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SKELETAL MUSCLE METABOLISM IN CRITICALLY ILL PATIENTS

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To my family

ABSTRACT

The loss of skeletal muscle mass is of importance for the length of the hospital stay and recovery in critically ill patients admitted to the intensive care unit (ICU). In the acute illness, the export of amino acids from skeletal muscle provides substrates for vital functions and thus enhances survival. As there is no inactive store of protein in the body, the protein being depleted represents functional tissue. Eventually the role of muscle tissue as an exporter of substrates as well as the functional properties of muscle may become impaired. Hence, in long term ICU patients the muscle depletion may become a limiting factor for survival. The aims of this project were to evaluate efforts to limit skeletal muscle wasting in ICU patients staying for a prolonged time in the unit and also to develop a protocol for interventional studies on this group of patients. These aims were approached by characterising the biochemical markers of muscle protein depletion and then investigating the effects of interventions.

The changes in the biochemical markers for muscle protein catabolism were established early on in the course of the critical illness and there was a characteristic pattern of changes in relation to the length of stay for several markers. Despite the heterogeneity of the acute clinical appearance of the patients, muscle biochemistry shows similarities in the long-term critically ill patients. Furthermore the scatters of several of the markers do not differ from the scatter in a reference population. The results made it possible to design a protocol to be used in ICU patients, as well as in healthy volunteers to evaluate interventions. The protein content was lower in patients sampled on day 5 or later of the ICU stay in comparison with the patients sampled earlier. In paired samples, the decrease over time was found to be 10 % per 5 days. Muscle protein synthesis was not different in ICU patients compared to an age matched reference group, however, the scatter was large with outliers in the high as well as the low range. The persisting catabolism leading to loss of muscle tissue is presumably of great importance for the prognosis of the disease and for the length of hospital stay and recovery. The free glutamine concentration in muscle decreased down to 25% of reference levels, with all values outside the 95% confidence interval of the reference group. The decrease was established already in the biopsies taken at the earliest time point of ICU stay, and there was no further change detected in relation to length of stay. Branched chain and aromatic amino acids concentrations in muscle were high compared to the reference group, but no further increase over time was observed. In the total patient series the concentrations remained at the same level and no further increase over time was observed. The total muscle water content was elevated in the ICU patients due to doubling of the extracellular water. In addition the intracellular water content decreased between the paired biopsies. Growth hormone treatment was shown to increase the intracellular water by 6 %. Among the energy-rich phosphates the ATP and phosphorylated creatine concentrations were low as compared to the levels in the reference group with no relation to length of stay demonstrated.

The effect of treatment with growth hormone, 0.3 U /kg/day, was marginal on protein content but increased muscle protein synthesis by 33 % ($p < 0.01$) and the free glutamine concentration by 100% ($p < 0.05$).

Supplementation with glutamine or α-ketoglutarate (0.28g/ kg bw/24 h) on the other hand, was not shown to affect protein content and showed a marginal effect on muscle protein synthesis and the free glutamine concentration. The statistical correlation between the free glutamine and muscle protein synthesis was very weak but attained statistical significance, $r^2 = 0.09$ ($p < 0.05$). Immobilization by unloading of one leg for 10 days in healthy volunteers resulted in a decrease of the RNA concentration and an increase in the concentration of branched chain amino acids indicative of muscle protein catabolism.

In summary changes in biochemical markers for muscle protein catabolism are established early in the course of critical illness and there is a characteristic pattern of changes with a relation to the length of stay for several markers. Despite the heterogeneity of the acute clinical appearance of the patients, muscle biochemistry show similarities in long-term patients in the ICU. Growth hormone was shown to affect muscle metabolism with an increase in protein synthesis rate and glutamine concentration and in addition a decrease of intracellular water content. Supplementation with glutamine or α-ketoglutarate was shown to increase protein synthesis rate and glutamine concentration only marginally. These results indicate a potential to save muscle proteins in long term ICU patients.

The thesis is based on the following papers, which are referred to by their Roman numerals:

I. Gamrin L, Essén P, Forsberg AM, Hultman E, Wernerman J (1996) A descriptive study of skeletal muscle metabolism in critically ill patiens: Free amino acids, energy-rich phosphates, proteine, nucleic acids, fat, water, and electrolytes. *Crit Care Med* 24(4):575-583

II. Gamrin L, Andersson K, Hultman E, Nilsson E, Essén P, Wernerman J (1997) Longitudinal changes of biochemical parameters in muscle during critical illness. *Metabolism* 46(7):756-762

III. Gamrin L, Berg HE, Essén P, Tesch PA, Hultman E, Garlick PJ, McNurlan MA, Wernerman J (1998) The effect of unloading on protein synthesis in human skeletal muscle. *Acta Physiol Scand* 163:369-377

IV. Gamrin L, Essén P, Hultman E, McNurlan MA, Garlick PJ, Wernerman J (1999) The protein sparing effect in skeletal muscle of growth hormone treatment in critically ill patients. *Ann Surg. Accepted for publication*

V. Gamrin L, Essén P, Hultman E, Hammarqvist F, McNurlan MA, Garlick P, Wernerman J (2000) Effects on skeletal muscle metabolism by glutamine and α-ketoglutarate supplementation in critically ill patients. *Manuscript*

ABBREVATIONS

arg	Arginine
ASP	Alkali soluble protein
ATP	Adenosine triphosphate
COPD	Chronic obstructive pulmonary disease
DNA	Deoxy ribonucleic acid
FSR	Fractional synthesis rate, % per day
GH	Growth hormone
hist	Histidine
ICU	Intensive Care Unit
leu	Leucine
ile	Isoleucine
lys	Lysine
mRNA	Messenger ribonucleic acid
PCr	Phosphocreatiene
phe	Phenylalanine
RNA	Ribonucleic acid
SD	Standard deviation
TCr	Total creatine
tyr	Tyrosine
val	Valine

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INTRODUCTION

Patients are admitted to the intensive care unit (ICU) with threatening or established failure in one or several vital organ functions after being subjected to trauma, complications following surgery and/ or severe infections. At admission to the ICU the patients exhibit a wide variety of diagnoses. The majority have surgical and internal medical diagnoses often with predominating infectious signs. Most patients, (>90%), stay in ICU less than 5 days, either recovering or expiring during this period of time. The patients surviving the acute phase of critical illness are sometimes subjected to repeated insults leading to the progressive failure of one or more organ systems turning the condition into the sustained critical condition known as multiple organ failure (Baue, 1991).

Although clinically heterogeneous at admission, the patients with sustained long-term critical illness in the ICU show an increasing similarity of the clinical picture over time. Hence, the current condition may originate from other organ systems than the one/ones involved in the primary acute disease. Consequently, the diagnosis at admission may become less related to the current treatment. Catabolism with loss of muscle mass presumably has effects on survival beyond the acute phase of the disease (Hill, 1998). Although the patients requiring long-term intensive care comprise less than 10% of the total number of patients in the ICU (Carson, 1999; Niskanen, 1999), more than 50% of total ICU resources are consumed by this group (Takala, 1999a). These patients treated in the ICU, or later on general wards, represent a large amount of health care costs (Heyland, 1998). The recovery back to independent ambulatory function is difficult and laborious for the individual patient and its duration is longer than the disease itself. Indeed, this group of patients constitutes a medical challenge as today there is no efficient prophylaxis or treatment for the muscle depletion.

Severe acute illness demands support beyond the capacity of physiological mechanisms when the patient temporarily becomes dependent on ventilatory, hemodynamic and renal function assistance. In a longer perspective, the improvements in general treatment of ICU patients has led to the survival of patients in severe conditions who would not have survived 15-20 years ago (Souba, 1994) (van den Berghe, 1998). At admission, the patients exhibit symptoms of vital organ failure of varying severity. During the acute illness, skeletal muscle proteins provids substrates for vital functions in other organs, thus promoting recovery and survival (Hasselgren, 1987), (van den Berghe, 1998). Persistent catabolism after the acute phase of severe illness with loss of muscle mass is a prominent feature of the long-term ICU patient (Hill, 1997). The outcome in the acute phase is determined by the the acute pathology and the underlying health condition but, in sustained critical illness, the depletion of skeletal muscle is of increasing importance for morbidity and mortality (Cerra, 1987) (Griffiths, 1996; Wilmore, 1998).

Energy expenditure was reported to increase by 30-60% in critical illness 30 years ago (Kinney, 1974; Kinney, 1975) and an increase up to twice the normal level was reported in patients with extensive burns. With modern treatment strategies, an increase in energy expenditure of more than 20- 30% is very rarely seen, except in extensive burns, (Douglas, 1989). Standard procedures in general ICU treatment have

been shown to affect energy expenditure, with a decrease in energy expenditure by analgesia, sedation, ventilator treatment and increased energy expenditure by inotropic drugs, physiotherapy, pain (Chiolero, 1997).

The negative nitrogen balance and loss of muscle mass in association with trauma was first described by Cuthbertson (Cuthbertson, 1932) and was also demonstrated in septic patients (Clowes, 1980). Cuthbertson also demonstrated that the proportion of elements in the urine of catabolic patients is in conformity with that of skeletal muscle. Catabolism is characterised by weight loss and reduction of muscle mass and strength (Hasselgren, 1987). The loss of muscle mass has also been described as autocannibalism (Cerra, 1980). In sepsis and after severe trauma, there is a pronounced increase in amino acid release from skeletal muscle (Aulick, 1979) (Clowes, 1980) (Clowes, 1985). The export of amino acids from the periphery provides energy substrates and substrates for protein synthesis and gluconeogenesis to the splanchnic area where the uptake of amino acids is increased (Gelfand, 1983; Wilmore, 1979; Wilmore, 1980). Postoperatively, the loss of muscle mass has been shown to be related to a reduction in protein synthesis (Rennie, 1983) (Essén, 1993) and, to a lesser extent, to increased breakdown. On the other hand, in critically ill patients, an increase in whole body protein breakdown predominates over an increased protein synthesis rate (Arnold, 1993). An important part of this is of course a loss of muscle proteins. However, so far there have been very few reports concerning synthesis and degradation of muscle proteins. In acute infections there is an increased efflux of 3-methylhistidine, indicating an increase in protein breakdown (Sjölin, 1989b). However, the protein synthesis rate has been described less. The decrease seen postoperatively cannot be extended to intensive care patients as the regulatory mechanisms are perhaps different.

The increase shown in whole body protein metabolism and in whole body energy expenditure clearly indicates that the metabolic activity is increased in other organs than skeletal muscle (Douglas, 1989). The liver produces acute-phase reactants, immunocompetent cells proliferate and produce surface markers and export proteins (Fleck, 1985) and metabolic activity, also in enterocytes and in fibroblasts in the wound area, is stimulated (Hill, 1998). All of these processes are fed by substrates exported from skeletal muscle (Wernerman, 1987). It is a well known fact that a depleted patient exhibits a less pronounced general trauma response, which is associated with a compromised ability to handle these challenges. In clinical terms, a depleted individual will have a higher morbidity and mortality in nearly every given situation (Giner, 1996). This may be interpreted as an insufficient ability to mobilise substrates in the periphery or processes of priority in central organs.

A patient with an insufficient trauma response will mobilise insufficient amounts of substrates to meet the increase in demands. It is obvious that the ability to react on exogenous challenge in trauma or infection in this way is beneficial as the ability to cope with the challenge increases (Tucker, 1996). If treatment aimed at decreasing muscle depletion results in decreased substrate mobilisation this may be harmful to

the patient. Therefore, any such treatment should be combined with a supply of sufficient amounts of substrates in the nutritional support given.

The depletion of skeletal muscle in critical illness corresponds to an altered protein metabolism with catabolism and loss of protein. The biochemical changes also include alterations in the content of energy-rich phosphates (Tresadern, 1988) and water (Bergström, 1981). The clinical problems of skeletal muscle depletion are related (i) to the risk of substrate deficiency for vital processes throughout disease and (ii) to the reduced muscle strength. If the substrates exported from skeletal muscle to the splanchnic area become insufficient the patient must rely on exogenous supply only. The loss of muscle strength in patients with durations of disease of more than 2-3 weeks has implications for the recovery phase when the patient has to regain control of the daily ambulatory functions. The loss of muscle strength may prolong the time needed for weaning off ventilator and increase the risk of recurrent pneumonia. In addition, muscle depletion will increase the morbidity of patients in case of any additional challenge. An increased risk for postoperative pneumonia has been reported in patients with preoperative protein depletion (Windsor, 1988a) and also in patients with preoperative weight loss (Windsor, 1988b).
The recovery time after critical illness and the stay in the ICU is considerably longer than the time of the acute muscle losses. In postoperative patients, for example, body composition has not returned to normal even 6 months after major elective uncomplicated surgery (Hill, 1993a).

The skeletal muscle depletion in critically ill patients has been suggested to be a determinant of outcome (Griffiths, 1996; Wilmore, 1998). Although considerable advances have been made in critical care, leading to decreased mortality due to acute circulatory and respiratory failure, muscle protein catabolism complicates recovery in the patients with multiple organ failure. The progressive depletion of muscle mass is a significant problem and treatment strategies to reduce loss of protein are vital to this patient group. (Takala, 1999a). When therapeutic efforts are evaluated, the ultimate outcome is of course survival of the patient, which is likely to be associated with a decrease in morbidity. To attain this therapeutic goal, improvements in a number of functions will be necessary. Gut function, immunocompetence and muscle strength are often considered to be of particular importance. For each of these organ systems specific measures and markers are needed to evaluate the therapy.

MARKERS OF PROTEIN METABOLISM

Protein metabolism is a dynamic process integrating the inflow and outflow of free amino acids, their metabolism – transamination, deamination in the tissue and the balance of protein synthesis and protein degradation. In healthy adults, protein synthesis equals protein degradation. It has long been recognised that trauma leads to wasting of skeletal muscle. Different approaches may be used to study protein metabolism. The avaiblable techniques differ in feasibility in the intensive care setting.

Protein metabolism in general

Nitrogen balance

Nitrogen balance is an estimate of whole body protein balance. In healthy individuals the recorded intake and excretion of nitrogen depends on the accuracy of dietary records and of the completeness of the urinary collection (Bier, 1989; Woolfson, 1986). In critically ill patients, there are a number of factors contributing to the uncertainty of the calculations of nitrogen balance. The nitrogen intake from enteral or parenteral nutrition is easy to measure, but the inclination to retain provided nutrients is heavily dependent on the adaptation to a given level of intake, with regard to amino acids as well as calories. In addition, nitrogen is provided by blood products such as packed red cells, albumin and also by antibiotics. The degradation of haematomas or gastrointestinal blood may also be a source of nitrogen contributing to the nitrogen balance. Hence, it is almost impossible to indicate a time during which this ”intake” should be considered. The excretion of nitrogen may be affected by impaired bowel function with losses through drainage of the gastrointestinal tract and also retention of large quantities of intestinal fluid. Impaired renal function also diminishes nitrogen excretion. In critically ill patients the calculation of the nitrogen balance may be affected depending on the incorporation or not of supplemented blood products. The advantages of using nitrogen balance is that it represents an estimate of whole body protein metabolism, the technique is non-invasive and the analysis is relatively simple. Thus, it must stilll be regarded as a golden standard in many situations. The disadvantage of the method is the great difficulty involved in making correct estimates of intake and excretion and also the information not reflecting changes in protein metabolism at the organ level.

Protein turnover measurements .

Amino acids can be labelled with stable isotopes and the isotopic enrichment may be measured in one or more body compartments, e.g plasma, urine, tissues. The metabolic pathway of the labelled amino acid or of total nitrogen is often represented by a metabolic model for the analyses of data. A two-compartment model is most frequently utilized, assuming that the amino acids are part of either the whole body free amino acid pool or the protein-bound pool. The interaction between the two pools is protein synthesis and degradation. Some important underlying assumptions of the model are the balance and the homogeneity of the free amino acid pool (Garlick, 1994; Rennie, 1994). Total entry and exit into the pool should be in balance and other ways of entry or exit should be negligible. Thus, it is necessary to consider dietary or intravenous intake, de novo synthesis, oxidation and conversion to other metabolites. The turnover or rate of fluxes of an amino acid is estimated from isotopic enrichment data and it is possible to calculate protein synthesis and degradation. In the most frequently used amino acid, ^{13}C leucine, the oxidation of the labelled amino acid transfers the ^{13}C to CO_2 and the expired CO_2 is sampled. When using ^{15}N Glycine turnover, the oxidation of the labelled amino acid transfers the ^{15}N to the end products urinary urea and ammonia (Picou, 1969). Measurements are made of the incorporation into ammonium or urea. However, the protein turnover rates derived from urea and ammonia often differ (Garlick, 1985; Waterlow, 1978). The measurement of amino acid turnover gives a quantitative estimate of protein synthesis and degradation. However, the use of labelled amino acids has the disadvantage of a number of underlying

assumptions concerning the precursor pool and steady state. The determination of the enrichment of label is exact, but it requires access to a masspectrometer, which is expensive.

