Discussion
Scope of the study

The subject of this thesis is the catabolic stress response. This metabolic response to injury, infection or surgery is commonly seen in critically ill patients and derives its name from the net protein breakdown and concomitant loss of lean body mass that ensues.

Recent years have seen major developments in the field of dietary interventions and specific nutrients in critically ill patients. Notably, Van de Berghe et al. found that strict glycaemic control in ICU patients almost halved mortality in adult post-operative ICU patients.\cite{159} This study underlines the therapeutic potential of interventions aimed at the metabolic stress response. It is also exemplary of the complex interplay between the (neuro-)endocrine factors, substrate utilisation, nutrition and immune response at play in critically ill patients.\cite{153}

Two separate aspects of the metabolic stress response are addressed in this thesis. The first three chapters (Chapters 2, 3 and 4) focus on the methodological aspects of protein catabolism measurements by means of urea tracer experiments. The second part of this thesis (Chapters 5, 6 and 7) explores clinical aspects of the metabolic stress response and describes a nutritional supplement trial.

Part I (Chapters 1, 2 and 3)

a. Protein metabolism measurements

Various methods can be applied to study in vivo protein metabolism.\cite{1} These methods are discussed in more detail in the introduction of this thesis (Chapter 1). In short, the most accessible method involves measurement of total urinary nitrogen excretion. This method is readily applicable and non-invasive and has therefore found widespread application in the field of nutritional research,\cite{8,32,40,129,223,225,227,239,240,247,275} but is hampered by concerns regarding the completeness of urine collections, especially in children and patients with no urinary catheter in situ. The validity of this method is further undermined when urinary urea is used as a proxy of urinary nitrogen content\cite{27,32-38,205} or in the event of significant non-urinary nitrogen losses.\cite{39,40}

b. Urea metabolism measurements

Any surplus nitrogen resulting from protein ingestion and the balance between protein degradation and synthesis is condensed in the urea cycle. Consequently, urea production rate is commonly used as a measure of amino acid catabolism. In a normal subject with regular nitrogen intake, 80 to 90% of the urea produced is excreted in the urine.\cite{71,72,165,189,196,276,277} Under most circumstances therefore, urinary excretion of urea closely reflects its rate of production. As a research tool to investigate protein metabolism, this technique is not versatile enough. It is ineffective in several clinical situations, especially when urea production must be measured accurately over short periods of time. For instance, in order to obtain a representative rate of urea excretion, urine has to be collected over extended amounts of time, usually 2-3 days, to account for fluctuations due to food intake and diurnal rhythms.\cite{71,190} Therefore, any acute variation in
protein catabolism (and thus, in urea synthesis) due to physiological or pathological perturbation may not be reflected in the urinary urea excretion rate. This method is therefore unsuitable to quantify urea production in any state characterized by acute changes. Furthermore, it is not practical for use in any pathological state in which renal failure is manifested, because urea clearance may be grossly altered due to reduction in glomerular filtration rate and rate of urine flow. This is further complicated in clinical situations such as severe thermal injury (and in other situations), in which an alteration in urine output, and hence urea excretion, due to massive shifts in body fluids, may be compounded by significant losses of urea in wound exudate. This method is therefore unsuitable to quantify urea production in any state characterized by acute changes. Furthermore, it is not practical for use in any pathological state in which renal failure is manifested, because urea clearance may be grossly altered due to reduction in glomerular filtration rate and rate of urine flow. This is further complicated in clinical situations such as severe thermal injury (and in other situations), in which an alteration in urine output, and hence urea excretion, due to massive shifts in body fluids, may be compounded by significant losses of urea in wound exudate.

