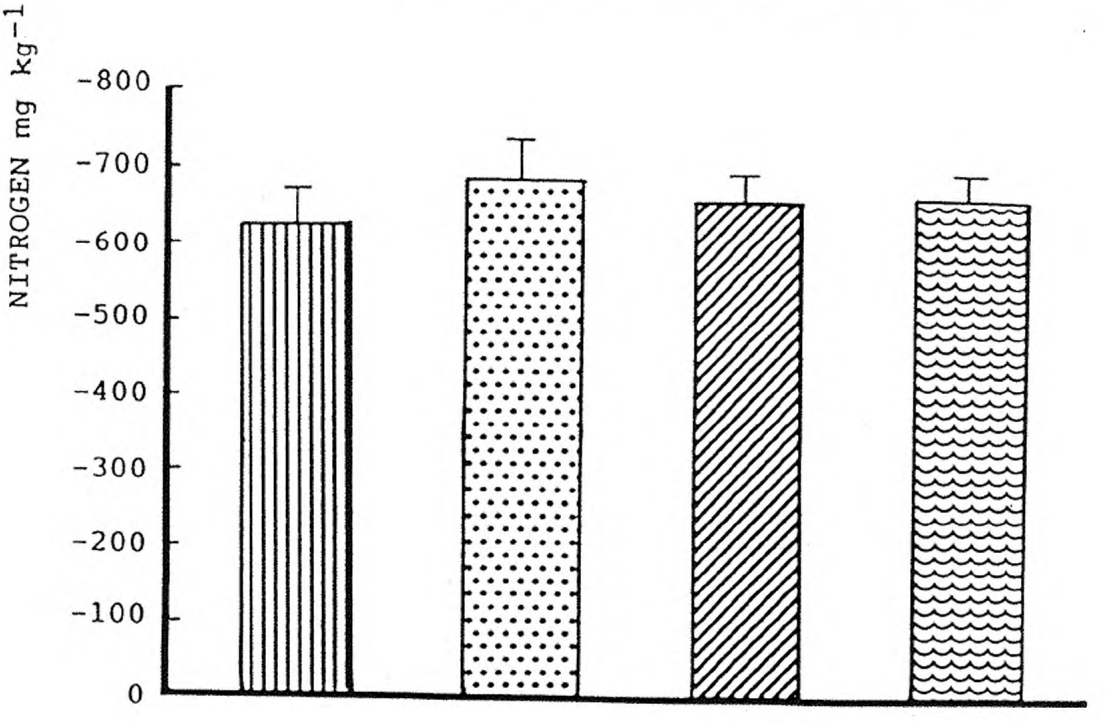


TABLE:9-2 CUMULATIVE 3 AND 6-DAY URINARY MEASUREMENTS IN THE ISOTONIC AMINO ACID GROUP

		Control		Naftidrofuryl	
		mean ± sem	n	mean ± sem	n
Urine volume (ml)	3-day	5290 ± 420	18	5384 ± 284	18
	6-day	13578 ± 543	15	15385 ± 1100	15
Nitrogen intake ($mg\ kg^{-1}$)	3-day	360 ± 9	18	362 ± 5	18
	6-day	812 ± 10	15	806 ± 9	15
Nitrogen balance ($mg\ kg^{-1}$)	3-day	-241 ± 26	18	-249 ± 24	18
	6-day	-552 ± 48	15	-549 ± 61	15
Creatinine ($\mu mol\ kg^{-1}$)	3-day	439 ± 22	18	483 ± 29	15
	6-day	903 ± 43	15	931 ± 66	15
Urea ($mmol\ kg^{-1}$)	3-day	17.57 ± 0.91	18	18.41 ± 0.87	18
	6-day	40.68 ± 1.79	15	41.56 ± 2.12	15
Potassium balance ($mmol\ kg^{-1}$)	3-day	-1.05 ± 0.20	18	-0.62 ± 0.17	18
	6-day	-1.14 ± 0.30	15	-0.32 ± 0.18	15*

Significance vs control: * $p<0.05$

FIGURE:9-2 CUMULATIVE 3 AND 6-DAY NITROGEN BALANCE IN THE ISOTONIC AMINO ACID GROUP

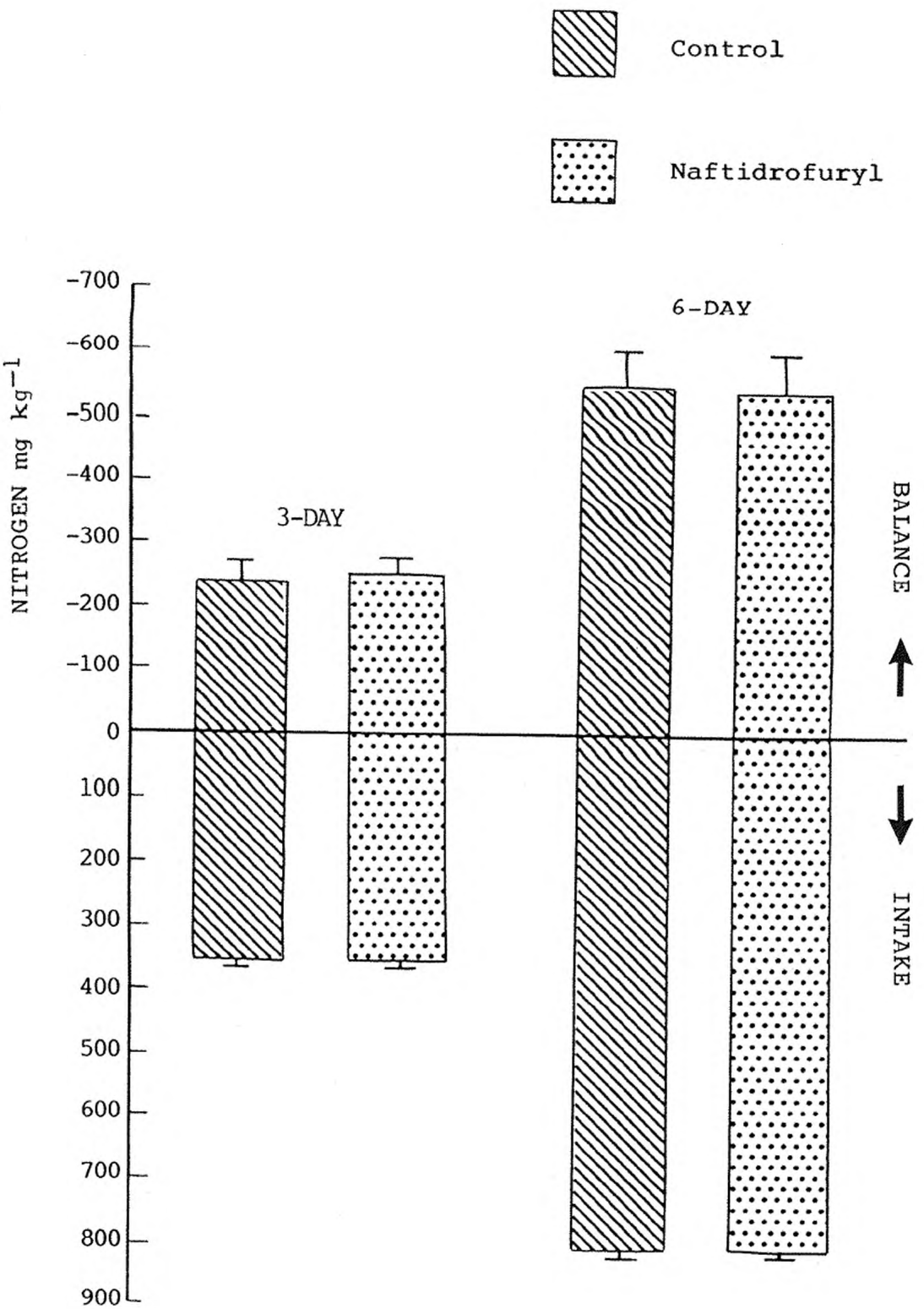


TABLE:9-3 CUMULATIVE 3 AND 6-DAY URINARY MEASUREMENTS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Control		Naftidrofuryl	
		mean ± sem	n	mean ± sem	n
Urine volume (ml)	3-day	5145 ± 430	17	5743 ± 374	18
	6-day	13828 ± 811	15	15740 ± 963	15
Nitrogen intake ($mg\ kg^{-1}$)	3-day	364 ± 5	17	373 ± 6	18
	6-day	807 ± 10	15	813 ± 8	15
Nitrogen balance ($mg\ kg^{-1}$)	3-day	-118 ± 28	17	-138 ± 18	18
	6-day	-265 ± 69	15	-271 ± 38	15
Creatinine ($\mu mol\ kg^{-1}$)	3-day	459 ± 25	17	467 ± 21	18
	6-day	921 ± 53	15	955 ± 31	15
Urea ($mmol\ kg^{-1}$)	3-day	13.74 ± 0.83	17	14.42 ± 0.44	18
	6-day	30.26 ± 1.97	15	29.66 ± 0.75	15
Potassium balance ($mmol\ kg^{-1}$)	3-day	-1.10 ± 0.18	17	-1.35 ± 0.23	18
	6-day	-1.11 ± 0.30	15	-1.44 ± 0.41	15

FIGURE:9-3 CUMULATIVE 3 AND 6-DAY NITROGEN BALANCE IN THE TOTAL PARENTERAL NUTRITION GROUP

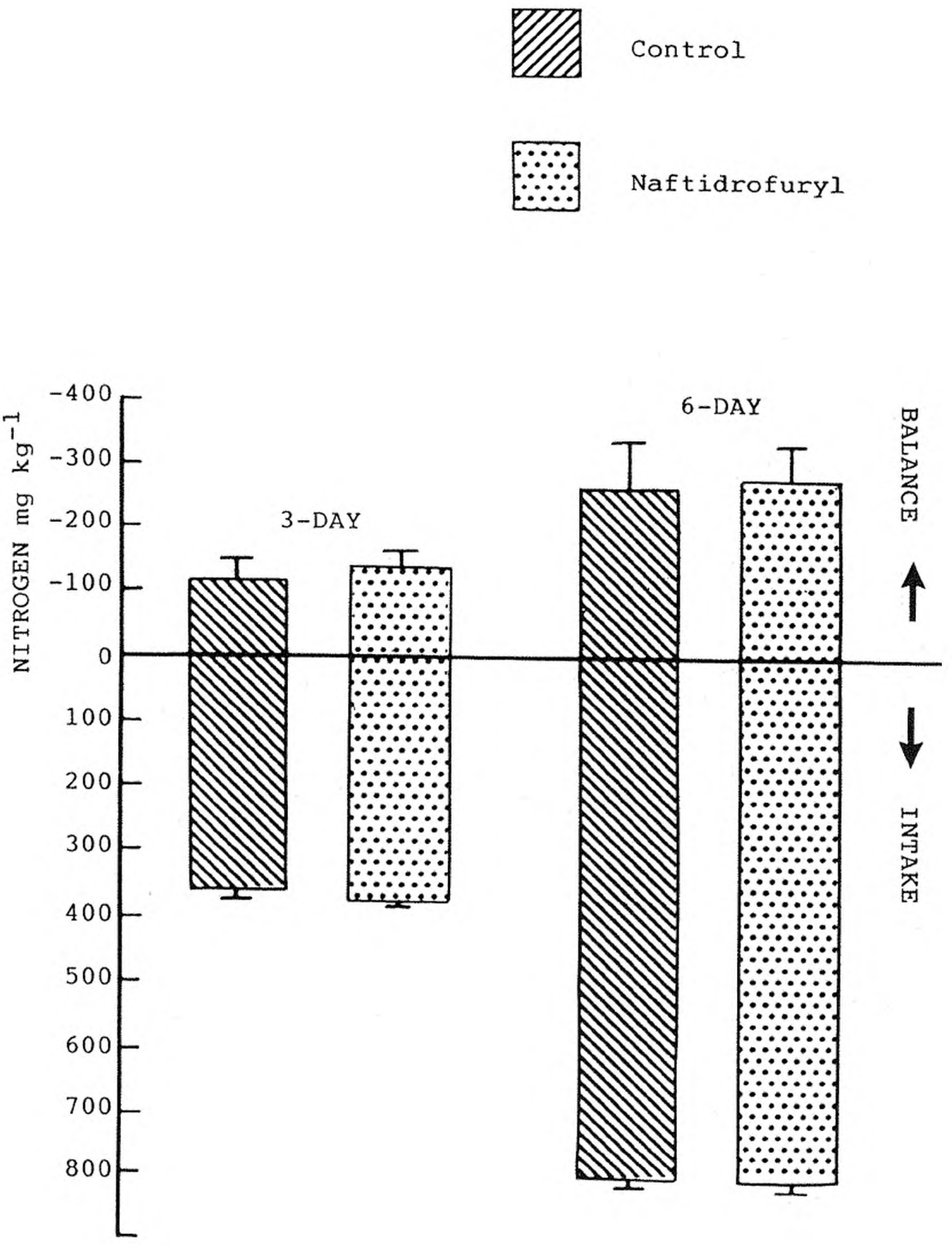


TABLE:9-4 DAILY NITROGEN BALANCE (MEAN ± SEM; mg kg^{-1}) IN THE THREE INFUSION GROUPS*

	Day (0+1)/2		Day 2		Day 3		Day 4		Day 5	
Control	-91 ± 6	16	-109 ± 11	16	-115 ± 8	16	-113 ± 9	16	-104 ± 11	16
Dextrose-saline										
Naftidrofuryl$	-106 ± 8	19	-120 ± 11	19	-114 ± 8	19	-116 ± 9	19	-107 ± 8	19
Control	-60 ± 9	15	-112 ± 18	15	-112 ± 13	15	-111 ± 11	15	-93 ± 11	15
Isotonic amino acid										
Naftidrofuryl	-64 ± 12	15	-114 ± 12	15	-103 ± 16	15	-111 ± 13	15	-89 ± 13	15
Control	-34 ± 12	15	-50 ± 18	15	-41 ± 18	15	-49 ± 16	15	-50 ± 16	15
Total parenteral nutrition										
Naftidrofuryl	-43 ± 8	15	-51 ± 8	15	-29 ± 13	15	-53 ± 10	15	-46 ± 7	15

* = Data for patients who have completed the full 6 days of the study

$ = Combined Naftidrofuryl-pre and Naftidrofuryl-post groups

TABLE:9-5

DAILY NITROGEN INTAKE (MEAN ± SEM; mg kg^{-1}) IN THE ISOTONIC AMINO ACID AND TOTAL PARENTERAL NUTRITION GROUPS*

	Day (0+1)/2		Day 2		Day 3		Day 4		Day 5	
Control	113 ± 2.9	15	140 ± 6.3	15	150 ± 1.2	15	146 ± 3.4	15	149 ± 1.2	15
Isotonic amino acid										
Naftidrofuryl	109 ± 2.3	15	147 ± 1.8	15	149 ± 1.4	15	146 ± 2.1	15	145 ± 2.7	15
Control	108 ± 1.6	15	147 ± 2.6	15	148 ± 1.7	15	147 ± 1.5	15	148 ± 1.6	15
Total parenteral nutrition										
Naftidrofuryl	115 ± 2.2	15	149 ± 1.0	15	149 ± 1.2	15	141 ± 5.8	15	145 ± 2.5	15

* = Data for patients who have completed the full 6 days of the study

FIGURE:9-4A DAILY NITROGEN BALANCE IN THE DEXTROSE-SALINE GROUP

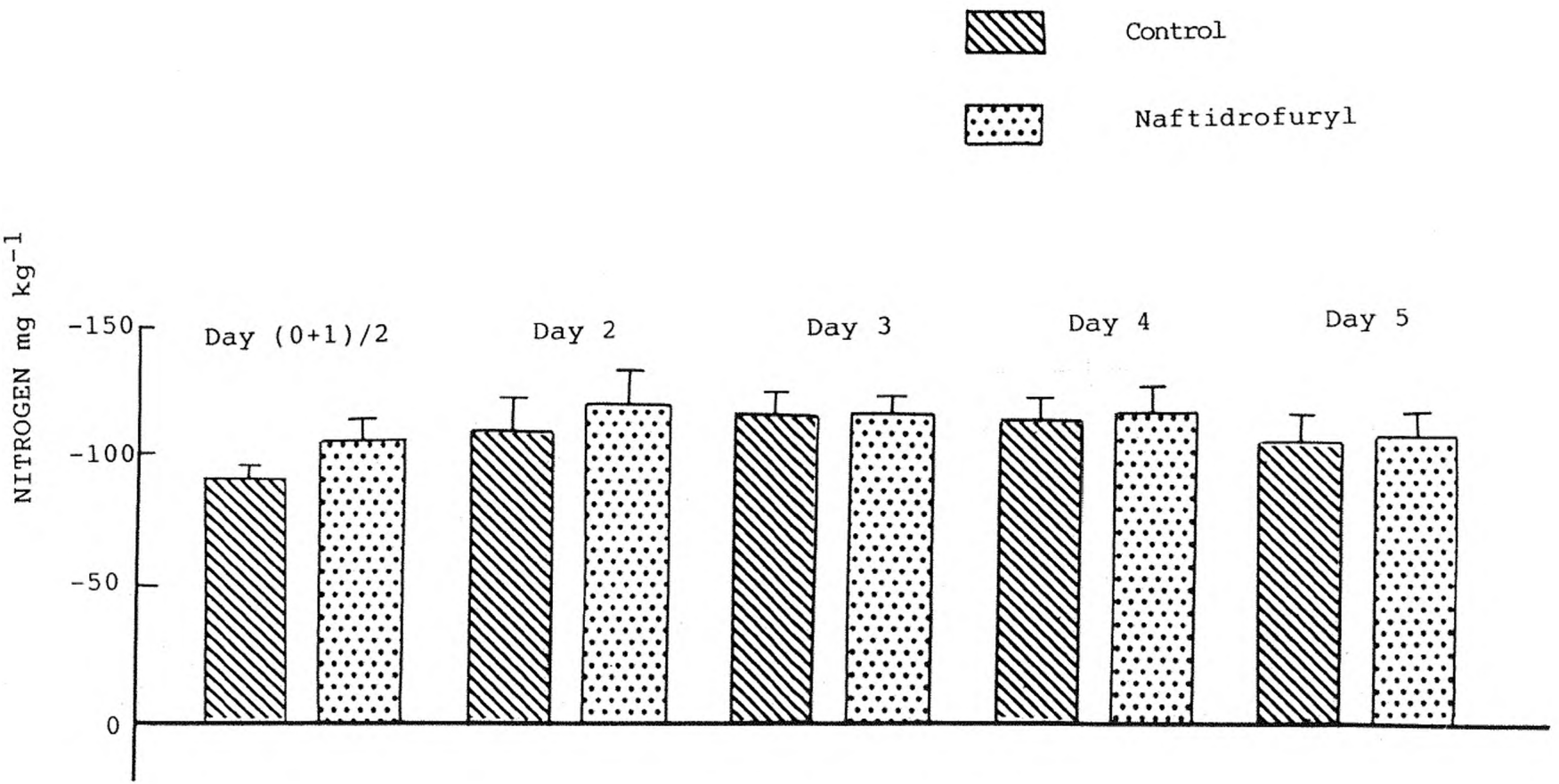


FIGURE:9-4B

DAILY NITROGEN BALANCE IN THE ISOTONIC AMINO ACID GROUP

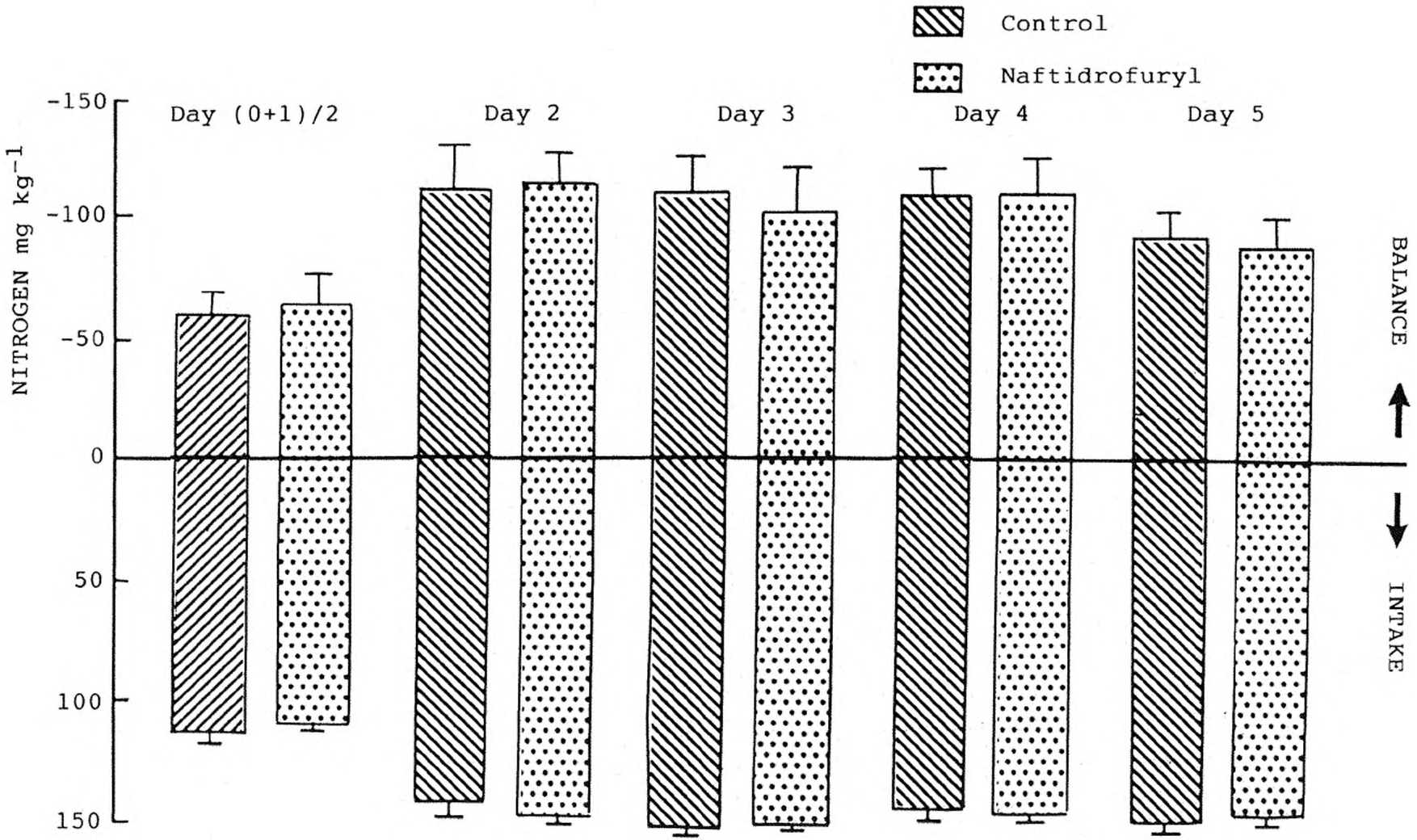


FIGURE:9-4C DAILY NITROGEN BALANCE IN THE TOTAL PARENTERAL NUTRITION GROUP

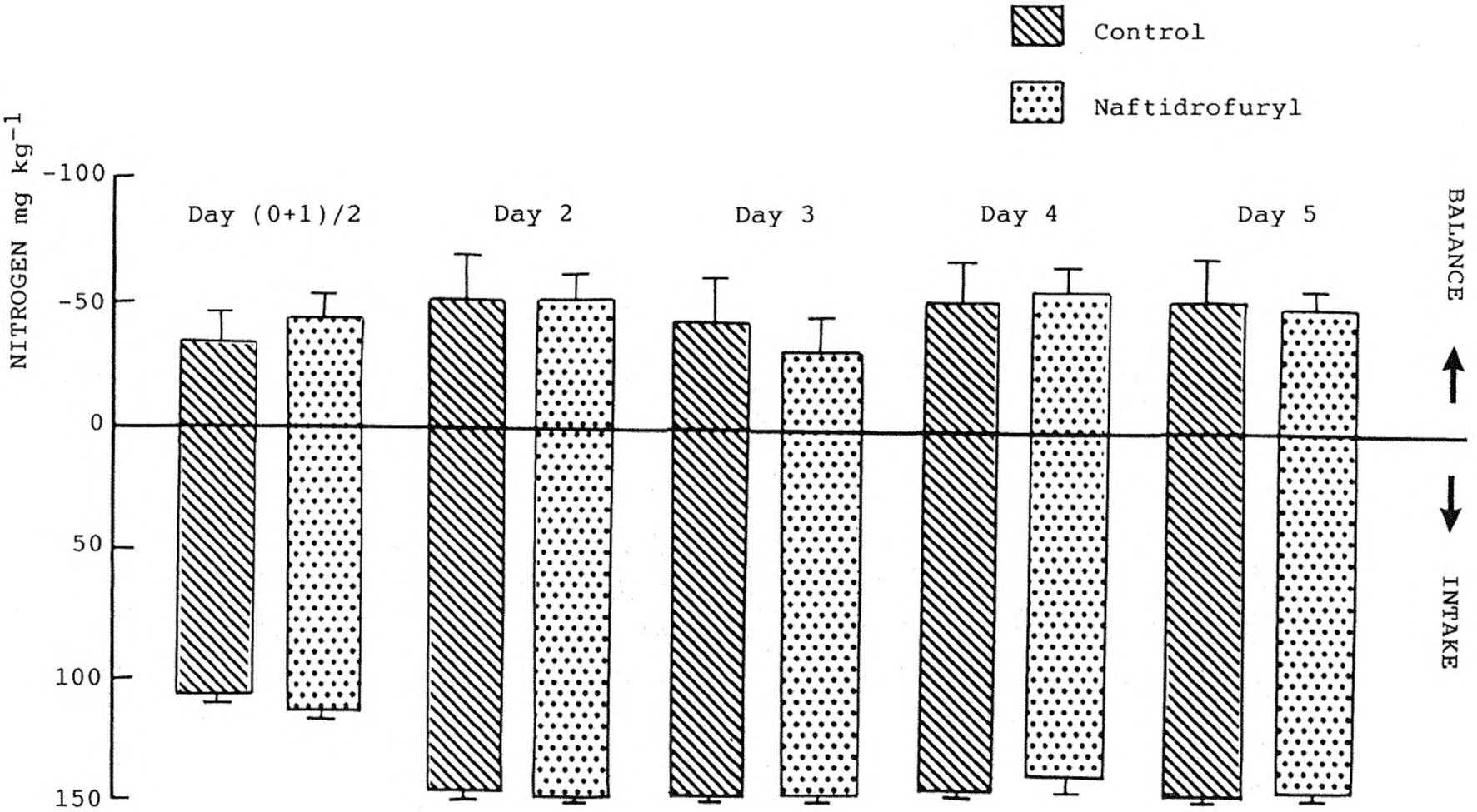


TABLE:9-6 **FLUID VOLUME (ml) RECEIVED BY THE THREE INFUSION GROUPS**

		Control mean ± sem	n	Naftidrofuryl mean ± sem	n
Dextrose-saline group*	3-day	8092 ± 218	16	8412 ± 267	19
	6-day	16685 ± 437	16	17504 ± 478	19
Isotonic amino acid group	3-day	8243 ± 198	18	8432 ± 174	18
	6-day	21006 ± 454	15	21904 ± 502	15
Total parenteral nutrition group	3-day	8738 ± 229	17	8987 ± 241	18
	6-day	22286 ± 569	15	23841 ± 618	15

* Naftidrofuryl refers to Naf-All group (Naf-pre + Naf-post)

TABLE:9-7 **DAY 5 CREATININE CLEARANCE (ml min^{-1}) IN THE THREE INFUSION GROUPS**

	Control mean ± sem	n	Naftidrofuryl mean ± sem	n
Dextrose-saline group*	87.5 ± 5.6	10	87.7 ± 8.5	10
Isotonic amino acid group	114.3 ± 10.9	10^a	112.6 ± 7.6	10^a
Total parenteral nutrition group	118 ± 9.7	10^b	120.3 ± 7.7	10^b

* Naftidrofuryl refers to Naf-pre group

Isotonic amino acid group vs Dextrose-saline group: a $p<0.05$

Total parenteral nutrition group vs Dextrose saline group: b $p<0.02$

TABLE:9-8 **URINARY CREATININE EXCRETION (μmol kg(LBM)$^{-1}$) IN THE DEXTROSE-SALINE GROUP**

	Cumulative 3-day			Cumulative 6-day		
	mean ± sem		n	mean ± sem		n
Control	539 ±	25	16	1118 ±	35	16
Naftidrofuryl-post	564 ±	55	8	1138 ±	51	8
Naftidrofuryl-pre	600 ±	52	11	1196 ±	84	11
Naftidrofuryl-all	582 ±	37	19	1167 ±	48	19

LBM = Lean body mass

TABLE:9-9 **DETAILED 6-DAY POTASSIUM BALANCE (mmol kg^{-1}) IN THE ISOTONIC AMINO ACID GROUP**

	Control			Naftidrofuryl		
	mean ± sem		n	mean ± sem		n
Potassium excreted	6.06 ±	0.3	15	5.36 ±	0.22	15
Potassium received	4.92 ±	0.06	15	5.04 ±	0.16	15
Potassium balance	-1.14 ±	0.30	15	-0.32 ±	0.18	15*

Significance vs Control group: * $p<0.05$

CHAPTER 10

EFFECT OF NAFTIDROFURYL WITH 4% DEXTROSE-SALINE INFUSION ON THE METABOLIC RESPONSE TO SURGERY.

PATIENTS AND METHODS

The patients and methods are those described in section II (chapters 7 & 8). The patients received a 4% dextrose-saline regimen which delivered 6 kcal (25 kJ) kg^{-1} day^{-1} and were randomised to receive a twice daily intravenous infusion of either placebo (control group = 16 patients) or 200 mg of naftidrofuryl oxalate (Naf-pre group = 11 patients) starting with the induction of anaesthesia. A third group (Naf-post = 8 patients) was also studied to determine whether naftidrofuryl oxalate would maintain its effect when given post-operatively as would be the case in non-surgical injury. This group was not blind and the patients received the first dose of naftidrofuryl oxalate on the first post-operative morning. The groups were matched for age, sex, anthropometric measurements and the operative procedure (see chapter 8, table:8-3).

RESULTS

Urine (see chapter 9, table:9-1)

The cumulative 3 and 6-day urinary volume, urea excretion and nitrogen balance showed no significant between group differences.

Glucose, insulin and gluconeogenic substrates
(table:10-1 & figure:10-1)

Blood glucose and insulin concentrations were raised in all groups on Day 1 and then fell, during the study period, towards original pre-operative values.

Peak blood lactate concentration occurred on Day 1 in all groups. Concentrations in the group given naftidrofuryl before the operation (Naf-pre) were significantly lower than those in the controls on all post-operative days (Day 1, $p<0.005$; Days 3 & 5, $p<0.05$) and were also lower than those in the group in which naftidrofuryl was started on the morning after the operation (Naf-post), although significantly so only on Day 3 ($p<0.005$). On Day 0, however, the concentration in the Naf-pre group was lower than that in the other 2 groups (vs control group, $p<0.05$; vs Naf-post group, $p>0.05$). As the patients in the Naf-post group did not receive their first infusion of naftidrofuryl until after the blood sampling on Day 1, they were pooled with controls to examine the changes occurring between Days 0 and 1. Lactate concentrations in this pooled control group were significantly higher on both days than those in the Naf-pre group (Day 0, $p<0.05$; Day 1, $p<0.001$). Furthermore, between group comparison of the magnitude of increase in lactate concentration, occurring between Days 0 and 1, showed that the increase in the Naf-pre group was significantly smaller than the increase in the pooled control group (Control + Naf-post groups; $p<0.05$) and there was no correlation between the increase and the original concentrations on Day 0 ($p>0.01$).

Peak blood pyruvate concentrations occurred on Day 1 and although the mean concentrations in the Naf-pre group, throughout the study period, were lower than that in the other 2 groups, the differences were not significant ($p>0.05$).

The ratio of lactate to pyruvate concentrations (L/P) in the Naf-pre group was significantly lower than that in the other 2 groups on all days (Day 0, vs control $p>0.05$, vs Naf-post $p<0.02$; Days 1,3 & 5, vs control and Naf-post $p<0.05$). The control and Naf-post groups showed no significant change, throughout the study period, in the L/P ratio. However, this ratio fell post-operatively in the Naf-pre group, although only significantly so on Day 1

($p<0.05$).

Peak total gluconeogenic substrates (Lactate, pyruvate, alanine and glycerol) concentrations occurred on Day 1 and by Day 5 the values were not different from the pre-operative concentrations in all groups. The mean concentrations in the Naf-pre group were lower than those in the control group, not only on all post-operative days ($p<0.02$) but also on Day 0 ($p<0.02$). Therefore, the magnitude of change between Days 0 and 1 was analysed. This increase was significantly lower in the Naf-pre group when compared to the control group ($p<0.05$) or the pooled control and Naf-post groups ($p<0.05$) and there was no correlation between the increase and the starting concentration on Day 0 ($p>0.05$).

Lipid metabolites (table:10-2 & figure:10-2)

Total ketones (3-hydroxybutyrate + acetoacetate) showed no between group differences although some general trends emerged. Concentrations in the Naf-pre group were all below 0.2 mmol/l on Day 1 and had increased by Day 3. However, 6 out of 23 patients who had not received naftidrofuryl (control and Naf-post patients) had total ketone concentrations above 0.2 mmol/l on Day 1. The mean increase in the total ketone concentration between Days 0 and 1 in the control group (0.046 mmol/l) was not significantly different from the mean increase in the Naf-pre group (- 0.059 mmol/l) over the same period ($p>0.05$). There was a significant negative linear correlation between log[total ketones] and lactate concentrations post-operatively in all groups ($r=-0.6273$, $n=48$, $p<0.001$ in the control group; $r=-0.4603$, $n=33$, $p<0.01$ in the Naf-pre group; $r=-0.5577$, $n=24$, $p<0.01$ in the Naf-post group).

Serum total triglycerides showed no between group differences. Trough concentrations occurred on Day 1 and returned to pre-operative values by Day 5.

Plasma free fatty acid (FFA) concentrations fell post-operatively but only significantly so in the control

group on Day 3 ($p<0.05$). Although lower, the FFA concentrations on Day 5 were not significantly different from the pre-operative values in all groups.

The post-operative plasma glycerol concentrations were not significantly different from the pre-operative values in all groups.

Haemoglobin, packed cell volume, albumin and total protein (table:10-3 & figure:10-3)

Haemoglobin and packed cell volume ratio fell post-operatively in all groups with the concentrations on Day 5 significantly lower than Day 0.

Serum albumin and total protein concentrations were significantly reduced on Days 1 and 3, in all groups, and then rose towards the starting pre-operative values. There were no between group differences in any of these variables.

Creatinine, urea and electrolytes (table:10-4 & figure:10-4)

Serum creatinine concentrations were lower, in all groups, on Days 3 and 5 than pre-operatively.

Serum urea concentrations were also lower on Days 3 and 5 when compared to the pre-operative values but only significantly so in the Naf-pre and Naf-post groups. However, there were no between group differences either in the magnitude of this fall ($p>0.1$) or the cumulative 3 and 6-day urinary urea excretion ($p>0.1$; table:9-1).

Serum bicarbonate fell on Day 1 but the concentrations on all post-operative days were not significantly different from pre-operatively, in all groups.

Serum concentrations of sodium and potassium fell post-operatively and showed no between group differences.

Venous plasma free amino acids

The mean concentration of individual plasma amino acids showed considerable fluctuations within each group.

The only significant between group difference was found for alanine (see below: non-essential amino acids (table:10-7A).

Branched-chain amino acids (BCAA) (table:10-5 & figure:10-5)

Valine, leucine and isoleucine concentrations fell on Day 1 and rose thereafter. On Day 5, leucine and isoleucine concentrations were higher than Day 0 whereas valine concentrations were not different from the pre-operative values. Trough total BCAA occured on Day 1 and by Day 5 the concentrations were generally higher than on Day 0 (only significant in the Naf-pre group, $p<0.05$). However, there were no between group differences in the magnitude of change during the study period (Days 0-1, $p>0.1$; Days 0-5, $p>0.05$ for all groups).

Essential amino acids (EAA) (table:10-6 & figure:10-6)

All groups showed similar changes. Day 1 tryptophan and Day 0 and Day 1 total EAA concentrations were lower in the Naf-pre group when compared to the control group ($p<0.05$). However, the trend and magnitude of change were similar in all groups.

The post-operative concentrations of methionine and tryptophan were not different, in all groups, from the pre-operative values. Trough lysine and threonine concentrations occurred on Day 1 and then rose to pre-operative values by Day 5. Peak phenylalanine concentrations occurred on Day 1 and then fell towards Day 0 values. Trough total EAA concentrations occurred on Day 1 in all groups. Day 5 concentrations were higher than pre-operatively but failed to reach statistical significance.

Non-essential amino acids (NEAA) (tables:10-7A & 10-7B and figures:10-7A, 10-7B & 10-7C)

The Day 0 alanine, proline, serine and total NEAA concentrations and the Day 1 alanine, citrulline, arginine, ornithine, glutamine, asparagine, alpha-aminobutyrate, cystine, serine and total NEAA

concentrations were lower in the Naf-pre group when compared to the control group. However, the change occurring between Days 0 and 1, for each of these variables, was similar in all groups.

Aspartate and tyrosine had no significant post-operative changes. On Day 1 all other NEAA fell with the exception of taurine and alpha-aminobutyrate whose concentrations were not different from Day 0. On Day 5 the individual amino acid concentrations returned to pre-operative values or higher with the exception of alanine and taurine which fell, histidine and citrulline which underwent no further change and arginine, ornithine, proline, glutamine and glycine whose values remained lower than Day 0. Trough total NEAA concentrations occurred on Day 1 in all groups and on day 5 the concentrations were lower than pre-operatively.

The only significant between group difference was found in alanine. The magnitude of fall in alanine concentration between Days 0 and 1 and Days 0 and 5 was less in the Naf-pre group than in the control group (Days 0-1, $p>0.05$; Days 0-5, $p<0.05$).

DISCUSSION

Table:9-1 and figure :9-1 (Chapter 9) show that a twice daily intravenous infusion of 200mg naftidrofuryl oxalate does not result in any measurable nitrogen sparing effect in patients who have undergone elective abdominal surgery and received 4% dextrose-saline as the sole nutritional support. These results complement the findings of Inglis et al. (1983), who failed to show any nitrogen sparing effect when naftidrofuryl 400 mg daily was given as a constant intravenous infusion. However, the authors indicated that this result might have been due to their use of a constant infusion of naftidrofuryl as opposed to a twice daily slow bolus (200 mg over 2 hours) as used by Burns et al. (1981) and in this study.

In addition to the evidence from urinary

measurements, there were no differences in albumin and total protein concentrations between the control and treatment groups. After the initial fall in albumin and total protein concentrations on Day 1, due mainly to the increased vascular permeability and catabolism associated with injury (Arturson and Jonsson, 1979; Davies et al. 1962; Fleck et al. 1985), the concentrations returned to pre-operative values as these effects diminished (Salo, 1982).

In studies of the metabolic response to surgical injury, it has always been important to distinguish between effects due to injury and effects due to the nutritional support regimen (Foster et al., 1979). The results in this chapter represent those after elective abdominal surgery with a minimal carbohydrate infusion (6 kcal $kg^{-1}day^{-1}$). The post-operative blood results are those of samples following 6-7 hours nutrient-free time during which the patients received a 0.9% w/v sodium chloride infusion to maintain venous access.

The rise in blood glucose and insulin concentrations, on Day 1, indicates the usual hyperglycaemia with insulin resistance that follows injury. The concentrations later fell towards pre-operative values.

No reason was apparent for the differences on Day 0, between the Naf-pre and control groups concentrations, in lactate, alanine, proline, serine, total gluconeogenic substrates, total essential amino acids, total non-essential amino acids, and may have been an artefact of selection. It should be noted that the Day 0 concentrations of the above variables fell within the Day 0 laboratory reference range (see appendix 1). In each of these cases, the daily between group difference was tested for the magnitude of change by paired analysis. Differences were only found for lactate and alanine (see result section above).

Interpretation of changes in venous plasma amino

acid concentrations following injury is difficult. A multitude of factors influence these changes including: a) The dietary intake, particularly the amount and composition of protein in the diet and its route and rate of delivery to tissues. b) The kinetic properties of the transport systems of individual amino acids and their intracellular fate. This, in turn, depends on activities of key enzymes and the hormonal milieu. c) The relative rates of protein synthesis and degradation and interconversion of amino acids. It is clear from the complexity of these regulatory systems that the concentrations of plasma amino acids give limited information on amino acid fluxes or on the physiological state of the individual. However, serial measurements of venous plasma amino acids and persistent changes in their concentrations may give an indication of the overall metabolic alterations occurring.

The changes in venous plasma free amino acids, described in this chapter, follow the same trends as those reported by others (Dale et al., 1977; Johnston et al., 1980; Askanazi et al., 1980a). The observed changes, and in particular the general pattern of decrease on Day 1 (with the exception of phenylalanine), do not appear to be the result of any single factor and may well be related to the endocrine changes known to occur in response to surgery (Johnston, 1972).

Many workers have shown that alanine concentration falls rapidly after surgery (Schønheyder et al., 1974; Dale et al., 1977; Askanazi et al., 1980a). This could be due to either a decreased production or an increased hepatic extraction. There is an increased output of alanine from muscle after injury and high levels have been reported in burns (Aulick and Wilmore, 1979). Furthermore, the increased adrenocortical activity after injury stimulates this release of alanine. Therefore, the falling plasma concentration of alanine may be explained by the increased hepatic uptake. These changes in alanine, between Days 1 and 5, appear to be the inverse of

those of the branched-chain amino acids (BCAA). Perhaps these changes are related to the cycle linking the metabolism of alanine with the BCAA (alanine shuttle) to provide gluconeogenic substrates.