Tissue protein content, ASP/DNA

Tissue protein content can be regarded as a reflection of the net protein balance in the tissue. The protein content of tissue samples is assayed using standard techniques (Lowry, 1951; Munro, 1964). The available methods are spectrophotometric and the reproducibility depends on precision in the point of time for reading and a standardised way of reading. The Lowry method has been employed in human studies in different conditions: in health (Forsberg, 1991) and critically ill patients (Soop, 1989). The coefficient of variation for analysis is 3-7% in repeated measurements. The conventional reference base for protein is DNA as an index of cell number rather than the fat free solid, which to a large extent consists of protein. The protein content has the advantage of providing a measure of the net protein balance. Furthermore, in a biopsy it is also gives information of status in the individual organ. The disadvantages of the protein content is that the measurement represents a mixture of proteins, that the analysis is technically difficult and the sampling technique is invasive.

Amino acid fluxes across organs

The arteriovenous difference of amino acids makes it possible to obtain information on the combined protein synthesis and degradation. The measurements are made across an organ and have been utilised for measurements across kidney (Owen, 1963), brain, splancnic area (Felig, 1969), and peripheral tissue such as the forearm (Felig, 1971) (London, 1965) (Pozefsky, 1969) and the leg (Felig, 1971). To obtain information of the exchange across the specific organ the blood flow must be included in the calculations. Blood flow measurements usually require a steady state. The measurements are often highly variable in healthy volunteers (Wernerman, 1987) and, in the ICU patient, there are a number of potential factors affecting blood flow. After the acute phase of critical illness, when haemodynamic stability is established, the regional blood flow is regulated by normal regulatory mechanisms. The regulation by the autonomous nervous system responds to stimuli such as pain, body temperature, and the ambient temperature. Even if efforts are made to acheive a steady state, there are some factors, for instance, the tone of the autonomous nervous system, which are very difficult to control. If dye dilution techniques are employed, there is sometimes uncertainty about the confinement of the dye to the intravascular space as capillary leakage is a well known feature in critically ill patients (Essén, 1998). The advantage of the arteriovenous difference is the ability to quantitate synthesis and degradation at the tissue level and the drawbacks of the method are related to the blood floow measurements, in particular the reproducibility of the steady state.

Tissue free mino acids

The amino acid content in skeletal muscle has been well characterised in man. The changes after severe trauma and injury are highly reproducible, with a profound decrease in glutamine concentration and a less marked decrease in glutamate concentration and an increase in esssential amino acids, especially the branched chain (BCAA) and aromatic acids, (AAA) (Askanazi, 1980b) (Roth, 1982). The changes in the

free amino acid concentrations have been utilized as a prognostic index for survival in severely ill patients (Roth, 1986). However, the amino acid content does not provide any information about the dynamics of protein metabolism or the underlying mechanisms. Although there is no generally accepted interpretation of the significance of the changes in amino acid content. The major advantages of the analys of free amino acids is the high reproducibility of the results. The disadvantages are the difficulties in interpretation and the invasiveness of the muscle biopsy.

Whole body protein has been measured with gamma in vivo neutron activation scanner. This requires specific eqiupment which has limited availability and is expensive (Hill, 1993b). Furthermore, small changes are difficult to dectect.

Tissue protein synthesis

Incorporation of labelled amino acids into protein.

Amino acids are incorporated into proteins by the aminoacyl-tRNA of the respective amino acid. tRNA is extremely short-lived and difficult to analyse (Watt, 1991). For measurements of protein synthesis a reliable estimate of the precursor pool has to be made. For leucine, which is one of the most frequently utilised amino acids, the enrichment of the label in the estimated precursor pool has been shown to differ from that of the tRNA (Ljungqvist, 1997). There are two major approaches to the administration of the labelled amino acid. The labelled amino acid can be given with a large dose of the amino acid, the flooding method, which is rapidly distributed to all possible amino acid compartments, including the precursor pool for protein synthesis (Garlick, 1989). This enables measurements during a relatively short period of time for muscle tissue, usually 90 min. The labelled amino acid can also be supplied in tracer amounts- the constant infusion technique (Rennie, 1982). After an initial bolus dose a low infusion rate is used to obtain a steady state when the amount of incorporated amino acid equals the disappearance from the pool. The period of time required for estimating muscle protein synthesis is usually 4-6 hours. The advantage of the method is semi-quantitative measurements of protein synthesis. The two methods of administering the labelled amino acid both have drawbacks. The constant infusion technique requires a steady state and the enrichment of the precursor is uncertain. The flooding method causes hyperaminoacidaemia, as the labelled amino acid is not given in tracer amounts. However, the label of the precursor pool is more certain. For investigations in the critically ill patients, the flooding method is chosen because it does not require a steady state, the measurements can be made in a shorter time and the label is more certainly distributed to the precursor pool (Essén, 1998). The two techniques have been employed to study volunteers and patients in stable conditions. In the fed state, the levels of incorporation are similar, while, in the basal state, values obtained by the constant infusion technique tend to be higher. When measured by the constant infusion technique, a flood gives an instantaneous doubling of the incorporation rate (Tjader, 1996b). This artefact may be interpreted as an indication of the lack of control over the precursor pool when using the constant infusion technique or an indication that the hyperaminoacidaemia used in the flooding technique invalidates the measurement. The lack of agreement between plasma enrichment and the tRNA pool during a constant infusion has been clearly demonstrated

(Ljunqvist, 1997). Postoperatively, FSR decreases by 30% immediately following elective medium scale surgery and remains low on the third postoperative day. Conventional postoperative nutrition influences FSR only very marginally (Essén, 1993). In a pilot study of ICU patients a larger scatter of the very low as well as the very high values are seen. However, the mean values are not very different from those of reference groups (Essén, 1998).

Ribosome analysis

Ribosomes are the intracellular particles on which protein synthesis occur. The ribosomes assemble in reading mRNA. The total number of ribosomes reflects the capacity for protein synthesis, while the polyribosome reflects the activity of the protein synthesis. In tissue samples the ribosomes can be isolated on a sucrose gradient and the proportion of polyribosome aggeregates can be assessed.

Concentration and size distribution of ribosomes gives qualitative information on the capacity for protein synthesis. Ribosome analyses are qualitative in nature, but the measurement does not require a steady state and repetitive measurements can be made (Wernerman, 1985). Although very useful in studies of volunteers and postoperative patients, ribosome analysis of muscle biopsies from ICU patients have not been used.

Since 80% of the RNA is contained in ribosomes, also the RNA content may be utilized as a reflection of the capacity for protein synthesis.

Tissue protein degradation.

No methods for quantification of components of intracellular protein degradation systems are available. However, degradation is of major importance for protein metabolism in severe illness. Intracellular protein degradation comprises several distinct proteolytic pathways, lysosomal and non-lysosomal. The lysosomes contain proteases, the cathepsines (B, D, E, H and L) are mainly involved in the degradation of non-myofibrillar proteins. Lysosomes also contain several peptidases for protein degradation. The non-lysosomal protein degradation mechanisms are the Ca^{++}- dependent proteases and also the cytoplasmic ubiquitin-proteasome-dependent pathways which are involved in the degradation of myofibrillar protein. The degradation of the ubiquitin-protein complex occurs in the proteasome. The ubiquitin-proteasome-dependent pathways have recently been suggested to be the main regulator of muscle protein degradation in injury and sepsis (Hasselgren, 1999).

In vitro protease activity

Cathepsins of the lysosomal protein degradation pathway may be analysed *in vitro*. Elevated levels have been reported in catabolic states (Guarnieri, 1988). Proteinase activity has been measured in humans and the activity of acid proteases decreases postoperatively (Hammarqvist, 1992b). Proteasome enzymatic activity can also be measured in human skeletal muscle and liver tissue (Andersson, 1995). Such in vitro measurements of enzymatic activity give qualitative information. However, it has not yet been demonstrated wether changes in *in vitro* activity actually reflect the change in protein degradation seen *in vivo*. Furthermore, the technique is invasive as it requires biopsy material.

3-methylhistidine

3-methylhistidine is an integral part of actin in all cells and of myosin in the white fibers. As 3-methylhistidine is not reutilised in protein synthesis and is not considered to be metaboliszed to any substantial degree and is rapidly excreted in the urine, it may be used as an indicator of the breakdown of contractile protein (Sjölin, 1989a). Efflux of 3-methylhistidine from muscle and its urinary excretion have been used as estimates of the skeletal muscle protein degradation (Sjölin, 1989b). 3-methylhistidine has the advantage of making it possible to get a qualitative estimate of protein degradation. However, the disadvantages are that it only reflects contractile protein and if the urinary excretion is used the 3-methylhistidine may originate from other tissues beside the muscle. When the efflux from the leg is used problems involved with arteriovenous difference come up and in addition the concentration of 3-methylhistidine in plasma is very low, which requires a high precision in the analyses. Urinary excretion of 3-methylhistidine is used in a number of studies. Efflux from the leg has demonstrated an increase in degradation in acute infection.

Molecular biology techniques

The genetic expression of the components of the proteolytic pathways may be studied using molecular biology techniques. Increased mRNA levels of components of lysosomal, Ca^{++} -activated and ubiquitin-dependent proteolytic pathways have been reported in head trauma patients (Mansoor, 1996) and for the ubiquitin-dependent pathways in septic patients (Tiao, 1997). Increased proteolytic expression and enzyme activity of the ubiquitin-proteasome-dependent pathway has been demonstrated in septic rodents (Hobler, 1999). Increased activity and expression of different components of the proteolytic systems are qualitative measures of protein degradation. Enzyme activity and the genetic expression of components used together have the advantage of measuring activity on different levels of the degradation systems. Although the measurements are only indirect markers of the actual protein degradation this may give information on the regulation of protein breakdown. A drawback is the invasiveness of the method.

Tissue water distribution

In tissue samples, the water content can be measured employing the chloride method. Two major points concerning the chloride method must to be considered. First the risk of contamination of the sample with chlorides. Local anesthetic contain chloride and great precaution must be taken to keep the local anaestehetics outside the fascia of the muscle. Also, the equipment used in sampling must be cleaned meticulously from chloride. Secondly, the membrane potential is assumed to be normal. However, there are reports of changes in the membrane potential in ICU patients. The cloride titration in experienced hands has a CV of 2-4 %. Despite the problems involved in the chloride method, all alternatives seem to present even greater difficulties in ICU patients.

Measurements of whole body water may be done employing dilution techniques. Deuteriated water, ethanol and bromide have been used. The employment of dilution techniques in critically ill patients carries the uncertainty of distribution space and equilibration time as compared to measurements in healthy individuals.

Measurements of bioimpedance have the advantage of being non-invasive. However, there are uncertainties about the representativity of measurements in one part of the body compared to parts of different composition, wrist-ankle vs trunk composition. Furthermore, there are problems in interpreting data in patients with water retention.

Energy rich phosphates

ATP is the immediate initial source of energy for muscle contraction while PCr constitutes a store of energy rich phosphates that can be readily utilised for muscle contraction via ATP. PCr is resynthesised after work within a few minutes. The PCr content of skeletal muscle is high compared to other tissues. Most active processes in the tissues require energy. In man, an anoxic pattern of change with a greater relative loss of PCr than of ATP has been demonstrated after exercise. In sepsis and trauma the loss is characterised by a relative greater loss of ATP and loss of PCr to a lesser extent. In malnourished septic patients the changes in ATP and PCr were more marked (Tresadern, 1988). In patients with COPD and acute respiratory failure the low levels of ATP and PCr at admission had returned to normal after treatment (Gertz, 1977).

Energy-rich phosphates are assayed by an enzymatic method (Harris, 1974). Metabolites such as ADP, AMP can be analyzed when the sample is immediately frozen in liquid nitrogen. However, it has been shown that the content of PCr after delayed freezing of 1-2 minutes corresponds better to the level in fresh muscle, and the content of ATP in unaffected by delayed freezing up to 5 min (Söderlund, 1986).

BACKGROUND

Markers of muscle protein metabolism

At the start of the project the available information on metabolic parameters in the skeletal muscle of intensive care patients was limited. In previous studies measurements had been made of free amino acids, energy-rich phosphates and water. The free amino acid concentrations has been shown to exhibit similar changes in different conditions, such as postoperatively (Askanzi, 1978), after injury and trauma (Askanazi, 1980b) (Roth, 1982) and in sepsis (Milewski, 1982). The changes in free amino acid concentrations include decreased concentrations of glutamine and the basic amino acids (lys, hist, arg) together with increased concentration of branched chain (val, ile, leu) and aromatic amino acids (tyr, phe), with more pronounced changes in septic than non-septic patients (Askanazi, 1980b). The content of energy-rich phosphates, ATP and PCr, decreases in patients with severe trauma and sepsis (Liaw, 1980), with a more pronounced decrease in patients with a prolonged disease (Bergström, 1976). In repeated sampling in severely injured patients, the decrease in ATP and PCr appeared on day 8, with a further decrease in ATP to day 30 (Larsson, 1984; Larsson, 1985). No significant changes are reported in postoperative patients and in patients with moderate injury (Liaw, 1980). The water distribution in skeletal muscle has been investigated following surgical injury when an increase in total muscle water is seen(Bergström, 1981) (Stillström, 1987) as well as in patients with sustained surgical sepsis (Soop, 1990). The whole body water content has been estimated in ventilator-dependent patients and showed a decrease in total water content over 10 days after establishing hemodynamic stability (Streat, 1987). Data on skeletal muscle protein content, the fractional synthesis rate of protein and the changes over time in skeletal muscle biochemical markers during long-term ICU stay had not been reported in the literature at the start if the present project.

Possible treatment strategies

In order to conteract muscle protein depletion and to improve nitrogen economy a number of strategies have been suggested over the years

Nutrition has an important role in the general therapy of critically ill patients. Before total parenteral nutrition was available patients who could not be fed enterally were given glucose as intravenous nutrition. Thus if sufficient nutrition could not be provided and the patient's inability to use enteral nutrition was persisting, the patient starved to death. Overenthusiastic intravenous nutritional support, sometimes called hyperalimentation, with glucose calories in excess of demand cause adverse affects such as hyperglycemia, a high CO_2 production, an increased energy expenditure, and elevated body temperature (Askanazi, 1980a; Askanazi, 1980c; Burke, 1979). Subsequently the development of intravenous amino acid solutions and fat emulsions made it possible to prevent starvation in the ICU. The provision of parenteral nutrition in the high range of the estimated demand leads to an increase in the whole body fat content (Streat, 1987). Increase of the protein supplementation up to 0.2gN/kg bw/day leads to an increased nitrogen retention but supplementation above this level does not improve nitrogen balance (Larsson, 1990).

Enteral nutrition has always been the primary alternative to nutriate critically ill patients. Recently enteral nutrition has been advocated as the superior mode of nutrition. The importance of the gut as a part of the systemic response to injury and trauma has been recognized (Souba, 1990), (Lacey, 1990). Disuse of the gastrointestinal tract leads to changes in the gut microflora, impaired gut immune barrier, and disruption if the mucosal barrier. The maintenace of gut function is suggested to prevent septic complications and organ failure (Souba, 1994). However, enteral nutrition has not been shown to prevent organ system failure (Cerra, 1988). So far, it has not been possible with conventional nutrition to achieve one of the main goals for nutritional support: to prevent further loss of protein and to reduce the negative nitrogen balance. Although patients are no longer starving in the ICU, the catabolism is persisting in long term critically ill patients even though nutrition is provided.

Anabolic steroids have been used in order to modulate the metabolic response to surgery. However, the impact on postoperative nitrogen balance has been variable in the published studies (Michelsen, 1982) (Yule, 1981).

A statistical correlation between protein synthesis and glutamine content in muscle has been demonstrated in animal studies (Jepson, 1988) (McLennan, 1987). Also, in human skeletal muscle, a correlation between the change in glutamine content and the change in protein synthesis has been demonstrated in the immediate postoperative period (Wernerman, 1990). Furthermore, supply of glutamine postoperatively spares free glutamine, counteracts the fall in the protein synthesis of muscle and improves the whole-body nitrogen balance (Hammarqvist, 1991). α-ketoglutarate is the ketoacid corresponding to glutamate and it has the same carbon skeleton as glutamine. Supplementation with α-ketoglutarate has been shown to have effects similar to those of glutamine in postoperative patients (Hammarqvist, 1991).

Biosynthetc growth hormone might reverse or attenuate muscle wasting in several patient groups. In postoperative patients, growth hormone improved the nitrogen balance after major gastrointestinal surgery when the patients were given hyponitrogenous, hypocaloric intravenous nutrition (Ponting, 1988), (Jiang, 1989), as well as in patients given adequate caloric intake (Mjaaland, 1991), (Hammarqvist, 1992a). In addition the concentration of free glutamine and the rate of protein synthesis in skeletal muscle are preserved after elective open cholecystectomy (Hammarqvist, 1992a), and the efflux of free glutamine is decreased (Mjaaland, 1993). In patients with burn injuries, growth hormone administration improves the nitrogen balance (Ziegler, 1990). Moreover, in children with severe burns, the time for the healing of skin graft donor sites is reduced and the hospital stay is shortened (Herndon, 1990).

