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Introduction
Introduction

Anyone who has ever broken a leg or arm and had it plastered will attest to the speed and extent of muscle wasting brought about by the consequent immobilisation. This may seem an inevitable reaction of the body, but not every organ or tissue is immediately broken down once not in use; consider the brain and liver, both organs with a capacity which vastly outweighs their requirements. And fat tissue does not shrivel as fast as one might wish for either.

In fact, the loss of muscle tissue following immobilisation is characteristic for the way the body handles protein, the muscle’s main constituent. Unlike body fat, a highly efficient form of fuel storage, body protein cannot be stored but makes up functional or structural tissues. As a consequence, protein tissue is constantly being broken down and resynthesized, be it into muscle tissue, clotting factors or immunoglobulins, to attend to the body’s requirements. In an adult of average size and under normal conditions, this process involves up to 3-4% of the whole-body protein content per day. After puberty, when growth has ended, protein synthesis and breakdown are thus kept in strict balance. Any surplus arising from dietary protein intake is simply used as metabolic fuel.

Only in extreme situations will this delicate balance between protein synthesis and breakdown falter. For instance, in case of prolonged starvation, the body will increase protein oxidation for energy generation. As a consequence the whole-body protein content diminishes, a situation referred to as catabolism. Loss of protein tissue inherently means loss of function and leads to a compromised immune function, a decrease or delay in wound healing and tissue repair and a loss of muscle strength and diminished activity. A similar situation can arise secondary to illness.

The response of the human body to any trauma or infection is known as inflammation. One prominent clinical feature of this response is fever. Another is the loss of whole body protein, a fact first acknowledged by Cuthbertson in 1932. The effort to fight off infection and repair tissue requires the production of vast amounts of protein. In the acute situation, this can only be achieved at the expense of other protein tissue.

Rapid weight loss, body fat mostly and some skeletal muscle mass, frequently accompanies short-term, self-limiting diseases such as the flu. The loss of body tissue will be minimal and of little consequence in most patients. But when the disease is prolonged, the catabolic state can itself be damaging and prolong the disease state. Poor dietary intake and inactivity, both common in the hospital patient, will further exacerbate this. The patient will be “consumed”, not only by the disease but also by the effort to fight it.

Critically ill children, for instance patients with burns or following major surgery, are especially prone to this course of events. Protein turnover increases several fold in these patients, whereas they have reduced macronutrient reserves with less than half the protein content of adults. It is the challenge of paediatric intensive care medicine to prevent and counteract this.
The metabolic stress response

The metabolic stress response to injury or infection involves a number of well-described neuro-hormonal events that include activation of the sympathetic nervous system, stimulation of the hypothalamic-pituitary-adrenal axis, and an increase in the secretion of glucagon relative to that of insulin. These neuro-humoral signals are manifested by the clinical signs and metabolic events that characterize the stress response: tachycardia, tachypnoea, hyperglycaemia, mobilisation of body fat, and net breakdown of skeletal muscle protein. The resulting alterations in energy and substrate metabolism are in essence catabolic. Whole body oxygen consumption and energy expenditure (EE) is generally increased, although with substantial variability, and may even be decreased in some patients. Substantial increments in EE are generally observed in paediatric burn patients and patients suffering from neurotrauma and sepsis.

Whole-body protein catabolism is observed. Protein breakdown is increased, as is protein synthesis. Skeletal muscle protein is catabolised, resulting in the oxidation of branched chain amino acids and net efflux of amino acids from the muscle. The caloric contribution of protein oxidation to energy generation is limited and will under the most extreme circumstances not surpass 20% of resting metabolic rate. The amino acids released by skeletal muscle are essentially used in the liver for synthesis of acute phase proteins and for production of glucose as the preferred energy substrate for the brain, erythrocytes, and renal medulla.

Table 1. Major metabolic alterations and neuroendocrine mediators in critical illness

<table>
<thead>
<tr>
<th>Changes</th>
<th>Energy metabolism</th>
<th>Glucose metabolism</th>
<th>Lipid metabolism</th>
<th>Protein metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased energy expenditure</td>
<td>Increased glucose production</td>
<td>Increased lipolysis</td>
<td>Increased proteolysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased gluconeogenesis</td>
<td>Increased lipid oxidation</td>
<td>Net protein breakdown</td>
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<tr>
<td></td>
<td></td>
<td>Increased lactate production</td>
<td></td>
<td>Synthesis of acute phase proteins</td>
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<td></td>
<td>Hyperglycaemia</td>
<td>Insulin resistance</td>
<td></td>
<td></td>
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<tr>
<td>Main mediators</td>
<td>Fever, pyrogens</td>
<td>Cortisol</td>
<td>Cortisol</td>
<td>Cortisol</td>
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<tr>
<td></td>
<td>Epinephrine</td>
<td>Glucagon</td>
<td>Epinephrine</td>
<td>Cytokines</td>
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<td></td>
<td>Cytokines</td>
<td>Glucagon</td>
<td>Growth hormone</td>
<td>Cytokines</td>
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</table>