Several possible mechanisms can be proposed to explain the observed increases in plasma phenylalanine concentrations. These include: a) inhibition of phenylalanine 4-hydroxylase which has been considered to be the rate-limiting enzyme in the metabolic conversion of phenylalanine to tyrosine (McGee et al., 1972); b) decreased utilisation of phenylalanine for protein synthesis; and/or d) increased release of this amino acid into the extra-cellular compartment because of protein breakdown. High plasma phenylalanine concentrations are frequently found in catabolic situations and it has been suggested that this rise might be due to an increased flux of this amino acid, through the plasma compartment, as the result of increased skeletal muscle catabolism as opposed to an inhibition of hepatic phenylalanine hydroxylase (Wannemacher et al., 1976; Woolf et al., 1976). Furthermore, the rate of appearance of tyrosine (the phenylalanine metabolite) following an oral phenylalanine load was normal in uncomplicated trauma patients (Herndon, 1978) and the rate of hepatic uptake of phenylalanine was increased following injury (Wilmore et al., 1980). Thus, the rise in plasma phenylalanine concentration reflects the rapid skeletal muscle catabolism that occurs following injury and does not necessarily reflect altered hepatic handling of this amino acid as suggested by Dale et al. (1977). The liver is the primary site of degradation of the aromatic and sulphur-containing amino acids. No changes were observed, in the results reported in this chapter, in the aromatic amino acids tyrosine and tryptophan. Furthermore, the sulphur-containing amino acids methionine and cystine returned to pre-operative concentration by Day 5.

Phenylalanine is hydroxylated in the liver to form tyrosine and cystine can be synthesised from methionine.

Dale et al. (1977) and Johnston et al. (1980) have suggested that the efficacy of these pathways may be impaired in periods of metabolic stress and therefore tyrosine and cystine may become essential. The rise in phenylalanine/tyrosine ratio reported here (table:10-8 & figure:10-8) with no change in tyrosine and a rise in phenylalanine concentration is not consistent with impairment of the efficacy of the above mentioned metabolic pathway. The trans-sulphuration pathway (figure:10-10) leads from methionine via homocysteine and cystathionine to the end-products sulphate and taurine. This sequence operates primarily in the liver and is the major route for degradation of methionine. It also participates in the biosynthesis of several physiologically indispensable compounds: cyst(e)ine, glutathione, taurine, choline, carnitine and creatine. These substances are not considered essential nutrients for healthy subjects because they can be readily synthesised by the trans-sulphuration pathway and associated reactions. However, investigators have recently reported inadequate biosynthesis of several products of the trans-sulphuration pathway in fasted subjects and patients receiving long term parenteral nutrition (Chawla et al., 1985; Geggel et al., 1985; Mårtensson, 1986).

A block in the trans-sulphuration pathway could theoretically cause depletion of both cystine and taurine. However, on Day 5 the cystine concentration was not different from pre-operatively whereas taurine fell significantly. The Day 5 methionine/cystine ratio was not different from Day 0 whereas the methionine/taurine ratio rose significantly and this rise was parallel to the changes in the cystine/taurine ratio (table: 10-8). This data is consistent with a block of the trans-sulphuration pathway between cystine and taurine. However, taurine is the most abundant free intracellular amino acid (Soupart, 1962; Jacobsen & Smith, 1968) and increased excretion of this compound, in the early catabolic phase, has been

reported (Jacobsen & Smith, 1968; Mårtensson et al., 1985). Furthermore, the results reported here are those of venous plasma samples and do not necessarily reflect intracellular changes and the urinary excretion of these substrates was not measured. Therefore, although no firm conclusion can be made, the above results represent an interesting finding which needs further evaluation.

Smith et al. (1975) have reported that early keto-adaptation is associated with improved conservation of nitrogen. Wedge et al. (1976) have shown, in a heterogenous group of normally nourished patients following accidental injury, that the increase in the concentration of branched-chain amino acids (BCAA) was significantly less in those patients with initial hyperketonaemia (defined as more than 0.2 mmol/l) than in those with normoketonaemia, and was accompanied by lower urinary nitrogen excretion throughout the study period. In the patients studied in this chapter, there was a negative linear correlation between Day 1 total ketone and BCAA concentrations (with the exception of the Naf-pre group). However, there were no correlations between the cumulative 6-day nitrogen balance, Day 1 total ketone concentrations, Day 1 BCAA concentrations or the magnitude of change in these variables between days 0 and 1 (table:10-9).

The ratio of lactate to pyruvate concentrations (L/P) is defined by the direction of the cytoplasmic reaction:

$$\text{Pyruvate} + \text{NADH}_2 \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}$$

which is catalysed by lactate dehydrogenase (LDH). The ratio of NADH/NAD indicates the reduction-oxidation (redox) state and since the L/P ratio is related to the

NAD/NADH ratio by the reaction equilibrium constant, it is a measure of the oxidative capacity of the tissue. Therefore, an increase or decrease in L/P ratio reflects a decrease or an increase, respectively, of the oxidative capacity of the tissue. The observed decrease in L/P ratio in the Naf-pre group, on Day 1, is consistent with an increased oxidative capacity of the cell and this may be related to a facilitatory action of naftidrofuryl in the oxidation of substrates within the tricaboxylic acid cycle.

Catecholamines cause an increased production of lactate from muscle and an acceleration of the alanine shuttle. If the critical factor after injury is a cellular, rather than a hormonal, modification affecting nitrogen conservation then the utilisation of pyruvate decarboxylation and fatty acid beta-oxidation as sources of acetyl CoA may be reduced. Under the conditions prevailing in immediate post-operative period- namely, increased muscle lactate production from glycogen, lipolysis and glucose intolerance-, a rise in blood ketones, lactate and pyruvate concentrations would be expected. In contrast, an agent that is able to increase the utilisation of carbohydrate and fat in the tricarboxylic acid cycle and reduce the need for protein degradation, as has been suggested for naftidrofuryl (Burns et al. 1981), would therefore be expected to reduce the increases in both lactate and total ketone concentrations that occur at this stage. This would indicate a resetting of the regulation that links lactate and pyruvate with ketone production, and the usual inverse relations would still operate. The results reported here for the diminished rise in lactate concentration, with a decrease in L/P ratio, and the possibility of a diminished rise in total ketone concentration on Day 1 are consistent with this mode of action on the first post-operative day. Furthermore, the lesser fall in alanine concentration without a fall in BCAA and the smaller increase in total gluconeogenic substrates in the Naf-pre group may be a

consequence of reduced need for gluconeogenesis thus reducing the demands for protein catabolism.

Admittedly, the results reported here are those of venous blood samples and do not accurately reflect the changes at cellular level. Furthermore, these potentially beneficial changes had no effect on the nitrogen balance, urinary excretion of urea and creatinine (table:9-1) and the venous concentrations of insulin, glucose, branched-chain amino acids, albumin and total protein.

No measurable naftidrofuryl effect was detected when the first dose of the drug was given post-operatively (Naf-post), as examplified in non-surgical (traumatic) injury.

In conclusion, in spite of these interesting metabolic changes, the twice daily infusion of 200mg naftidrofuryl did not result in a measurable nitrogen sparing effect in patients undergoing elective abdominal surgery and receiving 4% dextrose-saline. The next two chapters will report the effect of this drug in patients receiving different nutritional regimens.

TABLE:10-1

BLOOD ANALYTE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Glucose mmol/l	Control	4.8 ± 0.15	16	7.5 ± 0.44	16***	6.0 ± 0.36	16**	5.1 ± 0.17	16
	Naf-post	4.3 ± 0.21	8	7.4 ± 0.48	8***	6.4 ± 0.44	8**	5.8 ± 0.57	8*
	Naf-pre	4.5 ± 0.21	11	6.9 ± 0.53	11***	5.8 ± 0.51	11*	5.0 ± 0.27	10
Insulin mU/l	Control	14.2 ± 0.85	16	42.0 ± 7.07	16**	24.1 ± 4.57	16*	18.4 ± 2.44	15
	Naf-post	16.4 ± 1.71	8	50.3 ± 12.1	8**	33.3 ± 7.55	8*	19.7 ± 3.01	7
	Naf-pre	12.7 ± 1.33	11	39.7 ± 10.13	11**	15.8 ± 3.92	11	11.6 ± 1.67	10
Pyruvate µmol/l	Control	67 ± 3	15	138 ± 15	15***	80 ± 7	15	67 ± 5	14
	Naf-post	65 ± 7	8	106 ± 18	8*	78 ± 8	8	58 ± 8	8
	Naf-pre	59 ± 6	10	103 ± 18	10*	69 ± 6	10	71 ± 8	10
Lactate µmol/l	Control	766 ± 52	16	1457 ± 122	16***	863 ± 95	16	777 ± 52	16
	Naf-post	764 ± 71	8	1194 ± 163	8*	957 ± 116	8	711 ± 75	8
	Naf-pre	a610 ± 44	11	aaa847 ± 114	11*	a572 ± 51	11	a584 ± 29	11
L/P	Control	11.6 ± 0.78	15	11.1 ± 0.97	15	11.2 ± 0.72	15	12.1 ± 1.09	14
	Naf-post	11.9 ± 0.61	8	11.6 ± 1.4	8	12.6 ± 1.03	8	12.7 ± 1.91	8
	Naf-pre	10.1 ± 0.45	10	a8.4 ± 0.37	10*	aa8.7 ± 0.41	10	a8.6 ± 0.59	10
T.G.S. mmol/l	Control	1.29 ± 0.06	15	2.05 ± 0.17	15***			1.14 ± 0.07	14
	Naf-post	1.25 ± 0.09	8	1.71 ± 0.24	8			1.06 ± 0.09	7
	Naf-pre	aa1.03 ± 0.05	10	aaa1.22 ± 0.14	10			a0.88 ± 0.05	10

Naf-post = group which received 1st dose of naftidrofuryl on the 1st post-operative day
Naf-pre = group which received 1st dose of naftidrofuryl with the induction of anaesthesia
L/P = Lactate to pyruvate ratio T.G.S. = Total gluconeogenic substrates
Significance vs pre-operative concentrations (paired data): * p<0.05 ** p<0.01 *** p<0.001
Significance vs Control: a p<0.05 aa p<0.01 aaa p<0.001

FIGURE:10-1 BLOOD ANALYTE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

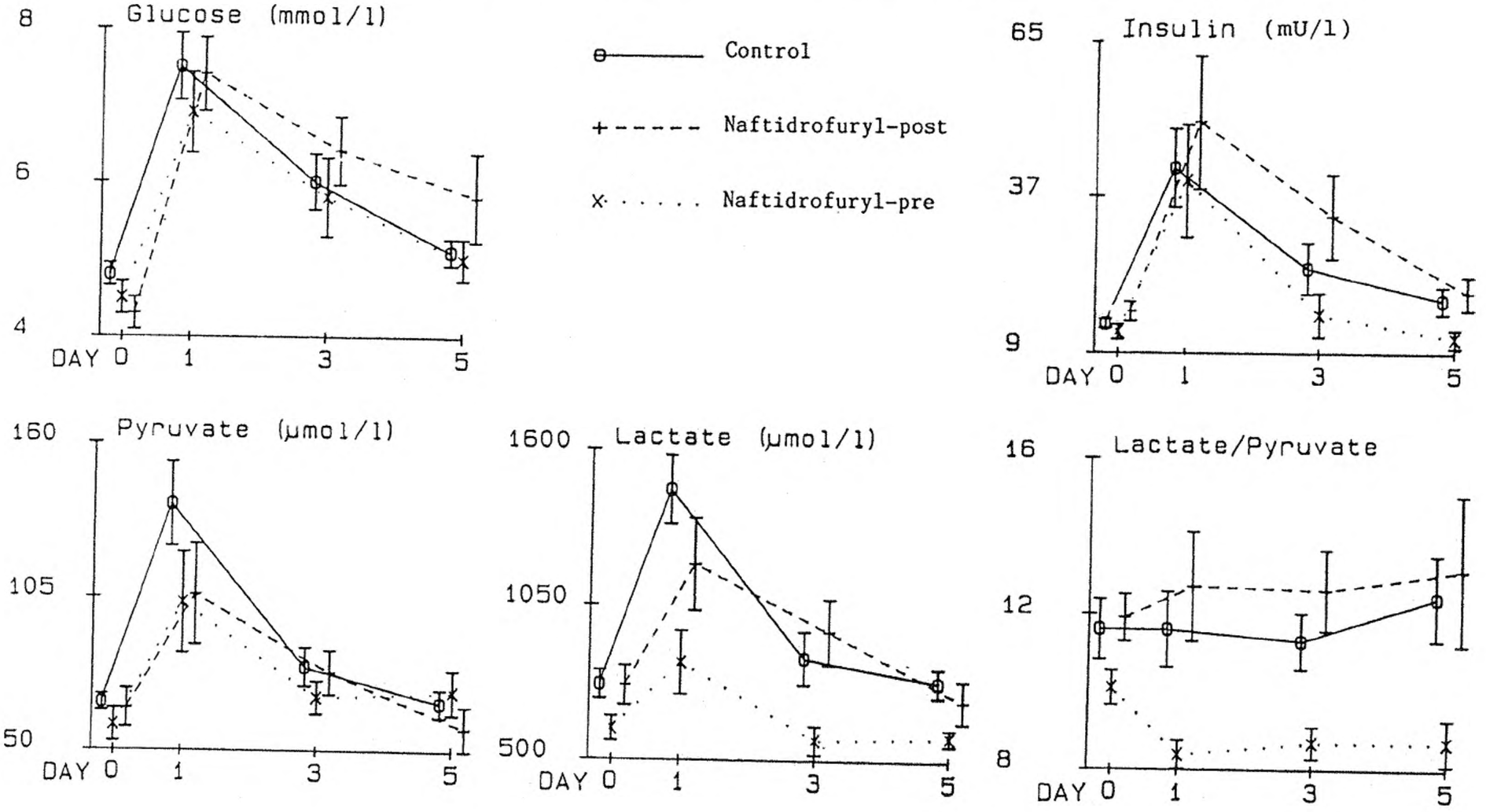


TABLE:10-2

LIPID METABOLITE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
T.K.$ µmol/l	Control	82 (69 - 99)	16	87 (68 - 113)	16	131 (102 - 169)	16	212 (169 - 265)	16**
	Naf-post	91 (60 - 139)	8	114 (76 - 172)	8	160 (103 - 251)	8	267 (199 - 359)	8*
	Naf-pre	80 (59 - 108)	11	60 (47 - 75)	11	253 (189 - 338)	11	289 (224 - 372)	11*
T.T.G. mmol/l	Control	1.40 ± 0.11	16	0.77 ± 0.06	16***	1.13 ± 0.10	15*	1.36 ± 0.09	15
	Naf-post	1.42 ± 0.15	8	0.72 ± 0.08	8***	1.13 ± 0.06	8*	1.36 ± 0.1	7
	Naf-pre	1.34 ± 0.10	11	0.82 ± 0.07	11***	1.11 ± 0.07	11*	1.34 ± 0.05	10
F.F.A. µmol/l	Control	861 ± 99	16	732 ± 137	15	624 ± 32	15*	806 ± 83	15
	Naf-post	1018 ± 132	8	755 ± 131	8	632 ± 191	7	825 ± 111	7
	Naf-pre	932 ± 102	11	704 ± 81	11	a827 ± 55	11	825 ± 92	11
Glycerol µmol/l	Control	80 ± 8	16	82 ± 8	16	84 ± 12	16	86 ± 10	15
	Naf-post	105 ± 18	8	89 ± 7	8	64 ± 8	8	82 ± 12	8
	Naf-pre	76 ± 5	11	64 ± 7	11	71 ± 8	11	65 ± 4	10

T.K. = Total ketones T.T.G. = Total triglycerides F.F.A. = Free fatty acids

Naf-post = group which received 1st dose of naftidrofuryl on the 1st post-operative day

Naf-pre = group which received 1st dose of naftidrofuryl with the induction of anaesthesia

$ = Figures derived from logarithmic transformation (geometric mean and range of 1 sem about the mean)

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Significance vs control: a $p<0.05$

FIGURE:10-2 LIPID METABOLITE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

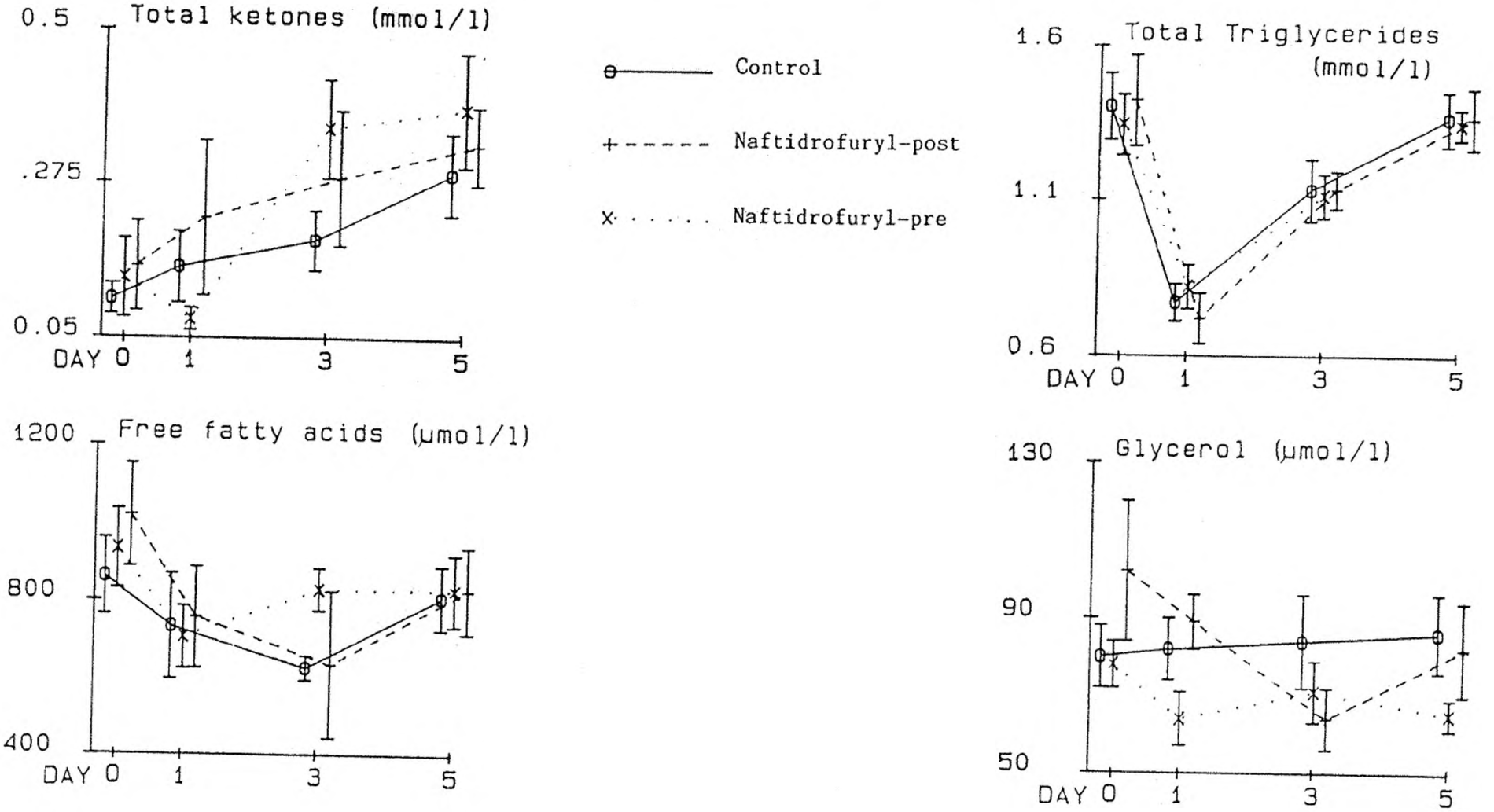


TABLE:10-3 HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Haemoglobin g/dl	Control	13.6 ± 0.36	16	13.1 ± 0.26	16	12.2 ± 0.41	15***	12.1 ± 0.55	15***
	Naf-post	14.6 ± 0.52	8	14.0 ± 0.68	7	12.9 ± 0.6	8***	13.1 ± 0.7	7***
	Naf-pre	13.9 ± 0.38	11	13.1 ± 0.34	11	12.2 ± 0.41	11***	12.2 ± 0.36	10***
P.C.V. l/l	Control	0.406 ± 0.010	16	0.395 ± 0.008	16	0.373 ± 0.009	15***	0.362 ± 0.018	15**
	Naf-post	0.434 ± 0.012	8	0.421 ± 0.017	7	0.390 ± 0.016	8**	0.386 ± 0.022	7**
	Naf-pre	0.406 ± 0.011	11	0.390 ± 0.012	11	0.360 ± 0.012	11**	0.356 ± 0.011	10**
Albumin$ g/l	Control	43.5 ± 0.63	16	40.0 ± 0.64	16***	38.1 ± 1.60	15***	39.4 ± 1.21	15**
	Naf-post	43.8 ± 1.11	8	39.5 ± 0.78	7***	38.8 ± 0.73	8***	40.8 ± 1.60	7*
	Naf-pre	43.3 ± 1.10	11	38.9 ± 0.92	11***	39.1 ± 0.81	11***	40.0 ± 0.60	10*
Total protein$ g/l	Control	69.9 ± 1.08	16	63.8 ± 1.20	16***	64.8 ± 1.91	15**	67.7 ± 1.83	15
	Naf-post	73.0 ± 1.55	8	66.1 ± 0.51	7***	67.3 ± 0.83	8**	71.4 ± 1.61	7
	Naf-pre	71.1 ± 1.14	11	64.0 ± 1.2	11***	66.9 ± 1.40	11**	68.3 ± 1.20	10

Naf-post = group which received 1st dose of naftidrofuryl on the 1st post-operative day

Naf-pre = group which received 1st dose of naftidrofuryl with the induction of anaesthesia

P.C.V. = Packed cell volume ratio

$ = Concentrations corrected for changes in packed cell volume

Significance vs pre-operative values: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:10-3

HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP.

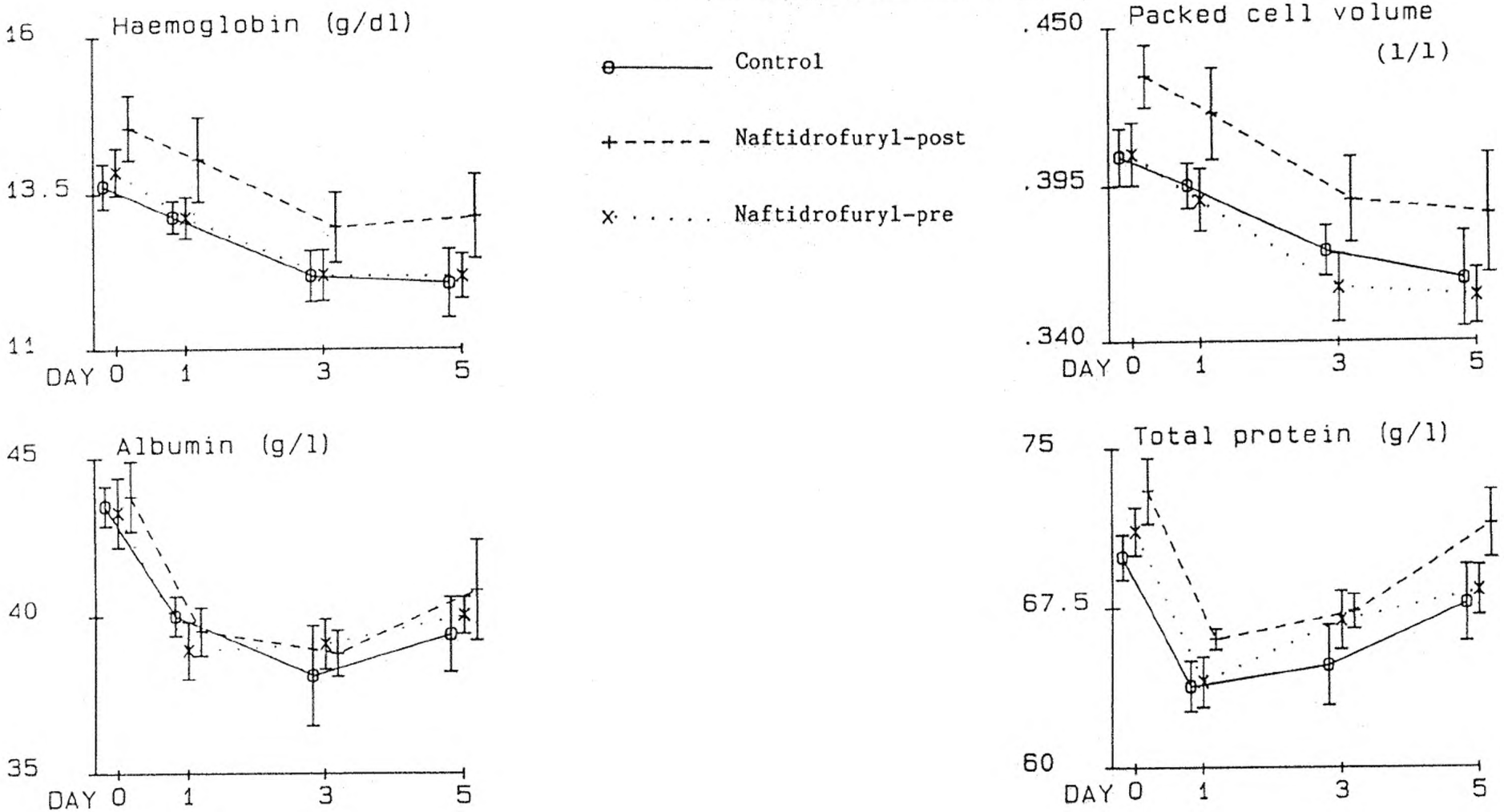


TABLE:10-4 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
	Control	82.5 ± 5.42	16	82.5 ± 10.17	16	71.1 ± 6.37	16***	69.9 ± 6.47	16**
Creatinine	Naf-post	87.9 ± 4.29	8	88.0 ± 7.17	8	74.1 ± 5.22	8**	71.5 ± 5.23	8*
µmol/l	Naf-pre	84.7 ± 6.43	11	80.0 ± 6.12	11*	69.9 ± 4.20	11**	70.0 ± 4.66	11*
	Control	4.4 ± 0.43	16	5.6 ± 0.66	16	3.6 ± 0.54	16	3.7 ± 0.48	16
Urea	Naf-post	4.5 ± 0.30	8	4.6 ± 0.36	8	3.6 ± 0.48	8	2.9 ± 0.42	8*
mmol/l	Naf-pre	5.0 ± 0.25	11	4.6 ± 0.45	11	3.3 ± 0.40	11**	3.7 ± 0.30	11**
	Control	28.6 ± 0.80	16	26.7 ± 0.64	16	28.1 ± 0.62	16	26.4 ± 1.22	16
Bicarbonate	Naf-post	26.6 ± 1.31	8	25.5 ± 0.63	8	28.1 ± 0.91	8	27.3 ± 1.31	8
mmol/l	Naf-pre	30.4 ± 0.69	11	28.9 ± 0.79	11	29.4 ± 0.65	11	29.2 ± 0.77	11
	Control	141 ± 0.36	16	137 ± 0.67	16***	138 ± 0.66	16***	139 ± 0.81	16**
Sodium	Naf-post	141 ± 0.63	8	135 ± 1.03	8**	137 ± 0.80	8**	137 ± 0.65	8**
mmol/l	Naf-pre	140 ± 0.56	11	136 ± 0.96	11**	139 ± 0.61	11	138 ± 0.98	11
	Control	4.1 ± 0.11	16	4.1 ± 0.16	16	3.8 ± 0.14	16	3.8 ± 0.08	16
Potassium	Naf-post	4.3 ± 0.07	8	4.3 ± 0.09	8	4.0 ± 0.10	8	4.0 ± 0.11	8
mmol/l	Naf-pre	4.3 ± 0.08	11	4.0 ± 0.11	11*	3.8 ± 0.09	11**	3.9 ± 0.14	11

Naf-post = group which received 1st dose of naftidrofuryl on the 1st post-operative day

Naf-pre = group which received 1st dose of naftidrofuryl with the induction of anaesthesia

Significance vs pre-operative concentrations: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:10-4 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE DEXTROSE-SALINE GROUP

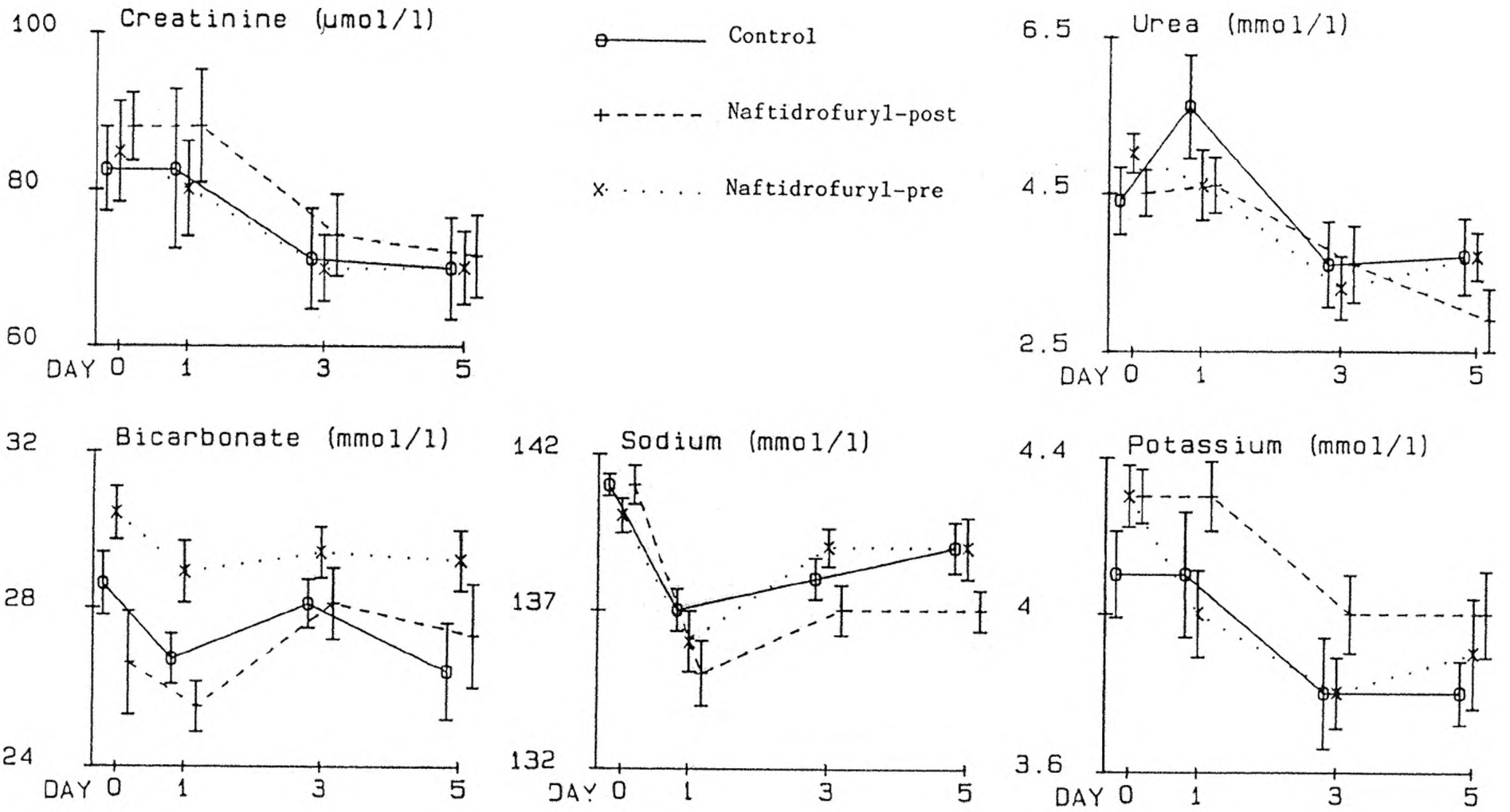


TABLE:10-5 BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (μmol/l) IN THE DEXTROSE-SALINE GROUP

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Valine	Control	214 ± 10.5	16	175 ± 10.4	16**	211 ± 9.3	16
	Naf-post	212 ± 12.3	8	182 ± 23.8	8*	216 ± 14	8
	Naf-pre	194 ± 5.0	11	154 ± 9.6	11***	218 ± 13.9	11
Leucine	Control	126 ± 6.7	16	107 ± 6.8	16*	143 ± 5.3	16*
	Naf-post	121 ± 7.6	8	106 ± 14.6	8*	142 ± 10.6	8*
	Naf-pre	113 ± 3.8	11	92 ± 5.8	11***	138 ± 8.6	11**
Isoleucine	Control	63 ± 2.9	16	42 ± 3.2	16***	73 ± 3.3	16*
	Naf-post	61 ± 3.9	8	47 ± 6.7	8*	82 ± 6.3	8**
	Naf-pre	55 ± 2.3	11	37 ± 2.8	11***	75 ± 4.9	11**
Total BCAA	Control	403 ± 18.9	16	324 ± 19	16**	422 ± 15.7	16
	Naf-post	394 ± 21.7	8	336 ± 43.9	8*	440 ± 29.9	8
	Naf-pre	362 ± 10.2	11	283 ± 17.5	11***	432 ± 25.7	11*

BCAA = Branched-chain amino acids

Naf-post group = 1st dose of naftidrofuryl given on Day 1

Naf-pre group = 1st dose of naftidrofuryl given with induction of anaesthesia

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:10-5 BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (µmol/l) IN THE DEXTROSE-SALINE GROUP

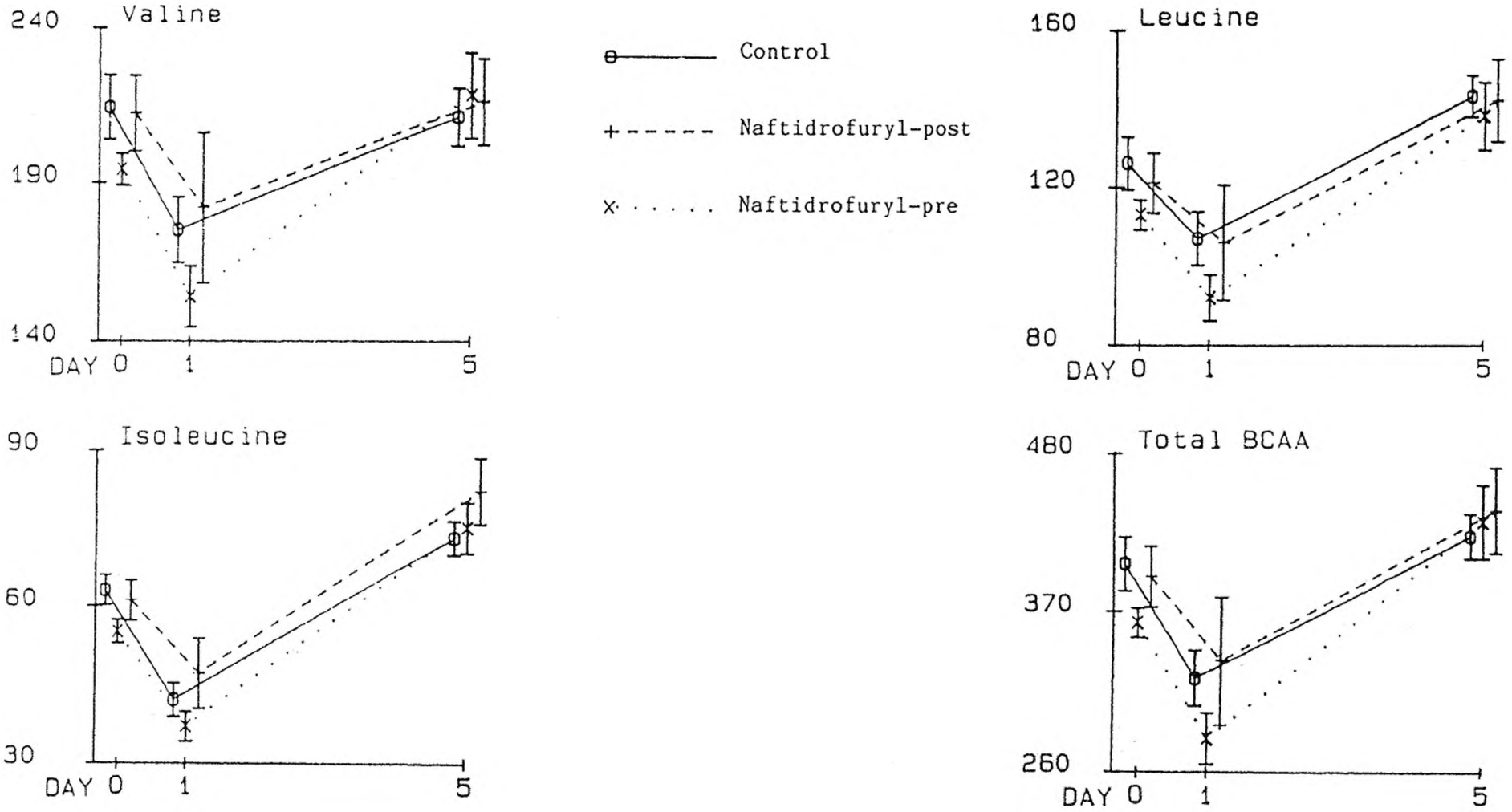


TABLE:10-6 CONCENTRATIONS OF ESSENTIAL AMINO ACIDS (µmol/l)

IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Lysine	Control	195 ± 8.8	16	131 ± 8.0	16***	195 ± 11.6	16
	Naf-post	192 ± 17.8	8	119 ± 6.9	8***	198 ± 12.2	7
	Naf-pre	178 ± 6.1	11	107 ± 8.8	11***	158 ± 13.5	11
Threonine	Control	135 ± 10.3	16	84 ± 6.9	16**	137 ± 10.7	16
	Naf-post	139 ± 14.1	8	79 ± 1.9	8**	137 ± 9.9	7
	Naf-pre	118 ± 6.9	11	65 ± 4.9	11***	112 ± 11.2	11
Methionine	Control	30 ± 2.0	16	29 ± 2.2	16	33 ± 1.9	16
	Naf-post	27 ± 2.6	8	25 ± 0.9	8	34 ± 4.2	8
	Naf-pre	24 ± 1.2	11	23 ± 1.3	11	29 ± 1.9	11
Tryptophan	Control	23 ± 3.1	16	24 ± 3.0	16	21 ± 3.3	16
	Naf-post	28 ± 9.1	8	20 ± 3.9	8	20 ± 4.2	8
	Naf-pre	17 ± 1.4	11	a15 ± 1.6	11	18 ± 2.4	11
Phenylalanine	Control	63 ± 3.2	16	75 ± 4.1	16**	69 ± 2.7	16
	Naf-post	60 ± 2.5	8	70 ± 2.3	8***	66 ± 3.7	8
	Naf-pre	59 ± 2.8	11	68 ± 2.7	11**	67 ± 5.8	11
Valine	Control	214 ± 10.5	16	175 ± 10.4	16**	211 ± 9.3	16
	Naf-post	212 ± 12.3	8	182 ± 23.8	8*	216 ± 14	8
	Naf-pre	194 ± 5.0	11	154 ± 9.6	11***	218 ± 13.9	11
Leucine	Control	126 ± 6.7	16	107 ± 6.8	16*	143 ± 5.3	16*
	Naf-post	121 ± 7.6	8	106 ± 14.6	8*	142 ± 10.6	8*
	Naf-pre	113 ± 3.8	11	92 ± 5.8	11***	138 ± 8.6	11**
Isoleucine	Control	63 ± 2.9	16	42 ± 3.2	16***	73 ± 3.3	16*
	Naf-post	61 ± 3.9	8	47 ± 6.7	8*	82 ± 6.3	8**
	Naf-pre	55 ± 2.3	11	37 ± 2.8	11***	75 ± 4.9	11**
Total EAA	Control	849 ± 33.8	16	666 ± 33.5	16***	877 ± 32.1	16
	Naf-post	840 ± 45.4	8	648 ± 48.3	8**	894 ± 50.8	7
	Naf-pre	a758 ± 20.1	11	a560 ± 30.2	11**	816 ± 41.9	11