PROTOCOL CONSIDERATIONS

The evaluation of metabolic and nutritional treatment in long-term intensive care patients involves several methodological problems. The patient group is heterogeneous, and therefore it is difficult to obtain study groups of a reasonable size. The number of patients available is not very large. In medium sized units, usually fewer than 100 patients/year stay longer than 5 days in the unit. This means that groups of more

than 20 patients are often are unrealistic. If the endpoints are clinical or comparatively easy to sample, multi-center studies may be used. For more invasive measurements, however, a protocol in a single centre is often the only possibility. Another difficulty is the length of the study period. If a certain treatment is instituted, a detectable result is more likely, the longer the study period. However, extending the study period from 5 to 10 days means that the number of patients that it is possible to recruit decreases dramatically. The number of patients staying for more than 10 days is definitely less than 50 each year in a medium-sized unit.

Over the years there has been an intensive discussion as to whether or not the results in postoperative patients are applicable to ICU patients. There is an extensive amount of literature on postoperative patients, and in that situation, it is possible to design fairly homogenous patient groups of reasonable size. In the field of metabolism and nutrition, it has been shown that nutrition improves nitrogen balance, that glutamine supplementation prevents the decrease in muscle free glutamine and further improves the nitrogen balance, that growth hormone, given as an adjunct to nutrition improves muscle protein synthesis, prevents the decrease in muscle free glutamine and also improves the nitrogen balance among other examples. It is also possible to show that a certain treatment affects morbidity resulting an a shortened hospital stay. This measure of morbidity is highly controversial and it is currently discussed at almost every single congress. Nevertheless, in order to conduct controlled studies in homogenous groups of patients, such a measure may have a meaning. With respect to parenteral nutrition, this is not the treatment of choice in either postoperative patients or intensive care patients. However, it has the advantage over enteral nutrition that the prescribed dose of nutrients is actually given, in more than 95% of cases. This is much more problematic for enteral nutrition. Study protocols involving postoperative patients given parenteral nutrition including some manipulation do therefore have a rationale although they can not be considered as a treatment recommendation. If one look at metabolism in muscles, however, there are several features that show a difference between postoperative patients and long-term ICU patients. For example, muscle glutamine depletion is much more extensive in ICU patients, and the decrease seen in muscle protein synthesis postoperatively is not a uniform finding in ICU patients. The reported increase in protein breakdown in ICU patients is not found postoperatively in most cases. Therefore the results obtained in postoperative patients can only constitute evidence with limited implications for the ICU situation. As for animal studies, no models for long term critical illness are at hand. There is of course a limitation in time for most animal experiments involving invasive procedures. The most frequently used animals, such as rodents or pigs present a number of severe problems when studies are extended over time and involve sepsis, SIRS or sustained infection. In acute situations, animal studies give valuable information concerning regulatory mechanisms but when it comes to long-term studies, the implications for intensive care patients are much less obvious.

Against this background, there are very few other options than to conduct this type of pilot studies in intensive care patients. The effort to make a clinical outcome study that has a reasonable statistical power to detect differences in morbidity and mortality involves a very large number of patients. Therefore, the

rationale for studies in smaller groups of patients in which markers of therapeutic effects are the end points is important for building up good hypotheses for the outcome studies. From this perspective, the rationale to try to develop a protocol with a suitable clinical design is an obvious option.

The clinical condition of the critically ill ICU patients cannot be described as stable. At admission, the patients are often haemodynamically unstable due to dehydration, sepsis, and/or cardiovascular failure. When they are being treated and their condition is improving, there is increasing stability in their general condition. Nevertheless, when the acute phase in over, there are still variations in hemodynamic, respiratory and renal functions that occur within hours, sometimes within minutes throughout the stay.

The available biochemical markers of muscle protein metabolism were evaluated with regard to the information to be obtained in relation to the invasiveness of the sampling procedure and the strengths and weaknesses of the methods. The results of arteriovenous difference studies reported in the literature are often difficult to interpret, and especially the blood flow measurements in the intensive care setting are difficult to reproduce.

The muscle biopsy technique provides the opportunity for sampling with (i) several analyses to be made in the same tissue sample, (ii) repeated sampling and (iii) no steady state required. In analyses peformed on biopsy material, the scatter is often not very marked, which is a condition for conclusions to be drawn in small groups of patients. The disadvantage is the invasiveness of the method. We chose to utilise the percutaneous muscle biopsy technique, including analyses of amino acids, protein, nucleic acids, water, energy-rich phosphates, and subsequently, the protein synthesis rate.

AIMS

In general terms, the aim was to evaluate the possibility of nutritional and metabolic measures to limit skeletal muscle wasting in ICU patients with multiple organ failure staying for a long time in the unit. This aim was approached in a stepwise fashion, starting with characterisation of the biochemical markers and then investigating the effects of the interventions.

The biochemical markers in skeletal muscle were characterized in order to develop a clinical protocol enabling evaluation of interventions in small groups of patients. This includes the choice of parameters to be used as markers, the evaluation of a suitable patient group to be studied and the length of the study.

Specific aims for the separate studies

Study I: To characterise biochemical markers for muscle catabolism in critically ill patients in relation to the length of the ICU stay.

Study II: To describe the changes in biochemical markers of muscle catabolism over time in paired samples from ICU patients.

The main effect variables and the time for sequential sampling in studies III, IV and V were based on the findings in studies I and II.

Study III: To investigate the effect of unloading/immobilisation *per se* on the changes in biochemical markers of skeletal muscle in healthy volunteers.

Study IV: To investigate wether pharmacological doses of growth hormone attenuates muscle protein catabolism in long term ICU patients.

Study period V: To investigate wether glutamine and α-ketoglutarate supplementation counteracts muscle glutamine depletion and muscle protein catabolism in long term ICU patients.

METHODS

Patients and reference groups

The patients were investigated after being adequately resuscitated to obtain haemodynamic stability. In addition, there were sometimes disturbances of coagulation parameters during the initial period of the ICU stay, either as a feature of sepsis or due to anticoagulant treatment in renal replacement therapy, which made percutaneous muscle biopsies impossible due to the potential risk of bleeding.

In studies I and II, the patients were studied at different points in time during the disease and in study IV and V the patients were investigated after establishing hemodynamic stability after the acute phase of critical illness. The patients were long-term in the ICU with multiple organ failure during the course of the disease. The total patient series is comparable regarding severity of disease (APACHE-II score at admission), length of ICU stay at the time of the investigation, age, gender, body weight. (table 1)

Table 1

Study	n	Age	Gender, m/f	kg bw	ICU day study start	s/ns	APACHE-II, at admission
I	20	61 ± 18	14/7	79 ± 21	6 ± 3	15/5	20 ± 5
II	9	55 ± 15	5/2	81 ± 22	9 ± 4	7/2	20 ± 6
IV	10 +10	56 ± 15	17/3	77 ± 14	11 ± 10	13/7	16 ± 5
V	9+ 9+ 9	60 ± 15	15/12	69 ± 12	9 ± 3	20/7	19 ± 6

The patients in the present studies are a highly selected group out of the total patient series. They constitute less than 10% of the total number of patients in the ICU and are the ones most likely to benefit from an improved nutritional therapy as the loss of muscle mass is presumably of importance for morbidity and for the length of hospital stay and recovery.

The patients or, if communication with the patients was not possible, their next of kin were informed about the purpose, procedure and possible risks involved in the study and in the sampling procedure before obtaining their informed consent. The study protocols were approved by the Ethics Committee of the Karolinska Institutet, in Stockholm, Sweden.

The reference groups are taken from other studies analysed in close connection in time in the same laboratories and by the same staff as the analyses in studies I, II, III, IV, and V. The reference groups were all sampled in the postabsorptive state after fasting overnight. As there is no complete age-matched reference material for all the biochemical markers involved, the reference groups for the biochemical markers (a- d) are taken from the following studies:

a. The reference material for free amino acids in muscle consists of data from metabolically healthy patients scheduled for elective cholecystectomy and colorectal surgery (n= 17+ 16) of an age similar to that of the critically ill patients (Hammarqvist, 1995; Hammarqvist, 1992a). No correlation between age and the free amino acid content has been demonstrated in the present study or in previous studies.

b. The reference group for alkali soluble protein (ASP), DNA, RNA and water in muscle consists of healthy subjects of similar age as the critically ill patients, n= 20, analyses were made in the same

laboratory by the same staff as the samples from the patients. A statistically significant decrease in protein content has been demonstrated with increase age (Forsberg, 1991).

c. Energy-rich phosphates in muscle from healthy male volunteers, n= 12, who were not age matched (study III and unpublished data) This reference group was chosen because of the close connection in time of the analyses. However, a reference group of patients of similar age (mean age 65 years), has been reported with ATP and PCr levels similar to those in the reference group (Lennmarken, 1986). Content of energy-rich phosphates is not regarded as being age related.

d. FSR for muscle protein from metabolically healthy patients scheduled for elective cholecystectomy (Hammarqvist, 1992a; Tjader, 1996a) minor surgery (Tjader, 1993), or colorectal surgery (Hammarqvist, 1995) n= 39, with age similar to that of the critically ill patients and 12 healthy volunteers, study III. In the combined reference group, n= 50, there is a correlation with age (fig 1).

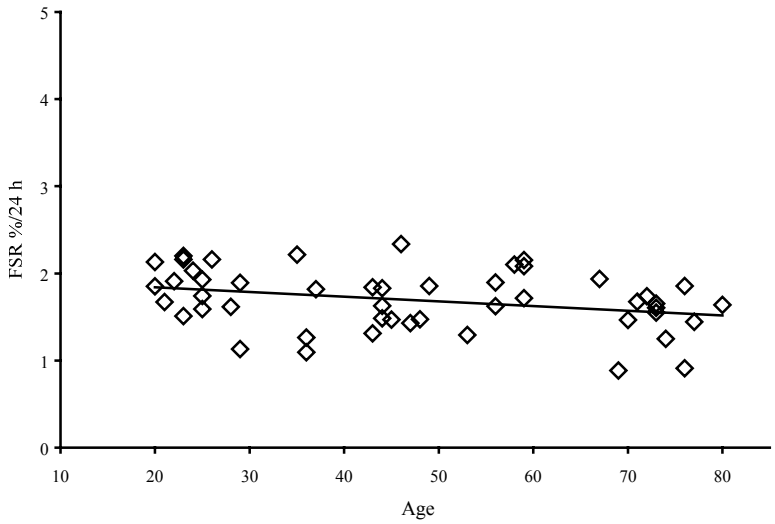

Fig. 1
Correlation between protein synthesis rate, FSR, and age in a reference group of healthy volunteers and patients scheduled for abdominal or minor surgery, n=50, $p < 0.05$, $r^2 = 0.09$. Data from study III and unpublished data, (Tjader, 1993, 1996, Hammarqvist, 1995).

Clinical routines

The investigated patients were recruited at Huddinge University Hospital ICU (n= 54, studies I, II, IV and V), and at S:t Görans Hospital ICU (n= 13, studies IV and V). The patients were comparable regarding APACHE-II score at admission, age. (tab 2). Although a University Hospital and a General Hospital the wards have similar medical and general care routines due to the same person being responsible for both wards at different points in time. All aspects of clinial routines discussed below apply to both units. The wards have 24 hour coverage by residents or fellows in anaesthesiology and intensive care in attendance on a daily basis and on call for emergencies and for attending the patients in the ICU.

Table 2

Hospital	n	Age	Gender, m/f	kg bw	ICU day	APACHE-II, admission
Huddinge University Hospital	34	59 ± 14	22/12	73 ± 12	9 ± 7	17 ± 6
S:t Görans Hospital	13	60 ± 17	11/2	71 ± 16	10 ± 8	17 ± 5

Haemodynamic instability was initially treated with fluid resuscitation and/or inotropic drugs. After the acute haemodynamically unstable phase, diuretics are given to facilitate excretion of the fluid overload. The daily fluid balance in long-term patients is aimed at being slightly negative to prevent further fluid retention, thus diuretics are perscribed daily in low doses.

The standard routines of the ward were employed. The daily care of the patients includes physiotherapy for pulmonary function and prophylaxis against muscular contractures several times daily, and also mobilisation from the bed as soon as the condition of the patient allows.
If patients were expected to require ventilatory treatment for more than 5-7 days, a tracheostomy was often performed after the first week to facilitate suction of secretions from the airways and also to have the patient as consciuos as possible, so as to prevent sedation due the discomfort of an oral endotracheal tube.
After the acute phase of illness, the aim is to let the patient be as awake as possible, the pain, anxiety and agony of the patient being evaluated and treated prior to this. When required, the patients were sedated with benzodiazepines or propofol. Patients were treated with analgetics when indicated, analgesia was provided by systemic administration of paracetamol and often opioids. During the past few years, there has been increased utilisation of epidural analgesia with bupivacaine and sufentanil to control abdominal and/or thoracic pain. No patients received continuous muscle relaxants; when indicated muscle relaxants were given as part of general anaesthesia during short procedures such as tracheostomy.

Corticosteriods were supplied to a small number of patients due to chronic obstructive pulmonary disease, COPD, and as part of treatment of severe obstructivity, low-dose hydrocortisone or betamethason was given in decreasing doses. Patients treated with steroids were included in all the groups.

The nutrition was given according to general routines as described in the studies. In studies IV and V the amino acid supply was perscribed by the investigator, furthermore the nutrition was standardised in all patients for 12 h prior to sampling when nutrition was discontinued and a low-calorie glucose solution was supplied for the sampling to be made in the postabsorptive state. In case of hyperglycaemia short acting insulin was provided as a continuous infusion to avoid glucose levels of 12 mmol/L or higher. The number of patients receiving insulin was equal in all groups, except for the GH treated patients who required higher doses in a slightly increased number of patients.
The total series was collected over a period of 6 years. During this period of time there were a number of changes in the general stategies in the treatment of critically ill patients, which could not be standardised for throughout the period. The ventilatory strategy has developed towards increased utilisation of non-invasive ventilatory strategies, mainly continuous positive airway pressure (CPAP), which was used as an alternative to ventilator treatment with endotracheal intubation in a small number of patients of during the latter part of the studies. The reduced intrathoracic pressure is unlikely to influence the results. Enteral feeding has been more used frequently both in the administration of small amounts of enteral feeds and also in patients receiving the major part of the estimated caloric requirement enterally. In studies IV and

V, a few patients received more than 50% of their caloric intake enterally, wheareas no patients recieved the main part of the caloric support enterally in studies I and II. The treatment of pain has developed towards more freqent utilisation of epidural analgesia for pain relief. Epidural analgesia might reduce administration of opioids which probably has implications on the success rate of enteral feeding. However, the influence of these developments in general treatment have not been shown to have a major impact on the results.

Twenty patients were investigated in study I. The decrease in protein content was shown to be established from day 5 of the ICU stay. The paired samples of study II showed that a reduction of approximately 10% per week in ASP/DNA is the ”natural course” for long-staying patients in the ICU with multiple organ failure. This finding was the basis for choosing the 5 day study period. The calculation of the size of patient groups was also made on the basis of the reduction in protein content. A change of 5% over 5 days in the protein content could be demonstrated in 10 patients with 80% power.
The patients who could be included in the studies were, as mentioned before, only a small fraction of the total number of patients in the ICU. The number of long term patients in the ICU are on average 4- 5 per month, but all of these are not eligible due to underlying diseases with disturbances in the coagulatory system preventing muscle biopsy, such as renal failure, haematological malignancies, and liver dysfunction. The advantage of including a larger number of patients must be contrasted with the disadvantage of running a study over an extended period of time.

Study protocol

The study protcol of study IV and V is including provision of growth hormone or glutamine/α-ketoglutarate for 5 days. Muscle biopsies were taken before and after the study period. On each occasion two biopsies were taken 90 minutes apart. At the first biopsy, muscle tissue was taken for analysis of protein, nucleic acids, amino acids, energy-rich phosphate, and electrolytes. Immediately following the first biopsy a flooding dose of L-[$^2H_5$]phenylalanine was given. After 90 minutes a second biopsy for determination of the incorporation of L-[$^2H_5$]phenylalanine in muscle was taken, (fig 2).