Stable isotope protocols provide a more accurate way of studying metabolic processes, yet require multiple (blood) samples and mass spectrometry instrumentation and sometimes involve complex mathematics. This limits the application of stable isotope measurements in clinical care. Our ultimate aim was to develop a stable isotope protocol suitable for protein metabolism measurements in the paediatric intensive care setting. Ideally, this should involve a simple protocol of short duration and with a minimally invasive character, for instance consisting of a single oral tracer dose and with enrichments determined in a limited number of (urine) samples. However, when developing a tracer protocol, numerous practical and theoretical considerations intertwine, limiting the options of the protocol to a large extent. For example, the metabolic process under study will determine the choice of tracer. Tracer choice, on the other hand, is also fully dependent on the mass spectrometry equipment available. The metabolic process under study will further dictate the sampling route and the number of samples required to obtain valid modelling mathematics. When working in a clinical setting, and especially with children, limitations on the choice of sampling site, sample number and duration of measurement will further challenge the applicability and validity of available protocols.

We chose urea as tracer for the measurement of protein catabolism (Chapters 3, 4 and 5). As discussed before (Chapter 1), both amino acid and urea stable isotope tracers can be applied for measuring protein metabolism. One major advantage of amino acid tracers is that both protein breakdown and protein synthesis rate (and consequently, whole body protein flux) are determined. In addition, amino acid oxidation rates can be calculated, albeit only when VCO₂ and ¹³CO₂ enrichment in expired air (or the ¹³C-enrichment of blood bicarbonate) are measured. Urea tracer protocols will yield only urea synthesis rate, which is considered to be a true indicator of protein catabolism. No information regarding protein synthesis rate, protein breakdown rate or flux is obtained.
The choice for the urea tracer was taken for both theoretical and practical reasons. Firstly, there is ample experience with urea tracers in literature. Secondly, tracer choice was determined by the availability of gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS) equipment. This mass spectrometry method has high sensitivity and accuracy for the measurement of $[^{13}C]_\text{urea}$ enrichments (Chapter 2), which allowed us to study the accuracy of urea kinetics calculations. Also, the universal application of the $[^{13}C]_\text{urea}$ tracer for the detection of gastric Helicobacter pylori infection makes this a low cost and easily accessible tracer. Finally, there was previous experience with the application of oral urea isotope protocols, wherein tracer enrichment is determined in urinary urea. This was of importance to us, as we envisaged the application of $[^{13}C]_\text{urea}$ in an oral tracer protocol.

Chapter 2 describes the development of an analytical method for the conversion of urea in plasma into 2-methoxypyrimidine. In essence, this new derivatisation procedure enables both gas chromatography mass spectrometry (GCMS) and GC-c-IRMS analysis of urea enrichment in blood samples. Also, the required sample volume is reduced to a minimum (less than 500 µl plasma volume), enabling application in young children and neonates.

Previous assays have been described for the analysis of stable isotope labelled urea enrichments in body fluids by GCMS and GC-c-IRMS. Urea enrichments determined by GCMS have a standard deviation that is approximately 10-50 times that of measurements obtained by GC-c-IRMS. The accuracy of GCMS however will suffice for most urea tracer protocols, e.g. in the primed, constant rate infusion of $[^{15}N]_\text{urea}$. The use of GC-c-IRMS offers the advantage of high accuracy and hence allows for the use of low tracer doses. Although the high costs of investment for GC-IRMS equipment limit its availability, the high accuracy of GC-IRMS analysis is required when working with low enrichments and small changes in isotopic ratios, as seen for example in single tracer bolus experiments. Measurement by GCMS would require a significantly higher tracer dose (150 or 250 µmol per kg as opposed to 25 µmol per kg) to obtain adequate signal-to-noise ratios by the protocol’s end. Such dose requirements preclude their application, as they would influence the metabolic system under study.

The derivatisation method described in Chapter 2 is suitable for GC-IRMS (and GC-c-IRMS) measurements, making it possible to measure $[^{13}C]_\text{urea}$ enrichments (in plasma and urine) directly. Also, it is possible to use the same derivatised sample for GCMS analysis. The GCMS analysis can then be applied to determine urea concentration or a $^{15}N$ (and/or $^{18}O$) label simultaneously. The derivatisation method described in Chapter 2 was applied in an experimental piglet model with the simultaneous administration of a $[^{13}C]_\text{urea}$ bolus and a $[^{15}N]_\text{urea}$ primed constant infusion. Deconvolution of GCMS and GC-c-IRMS measurements resulted in the enrichments for $[^{18}N]_\text{urea}$, $[^{13}C]_\text{urea}$ and $[^{15}N]_\text{urea}$ separately.

