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MUSCLE PROTEIN SYNTHESIS – EFFECTS OF METABOLIC STRESS AND FEEDING

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“ Nec scire fas est omnia”

Horatius

ABSTRACT

Surgical trauma and critical illness are pathophysiological conditions where the metabolic stress leads to an imbalance between protein synthesis and protein breakdown, resulting in a net loss of body proteins. A major part of the protein losses comes from skeletal muscle. Muscle depletion is associated with a high morbidity and mortality. To understand the changes that occur during critical illness and following trauma it is therefore necessary to study protein metabolism on the tissue level.

The aim of this thesis work was to investigate the impact of metabolic stress and feeding on muscle protein synthesis. Healthy patients undergoing elective surgery and critically ill patients in the intensive care unit were investigated by the flood technique, employing L-[²H₅] phenylalanine, for quantification of muscle protein synthesis rate.

The effect of continuous and ongoing total parenteral nutrition was investigated in healthy patients scheduled for elective abdominal surgery of medium size. Muscle protein synthesis rate was determined before surgery and 24 hours after surgery with continuous and ongoing total parenteral nutrition. Conventional TPN could not prevent the decrease in muscle protein synthesis rate as compared to a control group receiving saline. Whereas conventional total parenteral nutrition is not effective in the immediate postoperative period it is shown that provision of glutamine can attenuate the decrease in muscle protein synthesis. However, glutamine is only marginally effective on muscle protein synthesis in ICU patients. To evaluate if a large enough dose of intravenous glutamine supplementation would influence muscle protein synthesis, ICU patients were randomized to receive 0, 20, 40 or 60 g of glutamine per kg body weight and day for a five-day study period. The main result was that plasma glutamine concentrations were normalized in all glutamine treated groups. No increase was seen in muscle glutamine concentration and therefore glutamine had no effect on muscle protein synthesis rate. The effect of difference size of trauma on muscle protein synthesis rate was investigated in patients undergoing elective minor surgery and major surgery before and immediately after surgery. Minor surgery did not have an impact on muscle protein synthesis rate, as expected. However, muscle protein synthesis rate following major surgery was also unaltered. Intensive care patients have an on average normal muscle protein synthesis rate but with a larger variation than in healthy individuals. In order to evaluate if artefacts or muscle tissue heterogeneity can explain this large scatter, muscle protein synthesis rate was determined simultaneously in both legs of ICU patients. Muscle protein synthesis rate was on average normal, and similar in the two legs. The variation between the legs was smaller than the variation between individuals and muscle morphology revealed no local differences.

In summary the immediate effect of surgical trauma on muscle protein synthesis rate was not demonstrated to be size dependent. The decrease in muscle protein synthesis rate observed following medium size abdominal surgery could not be prevented by continuous and ongoing conventional total parenteral nutrition. In ICU patients glutamine supplemented TPN normalized plasma glutamine concentrations but muscle glutamine concentrations were not affected and no effect were seen on muscle protein synthesis. No local differences were found in leg muscle morphology of ICU patients, confirming earlier results showing a large variation in muscle protein synthesis rate between individuals. ICU patients have an on average normal muscle protein synthesis rate with a low intra-individual variation.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I – IV.

- I. **Muscle protein synthesis rate decreases 24 hours after abdominal surgery irrespective of total parenteral nutrition.** Tjäder I, Essén P, Thörne A, Garlick P J, Wernerman J, McNurlan M A: JPEN, Vol.20, No.2, 135-138, 1996.
- II. **Effects on skeletal muscle of intravenous glutamine supplementation to ICU patients.** Tjäder I, Rooyackers O, Forsberg A-M, Vesali RF, Wernerman J. Intensive Care Med 30, 266-275. 2004.
- III. **Impact of surgical trauma on human skeletal muscle protein synthesis.** Tjäder I, Essen P, Garlick P J, McMurlan M A, Rooyackers O, Wernerman J. Clin Sci.107.6: 601-7. 2004
- IV. **Reproducibility in skeletal muscle protein synthesis rate in ICU patients.** Inga Tjäder, Olav Rooyackers, Maria Klaude, Inger Nennesmo, Jan Wernerman. Manuscript

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LIST OF ABBREVIATIONS

APACHE	Acute physiology, age, chronic health evaluation
ASP	Alkali soluble protein
ICU	Intensive care unit
DNA	Deoxy ribonucleic acid
FSR	Fractional synthesis rate
GLN	glutamine
HPLC	High performance liquid chromatography
Leu	leucine
mRNA	messenger ribonucleic acid
Phe	phenylalanine
TPN	Total parenteral nutrition
Val	valine

1 INTRODUCTION

Long-staying ICU patients have a 6-month mortality approaching 50 %. Half the deaths occur in the unit, but in addition this number is doubled during the following 6 months. In total this is more than 1000 deaths per year in Sweden. Is this mortality inevitable, or can the care and treatment of patients be improved? If improvements are possible, what strategies should be adapted?

The economic constraints upon health care, which have reduced the number of hospital beds by nearly 50 %, and decreased the length of hospital stay from 11 days to 6 days during a 30-year period (Molin and Johansson, 2004) have not fully reached the intensive care unit. Nevertheless, strategies to improve outcome must be formulated and tools to evaluate such strategies must be developed.

An important consequence of trauma and disease is the reduced muscle mass and muscle strength, which leads to delayed mobilization and prolonged hospital stay. The time of rehabilitation outside hospital is long and the return to work is late. In particular the reduced muscle mass is suggested to increase morbidity and mortality in ICU patients (Griffiths, 1996). Knowledge of the mechanisms behind the loss of muscle mass is necessary to generate strategies to counteract this reduction and thereby improving, not only the individual state of health, but also health economy.

The aim of this thesis is to investigate and characterize muscle protein synthesis in relation to surgical trauma and critical illness and to evaluate the effect of nutritional interventions.

1.1 PROTEIN METABOLISM AND TRAUMA

In growing individuals protein synthesis predominates over protein degradation, whereas in healthy adults there is a balance between protein synthesis and protein breakdown. During pathophysiological conditions with metabolic stress, such as trauma or critical illness, changes in protein metabolism leads to an imbalance between protein synthesis and protein breakdown resulting in a net loss of body protein.

Following trauma there is an increase in whole-body protein synthesis together with an even larger increase in whole-body protein breakdown resulting in a catabolic situation with a negative nitrogen balance (Cuthbertson et al., 1972). A major part of the protein loss comes from skeletal muscle (Arnold et al., 1993). A similar situation is found in the septic patient (Clowes et al., 1980). However, after elective surgery whole-body protein synthesis is not affected, but it may increase if complications to surgery arise. On the other hand whole-body protein degradation increases in relation to severity of the surgical trauma (Tashiro et al., 1996a; Yamamori et al., 1987).

The changes in whole-body protein metabolism seen after trauma and other pathophysiological insults are not uniformly distributed among tissues. Measurements in the whole body do therefore not adequately reflect the

changes in synthesis and breakdown in individual tissues. Studies on organ level are therefore necessary to better understand the metabolic changes in protein metabolism that occur following trauma and critical illness.

Skeletal muscle is our largest organ containing 40 % of the total amount of body proteins. Muscle represents as much as 50 % of the whole body protein synthesis. In catabolic states, skeletal muscle proteins are degraded and amino acids are transported from peripheral tissues to other organs with increased metabolic activity (Wernerman and Vinnars, 1987). The amino acids are utilized as substrates for gluconeogenesis, ureagenesis, oxidation, and protein synthesis in the liver, the intestinal mucosa and in immunocompetent cells (Clowes et al., 1980; Deutz et al., 1992; Rosenblatt et al., 1983; Souba and Wilmore, 1983). It is known that the ability to respond to trauma is dependent on available muscle mass and that a depleted patient has a higher morbidity and mortality (Griffiths, 1996). On a whole-body level, protein metabolism in response to trauma is quite well characterized, but in skeletal muscle it is less investigated.

1.2 METHODS TO STUDY MUSCLE PROTEIN METABOLISM

Tissue protein content in biopsies can be measured as alkali soluble protein (ASP) per DNA (Lowry et al., 1951), and it is a reflection of net protein balance in tissue. However, the exactness of such biochemical analyses or of imaging techniques (Lukaski, 1987; Nair, 1995; Welle, 1999), is insufficient to be able to elucidate small changes in protein mass of individual subjects. In addition estimates of muscle protein content do not give any information about regulation of muscle protein metabolism. To be able to study the effect of clinical interventions upon muscle proteins, it is necessary to have methods that give quantitative results.

Below techniques used to study muscle protein metabolism are described and discussed.

1.2.1 Intracellular concentration of amino acids

The concentration of free amino acids in skeletal muscle is well characterized. The intracellular amino acid pattern is only marginally affected by food intake (Bergstrom et al., 1990), but characteristic changes occur after trauma including a marked decrease in glutamine concentration (Hammarqvist et al., 1989; van Acker et al., 2000a; Vinnars et al., 1976). The decrease in muscle glutamine concentration correlates with the decrease in muscle protein synthesis seen after surgical trauma (Essen et al., 1992b; Wernerman et al., 1986b). Similar changes in muscle free amino acids are seen in conjunction with starvation and malnutrition (Jepson et al., 1988). The changes occurring after trauma are normalized rather slowly and glutamine muscle concentration is not back to normal until several weeks after elective surgery (Petersson et al., 1992). After short-term starvation on the other hand, muscle glutamine concentration is rapidly restored (Hammarqvist et al., 2005). In ICU patients the total amino acids are reduced by 60 % and the muscle glutamine concentration by 70 % (Gamrin et al., 1996). Determinations of tissue free amino acids are

highly reproducible, but the measurements remain an indirect method to assess muscle protein metabolism.