Naf-post group = 1st dose of naftidrofuryl given on Day 1

Naf-pre group = 1st dose of naftidrofuryl given with induction of anaesthesia

EAA = Essential amino acids

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.01$ ** $p<0.001$

Significance vs Control: a $p<0.05$

FIGURE:10-6 ESSENTIAL AMINO ACID CONCENTRATIONS (μmol/l) IN THE DEXTROSE-SALINE GROUP

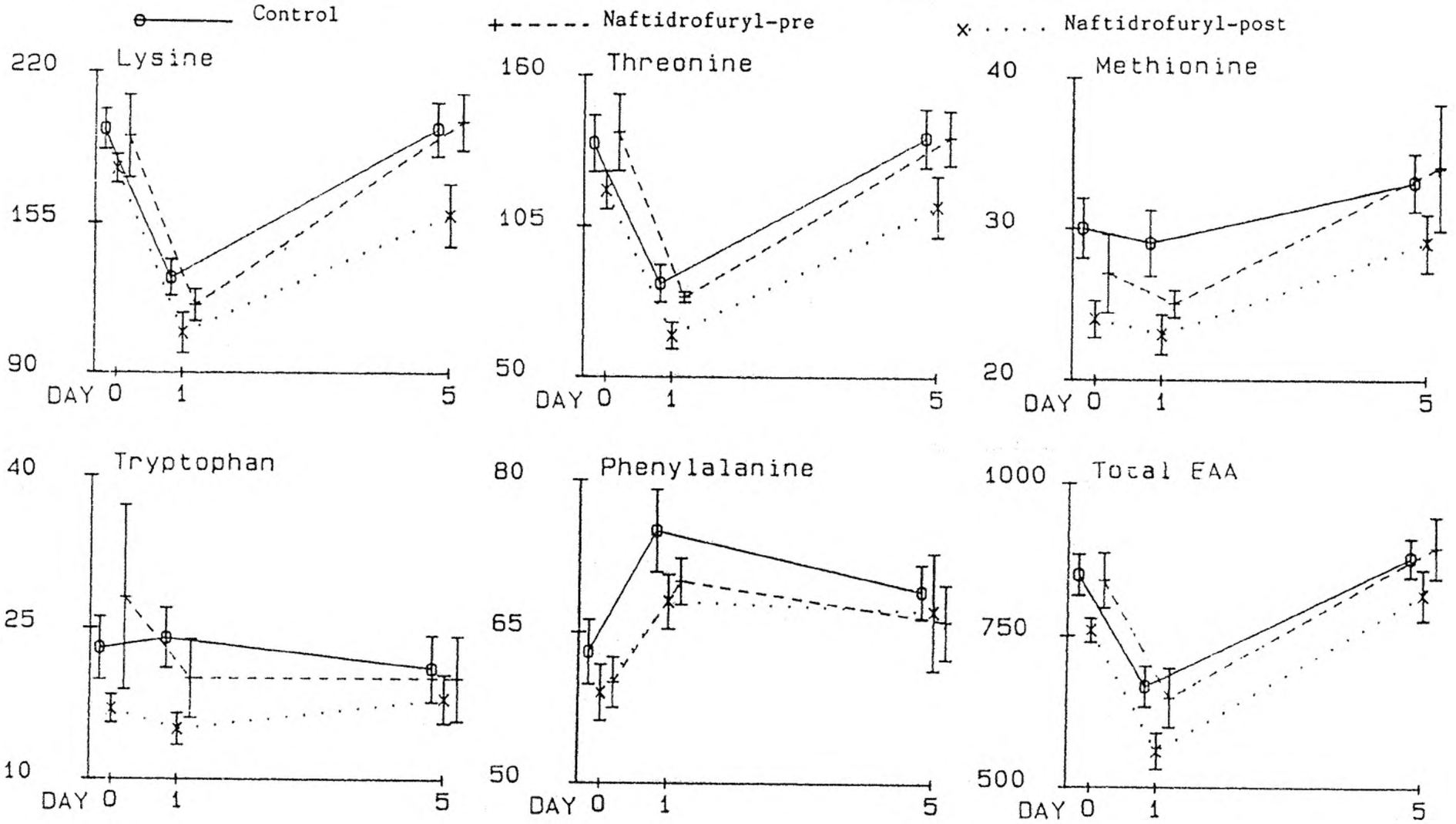


TABLE:10-7A

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (μmol/l) IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Alanine	Control	363 ± 29.7	16	311 ± 22	15*	216 ± 16.1	16***
	Naf-post	320 ± 30.4	8	290 ± 30.3	8	209 ± 16.2	8**
	Naf-pre	a281 ± 16.6	11	aa214 ± 21.8	11**	182 ± 10.2	11***
Taurine	Control	50 ± 1.8	16	55 ± 5.9	16	32 ± 2.0	16***
	Naf-post	53 ± 4.2	8	51 ± 3.2	8	36 ± 4.3	8***
	Naf-pre	52 ± 3.6	11	50 ± 2.5	11	33 ± 1.8	11***
Histidine	Control	83 ± 4.4	16	69 ± 4.3	16**	66 ± 4.0	16**
	Naf-post	79 ± 7.1	8	58 ± 3.7	8**	68 ± 4.7	8
	Naf-pre	83 ± 4.2	11	60 ± 1.8	11***	56 ± 2.7	11***
Citrulline	Control	46 ± 3.3	16	24 ± 1.5	16***	20 ± 1.7	16***
	Naf-post	44 ± 4.0	8	22 ± 2.2	8***	23 ± 3.3	8***
	Naf-pre	42 ± 4.0	11	a18 ± 2.1	11***	18 ± 1.6	11***
Arginine	Control	102 ± 6.4	16	57 ± 4.5	16***	79 ± 5.3	16**
	Naf-post	106 ± 11.4	8	53 ± 6.3	8***	88 ± 8.9	8*
	Naf-pre	94 ± 6.0	11	a44 ± 2.7	11***	71 ± 5.5	11**
Ornithine	Control	68 ± 4.8	16	37 ± 2.4	16***	47 ± 4.1	16**
	Naf-post	58 ± 6.0	8	38 ± 5.9	8**	53 ± 7.9	8
	Naf-pre	60 ± 4.1	11	a30 ± 2.6	11***	43 ± 3.3	11**
Proline	Control	180 ± 10.1	16	114 ± 8.6	16***	135 ± 7.0	16***
	Naf-post	168 ± 23.9	8	110 ± 8.4	8**	142 ± 8.5	8*
	Naf-pre	a144 ± 6.6	11	102 ± 13.8	11**	122 ± 6.8	11***
Glutamine	Control	680 ± 31.1	16	534 ± 25.4	16***	550 ± 31.8	16**
	Naf-post	607 ± 34.1	8	449 ± 28.1	8***	529 ± 33.4	8*
	Naf-pre	617 ± 22.2	11	aa424 ± 16.9	11***	495 ± 23.1	11***

Naf-post = 1st dose of naftidrofuryl given on Day 1

Naf-pre = 1st dose of naftidrofuryl given with induction of anaesthesia

Significance vs pre-operative concentrations: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Significance vs control: a $p<0.05$ aa $p<0.005$

TABLE:10-7B

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (μmol/l) IN THE DEXTROSE-SALINE GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Glutamate	Control	32 ± 5.0	16	23 ± 3.0	16	25 ± 4.1	16
	Naf-post	34 ± 5.3	8	24 ± 4.3	8**	27 ± 5.5	8
	Naf-pre	27 ± 2.8	11	25 ± 2.3	11	27 ± 4.4	11
Glycine	Control	231 ± 16.1	16	176 ± 15.5	16**	192 ± 14.2	16*
	Naf-post	216 ± 18.2	8	151 ± 9.9	8***	196 ± 10.2	8
	Naf-pre	203 ± 23.1	11	136 ± 10.8	11**	159 ± 15.9	11**
Tyrosine	Control	61 ± 2.9	16	55 ± 2.2	16	58 ± 1.8	16
	Naf-post	58 ± 4.4	8	52 ± 2.2	8	63 ± 7.9	8
	Naf-pre	56 ± 3.5	11	49 ± 3.2	11	53 ± 3.6	11
Aspartate	Control	1.6 ± 0.2	15	1.6 ± 0.2	15	1.5 ± 0.2	14
	Naf-post	2.0 ± 0.3	7	1.5 ± 0.2	7	1.7 ± 0.2	5
	Naf-pre	1.7 ± 0.3	10	1.7 ± 0.3	10	1.4 ± 0.2	10
Asparagine	Control	48 ± 2.3	16	37 ± 2.6	16**	46 ± 2.4	16
	Naf-post	50 ± 6.1	8	37 ± 1.8	8*	52 ± 3.4	8
	Naf-pre	42 ± 2.5	11	a29 ± 1.3	11***	42 ± 2.6	11
AAB	Control	23 ± 1.9	16	24 ± 1.8	16	32 ± 1.7	16***
	Naf-post	60 ± 4.6	8	25 ± 1.9	8	39 ± 3.6	8*
	Naf-pre	20 ± 1.4	11	aa17 ± 1.8	11	39 ± 3.1	11***
Cystine	Control	60 ± 3.0	16	45 ± 2.3	16***	58 ± 3.4	16
	Naf-post	60 ± 3.2	8	43 ± 2.4	8***	61 ± 4.3	8
	Naf-pre	52 ± 3.2	11	a37 ± 2.5	11***	52 ± 3.5	11
Serine	Control	116 ± 6.3	16	73 ± 6.5	16***	100 ± 6.3	16
	Naf-post	112 ± 9.4	8	66 ± 3.8	8***	99 ± 7.0	8
	Naf-pre	a92 ± 4.7	11	a58 ± 4.6	11***	81 ± 6.1	11
Total NEAA	Control	2123 ± 80.3	15	1639 ± 76.7	15***	1688 ± 81.1	14***
	Naf-post	2079 ± 136.4	7	1497 ± 131.4	7***	1545 ± 100.4	5**
	Naf-pre	a1825 ± 67.7	10	aa1335 ± 58.3	9***	1462 ± 72.6	10***

NEAA = Non-essential amino acids AAB = Alpha-aminobutyrate

Naf-post = 1st dose of naftidrofuryl given on Day 1

Naf-pre = 1st dose of naftidrofuryl given with induction of anaesthesia

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Significance vs control: a $p<0.05$ aa $p<0.01$

FIGURE:10-7A NON-ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE DEXTROSE-SALINE GROUP

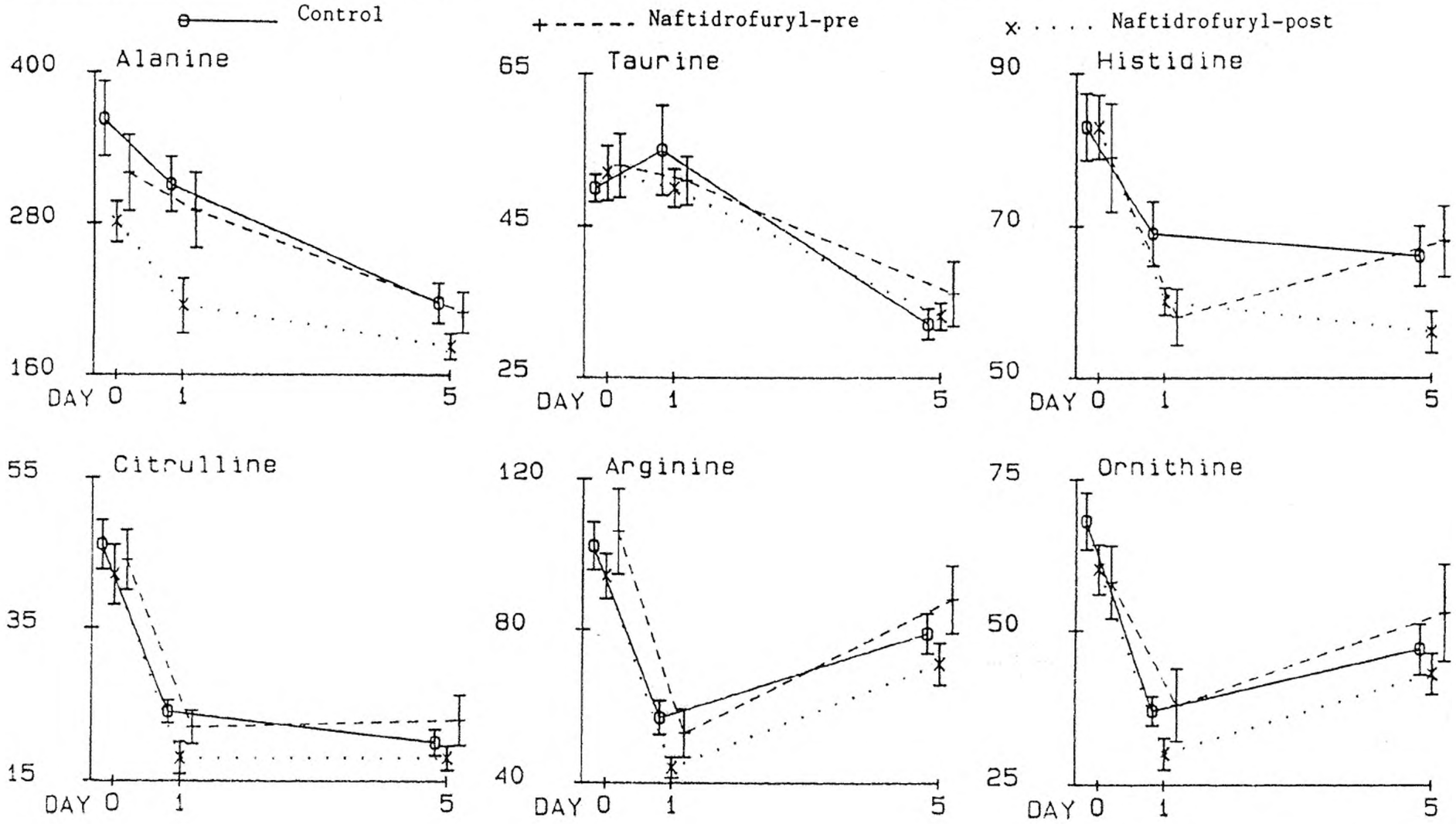


FIGURE:10-7B NON-ESSENTIAL AMINO ACID CONCENTRATIONS (μmol/l) IN THE DEXTROSE-SALINE GROUP

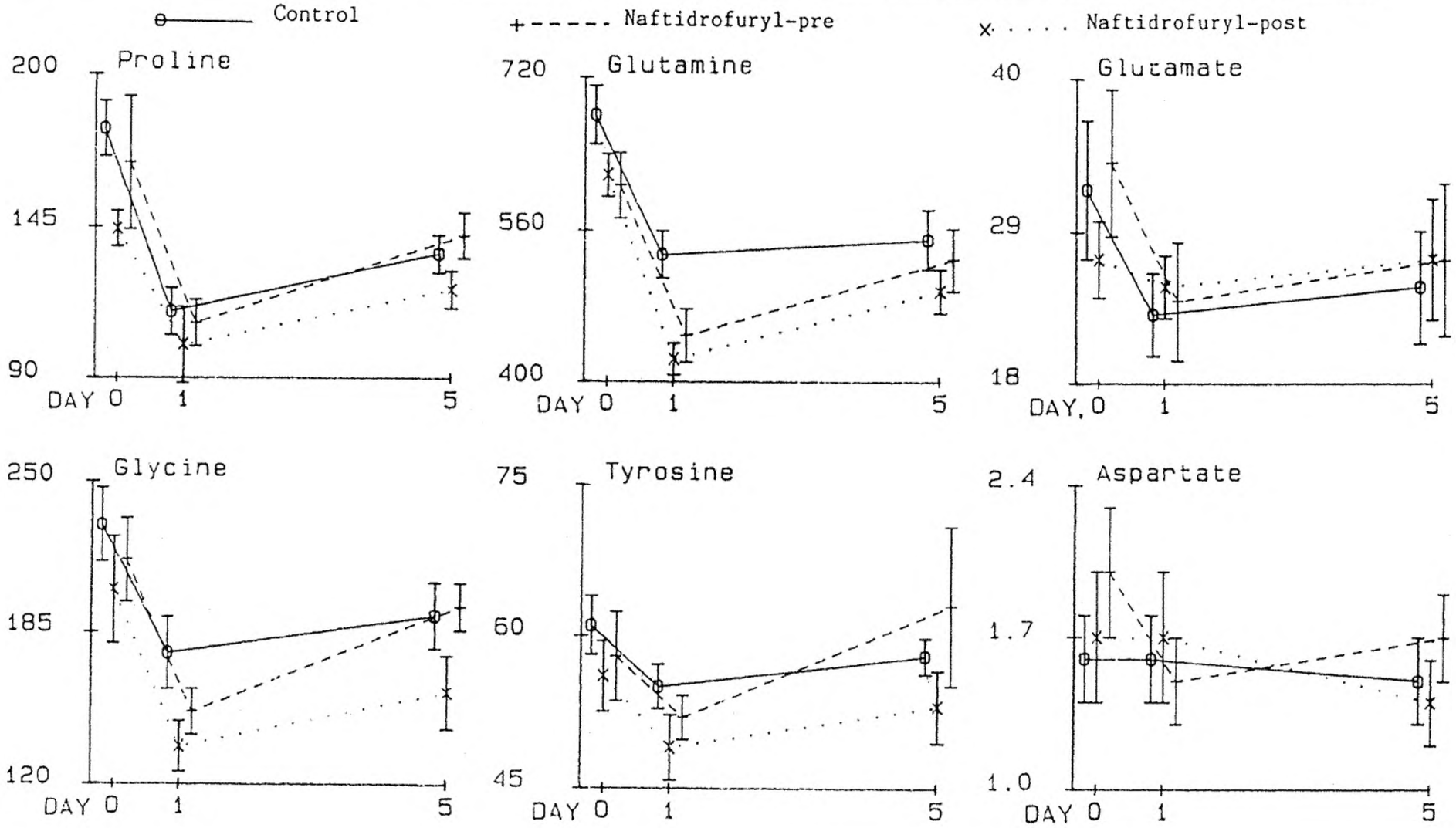


FIGURE:10-7C NON-ESEENTIAL AMINO ACID CONCENTRATIONS (µmol/1) IN THE DEXTROSE-SALINE GROUP

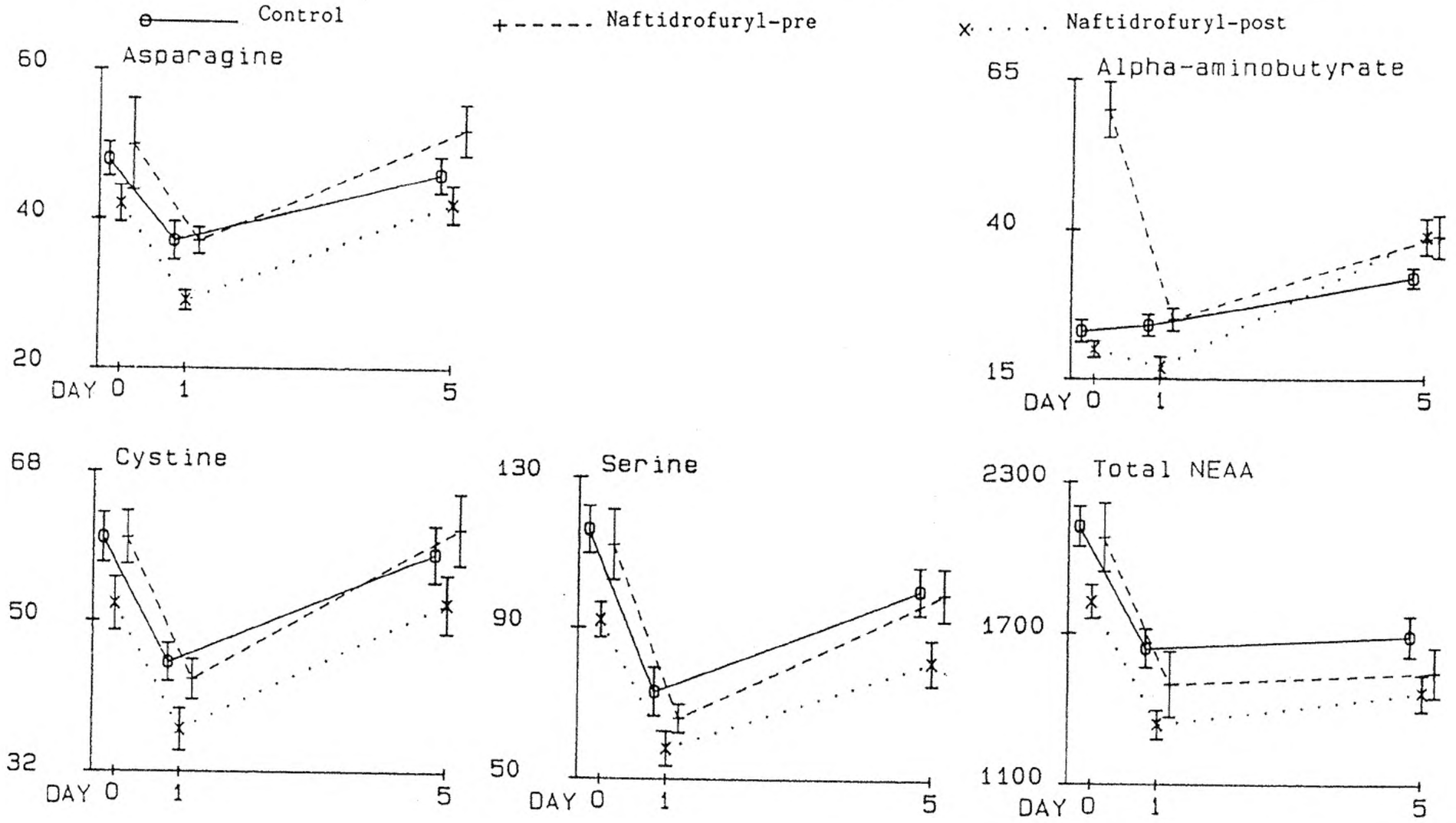


TABLE:10-8 PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE, AND CYSTINE/TAURINE RATIOS IN THE DEXTROSE-SALINE GROUP

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Phe/Tyr	Control	1.05 ± 0.05	16	1.37 ± 0.07	16***	1.20 ± 0.05	16**
	Naf-post	1.06 ± 0.07	8	1.37 ± 0.06	8**	1.14 ± 0.08	8
	Naf-pre	1.10 ± 0.10	11	1.42 ± 0.08	11**	1.27 ± 0.10	11*
Met/CySS	Control	0.52 ± 0.03	16	0.65 ± 0.05	16	0.58 ± 0.03	16
	Naf-post	0.46 ± 0.04	8	0.59 ± 0.05	8	0.56 ± 0.05	8
	Naf-pre	0.48 ± 0.03	11	0.61 ± 0.06	11	0.58 ± 0.05	11
Met/Tau	Control	0.62 ± 0.04	16	0.59 ± 0.05	16	1.06 ± 0.07	16***
	Naf-post	0.52 ± 0.05	8	0.49 ± 0.03	8	1.04 ± 0.14	8**
	Naf-pre	0.49 ± 0.05	11	0.46 ± 0.05	11	0.88 ± 0.07	11**
CySS/Tau	Control	1.21 ± 0.06	16	0.92 ± 0.08	16***	1.90 ± 0.16	16***
	Naf-post	1.19 ± 0.11	8	0.87 ± 0.09	8***	1.87 ± 0.20	8***
	Naf-pre	1.04 ± 0.09	11	0.79 ± 0.08	11***	1.56 ± 0.09	11***

Naf-post = 1st dose of naftidrofuryl given on Day 1

Naf-pre = 1st dose of naftidrofuryl given with induction of anaesthesia

Phe/Tyr = Phenylalanine to tyrosine ratio Met/CySS = methionine to cystine ratio

Met/Tau = Methionine to taurine ratio CySS/Tau = cystine to taurine ratio

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.005$ *** $p<0.001$

FIGURE:10-8

PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE AND CYSTINE/TAURINE RATIOS IN THE DEXTROSE-SALINE GROUP

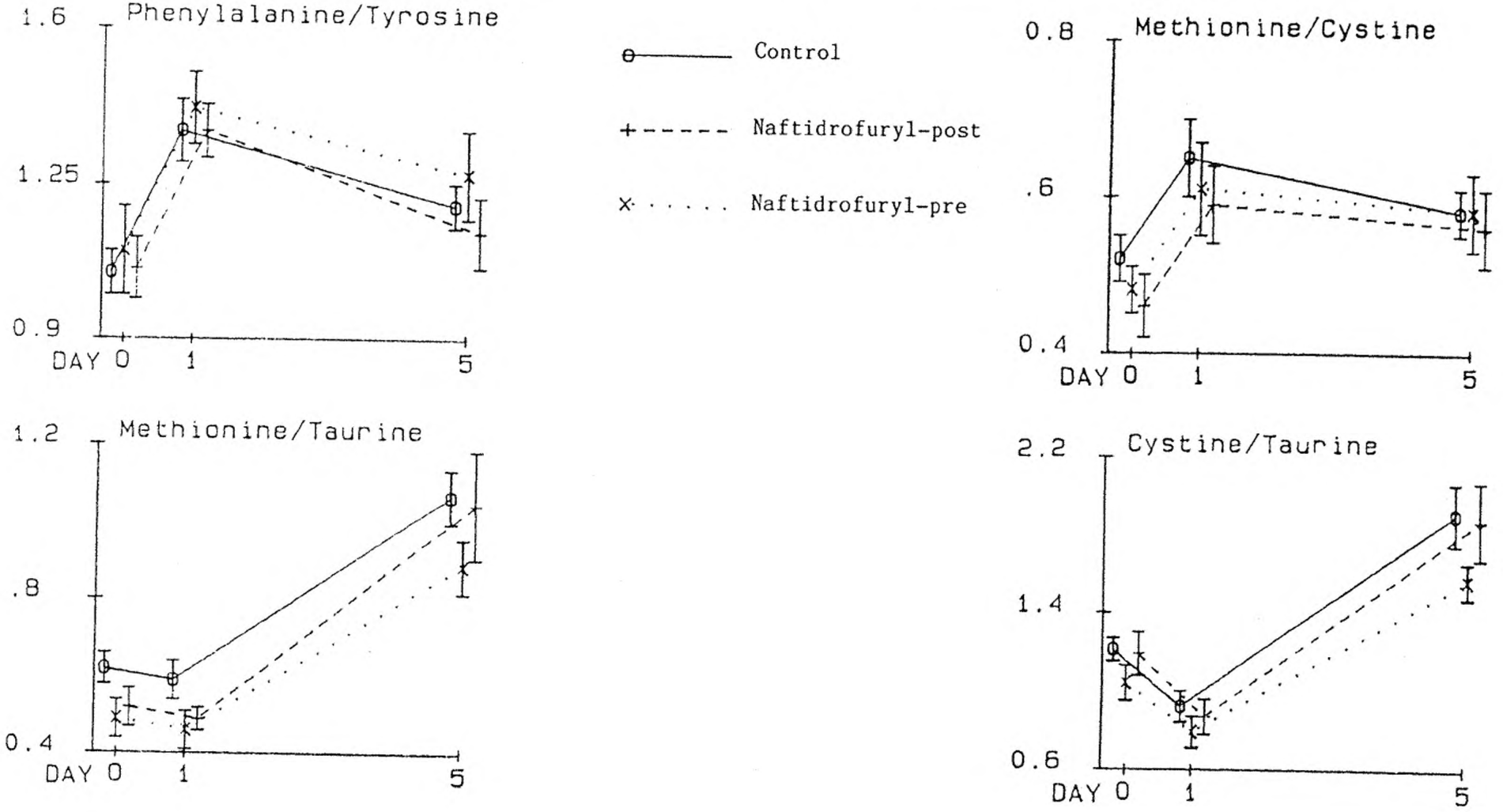


TABLE:10-9 LINEAR CORRELATIONS BETWEEN TOTAL KETONE AND BRANCHED-CHAIN AMINO ACID CONCENTRATIONS AND THE MAGNITUDE OF CHANGE BETWEEN DAYS 0 & 1 OF THESE SUBSTRATES AND THE 6-DAY NITROGEN BALANCE

	Group	r	n	p	
	Control	0.5491	16	p<0.05	
[Day 1 TK] mmol/l	Naf-post	0.9668	8	p<0.001	
vs	Naf-pre	0.1441	11	p>0.1	ns
[Day 1 BCAA] µmol/l	All patients	0.7375	35	p<0.001	
	Control	0.4896	16	p>0.05	ns
[Day 1 - Day 0 TK] mmol/l	Naf-post	0.7041	8	p>0.05	ns
vs	Naf-pre	0.4026	11	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	All patients	0.4285	35	p<0.05	
	Control	0.0806	16	p>0.1	ns
[Day 1 TK] mmol/l	Naf-post	0.0372	8	p>0.1	ns
vs	Naf-pre	0.1851	11	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.0454	35	p>0.1	ns
	Control	0.0409	16	p>0.1	ns
[Day 1 BCAA] µmol/l	Naf-post	0.3474	8	p>0.1	ns
vs	Naf-pre	0.1519	11	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.1474	35	p>0.1	ns
	Control	0.0104	16	p>0.1	ns
[Day 1 - Day 0 TK] mmol/l	Naf-post	0.7152	8	p>0.05	ns
vs	Naf-pre	0.4336	11	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.2194	35	p>0.1	ns
	Control	0.0027	16	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	Naf-post	0.4075	8	p>0.1	ns
vs	Naf-pre	0.3463	11	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.1475	35	p>0.1	ns

TK = total ketones

BCAA = Branched-chain amino acids

r = Coefficient of correlation

p = Level of significance

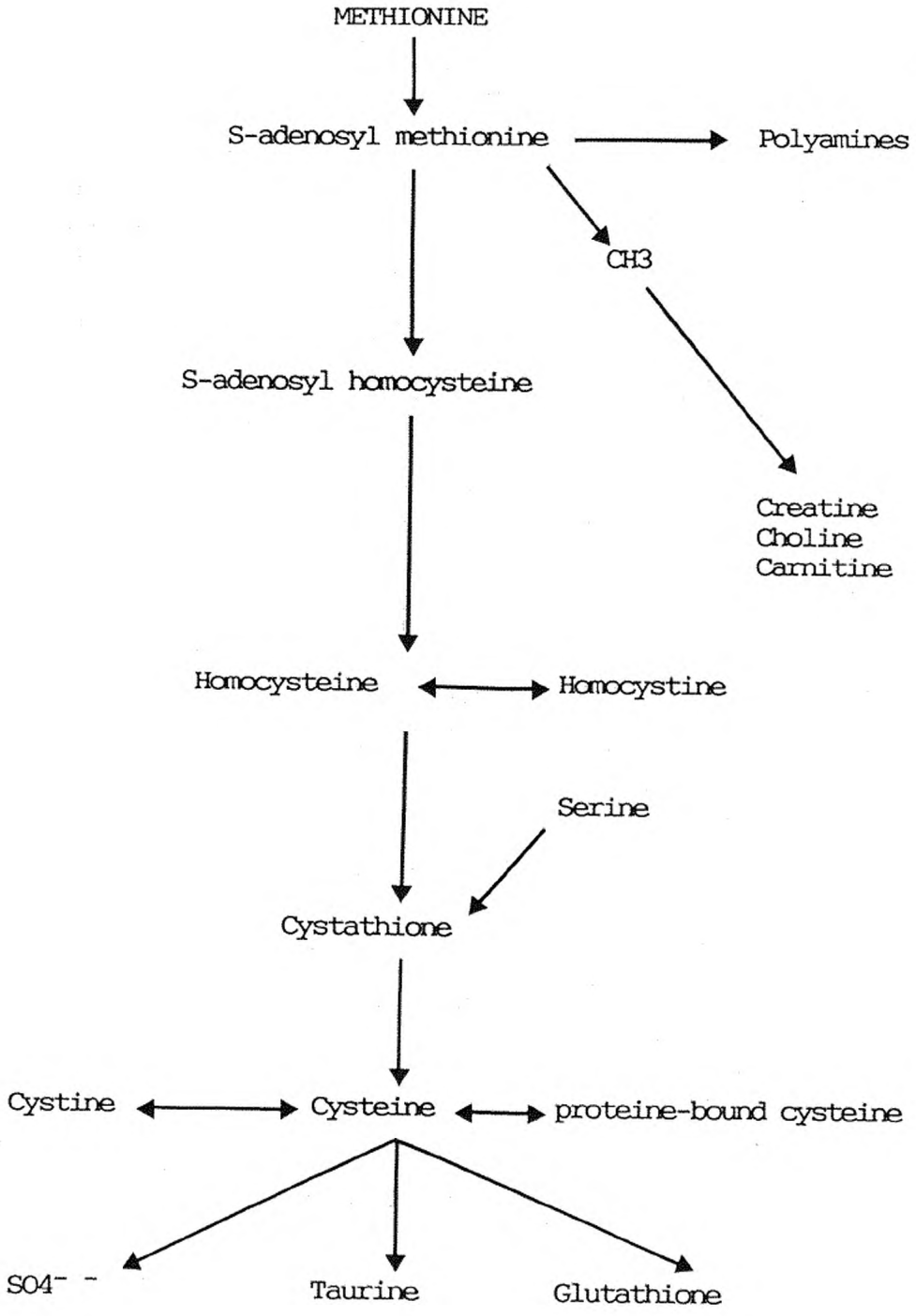
[ ] = Concentration

ns = not significant

Naf-post = 1st dose of naftidrofuryl given on Day 1

Naf-pre = 1st dose of naftidrofuryl given with induction of anaesthesia

FIGURE:10-10 THE TRANS-SULPHURATION PATHWAY



CHAPTER 11

EFFECT OF NAFTIDROFURYL WITH ISOTONIC AMINO ACID INFUSION ON THE METABOLIC RESPONSE TO SURGERY

PATIENTS AND METHODS

The patients and methods are those described in chapters 7 and 8. 36 patients were randomised to receive a twice daily infusion of either placebo (control group = 18 patients) or 200 mg naftidrofuryl oxalate (naftidrofuryl group = 18 patients). All patients also received a peripheral intravenous infusion of isotonic amino acids which delivered 0.15 g nitrogen kg^{-1} day^{-1}. The groups were matched for age, sex, anthropometric measurements and the type of surgical procedure (see chapter 8, table:8-4).

RESULTS

Urine (see chapter 9, table:9-2)

The cumulative 3 and 6-day urinary volume, urea excretion and nitrogen balance showed no significant between group differences.

Glucose, insulin and gluconeogenic substrates
(table:11-1 & figure:11-1)

Peak blood glucose concentration occurred on Day 1 and by Day 3 had returned to pre-operative values. Thereafter, no further changes occurred and there were no between group differences.

Peak insulin concentration occurred on Day 1 and fell thereafter. The Day 5 concentrations were lower in both groups than the pre-operative values. The naftidrofuryl group had a higher mean insulin concentration than the control group on all post-operative days. However, as the mean Day 0 insulin concentration was also

higher in the naftidrofuryl group than in the control group, between group comparisons of the magnitude of change between days revealed no differences ($p>0.1$). The trend and the magnitude of change were similar in both groups.

Peak blood pyruvate and lactate concentrations occurred on Day 1 in the naftidrofuryl group and then fell towards pre-operative concentrations. However, there were no significant between day differences in the control group, throughout the study period, in either of these variables. The mean Day 5 concentrations of these substrates, though not significantly different, were lower in the control group and higher in the naftidrofuryl group than the mean Day 0 concentrations ($p>0.1$).

The ratio of lactate to pyruvate concentrations showed no between group differences and there were no changes, in each group, throughout the study period.

Total gluconeogenic substrates (Lactate, pyruvate, alanine and glycerol) concentration showed no significant changes in the naftidrofuryl group but fell post-operatively in the control group (Day 5 < Day 0, $p<0.05$). Furthermore, the change in concentrations between Days 0 and 1 was significantly greater in the naftidrofuryl group (mean change = 0.159 mmol/l) when compared to the control group (mean change = -0.151 mmol/l, $p<0.05$).

Lipid metabolites: (table:11-2 & figure:11-2)

Total ketones (3-hydroxybutyrate + acetoacetate) showed no between group difference. Their concentrations rose steadily between Days 0 and 1 and between Days 1 and 3 but the increase between Days 3 and 5 was not significant.

Serum total triglycerides showed no between group difference. Trough concentrations occurred on Day 1 in both groups and rose thereafter, the mean Day 5 concentrations not being different from mean Day 0 concentrations.

Plasma free fatty acid concentrations rose between

Days 0 and 1 and thereafter showed no change in the control group, whereas in the naftidrofuryl group a further rise occurred between Days 3 and 5.

Glycerol showed no significant between day changes in the control group, whereas in the naftidrofuryl group the mean Day 5 concentration was significantly higher than pre-operatively.

Haemoglobin, packed cell volume, albumin and total protein (table:11-3 & figure:11-3)

Haemoglobin and packed cell volume ratio fell progressively between Days 0 and 1 and between Days 1 and 3. Thereafter, no significant changes occurred and there were no between group differences.

There were no between group differences in the serum albumin and total protein concentrations. They fell sharply on Day 1 and rose thereafter, Day 3 and Day 5 concentrations remaining lower than pre-operatively.

Creatinine, urea and electrolytes
(table 11-4 & figure:11-4)

Serum creatinine concentrations were lower on Days 3 and 5 than pre-operatively and showed no between group differences.

Serum urea concentrations rose on Day 1 with no between group differences and thereafter showed no significant changes.

Day 5 serum bicarbonate concentrations were lower than pre-operatively in both groups (Day 5 < Day 0, $p<0.05$) and showed no between group differences.

Serum sodium concentrations fell on Day 1 ($p<0.001$ in both groups) and thereafter showed no significant changes.

Serum potassium concentrations showed no significant between day or between group differences.

Venous plasma free amino acids

The mean concentration of individual plasma amino

acid showed considerable fluctuations within each group. The only between group differences were found in alanine, glycine and serine (see below: non-essential amino acids, tables:11-7A & 11-7B).

Branched-chain amino acids (BCAA) (table:11-5 & figure:11-5)

Valine, leucine and isoleucine concentrations showed no significant changes on Day 1 and rose sharply thereafter, Day 5 concentrations being higher than pre-operatively in both groups. Total BCAA followed the same pattern with no between group differences.

Essential amino acids (EAA) (table:11-6 & figure:11-6)

The naftidrofuryl and control groups showed similar changes throughout the study period. There were no significant post-operative changes in tryptophan concentrations. Trough lysine, methionine and threonine concentrations occurred on Day 1 and rose thereafter. The Day 5 lysine and threonine concentrations remained lower than Day 0 concentrations, whereas the Day 5 methionine concentrations were higher than pre-operatively. Peak phenylalanine concentrations occurred on Day 1 and then fell with the Day 5 concentrations remaining higher than pre-operatively. Trough essential amino acids concentrations occurred on Day 1 and rose thereafter, the Day 5 concentrations being higher than pre-operatively.

Non-essential amino acids (NEAA) (tables:11-7A & 11-7B and figures: 11-7A & 11-7B & 11-7C)

On Day 1 individual non-essential amino acid concentrations fell with the exception of taurine which showed no change and alpha-aminobutyrate which increased. Thereafter, the concentrations rose with the exception of alanine and taurine which fell and aspartate, citrulline and glutamate which showed no further changes. On Day 5, the individual amino acid concentrations were lower than pre-operatively with the exception of proline and alpha-aminobutyrate which were higher than pre-operatively and

ornithine, tyrosine and cystine which were not different from Day 0.

Between group differences were found in alanine, glycine and serine. The Day 5 alanine and serine concentrations were higher in the naftidrofuryl group when compared to the control group ($p<0.05$). The Day 5 glycine concentration was significantly lower than the Day 0 concentration in the control group ($p<0.02$), whereas it was not different from Day 0 in the naftidrofuryl group. Furthermore, the decreases in alanine and glycine concentrations, between Days 0 and 5 were significantly smaller in the naftidrofuryl group (mean [Day 0 - Day 5] = 91.43 µmol/l for alanine and -16.43 µmol/l for glycine) when compared to the control group (mean [Day 0 - Day 5] = 174.19 µmol/l for alanine, $p<0.01$, and 36.13 µmol/l for glycine, $p<0.05$). However, the decrease in serine concentration over the same period showed no significant between group difference (mean [Day 0 - Day 5] = 13.44 µmol/l for the control group and -2 µmol/l for the naftidrofuryl group, $p>0.05$).

Total NEAA showed similar changes in both groups. The concentrations fell on Day 1 and rose thereafter, Day 5 concentration remaining lower than pre-operatively.

DISCUSSION

Table:9-2 and figure:9-2 show that a twice daily infusion of 200 mg naftidrofuryl oxalate does not result in any measurable nitrogen sparing effect in patients who underwent elective abdominal surgery and received peripheral isotonic amino acids as their sole nutritional support. This result does not agree with the findings of Burns and Galloway (1984) who have found that a similar dose of naftidrofuryl oxalate improved nitrogen balance in such patients. The patients studied by Burns and Galloway received a fixed amount of isotonic amino acids regardless of the body weight (15 g nitrogen daily; Perifusin$^{(R)}$), whereas the patients studied in this chapter received a similar solution which delivered 0.15 g nitrogen kg^{-1}

day^{-1} and resulted in a lower nitrogen intake (10.5 g nitrogen day^{-1} for a 70 kg patient; mean (sem) body weight = 68 (3.4) kg for the control group and 70.3 (2.6) kg for the naftidrofuryl group, table:8-4). However, this does not account for the different findings.

In addition to the evidence for this conclusion from urinary measurements, there were no differences in albumin and total protein concentrations between the control and naftidrofuryl groups. After the initial fall in albumin and total protein concentrations on Day 1, due mainly to the increased vascular permeability and catabolism associated with injury (Arturson and Jonsson, 1979; Davies et al., 1962; Fleck et al., 1985), the concentrations returned towards pre-operative values as these effects diminished.

The results in this chapter represent those after elective abdominal surgery with the patients receiving isotonic amino acids only (0.15 g nitrogen $Kg^{-1}day^{-1}$). The post-operative blood results are those of samples following 6-7 hours nutrient-free time during which the patients received a 0.9% w/v sodium chloride infusion to maintain venous access.

No reason was apparent for the between group difference in insulin concentration on Day 0, and it may be due to an artefact of selection. It should be noted that the Day 0 insulin concentrations, in the control and treatment groups, were within the Day 0 laboratory reference range (see appendix 1). Therefore, the magnitude of change between days was tested for between group difference and found to be not significant.

The rise in blood glucose and insulin concentrations, on Day 1, indicates the usual hyperglycaemia with insulin resistance that follows injury. The concentrations later fell to values lower than pre-operatively (Day 5 < Day 0, $p<0.05$ for insulin and Day 5 < Day 0, $p>0.05$ for glucose) as the patients had no carbohydrate intake.

There were significant negative linear

correlations between log [total ketones] and lactate concentrations post-operatively in both groups ($r= -0.2797$, $n=51$, $p<0.05$ in the control group; $r= -0.4512$, $n=50$, $p<0.01$ in the naftidrofuryl group). However, there were no significant correlations between Day 1 total ketone and BCAA concentrations nor between the cumulative 6-day nitrogen balance and the Day 1 total ketone concentration, the Day 1 BCAA concentration or the magnitude of change in these variables between Days 0 and 1 (table:11-9). This does not agree with the report of Wedge et al. (1976) that injured patients with initial hyperketonaemia have a smaller increase in the concentration of branched-chain amino acids and a lower urinary nitrogen excretion than those with normoketonaemia. However, the patients studied in this chapter are not comparable to those studied by Wedge et al. (1976). Their patients had accidental injuries which varied from a long bone fracture to multiple trauma and the timing of their last pre-injury nutritional intake was not documented.

The fall on Day 1 in total triglyceride concentrations is due to the catecholamine-induced activation of hormone-sensitive lipase (Steinberg et al., 1975). Increases in tissue lipolysis following injury have been well documented (Allison et al., 1969; Wiklund and Jorfeldt, 1975; Äärimaa et al., 1978). The released free fatty acids and glycerol are transported via plasma to the liver and other tissues. The glycerol is taken up by the liver, at a rate dependant upon its concentration (Shaw et al., 1976). The free fatty acids have essentially two fates. They may be esterified to form triglycerides or they may be oxidised to form acetyl CoA. If the rate of fatty acid oxidation is high, overproduction of ketones may result (McGarry and Foster, 1976). The findings reported in this chapter of the progressive increase in total ketones (Day 0 < Day 1 < Day 3 = Day 5) with the post-operative rise in free fatty acids (Day 0 <Day 1= Day 3 = Day 5) and no change in

glycerol concentrations (Day 0= Day 1= Day 3 = Day 5) are suggestive that the ketonaemia resulted from diversion of fatty acids esterification to beta-oxidation but with no increase in adipose tissue lipolysis. In support of this are the findings of Foster et al. (1979) who have shown that fasted surgical patients had lower blood ketones, glycerol and free fatty acid concentrations than healthy volunteers fasted over the same period. Indeed, the total ketones concentrations reported here at Day 5 (1.675 mmol/l for the control group; 1.346 mmol/l for the naftidrofuryl group) were much less than the concentration after 4 days in the fasted uninjured subjects in their study (5.68 mmol/l). Admittedly, this assumption cannot be made with any degree of certainty as turnover measurements are not available and venous blood concentrations do not necessarily correlate with turnover measurements in the injured subject.

If the above hypothesis is true, then the diversion of free fatty acids from esterification to beta-oxidation is presumably due to the depletion of glycogen stores and, with no carbohydrate intake, to a reduction in acetyl CoA from carbohydrate sources.