Carbohydrate metabolism in the critically ill patient presents with insulin resistance and increased glycolysis, gluconeogenesis and hyperglycaemia. Glucagon, cortisol, epinephrine and growth hormone are involved in these alterations of glucose homeostasis. In addition, pro-inflammatory
cytokines exert effects on glucose metabolism. Plasma glucose concentrations are raised in the fasted patient and may even further increase in response to artificial (enteral or parenteral) nutrition. Extensive literature reports on the toxic effects of glucose exist. Finally, alterations in the lipid metabolism include the inhibition of lipoprotein lipase, resulting in hypertriglyceridaemia, and increased lipolysis. The latter results in higher rates of \( \beta \)-oxidation, yielding ketone bodies as substrate for the citric acid cycle.

**Measurement of protein metabolism**

**a. Protein metabolism**

Protein metabolism is a dynamic process of continuous protein breakdown and synthesis. As the human body is unable to store protein, it is dependent on dietary intake and protein breakdown for the supply of amino acids. In fact, only 20% of amino acids used for protein synthesis originate from dietary sources, the remaining 80% are derived from recycling of amino acids from protein breakdown.\(^{17}\)

**Figure 1   Graphical representation of the deamination process of amino acids**

Amino acids not used for protein synthesis are degraded in the liver. The first step of amino acid degradation is deamination, a process whereby the amino group is removed and that is catalysed by enzymes called transaminases. The removal of the amino group begins with the transfer of the amino group to alpha-ketoglutarate. The product of this transfer is an alpha-keto acid formed from the amino acid and glutamate (formed from the addition of the amino group to alpha-ketoglutarate). Glutamate then has its amino group removed by oxidative deamination. This reaction reforms alpha-ketoglutarate with the other product being ammonia (\( \text{NH}_4^+ \)). Ammonia is toxic to the nervous system and its accumulation rapidly causes death. Therefore it must be detoxified to a form, which can be readily removed from the body. Through the Krebs-Henseleit cycle ammonia is converted to urea, which is water-soluble and is readily excreted via the kidneys in urine.

Once the amino acid has lost its amino group, the remaining carbon skeleton is converted into a major metabolic intermediate. It can be converted into acetyl CoA, pyruvate or one of the intermediates of the citric acid cycle. Acetyl CoA will either be immediately oxidised via the citric
acid cycle or may be converted to ketone bodies. Carbon skeletons ending up as either pyruvate or a citric acid cycle intermediate may be used for energy production or for gluconeogenesis. Whereas glucose and fat are the main sources of energy for the body, the oxidation of amino acids contributes approximately 10% to total energy production in humans.

b. Urea, nitrogen and nitrogen balance studies
As described above, amino acid degradation yields nitrogen in the form of ammonia, which is then converted into urea for excretion in the urine. This makes urinary urea the most accessible marker of protein degradation, and by inference, of muscle catabolism. Indeed, the first study to describe what is now known as the metabolic stress response, reported on the enhanced nitrogen content of urine following skeletal trauma.²

It is of interest to make a clear distinction between urinary urea nitrogen (UUN) and total nitrogen content of urine, or total urinary nitrogen (TUN). In the healthy subject, 80% to 90% of total urinary nitrogen is accounted for by urea nitrogen.¹⁸,¹⁹ Other nitrogen containing compounds in urine are predominantly ammonia and ammonium salts.²⁰ Due to its relative ease, low laboratory costs and rapid turnaround time, analysis of urinary urea concentration is broadly used as a substitute for total urinary nitrogen content. In the past decades, the measurement of total urinary nitrogen content has evolved from the time-consuming Kjehldahl method, which involved the use of corrosive materials, to more modern methods such as pyrochelominescence techniques. However, these methods are not readily available as a routine clinical tool in many institutions, and so the main body of clinical nutrition research has reported on urinary urea nitrogen, or an adjustment of UUN, to approximate nitrogen losses.