1.2.2 Ribosome analyses

Polyribosomes are aggregates of ribosomes and their connected proteins bound to mRNA molecules. Ribosomes that are not bound to mRNA are not involved in protein synthesis whereas those bound to mRNA are synthesizing proteins. Concentration and size distribution of ribosomes give qualitative information about the capacity for protein synthesis. The method has been used in studies of human muscle (Wernerman et al., 1985; Wernerman et al., 1986a). The method has an advantage over tracer methods as no assumptions about precursor pool enrichment are involved, no steady state is required and repetitive measurements are possible. However, the measurements are merely qualitative in character and do only allow to reveal the direction of changes in protein synthesis.

1.2.3 Protein degradation

Protein degradation may be studied by measuring enzyme activity of lysosomal proteases and of the 26S-proteasome pathway (Klaude et al., 2005) or the gene expression of components involved in these pathways (Biolo et al., 2000; Mansoor et al., 1996; Tiao et al., 1997). Such estimates are qualitative in character, and may give information about the direction of changes.

1.2.4 3-methylhistidine

Free 3-methylhistidine derived from contractile proteins cannot be re-used for protein synthesis and the only route for elimination is via urinary excretion. Consequently it can act as an index of the rate of actin and myosin degradation. The measurements of urinary 3-methylhistidine excretion have been used in clinical studies of starvation, surgical trauma and infections (Giesecke et al., 1989; Sjolín et al., 1990; Sjolín et al., 1989; Tashiro et al., 1996b). However, as 3-methylhistidine excretions in urine may originate from contractile proteins in other tissue than skeletal muscle it is a qualitative measurement of limited value (Rennie and Millward, 1983). When 3-methylhistidine release from a leg or arm is measured as an AV-difference or appearance rate, quantitative measurements of the degradation rate of contractile proteins can be obtained (Vesali et al., 2004).

1.2.5 Amino acid balance across organs

The arterio-venous differences of free amino acids can be used to evaluate protein metabolism in limbs and organs. Limbs in this kind of studies represent skeletal muscle tissue. The method includes the assessment of blood flow, and catheterisation of an artery and a vein that drains the organ of interest are necessary. Conclusions about protein balance can be made when the studied amino acid is not de novo synthesised or metabolised in the organ of interest.

The amino acid net balance obtained with this approach is only representing a measure of net protein balance.

To be able to differentiate between rates of protein synthesis and protein breakdown in an organ, the amino acid balance method must be combined with a tracer infusion (Nair et al., 1992). The advantage of this method is that estimates of both protein synthesis and degradation can be obtained. The main disadvantage is that it has many underlying assumptions, necessitating both a physiological and a tracer steady state during the measurements to obtain reliable results. Furthermore, calculation of protein synthesis and protein degradation is strongly related since they are based on the same measurements and assumptions. These problems can partly be overcome by additional measurement of the intracellular tracer amount, which is possible in limb studies. However, this makes the method even more invasive.

1.2.6 Incorporation of a labelled amino acid into protein

The most reliable method to study muscle protein synthesis rate quantitatively is to determine the incorporation rate of an isotopically labelled amino acid into tissue proteins. The method can be used to investigate protein synthesis in any tissue, if biopsies of that tissue can be obtained. There are two approaches to administer the labelled amino acid. The flooding method, where a large dose of the labelled amino acid is given over a short period of time (minutes) flushing through and thus equilibrating all amino acid compartments. With the constant infusion method the labelled amino acid is given in tracer amounts as an infusion over a longer period of time (hours), until an isotopic steady state is reached. The main objection to the constant infusion technique is related to the control of the precursor pool. The estimate of the enrichment in the tissue precursor pool given by the enrichment in plasma amino acids or plasma keto acids tends to underestimate protein synthesis. To receive a better control of the precursor pool, repetitive muscle biopsies are needed during the tracer infusion period, and this makes the constant infusion technique more invasive. The objection against the flooding technique is that a large dose of amino acids would stimulate protein synthesis. This has been shown when determination of muscle protein synthesis was made by the constant infusion method when a flood of leucine was given (Smith et al., 1992). The higher rate was the same rate as that calculated from the flood itself and may be related to lack of control of precursor pool during the constant infusion. The choice of the flooding technique to measure muscle protein synthesis in this thesis is based on the reliable estimate of precursor pool and the comparatively short measurement period, which enables studies of acute changes under physiological non steady state conditions such as surgery and critical illness. This thesis focuses on muscle protein synthesis, which may give information not only on the direction of changes, but also on the size of changes and the mechanism behind the changes. It would have been preferable to make measurements of muscle protein degradation in parallel, but no acceptable quantitative technique was available at the time.

1.3 THE EFFECT OF FEEDING

The effects of feeding and starvation on muscle protein synthesis rate are still not sufficiently characterized, mainly because results are related to the technique used for assessment. Healthy volunteers starved for three days show a 13 % decrease in muscle protein synthesis rate as compared to an overnight fast (Essen et al., 1992a). After three days of starvation there is a 25 % decrease in the polyribosome concentration (Wernerman et al., 1986a). The effect of feeding, investigated in healthy volunteers given hourly meals for ten hours, is an increase by 20 % in muscle protein synthesis rate as compared to the basal state after an overnight fast (McNurlan et al., 1993). A comparison between two groups of volunteers fasted for ten hours or fed for the same period did not show any difference in fractional synthesis rates between the groups, indicating that feeding may have a slow effect upon protein synthesis rate (McNurlan et al., 1993). Also, the decrease in total ribosome and polyribosome concentration observed after three days of starvation was not restored by two days of refeeding (Wernerman et al., 1986a). When employing the constant infusion technique, on the other hand, a doubling of muscle protein synthesis rate is reported after hourly meals of liquid food for 15 hours as compared to another group starved for 15 hours (Rennie et al., 1982). In this study the two groups of volunteers were compared without any measurements of the initial protein synthesis rate. The effect of feeding and starvation in the respective group were not investigated. So here we are left with conflicting results related to the technique chosen to assess muscle protein synthesis rate.

1.4 THE COMBINED EFFECT OF TRAUMA AND FEEDING

The decrease in muscle protein synthesis following surgery can be either an effect of the surgical trauma, an effect of starvation, or a combination. Before elective surgery, patients are usually fasted over night and moreover not sufficiently fed in the immediate postoperative period. Surgical trauma results in a 30 % reduction in polyribosome concentration in skeletal muscle on the third postoperative day, regardless provision of parenteral nutrition (Wernerman et al., 1986b). The same result is obtained using the flooding technique when parenteral nutrition is given for three postoperative days, the 50 % decrease in muscle protein synthesis is not counteracted (Essen et al., 1993). In both studies the parenteral nutrition was given during daytime, while the determination of muscle protein synthesis on the third postoperative day was performed in the morning after an overnight fast to make the postoperative measurement comparable to the preoperative measurement. Could the absence of an effect of feeding after a standardized trauma be due to fact that measurements were made in the postabsorptive state?

1.5 THE EFFECT OF GLUTAMINE

Glutamine is the most abundant free amino acid in skeletal muscle. In severe catabolic states, such as critical illness, muscle free glutamine decreases to 25 % of normal levels. Glutamine is constantly exported from the muscle to the splanchnic region to serve as an oxidative substrate for rapidly dividing cells in the immune system and in the intestinal mucosa. It is also a precursor for the synthesis of nucleotides. Glutamine is a non-essential amino acid, but under catabolic conditions with increased demands, endogenous production is insufficient, and glutamine is suggested to become a conditionally essential amino acid (Lacey and Wilmore, 1990). Depletion of plasma glutamine is related to a poor outcome in intensive care patients (Oudemans-van Straaten et al., 2001), while intravenous glutamine supplementation has beneficial effect on morbidity and mortality in intensive care patients (Goeters et al., 2002; Griffiths et al., 1997; Novak et al., 2002).

Surgical trauma leads to muscle glutamine depletion and to a decrease in muscle protein synthesis, which cannot be counteracted by provision of conventional parenteral nutrition. On the other hand, provision of intravenous glutamine postoperatively counteracts the decrease in muscle free glutamine and in protein synthesis, as assessed by ribosome analysis (Hammarqvist et al., 1989). This attenuation disappears if glutamine is discontinued (Pettersson et al., 1994). Glutamine supplementation is not effective if started up later in the postoperative course (Januszkiewicz et al., 1996). In intensive care patients, parenteral nutrition supplemented with 20 g glutamine per day during a five-day study period, was only marginally effective on muscle free glutamine concentration and on muscle protein synthesis, (Gamrin et al., 2000a). On the other hand, there are case reports showing an effect on muscle free glutamine concentration after intravenous supplementation of 40 and 60 grams of glutamine per day (Roth et al., 1992). Intensive care patients have increased demands of glutamine; the marginal effect on muscle protein synthesis may therefore be related to an insufficient dose of glutamine.