The post-operative rise in serum urea concentrations, in the absence of carbohydrate intake, is due partly to transamination and oxidation of the infused amino acids to provide energy and partly to the increased gluconeogenesis from amino acid sources.

The changes in venous plasma free amino acids followed the same trends as those reported by Askanazi et al. (1980a) in patients who have undergone total hip replacement and received isotonic amino acids only (3.5% Freamine$^{(R)}$). The single exception was tryptophan concentration which showed no significant change in the patients described in this chapter but increased in the patients studied by Askanazi. This difference in tryptophan concentration, between the two studies, is difficult to explain and may be related to the degree of severity of injury with elective abdominal surgery being

less traumatic than total hip replacement.

The observed changes and in particular the general decreasing pattern on Day 1 of most amino acids (with the exception of phenylalanine, alpa-aminobutyrate and branched-chain amino acids), do not appear to be the result of a single factor and may well be related to the extensive endocrine changes known to occur in response to surgery (Johnston et al., 1972).

The rise in plasma phenylalanine concentrations reflects, as already discussed in chapter 10, the rapid skeletal muscle catabolism that occurs following injury and does not necessarily reflect altered hepatic handling of this amino acid. Furthermore, there were no increases in the concentrations of the aromatic amino acids tryptophan and tyrosine and the sulphur-containing amino acid cystine, which are catabolised in the liver, to support the theory of altered hepatic handling of the amino acids in the patients studied in this thesis.

The rise in phenylalanine to tyrosine (Phe/Tyr) and methionine to cystine (Met/CySS) ratios (Table: 11-9 & figure:11-9), with Day 5 concentrations of tyrosine and cystine being not different from Day 0, is due to the increased Day 5 concentrations of phenylalanine and methionine. This does not necessarily support the view that tyrosine and cystine may become essential in response to surgical injury (Dale et al., 1977; Johnston et al., 1980). The significant increases in the methionine/cystine, methionine/taurine and cystine/taurine ratios, with Day 5 taurine concentrations being lower than pre-operatively, are consistent with a probable block in the trans-sulphuration pathway as already discussed in the previous chapter. However, this assumption cannot be made with certainty as tissue concentrations and urinary excretion of these substrates were not measured.

The only between group differences were found in lactate, pyruvate, alanine, serine and glycine concentrations. If, as postulated, naftidrofuryl improves substrate utilisation in the tricarboxylic acid cycle

(Burns et al., 1981), then one would expect a lower concentration of pyruvate and lactate as opposed to the higher concentrations reported here.

Raised levels of pyruvate together with decreased citrate and increased alpha-oxoglutarate concentrations have been reported in normal post-absorptive mice livers in response to the administration of naftidrofuryl (Burns, 1982). The low citrate was explained by its increased rate of conversion to alpha-oxoglutarate; this being consistent with improved rate of cycling of the tricarboxylic acid cycle. In the post-absorptive state with intact glycogen stores, the glycolytic rate would increase in response to lowered citrate which would relieve phosphofructokinase from the feedback inhibition caused by citrate accumulation; thus resulting in increased pyruvate concentrations. Shaw and Johnson (1975) have also reported increased blood pyruvate concentration with no change in lactate concentration, leading to a decrease in lactate/pyruvate ratio, after exercise in healthy volunteers receiving naftidrofuryl. They suggested that this was due to an enhancement of the cellular oxidative capacity. However, metabolism in the fasting and post-surgical state is different from that of the post-absorptive or post-exercise states in healthy individuals. Therefore, the results reported by Shaw and Johnson (1975) and Burns (1982) are not applicable to the patients studied in this chapter and the findings of raised pyruvate and lactate concentrations remain pertinent to our data.

Under the conditions prevailing at the time of blood sampling, namely 18-24 hours following surgery and no caloric intake for approximately 36 hrs, the glycogen stores are presumed depleted and the increased pyruvate concentration in the naftidrofuryl group could be due to either reduced utilisation or increased production through increased gluconeogenesis. Reduced utilisation will not support the view that naftidrofuryl increases tricarboxylic acid cycle activity. Following injury, the

rate of gluconeogenesis generally correlates with the rate of ureagenesis, and there is no evidence of between group differences in serum urea and/or blood glucose concentrations (tables: 11-1 & 11-4) and urinary urea excretion (Table:9-2). Therefore, increased pyruvate cannot be attributed to increased gluconeogenesis.

Given the present data, it is difficult to postulate a pathway explaining the increased pyruvate and lactate concentrations, on Day 1, in response to naftidrofuryl.

The smaller post-operative fall in glycine, serine and alanine concentrations in the naftidrofuryl group when compared to the control group, in the presence of raised pyruvate and no between group difference in the branched-chain amino acids, may be the consequence of reduced need for gluconeogenesis from amino acid sources thus reducing the need for protein breakdown. This is, however, not borne out in the results of nitrogen balance or urea excretion.

In conclusion, the twice daily infusion of 200mg naftidrofuryl oxalate did not result in any measurable nitrogen sparing effect in patients who underwent elective abdominal surgery and received isotonic amino acids as their sole nutritional support. The next chapter will report the effect of this drug in similar patients receiving amino acids, carbohydrate and fat.

TABLE:11-1

BLOOD ANALYTE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Glucose mmol/l	Control	4.4 ± 0.12	18	5.5 ± 0.16	18***	4.1 ± 0.18	18	4.2 ± 0.14	15
	Naf.	4.7 ± 0.16	18	5.7 ± 0.19	18***	4.4 ± 0.17	18	4.3 ± 0.21	15
Insulin mU/l	Control	11.3 ± 0.55	17	16.0 ± 1.22	17***	9.4 ± 0.79	17*	9.2 ± 0.65	15*
	Naf.	aa14.2 ± 0.96	18	a20.7 ± 2.0	18***	aa13.2 ± 1.18	18	a11.4 ± 0.78	15*
Pyruvate µmol/l	Control	73 ± 6	18	73 ± 4	18	66 ± 5	17	64 ± 4	14
	Naf.	65 ± 4	18	81 ± 5	18**	76 ± 6	17	73 ± 12	15
Lactate µmol/l	Control	776 ± 69	18	789 ± 76	18	687 ± 47	18	641 ± 42	15
	Naf.	703 ± 49	18	867 ± 71	18*	689 ± 51	18	727 ± 59	15
L/P	Control	10.8 ± 0.62	18	10.7 ± 0.56	18	10.6 ± 0.49	17	10.2 ± 0.42	14
	Naf.	10.9 ± 0.27	18	10.6 ± 0.52	18	9.7 ± 0.39	17	10.9 ± 0.79	15
T.G.S. mmol/l	Control	1.30 ± 0.11	18	1.17 ± 0.10	17			0.97 ± 0.06	15*
	Naf.	1.16 ± 0.07	18	1.27 ± 0.09	18			1.14 ± 0.09	14

Naf. = Naftidrofuryl group L/P = Lactate to pyruvate ratio T.G.S. = Total gluconeogenic substrates
Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.005$ *** $p<0.001$
Significance vs control: a $p<0.05$ aa $p<0.025$

FIGURE:11-1 BLOOD ANALYTE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

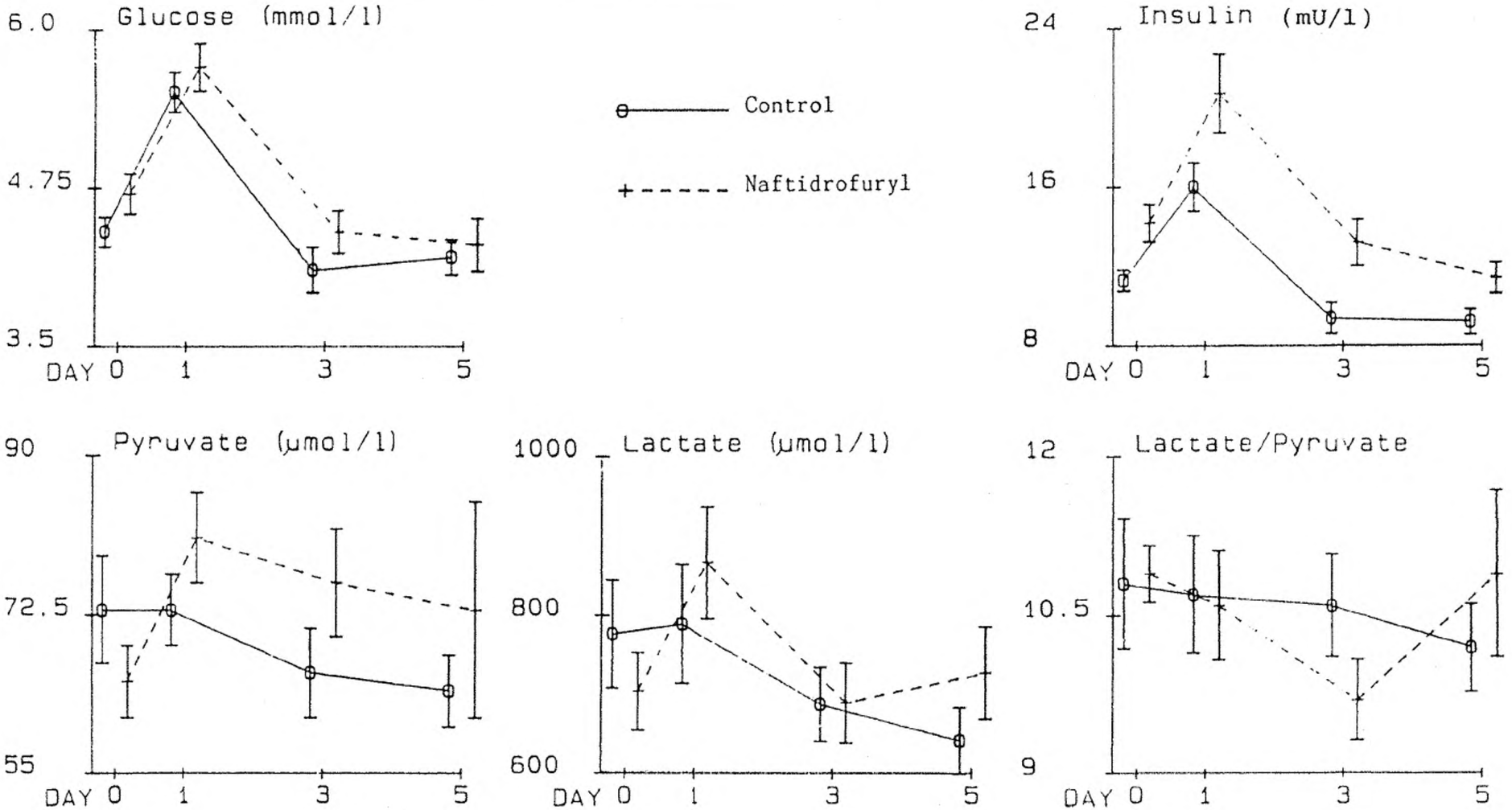


TABLE:11-2 LIPID METABOLITE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
T.K.$ µmol/l	Control	97 (82 - 115)	18	352 (290 - 428)	18**	1238 (1069 - 1435)	18***	1437 (1239 - 1667)	15***
	Naf.	103 (86 - 123)	18	279 (231 - 337)	18***	998 (850 - 1172)	17***	1130 (954 - 1338)	15**
T.T.G. mmol/l	Control	1.72 ± 0.11	18	0.83 ± 0.05	18***	1.47 ± 0.08	18*	1.68 ± 0.10	15
	Naf.	1.48 ± 0.16	18	0.83 ± 0.07	18***	1.37 ± 0.09	18	1.74 ± 0.06	15
F.F.A. µmol/l	Control	710 ± 56	17	860 ± 56	17**	838 ± 43	17*	859 ± 69	15*
	Naf.	685 ± 78	17	800 ± 60	17*	862 ± 71	17*	955 ± 101	15*
Glycerol µmol/l	Control	81 ± 6	18	86 ± 6	18	91 ± 9	18	80 ± 10	15
	Naf.	75 ± 8	18	89 ± 5	18	99 ± 14	18	101 ± 10	15*

Naf. = Naftidrofuryl group

T.K. = Total ketones T.T.G. = Total triglycerides F.F.A. = Free fatty acids

$ = figures derived from logarithmic transformation (geometric mean and range of 1 sem about the meam)

Significance vs pre-operative concentrations (paired data): * p<0.05 ** p<0.01 *** p<0.001

FIGURE:11-2 LIPID METABOLITE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

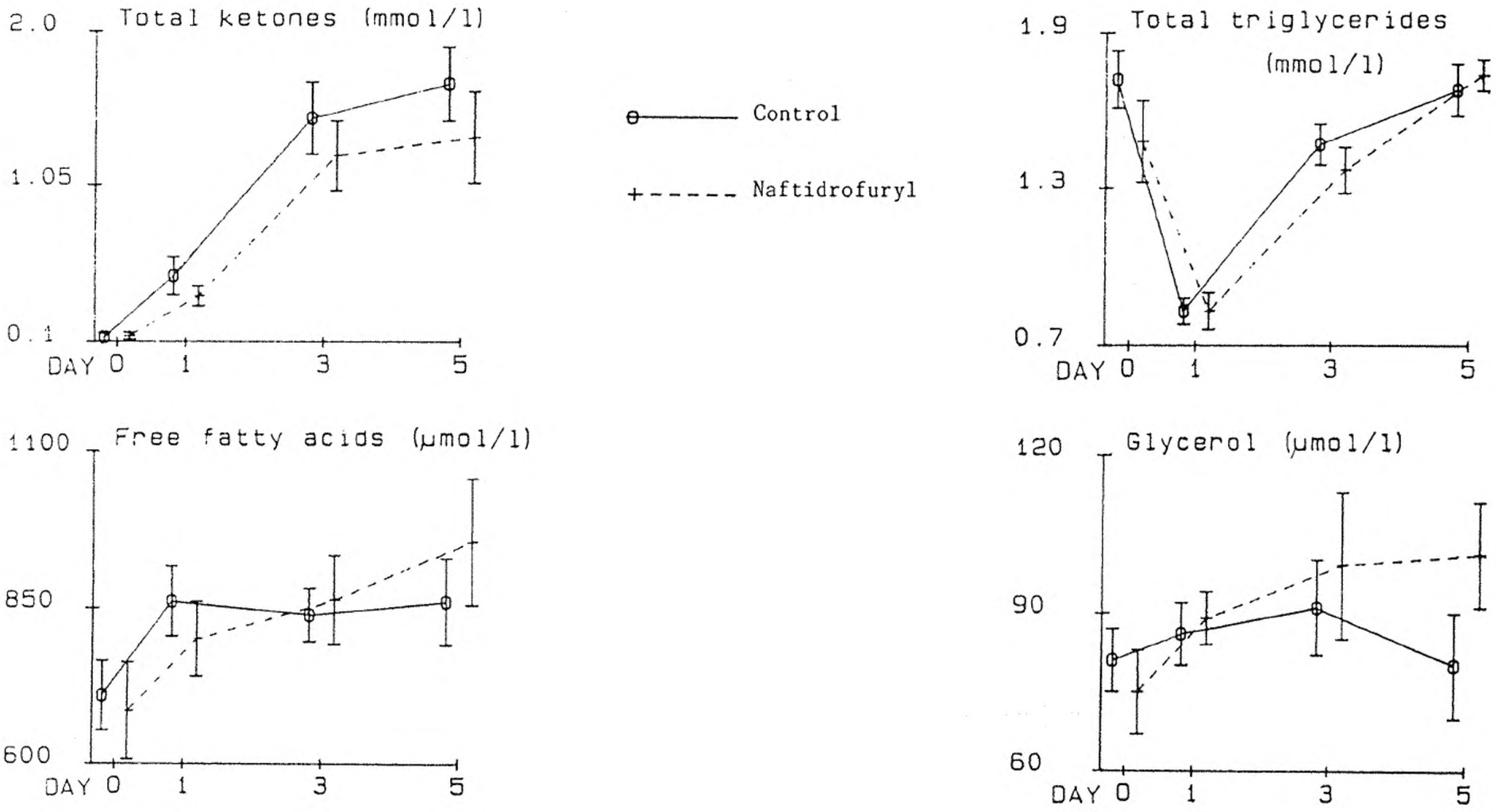


TABLE:11-3 HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Haemoglobin g/dl	Control	14.6 ± 0.24	18	13.5 ± 0.23	18***	12.5 ± 0.29	18***	12.3 ± 0.36	15***
	Naf.	14.0 ± 0.29	18	13.6 ± 0.29	18*	12.5 ± 0.31	18***	12.5 ± 0.34	15***
P.C.V. l/l	Control	0.433 ± 0.007	18	0.411 ± 0.010	18**	0.368 ± 0.011	16***	0.366 ± 0.013	15***
	Naf.	0.424 ± 0.007	18	0.409 ± 0.008	18*	0.384 ± 0.009	16***	0.380 ± 0.008	15***
Albumin$ g/l	Control	42.2 ± 0.78	18	38.7 ± 0.76	18***	39.4 ± 0.68	16***	40.8 ± 0.83	15*
	Naf.	43.0 ± 0.55	18	39.9 ± 0.77	18***	40.3 ± 0.65	16***	41.2 ± 0.94	15*
Total protein$ g/l	Control	69.0 ± 1.14	18	62.8 ± 1.28	18***	66.9 ± 1.38	16*	67.8 ± 1.58	15
	Naf.	70.8 ± 1.04	18	65.8 ± 1.37	18***	68.5 ± 1.12	16*	69.0 ± 1.66	15

Naf. = Naftidrofuryl group P.C.V. = Packed cell volume ratio

$ = Concentrations corrected for changes in packed cell volume

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.005$ ** $p<0.001$

FIGURE:11-3

HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

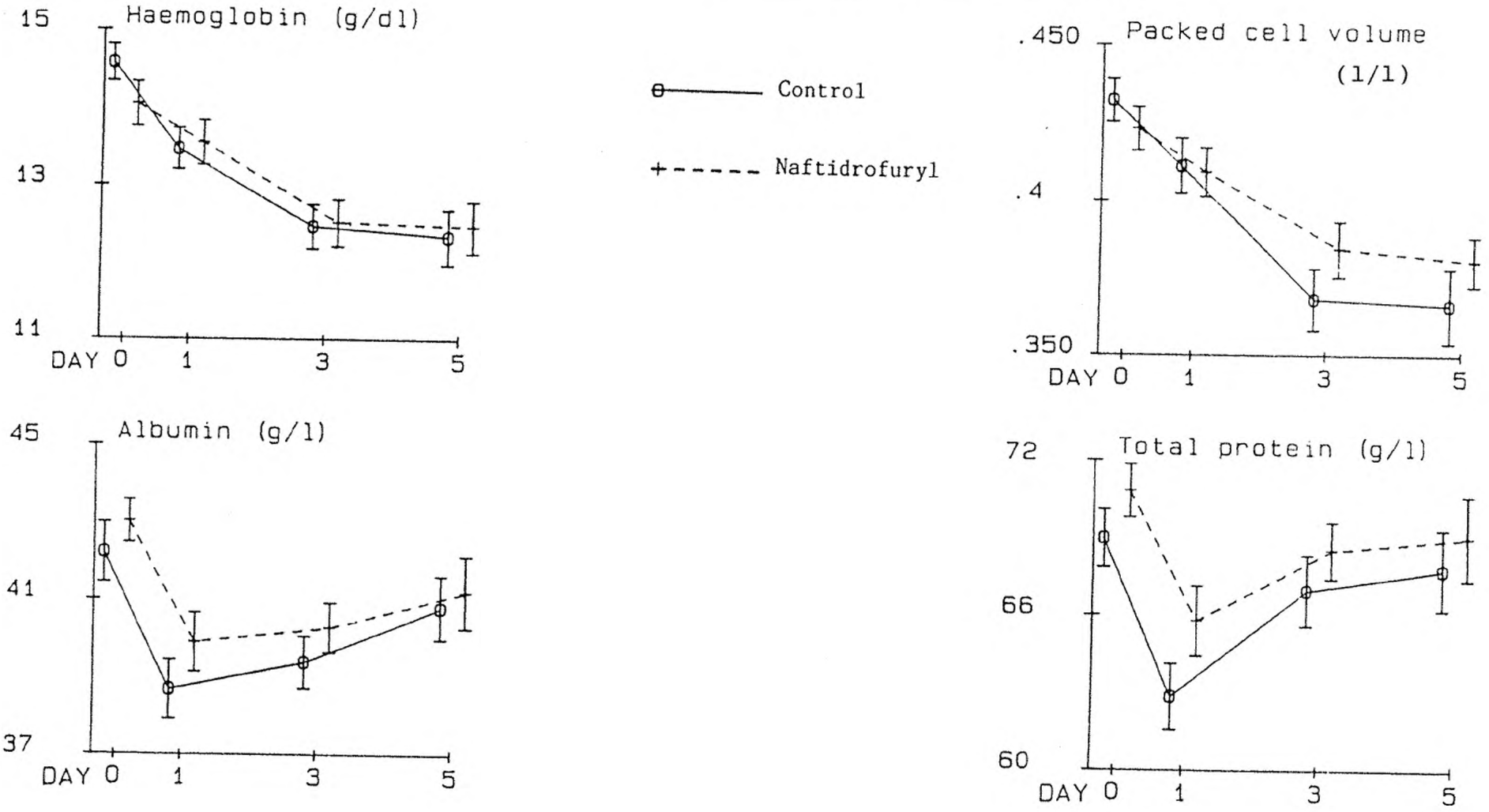


TABLE:11-4 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Creatinine µmol/l	Control	85.9 ± 3.96	18	82.3 ± 4.59	18	77.7 ± 2.89	18*	75.9 ± 4.40	15*
	Naf.	86.0 ± 3.47	18	88.1 ± 3.46	18	75.6 ± 2.78	18**	78.0 ± 3.58	15*
Urea mmol/l	Control	4.9 ± 0.28	18	6.1 ± 0.38	18***	6.3 ± 0.33	18***	6.2 ± 0.47	15**
	Naf.	5.1 ± 0.31	18	7.0 ± 0.52	18***	6.2 ± 0.31	18**	6.2 ± 0.47	15*
Bicarbonate mmol/l	Control	29.6 ± 0.58	18	28.5 ± 0.73	18	28.2 ± 0.83	17	26.8 ± 0.78	15**
	Naf.	29.9 ± 0.50	18	29.1 ± 0.63	18	29.0 ± 0.58	18	27.9 ± 0.85	15**
Sodium mmol/l	Control	141 ± 0.63	18	138 ± 0.58	18***	138 ± 0.57	18***	138 ± 0.70	15*
	Naf.	141 ± 0.60	18	139 ± 0.44	18***	138 ± 0.64	18***	139 ± 0.81	15*
Potassium mmol/l	Control	4.3 ± 0.07	18	4.4 ± 0.11	18	4.3 ± 0.10	18	4.5 ± 0.12	15
	Naf.	4.1 ± 0.09	18	4.2 ± 0.07	18	4.1 ± 0.07	18	4.4 ± 0.12	15

Naf. = Naftidrofuryl group

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:11-4 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE ISOTONIC AMINO ACID GROUP

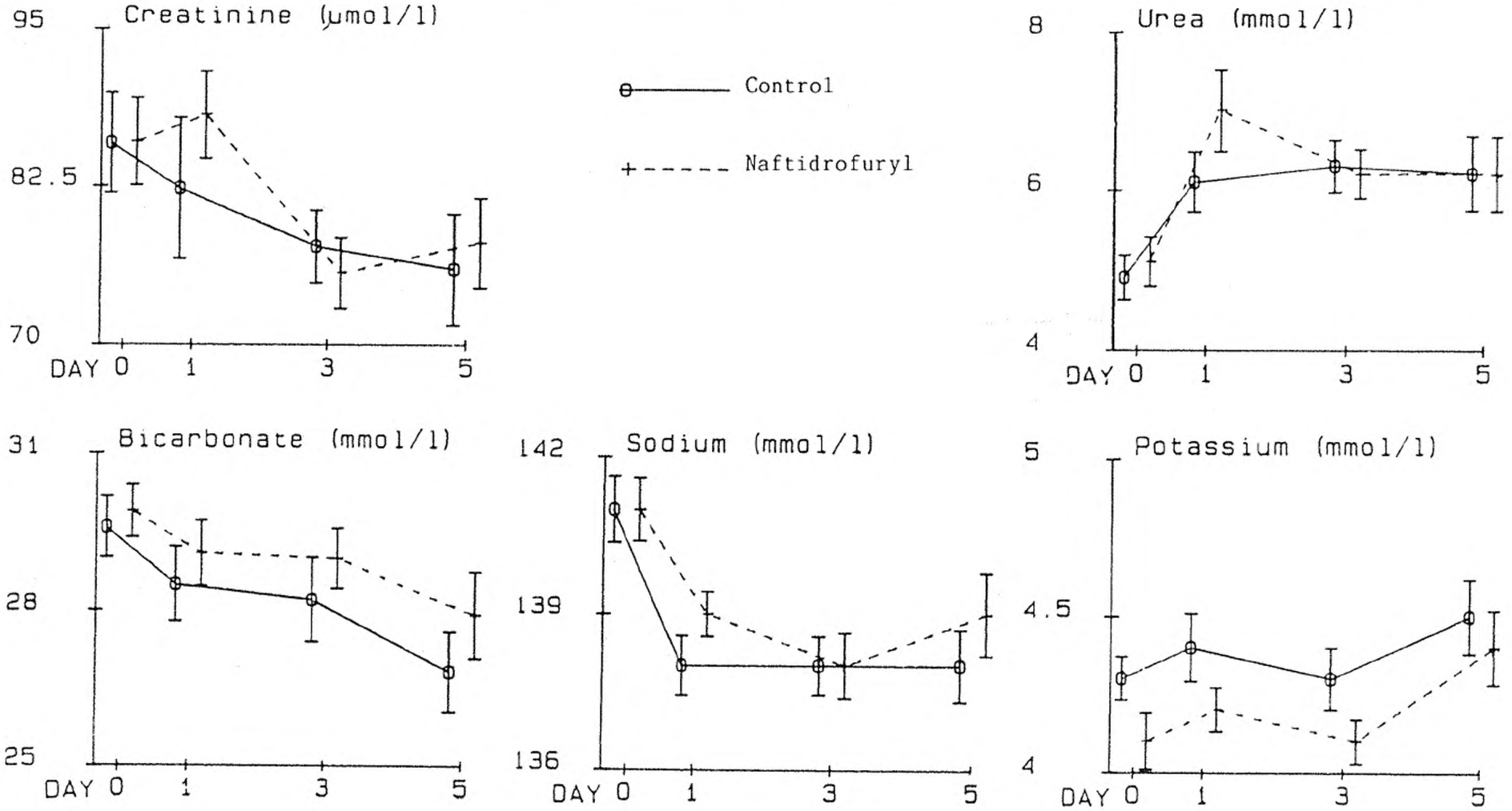


TABLE:11-5 BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (μmol/l)

IN THE ISOTONIC AMINO ACID GROUP

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Valine	Control	222 ± 7.6	18	225 ± 10.3	17	390 ± 15.6	15**
	Naf.	219 ± 6.2	18	233 ± 7.4	18	386 ± 13.0	14**
Leucine	Control	135 ± 5.5	18	141 ± 6.3	17	257 ± 11.7	15**
	Naf.	132 ± 3.8	18	150 ± 5.1	18*	261 ± 8.4	14**
Isoleucine	Control	66 ± 2.9	18	58 ± 2.7	17	146 ± 6.6	15**
	Naf.	64 ± 2.6	18	65 ± 3.5	18	153 ± 6.2	14**
Total BCAA	Control	423 ± 15.3	18	424 ± 18.4	17	793 ± 33.0	15**
	Naf.	415 ± 11.8	18	448 ± 15.2	18	806 ± 25.3	14**

Naf. = Naftidrofuryl group

BCAA = Branched-chain amino acids

Significance vs pre-operative values (paired data): * $p<0.005$ ** $p<0.001$

FIGURE:11-5 BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (µmol/l) IN THE ISOTONIC AMINO ACID GROUP

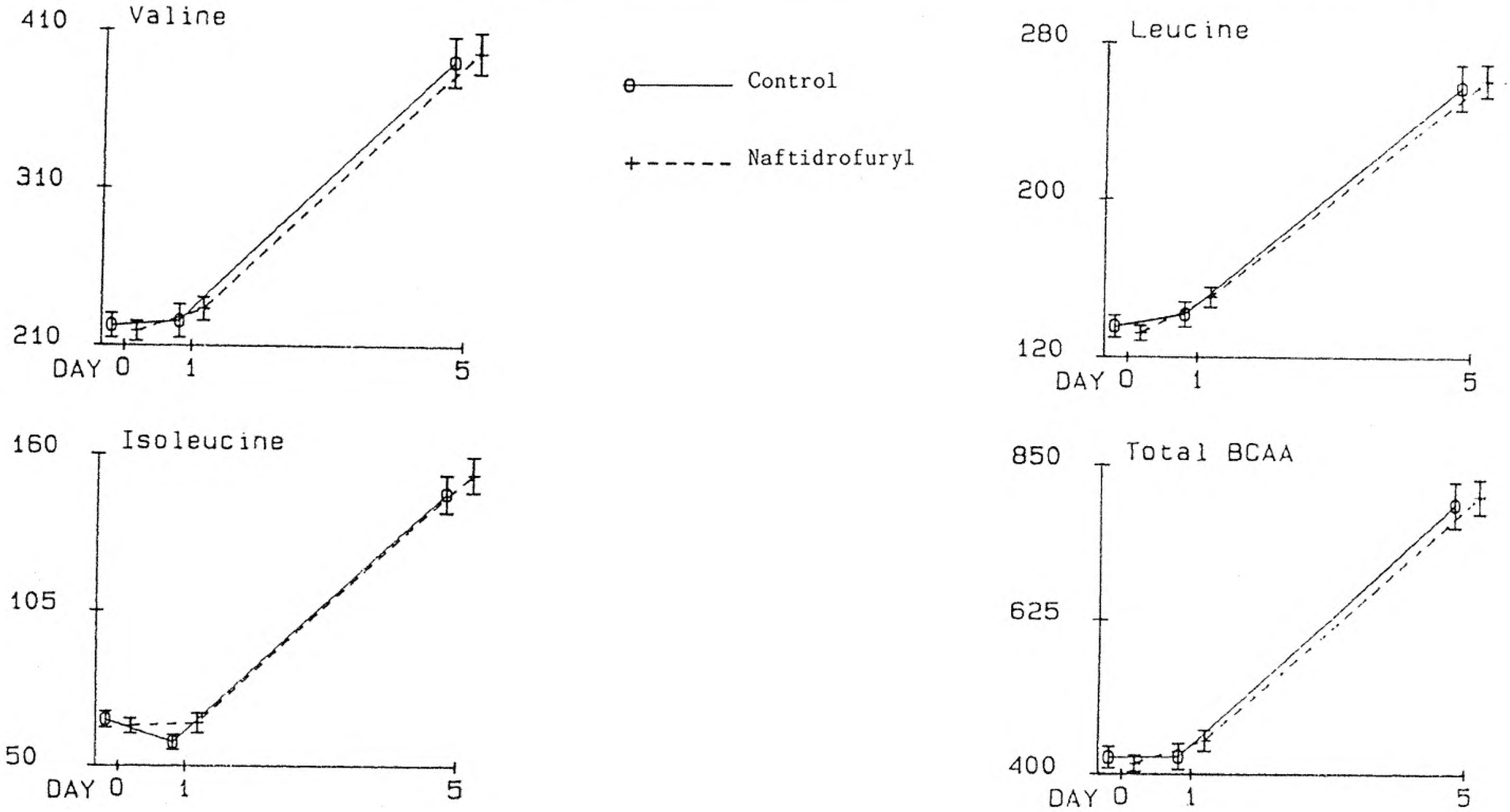


TABLE:11-6 CONCENTRATIONS OF ESSENTIAL AMINO ACIDS (μmol/l) IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
lysine	Control	187 ± 5.9	18	125 ± 5.4	17***	160 ± 9.2	15***
	Naf.	186 ± 6.8	18	129 ± 6.1	18***	168 ± 9.2	14*
Threonine	Control	128 ± 9.3	18	74 ± 3.2	17***	81 ± 5.4	15***
	Naf.	121 ± 7.0	18	76 ± 3.7	18***	90 ± 4.6	14*
Methionine	Control	29 ± 1.5	18	26 ± 1.6	17*	32 ± 2.0	15*
	Naf.	31 ± 1.5	18	31 ± 1.6	18	34 ± 1.7	14*
Tryptophan	Control	15 ± 1.9	18	15 ± 1.8	17	14 ± 2.2	15
	Naf.	11 ± 1.1	18	11 ± 1.2	18	13 ± 1.8	14
Phenylalanine	Control	64 ± 2.3	18	77 ± 2.7	17***	71 ± 2.9	15**
	Naf.	62 ± 1.9	18	79 ± 2.9	18***	77 ± 4.4	14*
Valine	Control	222 ± 7.6	18	225 ± 10.3	17	390 ± 15.6	15***
	Naf.	219 ± 6.2	18	233 ± 7.4	18	386 ± 13.0	14***
Leucine	Control	135 ± 5.5	18	141 ± 6.3	17	257 ± 11.7	15***
	Naf.	132 ± 3.8	18	150 ± 5.1	18**	261 ± 8.4	14***
Isoleucine	Control	66 ± 2.9	18	58 ± 2.7	17	146 ± 6.6	15***
	Naf.	64 ± 2.6	18	65 ± 3.5	18	153 ± 6.2	14***
Total EAA	Control	846 ± 24.3	18	741 ± 23.7	17***	1153 ± 45.9	15***
	Naf.	825 ± 21.2	18	774 ± 25.6	18*	1189 ± 33.9	14***

Naf. = Naftidrofuryl group

EAA = Essential amino acids

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.005$ *** $p<0.001$

FIGURE:11-6 ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE ISOTONIC AMINO ACID GROUP

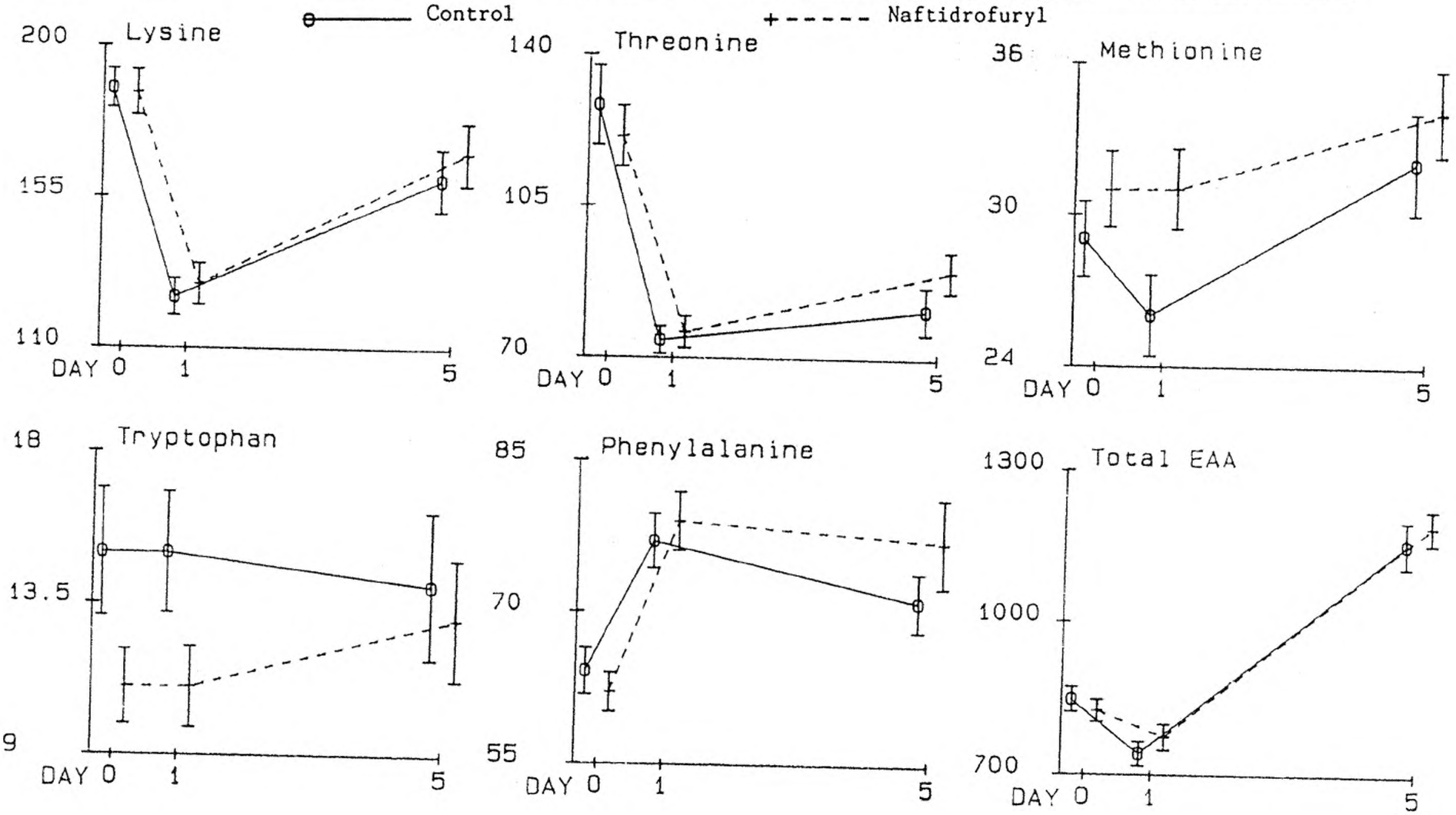


TABLE:11-7A

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (µmol/l) IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Alanine	Control	342 ± 21.8	18	249 ± 20.1	17***	176 ± 15.2	15***
	Naf.	322 ± 19.9	18	258 ± 17.5	18*	a224 ± 13.4	14**
Taurine	Control	48 ± 2.4	18	49 ± 2.1	17	36 ± 3.3	15***
	Naf.	48 ± 2.5	18	48 ± 2.9	18	37 ± 3.1	14***
Histidine	Control	86 ± 2.4	18	66 ± 3.0	17***	65 ± 2.3	15***
	Naf.	82 ± 2.9	18	63 ± 2.8	18***	64 ± 2.8	14***
Citrulline	Control	47 ± 2.2	18	24 ± 1.0	17***	22 ± 1.0	15***
	Naf.	44 ± 2.6	18	24 ± 1.8	18***	24 ± 1.8	14***
Arginine	Control	102 ± 4.7	18	57 ± 3.1	17***	73 ± 5.0	15**
	Naf.	100 ± 4.7	18	60 ± 3.9	18***	80 ± 4.2	14*
Ornithine	Control	60 ± 2.9	18	40 ± 2.2	17***	51 ± 4.2	15
	Naf.	55 ± 2.3	18	39 ± 1.8	18***	54 ± 3.6	14
Proline	Control	186 ± 9.9	18	183 ± 19.4	17	220 ± 28.5	15*
	Naf.	175 ± 11.3	18	168 ± 12.1	18	223 ± 22.2	14*
Glutamine	Control	623 ± 20.0	18	494 ± 23.5	17***	505 ± 24.1	15***
	Naf.	626 ± 17.8	18	485 ± 18.8	18***	504 ± 19.4	14***

Naf. = Naftidrofuryl group

Significance vs Day 0 (paired data): * p<0.05 * p<0.01 *** p<0.001

Significance vs Control: a p<0.05

TABLE:11-7B

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (μmol/l) IN THE ISOTONIC AMINO ACID GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Glutamate	Control	34 ± 4.6	18	24 ± 3.1	16*	25 ± 2.2	15*
	Naf.	27 ± 2.6	18	21 ± 2.6	18*	20 ± 2.0	14**
Glycine	Control	226 ± 10.9	18	182 ± 7.8	17***	187 ± 13.4	15***
	Naf.	218 ± 12.9	18	177 ± 8.7	18***	220 ± 22.5	14
Tyrosine	Control	61 ± 3.0	18	55 ± 3.5	17*	59 ± 3.0	15
	Naf.	61 ± 2.8	18	56 ± 3.0	18*	64 ± 4.4	14
Aspartate	Control	2.3 ± 0.15	18	1.7 ± 0.18	17*	1.7 ± 0.20	15*
	Naf.	2.1 ± 0.11	18	1.7 ± 0.17	18*	1.5 ± 0.11	14*
Asparagine	Control	52 ± 3.3	18	31 ± 2.6	17***	42 ± 2.4	15
	Naf.	47 ± 2.4	18	35 ± 2.1	18***	43 ± 2.2	14
AAB	Control	25 ± 2.4	18	34 ± 1.7	17**	70 ± 6.2	15***
	Naf.	25 ± 1.3	18	33 ± 1.9	18***	71 ± 4.7	14***
Cystine	Control	60 ± 2.8	18	42 ± 2.1	17***	56 ± 3.4	15
	Naf.	59 ± 2.8	18	45 ± 1.8	18***	59 ± 2.7	14
Serine	Control	99 ± 4.0	18	69 ± 3.7	17***	89 ± 4.3	15*
	Naf.	108 ± 6.7	18	73 ± 4.1	18***	102 ± 5.3	14
Total NEAA	Control	2054 ± 50.3	18	1591 ± 61.8	16***	1675 ± 78.9	15***
	Naf.	1998 ± 63	18	1588 ± 58.6	18***	1792 ± 76.4	14***

Naf. = Naftidrofuryl group

NEAA = Non-essential amino acids

AAB = Alpha-aminobutyrate

Significance vs Day 0 (paired data): * $p<0.05$ * $p<0.01$ *** $p<0.001$

FIGURE:11-7A NON-ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE ISOTONIC AMINO ACID GROUP