When nitrogen excretion measurements are combined with protein (nitrogen) intake data, nitrogen balance can be calculated. For decades, balance studies of nitrogen and other elements like potassium and sulphur, were the main instrument available to nutritional research. The mathematics involved are simple. Nitrogen intake is calculated using the mean nitrogen content of protein (grams of nitrogen = grams of protein / 6.25) and total nitrogen excretion is then subtracted from nitrogen intake. Whole-body nitrogen loss and whole-body nitrogen retention can thus be assessed and as loss of protein tissue represents loss of lean body mass, have come to define catabolism and anabolism, respectively.²¹,²²

There are however some limitations to this elegant method. One principal objection, inherent to all balance studies, is that nitrogen balances can only indicate whether there are changes in whole body nitrogen content, without revealing the ways in which these changes are brought about.³,²³ Furthermore, as the body can adjust to prolonged alterations in protein intake, nitrogen balances studies can yield similar results over a range of protein intakes. They therefore do not necessarily provide an adequate measure of the status of body protein nutriture. For the same reason, their application as a means of establishing values for protein or amino acid requirements is limited.²³

The validity of nitrogen balance studies depends on the completeness of nitrogen excretion measurement. Many clinical studies assess whole body nitrogen loss by measuring urinary
nitrogen excretion. As a rule, non-urinary nitrogen losses are neglected or accounted for by a formula that converts TUN or UUN to total nitrogen excretion.\textsuperscript{18, 24} For obvious reasons, obtaining complete 24-hour urine collections is a prerequisite in these studies. However, this can be an issue of concern, especially in free-living study populations. Also, in newborns and infants, acquiring accurate nitrogen excretion data is particularly difficult. In this population, the relatively small volumes of the excreta may result in relatively large effects of sampling errors.

Concerns regarding the completeness of urine collections aside, there are limitations to the use of urinary urea nitrogen as a proxy of total urinary nitrogen. As mentioned before, measurement of urinary urea nitrogen has found widespread application, mostly for practical reasons. The measured urinary urea nitrogen is then increased by 25\% to account for the non-urea nitrogen components of urine and non-urinary nitrogen losses. Other correction methods, sometimes involving measurement of urinary ammonia, have also been applied.\textsuperscript{18, 25, 26} The validity of this practice has been the subject of continuous debate.\textsuperscript{19, 25, 27-31} Application in patients is compromised by the fact that the contribution of urea to total urinary nitrogen content can vary significantly from that seen in healthy individuals. For instance, Boehm et al. demonstrated that calculation of TUN from either urinary urea nitrogen alone or in combination with urinary ammonia in critically ill children could lead to an underestimation of TUN of up to 30\%.\textsuperscript{32} Similar discrepancies between UUN and TUN have also been reported in critically ill\textsuperscript{33, 34} or postoperative patients,\textsuperscript{27, 35-37} and malnourished subjects.\textsuperscript{38}

Completeness of nitrogen excretion measurements is further compromised in the event of significant non-urinary losses of nitrogen. Under laboratory circumstances, absolute nitrogen output determination involves the measurement of nitrogen content from all bodily excrement, including urine, faeces, sweat, hair and dermal tissue. In the clinical setting this is not feasible and often unnecessary. In healthy children, faecal loss of nitrogen averages 15\% of urinary nitrogen excretion.\textsuperscript{39} For patients, this is generally assumed to be the same. Other sources of nitrogen loss are considered negligible. This may not be the case in patients with surgical wounds, intestinal stomata, chest drains and burns. To date, only one study has reported on this in the paediatric population. Albers et al. studied non-urinary nitrogen losses in newborns and infants on parenteral nutrition following digestive tract surgery. In these patients, non-urinary nitrogen losses were variable in size, and could amount to up to 110\% of urinary nitrogen losses.\textsuperscript{40}

c. Stable isotopes

The use of stable isotope tracers for the measurement of protein kinetics dates back to as far as 1939.\textsuperscript{41} Not until 1969 were stable isotopes first applied in humans, when Picou et al. reported on the whole body protein synthesis of malnourished children.\textsuperscript{42} Since then, stable isotope techniques have found widespread application in the study of human metabolism in vivo. This can be attributed to advances in the analysis of stable isotope enrichment by mass spectrometry (e.g. the development of quadrupole gas chromatograph-mass spectrometry and selective ion monitoring) and an increasing availability of stable isotopes. The intensified research has spawned
major advances in both nutritional and metabolic research, from daily dietary recommendations of protein and amino acids\textsuperscript{13} to detailed studies of children afflicted by inborn errors of metabolism.\textsuperscript{44} Stable isotopes have an obvious ethical advantage over radioactive isotopes, especially when paediatric patients are involved. The multiple practical advantages associated with the use of stable isotopes should also be appreciated. For instance, the non-radioactive character of the tracer allows for the simultaneous and repeated administration in one subject. Again, in paediatric investigations, this is no trivial advantage. Secondly, the mass spectrometry analysis of stable nuclide molecular position and isotopic enrichment in biological molecules is both highly specific and extraordinarily precise. Furthermore, the in vivo effects consequent to the use of stable isotope are less than those measured with the corresponding radiotracer. Finally, for certain elements there are simply no practical radioactive alternatives, most notably nitrogen.\textsuperscript{45}