1.6 THE EFFECT OF GENERAL ANAESTHESIA

Anaesthesia is an important part of the surgical procedure and for interpretation of results from studies of the effect of surgical trauma on muscle protein synthesis it is necessary to determine the effect of general anaesthesia on muscle protein synthesis. Whole-body protein synthesis and degradation are decreased by halothane (Rennie and McLennan, 1985) while another volatile agent enflurane does not seem to affect whole-body protein synthesis or degradation (Carli and Elia, 1991). Whole-body protein synthesis and degradation is also unaffected by intravenous anaesthesia (Carli et al., 1990). The isolated effect of general anaesthesia on muscle protein synthesis rate has been less investigated, but modified neurolept anaesthesia has no effect on muscle protein synthesis (Essen et al., 1992b).

1.7 THE EFFECT OF SURGICAL TRAUMA

Elective open cholecystectomy, a trauma of moderate severity, results in a 30 % decrease in muscle protein synthesis when assessed by the flood technique, immediately after surgery (Essen et al., 1992b). A decrease of similar magnitude is also seen immediately following laparoscopic cholecystectomy (Essen et al., 1995). On the third postoperative day after open cholecystectomy a further decrease in muscle protein synthesis to 50 % of the preoperative value is seen (Essen et al., 1993). Another marker of protein synthesis, the total ribosome concentration, decreases by 12 %, when assessed 24 hours after hip replacement surgery (Blomqvist et al., 1995) and by 40 % three days after cholecystectomy (Wernerman et al., 1986b). We know that muscle protein synthesis is decreased by 12 % 24 hours following elective hip replacement, a comparatively less stressful surgical procedure. Immediately after surgery of medium severity, open cholecystectomy, muscle protein synthesis is decreased by 30 %. It is not known if the drop in muscle protein synthesis is influenced by the magnitude of surgical trauma. Surgical trauma increases whole-body protein degradation (Clague et al., 1983), while whole-body protein synthesis is less affected by moderate stress and is increased by severe surgical stress (Tashiro et al., 1991; Tashiro et al., 1996a; Yamamori et al., 1987). Taken together these changes lead to a period of negative nitrogen balance. Whole-body nitrogen loss following surgical trauma is proportional to the severity of the surgery (Cuthbertson et al., 1972). However, as mentioned earlier these changes are whole-body measurements and the result represents changes of all tissues. Other metabolic markers that demonstrate changes proportional to the severity of surgery are urinary excretion of 3-methylhistidine (Tashiro et al., 1996b) and reduction in insulin sensitivity (Thorell et al., 1993). Also, the cytokine response of IL-6, a marker of tissue damage correlates with severity and duration of trauma (Cruickshank et al., 1990).

1.8 MUSCLE PROTEIN SYNTHESIS IN ICU PATIENTS

Intensive care patients are a heterogeneous group of individuals, although they all share a critical illness with multiple organ failure. In contrast to medium size surgery patients with a 30 % decrease in protein synthesis rate postoperatively, intensive care patients have a mean protein synthesis rate, which is similar as to what is found in healthy individuals (Gamrin et al., 2000b; Garlick et al., 1989). However, the variation is higher in intensive care patients, between 0.46 – 3.86 % per 24 h (Essen et al., 1998; Gamrin et al., 2000a; Gamrin et al., 2000b). Muscle protein synthesis rate in healthy volunteers is 1.65 % per day ranging from 1.33 – 2.28 % per 24 h (McNurlan et al., 1991), which is not different from muscle protein synthesis rate in elective surgery patients (Essen et al., 1995).

The large scatter in muscle fractional synthesis rate in ICU patients is not related to any clinical feature. In subgroups a relation to acidosis/alkalosis is reported (Caso et al., 2004; Vosswinkel et al., 2000), also a weak correlation to

the length of stay in the ICU is seen (Gamrin et al., 2000b). Within the larger scatter singular patients with a marked elevated fractional synthesis rate are noted. In healthy volunteers a good agreement between muscle protein synthesis rates determined from both legs simultaneously is seen (McNurlan et al., 1991). May therefore heterogeneity or artefacts explain the larger scatter seen in ICU patients?

2 AIMS

In general terms the aim of the thesis was to investigate some new aspects of the impact of metabolic stress and feeding on muscle protein synthesis following surgical trauma and during critical illness.

The specific aims of the thesis were:

- To determine whether the absence of effect of feeding on muscle protein synthesis rate in the postoperative period is due to the fact that the measurements were made in the postabsorptive state after an overnight fast, and not during ongoing feeding.
- To evaluate the effect of intravenous glutamine supplementation in three different doses on muscle protein synthesis rate in intensive care patients
- To investigate the influence of different size of surgical trauma on muscle protein synthesis rate
- To investigate the reproducibility of muscle protein synthesis rate and muscle morphology in intensive care patients

3 MATERIALS AND METHOD

3.1 PATIENTS

Metabolically healthy patients (n = 41) undergoing elective surgery were included in studies I and III. Preoperatively all had normal routine blood chemical analyses for haemoglobin, leucocytes, liver enzymes and renal function tests. The intention was that these patients would represent a human model of trauma.

The patient model of open cholecystectomy has been very useful in a number of studies (Essen et al., 1993; Essen et al., 1992b). It was natural to choose this model in study I, and the study was done at a time-point when elective open cholecystectomy was still a frequent procedure. Today elective abdominal surgery is most often done by laparoscopic technique and in addition anaesthesia procedures are different. Open colonic resection has also been used as a human trauma model, but the results are more scattered and less well reproducible as compared to open cholecystectomy (Flaring et al., 2003).

When looking for patient-groups suitable to represent minor and major surgical trauma, the choice for minor surgery fell upon partial mastectomy, a minor surgical procedure, outside the abdominal cavity in otherwise healthy individuals. The possible disadvantages were the uneven gender distribution and the comparatively younger age group. For major surgery several different procedures were intentionally used. The difficulty was to find patients that were not weight losers, and who were not on medication. Here the methodological problem was the large scatter in operation time and bleeding, independent of identical procedures.

Patients (n = 57) admitted to the multi-disciplinary ICU at Karolinska University Hospital at Huddinge were included in the intensive care studies II and IV. Here the purpose was to include patients with multiple organ failure representative of the long-stayers in the ICU. Some patient-groups were left out; the immunosuppressed patients, in whom muscle biopsies could often not be performed. Also patients on renal replacement therapy were left out for the same reason. In study II patient inclusion was problematic as a 5-day treatment period in the ICU was a part of the protocol.

The patients (I, III), or if communication was not possible as in the ICU patients, the relatives (II, IV), were informed about the nature, purpose, procedures and potential risks involved in the study before obtaining their voluntary consent to participate in the studies. In total 98 patients were investigated (Table 1). The Ethical Committee at Karolinska Institutet, Stockholm, Sweden, approved all experiments.

Table 1.

Characteristics of the patients participating in the studies. Means \pm SD.

Study		n =	Gender (male/female)	Age (year)	Height (meter)	Weight (kilogram)
I	NaCl	7	3 / 4	42 \pm 12	1.68	70 \pm 12
	TPN	7	5 / 2	43 \pm 15	1.76	78 \pm 13
II	controls	10	9 / 1	64 \pm 10	1.75	77 \pm 15
	20 g GLN	10	5 / 5	65 \pm 13	1.72	73 \pm 14
	40 g GLN	10	6 / 4	62 \pm 15	1.69	73 \pm 13
	60 g GLN	10	6 / 4	65 \pm 16	1.74	71 \pm 19
III	Minor	8	0 / 8	50 \pm 6	1.65	67 \pm 8
	Major	19	10 / 9	58 \pm 10	1.72	70 \pm 17
IV		17	8 / 9	64 \pm 13	1.72	84 \pm 19

3.2 CLINICAL TREATMENT

3.2.1 General anaesthesia

In order to obtain results comparable with those reported by Essén et al (Essen et al., 1992b), a modified neurolept general anaesthesia was used in study I as described in the article. Today this type of general anaesthesia is abandoned related to a relative high frequency of awareness during anaesthesia and also the relative poor intra-operative pain-control obtained.

General anaesthesia for minor and major surgery in study III, was carried out with modern volatile agents after induction with propofol. The major surgery group was anesthetized in two slightly different ways as described in detail in the manuscript. Anaesthesia for minor surgery and major surgery was carried out as described in the article (III).

All routine perioperative proceedings such as anaesthesia time, operating time, blood loss, blood products, fluid therapy and urinary flow were registered. At the end of surgery postoperative temperature were noted for all patients.

3.2.2 Nutrition

Parenteral nutrition was given in study I, II and IV. In study I and II the quantity and composition of nutrition were decided by the investigators, whereas in study IV the nutrition were prescribed by the intensivist in charge according to the routine of the unit.

The strategy to feed the critically ill patient has changed over the years and the patients are more often and to a greater degree feed by the enteral route today. In study IV parenteral and enteral nutrition were combined for most patients.

3.2.3 Intensive care treatment

Residents or fellows in Anaesthesiology and Intensive care Medicine attending the patients in the ICU on a daily basis or on call, cover the ICU at Karolinska University Hospital 24 hours. The patients in study II and IV were in every aspect, except for the purpose of the studies, treated according to the routines of the unit.

3.3 STUDY PROTOCOLS

Determinations of muscle protein synthesis rate were performed employing the same protocol in all studies (Figure 1). In study no I, II and III muscle protein synthesis was determined twice, before and after surgery (I and III) or before and after a five-day treatment period (II). Muscle protein synthesis rate was determined in both legs in study IV and therefore one of the measurement periods had to be extended by three minutes.

3.3.1 Randomization

In study I and II the patients were randomized to the different treatment strategies by the closed envelope system.