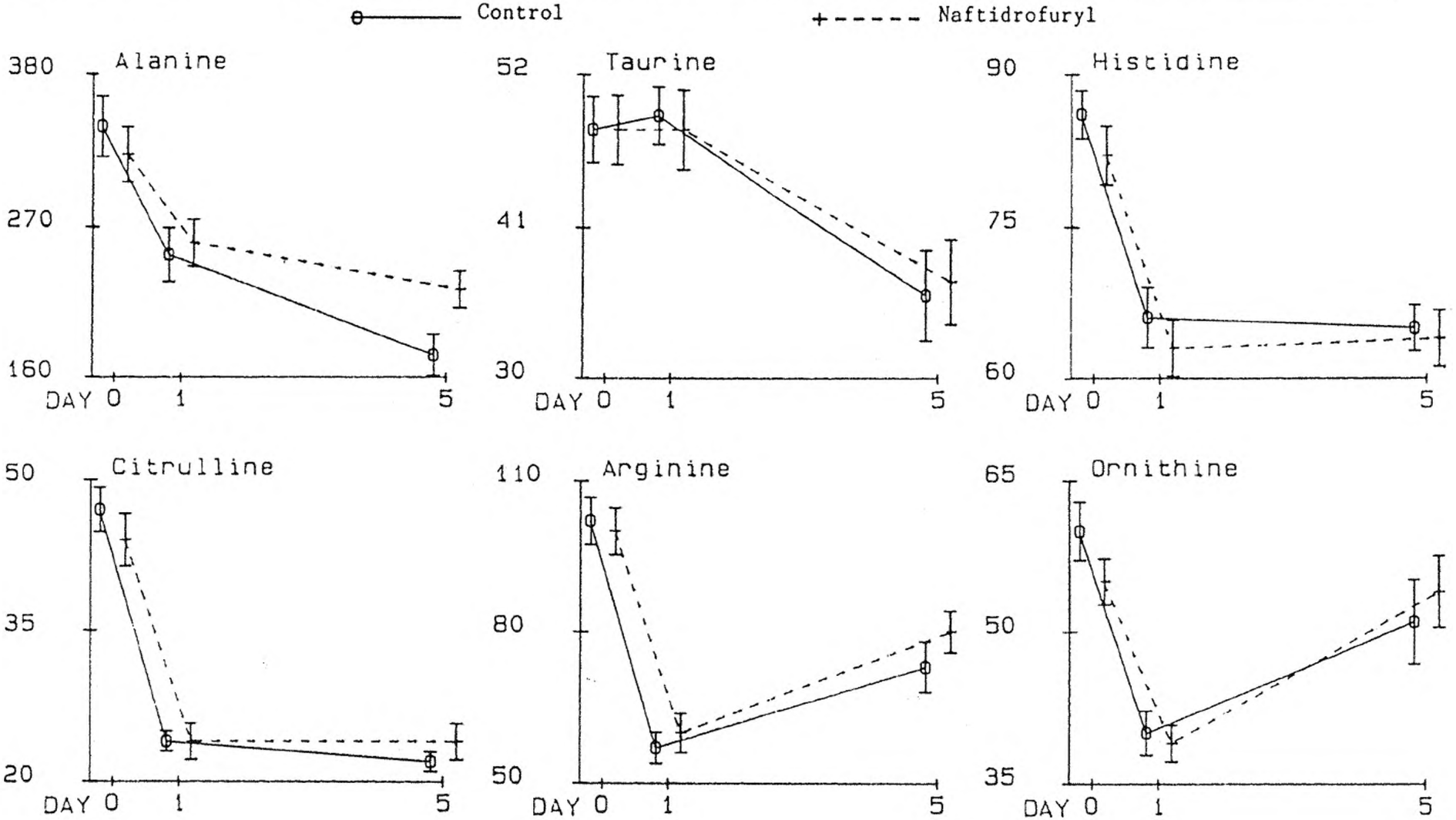


FIGURE:11-7B NON-ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/1) IN THE ISOTONIC AMINO ACID GROUP

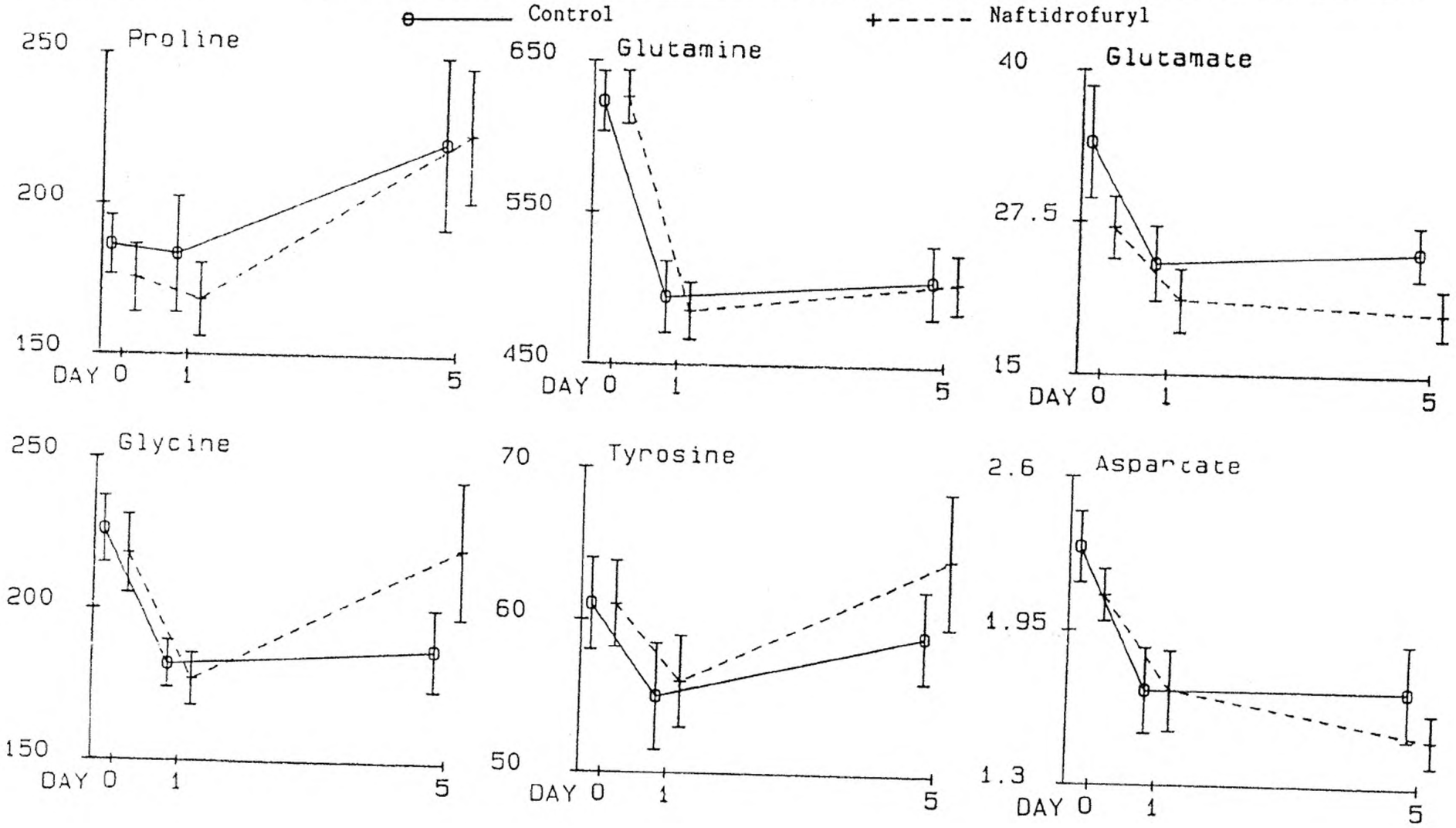


FIGURE:11-7C NON-ESEENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE ISOTONIC AMINO ACID GROUP

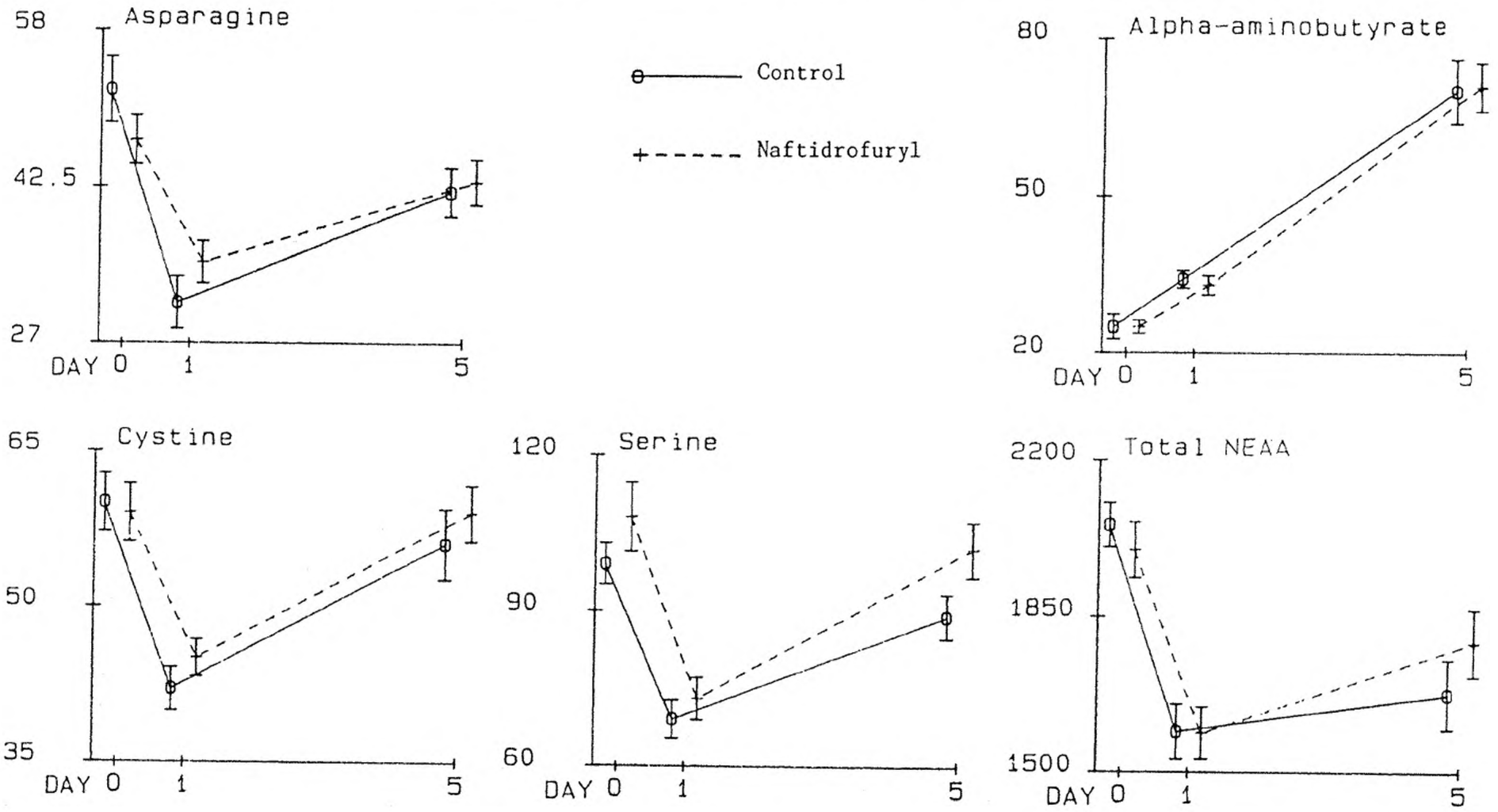


TABLE:11-8 PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE, AND CYSTINE/TAURINE RATIOS IN THE ISOTONIC AMINO ACID GROUP

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Phe/Tyr	Control	1.07 ± 0.04	18	1.44 ± 0.06	17***	1.21 ± 0.04	15*
	Naf.	1.05 ± 0.04	18	1.42 ± 0.06	18***	1.24 ± 0.07	14**
Met/CySS	Control	0.49 ± 0.02	18	0.62 ± 0.03	17***	0.61 ± 0.03	15***
	Naf.	0.53 ± 0.02	18	0.69 ± 0.04	18***	0.63 ± 0.04	14***
Met/Tau	Control	0.63 ± 0.05	18	0.54 ± 0.04	17	0.98 ± 0.08	15***
	Naf.	0.68 ± 0.05	18	0.68 ± 0.06	18	1.08 ± 0.09	14***
CySS/Tau	Control	1.31 ± 0.10	18	0.88 ± 0.06	17***	1.63 ± 0.11	15***
	Naf.	1.29 ± 0.08	18	0.99 ± 0.06	18***	1.72 ± 0.11	14***

Naf. = Naftidrofuryl group

Phe/Tyr = Phenylalanine to tyrosine ratio Met/CySS = Methionine to cystine ratio

Met/Tau = Methionine to taurine ratio CySS/Tau = Cystine to taurine ratio

Significance vs Day 0 (paired data): $p<0.02$ ** $p<0.01$ *** $p<0.001$

FIGURE:11-8

PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE AND CYSTINE/TAURINE RATIOS IN THE ISOTONIC AMINO ACID GROUP

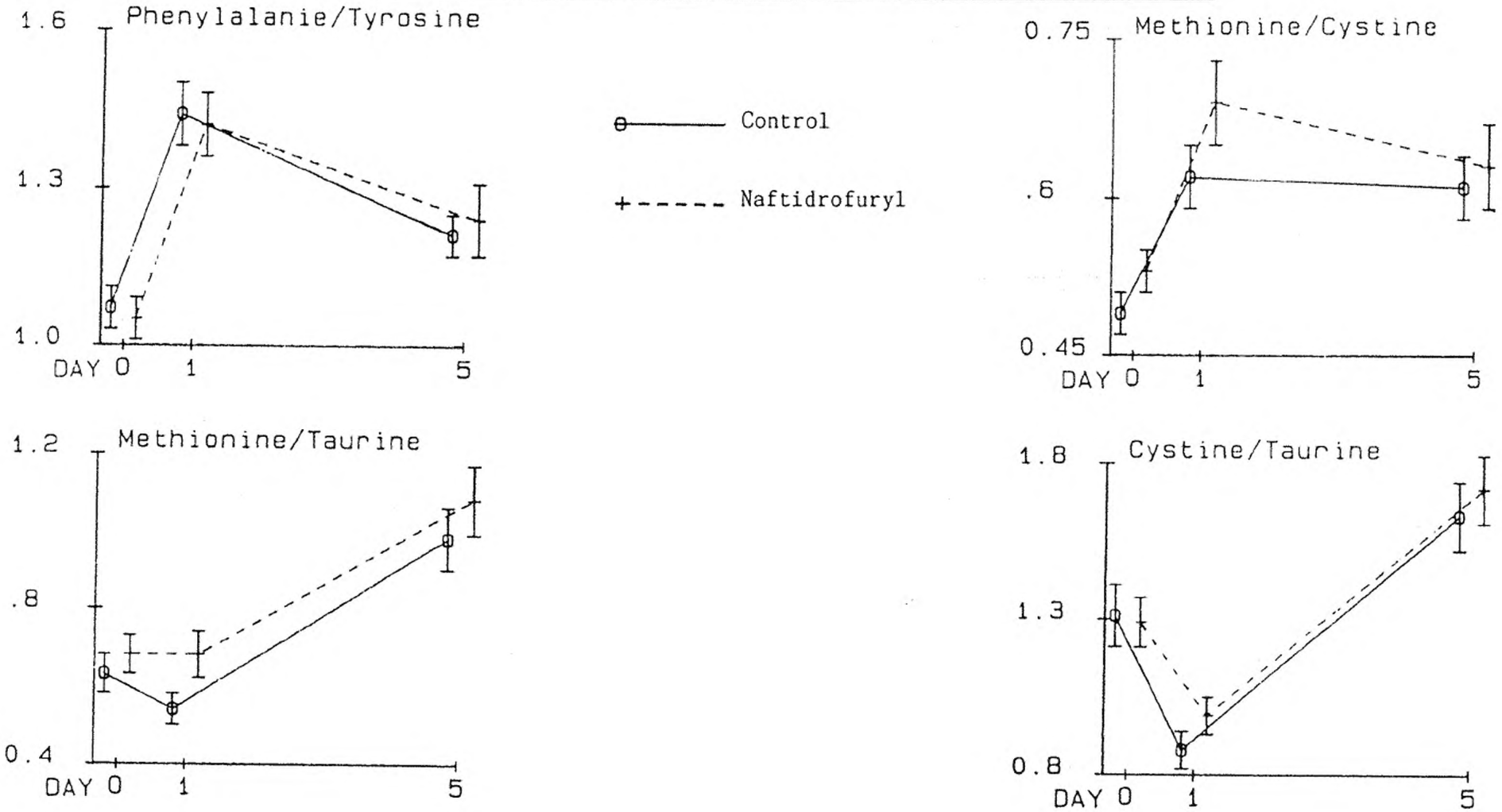


TABLE:11-9 LINEAR CORRELATIONS BETWEEN TOTAL KETONE AND BRANCHED-CHAIN AMINO ACID CONCENTRATIONS AND THE MAGNITUDE OF CHANGE BETWEEN DAYS 0 & 1 OF THESE SUBSTRATES AND THE 6-DAY NITROGEN BALANCE

	Group	r	n	p	
[Day 1 TK] µmol/l	Control	0.4195	17	p>0.05	ns
vs	Naf.	0.2946	18	p>0.1	ns
[Day 1 BCAA] µmol/l	All patients	0.3357	35	p>0.05	ns
[Day 1 - Day 0 TK] µmol/l	Control	0.2668	17	p>0.1	ns
vs	Naf.	0.2297	18	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	All patients	0.0911	35	p>0.1	ns
[Day 1 TK] µmol/l	Control	0.2425	15	p>0.1	ns
vs	Naf.	0.1814	15	p>0.1	ns
6-Day nitrogen balance mg kg^{-1}	All patients	0.0835	30	p>0.1	ns
[Day 1 BCAA] µmol/l	Control	0.3357	15	p>0.1	ns
vs	Naf.	0.3818	15	p>0.1	ns
6-Day nitrogen balance mg kg^{-1}	All patients	0.3601	30	p>0.05	ns
[Day 1 - Day 0 TK] umol/l	Control	0.2793	15	p>0.1	ns
vs	Naf.	0.3361	15	p>0.1	ns
6-Day nitrogen balance mg kg^{-1}	All patients	0.2448	30	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	Control	0.2676	15	p>0.1	ns
vs	Naf.	0.0425	15	p>0.1	ns
6-Day nitrogen balance mg kg^{-1}	All patients	0.1349	30	p>0.1	ns

NAf. = Naftidrofuryl group

TK = Total Ketones

[ ] = Concentration

p = Level of significance

BCAA = Branched-chain amino acids

n = number of patients

r = Coefficient of correlation

ns = not significant

CHAPTER 12

EFFECT OF NAFTIDROFURYL WITH GLUCOSE, FAT AND AMINO ACID INFUSION ON THE METABOLIC RESPONSE TO SURGERY

PATIENTS AND METHODS

The patients and methods are those described in section II (chapters 7 & 8). 35 patients were randomised to receive a twice daily infusion of either placebo (control group = 17 patients) or 200 mg naftidrofuryl oxalate (naftidrofuryl group = 18 patients). All patients also received a central venous infusion of glucose, amino acids and fat which delivered 0.15 g nitrogen and 24 non-protein kcal (50% glucose and 50% fat) kg^{-1} day^{-1}. The mean weight and lean body mass were heavier in the control than in the naftidrofuryl group ($p<0.05$). Otherwise, the groups were matched for age, sex, height, mid-arm muscle circumference and the type of surgical procedure (see chapter 8, table: 8-5).

RESULTS

Urine (see chapter 9, table:9-3)

The cumulative 3 and 6-day urinary volume, urea and creatinine excretions, and potassium and nitrogen balances showed no significant between group differences.

Glucose, insulin and total gluconeogenic substrates: (table:12-1 & figure:12-1)

Peak blood glucose and insulin concentrations occurred on Day 1 in both groups and fell thereafter, Day 5 concentrations being higher than the starting Day 0 values with no between group differences.

In the control group, peak blood pyruvate and lactate concentrations occurred on Day 1 and then fell, Day 5 concentrations remaining higher than

pre-operatively. However, there were no significant changes in the naftidrofuryl group, throughout the study period, in either lactate or pyruvate concentrations. Moreover, on Day 1, lactate concentration was significantly lower in the naftidrofuryl group when compared to the control group ($p<0.05$).

The lactate to pyruvate ratio (L/P) fell post-operatively in the naftidrofuryl group (Day 1 = Day 3 = Day 5 < Day 0), whereas there were no significant changes in the control group. On Day 1 this ratio was significantly lower in the naftidrofuryl group when compared to the control group.

Total gluconeogenic substrates (Lactate, pyruvate, alanine and glycerol) concentrations rose post-operatively in the control group (Day 1, $p<0.05$; Day 5, $p>0.05$) and showed no changes in the naftidrofuryl group.

Lipid metabolites (table:12-2 & figure:12-2)

Total ketone concentrations showed no significant changes between consecutive days in both groups, although Days 3 and 5 were lower than Day 0 in the control group.

Trough total triglyceride concentrations occurred on Day 1 and rose thereafter, with no between group differences, Days 3 and 5 remaining lower than pre-operatively.

Free fatty acids fell after Day 1, in both groups, so that Days 3 and 5 were lower than Days 0 and 1.

Glycerol concentrations showed no significant changes between consecutive days, although Day 5 concentrations were lower than pre-operatively in both groups.

Haemoglobin, packed cell volume, albumin and total protein (table:12-3 & figure:12-3)

Haemoglobin and packed cell volume ratio fell post-operatively with no between group differences.

Albumin concentration fell on Day 1 and showed no significant change thereafter (Day 1 = Day 3 = Day 5 < Day

0, p<0.001). Total protein concentrations fell on Day 1 (Day 1< Day 0, p<0.001) and rose thereafter, Day 5 concentrations remaining lower than pre-operatively (Day 5 < Day 0, p<0.05). There were no between group differences in either albumin or total protein concentrations.

Creatinine, urea and electrolytes

(table:12-4 & figure:12-4)

There were no between group differences in any of these measured variables.

Serum creatinine and bicarbonate concentrations were lower on all post-operative days than pre-operatively.

Peak serum urea concentration occurred on Day 1 in both groups and then returned to pre-operative values.

Serum sodium and potassium concentrations fell post-operatively but by Day 5 the concentrations had returned to pre-operative values.

Venous plasma free amino acids

The mean concentration of individual plasma amino acids showed considerable fluctuations within each group. The only between group difference was found in alanine.

Branched-chain amino acids (BCAA) (table:12-5 & figure:12-5)

Valine, leucine and isoleucine concentrations fell on Day 1 and rose thereafter, Day 5 concentrations being higher than pre-operatively in both groups. Total BCAA followed the same pattern with no between group differences.

Essential amino acids (EAA) (table:12-6 & figure:12-6)

The naftidrofuryl and control groups showed similar changes throughout the study period. Trough lysine and threonine concentrations occurred on Day 1 and rose thereafter, returning to pre-operative values. Day 5 methionine concentrations were higher than pre-operatively (Day 0 = Day 1 < Day 5, p<0.05). Phenylalanine and

tryptophan concentrations rose on Day 1 and showed no significant changes thereafter. Trough essential amino acid concentrations occurred on Day 1 and rose thereafter, Day 5 concentrations being higher than pre-operatively.

Non-essential amino acids (NEAA) (tables:12-7A & 12-7B and figures:12-7A, 12-7B & 12-7C)

On Day 1, individual non-essential amino acid concentrations fell with the exception of aspartate and proline which showed no significant change and alpha-aminobutyrate which increased. Thereafter, the concentrations rose with the exception of taurine which fell and histidine, citrulline and aspartate which showed no further changes. On Day 5, the individual amino acid concentrations were lower than pre-operatively with the exception of ornithine, proline, and alpha-aminobutyrate which were higher than pre-operatively and arginine, glutamate, tyrosine, asparagine, aspartate, cystine and serine which were not different from Day 0.

Taurine concentrations fell on Day 1 but only significantly so in the naftidrofuryl group. However, there were no between group differences post-operatively and the magnitude and direction of change, between Days 0 and 1, were similar in the control and naftidrofuryl groups.

Day 0 asparagine concentration was lower in the naftidrofuryl group when compared to the control group. However, no differences were detected, for the same variable, in the post-operative period and the magnitude and direction of change were similar in both groups throughout the study period.

A between group difference was found only in alanine. Day 1 alanine concentration was lower in the naftidrofuryl group when compared to the control group and the Day 5 alanine concentration was lower than pre-operatively but only significantly so in the naftidrofuryl group ($p<0.05$). However, the magnitude of decrease in alanine concentration between Days 0 and 1 and days 0 and 5 showed no between group difference.

Total NEAA showed similar changes in both groups. The concentrations fell on Day 1 and rose thereafter, Day 5 concentrations being not different from pre-operatively.

DISCUSSION

the weight and lean body mass were heavier in the control group when compared to the naftidrofuryl group (table: 8-5 , $p<0.05$). This was almost certainly due to a bias within the random selection and should not affect the interpretation of the data as the results are expressed per kg body weight.

Table: 9-3 shows that a twice daily intravenous infusion of 200 mg naftidrofuryl does not result in any measurable nitrogen sparing effect in patients who have undergone elective abdominal surgery and received a central venous infusion which delivered 0.15 g nitrogen kg^{-1} day^{-1} and 24 non-protein kcal (50% fat, 50% glucose) kg^{-1} day^{-1}. Furthermore, there were no differences in albumin and total protein concentrations between the control and treatment groups. The only significant between group differences were found in total ketones, alanine, lactate and pyruvate concentrations and L/P ratio.

There was no apparent reason for the between group difference in asparagine concentration on Day 0, and it may be due to an artefact of selection. Furthermore, it should be noted that the Day 0 asparagine concentrations, in the control and naftidrofuryl groups, were within the Day 0 laboratory reference range (see appendix 1). Therefore, between group differences were tested for the magnitude of change between days and no differences were detected.

Ketogenesis is usually suppressed in the fed state. The patients studied in this chapter received 24 non-protein kcal kg^{-1} day^{-1} (50% fat, 50% glucose) and had raised insulin concentration on all post-operative days. These conditions do not favour tissue lipolysis and acetyl

coenzyme A would be preferentially utilised in the tricarboxylic acid cycle, by combination with the readily available oxaloacetate, as opposed to yielding ketone bodies. This explains the suppressed ketogenesis reported in this chapter. The observed between group differences in total ketone concentrations may be regarded as not significant since the concentrations are low and the direction and magnitude of post-operative changes are similar in the control and naftidrofuryl groups.

There were no significant correlations between Day 1 total ketones and Day 1 BCAA concentrations, nor between the cumulative 6-day nitrogen balance and Day 1 total ketones concentrations, Day 1 BCAA concentrations or the magnitude of change in these variables between Days 0 and 1 (table: 11-9). This is in agreement with the findings in the previous two chapters. However, there were no significant correlations between the post-operative log [total ketones] and lactate concentrations in the control and naftidrofuryl groups. This is not in agreement with the negative linear correlations reported in the previous 2 chapters and is probably due to the suppressed ketogenesis in this infusion group which received a large dose of glucose.

After the initial fall on Day 1, alanine concentration rose in response to the glucose-containing feeding regimen (Jeejeebhoy et al., 1976; Wolfe et al., 1977; O'Connell et al., 1974; Waterhouse and Keilson, 1978). In spite of the rise between Days 1 and 5, the Day 5 alanine concentration remained lower than pre-operatively but only significantly so in the naftidrofuryl group ($p<0.05$). However, the direction and the magnitude of change between Days 0 and 1, Days 1 and 5 and Days 0 and 5 showed no significant between group differences. Therefore, no conclusion may be drawn from the observed small between group difference in alanine concentration.

The observed rise in phenylalanine to tyrosine (Phe/Tyr) and methionine to cystine (Met/CySS) ratios

(table:12-9), with Day 5 tyrosine and cystine concentrations being not different from Day 0, is probably due (as already discussed in the previous two chapters) to the increased Day 5 concentrations of phenylalanine and methionine. The significant increases in methionine to cystine (Met/CySS), methionine to taurine (Met/Tau) and cystine to taurine (CySS/Tau) ratios (table:12-9), with Day 5 taurine concentrations being lower than pre-operatively, are consistent with a probable block in the trans-sulphuration pathway. However, as discussed previously, this assumption cannot be made with any degree of certainty as tissue concentrations and urinary excretion of these substrates were not measured.

The lack of the post-operative rise in lactate and pyruvate concentrations in the naftidrofuryl group may be due to either increased utilisation or reduced production of these substrates. Injury is associated with hormonal changes which cause increased production of lactate from muscle and an acceleration of the alanine shuttle. Therefore, the lack of the post-operative rise in lactate and pyruvate concentrations is more likely to be due to increased utilisation of these substrates.

As already described in chapter 10, the observed post-operative decrease in L/P ratio in the naftidrofuryl group may be due to increased oxidative capacity of the cell. In the naftidrofuryl group, the mean concentrations of lactate and pyruvate showed no significant between day changes, throughout the study period. However, individual changes in lactate and pyruvate may bear no direct relation to changes in L/P ratio (Neill et al., 1969). Animal studies have indicated that naftidrofuryl may directly enhance tissue oxidative metabolism by activation of succinate dehydrogenase (Maynaud et al., 1973). However, this enzyme is not thought to be a rate-limiting enzyme for the tricarboxylic acid cycle (Ottaway, 1976), and the observed changes may be due to activation of other enzymes or to some as yet unknown effect of naftidrofuryl probably on other controlling points of the tricarboxylic

acid cycle. Nevertheless, any facilitatory action of naftidrofuryl in the oxidation of pyruvate within the TCA cycle would be expected to lower tissue and blood L/P ratios.

In conclusion, in spite the above described potentially beneficial biochemical changes, the twice daily infusion of 200 mg naftidrofuryl oxalate had no nitrogen-sparing effect in patients who had elective abdominal surgery and received a central venous infusion of glucose, amino acids and fat.

TABLE:12-1

BLOOD ANALYTE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Glucose mmol/l	Control	4.3 ± 0.12	17	6.2 ± 0.39	17***	5.3 ± 0.2	17***	5.5 ± 0.17	15**
	Naf.	4.5 ± 0.11	18	6.1 ± 0.35	18***	5.4 ± 0.33	18**	5.5 ± 0.48	15*
Insulin mU/l	Control	14.0 ± 1.35	17	35.8 ± 5.78	17***	22.8 ± 2.56	16***	18.5 ± 2.07	15*
	Naf.	14.3 ± 1.47	18	23.8 ± 3.25	18***	18.1 ± 1.97	18**	16.4 ± 1.86	15*
Pyruvate µmol/l	Control	60 ± 2	17	98 ± 14	17*	80 ± 5	16**	80 ± 6	15**
	Naf.	69 ± 5	18	79 ± 20	18	75 ± 7	18	80 ± 3	15
Lactate µmol/l	Control	765 ± 49	17	1170 ± 178	17*	926 ± 91	16	959 ± 57	15*
	Naf.	824 ± 71	18	a796 ± 52	18	750 ± 48	18	852 ± 60	15
L/P	Control	12.6 ± 0.70	17	11.4 ± 0.42	17	11.5 ± 0.53	16	11.3 ± 0.76	15
	Naf.	12.0 ± 0.48	18	aa10.1 ± 0.38	18**	10.4 ± 0.48	18***	10.5 ± 0.41	15**
T.G.S. mmol/l	Control	1.25 ± 0.06	17	1.63 ± 0.21	17*			1.42 ± 0.06	14
	Naf.	1.26 ± 0.09	18	1.17 ± 0.06	18			1.26 ± 0.65	15

Naf. = Naftidrofuryl group L/P = Lactate to pyruvate ratio T.G.S. = Total gluconeogenic substrates
Significance vs pre-operative concentrations (paired data): * p<0.05 ** p<0.01 *** p<0.001
Significance vs control: a p<0.05 aa p<0.01

FIGURE:12-1 BLOOD ANALYTE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

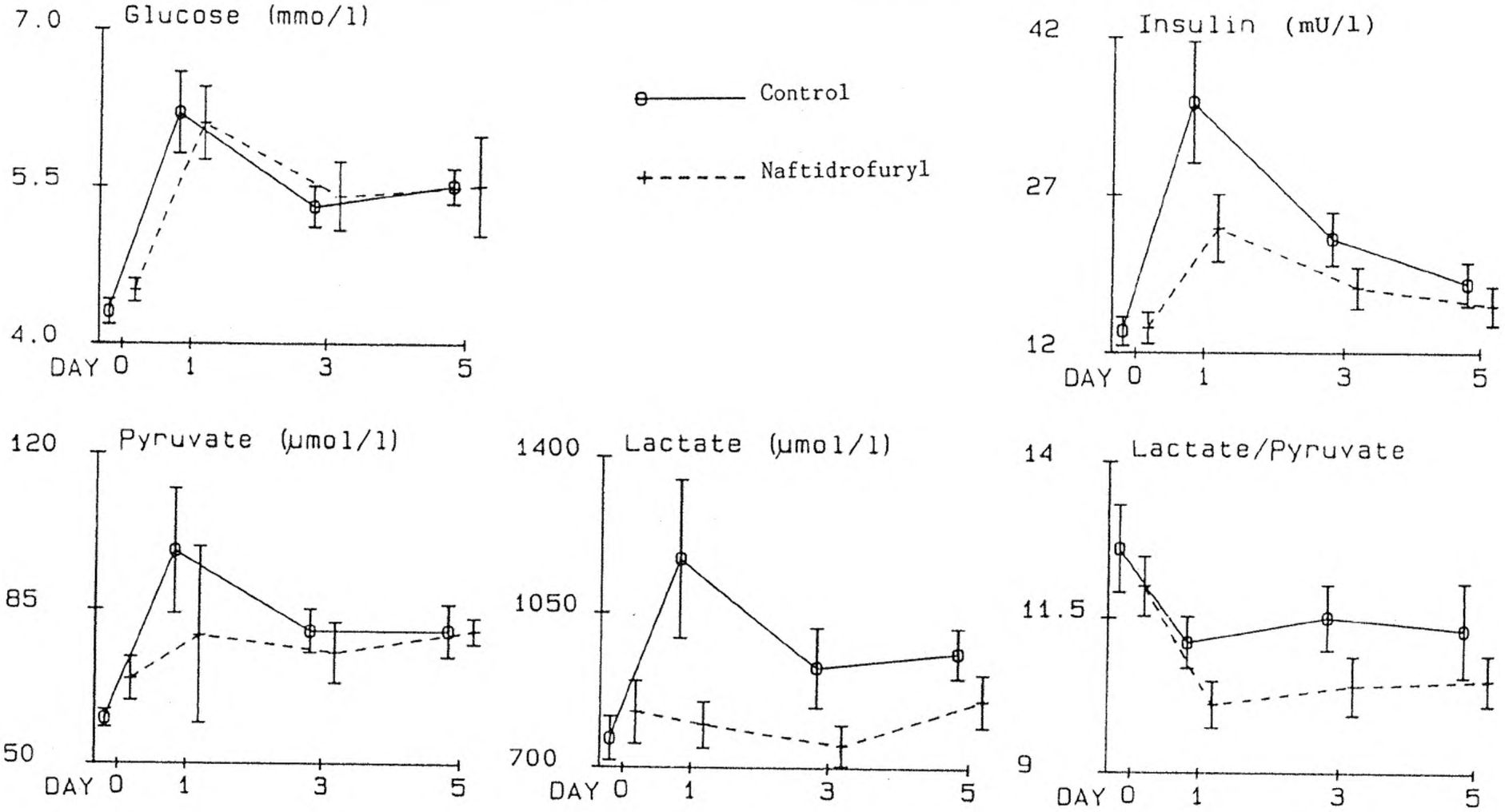


TABLE:12-2 LIPID METABOLITE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
T.K.$ µmol/l	Control	141 (116 - 172)	17	84 (69 - 101)	17	85 (70 - 103)	17**	62 (50 - 77)	15*
	Naf.	102 (82 - 128)	18	109 (91 - 131)	18	112 (94 - 133)	18	83 (69 - 100)	15
T.T.G. mmol/l	Control	1.55 ± 0.12	16	0.74 ± 0.06	16***	0.91 ± 0.06	15***	0.97 ± 0.07	15**
	Naf.	1.47 ± 0.16	18	0.82 ± 0.09	17***	0.97 ± 0.10	17***	1.08 ± 0.08	15*
F.F.A. µmol/l	Control	650 ± 60	17	660 ± 50	17	540 ± 40	15*	500 ± 40	14*
	Naf.	620 ± 50	17	670 ± 50	16	530 ± 30	16*	570 ± 40	14*
Glycerol µmol/l	Control	87 ± 10	17	88 ± 9	17	74 ± 6	16	70 ± 4	15*
	Naf.	88 ± 7	16	76 ± 5	16	68 ± 4	16	75 ± 4	15*

Naf. = Naftidrofuryl group

T.K. = Total ketones T.T.G. = Total triglycerides F.F.A. = Free fatty acids

$ = Figures derived from logarithmic transformation (geometric mean and range of 1 sem about the mean)

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.005$ *** $p<0.001$

FIGURE:12-2 LIPID METABOLITE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

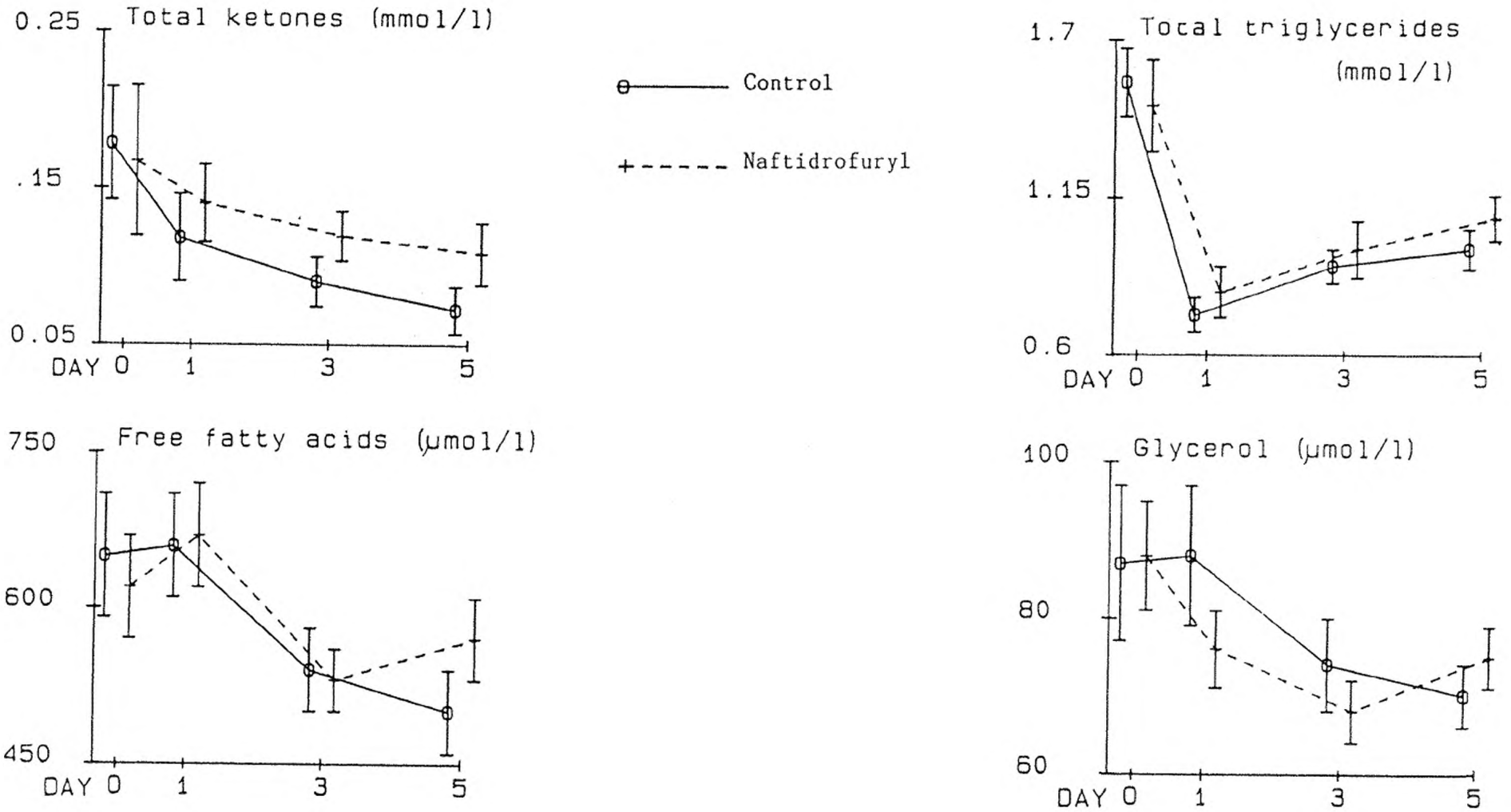


TABLE:12-3

HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Haemoglobin g/dl	Control	14.3 ± 0.39	17	13.8 ± 0.44	17*	12.6 ± 0.44	17***	12.4 ± 0.44	15***
	Naf.	14.4 ± 0.35	18	12.9 ± 0.38	18***	12.1 ± 0.36	18***	12.1 ± 0.40	15***
P.C.V. l/l	Control	0.430 ± 0.010	17	0.410 ± 0.012	17*	0.372 ± 0.011	15**	0.361 ± 0.009	15***
	Naf.	0.426 ± 0.010	18	0.392 ± 0.009	18*	0.368 ± 0.009	18***	0.368 ± 0.011	15***
Albumin$ g/l	Control	41.9 ± 0.82	17	37.8 ± 0.87	17***	37.6 ± 0.58	15***	38.4 ± 0.87	15***
	Naf.	42.4 ± 0.66	18	38.9 ± 0.78	18***	37.5 ± 1.33	18***	38.9 ± 1.15	15***
Total protein$ g/l	Control	69.4 ± 1.08	17	62.8 ± 1.09	17***	64.8 ± 0.88	15***	67.9 ± 1.27	15
	Naf.	71.8 ± 0.85	18	64.8 ± 1.50	18***	64.9 ± 2.15	18***	69.0 ± 1.56	15