In a stable isotope experiment, a molecule carrying the stable isotope nuclide or tracer is administered, either via the oral or intravenous route. As it enters the body, it is distributed over the metabolic pool of the system under study and mixes with the unlabelled endogenous molecule, or tracee. Samples are taken to determine the ratio of the labelled to the unlabelled molecule (tracer to tracee ratio) by mass spectrometry. Depending on the substrate, this can be done by gas chromatograph mass spectrometry (GCMS), isotope ratio mass spectrometry (IRMS), or a gas chromatograph isotope ratio mass spectrometry (GC-IRMS). Over the course of time, the tracer is lost from the sampled compartment and, as the tracer is not made endogenously, it is replaced by unlabelled endogenous material. This corresponds with the dilution of tracer enrichment seen over time after a single tracer administration. If the tracer is administered by continuous infusion, the ratio of tracer to tracee in the body will eventually stabilise or plateau. By injection of a priming dose of tracer prior to the start of the experiment, the time required to achieve isotopic equilibrium can be shortened. As will be discussed below, the isotopic equilibrium or isotopic steady state allows for the calculation metabolic processes in a practical and straightforward way.

**Modelling of stable isotopes experiments**

Inherent to the design and interpretation of tracer studies is the use of models. In order to calculate turnover rates from the isotopic enrichments obtained after tracer administration, a mathematical representation of the physiological system involved must be defined: a model.\textsuperscript{46,47} Figure 2 gives a representation a hypothetical biological system as defined in compartmental analysis. It exists of discrete entities (called pools or compartments) that, depending on the question, might be anatomic, functional or biochemical. The arrows indicate the exchange of material between the pools or into and out of the system. In compartmental analysis, it is assumed that the exchanges between compartments occur by first-order kinetics and that the rate constant is independent of concentration.\textsuperscript{1} A series of differential equations can then be formulated, the number of which is determined by the number of pools and whose parameters depend on the system’s structure, i.e. the relationship between the pools.
Figure 2  A hypothetical multiple pool biological system

The pools may represent structural, functional, or biochemical entities. The central pool represents an area (such as a vein) accessible for practical or ethical sampling of the system. The arrows reflect movement of materials between the pools or into and out of the system.

In an ideal experiment, all compartments of the system under study are sampled following tracer administration. In human in vivo studies, the number of accessible compartments is limited for both practical and ethical considerations. The majority of studies therefore use blood, urine and/or breath samples, although enrichments are sometimes determined by muscle, liver or bone marrow biopsies. One practical way to limit the number of possible compartments involved is by performing tracer studies on a single limb. The isotopic tracer is injected directly into the arterial supply of for instance a leg, and samples are obtained from the venous side. The metabolically active tissue, in this case muscle, is studied directly. In animal experiments, a similar approach can be applied to study the metabolic processes across the liver and portal drained viscera.

In paediatric subjects, the accessible or sampling pool is generally speaking either blood or urine. Unfortunately, these samples rarely represent the pool where the metabolic events of interest take place, commonly the intracellular space. Despite the limitations of sampling inherent to the paediatric population, valid results can be obtained under certain conditions. One approach is the so-called single pool model (Figure 3). This model simply ignores the presence of inaccessible components of the system. It is assumed that the tracer is administered into an instantly mixing, metabolically active compartment, which is also accessible to sampling.

In the steady state, when endogenous substrate concentration and flux are constant, sampling tracer dilution in an accessible single pool can accurately define two parameters of accessible compartment dynamics. These are the rate of appearance (Ra) of the unlabelled endogenous
material, and the rate of disappearance (Rd) of the tracee from the compartment. These are valuable parameters, and, in a few selected instances, accessible pool parameters also reflect the parameters of the system as a whole. The main application of the single pool model is in the primed-constant intravenous infusion of labelled amino acids for the study of whole-body protein metabolism. This approach is relatively quick, easy to apply, and requires only a few blood samples.

**Figure 3** A single pool model

![](image)

All entry into the system (rate of appearance Ra) is assumed to occur directly into the accessible sampling pool and all exit from the system (rate of disappearance Rd) occurs directly from the sampling pool. This approach completely ignores the remaining, hidden structure of the system.