3.3.2 Determination of fractional synthesis rate of protein

The flooding technique for the determination of muscle protein synthesis rate was used in all studies and the method is described in detail in the articles. The determination of fractional synthesis rate starts with an intravenous injection of L-[²H₅] phenylalanine given over a ten minute period. Blood samples were taken at regular intervals during the 90-minute incorporation period. At 90 minutes a percutaneous muscle biopsy was taken from the thigh muscle. At the second measurement of muscle protein synthesis rate the procedure was repeated but in addition, to determine the basal isotopic enrichment an initial muscle biopsy was taken before injection of labelled L-[²H₅] phenylalanine.

3.3.3 Blood sampling

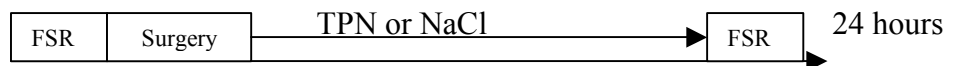
In patients undergoing elective open cholecystectomy (I) and minor surgery (III a) a venous line in the antecubital vein was used for blood sampling while the rest of the patients all had arterial lines in the radial artery for continuous blood pressure measurement and blood sampling. In study II additional blood sampling were done every morning for determination of plasma glutamine concentration and some of the parameters necessary to calculate the SOFA score.

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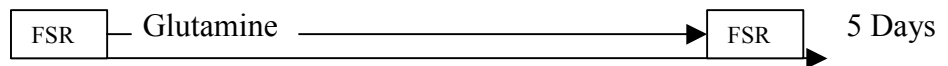
3.3.4 Urine collection

In study II urine was collected in 24-hours portions from between 06:00 to 06:00 from day one until the morning of day seven. Thus, the collection started 8 hours before study start and ended 16 hours after the end of the period. The extended period of urine collection was in accord with the routines of the ward in order to facilitate for the staff.

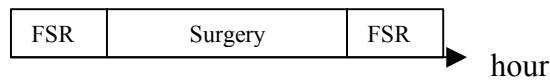
Study I.



Study II.



Study III.



Study IV. Measurement of Protein synthesis rate (FSR)

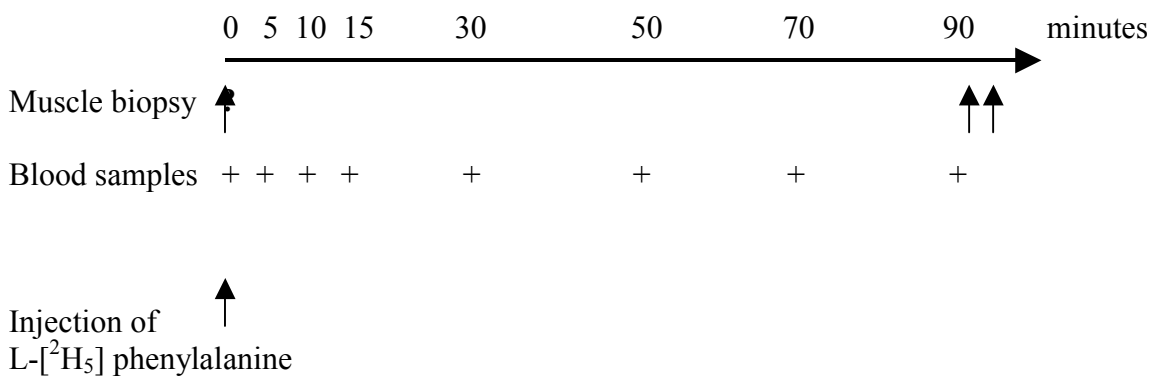


Figure 1. Experimental protocols of the different studies (I, II, III and IV). The protocol for muscle protein fractional synthesis rate (FSR) determinations in study IV is representative for the FSR determinations also in the other studies.

3.3.5 Muscle biopsies

In total 98 patients were investigated in the four different studies indicating a large number of muscle biopsies. Taking a muscle biopsy is an invasive procedure and it is associated with local pain and a possible risk for haemorrhage and infection. In spite of the fact that some 300 muscle biopsies have been taken, only 3 patients experienced any side effects. The side effects registered were minor and consisted of the change of bandage due to leakage of tissue fluid. The volunteers were awake during the biopsies and they experienced a short “kick of a horse” followed by muscle stiffness the next coming days. The technique has been described in detail elsewhere (Bergström, 1962). In brief percutaneous muscle biopsies were taken with the Bergström needle from the lateral portion of the quadriceps muscle, 15 – 20 cm above the knee. Additional biopsies were taken not less than 4 cm apart or from the other leg in order to avoid an effect of possible muscle damage made by previous biopsies. In the subjects that were awake, an injection of local anaesthesia was given into the skin before taking the biopsy. The intensive care patients were sedated with intravenous propofol and if needed supplementary morphine. The weight of the muscle aliquot for measurement of muscle protein synthesis rate was 25-50 mg. The total weight of the biopsy material was 200 – 400 mg. depending on the number of analyses. The tissue specimens were divided into portions and were frozen in liquid nitrogen within 1-2 minutes after sampling. All samples were stored at – 80 ° C until analysed.

3.4 ANALYTICAL TECHNIQUES

3.4.1 Blood analyses

Serum glucose and serum insulin were analysed in study I, and the methods are described in the article. Blood chemical parameters needed for SOFA score and APACHE II scores were analysed by the chemical laboratory at the hospital. All ICU patients were tested not having any coagulation disorders prohibiting muscle biopsies.

3.4.2 Urine analyses

The total nitrogen content of the urine (study II) was determined by a chemoluminescent nitrogen system described in study II. 3-methylhistidine in urine was analysed using HPLC (Wassner et al., 1980).

3.4.3 Tissue analyses

Thiols in human muscle tissue were determined by HPLC, as described by Luo et al (Luo et al., 1995). The assays for water, electrolyte, protein, nucleic acids, lactate and energy-rich phosphates have been described elsewhere in detail (Forsberg et al., 1991).

3.4.4 Measurement of fractional synthesis rate

The measurement of protein synthesis rate in muscle tissue by the flooding dose technique has been described in detail previously (McNurlan et al., 1991). In brief, the determination of L- [²H₅] phenylalanine enrichment in plasma samples, as well as in samples of hydrolyzed muscle protein was done by gas chromatography-mass spectrometry with electron impact ionization and selective ion monitoring. The enrichment in plasma was measured by monitoring the ions at masses of mass/charge 336 and 341 of the tertiary butyldimethylsilyl derivate of phenylalanine. The enrichment of phenylalanine from protein hydrolysates was measured following enzymatic decarboxylation to phenylethylamine and subsequent analysis of its heptafluorobutyryl derivative at masses of mass/charge 106 and 109.

The fractional rate of protein synthesis (FSR in %/day) was calculated from the formula:

$FSR = ([P(t) - P(0)] \times 100/A) \times 1440$, where P (0) and P (t) are the enrichments of phenylalanine in muscle protein at the beginning and at the end of the incorporation period (Molar Percent Excess) and A is the area under the curve for plasma phenylalanine enrichment (MPE x time in days).

3.5 STATISTICAL ANALYSES

In study no I data are given as individual values or mean values \pm SEM. In the other studies data are given as individual values or mean \pm SD if not indicated otherwise. Statistica for Windows (Statsoft, Tulsa, USA) was used for evaluation of correlations and for comparison of rates of protein synthesis by Student's t-test and ANOVA/MANOVA for repeated measure. In study no IV, Statistica with non-parametric statistics were employed. Differences were taken as statistically significant if $P < 0.05$.

4 DISCUSSION OF RESULTS

Over all this thesis deals with two major questions concerning muscle protein synthesis: 1) if the catabolic effects of trauma and sepsis can be attenuated by nutritional means, and 2) if the mechanisms behind this catabolic effects are related to the size of the insult or to the large inter-individual variation in muscle protein synthesis. Question 1 was addressed in study I and II and question 2 in study III and IV.

4.1 STUDY I

Muscle protein synthesis decreases following surgical trauma, and this is not prevented by conventional parenteral nutrition (Essen et al., 1993; Wernerman et al., 1988). However, the measurements of protein synthesis rate before as well as after surgery were made in the postabsorptive state after an overnight fast. The effect of continuous and ongoing nutrition has not been elucidated. To answer this question, in study I it was investigated if ongoing continuous total parenteral nutrition would attenuate the decrease in protein synthesis rate observed after elective abdominal surgery. Fractional synthesis rate in the group receiving NaCl decreased by 31 ± 20 % ($P < 0.01$) and the patients randomized to receive total parenteral nutrition decreased by 23 ± 13 % ($p < 0.006$) (Study I, Figure1). There were no significant differences between the two groups before or after surgery ($P = 0.37$). So, ongoing and continuous total parenteral nutrition during 24 hours could not prevent the decrease seen in muscle protein synthesis. Although, there was no significant difference between the two groups an attenuated effect on muscle protein synthesis cannot completely be ruled out due to the small number of patients investigated.

4.2 STUDY II

Conventional parenteral nutrition did not attenuate the decrease seen in muscle protein synthesis, addition of intravenous glutamine in the postoperative situation can attenuate the muscle glutamine depletion and the decrease in muscle protein synthesis (Hammarqvist et al., 1991; Petersson et al., 1994). A preliminary report indicates that this may also be the case in the ICU patients (Roth et al., 1992), although a 20 g glutamine supplementation for five days only gave a marginal effect on muscle protein synthesis in a similar patient group (Gamrin et al., 2000a). The main hypotheses in study II was that a large enough dose of glutamine given to ICU patients would have a positive effect on plasma glutamine concentration and further on influence muscle glutamine concentration and muscle protein synthesis rate.