Naf. = Naftidrofuryl group P.C.V. = Packed cell volume ratio

$ = Concentrations corrected for changes in packed cell volume

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:12-3

HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

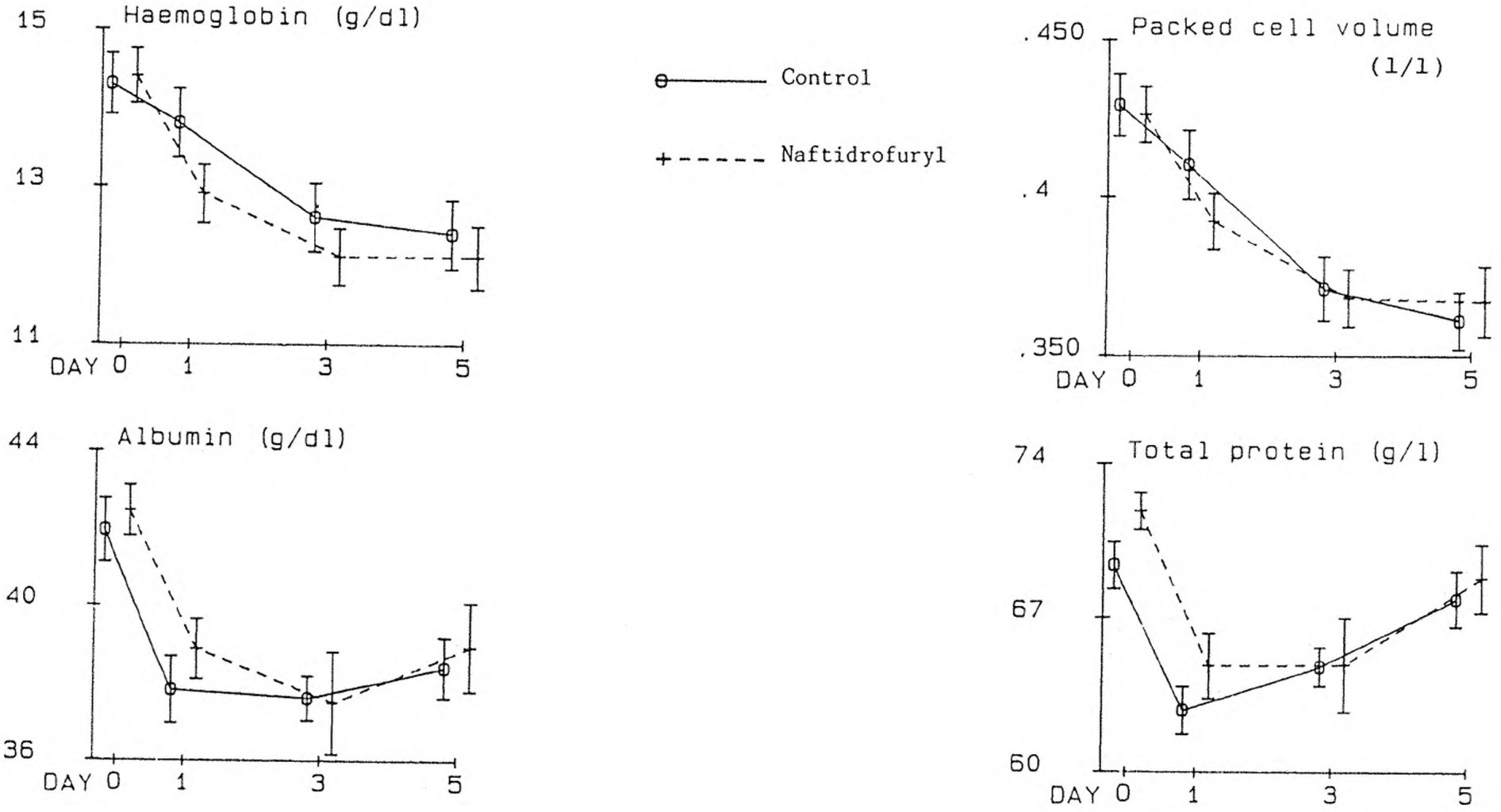


TABLE:12-4 UREA, CREATININE AND ELECTROLYTE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
Creatinine µmol/l	Control	92.7 ± 4.72	17	83.9 ± 4.58	17*	69.4 ± 3.59	17***	72.5 ± 3.65	15***
	Naf.	83.8 ± 3.58	18	76.6 ± 5.28	18*	65.6 ± 4.21	18***	70.1 ± 5.24	15***
Urea mmol/l	Control	4.8 ± 0.28	17	5.8 ± 0.27	17**	4.8 ± 0.35	17	5.2 ± 0.51	15
	Naf.	4.7 ± 0.25	18	5.5 ± 0.37	18*	3.9 ± 0.34	18	4.5 ± 0.34	15
Bicarbonate mmol/l	Control	28.7 ± 0.50	17	25.6 ± 0.74	17**	25.7 ± 0.47	16***	25.9 ± 0.42	15**
	Naf.	28.5 ± 0.85	18	25.2 ± 0.38	18***	25.5 ± 0.50	18**	26.4 ± 0.70	15*
Sodium mmol/l	Control	140 ± 0.44	17	137 ± 0.50	17***	138 ± 0.84	17*	139 ± 0.53	15
	Naf.	140 ± 0.62	18	138 ± 0.87	18*	140 ± 0.72	18	140 ± 0.65	15
Potassium mmol/l	Control	4.3 ± 0.07	17	4.2 ± 0.10	17	4.0 ± 0.11	17*	4.1 ± 0.16	15
	Naf.	4.2 ± 0.11	18	4.1 ± 0.11	18	3.8 ± 0.07	18*	4.0 ± 0.09	15

Naf. = Naftidrofuryl group

Significance vs pre-operative concentrations (paired data): * $p<0.05$ ** $p<0.05$ *** $p<0.001$

FIGURE:12-4 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE TOTAL PARENTERAL NUTRITION GROUP

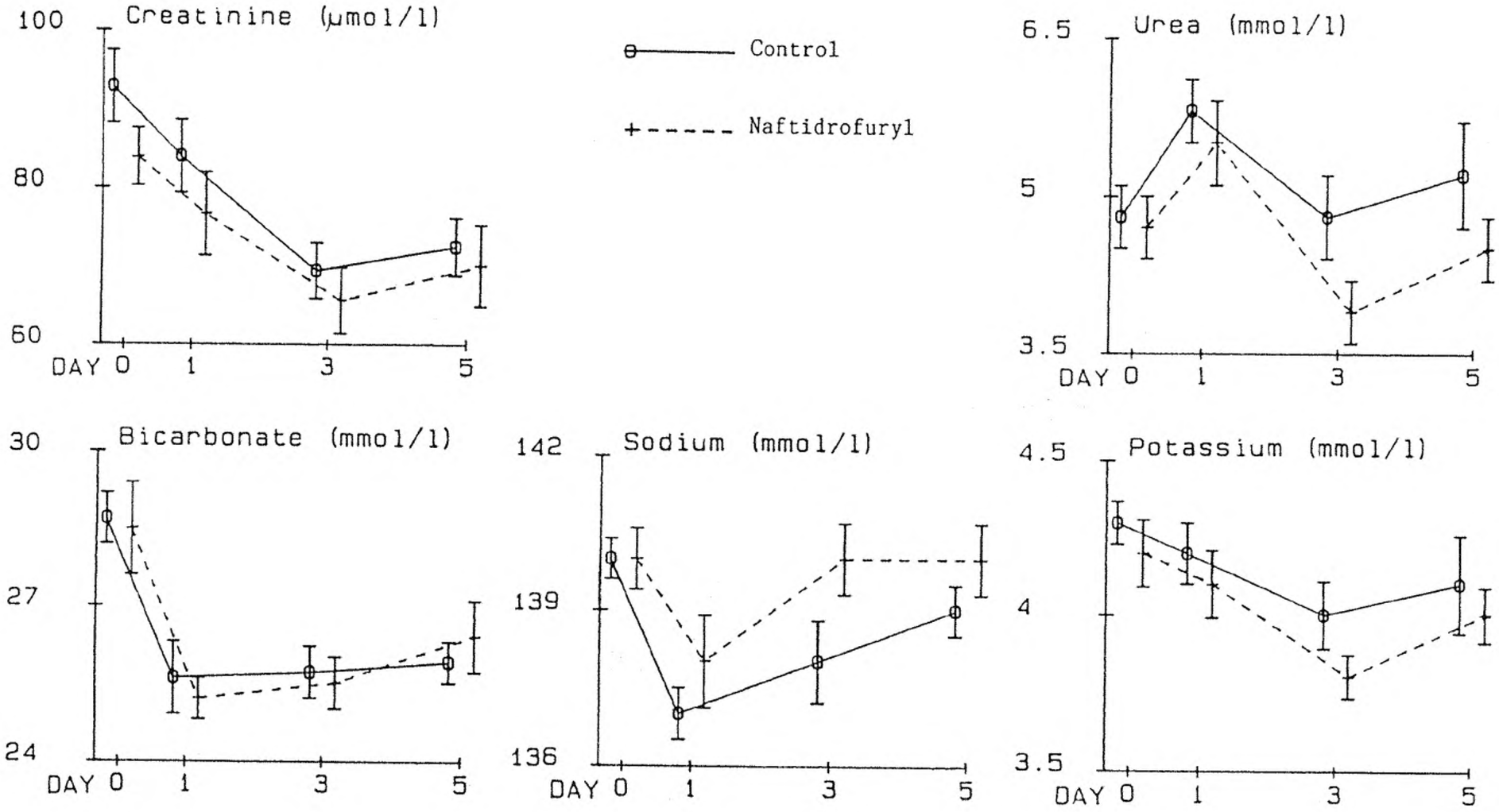


TABLE:12-5 **BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (μmol/l) IN THE TOTAL PARENTERAL NUTRITION**

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Valine	Control	232 ± 9.9	16	210 ± 12.8	16**	254 ± 12.7	14*
	Naf.	215 ± 9.4	18	198 ± 7.6	18*	242 ± 13.8	15**
Leucine	Control	142 ± 7.1	16	121 ± 10.0	16**	151 ± 10.1	14*
	Naf.	134 ± 6.1	18	115 ± 5.8	18**	149 ± 7.1	15*
Isoleucine	Control	71 ± 3.6	16	51 ± 4.6	16***	79 ± 4.4	14*
	Naf.	65 ± 3.3	18	50 ± 3.7	18**	78 ± 3.6	15**
Total BCAA	Control	445 ± 20.0	16	382 ± 26.1	16**	484 ± 24.4	14*
	Naf.	413 ± 18.3	18	363 ± 15.3	18**	470 ± 23.4	15**

Naf. = Naftidrofuryl group BCAA = Branched-chain amino acids

Significance vs Day 0 values (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:12-5 BRANCHED-CHAIN AMINO ACID CONCENTRATIONS (µmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

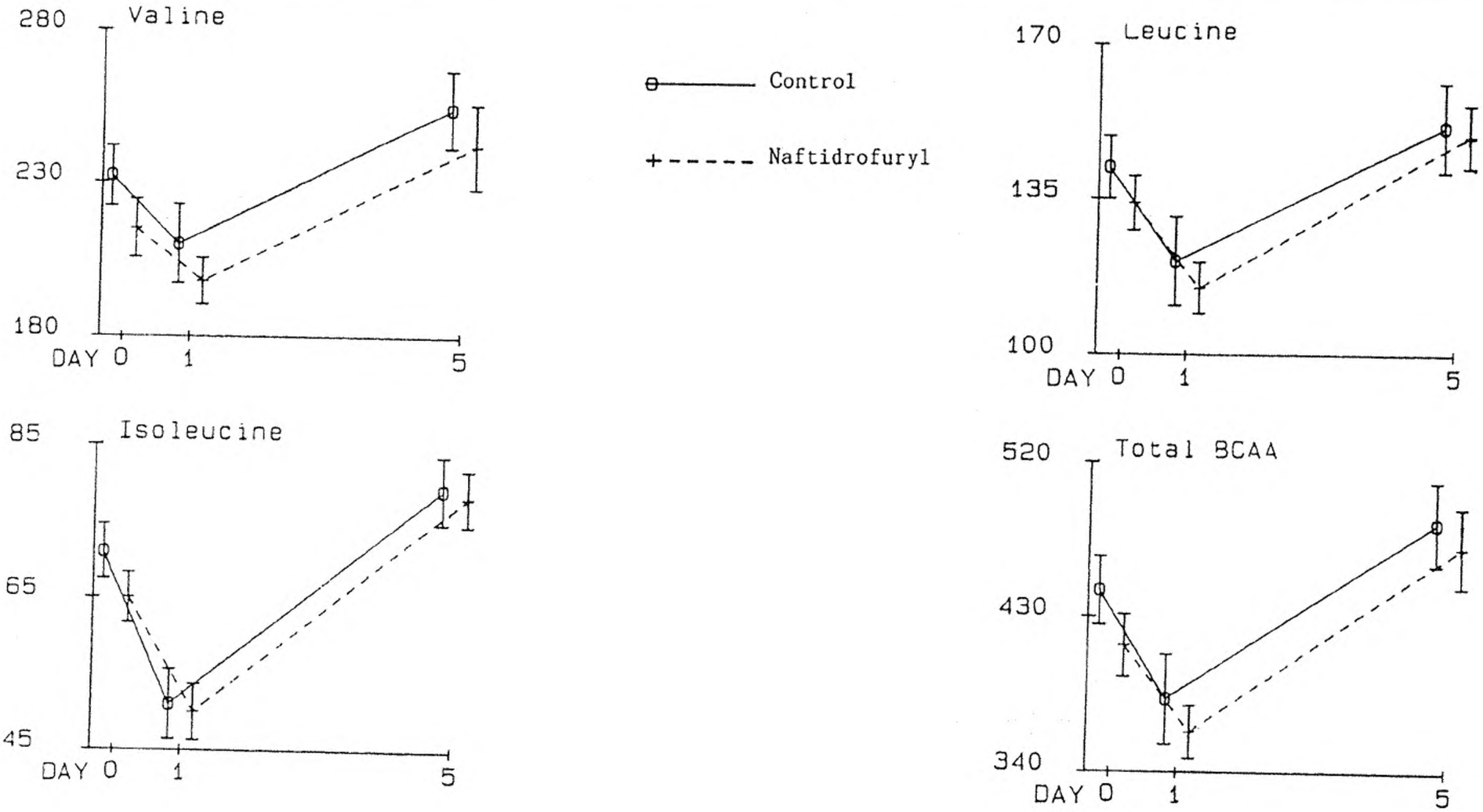


TABLE:12-6 ESSENTIAL AMINO ACID CONCENTRATIONS (μmol/l)

IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Lysine	Control	180 ± 5.0	16	109 ± 5.7	16***	163 ± 7.9	14
	Naf.	165 ± 7.0	18	94 ± 4.9	18***	163 ± 8.8	15
Threonine	Control	125 ± 8.4	16	77 ± 3.9	16***	125 ± 12.2	14
	Naf.	108 ± 7.2	18	70 ± 4.5	18***	125 ± 10.2	15
Methionine	Control	31 ± 2.0	16	27 ± 1.5	16	38 ± 2.7	14*
	Naf.	29 ± 1.3	18	26 ± 1.8	18	33 ± 1.7	15*
Tryptophan	Control	16 ± 1.1	16	20 ± 1.5	16**	19 ± 1.5	14**
	Naf.	15 ± 1.5	18	16 ± 1.3	18*	19 ± 1.8	15*
Phenylalanine	Control	65 ± 1.9	16	83 ± 2.7	16***	86 ± 3.9	14***
	Naf.	65 ± 2.7	18	81 ± 2.2	18***	81 ± 4.3	15**
Valine	Control	232 ± 9.9	16	210 ± 12.8	16**	254 ± 12.7	14*
	Naf.	215 ± 9.4	18	198 ± 7.6	18*	242 ± 13.8	15**
Leucine	Control	142 ± 7.1	16	121 ± 10.0	16**	151 ± 10.1	14*
	Naf.	134 ± 6.1	18	115 ± 5.8	18**	149 ± 7.1	15*
Isoleucine	Control	71 ± 3.6	16	51 ± 4.6	16***	79 ± 4.4	14*
	Naf.	65 ± 3.3	18	50 ± 3.7	18**	78 ± 3.6	15**
Total EAA	Control	862 ± 27.8	16	696 ± 35.7	16***	915 ± 38.6	14*
	Naf.	794 ± 28.3	18	650 ± 24.8	18***	891 ± 33.7	15**

Naf. = Naftidrofuryl group EAA = Essential amino acids

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

FIGURE:12-6 ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

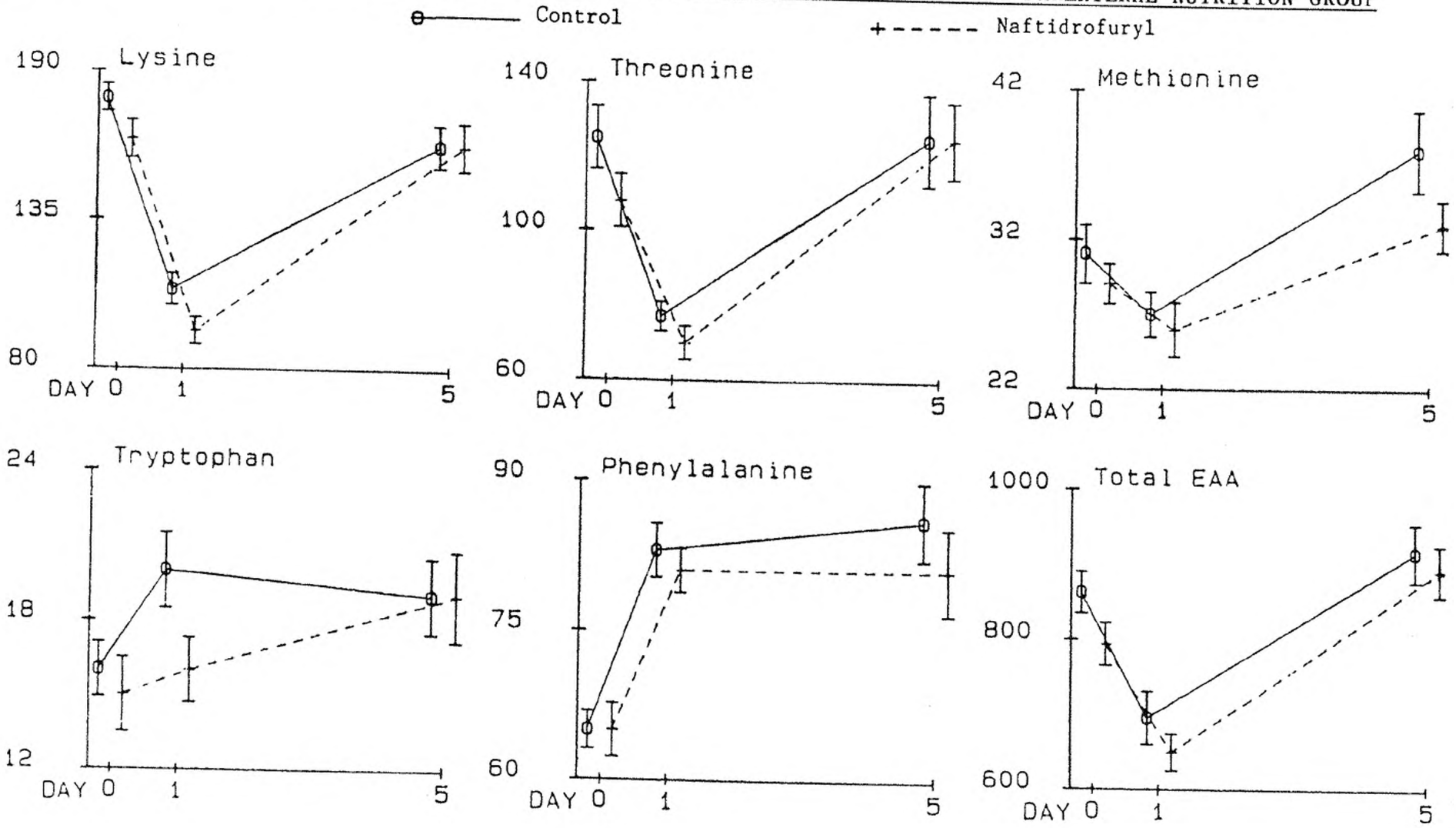


TABLE:12-7A

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (μmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Alanine	Control	327 ± 21.8	16	265 ± 16.1	16**	309 ± 19.5	14
	Naf.	305 ± 16.7	18	a219 ± 12.2	16***	258 ± 14.8	15**
Taurine	Control	53 ± 1.6	16	49 ± 2.6	16	33 ± 2.2	14***
	Naf.	48 ± 2.5	18	42 ± 1.6	18**	30 ± 1.4	15***
Histidine	Control	88 ± 3.1	16	69 ± 3.9	16***	67 ± 2.8	14***
	Naf.	87 ± 5.4	18	68 ± 3.7	18***	68 ± 2.6	15***
Citrulline	Control	44 ± 3.5	16	22 ± 1.5	16***	23 ± 1.7	14***
	Naf.	43 ± 3.3	18	23 ± 2.7	18***	22 + 1.7	15***
Arginine	Control	92 ± 5.0	16	49 ± 3.2	16***	88 ± 8.7	14
	Naf.	91 ± 5.4	18	47 ± 3.0	18***	99 ± 6.9	15
Ornithine	Control	68 ± 3.4	16	42 ± 2.6	16***	78 ± 6.0	14***
	Naf.	58 ± 3.5	18	37 ± 2.6	18*	76 ± 5.5	15**
Proline	Control	187 ± 20.1	16	179 ± 16.0	16	280 ± 38.8	14*
	Naf.	164 ± 12.1	18	160 ± 14.2	18	244 ± 26.2	15**
Glutamine	Control	601 ± 32.1	16	486 ± 23.4	16*	486 ± 32.8	14*
	Naf.	581 ± 17.1	18	422 ± 21.0	18***	497 ± 25.6	15*

Naf. = Naftidrofuryl group

Significance vs Day 0 (paired data): * p<0.05 ** p<0.01 *** p<0.001

Significance vs Control: a p<0.05

TABLE:12-7B

CONCENTRATIONS OF NON-ESSENTIAL AMINO ACIDS (µmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 5 mean ± sem	n
Glutamate	Control	33 ± 4.3	16	25 ± 2.5	16*	38 ± 4.3	14
	Naf.	34 ± 4.1	18	25 ± 3.2	18*	32 ± 2.6	15
Glycine	Control	204 ± 15.4	16	144 ± 10.5	16**	159 ± 10.0	14*
	Naf.	195 ± 9.0	18	138 ± 7.2	18***	161 ± 10.6	15**
Tyrosine	Control	64 ± 3.2	16	56 ± 2.5	16*	70 ± 5.3	14
	Naf.	60 ± 2.6	18	54 ± 2.5	18*	67 ± 4.0	15
Aspartate	Control	2.2 ± 0.20	16	1.8 ± 0.13	16	2.2 ± 0.17	14
	Naf.	2.0 ± 0.20	18	1.7 ± 0.17	18	2.1 ± 0.18	15
Asparagine	Control	55 ± 3.0	16	33 ± 1.6	16***	47 ± 2.8	14
	Naf.	a42 ± 1.8	18	29 ± 2.3	18***	39 ± 2.9	15
AAB	Control	28 ± 2.0	16	33 ± 2.6	16**	38 ± 2.9	14**
	Naf.	25 ± 2.3	18	27 ± 1.6	18	33 ± 2.3	15*
Cystine	Control	60 ± 2.4	16	41 ± 2.3	16***	56 ± 3.3	14
	Naf.	58 ± 2.4	18	39 ± 2.0	18***	54 ± 2.0	15
Serine	Control	109 ± 5.8	16	63 ± 3.7	16***	95 ± 5.1	14
	Naf.	96 ± 5.2	18	65 ± 4.7	18***	92 ± 5.4	15
Total NEAA	Control	2014 ± 73	16	1554 ± 53	16***	1869 ± 111	14
	Naf.	1890 ± 57	18	1403 ± 58	18***	1771 ± 72	15

Naf. = Naftidrofuryl group

AAB = Alpha-aminobutyrate NEAA = Non-essential amino acids

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Significance vs Control: a $p<0.01$

FIGURE:12-7A NON-ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

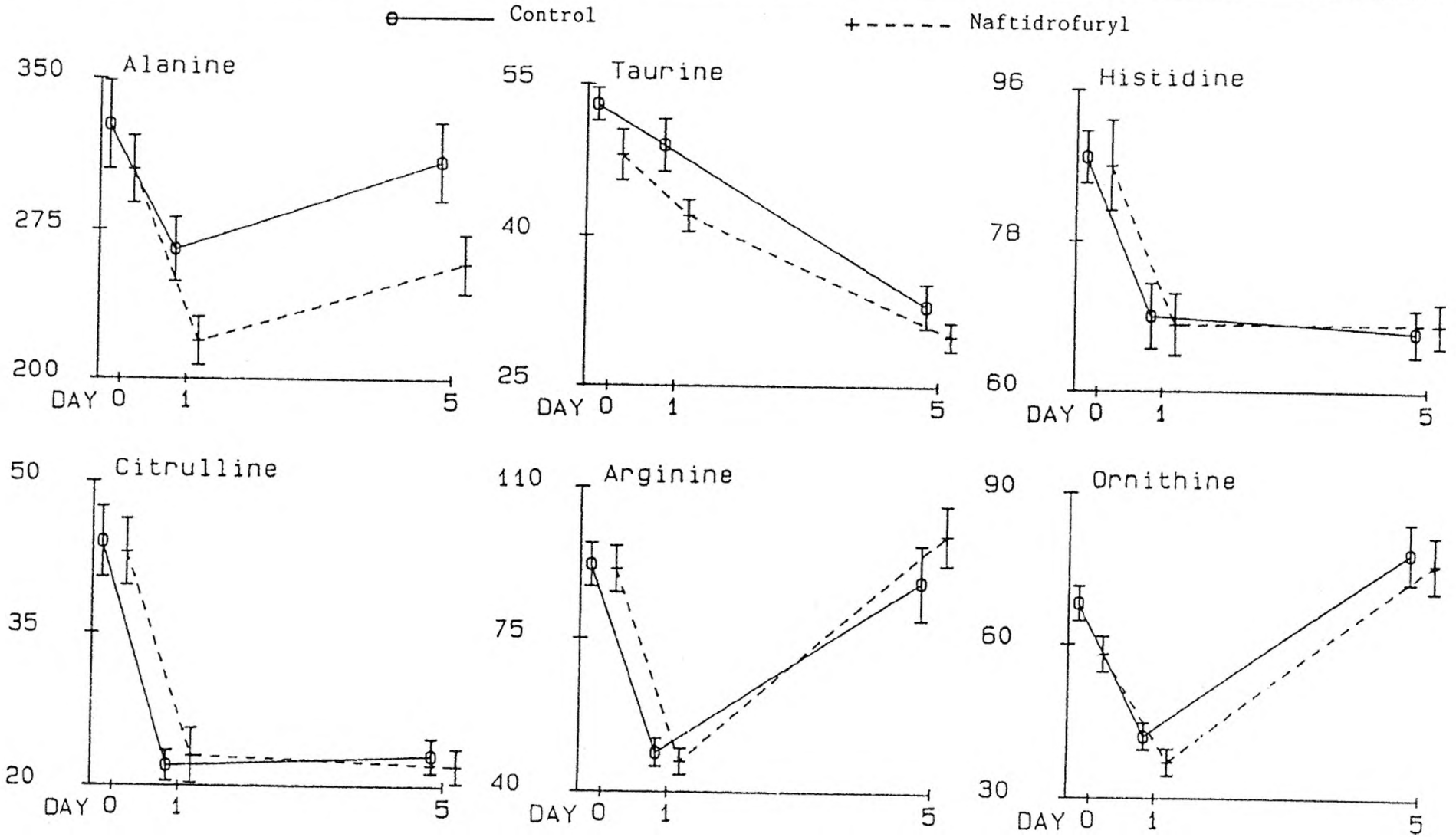


FIGURE:12-7B NON-ESSENTIAL AMINO ACID CONCENTRATIONS (µmol/l) IN THE TOTAL PARENTERAL NUTRITION GROUP

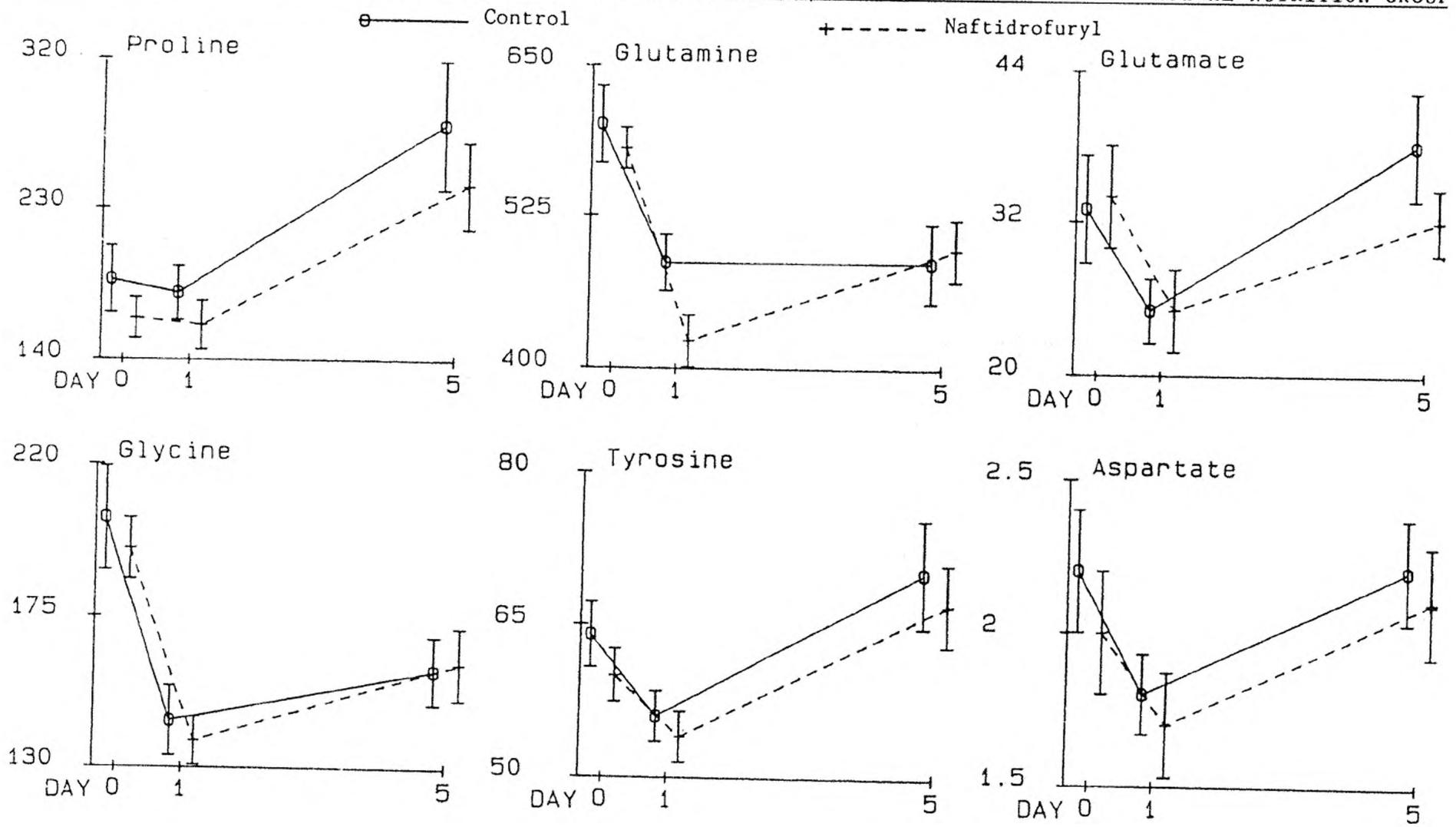


FIGURE:12-7C NON-ESEENTIAL AMINO ACID CONCENTRATIONS (µmol/1) IN THE TOTAL PARENTERAL NUTRITION GROUP

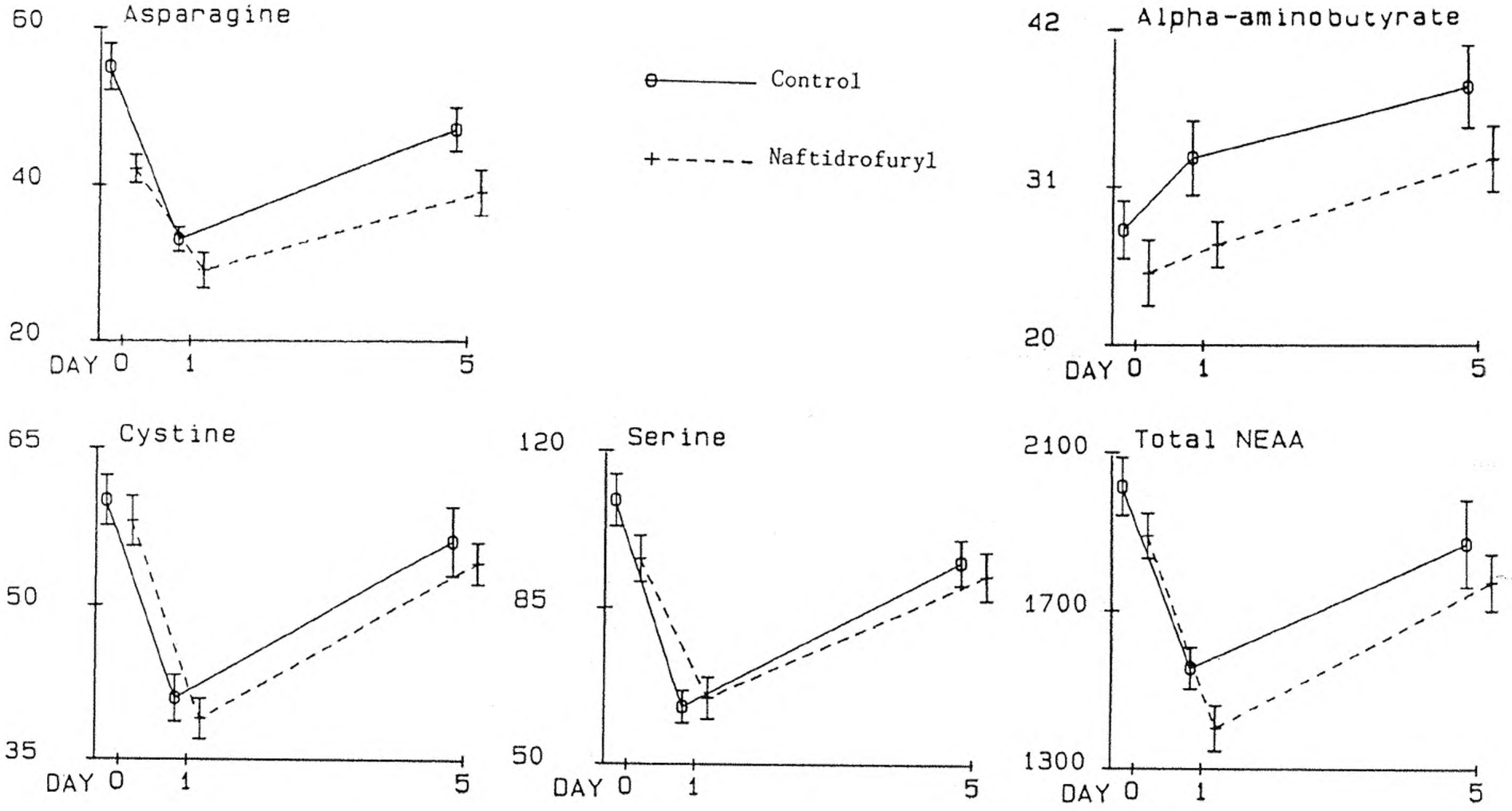


TABLE:12-8 PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE, AND CYSTINE/TAURINE RATIOS IN THE TOTAL PARENTERAL NUTRITION GROUP

		Day 0		Day 1		Day 5	
		mean ± sem	n	mean ± sem	n	mean ± sem	n
Phe/Tyr	Control	1.04 ± 0.03	16	1.52 ± 0.05	16***	1.16 ± 0.06	14**
	Naf.	1.09 ± 0.05	18	1.53 ± 0.07	18***	1.25 ± 0.07	15*
Met/CySS	Control	0.52 ± 0.03	16	0.68 ± 0.05	16*	0.76 ± 0.07	14**
	Naf.	0.51 ± 0.03	18	0.66 ± 0.04	18**	0.74 ± 0.10	15*
Met/Tau	Control	0.58 ± 0.04	16	0.58 ± 0.05	16	1.23 ± 0.12	14***
	Naf.	0.62 ± 0.04	18	0.61 ± 0.04	18	1.29 ± 0.19	15**
CySS/Tau	Control	1.15 ± 0.06	16	0.89 ± 0.07	16**	1.66 ± 0.13	14***
	Naf.	1.23 ± 0.09	18	0.97 ± 0.07	18**	1.78 ± 0.12	15***

Naf. = Naftidrofuryl group

Phe/Tyr = Phenylalanine to tyrosine ratio Met/CySS = Methionine to cystine ratio

Met/Tau = Methionine to taurine ratio CySS/Tau = Cystine to taurine ratio

Significance vs Day 0 (paired data): * $p<0.05$ ** $p<0.005$ *** $p<0.001$

FIGURE:12-8

PHENYLALANINE/TYROSINE, METHIONINE/CYSTINE, METHIONINE/TAURINE AND CYSTINE/TAURINE RATIOS IN THE TOTAL PARENTERAL NUTRITION GROUP

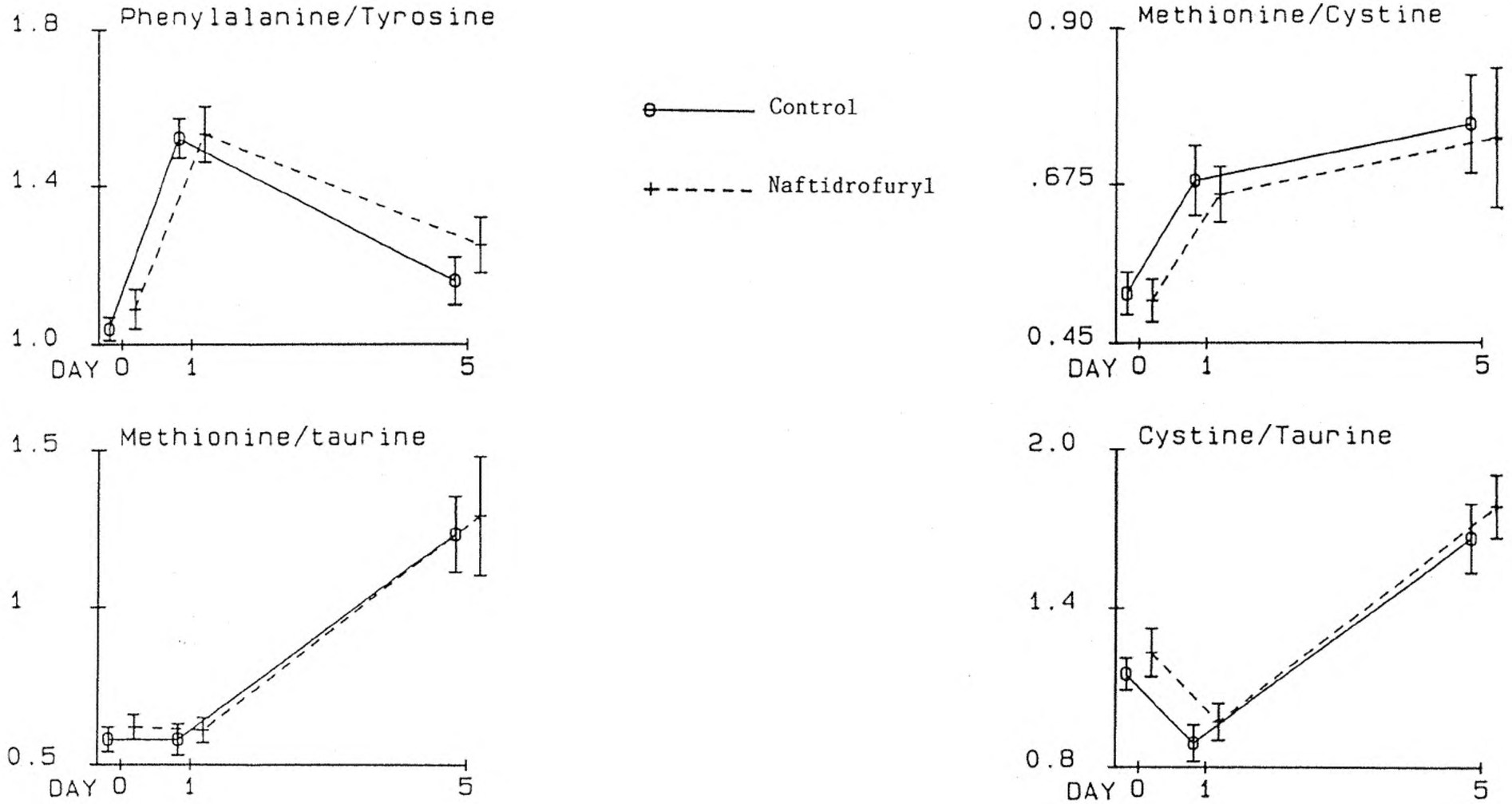


TABLE:12-9 LINEAR CORRELATIONS BETWEEN TOTAL KETONE AND BRANCHED-CHAIN AMINO ACID CONCENTRATIONS AND THE MAGNITUDE OF CHANGE BETWEEN DAYS 0 & 1 OF THESE SUBSTRATES AND THE 6-DAY NITROGEN BALANCE

	Group	r	n	p	
[Day 1 TK] µmol/l	Control	0.1437	16	p>0.1	ns
vs	Naf.	0.1954	18	p>0.1	ns
[Day 1 BCAA] µmol/l	All patients	0.1700	34	p>0.1	ns
[Day 1 - Day 0 TK] µmol/l	Control	0.0290	16	p>0.1	ns
vs	Naf.	0.1481	18	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	All patients	0.0911	34	p>0.1	ns
[Day 1 TK] µmol/l	Control	0.2428	15	p>0.1	ns
vs	Naf.	0.1895	15	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.0668	30	p>0.1	ns
[Day 1 BCAA] µmol/l	Control	0.1191	15	p>0.1	ns
vs	Naf.	0.2764	15	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.1459	30	p>0.1	ns
[Day 1 - Day 0 TK] µmol/l	Control	0.1946	15	p>0.1	ns
vs	Naf.	0.4055	15	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.2134	30	p>0.1	ns
[Day 1 - Day 0 BCAA] µmol/l	Control	0.1299	15	p>0.1	ns
vs	Naf.	0.4935	15	p>0.1	ns
6-Day nitrogen balance g kg-1	All patients	0.2050	30	p>0.1	ns

Naf. = Naftidrofuryl group

TK = Total Ketones

r = Coefficient of correlation

[ ] = Concentration

BCAA = Branched-chain amino acids

n = number of patients

p = Level of significance

ns = not significant

CHAPTER 13

EFFECT OF EXOGENOUS ENERGY SUBSTRATES ON THE METABOLIC RESPONSE TO SURGERY

The relationship of undernutrition to the pathogenesis of impaired cell-mediated immunity, increased susceptibility to infection and poor wound healing has been acknowledged in recent years and great improvements have been made in assessing nutritional deficiencies in patients and in initiating therapeutic interventions. A disturbingly high incidence of malnutrition has been reported in hospitalised patients (Bistrian et al. 1974 & 1975, Hill et al. 1977, Heymsfield et al. 1979). The benefits of nutritional support are well established and it is accepted that the enteral route should be used in preference to the parenteral route whenever possible. This is because parenteral nutrition is more expensive and is associated with complications related to central venous access, sepsis and metabolic abnormalities related to the rate or the nature of the nutrients infused. A possible solution to this nutritional predicament would be to infuse the equivalent amount of required nutrients in a less concentrated solution via peripheral veins. This is neither practical nor safe since it would require excessive fluid volume in order to meet the nutritional requirements of an individual (Rhodin et al. 1975). A simple technique to diminish loss of body cell mass was proposed by Blackburn et al. (1973) who advocated the use of isotonic amino acid solutions as post-operative peripheral intravenous feeding regimen. They showed that this solution produced a degree of metabolic adaptation similar to that seen in fasting man and patients were therefore able to mobilise fat stores and develop a starvation-like ketosis with concomitant reduction in net protein catabolism.