For most metabolic investigations, the single pool model is generally not satisfying since very few metabolic systems can be adequately described by a uniform single compartment. Multiple pool modelling, as is applied to analyse the isotopic decay curve following administration of a single tracer dose, requires many more samples, and the post hoc interpretation of the data is more complex. This approach does, however, yield additional kinetic information about the turnover of different compartments, even though ascribing these kinetic compartments to specific tissues is speculative.1, 53

**Stable isotopes: amino acids**

The use of amino acid isotopes for the in vivo measurement of protein kinetics is based on a model of amino acid metabolism,54, 55 which can be summarized as follows (Figure 4): amino acids from the diet (I) and from protein breakdown (B) enter a metabolic pool for protein synthesis (S) or for amino acid oxidation (O). Assuming that this pool is constant in size, the input of amino acids is equal to the output and corresponds to Q, which is the rate of amino acid flux through the amino acid pool:

\[
Q = I + B = S + O
\]
The fundamental measurement is the rate of appearance of the tracee amino acid (I + B in the model). In the post-absorptive or fasting state, when protein intake (I) is zero and with appropriate measurement of amino acid oxidation, this measurement can be used to calculate estimates of both whole-body protein synthesis and protein degradation.

**Figure 4  Model of protein kinetics**

![Model of protein kinetics](image)

**Precursor method**

The human body is unable to synthesize nine amino acids, and is therefore dependent on dietary intake to meet its daily requirements of these indispensable, or essential, amino acids. In the absence of dietary intake, the rate of appearance of these essential amino acids can wholly be attributed to release from protein breakdown. When the amount of amino acid per gram protein is known, the amount of protein breakdown follows. This is called the precursor method and any essential amino acid can in theory be applied to measure whole-body protein turnover according to this principle.\(^1\)

This method relies on the central assumption that the tracer enrichment, as determined in whole blood or plasma, represents the intracellular tracer enrichment. This is unlikely and almost certainly not the case for most amino acid tracers.\(^3\) An expression of this is the fact that different estimates of whole-body protein turnover are found when different essential amino acid tracers are used in the same individual.\(^57, 58\) This dilemma was solved for the tracer \([1-^{13}\text{C}]\text{leucine}\), when it was found that alpha-ketoisocapric acid (α-KIC) could serve as a surrogate for intracellular enrichment.\(^59\) Infusions of \([1-^{13}\text{C}]\text{leucine}\) with measurement of α-KIC enrichments have therefore become the standard application of the precursor method for protein metabolism measurements.

As \([1-^{13}\text{C}]\text{leucine}\) is broken down, \(^{13}\text{CO}_2\) is formed, which is excreted through respiration. With the measurement of \(^{13}\text{CO}_2\) enrichment in expired air and total carbon dioxide production (VCO₂) the
leucine oxidation rate can be calculated. VCO$_2$ can be determined either by indirect calorimetry$^{60}$ or by a separate, primed constant infusion of $[1^{-13}C]$bicarbonate.$^{61,62}$

Phenylalanine tracers are an alternative to leucine, with the advantage that its flux and oxidation rates can all be measured by plasma sampling, with no need for CO$_2$ measurement or IRMS.$^{63,64}$ It does on the other hand require the infusion of labelled tyrosine. In addition, the model assumptions involved with $[^{2}H(D_{5})]$phenylalanine infusions are complex, mainly due to the fact that no plasma amino acid derivative is available which might indicate precursor pool enrichment.

**End-product method**

This method is based on the measurement of tracer enrichment in the end products of protein metabolism following administration of a tracer, commonly $[^{15}N]$glycine. Various routes of tracer administration (intravenous vs. oral),$^{65,66}$ tracer dose (primed constant infusion vs. bolus),$^{42,67-69}$ sampling site (blood vs. urine),$^{70}$ applied calculations (compartmental modelling of the enrichment decay curve vs. analysis of cumulative excretion of isotope)$^{10}$ and end products (urea vs. ammonia)$^{65,68}$ have been described. The most practical approach is to measure the cumulative tracer enrichment of ammonia from a 9-h urine collection following a single, oral tracer bolus. This method was developed by Waterlow$^{68}$ and has since found common application, especially in the paediatric population.

**Stable isotopes: urea**

Any surplus nitrogen from food protein and from the balance between breakdown and synthesis of proteins in the tissues is condensed in the urea cycle. Measurement of urea synthesis by means of urea tracers can thus be used as a measure of net protein breakdown. No information regarding protein synthesis rate or protein flux is obtained.