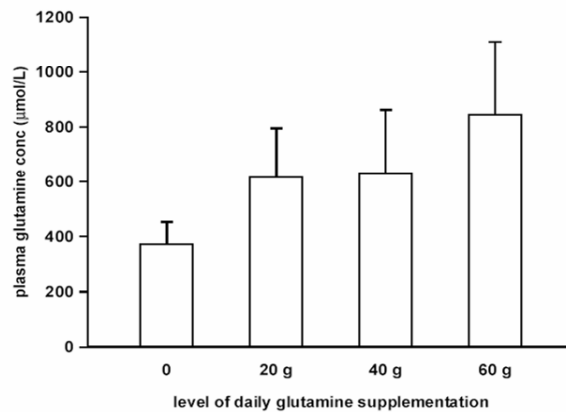


Figure 2. Plasma glutamine concentrations in ICU patients (n = 4x10) randomised to receive total parenteral nutrition supplemented with 0, 0.28, 0.57 or 0.86 gram of glutamine per kg body weight per day (approximately 0, 20, 40 or 60 g of glutamine per day) during a five-day study period. Data are expressed as means \pm SD.

The most important finding studying study II was that provision of intravenous glutamine normalized plasma glutamine concentration in all glutamine treated groups (figure 2). The restoration of plasma glutamine into the normal range was rapid and dose-dependent. Furthermore, the higher level remained throughout the five-day study period.

Plasma glutamine concentrations are low in intensive care patients with pancreatitis (Roth et al., 1985), burns (Parry-Billings et al., 1990), sepsis and stress (Roth, 1982). At the start of study II the mean plasma glutamine concentration was 408 ± 160 $\mu\text{mol/L}$ which is under the lower normal limit for plasma glutamine concentration (500 $\mu\text{mol/L}$). The present study, showing a normalization of plasma glutamine in 30 glutamine-supplemented patients, is too small for any conclusions about mortality rate.

Glutamine supplementation in three different doses 20, 40 or 60 g per day during five days did however not effect muscle glutamine concentration and consequently had no effect on muscle protein synthesis rate (Figure 3).

The patients were studied for five days and many additional parameters were investigated although they were not the main purpose of this study. These post-hoc analyses elucidated several possible effects of the extra glutamine on skeletal muscle protein degradation and energy metabolism.

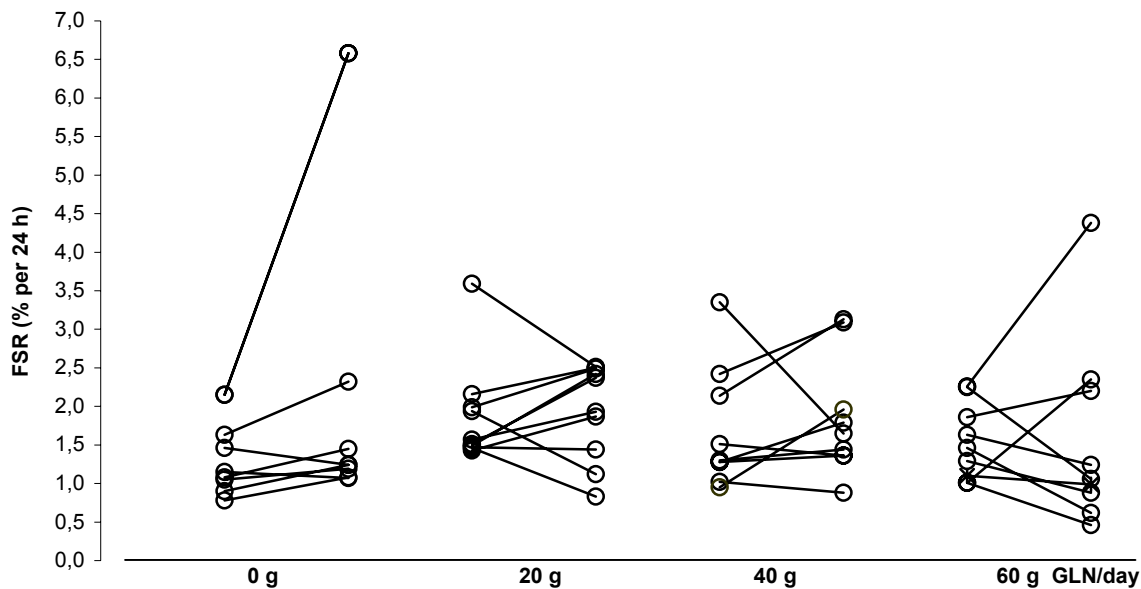


Figure 3. Muscle protein fractional synthesis rate before and after a five-day study period in ICU patients receiving total parenteral nutrition supplemented with 0, 0.28, 0.57 or 0.86 g of glutamine per kg body weight per day (approximately 0, 20, 40 or 60 g of glutamine per day).

3-Methylhistidine excretion in urine was not different between the four groups. However, when comparing the combined glutamine treated groups with the control group, a 30 % decrease in 3-methylhistidine excretion in urine was seen in the glutamine treated group (Study II, Figure 2). This post hoc result may indicate a positive effect of glutamine supplementation on muscle protein degradation in man. A similar effect of glutamine has been shown in glutamine perfused rat muscle resulting in decreased net protein loss and protein breakdown (MacLennan et al., 1987). It emphasizes the importance to find methods to study both protein synthesis and protein breakdown quantitatively. Employing an infusion of labelled 3-methylhistidine and measure the arterio-venous difference of tracer and unlabelled 3-methylhistidine is a new method for measurement of contractile protein breakdown possible to use together with the flood method for muscle protein synthesis (Vesali et al., 2004).

Phosphocreatine constitutes a store of energy rich phosphates that can be utilized for muscle contraction via ATP, which is the initial source of energy for muscle contraction. In healthy individuals phosphocreatine utilized during exercise is rapidly resynthesised. In the septic and traumatized patient phosphocreatine concentration in muscle is decreased and the decrease is more pronounced in patients with sustained disease (Bergstrom et al., 1976). In the present study all groups were low in initial phosphocreatine concentration in muscle. During the five-day study period the control group made a further decrease in both creatine and the phosphorylated fraction of creatine. The glutamine treated groups remained unaltered and this is in

accord with other intensive care patients given α -ketoglutarate or glutamine (Gamrin et al., 2000a).

Another secondary finding was that the ICU patients in this study had mean muscle lactate concentration of 18.8 ± 7.6 mmol/kg fat free solid, which is more than twice the normal level. The control group increased significantly in muscle lactate concentration for the period of the study whereas in the combined glutamine treated group muscle lactate remained unchanged (Figure 4). Glutamine and glutamate are important precursors for the synthesis of α -ketoglutarate, which is an intermediate in the Krebs cycle. A shortage of intermediates for the Krebs cycle may induce an elevated production of lactate from pyruvate in ICU patients. Decreased levels of Krebs cycle intermediates have been shown in an experimental animal model for catabolism (Rooyackers et al., 1996). This post hoc finding of a positive effect of glutamine on muscle lactate levels together with an attenuated decrease in total and phosphorylated creatine may suggest a positive effect of glutamine supplementation on muscle energy metabolism. An alternative explanation could be that extra glutamine provides substrate for gluconeogenesis and that muscle can decrease its production of lactate as a gluconeogenic substrate. An increased activity in the Cori cycle has been suggested in ICU patients (Tappy et al., 1998), and this might be inhibited by the extra glutamine. However, to confirm these post-hoc results, studies designed to specifically address these findings are needed.

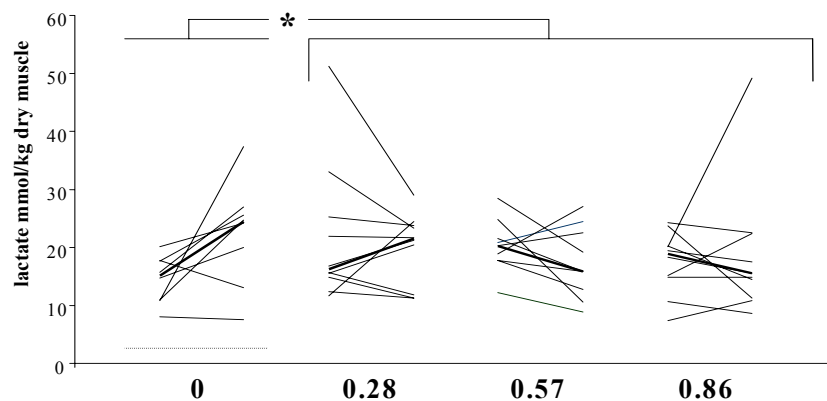


Figure 4. Muscle lactate concentration before and after a five-day study period in ICU patients receiving total parenteral nutrition supplemented with 0, 0.28, 0.57 or 0.86 g of glutamine per kg body weight per day. The filled lines denote mean values.