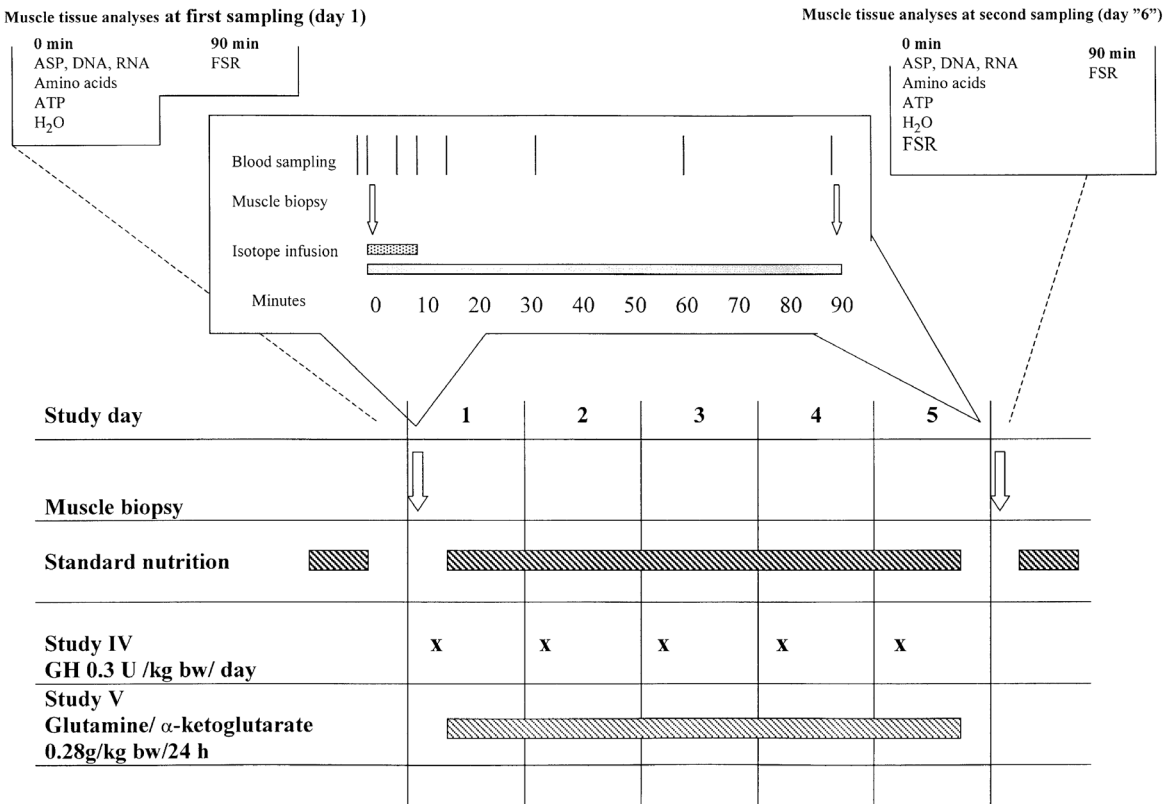

Fig. 2
Study protocol for study I, II, IV and V in critically ill patients.

Analytical procedures

Muscle biopsy

The technique is described in detail elsewhere (Bergström, 1962). In brief, after applying local anaesthetic to the skin, a muscle biopsy specimen was taken from the lateral portion of the quadriceps femoris muscle, approximately 15 cm above the knee. Special care was taken not to introduce the local anaesthetic into the muscle tissue since that would produce errors in the analyses of water and chloride. The tissue specimen was divided into portions for determination of protein, nucleic acids and water, for an amino acid determination, and in studies IV and V for determination of the protein synthesis rate. Each piece of muscle tissue was weighed three times within 24 seconds on an automatic electrobalance to calculate the wet weight extrapolated back to time zero. Specimens taken for analysis of water, protein, nucleic acids and energy-rich phosphates were frozen in liquid nitrogen 1- 2 minutes after sampling. It has been shown that the phosphocreatine content in muscle after a 1 minute delay in freezing corresponds to the level in fresh muscle and that the ATP content is unchanged after a delay in freezing of up to 6 min (Söderlund, 1986)]. All samples were stored at –80C^{o} until analysed.

Protein and nucleic acids

The frozen samples were freeze-dried, weighed, fat-extracted with petroleum ether, and reweighed to obtain the total muscle water and fat. The muscle sample was dissected free of blood and connective tissue, powdered and divided for analysis. After precipitation with perchloric acid, alkali-soluble protein was analysed with spectrophotometric analysis according to Lowry (Lowry, 1951). For the analysis of

RNA, the DNA and protein were precipitated and RNA was analysed by the direct spectrophotometric method of Fleck and Begg (Fleck, 1965).

Amino acids

The muscle specimens were homogenised and, after precipitation with sulfosalicylic acid and centrifugation, the free amino acids were separated and quantified by ion exchange chromatoghaphy and the amino acid derivatives were detected by fluorescence (Kedenburg, 1971).

Water content

The water distribution was calculated from the chloride and water content of the muscle specimen. Since chloride is freely diffusible across the the muscle cell membrane and follows Nernst's equation, the ratio of the intracellular to the extracellular chloride concentration will be 26:1, assuming a resting membrane potential of –87.2 mV. The extracellular chloride concentration was calculated from the plasma concentration, which was corrected for protein content and the Donnan equilibrium factor. Estimates of total water were based on these analyses and calculated as described elsewhere, (Bergström, 1983). Sodium, potassium and magnesium were determined in an atomic absorption spectrophotometer. Chloride was analysed by electrometric titration against silver nitrate, using a pH meter.

Energy-rich phosphates

ATP, PCr and total Creatine were analysed. Other metabolites such as ADP and AMP could not be analysed due to the chosen freezing procedure. The frozen samples were freeze-dried and the metabolites were extracted with perchloric acid. Enzymatic reactions were measured in a spectrophotometer (Harris, 1974).

Protein synthesis

The determination of the protein synthesis rate in human muscle by the flooding technique using L-[$^2H_5$]phenylalanine has been described previously (McNurlan MA, 1994) After intravenous injection of 45mg/kg body weight of L-[$^2H_5$]phenylalanine (15 mol% excess) into a vein over a 10 minute period, blood samples from an indwelling arterial or cubital vein catheter, inserted for the sampling, were taken from the other arm at 0, 5, 10, 15, 30, 60 and 90 min. A percutaneous muscle specimen was taken at 90 min. at the first sampling. At the second sampling, the procedure was repeated with L-[$^2H_5$]phenylalanine of higher enrichment (30 mol%excess) and percutaneous muscle biopsy specimens being taken at 0 and 90 min. Enrichment of phenylalanine in plasma samples was determined after acid precipitation. The enrichment of phenylalanine in muscle protein was determined after precipitation with 0.2 mol/l perchloric acid with extensive washing to remove free phenylalanine and hydrolysis with 6 M HCl at 110^o C for 24 h. The determination of L-[$^2H_5$]phenylalanine enrichment in both plasma samples and in samples of hydrolysed muscle protein was made by GC-MS under electron impact ionisation and selective ion recording (EL-SIR). The enrichment in plasma was measured by monitoring the ions at mass-to-charge ratio (m/z), 336 and 341 of the t-butyldimethylsilyl derivative and that in hydrolysates by

converting to phenethylamine and monitoring the ions at m/z 106 and 109 of the heptafluorobutyryl derivative (McNurlan MA, 1994)
The fractional rate of protein synthesis, expressed as a percentage of the protein pool synthesized per 24 h, was calculated from the enrichmentor change in enrichment of phenylalanine in protein divided by the area defined by the plasma phenylalanine enrichment time-curve.

Reference bases

The measurements are expressed in relation to a number of different reflections of the intracellular mass. The most frequently utlilised reference base is fat-free solid. This is the dry matter of the cell, up to 70% of which which is composed of protein. The reference base DNA is utilised as a marker for the number of cell units. The protein and RNA content is often related to DNA, which is considered to have a longer half-life than the majority of cellular components. The intracellular water content is used as a reference base for soluble compounds such as electrolytes and amino acids due to their small size and solubility. The total creatine content in muscle is utilized for energy-rich phosphates since it has a half life of about 30 days and is reported to be very resistant to changes (Bergström, 1976). The protein and water content has been shown to change over time, and although these changes may have implications for the results no better alternative reference bases have been identified. These reference bases are well-established concepts and are used when biochemical parameters in skeletal muscle are reported in the literature (Forsberg, 1991).

Age

With increasing age, there is a reduction in fibre size and number, which has been demonstrated in autopsy material. The atrophy begins at the age of 25 and accelerates thereafter. There is a loss of fibres with no predominant effect on any fibre type and, to a lesser extent, a reduction in fibre size, mostly in type 2 fibres (Lexell, 1988). The protein content, ASP/DNA, in skeletal muscle is known to be related to age with a reduction in protein content with increasing age (Forsberg, 1991).

Statistics

Values are given as means ± SD if not indicated otherwise. Student's t-test for paired samples was used to compare results between first and second biopsies. Student's t-test for unpaired samples was used to compare results between groups. Correlations were evaluated using Pearson's correlation coefficient.

DISCUSSION OF RESULTS

The primary end points were the biochemical markers of skeletal muscle depletion in critical illness. These are discussed in detail below, less significant markers are touched upon more briefly.

Protein content, ASP/DNA

In study I, the muscle protein content was lower in patients sampled on day 5 or later of the ICU stay, compared to sampling before day 5. Patients sampled before day 5 were shown to have protein content levels corresponding to the levels of the reference group (Forsberg, 1991). In the paired samples in study II the decrease over time was found to be approximately 10 % per week. The decrease in relation to the length of stay was confirmed in studies IV and V, when the group was extended with the initial sample from these patients ($p<0.001$, $r^2=0.33$). Furthermore, the observation of the decrease in the paired samples in study II was reproduced in the control patients of studies IV and V who exhibited a decrease of a similar magnitude, 10 % per 5 days ($p < 0.01$), n=19 (fig 3).

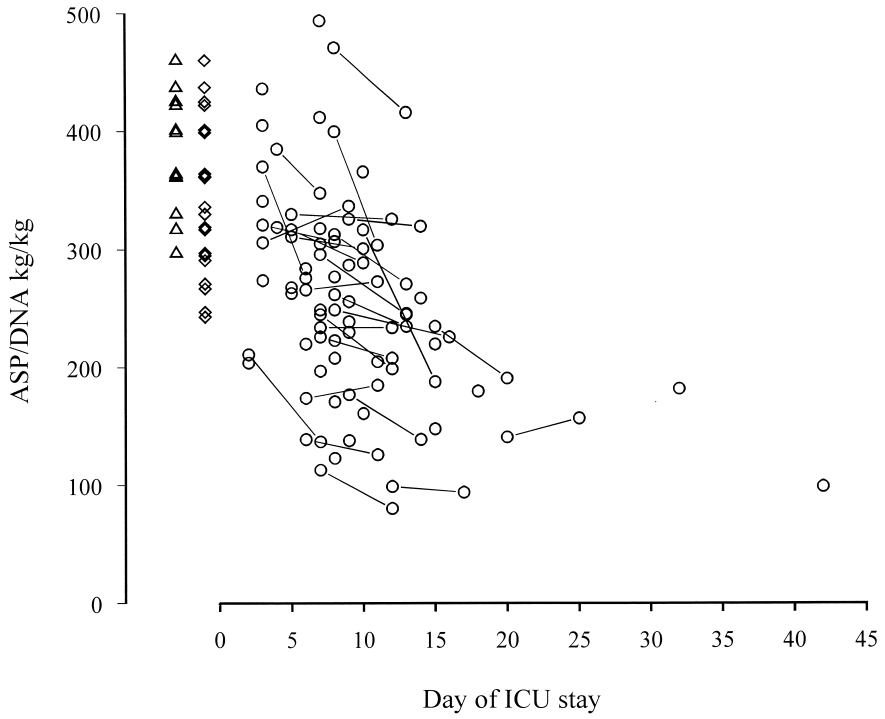

fig 3
Muscle protein content, ASP/DNA, in critically ill patients (n = 67) with repeated sampling in 28 patients and levels in a reference group, (n=33) of healthy volunteers and an age matched volunteers. o- patient data from study I, II, IV and V. Δ - data from study III and unpublished data, n= 12. - data from age matched volunteers, n= 21 (Forsberg 1991).

In study III, unloading of one leg did not significantly alter the protein content. In study IV the protein content decreased by 8% in the control patients ($p< 0.01$) with no significant change in the GH group. In study V, glutamine and α-ketoglutarate supplementation were not shown to affect the protein content. Extremely few observations of muscle protein content have been reported in the literature, except for pilot observations (Soop, 1989). In particular the change over time has not been described or quantified before.

Protein syntesis rate, FSR

The muscle protein synthesis fractional rate measured by the stable isotope technique has been shown to be slightly decreased (Essén, 1998) compared to a reference group. In studies IV and V the mean FSR of the initial biopsy, 1.73 ± 0.77 %/ 24 h, was not different from the rates in an age-matched reference material. (fig 4). However the scatter, in-between individuals is larger in the group of long-term ICU patients than in healthy subjects, with outliers in the high as well as in the low range. A similar pattern was seen in the previous study from our group (Essén, 1998), although the very high rates seen in a few subjects in the present study have not been noted previously. A positive statistical correlation, r^2= 0.33, ($p < 0.01$) between the day of ICU stay and the levels of FSR in the first biopsy was seen in the combined patient group from the previous (Essén, 1998) and present studies (IV and V) (fig 5). However, no significant change was seen between the paired samples of the control patients in studies IV and V.

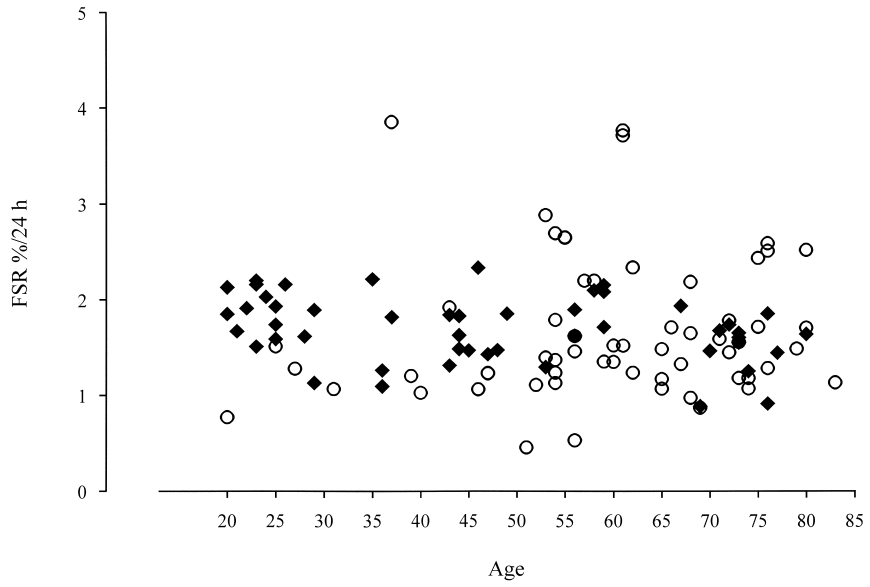

Fig. 4
FSR %/day in relation to age in critically ill patients, n=60, and a reference group, n=50. o- patient data from (Essén, 1998), and Gamrin: study IV and V, ◊ - reference group data from Gamrin study III and unpublished data, (Tjader 1993, 1996, Hammarqvist 1995)

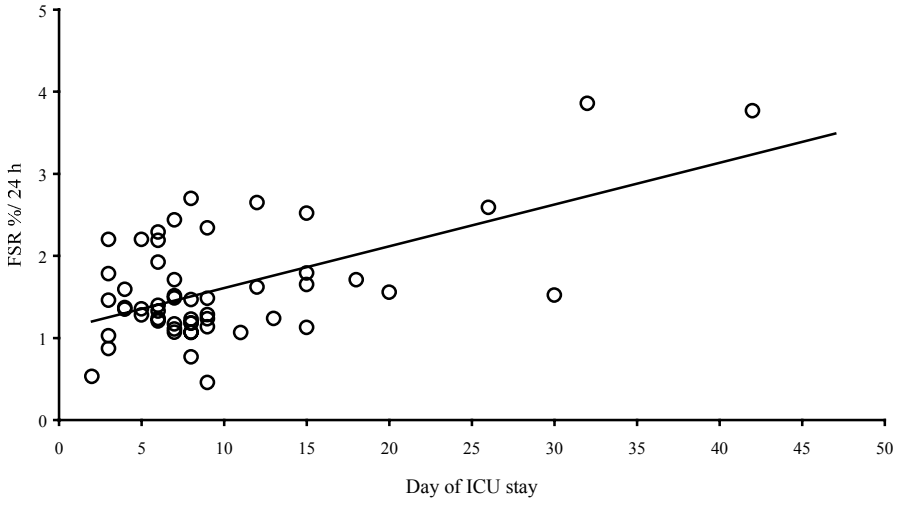

fig 5
Correlation between protein synthesis rate and length of stay in ICU in first sampling from critically ill patients, $p< 0.01$, $r^2 = 0.33$. Patient data from (Essén, 1998) and study IV and V.

Unloading of one leg did not affect FSR in study III. In study IV, the fractional synthesis rate of muscle proteins increased in the GH group by 33% ($p< 0.05$) while in the control patients no change was seen. No changes were demonstrated in the treatment groups or the control group in study V. However, when the calculations of protein synthesis rates were done in the two treatment groups together, there was an increase 76% ($p< 0.05$). The muscle protein synthesis rate in ICU patients has only been reported in a limited number of studies (Essén, 1998; Mansoor, 1997; Mittendorfer, 1999). In these studies the patients groups were highly selected (head trauma and non-adult burn patients) and no measures of the possible change over time has been reported.

Glutamine

Glutamine concentration decreased in study I down to 25% of the reference levels, with all values outside the 95% confidence interval of the reference group. The decrease was already established in the biopsies done at the earliest point in time, day 3, of ICU stay, and there was no further change detected in relation to the length of stay. The glutamine concentration remained at the same low level in the paired samples in study II. The first sample of the patients in studies IV and V confirmed the low intramuscular glutamine concentration, and no change was recorded in the paired samples of the control patients in the same situations (fig 6).