**c. Urea or nitrogen recycling**

All urea is produced in the liver and the major part is then excreted via the urine. A small proportion of urea however will enter the gastrointestinal lumen, where it is degraded into elemental nitrogen or ammonia by urease-positive bacteria. Once reabsorbed, nitrogen can be
reintegrated into urea or non-essential amino acids. This process of enterohepatic recycling of urea nitrogen can be quantified by protocols involving the doubly labelled urea tracer $^{15}$N$_2$urea.\textsuperscript{73, 188} Quantification of enterohepatic nitrogen recycling is interesting, as it has been suggested to contribute to the maintenance of nitrogen balance in conditions of low protein intake.\textsuperscript{74, 163, 165, 178, 186, 211, 276, 277, 280, 281}

The recycling of urea has consequences for stable isotope measurements of urea production. One is that singly labelled urea tracers can only give one value representing the total rate of urea synthesis. This value incorporates urea synthesized from both nitrogen recycled in the gastrointestinal lumen and nitrogen from amino acid oxidation. Protocols applying singly labelled urea tracers, as the $^{13}$Curea protocol described in Chapter 3, cannot discern between these two sources of nitrogen. This means that, when applied for the assessment of amino acid oxidation, these protocols will by definition overestimate whole body catabolism.\textsuperscript{71} Extrapolations from urea synthesis measurements to whole body protein oxidation rates should therefore be interpreted with caution when singly labelled urea tracers are applied.

d. Urea tracer protocols

The primed, constant rate intravenous infusion protocol of urea tracers has become the gold standard for the measurement of urea synthesis rate.\textsuperscript{73, 188} This infusion protocol has several inherent advantages, the most important ones being the relatively small number of samples required and simple mathematics involved. Also, the duration of infusion is limited, as isotopic plateau can be achieved after 2 to 3 hours if an adequate priming dose is administered.\textsuperscript{181, 199, 200} Low cost GCMS analysis can be applied in view of the high plasma enrichments obtained. Commonly, the doubly labelled urea tracer $^{15}$N$_2$urea is applied, as it also quantifies the enterohepatic recycling of urea nitrogen (from $^{15}$N$_1$urea enrichment). It has found widespread application in adults\textsuperscript{71, 180, 189-191, 282-284} and children.\textsuperscript{181, 192, 281}

In Chapter 4 addresses several methodological aspects of the primed, constant rate infusion of urea tracers. For this purpose, we performed a 10 h primed constant intravenous infusion of $^{15}$N$_2$urea in 8 fed and 6 fasted piglets. The calculation of urea synthesis rate during isotopic plateau is based on the assumption of a single homogenous pool with instant mixing of urea tracer and tracee. The validity of this assumption is challenged by empirical studies demonstrating two compartments for urea.\textsuperscript{165, 196, chapter 3} We compared plateau calculations and single and multiple pool compartmental models for calculation of urea pool size and total urea rate of appearance. No significant bias between methods was found. Also, we found that total body urea as determined by multiplying urea concentration with TBW (measured with $^2$H$_2$water) was equal to urea pool size estimated by compartmental modelling. This finding is in accordance with previous studies in non-ruminant mammals\textsuperscript{206} and humans\textsuperscript{196} and held true for both fasted and fed piglets, indicating no effect of feeding on the urea volume of distribution. These experimental observations thus support the assumptions underlying single pool calculations.