**Figure 5** *Representation of the gastrointestinal recycling of urea*
Following urea synthesis in the liver, not all urea is readily excreted through the urine. In a normal diet, approximately 30% of urea produced passes into the colon, where microbial flora break it down to ammonia. If reabsorbed by the gut, the ammonia can be recycled to urea, or enter the amino-nitrogen pool and be integrated into non-essential amino acids. This process is commonly referred to as the recycling of urea, or even salvage (Figure 5). It is the reason why urea excretion measurements will underestimate urea production rate by 20-30%. 71, 72

The recycling of urea also has consequences for stable isotope measurements of urea production. One is that the passage of urea in the gut will have to be accounted for in compartmental modelling. Secondly, only when the doubly labelled [$^{15}$N$_2$]urea tracer is applied, can the recycling of urea be quantified. 73 Quantification of recycling is interesting, as its role in and contribution to the maintenance of nitrogen balance remains unclear. 74

d. Alternative assessment methods of protein metabolism

In view of the complexity and invasiveness of stable isotope tracer experiments, not to mention the costs involved with mass spectrometry techniques and machines, alternative indicators of protein metabolism have been studied. One is the measurement of urinary 3-methylhistidine excretion. This compound is predominantly present in intact muscle tissue and released during muscle breakdown. 75 It is not reutilized for protein synthesis or oxidized for energy production 75, 76 and thus correlates with the catabolism of endogenous protein. Urinary 3-methylhistidine excretion (or the ratio of 3-methylhistidine to creatinine) has been used to quantify protein turnover and muscle breakdown in adults, children, and neonates in relation to nutritional state, surgery, and trauma.

Another way of studying protein metabolism, or more specifically protein synthesis, is to determine the total ribosome concentration or polyribosome concentration in muscle tissue. The number of ribosome or polyribosomes is considered a proxy of protein synthesis capacity of skeletal muscle. 83, 84 To obtain muscle tissue a needle biopsy is required. This method has been applied in a limited number of studies, two of which have reported correlations between ribosome number, nitrogen balance and intracellular glutamine concentrations. 85, 86

Malnutrition in critically ill children

a. Scope of the problem

The term malnutrition covers both under nutrition and overfeeding. The past two decades have seen an explosive rise in the prevalence of overweight and obesity, conditions currently affecting more than 50% of the United States population. 87 This epidemic has brought about a surge in research into this form of malnutrition. In the clinical setting though, malnutrition most commonly refers to under nutrition, although overfeeding has been linked to poor outcome in critically ill children and adults. 88
In the developing world, malnutrition remains a serious and ubiquitous problem, especially in the paediatric population. In its most extreme presentations, kwashiorkor (acute protein malnutrition) and marasmus (severe pannutrient deprivation), malnutrition is a clinical picture that catches the eye of the international media. Less obvious, chronic malnutrition however, is a plague affecting the life of generations. It is the underlying cause of 53% of all deaths in children younger than 5 years.

In developed countries, malnutrition is generally secondary to disease and caused by inadequate dietary intake, increased metabolic demands, impaired absorption or increased nutrient losses. Several of these factors may be present in ICU patients. The combined deficiency of calories and protein leads to the inappropriate loss of body cell mass and is called protein energy malnutrition.

Malnutrition can be found in 30 to 60% of hospitalized adult patients. It is a problem too among critically ill children. The first reports on malnutrition in hospitalized children and PICU patients date back to the early eighties. Pollack et al. found that acute protein-energy malnutrition was present in 16% of patients admitted to the PICU. Despite a surge in attention for nutrition and nutritional management in the decades since, these figures have not improved in recent studies.

b. Consequences of malnutrition

Studley was the first to note the detrimental effects of poor feeding status. In a study published in 1936, he described the fate of postoperative patients with chronic peptic ulcer. He observed a direct relationship between preoperative weight loss and mortality rate, independent of factors such as age, impaired cardiorespiratory function and type of surgery. Weight loss of more than 20% increased mortality by 33% in these patients, predominantly secondary to infectious complications.

Numerous studies have since reported on the consequences of malnutrition. Protein depletion from the body cell mass is a major contributor to morbidity and mortality and adversely affects tissue growth, wound healing, cell mediated immunity and the metabolic response to infection. Malnutrition on admission is an independent predictor of hospital length of stay in adults and the increased rate of complications is obvious even after one week of admission. Finally, severe, moderate as well as mild malnutrition in children are all correlated to increased mortality and morbidity.