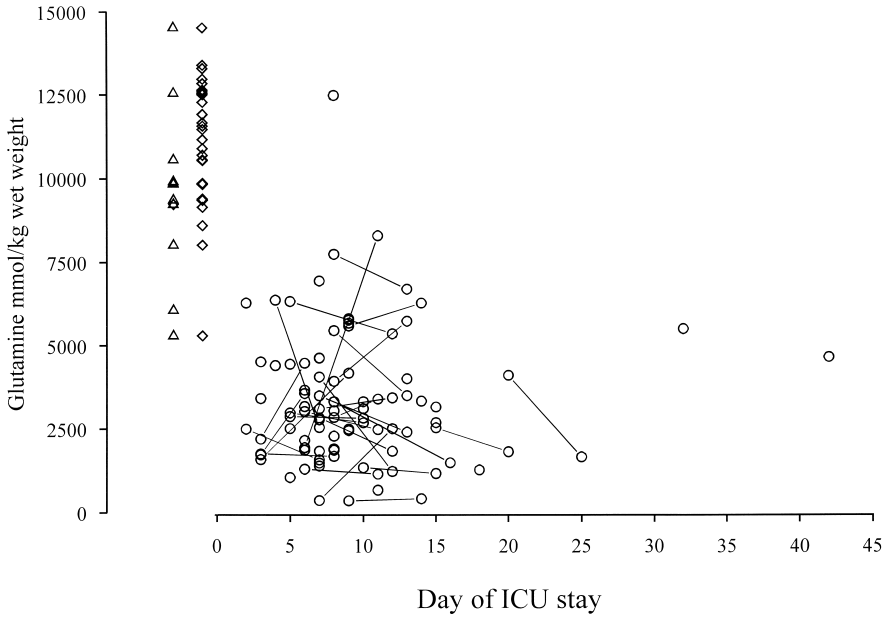

Fig. 6
Muscle free glutamine concentration in critically ill patients (n = 67) with repeated sampling in 28 patients and levels in a reference group, (n=42) of healthy volunteers and patients scheduled for abdominal surgery. o- patient data from study I, II, IV and V. Δ - data from study III and unpublished data, n= 12. ◊ - data from (Hammarqvist 1992, 1995), n= 30.

In study III, no effect on the glutamine concentration was detected after 10 days unloading. In study IV muscle free glutamine concentration increased by 100 % in the GH group ($p< 0.05$) while no significant change was seen in the control group. In study V the concentration of muscle free glutamine did not change in response to glutamine/α-ketoglutarate treatment. However, there was an increase in glutamine concentration approaching significance ($p= 0.08$) of the change in the combined treatment groups.

The profound reduction of glutamine concentration in skeletal muscle of ICU patients after injury and sepsis has been described previously by several investigators (Milewski, 1982), (Askanazi, 1980b), (Roth, 1982). In postoperative patients, the levels of glutamine have been shown to decrease within hours after surgical trauma (Essén, 1992), by 17% on sampling 24 hours after surgery (Blomqvist, 1995) and by 40% on day 2-3 postoperatively (Hammarqvist, 1989). Low intramuscular levels of glutamine in mechanically ventilated critically ill patients have been demonstrated. In burn patients investigated 12 days after the burn imjury the glutamine concentration was 30% of normal (Mittendorfer, 1999) and in patients studied 3 days after admission to the ICU, the glutamine concentration was reported to be 19% of the normal concentration (Palmer, 1996).

Branched chain amino acids, Aromatic amino acids

In study I the concentrations of branched chain and aromatic amino acids were high compared to the concentration in the reference group. In study II the concentrations remained at the same level and no further increase over time was observed. In studies IV and V, the levels of branched chain and aromatic amino acids remained at the high level also in the paired samples of the control patients. (fig 7). An increase in the branched chain acids, 48 %, concentration was seen ($p < 0.05$) in study III. In studies IV and V, the concentration of branced chain and aromatic amino acids did not respond to intervention with GH or supplementation with glutamine/α-ketoglutarate. Following injury and sepsis, branched chain and aromatic amino acids have been reported to increase (Milewski, 1982), (Askanazi, 1980b), (Roth, 1982). A decrease has been reported at 12 hours postoperatively with a subsequent increase at 24 hours (Essén, 1992), and after 2-3 days (Hammarqvist, 1992a).

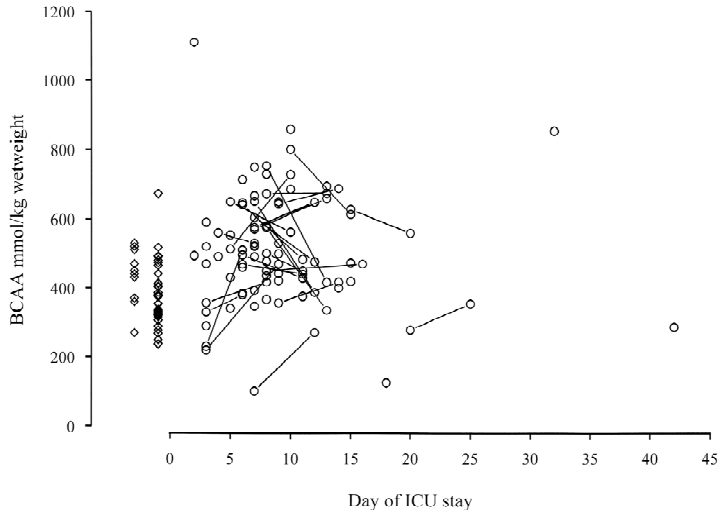

fig. 7a

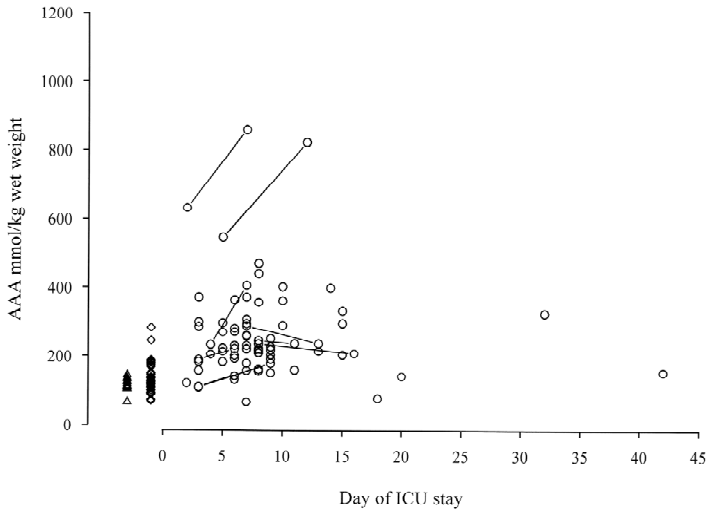

fig. 7b

fig 7
Muscle free amino acid concentration, a) BCAA and b) aromatic amino acid (AAA) in critically ill patients (n = 67) with repeated sampling in 28 patients and levels in a reference group, (n=42) of healthy volunteers and patients scheduled for abdominal surgery. o- patient data from study I, II, IV and V. Δ - data from study III and unpublished data, n= 12. ◊ - data from (Hammarqvist, 1992, 1995), n= 30.

Water distribution

In study I the total water content increased by 10% due to a doubling of the extracellular water content, compared to the reference group. The intracellular water content was not different from that in the reference group. No significant change in the paired samples was seen in study II. When the patient group was extended with the control patients of studies IV and V, the total water increased by 6% ($p < 0.05$) between biopsies due to an increase in extracellular water by 55% ($p < 0.01$), while the intracellular water content decreased between biopsies by 5% ($p < 0.05$) (fig 8). These findings are in accord with reports on the whole body water content in patients undergoing major surgery (Cheng, 1998) (Plank, 1998). Early on in the course of disease when fluid compartments in ICU patients are sometimes overexpanded due to fluid resuscitation, the whole body water changes have been reported to be largely accounted for by changes in the extracellular water (Finn, 1996) while the intracellular water decreases (Gatzen, 1992). Critically ill patients investigated later during the disease show a marked expansion of extracellular water and disturbance of the ratio between extracellular water and total water, as well as a reduction of intracellular water on a whole body basis (Gatzen, 1992). In study IV, the total water increased by 12 % in the control group, ($p < 0.05$), due to an increase in the extracellular water of 67 %, ($p < 0.05$). The intracellular water increased by 6 %, ($p < 0.05$) in the GH group.

In study V, the total water content decreased by 6 % in the α-ketoglutarate group ($p < 0.05$), but no changes were observed in water content in the glutamine and control groups.

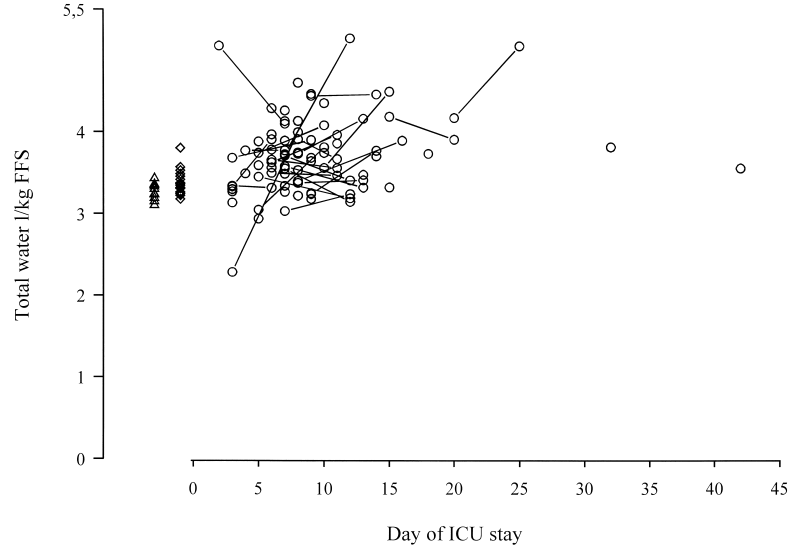

fig. 8a

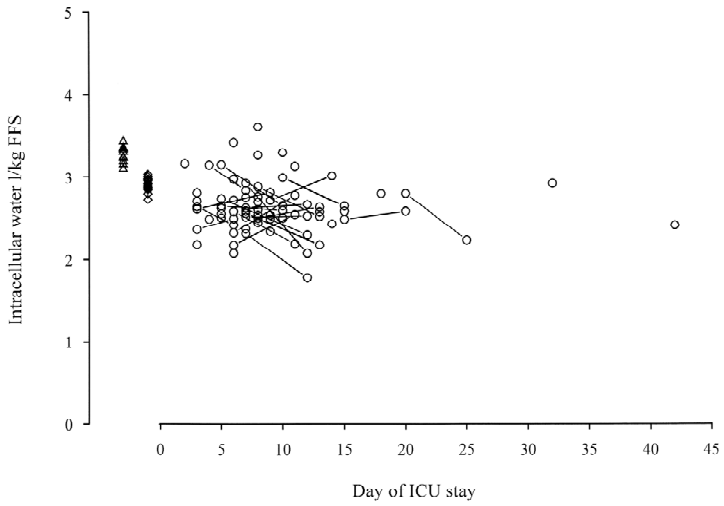

fig. 8b

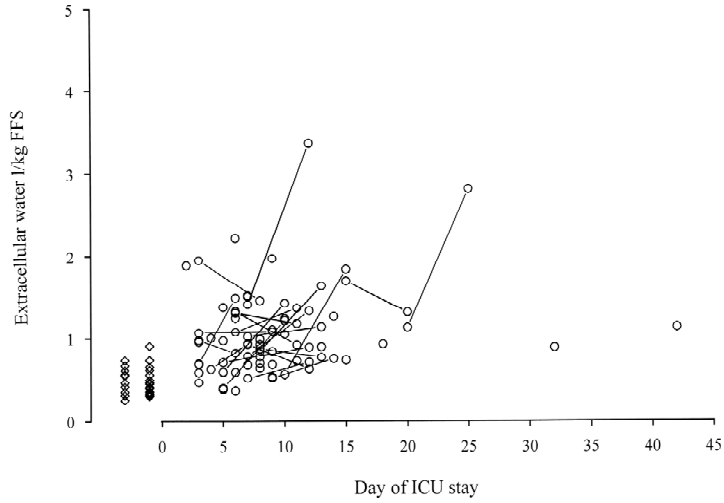

fig. 8c

Fig. 8 Water content: a) total water, b) intracellular water c) extracellular water in critically ill patients (n = 67) with repeated sampling in 28 patients and levels in a reference group, (n=33). o- patient data from study I, II, IV and V. Δ - data from study III and unpublished data, n= 12. ◊ - data from age matched volunteers, n= 21 (Forsberg, 1991)

Energy-rich phosphates

In study I, ATP and PCr were low compared to the levels in the reference group and no demonstrable relation to the length of stay. No change between the paired samples was seen in study II. ATP in relation to total creatine and the phosphorylated fraction of creatine showed similar changes to those when the compunds were related to fat free solid (fig 9). The content of energy-rich phosphates, ATP and PCr decreases in critically ill patients following severe trauma and sepsis (Liaw, 1980), with a more pronounced decrease in patients with a sustained disease (Bergström, 1976) and a pronounced decrease also in septic and non-septic malnourished patients (Tresadern, 1988). In repeated sampling in severely injured patients, the decrease in ATP and PCr was seen on day 8, with a further decrease in ATP to day 30 (Larsson, 1984; Larsson, 1985). No significant changes have been reported (Liaw, 1980) in postoperative patients and in patients with moderate injury. No effect on energy-rich phosphates of unloading was seen in study III. In study IV, GH treatment did not affect the content of energy-rich phosphates. In study V, phosphorylated creatinine decreased in the control group, but no changes in energy-rich phosphates were seen in the glutamine and α-ketoglutarate groups.

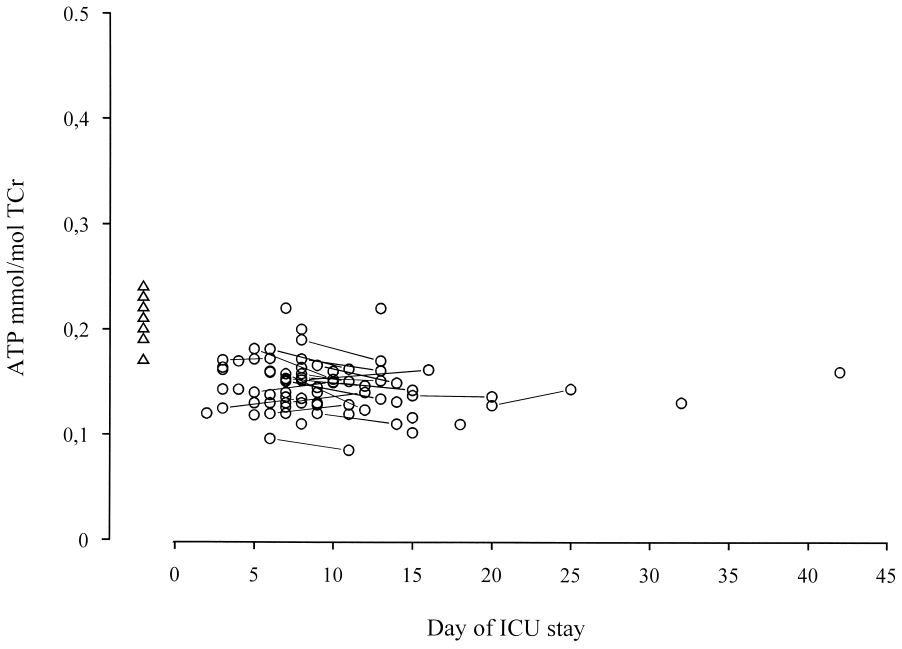

Fig. 9a

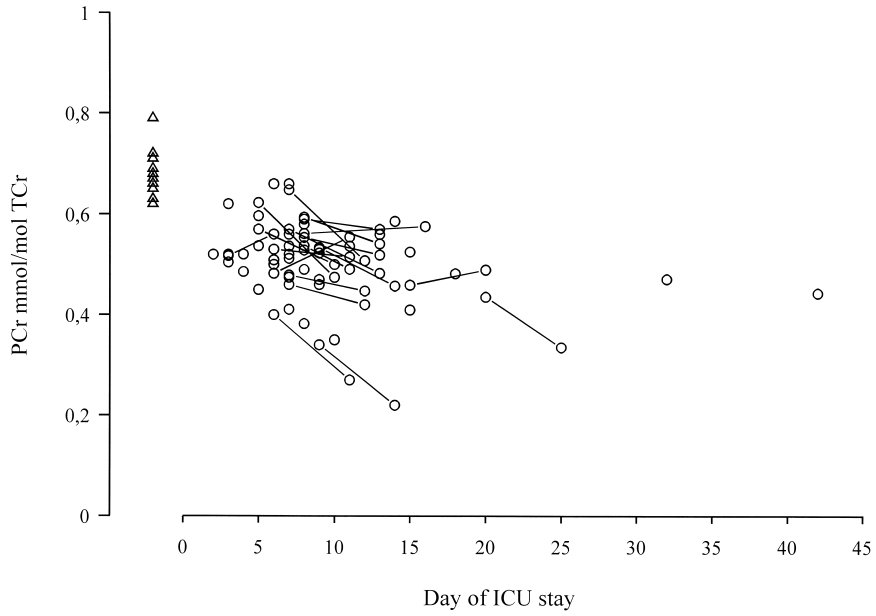

Fig. 9b
Fig. 9
Energy-rich phosphates a) ATP b) PCr related to total creatine content in critically ill patients (n = 67) study I, II, IV, V with repeated sampling in 28 patients from study II, IV, V and levels in a reference group, of healthy volunteers (n= 12, from study III and unpublished data); o- patients; Δ- reference group

Other markers

The RNA content is utilised as a reflection of the capacity for protein synthesis since 80% of the RNA is contained in the ribosomes. In studies I and II, the RNA/DNA was shown to correlate with the protein content (ASP/DNA). This correlation was reproduced in studies IV and V. In the combined patient series there was a statistical correlation between RNA and ASP in all patients at the first biopsy ($p< 0.001$, $r^2= 0.52$) (fig 10).