The previous 3 chapters have shown that naftidrofuryl oxalate has no nitrogen sparing effect in patients undergoing elective abdominal surgery. This chapter compares the control groups in the previously described infusion regimens, in order to study the effect of exogenous parenteral nutrients on the metabolic response to surgery with particular reference to the controversial nitrogen sparing effect of isotonic amino acid solutions.

PATIENTS AND METHODS:

The patients are those of the control groups in the previously described infusion regimens. Details of these regimens are described in chapters 7 and 8 and summarised in table 13-1 below.

Table:13-1 DAILY INFUSION REGIMENS.

	D/S group	IAA group	TPN group
Carbohydrate	6 kcal kg^{-1}	-	12 kcal kg^{-1}
Fat	-	-	12 kcal kg^{-1}
Nitrogen	-	0.15 g kg^{-1}	0.15 g kg^{-1}

D/S = Dextrose-saline
IAA = Isotonic amino acid
TPN = Total parenteral nutrition

The groups were matched for age, sex, anthropometric measurements and type of surgical procedure (Table:13-2).

The methods are those described in section II (chapter 7). The level of statistical significance for between infusion group significance was set at $p<0.02$ as already discussed in chapter 7.

RESULTS:

Urine: (table:13-3 & figure:13-3)

The cumulative 3 and 6-day nitrogen balances were

less negative in the TPN group compared to the IAA ($p<0.005$) and D/S ($p<0.001$) groups. There were no significant differences in these variables between the IAA and D/S groups.

The cumulative 3 and 6-day urinary urea excretion were lower in the D/S group than in the other two groups ($p<0.001$). The IAA group excreted significantly more urea when compared not only to the D/S group (3 and 6-day, $p<0.001$) but also to the TPN group (3-day, $p<0.005$; 6-day, $p<0.001$). There was no significant difference in nitrogen intake between the TPN and IAA groups.

The cumulative 3 and 6-day urinary creatinine excretion was lower in the D/S group compared to the other 2 groups ($p<0.005$) which were not different.

The cumulative urinary potassium balance was more negative in the D/S group compared to the other 2 groups (3-day, $p<0.005$; 6-day, $p<0.001$) which were not different.

Table 13-4 and figure 13-4 show the daily nitrogen intake and balance of the patients who have completed the full 6 days of the study. The results for the operative and first post-operative days were pooled due to the known post-operative urine retention thus rendering the first day collection unrepresentative (Day (0+1)/2). The IAA group had a less negative nitrogen balance than the D/S group in the first 2 days only (Day 0+1, $p<0.005$) and was not significantly different from that of the TPN group over the same period. For all the other days the TPN group had a much less negative nitrogen balance than the other two infusion groups which had similar balances. There was no difference in nitrogen intake, on all days, between the IAA and TPN groups.

Table 13-5 and figure 13-5 show the daily ureagenesis of the patients who completed the study. The ureagenesis on the first two days only (Day 0+1) was not significantly different between the TPN and IAA groups. For the rest of the study period the IAA group generated significantly more urea than the D/S and TPN groups

($p<0.001$).

Glucose, insulin and gluconeogenic substrates: (table:13-6 & figure:13-6)

Peak blood glucose concentration occurred on Day 1 in all groups and later fell to values not different from Day 0 in the D/S and IAA groups, whereas in the TPN group Day 5 concentration was still higher than pre-operatively ($p<0.01$).

Peak plasma insulin concentration occurred on Day 1 in all groups and later fell rapidly to Day 5 concentrations which were higher than Day 0 in the TPN group ($p<0.05$), not different from Day 0 in the D/S group and lower than Day 0 in the IAA group ($p<0.05$). The post-operative concentrations of glucose and insulin were lower in the IAA group compared to the other 2 groups which were not different.

Peak blood pyruvate concentration occurred on Day 1 in the D/S and TPN groups and later fell to values which were still higher than Day 0 in the TPN group ($p<0.01$) and not different from Day 0 in the D/S group. The IAA group showed no significant between day changes. Day 5 blood pyruvate concentration was higher in the TPN group when compared to the D/S ($p<0.02$) and IAA ($p<0.005$) groups.

Blood lactate concentrations showed similar changes to blood pyruvate, and lactate to pyruvate ratio (L/P) showed no significant between day or between group differences.

Peak total gluconeogenic substrates (Lactate, pyruvate, alanine and glycerol) concentration occurred on Day 1 in the D/S and TPN groups (D/S, $p<0.001$; TPN, $p<0.05$) and later returned to values not different from Day 0. However, in the IAA group the concentrations gradually fell to values lower than pre-operatively (Day 5 < Day 0, $p<0.05$).

Lipid metabolites: (table:13-7 & figure:13-7)

Total ketone concentrations showed no significant

changes between consecutive measurements in the TPN group, although Day 3 ($p<0.01$) and Day 5 ($p<0.05$) were significantly lower than Day 0. In the D/S group, total ketone concentrations increased after Day 1 so that Day 5 was significantly higher than pre-operatively ($p<0.01$). In the IAA group, total ketone concentrations increased between Days 0 and 1 ($p<0.01$) and Days 1 and 3 ($p<0.001$) but the increase between Days 3 and 5 was not significant. Between group analysis showed that total ketone concentrations in the IAA group were higher than in the other 2 groups on all post-operative days ($p<0.001$) and that the concentrations in the D/S group were higher than in the TPN group on Day 5 ($p<0.001$).

Trough total triglyceride concentrations (TTG) occurred on Day 1 in all groups and later rose to concentrations not different from Day 0 in the D/S and IAA groups, whereas in the TPN group the Day 5 concentration remained lower than pre-operatively ($p<0.01$). Between group analysis showed that the IAA group had higher concentrations than the other 2 groups on Days 3 and 5 (vs TPN, $p<0.001$; vs D/S, Day 3 $p<0.02$, Day 5 $p<0.05$). Day 5 TTG concentration was significantly lower in the TPN group when compared to the D/S and IAA groups (vs D/S group, $p<0.005$; vs IAA group, $p<0.001$).

In the IAA group, plasma free fatty acid (FFA) concentrations rose between Days 0 and 1 ($p<0.01$) and thereafter remained constant. In the TPN group, FFA concentrations fell after Day 1 so that Days 3 and 5 were lower than Day 0 ($p<0.05$). In the D/S group, Day 3 FFA concentration was lower than Day 0 ($p<0.05$) otherwise there were no significant between day differences. Between group analysis showed that the TPN group had lower concentrations than the D/S group on Days 3 and 5 ($p<0.005$) and lower concentrations than the IAA group on all post-operative days (Day 1, $p<0.02$; Day 3 and Day 5, $p<0.001$).

Glycerol concentrations showed no between day changes in the D/S and IAA groups whereas in the TPN group

Day 5 concentration was lower than pre-operatively ($p<0.05$). However, there were no significant between group differences in this measured variable.

Haemoglobin, packed cell volume, albumin and total protein (table:13-8 & figure:13-8)

Haemoglobin and packed cell volume ratio fell post-operatively and showed no between group difference.

Serum albumin and total protein concentrations fell post-operatively in all groups. Total protein concentration returned to pre-operative values by Day 5 whereas albumin concentration remained lower than pre-operatively (D/S group, $p<0.01$; IAA group, $p<0.05$; TPN group, $p<0.001$). There were no significant between group differences in these measured variables.

Creatinine, urea and electrolytes: (table:13-9 & figure:13-9)

Serum creatinine concentration fell post-operatively in all groups and showed no significant between group difference.

After the initial rise on Day 1, urea concentration returned towards pre-operative values in the D/S and TPN groups, whereas in the IAA group the concentrations remained higher than Day 0 ($p<0.01$). Post-operatively, the IAA group had higher urea concentration when compared to the D/S group (Days 3 and 5, $p<0.001$) and the TPN group (Day 3, $p<0.005$). Day 5 urea concentration in the TPN group was higher than that in the D/S group but failed to reach the statistical level of significance set at $p<0.02$ ($p<0.05$).

Serum bicarbonate concentration showed no significant between day changes in the D/S group whereas in the TPN group the concentrations, on all post-operative days, were lower than Day 0 (Days 1 & 5, $p<0.01$; Day 3, $p<0.001$). Day 5 concentration in the IAA group was lower than pre-operatively ($p<0.01$). Between group analysis showed that the TPN group had lower concentrations than

the IAA group on Days 1 and 3 ($p<0.02$) and lower concentration than the D/S group on Day 3 ($p<0.005$).

Serum sodium concentration fell post-operatively and showed no between group difference.

Serum potassium concentration showed no significant between day changes in the three groups. Between group analysis showed that on Day 5 the IAA group had higher serum potassium concentration than the other 2 groups.

DISCUSSION:

Blackburn et al. (1973) advocated the peripheral infusion of dextrose-free isotonic amino acids, as a method with fewer technical and metabolic problems than total parenteral nutrition, for sparing body nitrogen in post-operative patients. The resulting combined effects of decreased insulin and glucose concentrations, together with increased concentrations of free fatty acids and ketone bodies, would reduce the consumption of glucose and hence the need for gluconeogenesis from protein thus leading to protein sparing. Studies carried out to test this claim have produced conflicting results (Freeman et al, 1975; Tweedle et al., 1975; Hoover et al., 1975; Greenberg et al., 1976; Skillman et al., 1976; Craig et al., 1977; Freeman et al., 1977; Foster et al. 1978; Bozzetti et al., 1980; Miller et al., 1982; Harris et al., 1982; Garden et al., 1983; Thomson, 1985). Comparison of these results is difficult because different patient groups were studied, the amount and nature of infused protein was variable and different means were used to assess the response.

This present study showed that, for a similar nitrogen intake (0.15 g kg^{-1} day^{-1}), the TPN group had a less negative cumulative 3 and 6-day nitrogen balance than the IAA group but there were no significant differences in these measurements between the IAA and D/S groups. Analysis of the daily nitrogen balance and ureagenesis showed that, compared to 4% dextrose-saline, the infusion

of isotonic amino acids alone spared nitrogen on the first 2 days (operative and first post-operative days) only. Thereafter, it increased ureagenesis without improving nitrogen balance. The TPN infusion had a marked nitrogen sparing effect throughout the study period. This is in agreement with the findings of Wolfe et al. (1977) and McDougal et al. (1977) that nitrogen balance is most dependent on calorie intake but that the provision of amino acids and energy substrate is better than the provision of energy substrates alone. Injured patients have an obligatory need for fat energy and continue to oxidise fat despite being provided with sufficient glucose calories to meet their needs (Burke et al., 1979; Wolfe et al., 1979 and Askanazi et al., 1980b). More recently, Macfie et al. (1981) showed that the infusion of an amino acid, glucose and lipid mixture promoted nitrogen retention better than the infusion of an amino acid and glucose solution.

Most studies using solutions of amino acids alone have failed to demonstrate positive nitrogen balance although they usually showed a reduction in the negativity of this balance. Tweedle et al. (1975) compared healthy volunteers after an overnight fast to starved patients with complications several days after gastro-intestinal surgery. They suggested that the infusion of isotonic amino acids was an efficient method of protein conservation in starvation-adapted patients. However, they concluded that this adaptation requires many days to become established in patients, just as it does in total starvation (Owen et al., 1969). Craig et al. (1977) studied patients undergoing vagotomy and pyloroplasty for the operative and first 3 post-operative days and demonstrated that an infusion of 2.25 g kg^{-1} day^{-1} of amino acids resulted in a significantly less negative nitrogen balance than an isocaloric infusion (2.51 MJ) of dextrose or fat. They also showed that there were no between group differences in blood glucose, insulin and free fatty acid concentrations and concluded that the

short-term protein preservation achieved by a large amino acid infusion did not warrant the extra cost involved when compared with the results of dextrose infusion. Hoover et al. (1975) studied patients who received a peripheral infusion of either 90 g of amino acids or 120 g of dextrose per 70 kg body weight daily. They demonstrated significant nitrogen sparing in the amino acid group which had lower serum glucose and insulin concentrations and higher blood ketones and urea nitrogen than the dextrose group. Greenberg et al. (1976) infused amino acids alone (1 g kg^{-1} day^{-1}), 5% dextrose alone (150 g dextrose day^{-1}), amino acids and dextrose (1 g amino acids kg^{-1} day^{-1} and 150 g dextrose day^{-1}), or amino acids and fat (1 g amino acids kg^{-1} day^{-1} and 500 ml 10% Intralipid(^{R}) day^{-1}) to patients undergoing elective abdominal surgery. They demonstrated an improvement in nitrogen balance in patients infused with amino acids, but attributed no effect, deleterious or advantageous, to adding glucose or fat. They also concluded that the protein sparing effect of amino acids was a function of the infused amino acids alone and was not related to endogenous fat mobilisation. This view was later supported by Foster et al. (1978). Freeman et al. (1977a, 1977b) suggested that the improved nitrogen balance noted during peripheral amino acid infusion was not attributable to decreased insulin level alone but was also related to the availability of an adequate exogenous supply of amino acids and the ability of the body to mobilise endogenous lipid stores. More recently, Harris et al. (1982) failed to demonstrate any improvement in nitrogen balance and showed lower than predicted nitrogen-free respiratory quotient and increased 3-hydroxybutyrate excretion in post-operative patients receiving amino acids compared to those receiving 5% dextrose. They suggested that infusions of amino acids alone were associated with incomplete fat oxidation and inefficient use of body fat stores. Thomson (1985) showed an improvement in nitrogen balance and an increase in protein oxidation with a biochemical profile consistent

with starvation adaptation in post-operative patients receiving 3.5% amino acid infusion when compared to matched patients receiving 5% dextrose. He also attributed a higher resting energy expenditure and oxygen consumption in the amino acid group to the energy cost of increased protein synthesis and urea production. Garden et al. (1983) studied patients undergoing major abdominal surgery who received either isotonic amino acids (10 g nitrogen, 264 kcal day^{-1}) or 5% dextrose (2 litres, 380 kcal day^{-1}). They reported no significant between group difference in the 4-day mean nitrogen balance. However, analysis of the daily nitrogen balance showed that the amino acid infusion improved the nitrogen balance on the first two days of the study (only significantly so on Day 1). These results of Garden et al. are comparable to those described in this chapter where the IAA group significantly spared nitrogen on the first two days only compared to the D/S group. This early nitrogen-sparing effect may be attributed to glucose availability from the not yet depleted glycogen stores but this would be in conflict with the results of Greenberg et al. (1976, discussed above) who found no beneficial effect in adding glucose to amino acids. However, McDougal et al. (1977) reported that the effect of amino acid and glucose infusions were additive and the nitrogen balance was most dependent on calorie intake.

As would be expected, the D/S group generated significantly less urea than the two groups which received amino acids. The nitrogen balance and the urea production on the first two days only (Day 0+1) were not significantly different between the TPN and IAA groups. For the rest of the study period and in spite of having a similar amount of nitrogen (0.15 g kg^{-1} day^{-1}), the IAA group had a significantly more negative nitrogen balance and higher ureagenesis compared to the TPN group. Furthermore, if the ureagenesis in the D/S group is taken as a baseline, the mean excess ureagenesis, over the study period, accounts for 87% and 44% of the mean infused

nitrogen over the same period respectively in the IAA and TPN groups.

The excess ureagenesis in the IAA group may not solely be attributed to increased gluconeogenesis but also could be due to transamination and direct oxidation of the infused amino acids to provide energy. The much higher glycine and glutamic acid content of the isotonic amino acids solution compared to the TPN mixture (for a similar weight of infused nitrogen, table:7-3, chapter 7) may have also contributed to the above finding.

The greater cumulative urinary creatinine excretion in the TPN and IAA groups compared to the D/S group remained significant when expressed per kg lean body mass (Table:13-10).

Creatine and phosphocreatine are non-enzymatically dehydrated in muscle to creatinine which is quantitatively excreted in urine. At normal blood levels the creatinine is filtered at the renal glomerulus but not secreted or reabsorbed by the tubules. Therefore, its urinary excretion (in an otherwise normal kidney) is a function not only of muscle mass and its response to trauma, but mainly of the renal blood flow. The infusion of amino acids has been shown to increase renal blood flow and consequently the urinary creatinine excretion (Brenner et al.,1982; Graf et al., 1983; Eisenhauer et al., 1985; Ter Wee et al.,1986). This is further supported by the lower Day 5 creatinine clearance in the D/S group compared to the IAA and TPN groups (table:9-7).

The evidence of highly significant correlations, within each infusion group, between the urinary creatinine (a product of muscle catabolism) and the nitrogen balance, is consistent with the central role of muscle in the protein metabolic response to trauma (table:13-11).

The cumulative urinary potassium balance was more negative in the D/S group compared to the other 2 groups (table:13-12). The regulation of urinary potassium excretion is, as already discussed in chapter 9, multifactorial. Although, the infusion groups were

matched for age, sex, anthropometric measurements and the type of surgical procedure (table:13-2), they received different nutritional regimens resulting in different metabolic responses.

The provision of potassium seems to have improved the balance with the TPN and IAA group being in potassium equilibrium over the last 3 days of the study. The D/S solution provided no potassium replacement and resulted in a negative potassium balance. If this infusion was carried on for a longer period it would have resulted in potassium depletion and therefore potassium replacements should have been provided.

Potassium is primarily an intracellular ion. Cuthbertson et al. (1939) have reported an increased excretion of potassium in the traumatised rat. Wilkinson et al. (1950) and Le Quesne (1967) have also reported the increased excretion of potassium after abdominal injuries and the former showed that it is greater than that attributed to reduced food intake after partial gastrectomy.

There were positive linear correlations between the cumulative 3-day and 6-day total nitrogen balance and potassium balance with greater coefficient of correlations for the first half of the study (table:13-13). Similar correlations over the last 3 days were not significant. This is in agreement with the findings of Moore and Ball (1952) who have shown that in burn injuries the potassium loss may last only for 2-4 days.

Nitrogen balance is a measure of whole body protein metabolism and fails to identify changes in specific protein moities such as visceral, acute phase and muscle proteins. In this study, albumin and total protein concentrations showed no between group differences. After the initial fall in albumin and total protein concentrations on Day 1 due mainly to the increased vascular permeability and catabolism associated with injury (Arturson and Jonsson 1979, Davies 1982, Fleck et al. 1985), the concentrations returned to pre-operative

values as these effects diminished. These findings are in agreement with those of Hoover et al. (1975) who observed no difference in serum albumin and total protein concentrations in post-operative patients receiving either 5% dextrose or 3% amino acids and disagree with the reports of Skillman et al. (1976) and Garden et al. (1983). Skillman et al. (1976), using ^{14}C-labelled sodium carbonate, demonstrated increased albumin synthesis rate during amino acid infusion, the increase occurring when serum albumin concentrations fell. It is conceivable that their findings were related to increased rates of ureagenesis during amino acids infusion, rates upon which the calculation of albumin synthesis rate depends. Garden et al. (1983) reported higher concentrations of albumin and total protein on the 4th post-operative day in patients receiving amino acids alone compared to 5% dextrose. However, they showed no between group difference in the concentrations of transferrin and prealbumin. Furthermore, the mean fluid balance in the last two days of their study was less positive in the amino acid group which led the authors to conclude that the decreased fluid retention may explain the higher serum total protein and albumin concentrations in the amino acid group. In this present study the albumin and total protein concentrations were corrected for changes in packed cell volume which would take account of fluid changes in the intravascular compartment.

It is accepted that during starvation body protein and adipose tissue triglyceride are metabolised to provide energy requirements (Cahill 1970). With the added catabolism of stress, a marked increase in proteolysis occurs resulting in greater loss of protein. Insulin, besides having a marked antilipolytic activity, is a major anabolic hormone promoting amino acid uptake by cells (Cahill 1971, Tarrant and Ashmore 1965). Cahill (1971) has shown that the anabolic effects on muscle occur at very low insulin concentrations, whereas the inhibition of lipolysis requires higher levels. Therefore, a moderate

reduction in insulin concentration, in the injured patient, would allow active mobilisation of fat stores while still maintaining muscle anabolism. The released glycerol is utilised in gluconeogenesis and free fatty acids are either re-esterified or converted to ketone bodies, carbon dioxide and water by beta-oxidation. Starvation ketosis is controlled by insulin and does not induce the ketoacidosis that occurs in decompensated diabetes mellitus. Marked elevations of ketone bodies stimulate insulin secretion thus creating a feedback control to prevent ketosis from reaching pathological levels.

The blood measurements reported in this study were made following a 6-7 hrs nutrient-free period when only normal saline was infused and thus represent the patients' response in this 'fasted' state.

In keeping with theoretical considerations, the IAA group had lower concentrations of plasma insulin and blood glucose and higher concentrations of free fatty acids, and total ketones compared to the D/S and TPN groups. However, this biochemical profile did not result in a significant difference in the cumulative nitrogen balance or albumin and total protein concentrations between the D/S and IAA groups.

The TPN group showed similar changes to the D/S group in the concentrations of plasma insulin, and blood glucose, lactate and pyruvate and had lower Day 5 concentrations of free fatty acids and total ketones compared to the other two groups. Furthermore, the TPN group had a significantly less negative nitrogen balance than the D/S or IAA groups.

Glycerol turnover rate correlates with unidirectional lipolysis rate (Bortz et al., 1972) and free fatty acids turnover with net fat mobilisation (Armstrong et al., 1961). In uninjured subjects plasma glycerol and free fatty acids concentrations are directly related to their turnover. Carpentier et al. (1979) have reported that the latter relationship holds for injured

and infected patients receiving total parenteral nutrition but not if 5% dextrose is received. However, they studied injured and infected patients together and included turnover measurements later than 10 days post-injury so that the applicability of their findings to non-septic patients in the immediate post-operative period may be questioned.

In the IAA group the progressive increase in total ketones (Day 0 < Day 1 < Day 3 = Day 5) was not accompanied by any change in glycerol concentration and free fatty acids were higher post-operatively than on Day 0. In the TPN group there was no rise in total ketones and the glycerol concentrations were not different from the IAA group. There were no significant differences in glycerol concentrations between or within any of the three groups. Free fatty acid concentrations were lower on Days 3 and 5 than on Days 0 and 1 in the TPN group and higher post-operatively than on Day 0 in the IAA group.

Thus, the ketonaemia of the IAA group was not accompanied by any change in glycerol concentration and did not increase significantly between Days 3 and 5. This may be suggestive that the ketonaemia resulted from diversion of fatty acids from esterification to beta-oxidation with no increase in tissue lipolysis. Free fatty acid response to severe injury may be influenced by decreased tissue perfusion and albumin availibility. Nevertheless, the decrease in the post-operative concentration of free fatty acid in the TPN group may represent an increase in free fatty acid tissue re-esterification whereas in the IAA group the post-operative rise may represent reduced re-esterification. However, the above interpretation cannot be made with certainty since, as discussed in chapter 11, turnover measurements are not available and the direct relationship between venous concentrations of glycerol and free fatty acids and their turnover may be questioned.

The main stimuli to lipolysis are catecholamines

and glucagon in the presence of low insulin concentration. Of these hormones only insulin was measured and showed lower concentrations in the IAA group compared to D/S and TPN groups. The failure to significantly increase triglyceride lipolysis from adipose tissue, despite low insulin, may indicate tissue resistance to lipolysis under the conditions described.

This postulated inability to increase lipolysis may be relevant to the poor results of the infusion of isotonic amino acids alone in significantly reducing post-operative nitrogen loss reported in this chapter. This therapy is based on the assumption that fasting-induced mobilisation of fat from tissue stores in the presence of a suitable nitrogen intake can meet energy requirements and reduce protein degradation (Blackburn et al. 1973).

A number of factors may have contributed to the findings in this chapter being different from those reported by other investigators. The infusion requirements were given per kg body weight and delivered over a 16-hour period as opposed to a set amount daily infused over 24 hours. The quantity of amino acid infused (0.15 g nitrogen kg^{-1} day^{-1}; 10.5 g nitrogen per day for a 70 kg patient) was lower than in other studies. Furthermore, the infusion regimens were not strictly isocaloric, the IAA group received 4 kcal (protein) kg^{-1} day^{-1} whereas the D/S groups received 6 kcal (glucose) kg^{-1} day^{-1} and the TPN group received 28 kcal (12 kcal in glucose, 12 kcal in fat and 4 kcal in protein) kg^{-1} day^{-1}. This resulted in a 70 kg patient in the IAA group receiving 140 kcal (586 kJ) day^{-1} less than a similar weight patient in the D/S group. Another factor to consider is the amino acid profile of the solutions used. This is particularly pertinent to the IAA group as the solution used has a high glycine content and a low ratio of essential to non-essential amino acids (see chapter 7, table:7-3). Few workers have compared the effects of different amino acid solutions, although Tweedle et al.

(1972) have demonstrated that solutions low in glycine and alanine produce a better nitrogen balance than solutions with high concentrations of non-essential amino acids. Tweedle's study dealt with patients receiving total parenteral nutrition. However, the choice of an 'unbalanced' amino acid solution may have influenced the results of the present study. The solution (Perifusin$^{(R)}$, E. Merck ltd.) was used on the basis that it was the only commercially available isotonic amino acid solution.

In conclusion, the provision of a balanced carbohydrate, amino acids and fat emulsion solution has markedly spared nitrogen compared to the amino acids alone or the 4% dextrose-saline infusions. Although this study was not designed to investigate the clinical benefits of such nutritional regimens, it has demonstrated that there is no biochemical advantage associated with the peripheral intravenous infusion of isotonic amino acids alone (0.15 g nitrogen kg^{-1} day^{-1}) compared to 4% dextrose-saline (6 kcal (25kJ) kg^{-1} day^{-1}).

TABLE:13-2 PRE-OPERATIVE ANTHROPOMETRIC DATA AND SURGICAL PROCEDURES IN THE THREE INFUSION GROUPS

	DEXTROSE-SALINE GROUP mean ± sem	n	ISOTONIC AMINO ACID GROUP mean ± sem	n	TOTAL PARENTERAL NUTRITION GROUP mean ± sem	n
ANTHROPOMETRIC MEASUREMENTS						
HEIGHT (cm)	167.3 ± 2.35	16	170.7 ± 2.1	18	171.3 ± 2.3	17
WEIGHT (kg)	66.6 ± 2.5	16	68 ± 3.4	18	72.7 ± 2.5	17
MID-ARM CIRCUMFERENCE (cm)	24.3 ± 0.7	16	24.2 ± 0.7	18	25.1 ± 0.6	17
LEAN BODY MASS (kg)	45.2 ± 1.8	16	47.3 ± 2.0	18	50.2 ± 1.7	17
AGE (years)	54.7 ± 3.3	16	55 ± 3.3	18	58.7 ± 3.4	17
MEN:WOMEN	8:8		10:8		9:8	
SURGICAL PROCEDURES						
CHOLECYSTECTOMY	9		9		8	
RIGHT HEMICOLECTOMY	-		-		1	
TRANSVERSE COLECTOMY	1		-		-	
ANTERIOR RESECTION	-		2		1	
ANTERO-POSTERIOR RESECTION	1		1		1	
RESTORATION OF COLONIC CONTINUITY FOLLOWING HARTMANN'S PROCEDURE	-		-		1	
LAPAROTOMY	1		-		-	
HIGHLY SELECTIVE VAGOTOMY	1		1		1	
HIGHLY SELECTIVE VAGOTOMY AND NISSEN FUNDOPLICATION	-		-		1	
NISSEN FUNDOPLICATION	-		2		1	
PARTIAL GASTRECTOMY FOR CARCINOMA	1		1		1	
IVOR LEWIS OESOPHAGECTOMY	1		1		1	
AORTO-BIFEMORAL BYPASS GRAFT	1		1		1	

TABLE:13-3 CUMULATIVE 3 AND 6-DAY URINARY MEASUREMENTS IN THE THREE INFUSION GROUPS

		DEXTROSE-SALINE GROUP mean ± sem n	ISOTONIC AMINO ACID GROUP mean ± sem n	TOTAL PARENTERAL NUTRITION GROUP mean ± sem n
Urine volume (ml)	3-day	4633 ± 314 16	5290 ± 420 18	5145 ± 430 17
	6-day	11660 ± 607 16	13578 ± 543 15	13828 ± 811 15
Nitrogen intake ($mg\ kg^{-1}$)	3-day		360 ± 9 18	364 ± 5 17
	6-day		812 ± 10 15	807 ± 10 15
Nitrogen balance ($mg\ kg^{-1}$)	3-day	-284 ± 28 16	-241 ± 26 18*	-118 ± 28 17^{aa}
	6-day	-621 ± 55 16	-552 ± 48 15*	-265 ± 69 15^{aa}
Creatinine ($\mu mol\ kg^{-1}$)	3-day	342 ± 18 16^{b}	439 ± 22 18	459 ± 25 17^{aa}
	6-day	731 ± 33 16^{b}	903 ± 43 15	921 ± 53 15^{a}
Urea ($mmol\ kg^{-1}$)	3-day	8.07 ± 0.64 16^{bb}	17.57 ± 0.91 18*	13.74 ± 0.83 17^{aa}
	6-day	17.16 ± 1.35 16^{bb}	40.68 ± 1.79 15**	30.26 ± 1.97 15^{aa}
Potassium balance ($mmol\ kg^{-1}$)	3-day	-1.84 ± 0.15 16^{b}	-1.05 ± 0.20 18	-1.10 ± 0.18 17^{a}
	6-day	-3.21 ± 0.31 16^{bb}	-1.14 ± 0.30 15	-1.10 ± 0.30 15^{aa}

Isotonic amino acid group vs Total parenteral nutrition group: * $p<0.005$, ** $p<0.001$
Total parenteral nutrition vs Dextrose-saline group: a $p<0.005$, aa $p<0.001$
Dextrose-saline group vs Isotonic amino acid group: b $p<0.005$, bb $p<0.001$

FIGURE:13-3 CUMULATIVE 3 AND 6-DAY NITROGEN BALANCE IN THE THREE INFUSION GROUPS

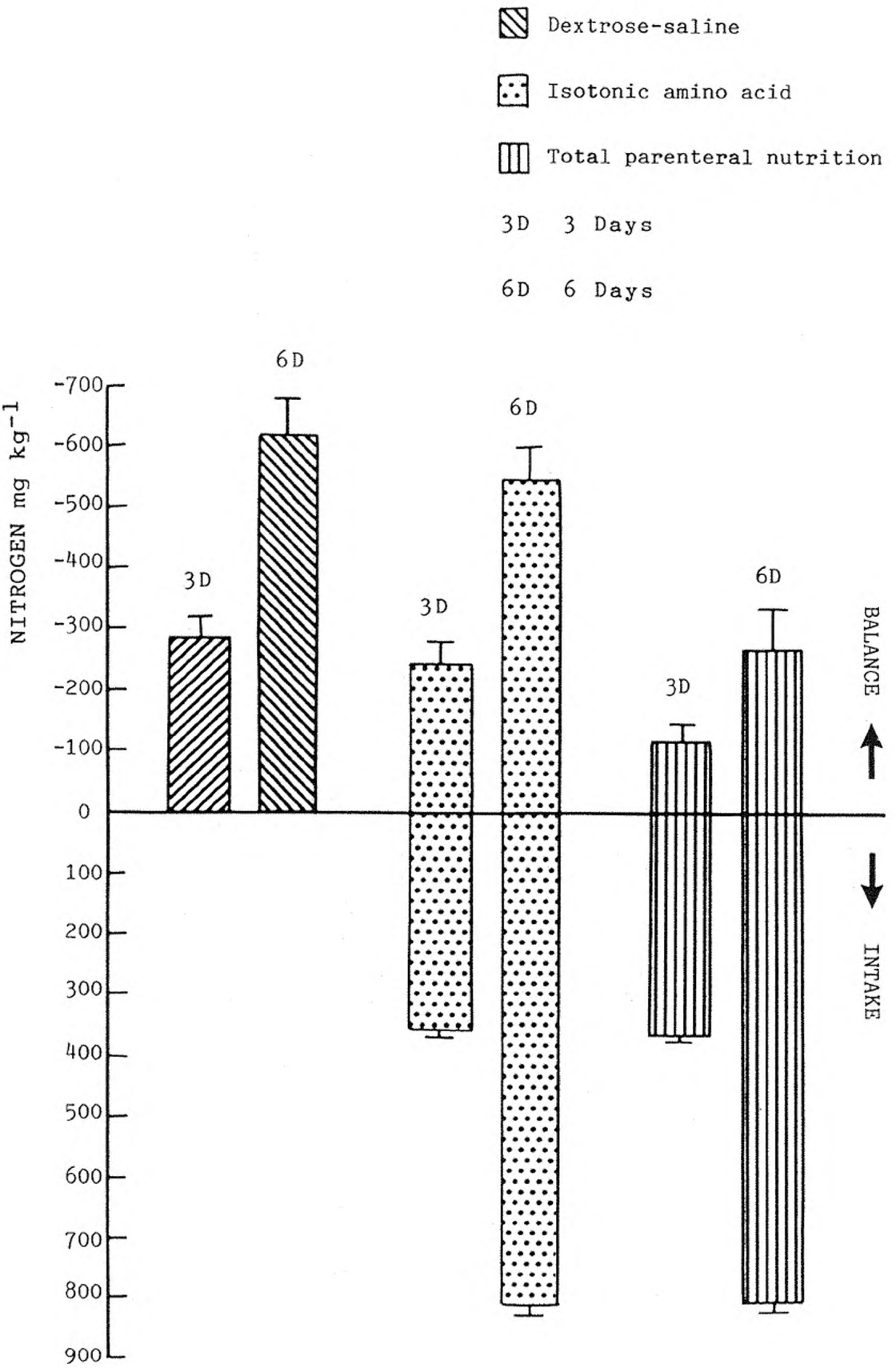


TABLE:13-4 DAILY NITROGEN INTAKE AND BALANCE (MEAN ± SEM; mg kg^{-1}) IN THE THREE INFUSION GROUPS[$]

	Day (0+1)/2		Day 2		Day 3		Day 4		Day 5	
Nitrogen intake	-		-		-		-		-	
Dextrose-saline group										
Nitrogen balance	-91 ± 6	16[b]	-109 ± 11	16	-115 ± 8	16	-113 ± 9	16	-104 ± 11	16
Nitrogen intake	113 ± 2.9	15	140 ± 6.3	15	150 ± 1.2	15	146 ± 3.4	15	149 ± 1.2	15
Isotonic amino acid group										
Nitrogen balance	-60 ± 9	15	-112 ± 18	15[*]	-112 ± 13	15[**]	-111 ± 11	15[***]	-93 ± 11	15
Nitrogen intake	108 ± 1.6	15	147 ± 2.6	15	148 ± 1.7	15	147 ± 1.5	15	148 ± 1.6	15
Total parenteral nutrition group										
Nitrogen balance	-34 ± 12	15[aaa]	-50 ± 18	15[aa]	-41 ± 18	15[aaa]	-49 ± 16	15[aaa]	-50 ± 16	15[a]

$ = Data for patients who have completed the full 6 days of the study

Significance Isotonic amino acid group vs Total parenteral nutrition group: * $p<0.02$, ** $p<0.01$, *** $p<0.005$

Significance Total parenteral nutrition group vs Dextrose-saline group: a $p<0.01$, aa $p<0.005$, aaa $p<0.001$

Significance Dextrose-saline group vs Isotonic amino acid group: b $p<0.005$

FIGURE:13-4 DAILY NITROGEN BALANCE IN THE THREE INFUSION GROUPS

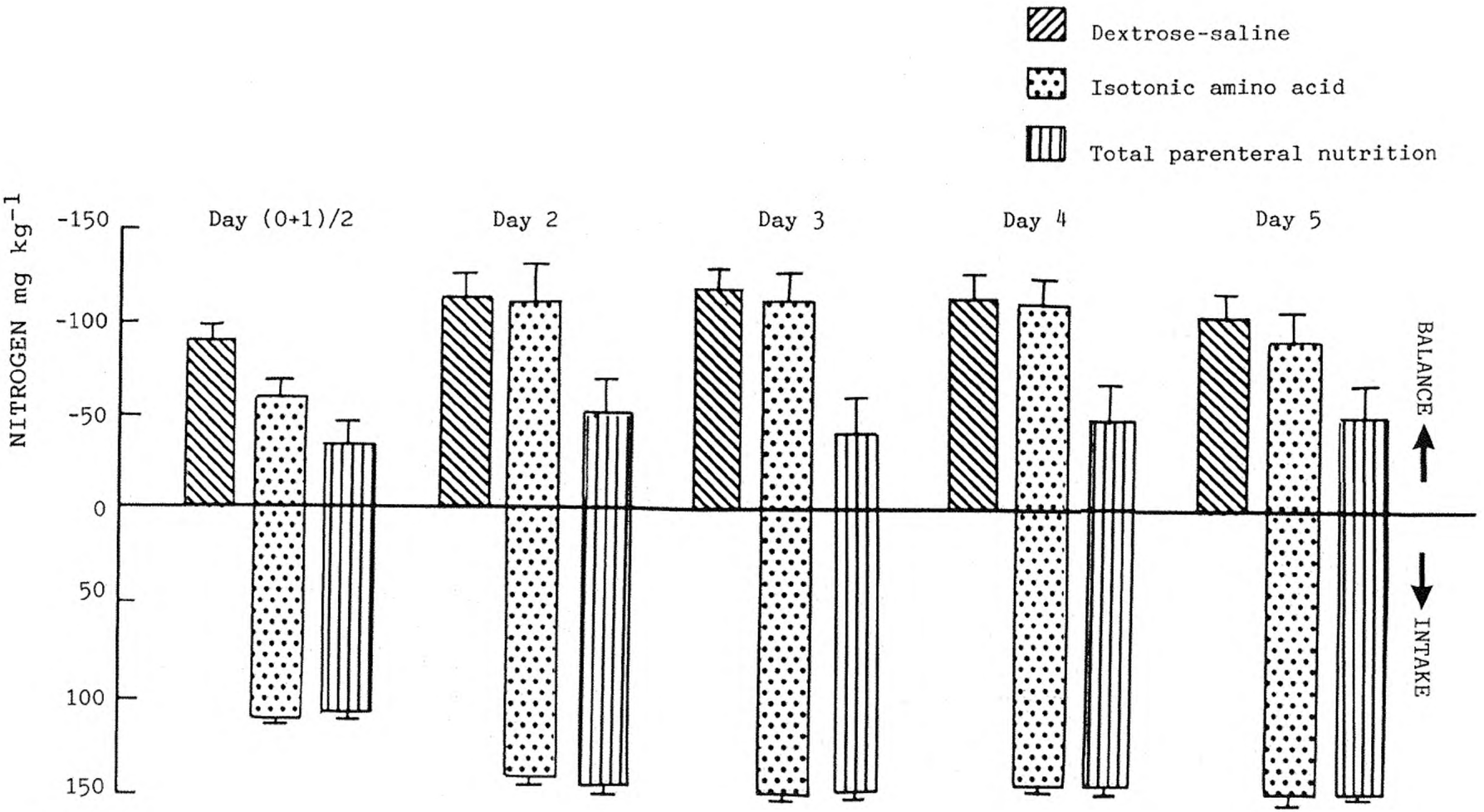


TABLE:13-5 DAILY UREAGENESIS (mmol kg^{-1}) IN THE THREE INFUSION GROUPS$^{\$}$

	DEXTROSE-SALINE GROUP		ISOTONIC AMINO ACID GROUP		TOTAL PARENTERAL NUTRITION GROUP	
	mean ± sem	n	mean ± sem	n	mean ± sem	n
DAY (0+1)/2	2.68 ± 0.17	16[b]	5.12 ± 0.26	15	4.48 ± 0.22	15[a]
DAY 2	3.19 ± 0.23	16[b]	7.73 ± 0.34	15[*]	5.46 ± 0.26	15[a]
DAY 3	2.96 ± 0.17	16[b]	8.09 ± 0.30	15[*]	4.93 ± 0.33	15[a]
DAY 4	2.92 ± 0.21	16[b]	8.07 ± 0.36	15[*]	5.47 ± 0.29	15[a]
DAY 5	2.69 ± 0.18	16[b]	7.65 ± 0.30	15[*]	5.56 ± 0.31	15[a]