4.3 STUDY III

The immediate effect of abdominal surgery of medium size on muscle protein synthesis rate is a decrease by 30 % (Essen et al., 1992b). Muscle protein synthesis rate in the minor surgery patients in study III was 1.72 ± 0.25 % per 24 h before surgery and 1.67 ± 0.29 % per 24 h after surgery ($P=0.68$). The patients in the minor surgery group, ($n=8$) had an operating time of 34 ± 13 min and a mean time between the two measurements of 168 ± 34 minutes. The major surgery patients ($n=20$) underwent surgery for of 211 ± 80 minutes and between the two measurements there was 360 ± 78 minutes. The hypothesis in this study was that the trauma response on fractional synthesis rate was size related. However, the major surgery patients had a basal muscle protein synthesis rate of 1.62 ± 0.30 % per 24 h before surgery and no statistically significant change were seen following surgery, 1.57 ± 0.40 % per 24 h ($P=0.59$) (Study III, Figure 1). This result was unexpected. Whole body protein breakdown and synthesis have been postulated to be depressed with mild injury but increase linearly with increasing severity of trauma (Clague, 1981). Similar to intensive care patients, major surgery patients are a heterogeneous group of patients and the surgical procedure as well as the anaesthesia management are not possible to standardize. Many confounding factors could be partly responsible for the absence of impact of a major surgical trauma on muscle protein synthesis rate.

Muscle protein synthesis rate is not influenced by modified neurolept anaesthesia (Essen et al., 1992b). Halothane, a volatile agent for anaesthesia and not very much employed today, has been reported to lower both protein synthesis and protein breakdown (Rennie and MacLennan, 1985). In study III anaesthesia was based on the volatile agent isoflurane or sevoflurane and no decrease were seen in muscle protein synthesis. Intravenous anaesthesia (propofol/remifentanil) or the combination desflurane/remifentanil does not influence whole-body protein synthesis (Schricker et al., 2001). Furthermore, the use of a continuous epidural blockade has been reported to attenuate the decrease in muscle protein synthesis 48 h postoperatively (Carli and Halliday, 1997). The indwelling epidural catheters in the present study were not activated during the study period. Anaesthesia as a confounding factor seems unlikely but methods to give anaesthesia have developed and an alternative explanation is that current anaesthesia routines may abolish the decrease in muscle protein synthesis rate earlier observed after surgery?

A descriptive survey of the influence of surgical trauma of different magnitudes and muscle protein fractional synthesis rate in relation to time following surgery is illustrated in figure 5. Another possible explanation to the absence of size related trauma response on muscle fractional synthesis rate might be that there is a variable time course following surgical trauma related to the magnitude of the trauma. Major surgery patients have not been investigated later on in the postoperative period. Furthermore, the postoperative effects may have a delayed onset, and an extended duration, since the changes in ribosomal

pattern seen after elective abdominal surgery are still not restored one month following surgery (Petersson et al., 1990). To fully understand muscle protein synthesis metabolism in the postoperative period, longitudinal studies of both protein synthesis and degradation are needed.

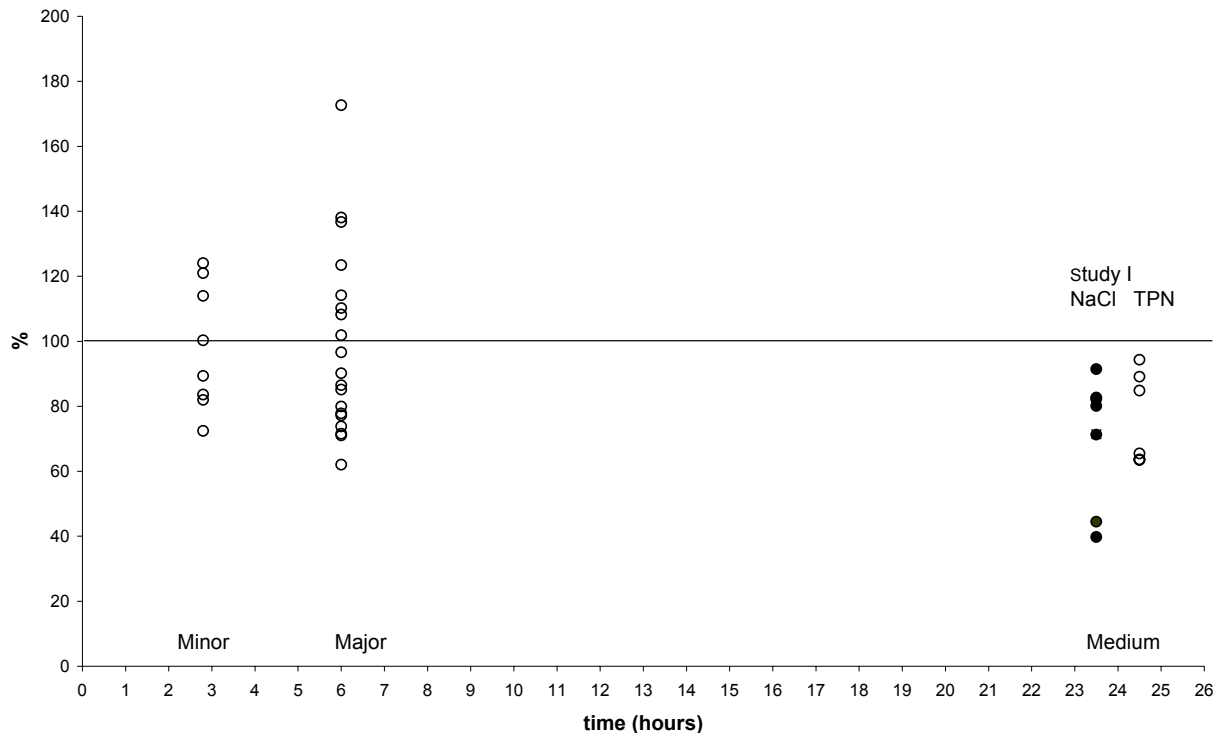


Figure 5. Change in muscle protein fractional synthesis rate in patients undergoing elective surgery. Postoperative values are given as percentage of preoperative values, and the mean time after start of surgery is indicated. Data from studies I and III.

4.4 STUDY IV

The variation in muscle fractional synthesis rate is addressed in study IV of this thesis (Figure 6). A possible explanation could be that local phenomena within the muscles of critically ill patients are responsible for this variation. Fractional synthesis rates were therefore measured simultaneously from both legs in critically ill patients with multi organ failure. Mean fractional synthesis rate determined on day 2 (1-42) of ICU stay was 2.64 ± 2.28 % per 24 h in the left leg and 2.79 ± 2.30 % per 24 h ($P=0.15$) in the right leg (Study IV, Figure1). The low intra-individual CV of 5.5 ± 3.6 % illustrates the homogeneity of muscle fractional synthesis rates in ICU patients measured at the same time in the two legs. Muscle morphology was examined and no obvious local differences were found between the two legs.

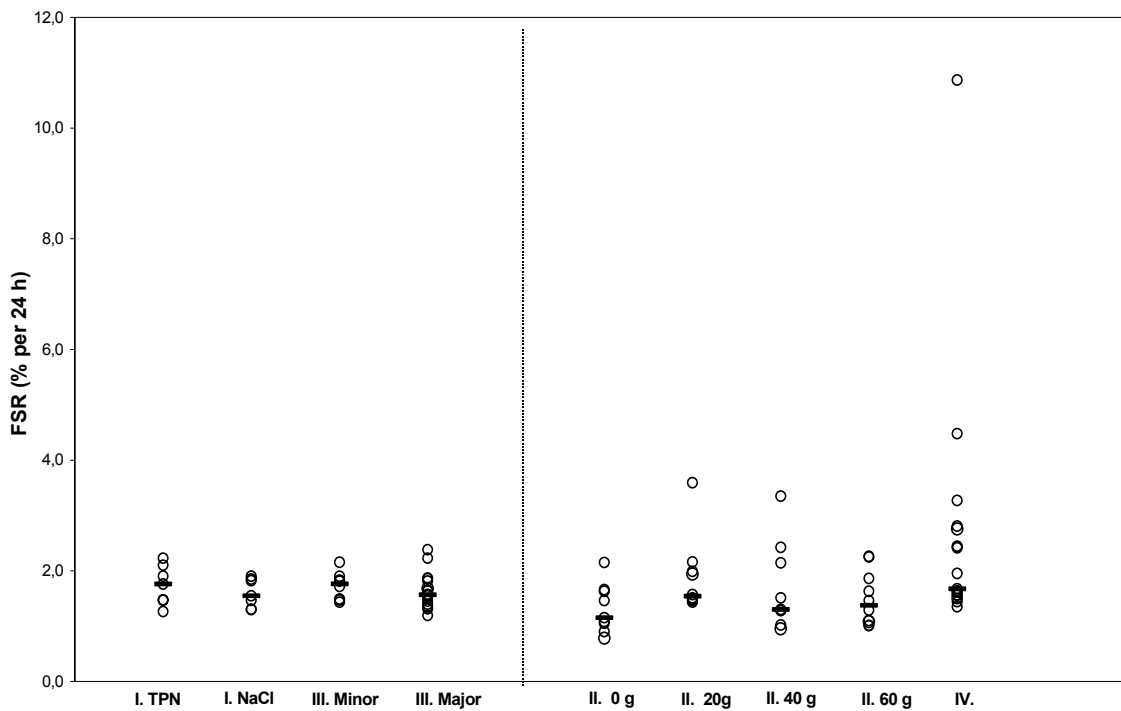


Figure 6. Muscle fractional synthesis rate preoperatively in patients scheduled for elective surgery (Studies I and III) and in intensive care patients before interventions (Studies II and IV). The horizontal bar indicates the median value.

The main result of this study is that muscle protein synthesis rate is similar in both legs and that artefacts and heterogeneity can be ruled out as explanation to the large scatter found in intensive care patients.