In study III, the unloading of one leg during 10 days reduced the content of RNA by 16 % in skeletal muscle ($p < 0.05$). RNA may be regarded as a measure of the capacity for protein synthesis.

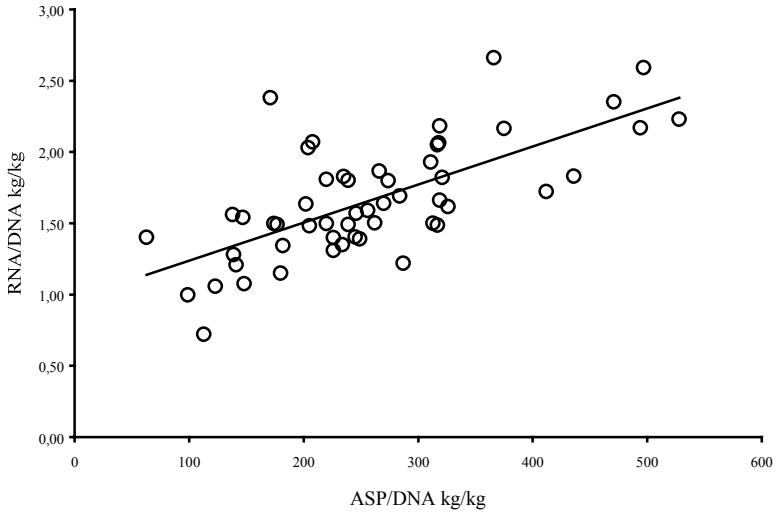

Fig. 10
Correlation between protein content ASP/DNA and RNA/DNA first sampling from critically ill patients, $p< 0.001$, $r^2 = 0.52$. Data from study I, II, IV, V

The concentrations of the basic amino acids (lys, hist, arg) and glutamate were low in studies I and II compared to levels in the reference group. The decreased levels occur throughout the studies.

In study I the fat content was not statistically different from that in the reference group. In study II, however, the fat content increased by 149% ($p < 0.01$) but in studies IV and V the level of the fat content showed no change over time. In the total patient series when all biopsies from the patients are included as single measurements there is a statistical correlation between fat content and the parenteral caloric intake ($p < 0.05$) (fig 11). This should be interpreted with caution as the caloric intake only represents the 24 hours period before the sampling.

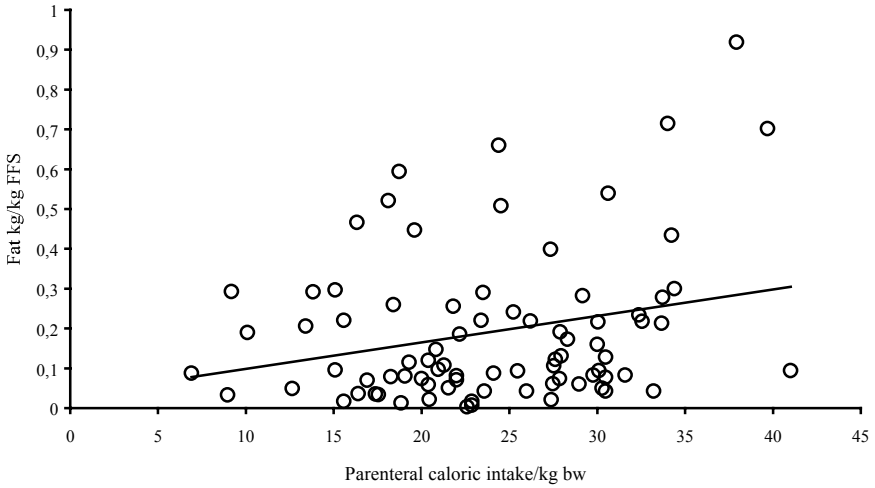

fig 11
Correlation between parenteral caloric intake and intramuscular fat content at first sampling from critically ill patients, $p< 0.05$, $r^2 = 0.33$. Data from study I, II, IV, V.

Reproducibility and scatter

Since the acute clinical picture is highly variable, and in view of the fact that very few reports on metabolism in this patient group are at hand, the reproducibility between the three control groups is a critical issue when this heterogenous group of patients is investigated. The present studies were of pilot nature with a limited number of patients in each study. As demonstrated for the markers presented and discussed, the three different control groups (studies II, IV, V) show a high level of agreement. The combined series therefore provides strong evidence that the reproducibility of the biochemical markers used is high in this group of long term ICU patients. The investigated markers showed scatters of the same magnitude, but at different levels in the critically ill patients compared to healthy subjects. This applies to ASP/DNA, glutamine and the H_2O content. The scatter of FSR, is however, larger in ICU patients than in healthy individuals.

GENERAL DISCUSSION

The patients studied are a selected group of all patients in the ICU. They were selected from the small group with sustained sepsis and multiple organ failure who stay in the ICU for a long time and in whom the muscle depletion might be a limiting factor for survival. Muscle protein depletion has an impact on the long-term prognosis, on the length of the hospital stay and on the recovery time for these patients (Griffiths, 1996; Takala, 1999a; Wilmore, 1998). Although the inclusion criteria were quite broad, (1. expected stay > 5 days and 2. possible to perform muscle biopsy), the number of patients included constitute < 2% of the total number of patients referred to the unit at Huddinge Hospital. In addition, more than 50% of the patients staying > 5 days in the ICU were not included, mainly due to failure to meet criteria 2 above. Furthermore, a few patients/relatives did not consent to the studies and a few patients were eligible but were not included for practical reasons. Therefore, it is not immediately obvious that the patients studied actually represent all long-term ICU patients with multiple organ failure. The unit is multidisciplinary with relatively few trauma patients but with a large proportion of patients undergoing immunosuppressive treatment from the Departments of Transplant Surgery and Haematology and it was not possible to include these patients. The representativity of the patient series has not been analysed in a strict sense but, nevertheless, it is our firm belief that the results obtained in the initial pilot studies I and II, and confirmed in the interventional studies IV and V reflect the situation of long stay ICU patients in general. The clinical observation of an increasing similarity of clinical features with increasing duration of the ICU stay is also supports the representativity of the investigated patient group. The hospital mortality for patients staying > 5 days in the ICU at Huddinge Hospital was 24 % in 1999, which compares with the 32 % hospital mortality of the studied patient series. This mortality rate is well in accord with informal information from all ICUs at university hospitals in Scandinavia.

The rationale for treating patients in the ICU for prolonged periods of time has been questioned from economic, medical and humanitarian standpoints. These issues have been addressed to some extent in the literature. The quality of life after prolonged intensive care has been reported to be fairly good with a largely subjective intact health status and daily physical performance (Niskanen, 1999) and also to be similar to the quality of life of patients having a short stay in the ICU (Heyland, 1998). This further underlines the importance of all efforts to improve outcome for this group of long-stay ICU patients.

The APACHE-II score at admission makes possible a grading of the acute condition of ICU patients, and it is also an indirect measure of the risk for complications and repeated insults in the sense that a more severely ill patient has a higher APACHE-II score. It needs to be emphasised that the APACHE-II score for an unselected group of ICU patients will distribute somewhat differently as compared to this group of long-stayers, (fig 12). In general terms, this difference reflects the possibility of intensive care medicine to enable survival also in extremely severe situations, but concurrently it also reflects the risk associated with intensive care such as iatrogenic infections and adverse effects of pharmacological therapy.

The general hypothesis of the studies was that biochemical markers may be useful to evaluate the effect of metabolic treatment on skeletal muscle. This hypothesis involves a number of assumptions. First, that it should be possible to study ICU patients as a group, in the sense that the scatter and heterogeneity

should not invalidate the conclusions. Second, the markers should be relevant in terms of reflecting the structure and function of muscles and, at the same time, sensitive enough to allow detection of differences also during a relatively short study period. It is of course crucial for the entire project that these assumptions are reasonably valid. The hetrogeneity of admission diagnosis of long stay ICU patients is widely known. The clinical features, of the long stay patients are however, often less related to the diagnosis at admission than to the concurrently ocurring complications, which are of similar nature in all critically ill patients. Furthermore, multiple organ failure as the ”final common path” has been extensively disccussed (Baue, 1991). After the pilot studies I and II, this question was addressed by an analysis of the recorded scatter of the biochemical markers in comparison with the scatter seen in the reference groups. The conclusion was drawn that the scatter in the pilot studies did not invalidate the assumptions made. In particular, the use of paired samples would make it possible to detect differences also in small groups. The arguments given above for the representativity of the patient series studied is another indirect evidence in favour of this assumption as well as the fact that all varibles studied were reproduced in the control groups of the intervention studies IV and V.

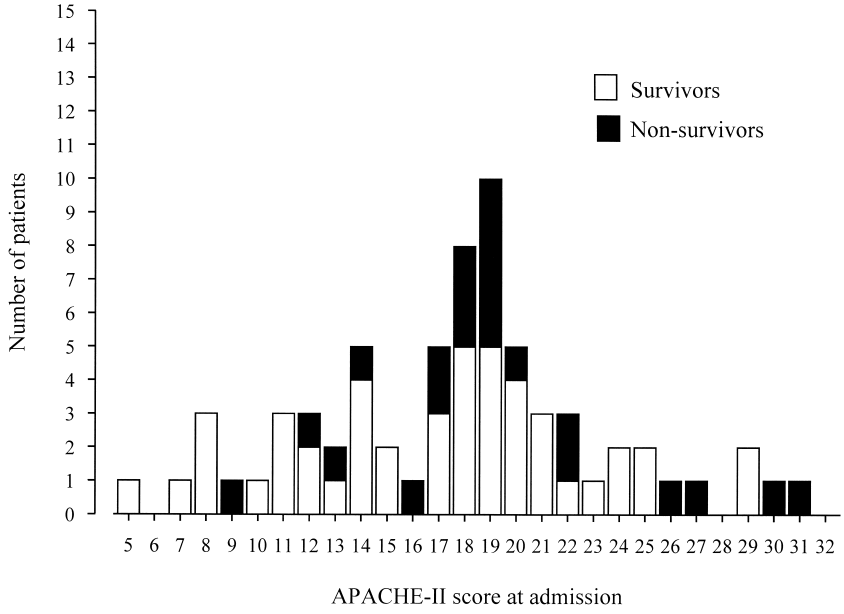

fig 12
Hospital mortality in critically ill patients in relation to APACHE-II score at admission. Data from study I, II, IV, V.

The choice of the muscle biopsy technique proved to enable sampling without the concerns necessary when a reproducible steady state is needed. The invasiveness was a comparatively small problem and the advantage of a sampling techniques not requiring a steady state became more obvious during the study. The quantifications of the markers were generally meaningful, and the reproducibility provided evidence in favour of the choice of sampling technique. The markers studied were of course chosen among the analyses that were available at the time. The depletion of muscle protein and the deterioration of muscle function were thought to be reflected in terms of protein metabolism and also in the determination of the water distribution and the energy-rich phosphagens. The pilot studies I and II suggested that the content of protein, ASP/DNA, had the potential of being a marker of skeletal muscle catabolism. The concentrations of some of the indispensable amino acids among free amino acids were discussed as

possible markers of the overall muscle protein metabolism without being able to differentiate the relative contributions of changes in synthesis and degradation. The extracellular water was another possible marker as water and protein content change in parallell and also from the perspective of ”cell swelling” being suggested as a regulatory mechanism of protein synthesis. The markers chosen are capable of reflecting general changes, while more specific and short-term changes may pass undetected. Although the coefficients of variation were not very large, the observed changes during the study period were sometimes too small to be significant in small groups of patients.

The depletion of muscle proteins over time in ICU patients was quantified to 10% per 5 days and was shown to be a highly reproducible finding. Other investigators have shown fibre atrophy occuring within 10 days of admission and sequential biopsies to show a mean reduction in fibre cross-sectional areas of 3-4%/day (Helliwell, 1998). Histopathological analysis of the neuromuscular disorders in critical illness reported generalised fibre atrophy, non-specific myopathy and necrotising myopathy (Coakley, 1993) Critical illness polyneuropathy has been described including axonal degeneration and denervation atrophy (Bolton, 1984). Loss of myosin has been reported in critically ill patients (Zochodne, 1994) and has been suggested to be related to administration of glucocorticoids and non-depolarising muscle relaxants. The loss of myosin has also been described in patients not being given these drugs (Deconinck, 1998). Skeletal muscle depletion may thus be interpreted as a part of the multiple organ failure, when the skeletal muscle tissue is regarded as an entity. The reduction of protein content in quantitative terms, as decribed here, gives a possibility to evaluate treatment by this marker, although this was not demonstrated within the frame work of this project. As the protein content is determined by the relation between de novo synthesis and degradation of proteins the estimation of these processes would add further information. Protein synthesis rate was quantified by the flooding dose technique, which gave information concerning the synthesis rate in a larger group of ICU patients for the first time. The different responses in postoperative and ICU patients is striking. Very few ICU patients have low protein synthesis rates similar to the protein synthesis rate of postoperative patients regardless if nutritional support was provided or not (Essén, 1993). In this project no measurements of protein degradation were included. The available information indicates that the degradation rate is elevated in ICU patients (Arnold, 1993), (Tiao, 1997). In postoperative patients protein metabolism seem to be regulated quite differently, a negative nitrogen balance and loss of skeletal muscle protein occur as a result of a decrease in muscle protein synthesis. In ICU patients muscle protein synthesis can obviously be low, intermediate or high, but the catabolism is presumably accompanied by a degradation rate higher than the synthesis. The interaction between protein synthesis and degradation and the regulation of these processes still remains to be described.

The low concentrations of glutamine in skeletal muscle have been demonstrated previously by several investigators, mostly on single sampling occasions. The very low glutamine levels were shown not to be related to the length of stay in the present series of patients. In contrast, in reports on subgroups of ICU patients, the glutamine concentration was reported to decrease over time in non-surviving patients with necrotizing pancreatitis (Roth, 1986). However, muscle glutamine depletion has been confirmed in

critically ill patients with similar low levels and also unaltered levels of skeletal muscle glutamine in paired samples (Palmer, 1996). The unaltered level of glutamine in paired samples was further confirmed in studies II, IV and V.

The effects of growth hormone were an increase in the skeletal muscle FSR and in the glutamine concentration and an increase in the intracellular water content. In surgical patients, growth hormone has been shown to improve nitrogen balance and to preserve levels of glutamine (Hammarqvist, 1992a). The improved nitrogen balance has been suggested to be due to increased protein synthesis rather than decreased protein degradation (Douglas, 1990). Growth hormone infusion into the brachial artery of healthy volunteers has been shown to stimulate protein synthesis in skeletal muscle, but not whole body protein synthesis (Fryburg, 1993).

The increase in muscle glutamine content may indicate an increased synthesis of glutamine in the muscle or a diminished efflux of glutamine from skeletal muscle in response to GH. The latter has been demonstrated in the response of postoperative patients to growth hormone treatment i (Mjaaland, 1993). It might be a potential disadvantage for the patient if the glutamine is retained in skeletal muscle at a time when an increased export to the splanchnic tissues would be required. Actually, this might be a major explanation of the increase in mortality reported in the multicentre study of ICU patients (Takala, 1999b). This is a hypothesis which is supported by the observation that GH is associated with increased splanchnic lactacidosis in a porcine sepsis model (Unneberg, 1996). To avoid potential tissue hazard, simultaneous provision of glutamine-supplemented nutrition has been advocatedwhen GH is administered (Hammarqvist, 1995). In postoperative patients, this also produces an additional effect on whole body nitrogen balance and biochemical markers of muscle protein metabolism (Hammarqvist, 1995) as well as on whole body protein metabolism, as reflected in leucine turnover in ICU patients (Carroll, 1999).
The increase in muscle intracellular water following GH administration is in accord with the suggestion that cell volyme is a regulator of protein synthesis, futhermore glutamine has also been suggested as a regulator if intracellular volume (Häussinger, 1991). Critically ill patients are known to have elevated levels of circulating GH and low levels of IGF-1(Ross, 1991). In addition, the pattern of GH secretion is converted from pulsatile diurnal secretion to that of a constantly high plasma concentration and a reduction of GH peaks both in number and amplitude (van den Berghe, 1998). A decreased metabolic response and a reduced IGF-1 response to physiological doses of exogenous GH has been reported in septic patients (Dahn, 1988). This condition has been defined as GH resistance. (Jenkins, 1996). The administration of GH increased the IGF-1 concentration in the ICU patients in study IV by 72%. Similar doses of GH in healthy volunteers have been reported to increase the IGF-1 concentration by 292% (Dahn, 1988). This emphasises the relative ”GH resistance” described in ICU patients.
However, at the present time,the results from a large multicentre trial, showing an increased mortality in growth hormone treated critically ill patients have inhibited GH administration for other purposes than substitution in GH deficiency (Takala, 1999b).