From a more public perspective, the complications of malnutrition increase quantity of care, hospital length of stay and thus the costs of health care. In one study of approximately 700 adult patients, the length of stay of underfed patients was 17 vs. 10 days in the appropriately fed patients. As a consequence, the malnourished patients represented an increase of cost up to 300%.
c. Causes of inadequate nutrition in the PICU setting

Inadequate nutrition in critically ill patients seems counterintuitive to the whole notion of intensive care. One common explanation is that the life threatening condition necessitating admission predominates care during the first days. Attention for nutritional therapy may therefore be delayed for a short yet significant interval.\textsuperscript{109} Even then, assessment of nutritional requirements and subsequent administration of nutrition in adequate amounts is not at all straightforward.\textsuperscript{119}

To begin with, there are no commonly accepted guidelines for nutritional management of critically ill paediatric patients. Secondly, the estimation of nutritional needs of critically ill children is challenging, as caloric requirements may vary considerably from those of healthy infants and children.\textsuperscript{100, 120-122} This can partly be attributed to the special conditions imposed by treatment in the intensive care setting, e.g. a temperature controlled environment, immobility, use of sedatives or muscle relaxants and mechanical ventilation.\textsuperscript{100, 123, 124} Also, critical illness itself may alter energy expenditure (EE). For instance, energy expenditure is elevated in children with burns,\textsuperscript{6, 125} neonatal sepsicaemia,\textsuperscript{126} congenital heart disease\textsuperscript{127} and head injury.\textsuperscript{7} Finally, EE may vary during the course of admission from an early “acute phase” to convalescence.\textsuperscript{128, 129}

To fully tailor nutrition to requirements, frequent measurements of EE by indirect calorimetry are required. Calorimetry equipment is costly and complex to handle and therefore rarely applied in the clinical setting. In stead, most PICU’s use weight or predictive equations to estimate energy demands.\textsuperscript{130} Many different predictive equations have been proposed and are currently in use.\textsuperscript{131-137} Most predictive equations are based on energy expenditure data of healthy infants and children and, consequently, lack accuracy on the individual level when applied in the clinical PICU setting.\textsuperscript{100, 120-122, 138} Adding to the inaccuracy of predictive equations are stress related corrective factors, known to grossly overestimate measured energy expenditure in critically ill children.\textsuperscript{139}

A second cause of inadequate nutrition is the insufficient delivery of nutrition, for instance due to fluid volume restrictions, gastrointestinal intolerance or procedural interruptions.\textsuperscript{119} In a retrospective study of nutrition and caloric intake in 95 critically ill children, Taylor et al. found that enteral nutrition was 88 times more likely to be interrupted than parenteral nutrition.\textsuperscript{102} Discrepancies between prescribed and actually administered feeding have been reported in adult ICU patients\textsuperscript{140, 141} and in critically ill children.\textsuperscript{142}

**Nutritional interventions**

Over the past decades, nutritional support has become a basic component of clinical management in the (paediatric) intensive care setting. There is also increased attention for nutrition in other vulnerable patient populations, notably the elderly and patients suffering from cancer. Despite these developments, there is no universal agreement on the definition of malnutrition, let alone on the optimal way of its prevention or treatment. Quantitative nutritional interventions aim at the administration of energy and substrates in adequate amounts, whereas
qualitative nutritional interventions aim to influence metabolism by means of specific nutrients or drugs.

d. Quantitative nutritional interventions

There is general consensus that excessive hypocaloric or hypercaloric feeding should be avoided. Also, protein restriction is not recommended, as it is associated with worse clinical outcomes. Adequate nutritional support and better nutritional status have been shown to improve physiologic stability and outcome in children and adults. It is recognized though, that aggressive nutritional support will not prevent substantial body protein loss during severe catabolic illness. These observations, albeit valuable, will not guide the clinician in choosing from the broad range of options available regarding amount of energy, and timing and route of administration. The variety in age and diagnoses of children admitted to the PICU makes this task even more daunting. Furthermore, the term adequate nutrition does require some scrutiny when considering the available observational studies. As is common in these studies, patients receiving very little or no nutrition are compared to the other patients. These, in their turn, are unlikely to receive an amount of energy matching their daily requirements or expenditure, as is the case with most patients admitted to the intensive care unit. Therefore, few valid conclusions can be drawn from the observational data apart from those addressing the complications of malnutrition.