In parallel with previous studies (Gamrin et al., 2000a; Gamrin et al., 2000b), outliers with very high muscle protein synthesis rate were found, and of a magnitude never reported before. Besides analytical errors, which are less probable as muscle fractional synthesis rate is similar in both legs, this high fractional synthesis rates may be associated to clinical parameters. Of all the tested clinical parameters, length of stay in the ICU was the only one showing a correlation. Adding the two studies (II, IV) performed in intensive care patients (n = 55) together, a statistically significant correlation was found between the mean fractional synthesis rate (study IV), fractional synthesis rate in first biopsy (study II) and length of ICU stay, $r^2=0.63$, $P<0.001$. Still, if the outliers (n = 5) were excluded a clear correlation exists $r^2= 0.14$, $P< 0.018$ (Figure 7). This correlation was also seen in a combined material of ICU patients from Essén and Gamrin (Gamrin et al., 2000a). In study II there were no statistically significant changes in fractional synthesis rate studied five days apart in the control group, in any of the glutamine treated groups or in all groups together if they were pooled ($p=0.14$).

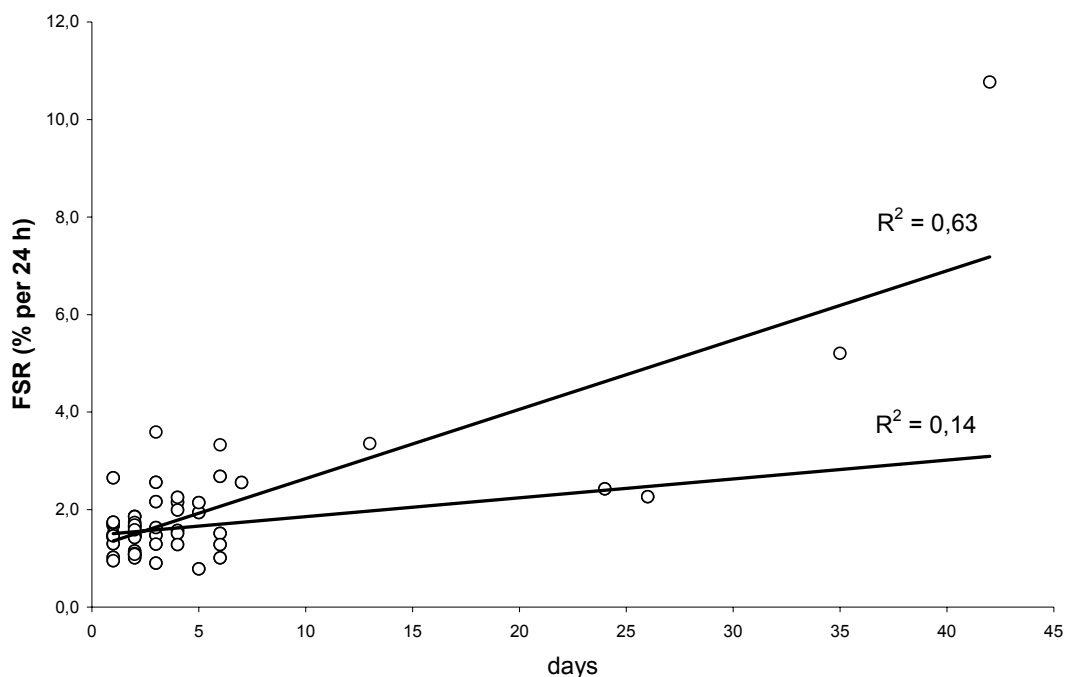


Figure 7. Correlation between day of intensive care unit stay and muscle fractional synthesis rate in critically ill patients ($n = 55$) $r^2 = 0.63$, $P < 0.001$. The correlation remains statistically significant also when outliers were excluded $r^2 = 0.14$, $P < 0.018$. Data from studies II and IV.

The statement implying that muscle fractional synthesis rate do not change over time may not be valid. A prospective longitudinal study at 10 – 20 - 30 days of ICU stay is necessary to solve this issue.

In study IV, two patients were obvious outliers and one had a mean muscle protein synthesis rate of the two legs of 10.77 ± 0.14 % per 24 h when studied on day 42 of ICU stay. In this patient, muscle morphology showed pathological changes characteristic for critical illness myopathy. These two patients were also examined by electromyography (EMG) and electroneurography (ENeG) outside the study protocol because of clinical signs of muscle weakness. EMG and ENeG showed axonal polyneuropathy with signs of denervation typical for critical illness neuropathy (Larsson et al., 2000; Stibler et al., 2003). These two cases with high fractional synthesis rate are anecdotic but they raise the question about the effect of the length of stay in the ICU and muscle pathology. Prospective studies of the longitudinal effect on muscle protein synthesis in ICU patients with persistent multiple organ failure are needed.

5 GENERAL DISCUSSION

This thesis focuses on muscle protein synthesis in relation to feeding, surgical trauma and sepsis. Whole body protein metabolism in the post-traumatic period is characterized by catabolism and negative nitrogen balance. Muscle protein content decreases by some 1.5 % per day during critical illness and this is not prevented by conventional nutrition (Gamrin et al., 1997). Food is known to be a good thing; what the protein losses over time would be without nutrition would be unethical to investigate today. There is indirect evidence for the benefit of adequate feeding in terms of a worse outcome in ICU patients with a large cumulated energy deficit (Bartlett et al., 1982; Singer et al., 2004). The imbalance between protein synthesis and protein breakdown, where protein breakdown predominates (Mansoor et al., 1996; Tiao et al., 1997), is the mechanism behind muscle protein depletion. To assess muscle protein metabolism, it is therefore necessary to be able to measure both protein synthesis and degradation quantitatively in parallel. Available methods to quantify in vivo muscle protein degradation rates depend on many assumptions that have not been well validated when these methods are applied to physiological unstable situations. However, the last 5 years new methods to assess muscle protein degradation have been developed and better validated. Application of these methods to ICU patients in the future will enable us to study protein metabolism in more detail and with better accuracy evaluate the effects of interventions. Quantitative measurements of protein synthesis were the focus of this thesis.

The quantitative determinations of muscle protein synthesis were made by incorporation of labelled amino acids into protein. This can be done by two main approaches, the constant infusion technique and the flood technique. The two techniques sometimes result in different rates of protein synthesis and a controversy has arisen concerning the accuracy of the measurements. In the fed state the two techniques give similar values for protein synthesis, while in the fasted state, determinations by the constant infusion method are lower (Garlick et al., 1994). Calculations of the protein synthesis rate by the constant infusion technique are made from the plasma enrichment as a surrogate estimate of the enrichment in the precursor pool and the enrichment in the tissue protein. For an accurate measurement of tissue protein synthesis rate, a reliable estimate of the true precursor pool (tRNA) is essential (Figure 8).

Tissue Amino Acid Pools

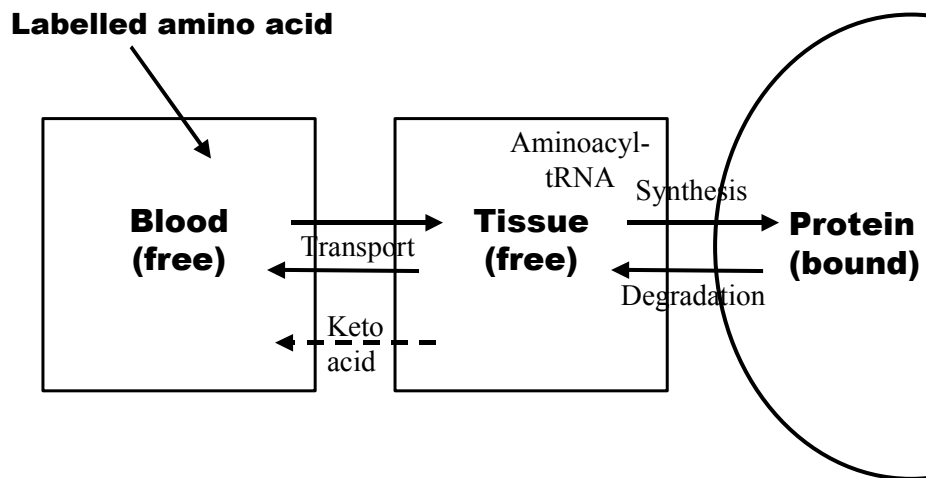


Figure 8. Schematic illustration of the different surrogate precursor pools for protein synthesis and the real precursor pool tRNA.

With the constant infusion method the labelled amino acid is given in tracer amounts over a period of 4-6 hours after an initial bolus. Protein synthesis rate is calculated by determination of the plateau value of plasma enrichment and the incorporation into protein of labelled amino acid at the end of the infusion. The method requires an isotopic steady state, where it is assumed that the isotopic enrichment of plasma free amino acid pools is representative of the true precursor pool for protein synthesis during the incorporation period. In a series of experiments in healthy volunteers, a constant infusion of two labelled amino acids during 6 hours was followed by a flood of another labelled amino acid (Smith et al., 1991; Tjader et al., 1996a; Tjader et al., 1996b). Measurements of the isotopic enrichment in the different surrogate pools to estimate the precursor pool enrichment for protein synthesis show that plasma enrichment is a poor estimate of tissue enrichment during the preflood period (Figure 9). During the postflood period free amino acid enrichment was closer in the surrogate pools. For the flooded amino acid there is a good agreement between the different surrogate precursor pools. An underestimation of muscle protein synthesis rate is seen where the surrogate precursor pools in plasma are compared to enrichment of the real precursor pool (tRNA) (Ljungqvist et al., 1997).