Glutamine supplementation to intravenous nutrition has been shown to reduce the infection rate and length of stay in bone marrow transplant patients (Ziegler, 1992) and postoperative patients (Jian, 1999; Morlion, 1998) and to reduce mortality from 71% to 42% in ICU patients (Griffiths, 1997). Glutamine is a preferred substrate for rapidly dividing cells of the immune system and the intestine (van Acker, 1999) (Hulsewé, 1999). Glutamine supplementation after surgery enhances DNA synthesis in T-lymphocytes (O'Riordain, 1994) and increases the lymphocyte count (Jacobi, 1997). The gut barrier function is enhanced by glutamine (van der Hulst, 1993). Since glutamine is constanly synthesised in the body, it has been regarded as a non-essential amino acid. It has been discussed if the endogenous capacity for glutamine synthesis is sufficient during severe catabolic states, and the term ”conditionally essential” has been suggested to emphasise the central role of glutamine as a substrate for cells with a critical role in vital processes (Lacey, 1990). The decreased glutamine content of skeletal muscle reflects an increasing demand for glutamine (Mittendorfer, 1999).

The muscle free glutamine concentration is maintained at a low level over time in long-stay ICU patients. There is no relation to the length of stay and no change in paired samples among the critically ill patients in the present studies. However, today there are no data on the net balance of glutamine synthesis and export in critically ill patients relating to the length of stay. The patient at risk for glutamine deficiency still needs to be defined.

In investigations with labelled glutamine and leucine an increase in whole body protein degradation and synthesis has been reported in critically ill patients, and also that glutamine from the protein degradation is insufficient to meet the increased demands (Jackson, 1999). In critically ill patients investigated 12 days after injury, an increase in glutamine synthesis is reported (Mittendorfer, 1999). However, an absence of glutamine steady state has been reported which is suggested to overestimate calculations of whole body glutamine flux (van Acker, 1998).

The hypothesised connection between free glutamine concentration and muscle protein synthesis discussed in relation to animal data was not confirmed in the separate studies of this project. However, when studies IV and V were combined, taking into the calculation the first biopsy in all patients and the second biopsy in the control patients, (53 samples from 40 patients), a weak statistical correlation was noted between the glutamine concentration and FSR ($p< 0.05$, $r^2= 0.09$) (fig 13).

Consequently the hypothesis that glutamine supplementation affects the muscle free glutamine concentration and thereby muscle protein synthesis was provided with some evidence from the combined patient series in studies IV and V. The supplementation may have to consist of higher doses of glutamine and/or be given for a longer period of time than the 5 days investigated here for such a relationship to be demonstrated in a small number of patients.

The demonstrated clinically beneficial effect of glutamine supplementation on survival and morbidity shown (Griffiths, 1997), (Ziegler, 1992), (Houdijk, 1998) may be explained by an immediate effect on muscle free glutamine and muscle protein synthesis, alternatively, by an effect in organs where glutamine is a vital substrate, i.e. the rapidly dividing cells of the immune system and the enterocytes in the splanchnic area. The reported reduction in mortality after 20 days in the ICU (Griffiths, 1997), indicates a diminished depletion of skeletal muscle and thus decreasing mortality after the acute phase of critical

illness. However, the enteral supplementation of glutamine in post-trauma ICU patients (Houdijk, 1998) implies a direct effect on the intestine, which to a large proportion consists of immunocompetent cells. A combined effect on central organs of the splancnic area and the periphery with an attenuation of muscle protein depletion may be the most likely explanation.

In critically ill patients, the wasting of muscle is presumably regulated by a number of factors including hormonal changes, cytokine effects and immobilisation *per se.* Therefore it was logical to try to elucidate the isolated effect of immobilisation. A protocol including a model in which the separate influence of unloading on skeletal muscle had previously shown a decrease in muscle strength and a decrease in the cross-sectional area of the thigh was used (Berg, 1991). In study III, unloading according to this model showed a reduction of the capacity for protein synthesis, RNA/DNA and increased concentrations of BCAA, which is seen when the protein breakdown is larger than the synthesis.
Prevention of muscle atrophy after immobilisation of one leg in plaster has been investigated used electrical stimulation. After cruciate ligament reconstruction, the reduction in quadriceps area was significantly less after electrical stimulation (Arvidsson, 1986). After tibial fracture electrical stimulation prevents the decrease in quadriceps cross-sectional area and protein synthesis on the immobilized leg (Gibson, 1988). However, these studies report of the combined effects of a local surgical procedure or trauma and immobilisation, while the ICU patient has a generalized alteration in metabolism.
In healthy volunteers, strict bed rest for 14 days decreased leg and whole body lean mass and decreased protein synthesis by 50%, measured by the arteriovenous difference and by direct incorporation techniques (Ferrando, 1996). Bed rest and muscle activity in the form of resistance exercise maintained the protein synthesis rate (Ferrando, 1997). This indicates that the contribution of inactivity to muscle protein depletion in long-stay ICU patients may be regulated differently compared to the situation in unstressed healthy volunteers. Nevertheless, muscle activity, either spontaneous or due to electrical stimulation show a preventive effect in several different situations.

The most obvious methodological extension of the protocol used in this project will be to try to include measurements of amino acid flux over muscle tissue, including arteriovenous difference measurements of isotopically labelled amino acids. Another possibility is the microdialysis technique, which can also be combined with isotopically labelled amino acids. Some pilot studies are now available in the literature (Rosdahl, 1998) and, from a technical point of view, such techniques may be employed. However, a number of methodological problems remain, among which the definition of a reproducible steady state and characterisation of the patients are two of the most important.
Our original intention was to continue to investigate the combined effect of GH and glutamine. Since GH can presently be given only as substitution therapy in conditions of GH deficiency, further studies on GH treatment in ICU patients are not possible. In the future when the underlying mechanism of GH effects have been better clarified, it may be possible to treat strictly defined patient groups. There are also some reports on testosterone treatment in healthy volunteers, which have a stimulatory effect on protein metabolism. This project has focused on skeletal muscle in ICU patients without regard to to other

tissues, which of course is a limited perspective. Future studies may be designed to link the pathology in skeletal muscle to that in other organs such as the intestine, liver and immune system.

CONCLUSION

The characterisation of biochemical markers in skeletal muscle of ICU patients with multiple organ failure staying in the unit for a long time showed a relationship with the length of stay for several parameters. These results were confirmed when paired samples were obtained. This made it possible to design a protocol to be used in this group of ICU patients, as well as in healthy volunteers, to evaluate interventions. The study period was 10 days for volunteers, and 5 days for ICU patients.

Conclusions drawn from the separate studies were:

Study I. A decrease over time was seen in ASP/DNA, while extracellular water and the concentrations of branched chain and aromatic amino acids increased. The concentration of glutamine was markedly reduced, but it did not change in relation to time.

Study II. The above mentioned findings were confirmed in paired samples and, in addition, an increase in fat content was seen.

On the basis of these results it could be assumed that a study period of 5 days would be sufficient to detect alterations in ASP/DNA, extracellular water and perhaps also glutamine and protein synthesis rates if these variables were affected.

Study III. An immobilisation period of 10 days was used in healthy volunteers. RNA/DNA decreased and the concentration of branched chain amino acids increased. The small size of the group rendered the results less conclusive than intended.

Study IV. Pharmacological doses of GH increased the muscle free glutamine concentration and protein synthesis rate and, in addition, the intracellular water. These results indicate that an addition of growth hormone has effects on biochemical markers in skeletal muscle, indicating a potential beneficial effect on muscle protein wasting.

Study V. Nutrition supplemented with glutamine/α-ketoglutarate showed a very marginal effect on muscle free glutamine concentration, which was the main hypothesis of the study. Although the relation to muscle protein synthesis was not obvious, a marginal effect was seen also in this marker. In addition, α -ketoglutarate prevented the increase in total muscle water.

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REFERENCES

Andersson K, Sang X, Ahlman B, et al. (1995) Determination of proteasome activity in human muscle and liver tissue. Clin Nutr suppl, 15, p 16.

Arnold J, Campbell I, Samuels T, et al. (1993) Increased whole body protein breakdown predominates over increased whole body protein synthesis in multiple organ failure. Clinical Science, 84, 655-661.

Arvidsson I, Arvidsson H, Eriksson E, et al. (1986) Prevention of quadriceps wasting after immobilization: an evaluation of the effect of electrical stimulation. Orthopedics (Thorofare, NJ), 9, 1519-1528.

Askanazi J, Carpentier Y, Elwyn D, et al. (1980a) Influence of total parenteral nutrition of fuel utilization in injury and sepsis. Ann Surg, 191, 40-46.

Askanazi J, Elwyn D & Kinney J (1980b) Muscle and plasma amino acids following injury. Ann Surg, 188, 797-803.

Askanazi J, Rosenbaums H, Hyman A, et al. (1980c) Respiraory changes induced by high loads of total parenteral nutrition. JAMA, 243, 1444-1447.

Aulick M & Wilmore D (1979) Increased peripheral amino acid release following burn injury. Surgery, 85, 560-565.

Baue A (1991) Nutrition and metabolism in sepsis and multisystem organ failure. Surgical Clinics of North America, 71, 549-565.

Berg H, Dudley G, Häggmark T, et al. (1991) Effects of lower limb unloading on skeletal muscle mass and function in humans. J Appl Phys, 70, 1882-1885.

Bergström J (1962) Muscle electrolytes in man. Scand J Clin Lab Invest, 168 (suppl 14), 11-13.

Bergström J, Alvestrand A & Fürst P (1983) Muscle intracellular electrolytes in patients with chronic uremia. Kidney Int, 24, S153-S160.

Bergström J, Boström H & Fürst P (1976) Preliminary studies of energy-rich phosphagens in muscle from severely ill patients. Critical Care Medicine, 4, 197-204.

Bergström J, Fürst P, Holmström B, et al. (1981) Influence of injury and nutrition on muscle water and electrolytes: effect of elective operation. Ann Surg, 193, 810-816.

Bier D (1989) Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. Diabetes Metabolism Reviews, 5, 111-132.

Blomqvist B, Hammarqvist F, Von der Decken A, et al. (1995) Glutamine and α–ketoglutarate prevents the decrease in muscle free glutamine concentration and influence protein synthesis after total hip replacement. Metabolism, 44, 1215-1222.

Bolton C, Gilbert J, Hahn A, et al. (1984) Polyneuropathy in critically ill patients. Journal of Neurology, neurosurgery & psychiatry, 47, 1223-1231.

Burke J, Wolfe R & Mullaney J (1979) Glucose requirements and possible hepatic and respiratory abnormalities following excessive glucose intake. Ann Surg, 190, 274.

Carroll P, Jackson N, Sönksen P, et al. (1999) Combined GH/IGF-1 treatment results in net protein anabolism in critically ill patients receiving TPN supplemeted with glutamine. Diabetes, Nutrition & Metabolism, 12, 172-173.

Carson S, Bach P, Brzozowski L, et al. (1999) Outcomes after long-term acute care. Am J Respir Crit Care, 159, 1568-1573.

Cerra B (1987) Hypermetabolism, organ failure, and organ support. Surgery, 101, 1-14.

Cerra F, McPherson J, Konstantinides F, et al. (1988) Enteral nutrition does not prevent multiple organ failure syndrome (MOFS) after sepsis. Surgery, 104, 727-733.

Cerra F, Siegel J & Coleman B (1980) Septic autocannibalism: A failure of exogenous nutrition support. Ann Surg, 192, 570.

Cheng A, Plank L & Hill G (1998) Prolonged overexpansion of extracellular water in elderly patients with sepsis. Arch surg, 745-751.

Chiolero R, Revelly JP, Tappy L. (1997) Energy metabolism in sepsis and injury. Nutrition, 13(9 Suppl):45S-51S.

Clowes G, Hirsch E, George B, et al. (1985) Survival from sepsis: the significance of altered protein metabolism regulated by protelysis inducing factor, the circulating cleavage product of interleukin 1. Ann Surg, 202, 446-458.

Clowes G, Randell H & Cha C-J (1980) Amino acid and energy metabolism in septic and traumatized patients. JPEN, 4, 195-203.

Coakley J, Nagendran K, Hoinavar M, et al. (1993) preliminary observations on the neuromuscular abnormalities in patients with organ failure and sepsis. Intensive Care Med, 19, 323-328.

Cuthbertson D (1932) Observations on the disturbance of metabolism produced by injury to the limbs. Q J Med, 1, 233.

Dahn M, Lange M & Jacobs L (1988) Insulin-like growth factor 1 production is inhibited in human sepsis. Arch Surg, 123, 1409-1414.

Deconinck N, Van Parijs V, Beckers-Bleukx G, et al. (1998) Critical illness myopathy unrelated to corticosteroids or neuromuscular blocking agents. Neuromuscular Disorders, 8, 186-192.

Douglas R, Humberstone D, Haystead A, et al. (1990) Metabolic effects of recombinant human growth hormone: isotopic studies in the postabsorptive state and during total parenteral nutrition. Br J Surg, 77, 785-790.

Douglas R & Shaw J (1989) Metabolic response to sepsis and trauma. British Journal of Surgery, 76, 115-122.

Essén P, McNurlan M, Gamrin L, et al. (1998) Tissue protein synthesis rates in critically ill patients. Crit Care Med, 26, 92-100.

Essén P, McNurlan M, Sonnenfeld T, et al. (1993) Muscle protein synthesis postoperatively- The effect of intravenous nutrition. Eur J Surg, 159, 195-200.

Essén P, Wernerman J, Sonnenfeld T, et al. (1992) Free amino acids in plasma and muscle during 24 hours postoperatively- a descriptive study. Clin Phys, 12, 165-179.

Felig P, Owen O, Wahren J, et al. (1969) Amino acid metabolism during prolonged starvation. Journal of Clinical Investigation, 48, 584-594.

Felig P & Wahren J (1971) Amino acid metabolism in excercising man. Journal of Clinical Investigation, 50, 2703-2714.

Ferrando A, Lane H, Stuart C, et al. (1996) Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. Am J Physiol, 270, E627-E633.

Ferrando A, Tipton K, Bamman M, et al. (1997) Resistance exercise maintains skeletal muscel protein synthesis during bed rest. J Appl Physiol, 81, 82-97.

Finn P, Plank L, CLark M, et al. (1996) Progressive cellular dehydration and proteolysis in criticall ill patients. Lancet, 347, 654-656.

Fleck A & Begg D (1965) The estimation of ribonucleic acid using ultaviolet absorption measuremnts. Biochim Biophys Acta, 108, 333-39.

Fleck A, Colley C & Myers M (1985) Liver export proteins and trauma. Br Med Bull, 41, 265-273.

Forsberg A, Nilsson E & Wernerman J (1991) Muscle composition in relation to age and sex. Clinical Science, 81, 249-256.

Fryburg D & Barrett E (1993) Growth hormone acutely stimulates skeletal muscle but not whole body protein synthesis in humans. Metabolism, 42, 123-1227.

Garlick P & Fern E (1985) Whole-body protein turnover: theoretical considerarions. In Substrate and energy metabolism (ed. J. H. Garrow, D), pp. 7-15. London: John Libbey.

Garlick P, McNurlan M, Essén P, et al. (1994) Measurement of tissue protein syntehsis in vivo: A critical analysis of contrasting methods. Am J Physiol, 266, E287-E297.

Garlick P, Wernerman J, McNurlan M, et al. (1989) Measurement of the rate of protein syntehsis in muscle of postabsorptive young men by injection of a "flooding dose " of -[1-13C]leucine. Clinical Science, 77, 329-336.

Gatzen C, Sheltinga M & Kimbrough T (1992) Growth hormone attenuates the abnormal distribution of body water in critically ill surgical patients. Surgery, 181-87.

Gelfand R, deFronzo R & Gusberg A (1983) Metabolic alterations associated with major injury or infection. In New aspects of clinical nutrition (ed. D. E. Kleinberger G.), pp. 221-239. Basel: Karger.

Gertz I, Hedenstierna G, Hellers G, et al. (1977) Muscle metabolism in patients with chronic obstructive lung disease and acute respiratory failure. Clin Sci and Mol Med, 52, 395-403.

Gibson J, Smith K & Rennie M (1988) Prevention of muscle atrophy by means of electrical stimulatio: maintenace of protein synthesis. Lancet, October 1, 767-769.

Giner M, Laviano A, Meguid MM et al. (1996) In 1995 a correlation between malnutrition and poor outcome in critically ill patients still exists. Nutrition, 12, 23-29,.

Griffiths R (1996) Muscle mass, Survival, and the Elderly ICU Patient. Nutrition, 12, 456-458.