To adequately compare adequate nutrition (randomly defined as an energy intake of 90 to 110% of requirements) with hypocaloric or hypercaloric regimens, randomised controlled trials are required. No such studies have been performed in critically ill children. A recent review of both observational and randomised controlled trials in critically ill adults concluded that provision of approximately 25% to 66% of goal calories may be sufficient. It also concluded that early aggressive use of enteral nutrition compared with delayed, less aggressive use of enteral nutrition, is associated with improved clinical outcome. However, energy provision with parenteral nutrition, either instead of or supplemental to enteral nutrition, did not offer additional benefits.

e. Qualitative nutritional interventions

The term quantitative nutritional interventions refers to a wide range of specific amino acids, lipids, hormones and drugs that are applied to alter the metabolic stress response in the patient’s benefit. Recent years have seen major developments in this field, with the improved understanding of the effects of compromised mitochondrial function, studies demonstrating outcome benefits of tight glucose control, new insights into the mechanisms behind insulin resistance, recognition of glutamine and antioxidants as key nutrients, and emerging knowledge concerning the interactions between metabolism and endocrinology.

Glutamine, arginine, L-ornithine-alpha-ketoglutarate (OKG) and citrulline are amino acids or amino acid salts that have found application in the field of nutritional therapy in view of their immunomodulatory and/or nitrogen sparing effects. Glutamine is an amino acid that has received considerable attention in the past years. It has been shown to be beneficial for the metabolically
stressed patient, especially the critically ill patient. For instance, glutamine provides substrate for glutathione synthesis, is an important fuel for immune cells, and preserves cell membrane integrity by enhancing the generation of heat shock protein. Arginine affects the immune system by stimulating direct or indirect proliferation of immune cells. Glutamine was shown to be beneficial in the surgical and critically ill patient, whereas arginine supplementation is still under debate.\textsuperscript{154} OKG has been applied in both chronically and acutely malnourished patients. Apart from improving nutritional status, OKG supplementation has been suggested to stimulate immune function and to promote wound healing and tissue repair.\textsuperscript{155} Citrulline may be a conditionally essential amino acid when intestinal function is compromised and have the ability to promote protein synthesis.\textsuperscript{156} Finally, the branched chain amino acids (BCAA) stimulate secretion of insulin and serve as a source of fuel for skeletal muscle. Despite unconvincing clinical trials in the past, there are calls for reappraisal of feeding supplemented with leucine, the metabolically most potent BCAA.\textsuperscript{157}

Lipids have long been exclusively considered as nutrients providing calories, essential fatty acids and fat-soluble vitamins. Their role as biologic regulators, involving also the inflammatory/immune response, have more recently been appreciated. There is now evidence that provision of fish oil fatty acids and/or $\gamma$-linolenic acid decreases harmful excessive inflammatory/immune activation and to improve clinical outcome. Similar effects have been reported for n-3 (or omega-3) fatty acid-enriched lipid emulsions in patients in sepsis.\textsuperscript{158}

The publications by Van den Berghe et al. on the beneficial effects for mortality and morbidity of strict glycaemic control in ICU patients,\textsuperscript{159, 160} have revived the attention for the application of hormonal therapy in critically ill patients. Previously, the administration of recombinant human growth hormone was shown to increase the mortality of critically ill adult patients.\textsuperscript{161} Van den Berghe et al. reported a mortality reduction of 43% in adult post-surgery patients by maintaining normoglycaemia with intensive insulin therapy.\textsuperscript{159} In medical ICU patients, this beneficial effect was limited to patients admitted for more than 3 days.\textsuperscript{160} No data exist on the application of strict glycaemic control in the paediatric age group. Nevertheless, the incidence of hyperglycaemia in the PICU population is high and associated with increased length of stay and mortality.\textsuperscript{15, 16}

**Aims and outline of the thesis**

The primary aim of the thesis was to investigate, quantify and find ways to modulate the protein catabolic response in critically ill children admitted to the paediatric intensive care unit.

The first three studies address the methodology of protein catabolism measurements by means of stable isotope studies. Chapter 2 describes a new assay for the analysis of stable isotope urea enrichments in body fluids using both GCMS and GC-c-IRMS. An oral tracer protocol for the measurement for urea synthesis rate is described in Chapter 3. Chapter 4 explores the accuracy of calculations of enterohepatic nitrogen recycling rates.
In the second part of this thesis, three clinical studies are presented. Chapters 5 and 6 are both set in the paediatric intensive care setting. The first addresses the energy expenditure and intakes of critically ill children. Chapter 6 explores the relation between nitrogen excretion rate and clinical characteristics and dietary intake in the same population. Chapter 7 describes an experiment in healthy males subjects that tests the influence of L-ornithine-alpha-ketoglutarate, a nutritional supplement, on urea synthesis rate.

In Chapter 8, the results and implications of the studies are discussed. Also, future perspectives are suggested. Finally, a summary of the thesis is provided in both English and Dutch.