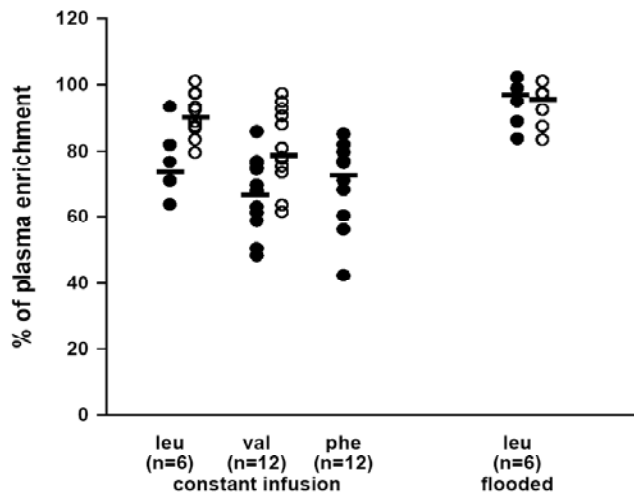


Figure 9. Isotopic enrichment of free amino acids in muscle tissue expressed as a percentage of the plasma enrichment in amino acids (filled symbols) and keto acids (open symbols) in healthy volunteers (n=18) given a constant infusion of the isotopically labelled amino acids for 360 minutes followed by a flood of another amino acid. Data from (Smith et al., 1991; Tjader et al., 1996a; Tjader et al., 1996b).

Even more problematic was the fact that nutritional intervention was able to change the relationship in enrichment between the different pools. The flooding technique equilibrates all possible amino acid compartments when given a large bolus dose (Davis et al., 1999; McNurlan et al., 1994). Furthermore, there is no difference between in results from the two methods when an isotopic steady state is documented in the precursor tRNA enrichment (Caso et al., 2002). Also when the enrichment in the tissue free amino acid pool is used as a surrogate for precursor enrichment, it is only marginal differences between the two techniques (Figure 10).

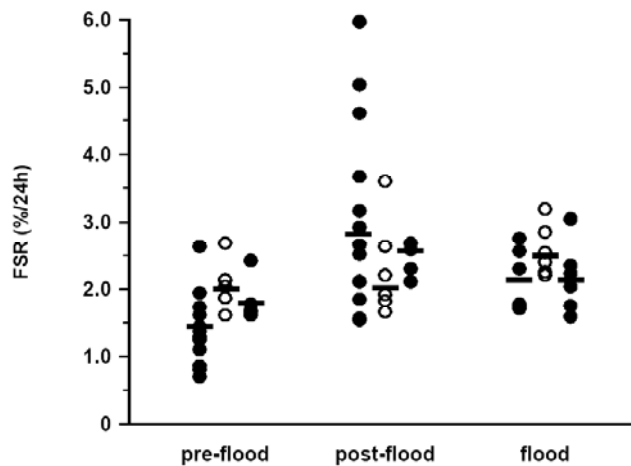


Figure 10. Muscle protein synthesis rate in healthy volunteers (n=18) in the basal state (filled symbols) and in the fed state (open symbols). Measurements are made by the constant infusion technique in the pre-flood and post-flood states, and by the flooding technique in the flood state. The horizontal bars indicate median values. Data from (Smith et al., 1991; Tjader et al., 1996a; Tjader et al., 1996b).

The main hypothesis in study II was that a large enough dose of exogenous glutamine supplementation would normalize plasma glutamine concentration, attenuate the decrease in muscle glutamine concentration, promote an increase in muscle protein synthesis and prevent the decrease in tissue protein content. In the present study plasma glutamine concentration was normalized, but exogenous glutamine had minimal effect on muscle glutamine concentration and hence had no significant effect on protein synthesis or protein content.

The result demonstrated that exogenous glutamine supplementation during five days did not influence muscle glutamine concentration in ICU patients although pilot studies had indicated an alteration to be possible (Roth et al., 1992). However, it cannot be excluded that longer intervention time will have effects on muscle metabolism.

Exogenous glutamine supplementation has also been suggested to have a protein saving effect by reducing the glutamine outflow from muscle and by that mechanism diminish the loss of muscle mass. However, in ICU patients undergoing renal replacement therapy exogenous glutamine supplementation did not influence the glutamine release from leg muscle during the initial day of

prospectively. These “responders” in terms of muscle free glutamine concentration did not, exhibit any response in terms of muscle protein synthesis more than those patients that did not “respond”. However, the increase in muscle glutamine content was correlated with the increase in muscle protein content, raising the hypothesis that in the “responders” in terms of muscle glutamine may also have a better preserved muscle protein content (Figure 12). As muscle protein degradation was not assessed, any possible response in that aspect of protein metabolism was not possible to detect.

The measurements of 3-methylhistidine excretion was not a before and after measurement, and can therefore not be used to evaluate such an effect. Still, post-hoc analyses indicated a beneficial effect on muscle protein degradation as indicated by the lower urinary excretion of 3-methylhistidine when the glutamine treated groups were combined, as compared to the control group.

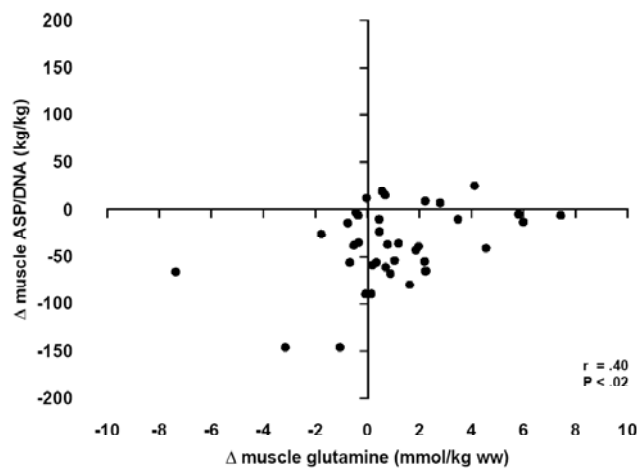


Figure 12. The change in muscle protein content (ASP/DNA) related to the change in muscle free glutamine concentration in ICU patients during a five-day study period. Data from study II.

In study IV muscle protein synthesis rate was quantitatively measured with a high reproducibility. In addition, muscle morphology was shown to be homogenous in the individual ICU patients. Muscle protein synthesis rate has a large variation in ICU patients (Essen et al., 1998; Gamrin et al., 2000b; Tjader et al., 2004). In comparison with earlier investigations study IV did not include

any patient with a low rate of muscle protein synthesis. Was this an accidental finding or could it be explained in terms of altered clinical routines? The patients in study IV were included during 2002 and the beginning of 2003. At that time-point the general routines in the ICU have changed in at least one aspect, which may in part explain the absence of low values for muscle protein synthesis rate. As normoglycemia and intensive insulin therapy reduces morbidity and mortality in surgical ICU patients (van den Berghe et al., 2001), this routine was adapted. This may have exerted an effect upon muscle protein synthesis rate in patients with a very low fractional synthesis rates. Still the effect of insulin upon muscle protein synthesis rate remains controversial. For example an insulin clamp (350 pmol) has no effect on muscle protein synthesis in healthy volunteers in the basal state (Nygren et al., 2002). Others have demonstrated an effect of insulin upon muscle protein degradation (Gelfand and Barrett, 1987). On the other hand, in burn victims a dramatic stimulation of insulin on muscle protein synthesis has been shown (Ferrando et al., 1999).

The low intra-individual coefficient of variation in the duplicate measurements of muscle FSR in Study IV, points out the high precision possible when repeated measurements are performed. In paired samples it will theoretically be possible to discover a 5.5 % change in 10 subjects and a 10 % change in 10 subjects with 80 % statistical power. This compares to the intra-individual variation of 8.4 % in repeated measurements in study III (minor surgery), which demonstrates that repeated measurements carry a higher standard deviation related to the repeated use of tracer during a relatively short period of time. This is in contrast to the inter-individual variation of 18 % in study I and 14 % for minor surgery in study III, which indicate that larger number of subjects will be needed to see differences between groups. For example to detect a difference at the level of the standard deviation between 2 groups 17 subjects in each group will be needed.

6 CONCLUSIONS

In this work 98 patients were investigated and the response in muscle protein synthesis in relation to surgical trauma or critical illness was evaluated. The conclusions from the different studies were:

- I. Continuous and ongoing total parenteral nutrition during the first 24 postoperative hours could not prevent a decrease in muscle protein synthesis following medium size surgery.
- II. Intravenous glutamine supplementation to intensive care patients in three different doses restored plasma glutamine concentration in a dose-dependent way but did not influence muscle glutamine concentration or muscle protein synthesis.
- III. The immediate impact of surgical trauma on muscle protein synthesis rate was demonstrated not to be size-related. Minor surgery, in a heterogeneous group of patients did not result in any immediate effect on muscle protein synthesis rate. However, the same result was also found also in patients undergoing major abdominal surgery.
- IV. Muscle protein synthesis rate in intensive care patients was on average not different from that in healthy individuals, although the inter-individual variation was higher. A high reproducibility of muscle protein synthesis rate determinations was demonstrated, with an intra-individual coefficient of variation of 5.5 % showing that heterogeneity does not explain the large variation.

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