$ = Data for patients who have completed the full 6 days of the study

Isotonic amino acid group vs Total parenteral nutrition group: * $p<0.001$

Total parenteral nutrition group vs Dextrose-saline group: a $p<0.001$

Dextrose-saline group vs Isotonic amino acid group: b $p<0.001$

FIGURE:13-5 DAILY UREAGENESIS IN THE THREE INFUSION GROUPS

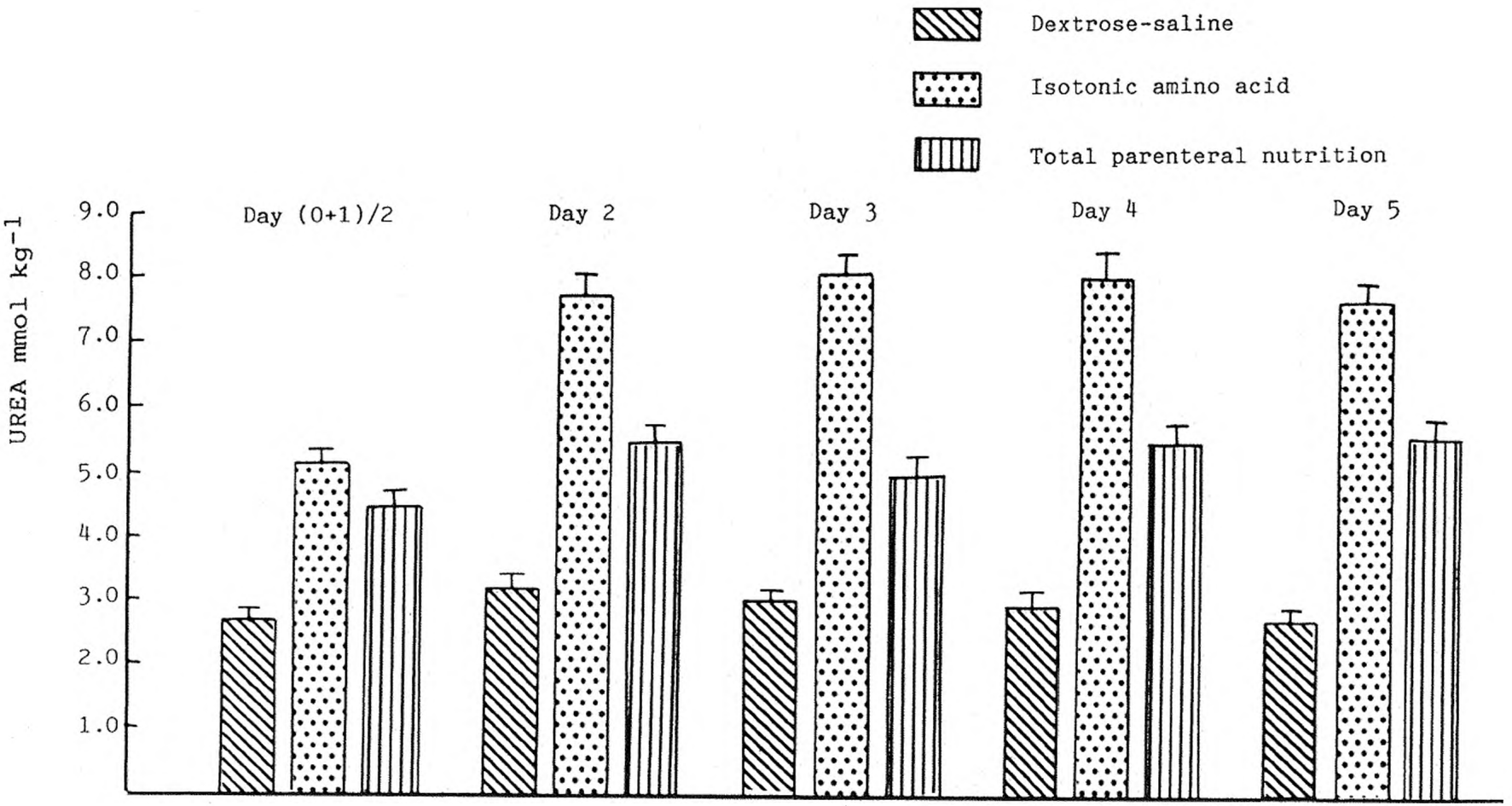


TABLE:13-6

BLOOD ANALYTE CONCENTRATIONS IN THE THREE INFUSION GROUPS

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
	D/S	4.8 ± 0.15	16	7.5 ± 0.44	16***	6.0 ± 0.36	16**	5.1 ± 0.17	16
Glucose	IAA	4.4 ± 0.12	18	aaa5.5 ± 0.16	18***	aaa4.1 ± 0.18	18	aaa4.2 ± 0.14	16
mmol/l	TPN	4.3 ± 0.12	17	6.2 ± 0.39	17***	bbb5.3 ± 0.20	17***	bbb5.5 ± 0.17	15**
	D/S	14.2 ± 0.85	16	42.0 ± 7.07	16**	24.1 ± 4.57	16*	18.4 ± 2.44	15
Insulin	IAA	11.3 ± 0.55	17	aa16.0 ± 1.22	17***	aa9.4 ± 0.79	15*	aa9.2 ± 0.65	15*
mU/l	TPN	14.0 ± 1.35	17	bb35.8 ± 5.78	17***	bbb22.8 ± 2.56	16***	bbb18.5 ± 2.07	15*
	D/S	67 ± 3	15	138 ± 15	15***	80 ± 7	15	67 ± 5	14
Pyruvate	IAA	73 ± 6	18	aaa73 ± 4	18	66 ± 5	17	64 ± 4	14
µmol/l	TPN	60 ± 2	17	98 ± 14	17*	80 ± 5	16**	a,bb80 ± 6	15**
	D/S	766 ± 52	16	1457 ± 122	16***	863 ± 95	16	777 ± 52	16
Lactate	IAA	776 ± 69	18	aaa789 ± 76	18	687 ± 47	18	641 ± 42	16
µmol/l	TPN	765 ± 49	17	1170 ± 178	17*	926 ± 91	16	bb959 ± 57	15*
	D/S	11.6 ± 0.78	15	11.1 ± 0.97	15	11.2 ± 0.72	15	12.4 ± 1.09	14
L/P	IAA	10.8 ± 0.62	18	10.7 ± 0.56	18	10.6 ± 0.49	17	10.2 ± 0.42	14
	TPN	12.6 ± 0.70	17	11.4 ± 0.42	17	11.5 ± 0.53	16	11.3 ± 0.76	15
	D/S	1.29 ± 0.06	15	2.05 ± 0.17	15***			1.14 ± 0.07	14
T.G.S.	IAA	1.30 ± 0.11	18	aaa1.17 ± 0.10	17			0.97 ± 0.06	15*
mmol/l	TPN	1.25 ± 0.06	17	1.63 ± 0.21	17*			a,bbb1.42 ± 0.06	14

D/S = Dextrose-saline group IAA = Isotonic amino acid group TPN = Total parenteral nutrition group
L/P = Lactate to pyruvate ratio T.G.S. = Total gluconeogenic substrates

Significance vs pre-operative values (paired data):	*	p<0.05	**	p<0.01	***	p<0.001
Significance IAA & TPN groups vs D/S group:	a	p<0.02	aa	p<0.005	aaa	p<0.001
Significance IAA group vs TPN group:	b	p<0.01	bb	p<0.005	bbb	p<0.001

FIGURE:13-6 BLOOD ANALYTE CONCENTRATIONS IN THE THREE INFUSION GROUPS

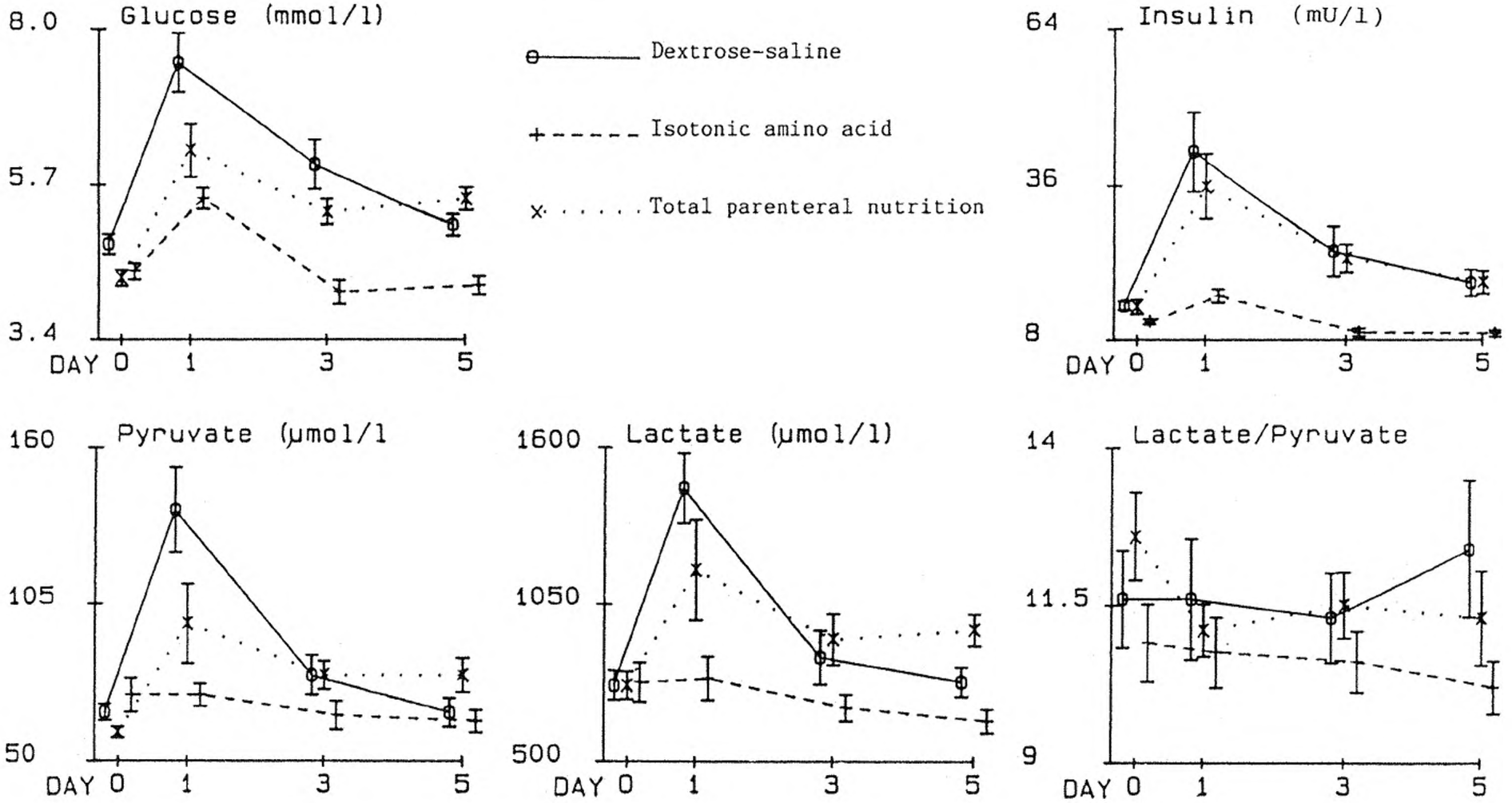


TABLE:13-7 LIPID METABOLITE CONCENTRATIONS IN THE THREE INFUSION GROUPS

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
T.K.$ µmol/l	D/S	82 (69 - 99)	16	87 (68 - 113)	16	131 (102 - 169)	16	212 (169 - 265)	16**
	IAA	97 (82 - 115)	18	aaa352 (290 - 428)	18**	aaa1238 (1069 - 1435)	16***	aaa1437 (1239 - 1667)	16***
	TPN	141 (116 - 172)	17	bbb84 (69 - 101)	17	bbb85 (70 - 103)	17**	aaa,bbb62 (50 - 77)	15*
T.T.G. mmol/l	D/S	1.40 ± 0.11	16	0.77 ± 0.06	16***	1.13 ± 0.10	15*	1.36 ± 0.09	15
	IAA	1.72 ± 0.11	18	0.83 ± 0.05	18***	a1.47 ± 0.08	18*	1.68 ± 0.10	16
	TPN	1.55 ± 0.12	16	0.74 ± 0.06	16***	bbb0.91 ± 0.06	15***	aa,bbb0.97 ± 0.07	15**
F.F.A. µmol/l	D/S	861 ± 99	16	732 ± 137	15	624 ± 32	15*	806 ± 83	15
	IAA	710 ± 56	17	860 ± 56	17**	aa838 ± 43	17*	859 ± 69	15*
	TPN	650 ± 60	17	b660 ± 50	17	aa,bbb540 ± 40	15*	aa,bbb500 ± 40	14*
Glycerol µmol/l	D/S	80 ± 8	16	82 ± 8	16	84 ± 12	16	86 ± 10	15
	IAA	81 ± 6	18	86 ± 6	18	91 ± 9	18	80 ± 10	16
	TPN	87 ± 10	17	88 ± 9	17	74 ± 6	16	70 ± 4	15*

D/S = Dextrose-saline group IAA = Isotonic amino acid group TPN = Total parenteral nutrition group

T.K. = Total ketones T.T.G. = Total triglycerides F.F.A. = Free fatty acids

$ = figures derived from logarithmic transformation (geometric mean and range of 1 sem about the mean)

Significance vs pre-operative values (paired data): * p<0.05 ** p<0.01 *** p<0.001

Significance IAA & TPN groups vs D/S group: a p<0.02 aa p<0.005 aaa p<0.001

Significance IAA group vs TPN group: b p<0.02 bb p<0.005 bbb p<0.001

FIGURE:13-7 LIPID METABOLITE CONCENTRATIONS IN THE THREE INFUSION GROUPS

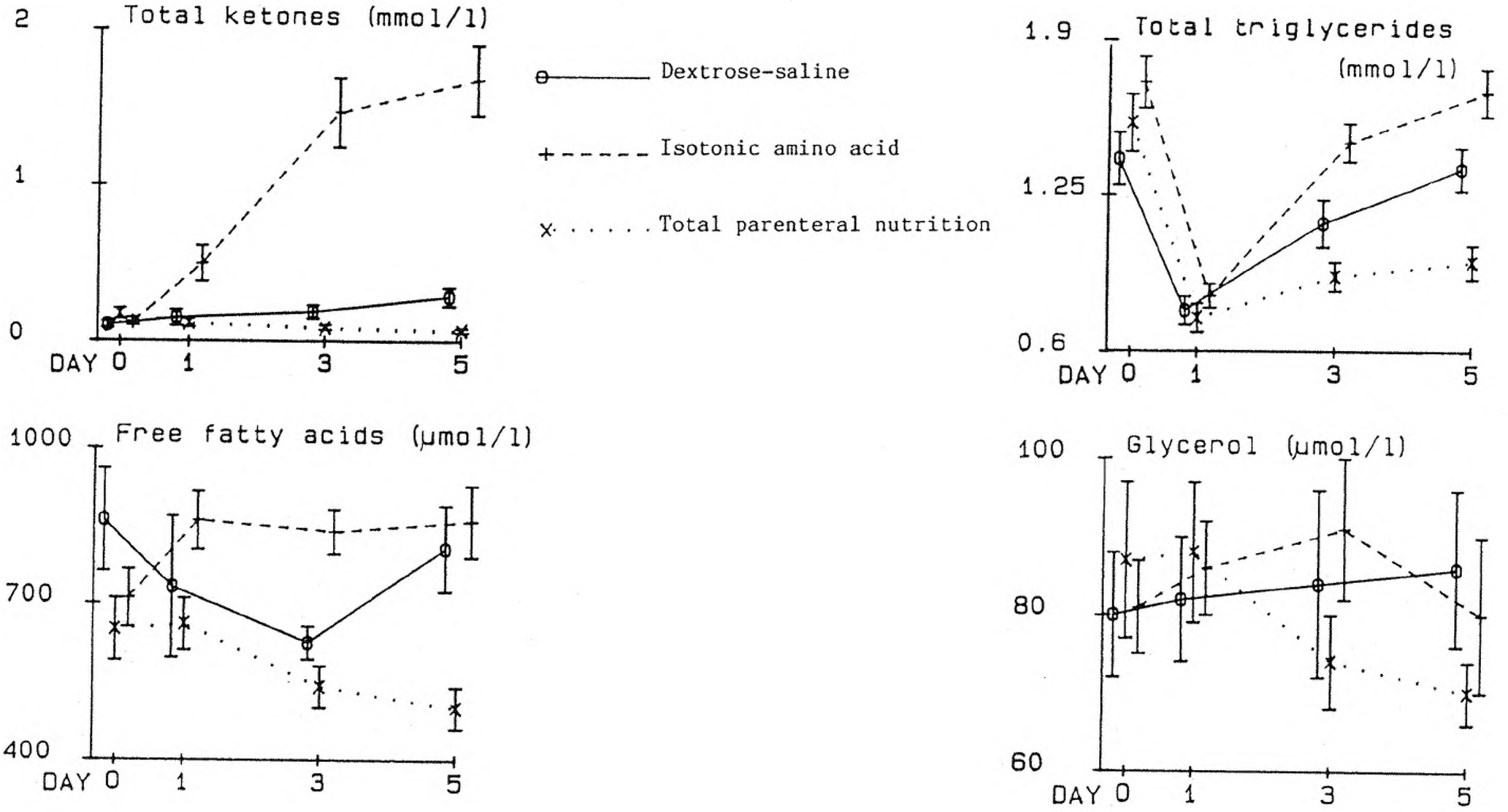


TABLE:13-8 HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE THREE INFUSION GROUPS

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
	D/S	13.6 ± 0.36	16	13.1 ± 0.26	16	12.2 ± 0.41	15***	12.1 ± 0.55	15***
Haemoglobin	IAA	14.6 ± 0.24	18	13.5 ± 0.23	18***	12.5 ± 0.29	18***	12.3 ± 0.36	16***
g/dl	TPN	14.3 ± 0.39	17	13.8 ± 0.44	17*	12.6 ± 0.44	17***	12.4 ± 0.44	15***
	D/S	0.406 ± 0.010	16	0.395 ± 0.008	16	0.373 ± 0.009	15***	0.362 ± 0.018	15**
P.C.V.	IAA	0.433 ± 0.007	18	0.411 ± 0.010	18**	0.368 ± 0.011	16***	0.366 ± 0.013	16***
l/l	TPN	0.430 ± 0.010	17	0.410 ± 0.012	17*	0.372 ± 0.011	15**	0.361 ± 0.009	15***
	D/S	43.5 ± 0.63	16	40.0 ± 0.64	16***	38.1 ± 1.60	15***	39.4 ± 1.21	15**
Albumin$	IAA	42.2 ± 0.78	18	38.7 ± 0.76	18***	39.4 ± 0.68	16***	40.8 ± 0.83	16*
g/l	TPN	41.9 ± 0.82	17	37.8 ± 0.87	17***	37.6 ± 0.58	15***	38.4 ± 0.87	15***
	D/S	69.9 ± 1.08	16	63.8 ± 1.20	16***	64.8 ± 1.91	15**	67.7 ± 1.83	15
Total protein$	IAA	69.0 ± 1.14	18	62.8 ± 1.28	18***	66.9 ± 1.38	16*	67.8 ± 1.58	16
g/l	TPN	69.4 ± 1.08	17	62.8 ± 1.09	17***	64.8 ± 0.88	15***	67.9 ± 1.27	15

D/S = Dextrose-saline group IAA = Isotonic amino acid group

TPN = Total parenteral nutrition group PCV = Packed cell volume ratio

$ = Concentrations corrected for changes in packed cell volume

Significance vs pre-operative values (paired data): * $p<0.05$ ** $p<0.01$ * $p<0.001$

FIGURE:13-8

HAEMOGLOBIN, PACKED CELL VOLUME, ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN THE THREE INFUSION GROUPS

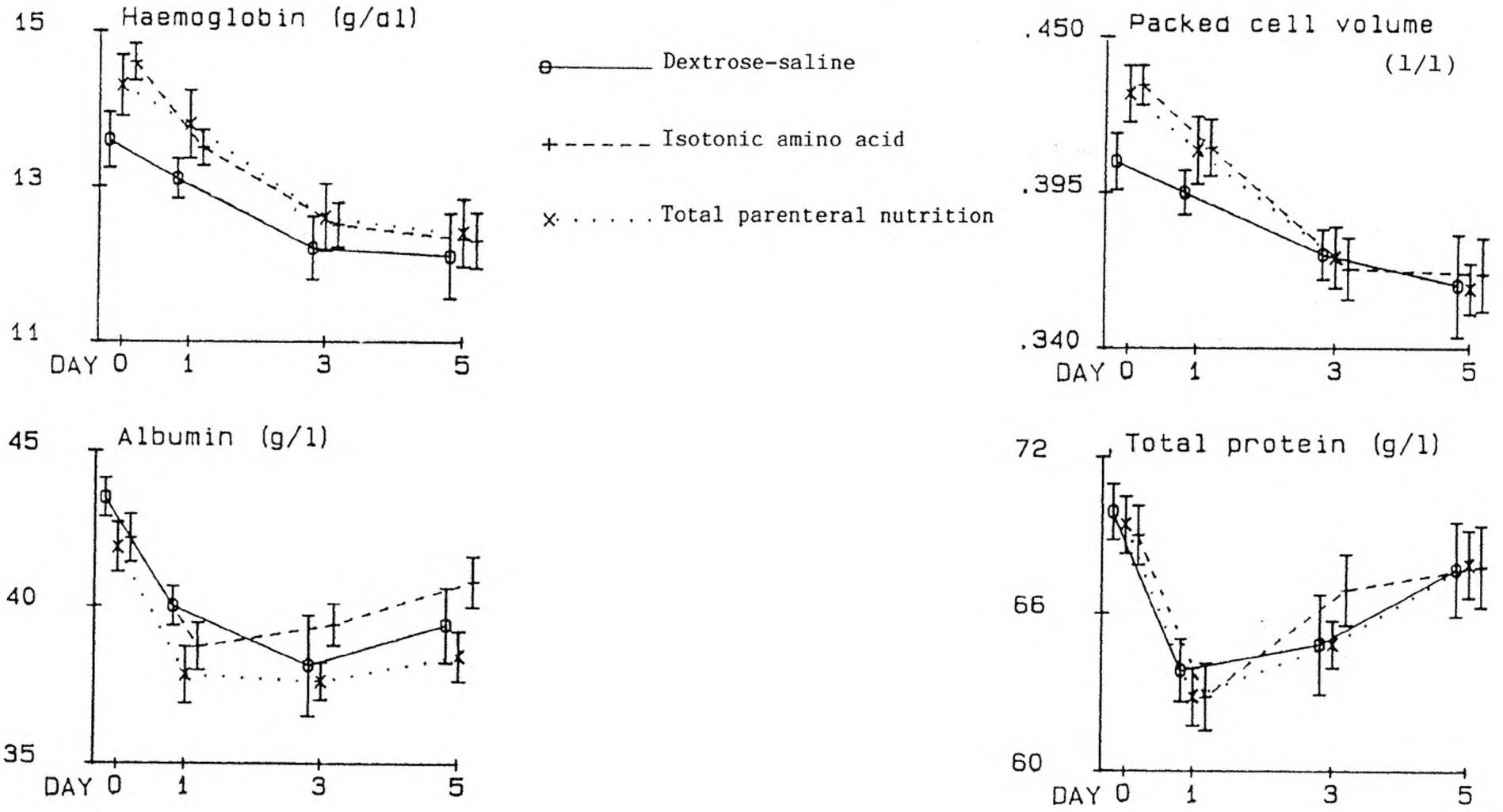


TABLE:13-9 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE THREE INFUSION GROUPS

		Day 0 mean ± sem	n	Day 1 mean ± sem	n	Day 3 mean ± sem	n	Day 5 mean ± sem	n
	D/S	82.5 ± 5.42	16	82.5 ± 10.17	16	71.1 ± 6.37	16***	69.9 ± 6.47	16**
Creatinine	IAA	85.9 ± 3.96	18	82.3 ± 4.59	18	77.7 ± 2.89	18*	75.9 ± 4.40	16*
µmol/l	TPN	92.7 ± 4.72	17	83.9 ± 4.58	17*	69.4 ± 3.59	17***	72.5 ± 3.65	15***
	D/S	4.4 ± 0.43	16	5.6 ± 0.66	16	3.6 ± 0.54	16	3.7 ± 0.48	16
Urea	IAA	4.9 ± 0.28	18	6.1 ± 0.38	18***	aaa6.3 ± 0.33	18***	aaa6.2 ± 0.47	16**
mmol/l	TPN	4.8 ± 0.28	17	5.8 ± 0.27	17**	bbb4.8 ± 0.35	17	5.2 ± 0.51	15
	D/S	28.6 ± 0.80	16	26.7 ± 0.64	16	28.1 ± 0.62	16	26.4 ± 1.22	16
Bicarbonate	IAA	29.6 ± 0.58	18	28.5 ± 0.73	18	28.2 ± 0.83	17	26.8 ± 0.78	16**
mmol/l	TPN	28.7 ± 0.50	17	bb25.6 ± 0.74	17**	aa,b25.7 ± 0.47	16***	25.9 ± 0.42	15**
	D/S	141 ± 0.36	16	137 ± 0.67	16***	138 ± 0.66	16***	139 ± 0.81	16**
Sodium	IAA	141 ± 0.63	18	138 ± 0.58	18***	138 ± 0.57	18***	138 ± 0.70	16*
mmol/l	TPN	140 ± 0.44	17	137 ± 0.50	17***	138 ± 0.84	17*	139 ± 0.53	15
	D/S	4.1 ± 0.11	16	4.1 ± 0.16	16	3.8 ± 0.14	16	3.8 ± 0.08	16
Potassium	IAA	4.3 ± 0.07	18	4.4 ± 0.11	18	a4.3 ± 0.10	18	aa4.5 ± 0.12	16
mmol/l	TPN	4.3 ± 0.07	17	4.2 ± 0.10	17	4.0 ± 0.11	17*	b4.1 ± 0.16	15

D/S = Dextrose-saline group IAA = Isotonic amino acid group TPN = Total parenteral nutrition group

Significance vs pre-operative values (paired data): * p<0.05 ** p<0.01 *** p<0.001

Significance IAA & TPN groups vs D/S group: a p<0.01 aa p<0.005 aaa p<0.001

Significance IAA group vs TPN group: b p<0.02 bb p<0.01 bbb p<0.005.

FIGURE:13-9 CREATININE, UREA AND ELECTROLYTE CONCENTRATIONS IN THE THREE INFUSION GROUPS

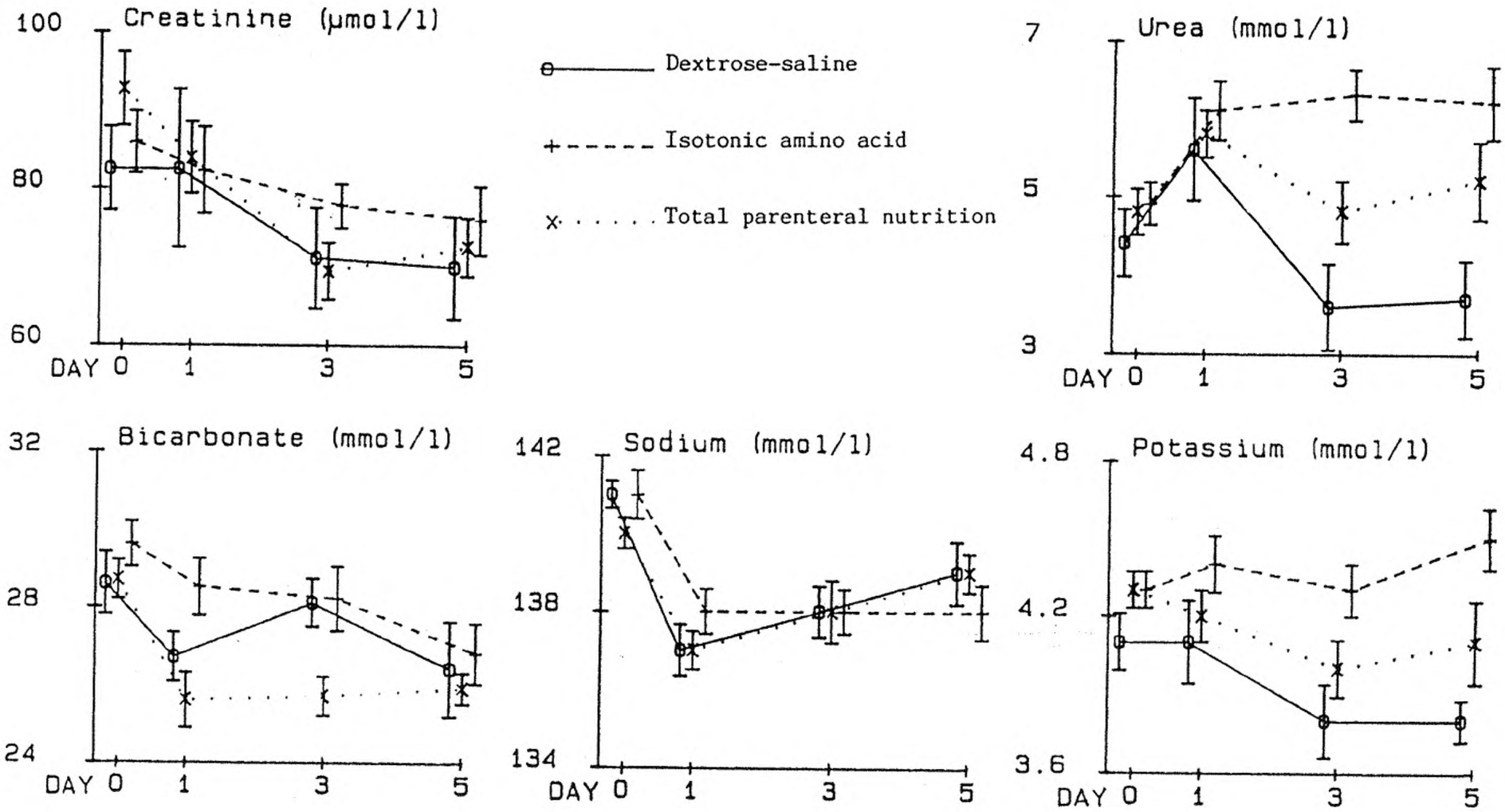


TABLE: 13-10 URINARY CREATININE EXCRETION (mmol kg(LBM)$^{-1}$).
IN THE THREE INFUSION GROUPS

	DEXTROSE-SALINE GROUP		ISOTONIC AMINO GROUP		TOTAL PARENTERAL GROUP	
	meam ± sem	n	mean ± sem	n	mean ± sem	n
Cumulative 3-day	0.54 ± 0.02	16[aa,b]	0.65 ± 0.02	18	0.66 ± 0.02	17
Cumulative 6-day	1.12 ± 0.03	16[a,b]	1.29 ± 0.04	15	1.34 ± 0.03	15

LBM = Lean body mass

Dextrose-saline group vs Isotonic amino acid group: a $p<0.005$, aa $p<0.001$

Dextrose-saline group vs Total parenteral nutrition group: b $p<0.001$

TABLE:13-11 LINEAR CORRELATIONS BETWEEN CREATININE EXCRETION AND NITROGEN BALANCE IN THE THREE INFUSION GROUPS

	INFUSION GROUPS	r	n	p
Cumulative 3-day	Dextrose-saline	0.6360	16	<0.01
	Isotonic amino acid	0.7524	18	<0.01
	Total parenteral nutrition	0.7735	17	<0.001
Cumulative 6-day	Dextrose-saline	0.5987	16	<0.05
	Isotonic amino acid	0.7987	15	<0.001
	Total parenteral nutrition	0.6674	15	<0.01

r = coefficient of correlation

n = number of patients

p = level of significance

Table:13-12 DETAILED POTASSIUM BALANCE (mmol kg^{-1}) IN THE THREE INFUSION GROUPS

		DEXTROSE-SALINE GROUP mean ± sem n	ISOTONIC AMINO ACID GROUP mean ± sem n	TOTAL PARENTERAL NUTRITION GROUP mean ± sem n
Cumulative first 3 days	K^+ excreted	1.92 ± 0.13 16^{aa}	3.15 ± 0.13 18	3.04 ± 0.16 17^{cc}
	K^+ received	0.07 ± 0.002 16^{aa}	2.10 ± 0.04 18^{b}	1.94 ± 0.02 17^{cc}
	K^+ balance	-1.84 ± 0.12 16^{a}	-1.05 ± 0.14 18	-1.10 ± 0.18 17^{c}
Cumulative 6 days	K^+ excreted	3.28 ± 0.2 16^{aa}	6.06 ± 0.2 15	5.33 ± 0.26 15^{cc}
	K^+ received	0.07 ± 0.002 16^{aa}	4.92 ± 0.09 15	4.22 ± 0.03 15^{cc}
	K^+ balance	-3.21 ± 0.2 16^{aa}	-1.14 ± 0.19 15	-1.11 ± 0.25 15^{cc}
Cumulative last 3 days	K^+ balance	-1.37 ± 0.14 16^{aa}	0.09 ± 0.09 15	0.01 ± 0.13 15^{cc}

Significance Dextrose-saline group vs Isotonic amino acid group: a $p<0.005$, aa $p<0.001$
Significance Isotonic amino acid group vs Total parenteral nutrition group: b $p<0.001$
Significance Total parenteral nutrition vs Dextrose-saline group: c $p<0.005$, cc $p<0.001$

TABLE:13-13 CORRELATIONS BETWEEN URINARY BALANCES OF NITROGEN AND POTASSIUM IN THE THREE INFUSION GROUPS

	INFUSION GROUPS	r	n	p
Cumulative first 3 days	Dextrose-saline	0.7908	16	<0.001
	Isotonic amino acid	0.7070	18	<0.01
	Total parenteral nutrition	0.5841	17	<0.05
Cumulative last 3 days	Dextrose-saline	0.3634	16	>0.01
	Isotonic amino acid	0.2037	15	>0.01
	Total parenteral nutrition	0.4585	15	>0.05
Cumulative 6-day	Dextrose-saline	0.5435	16	<0.05
	Isotonic amino acid	0.6628	15	<0.01
	Total parenteral nutrition	0.5329	15	<0.05

r = coefficient of correlation

n = number of patients

CHAPTER 14

CONCLUSIONS

The findings reported in this thesis allow the following conclusions to be drawn:

1. EFFECT OF NAFTIDROFURYL

The 12 hourly intravenous infusion of naftidrofuryl oxalate (200 mg in 250 mls of normal saline given over 2 hours) did not have any measurable nitrogen sparing effect in patients who have undergone elective abdominal surgery and received a peripheral intravenous infusion of 4% dextrose-saline (6 kcal; 25kJ) or isotonic amino acids (0.15 g nitrogen) or a central venous infusion of glucose (12 kcal; 50 kJ), amino acids (0.15 g nitrogen) and fat (12 kcal; 50 kJ) kg^{-1} day^{-1}. However, this drug had some effect on the intermediary metabolites in the glucose-containing infusion regimens (D/S and TPN groups) whereby it resulted in an attenuation in the expected post-operative rise in lactate and pyruvate concentrations and a decrease in the lactate to pyruvate ratio. Nevertheless, despite these potentially beneficial changes, naftidrofuryl oxalate had no effect on nitrogen balance.

This conclusion does not agree with the reports of Burns et al. (1981), Burns and Galloway (1984) and Galloway et al. (1983) but is in agreement with the findings of Inglis et al. (1983 & 1984). More recently, Rennie (1985) found no effect of naftidrofuryl oxalate on protein turnover or amino acid metabolism in vivo or in perfused rat muscle. However, he qualified his results on rat muscle with the caveat that rat plasma may contain an enzyme which rapidly destroys naftidrofuryl. Haggarty and Broom (1984) studied the effect of naftidrofuryl oxalate on respiration in isolated mitochondria and concluded that

the drug may stimulate oxidative metabolism by acting as a partial uncoupler of oxidative phosphorylation. If, indeed, this is the case, then the effects of naftidrofuryl oxalate are unlikely to be beneficial since electron transport and oxygen uptake will be stimulated with a lower than normal production of ATP. It is hard to understand how this could be linked to possible beneficial effects of the drug on nitrogen metabolism and might even be thought, from first principles, to have a likely deleterious effect. However, the results of the studies reported here show that naftidrofuryl oxalate has no measurable effect, either deleterious or beneficial on nitrogen balance.

2. EFFECT OF INFUSION REGIMENS

The provision of isotonic amino acids alone (0.15 g nitrogen kg^{-1} day^{-1}) increased ureagenesis without resulting in a significant improvement in nitrogen balance over a 3- and a 6-day period compared to the infusion of 4% dextrose-saline (6 kcal (25 kJ) kg^{-1} day^{-1}). However, the provision of a central venous infusion of glucose (12 kcal; 50 kJ), amino acids (0.15 g nitrogen) and fat emulsion (12 kcal; 50 kJ) kg^{-1} day^{-1} resulted in great improvement in nitrogen balance, over the same period, compared to the dextrose-saline and isotonic amino acids alone infusions.

3. OTHER OBSERVATIONS

Interesting changes were observed in the concentrations of venous plasma free amino acids. Surgery caused a rise in phenylalanine and a fall in taurine concentrations irrespective of the infusion regimen. These findings were not associated with any significant changes in tyrosine and cystine concentrations but phenylalanine/tyrosine, methionine/taurine and cystine/taurine ratios were raised. The rise in phenylalanine concentration is in agreement with the findings of Wannemacher et al. (1976) who attributed this

finding to an increased flux of this amino acid, through the plasma compartment, as the result of increased skeletal muscle catabolism. The fall in taurine concentration with the rise in methionine/taurine and cystine/taurine ratios was attributed to a probable block in the trans-sulphuration pathway. Recently, investigators have reported inadequate biosynthesis of several products of the trans-sulphuration pathway in fasted subjects and patients receiving long-term parenteral nutrition. However, taurine is the most abundant free intracellular amino acid (Soupart, 1962; Jacobsen and Smith, 1968) and its increased excretion, in the early catabolic phase, has been reported (Jacobsen and Smith, 1968; Mårtensson et al., 1985). Furthermore, as discussed previously, these venous plasma concentrations do not necessarily reflect intracellular or flux changes and the urinary excretion of the above substrates was not measured. Therefore, although no firm conclusion can be drawn, the above findings are interesting and need further evaluation. The work in this thesis did not address this problem and therefore it was not investigated further.

4. CLINICAL IMPLICATIONS

The literature review of the adverse effects of naftidrofuryl oxalate confirmed the manufacturer's claim that the drug is well tolerated in humans and the incidence of its adverse effects is low. In fact none of the patients studied here developed a side effect attributable to the drug. However, despite the widespread use of naftidrofuryl oxalate, there is only one published study which has examined the pharmacokinetics of this drug in humans (Lartique-Mattei et al., 1978) and that dealt solely with the pharmacokinetics following oral administration. The only information on the pharmacokinetics of this drug following intravenous administration in humans was available from an unpublished "in-house" study by the manufacturers (Lipha Pharmaceuticals Ltd., 1986). Furthermore, as discussed

previously, this drug does not have a recognised therapeutic serum level to aim for and there is no published data to indicate that there is any relation between its serum concentration and therapeutic effect in humans. Further studies are needed to clarify the above points.

The model of elective abdominal surgery was used as an easily controllable group on which the effect of naftidrofuryl oxalate could be studied, so that its use, if verified, could be extended to more seriously injured subjects in whom nitrogen loss presents a serious problem. Although the studies in this thesis were not designed to investigate the clinical effects of a drug or exogenous energy substrates, their findings do not support the use of naftidrofuryl oxalate or isotonic amino acids alone as nitrogen-sparing agents post-operatively. However the data confirm the use of amino acids in combination with glucose and fat emulsion as a beneficial nutritional regimen.

SECTION IV

APPENDICES AND BIBLIOGRAPHY

APPENDIX 1 Reference ranges

APPENDIX 2 Inter-assay variability

BIBLIOGRAPHY

APPENDIX 1

This appendix lists the reference ranges after overnight fast for the assays used.

Haemoglobin:	11.5 - 16.5	g/dl
Packed cell volume:	0.350 - 0.500	l/l
Blood glucose:	3.5 - 6.0	mmol/l
Plasma insulin:	4.5 - 20	mU/l
Blood lactate:	270 - 1190	µmol/l
Blood pyruvate:	30 - 120	µmol/l
Blood total ketones:	20 - 480	µmol/l
Serum total triglycerides:	0.4 - 2.6	mmol/l
Plasma free fatty acids:	150 - 1100	µmol/l
Plasma glycerol:	10 - 150	umol/l
Serum albumin:	37 - 49	g/l
Serum total protein:	62 - 80	g/l
Serum creatinine:	57 - 125	µmol/l
Serum urea:	2.5 - 7.2	mmol/l
Serum bicarbonate:	23 - 34	mmol/l
Serum sodium:	135 - 145	mmol/l
Serum potassium:	3.5 - 5.0	mmol/l
Plasma valine:	182 - 311	µmol/l
Plasma leucine:	107 - 184	µmol/l
Plasma isoleucine:	48 - 96	µmol/l
Plasma lysine:	152 - 271	µmol/l
Plasma threonine:	101 - 192	µmol/l
Plasma methionine:	26 - 42	µmol/l
Plasma tryptophan:	13 - 32	µmol/l
Plasma phenylalanine:	51 - 70	µmol/l
Plasma alanine:	264 - 443	µmol/l
Plasma taurine:	52 - 79	µmol/l
Plasma histidine:	86 - 163	µmol/l
Plasma citrulline:	38 - 57	µmol/l
Plasma arginine:	68 - 111	µmol/l
Plasma ornithine:	72 - 120	µmol/l
Plasma proline:	134 - 254	µmol/l

Plasma glutamine:	495 - 775	µmol/l
Plasma glutamate:	19 - 48	µmol/l
Plasma glycine:	211 - 360	µmol/l
Plasma tyrosine:	45 - 78	µmol/l
Plasma aspartate:	1 - 11	µmol/l
Plasma asparagine:	38 - 61	µmol/l
Plasma alpha-aminobutyrate:	21 - 42	µmol/l
Plasma cystine:	36 - 68	µmol/l
Plasma serine:	77 - 150	µmol/l
Plasma EAA:	680 - 1198	µmol/l
Plasma NEAA:	1657 - 2820	µmol/l
TGS:	761 - 1783	µmol/l

Total Ketones = sum of acetoacetate and 3-hydroxybutyrate.
NEAA = sum of non-essential amino acids.
EAA = sum of essential amino acids.
TGS = total gluconeogenic substrates.

APPENDIX 2

This appendix lists the between batch coefficient of variation for the assays used.

Blood glucose:	3.5%
Plasma insulin:	10.4%
Blood lactate:	2.7%
Blood pyruvate:	3.2%
Blood acetoacetate:	5.8%
Blood 3-hydroxybutyrate:	5.5%
Serum total triglycerides:	2.8%
Plasma free fatty acids:	8.1%
Plasma glycerol:	3.5%
Serum albumin:	1.1%
Serum total protein:	1.5%
Serum creatinine:	5.0%
Serum urea:	4.5%
Serum bicarbonate:	2.7%
Serum sodium:	0.6%
Serum potassium:	0.9%
Plasma valine:	4.1%
Plasma leucine:	2.1%
Plasma isoleucine:	3.0%
Plasma lysine:	9.0%
Plasma threonine:	6.3%
Plasma methionine:	4.7%
Plasma tryptophan:	6.6%
Plasma phenylalanine:	3.9%
Plasma alanine:	5.0%
Plasma taurine:	6.9%
Plasma histidine:	9.8%
Plasma citrulline:	7.2%
Plasma arginine:	7.0%
Plasma ornithine:	7.7%
Plasma proline:	9.0%
Plasma glutamine:	6.0%

Plasma glutamate:	5.2%
Plasma glycine:	6.9%
Plasma tyrosine:	2.8%
Plasma aspartate:	9.2%
Plasma asparagine:	7.0%
Plasma alpha-aminobutyrate:	6.3%
Plasma cystine:	8.4%
Plasma serine:	5.4%
Urinary nitrogen:	3.1%

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