Griffiths R, Jones C & Palmer T (1997) Six-month outcome of critically illpatients given glutamine-supplemented parenteral nutrition. Nutrition, 13, 295-302.

Guarnieri G, Toigo G, Situlin R, et al. (1988) Cathepsin Band D activity in human skeletal muscle in disease states. Advances in Experimental Medicine & Biology, 240, 243-256.

Hammarqvist F, Sandgren A, Andersson K, et al. (1995) Growth hormone added to glutamine-containing TPN prevents the changes in amino acid pattern postoperatively. Clin Nutr, 14(suppl), O43.

Hammarqvist F, Strömberg C, von der Decken A, et al. (1992a) Biosynthetic human growth hormone preserves both muscle protein synthesis and the decrease in muscle free glutamine, and improves whole-body nitrogen economy. Ann Surg, 216, 184-191.

Hammarqvist F, Wernerman J, Ali R, et al. (1989) Addition of glutamine to total parenteral nutrition after elective surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. Ann Surg, 209, 455-461.

Hammarqvist F, Wernerman J, von der Decken A, et al. (1991) Alpha-ketoglutarate preserves protein synthesis and free glutamine in skeletal muscle after surgery. Surgery, 109, 28-36.

Hammarqvist F, von der Decken A, Vinnars E, et al. (1992b) Proteinase activity in skeletal muscle following abdominal surgery. Clin Nutr, 11 suppl, 49.

Harris R, Hultman E & Nordesjö L-O (1974) Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand J Clin Lab Invest, 33, 109-120.

Hasselgren P (1999) Pathways of muscle protein breakdown in injury and sepsis. Curr Opin in Clin Nutr and Metab Care, 2, 155-160.

Hasselgren P-O & Fisher J (1987) Nutritional support in sepsis. In Nutritional support in critical care (ed. L. CE): Aspen systems corp. Rockville.

Helliwell T, Wilkinson A, Griffiths R, et al. (1998) Muscle fibre atrophy in critically ill patients is associated with the loss of myosin filaments and the presence of lysosomal enzymes and ubiquitin. Neuropathology and applied neurobiology, 24, 507-517.

Herndon D, Barrow R & Kunkle K (1990) Effects of recombinant human growth hormone on donor site healing in severely burned children. Ann Surg, 212, 424-29.

Heyland D, Konopad E, Noseworthy T, et al. (1998) Is it "worthwhile" to continue treating patients with a prolonged stay (>14 days) in the ICU? Chest, 114, 192-198.

Hill A, Hill G (1998) Metabolic response to severe injury. British Journal of Surgery, 85, 884-890.

Hill A, Plank L, Finn P, et al. (1997) Massive nitrogen loss in critical surgical illness. Annals of Surgery, 226, 191-197.

Hill G, Douglas R & Schroeder D (1993a) Metabolic basis for the management of patients undergoing surgery. World J surgery, 17, 146-153.

Hill G, Monk D & Plank L (1993b) Measuring body composition in intensive care patients. In Metabolic support of the critically ill patient, vol. 17 (ed. D. Wilmore), pp. 3-18. Berlin Heidelberg: Springer-Verlag.

Hobler S, Williams A, Fischer D, et al. (1999) Activity and expression of the 20S proteasome are increased in skeletal muscle during sepsis. Am J Physiol, 277, R434-R440.

Houdijk A, Rijnsburger E, Jansen J, et al. (1998) Randomized trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. Lancet, 352, 772-757.

Hulsewé K, van Acker B, von Meyenfeldt M, et al. (1999) Nutritional depletion and dietary manipulation: Effects on the immuns response. World J Surg, 23, 536-544.

Häussinger D, Hallbrucker C, vom Dahl S, et al. (1991) Cell volume is a major determinant of proteolysis control in liver. FEBS letters, 283, 70-72.

Jackson N, Carroll P, Russel-Jones D, et al. (1999) The metabolic consequenses of critical illness: acute effects on glutamine and protein metabolism. American journal of Physiology, 276, E163-E170.

Jacobi C, Ordemann J & Wenger F (1997) The influence of glutamine substitution in postoperative parenteral nutrition or immunologic function. First results of a prospective randomized trial. Shock, 7(S), 605.

Jenkins R & Ross R (1996) Aquired growth hormone resistance in ctabolic states. Baillières Clinical Endocrinology and Metabolism, 10(3), 411-419.

Jepson M, Bates P, Broadbent P, et al. (1988) Relationship betweeen glutamine concentration and protein synthesis in rat skeletal muscle. American Journal of Physiology, 255, E166-E172.

Jian Z, Cao J, Zhu X, et al. (1999) The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in patients: a randomized, double blind, controlled study of 120 patients. Jpen: journal of parenteral & enteral nutrition, 23(5suppl).

Jiang Z, He G & Zhang S (1989) Low-dose growth hormone and hypocaloric nutrition attenuate the protein catabolic response after major operation. Ann Surg, 210, 513-525.

Kedenburg C (1971) A lithium buffer system for accelerated single column amino acid anaysis in physiological fluids. Analytical Biochemistry, 40, 35-42.

Kinney J (1974) Energy requirements in injury and sepsis. Acta Anesthesiol Scand, 55(suppl).

Kinney J (1975) Enenrgy reqiurements of the surgical patient. In Manual of surgical nutrition (ed. A. c. o. surgeons), pp. 223-235. Philadelphia: WB Saunders.

Lacey J & Wilmore D (1990) Is glutamine a conditionally essential amino acid? Nutrition Reviews, 48, 297-309.

Larsson J, Lennmarken C, Martensson J, et al. (1990) Nitrogen requirements in severely injured patients. British Journal of Surgery, 77, 413-416.

Larsson J, Schildt B, Liljedahl S-O, et al. (1984) The effect of severe trauma on muscle energy metabolism in man. Acta Chirurgica Scandinavica, 150, 611-618.

Larsson J, Vinnars E & Liljedahl S-O (1985) Intracellular energy substrates in trauma. Acta Chir Scand, 522, 171-181.

Lennmarken C & Larsson J (1986) Skeletal muscle function and energy metabolites in malnourished surgical patients. Acta Chir Scand, 152, 169-173.

Lexell J, Taylor C & Sjöström M (1988) What is the cause of ageing atrophy? Journal of the neurological sciences, 84, 275-294.

Liaw K, Askanazi J, Michelsen C, et al. (1980) Effect of injury and sepsis on high-energy phosphates in muscle and red cells. Journal of trauma, 20.

London D, Foley T & Webb C (1965) Evidence for the release of individual amino acids from the resting human forearm. Nature, 208, 588-589.

Lowry O, Rosenborough N & Farr A (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem, 193, 265-275.

Mansoor O, Beaufrere B, Boire Y, et al. (1996) Increased mRNA levels for components of the lysosomal, Ca^{2+}-activated, and ATP-ubiquitin-dependent proteolytic pathways in skeletal muscle from head trauma patients. Proc Natl Acad Sci USA, 93, 2714-2718.

Mansoor O, Cayol M, Gachon P, et al. (1997) Albumin and fibrinogen synthesis increase while muscle protein synthesis decreases in head-injured patients. Am J Physiol, 273, E898-E902.

McLennan P, Brown R & Rennie M (1987) A positive relationship between protein synthetic rate and intracellular glutamine concentration in perfused rat skeletal muscle. FEBS letters, 215, 187-191.

Michelsen C, Askanazi J & Kinney J (1982) Effect of an anabolic steroid on postoperative nitrogen balance. J Trauma, 22, 410-413.

Milewski P, Threllfall C & Heath D (1982) Intracellular free amino acids in in undernourished patients with or without sepsis. Clin Sci, 62, 83-91.

Mittendorfer B, Gore D, Herndon D, et al. (1999) Accelerated glutamine synthesis in critically ill patients cannot maintain normal intramuscular free glutamine concentration. Journal of Parenteral and Enteral Nutrition, 2, 243-252.

Mjaaland M, Unneberg K & Hotvedt R (1991) Nitrogen retention caused by growth hormone in patients undergoing gastrointestinal surgery with epidural analgesia and parenteral nutriton. Eur J Surg, 157, 21-27.

Mjaaland M, Unneberg K, Larsson J, et al. (1993) Growth hormone after abdominal surgery attenuated forearm glutamine, alanine, 3-methylhistidine, and total amino acid efflux in patients receiving total parenteral nutrition(TPN). Ann Surg, 217, 413-422.

Morlion B, Stehle P, Wachtler P, et al. (1998) Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: a randomized, double-blind, controlled study. Ann Surg, 227, 302-308.

Munro H & AllisonJB (1964) Mammalian protein metabolism. New York: Academic Press.

Niskanen M, Ruokonen E, Takala J, et al. (1999) Quality of life after prolonged intensive care. Crit care med, 27, 1132-1139.

O'Riordain M, Fearon K, Ross J, et al. (1994) Glutamine-supplemented total parenteral nutrition enhances T-lymphocyte response in surgical patients undergoing colorectal resection. Ann Surg, 220, 212-221.

Owen O & Robinson R (1963) Amino acid extraction and ammonia metabolism in the human kidney during prolonged administration of ammionium chloride. Journal of Clinical Investiagtion, 42, 263.

Palmer T, Griffiths R & Jones C (1996) Effect of parenteral L-glutamine on muscle in the very severely ill. Nutrition, 12, 316-20.

Picou D, Taylor-Roberts T, Waterlow JC (1969) The measurement of total protein synthesis and nitrogen flux in man by constant infusion of 15N-glycine. J Physiol (Lond), 200, 52P-53P

Plank L, Conolly A & Hill G (1998) Sequential changes in the metabolic response in severely septic patients during the first 23 days after the onset of peritonitis. Annals of Surgery, 228, 146-158.

Ponting G, Halliday D, Teale J, et al. (1988) Postoperative positive nitrogen balance with intravenous hyponutrition and growth hormone. Lancet, feb 27, 438-439.

Pozefsky T, Felig P, Tobin J, et al. (1969) Amino acid balancs across tissue of the forearm in postabsorptive man. Effects of insulin at two dose levels. Journal of Clinical Investigations, 48, 2273-2282.

Rennie M, Edwards H, Emergy P, et al. (1983) Hypothesis: depressed protein synthesis is the dominante characteristic of muscle wasting and cachexia. Clin Physiol, 3, 378.

Rennie M, Edwards R, Halliday D, et al. (1982) Muscle protein synthesis measured by stable isotopes in man: the effect of feeding and fasting. Clinical Science, 63, 519-523.

Rennie M, Smith K & Watt P (1994) Measurement of human tissue protein synthesis: an optimal approach. Am J Physiol, 266, E 298-E307.

Rosdahl H, Samuelsson A, Ungerstedt U, et al. (1998) Influence of adrenergic agonists on the release of amino acids from rat skeletal muscle studied by microdialysis. Acta Physiologica Scandinavica, 163, 349-60.

Ross R, Miell J, Freeman E, et al. (1991) Critically ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of insulin-like growth factor-1. clinical endocrinology, 35, 47-54.

Roth E, Funovics J & Mühlbacher F (1982) Metabolic disorders in severe abdominal sepsis. Clin Nutr, 25-41.

Roth E, Zöch G, Mauritz W, et al. (1986) Metabolic changes of patients with acute necrotizing pancreatitis. Infusionstherapie, 13, 172-179.

Sjölin J, Stjernström H, Artursson G, et al. (1989a) Exchange of 3-methylhistidine in the splanchnic region in human infection. Am J Clin Nutr, 50, 1407-1414.

Sjölin J, Stjernström H, Henneberg S, et al. (1989b) Splanchnic and peripheral release of 3-methylhistidine in relation to its urinary excretion in human infection. Metabolism, 38, 23-29.

Soop M, Forsberg E, Thörne A, et al. (1990) Muscle and plasma amino-acid pattern in severely depleted surgical patients. Clin Nutr, 9, 206-213.

Soop M, Forsberg E, Thörne A, et al. (1989) Muscle alkali-soluble protein, carnitine, water and electrolytes in patients with persisting postoperative infection. Clin Nutr, 8, 237-245.

Souba W, Klimberg S & Plumley D (1990) The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. J Surg Res, 48, 383-391.

Souba W & Wilmore D (1994) Diet and nutrition in the care of the patients with surgery, trauma, and sepsis. In Modern nutrition in health and disease (eds M. Shils, Olson. JA, & M. Shike). Philadelphia: Lea& Febiger.

Stillström A, Persson E & Vinnars E (1987) Postoperative water and electrolyte changes in skeletal muscle: a clinical study with three different intravenous infusions. Acta Anaesthsiol Scand, 31, 284-288.

Streat S, Beddoe A & Hill G (1987) Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. The Journal of Trauma, 27, 262-266.

Söderlund K & Hultman E (1986) Effects of delayed freezing on the content of phosphagens in human skeletal muscle biopsy samples. Am J Phys, 61, 832-835.

Takala J (1999a) Progressive depletion in critically ill patients - an unsolved clinical problem that persists. Curr Opin Nutr Metab Care, 2, 153.

Takala J, Roukonen E, Webster N, et al. (1999b) Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med, 341, 785-792.

Tiao G, Hobler S, Wang J, et al. (1997) Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. J Clin Invest, 99, 163-168.

Tjader I, Essen P, Thorne A, et al. (1996a) Muscle protein synthesis rate decreases 24 hours after abdominal surgery irrespective of total parenteral nutrition. JPEN: Journal of Parenteral & Enteral Nutrition., 20(2), 135-138.

Tjader I, Essen P, Thorne A, et al. (1993) Minor surgery has no immediate effect on protein synthesis rate in human skelatal muscle. Clin Nutr, 12 S2, O20.

Tjader I, Essen P, Wernerman J, et al. (1996b) The effect of leucine administered as a flooding dose on the incorporation of constantly infused tracer valine and glycene inte skeletal muscle. Clin Nutr, 15(suppl1), O 42.

Tresadern J, Threlfall C, Wilford K, et al. (1988) Muscle adenosine 5'-triphosphate and creatine phosphate concentrations in relation to nutritional status and sepsis in man. Clin Sci, 75, 233-242.

Tucker H & Miguel S (1996) Cost containment through nutrition intervention. Nutrition Reviews, 54, 111-121.

Unneberg K, Balteskard L, Mjaaland M, et al. (1996) Growth hormone impaired compensation of hemorragic shock after trauma and sepsis in swine. J Trauma-Injury Infection & Critical Care, 41, 775-780.

van Acker B, Hulsewé K, Wagenmakers A, et al. (1998) Abscence of glutamine isotopic steady state: implications for the assessment of whole-body production rate. Clinical Science, 95, 339-346.

van Acker B, von Meyenfeldt M, van der Hulst R, et al. (1999) Glutamine: the pivot of our nitrogen economy. JPEN, 23, S45-S48.

van den Berghe G, deZegher F & Bouillon R (1998) Acute and prolonged critical illness as different neuroendocrine paradigms. Journal of Clinical Endocrinology and Metabolism, 83, 1827-1834.

van der Hulst R, van Kreel B, von Meyenfeldt M, et al. (1993) Glutamine and the preservation of gut integrity. Lancet, 341.

Waterlow J, Garlick P & Millward D (1978) Protein turnover in mammalian tissues and in the whole body. Amsterdam: North Holland.

Watt P, Lindsay Y, Scrimgeour C, et al. (1991) Isolation of aminoacyl-tRNA and its labelling with stable isotope tracers; use in studies of human tissue protein synthesis. Proc Natl Acad Sci USA, 88, 5892-5896.

Wernerman J, Hammarqvist F & Vinnars E (1990) α-ketoglutarate and postoperative muscle metabolism. Lancet, 335, 701-03.

Wernerman J & Vinnars E (1987) The effect of trauma and surgery on interorgan fluxes of amino acids in man. Clin Sci, 73, 129-133.

Wernerman J, von der Decken A & Vinnars E (1985) Size distribution of ribosomes in biopsy specimens of human skeletal muscle during starvation. Metabolism, 34, 665-669.

Wilmore D (1998) Does loss of body protein determine outcome in patients who are critically ill? Ann Surg, 228, 143-145.

Windsor J, Hill G (1988a) Risk factors for postoperative pneumonia. Annals of surgery, 208, 209-214.

Windsor J, Hill G (1988b) Weight loss with physiologic impairement. Annals of surgery, 207, 290-296.

Woolfson A (1986) Monitoring treatment. In Biochemistry of hospital nutrition (ed. A. Woolfson). London: Churchill Livingstone.

Yule A, Macfie J & Hill G (1981) The effect of an anabolic steroid on body composition in patients receiving intravenous nutrition. Aust NZ J Surg, 51, 581-584.

Ziegler T, Young L, Benfell K, et al. (1992) Clinical and metabolic efficacy of glutamine -supplemented parenteral nutrition after bone marrow transplantation. Annals of Internal medicine, 116, 821-828.

Ziegler T, Young L, Ferrari-Balivera E, et al. (1990) Use of human growth hormone combined with nutritional support in a critical care unit. JPEN, 14, 574-581.

Zochodne D, Ramsay D, Saly V, et al. (1994) Acute necrotizing myopathy of intensive care: electrophysiological studies. Muscle & Nerve, 17, 285-292.