However, some concerns regarding the validity of tracer plateau calculations remain. In the primed, constant infusion protocol, a priming dose is administered to speed up the attainment of
isotopic plateau. If the priming dose is adequate (commonly a dose of 6 to 9 times the hourly maintenance infusion rate is given), isotopic plateau can be achieved within 2 to 3 hours.\textsuperscript{189, 200, 284, 285} Urea is a metabolite with a slow fractional turnover rate. For instance, in humans the fractional turnover rate of leucine is ca. 120%/hour,\textsuperscript{61} compared with a urea pool fractional turnover rate of approximately 9%/hour.\textsuperscript{196, 286} When a priming dose is administered into a substrate pool with a very slow turnover, the priming dose will disappear slowly and can determine the plasma tracer enrichment during the first hours of infusion.\textsuperscript{287} When the size of the priming dose is inappropriate for pool size, this problem is further exacerbated. In both situations, short term sampling and inadequate priming dose, the apparent tracer enrichment plateau will reflect the priming dose and not the true metabolic production rate.

In our experiments, the size of the urea pool of individual piglets was not assessed prior to tracer administration, and so the priming dose could not be adjusted to urea pool size. Urea pool size showed a wide variation between piglets, which was mainly due to large differences in mean urea concentration between animals. Given the long half-life of urea (approximately 4.0 hours in the piglets), rapid attainment of the plateau was therefore unlikely. Indeed, in the majority of animals a slope in $[^{15}\text{N}]$urea enrichment over time was seen. This has also been reported in humans receiving prolonged infusions of $[^{15}\text{N}]$urea,\textsuperscript{201} whereas other studies have reported attainment of plateau enrichment with two urea tracers given at 50% difference in prime to constant infusion rate ratio.\textsuperscript{189}

As no stable isotopic plateau was attained, we resorted to the application of Steele’s equation.\textsuperscript{203} Overall, these calculations gave results similar to those from compartmental modelling, yet on the individual level differences were significant, as indicated by wide limits of agreement (± 36%). Whereas compartmental models and simulation analyses in the present study were fitted with non-linear assumptions, Steele’s equation assume linearity of changes between time points for interpolation. Non-linearity in urea synthesis rate may be present, for instance, due to tracer and tracee recycling kinetics. Therefore, the equation’s estimation in individual experiments may not necessarily reflect urea production and its elimination in the biological system.

e. Calculations of urea recycling rate

The above-mentioned concerns regarding the assumptions underlying isotopic plateau calculations are amplified in urea recycling rate measurements. Firstly, the plasma $[^{15}\text{N}]$urea enrichment at plateau is assumed to reflect urea recycling in the gut. Yet size and turnover rate of the gastrointestinal urea/nitrogen pool may be hard to fathom. In an experiment in two men with constant infusion of labelled urea, Wrong et al. found that the enrichment of ammonia in the colon was only approximately one tenth that of plasma urea.\textsuperscript{288} These results indicate a very high nitrogen flux through or in the colon many times greater than the entry of endogenous urea. Also, as no $[^{15}\text{N}]$urea prime is administered, long-term infusions of $[^{15}\text{N}]$urea may be necessary to obtain true $[^{15}\text{N}]$urea plateau enrichment. In a study assessing the effect of diet quality on the fate of urea in sheep, Lobley et al. gave continuous infusions for four days\textsuperscript{289} No priming dose was
given and isotopic enrichments were determined in faeces and urine samples. It was found that [$^{15}$N$_2$]urea enrichments were not stable until day 3 of infusion.

In our experiment, steady state for [$^{15}$N$_2$]urea was not present in most animals at the end of the 10 h protocol. Simulations indicated that an infusion of at least 33 h would be needed to obtain a plateau of [$^{15}$N$_2$]urea in individual animals for valid calculation of recycling rates. As a consequence, plateau calculations using actual [$^{15}$N$_2$]urea enrichments significantly underestimated urea recycling rate. One could reason that also in humans [$^{15}$N$_2$]urea tracer infusions of similar length would be required. Studies in young children seem to support this.

Millward and co-workers performed a study in which intermittent oral [$^{15}$N$_2$]urea boluses were administered in infants for 36 h, and measured [$^{15}$N]lysine enrichment in the urine as an indicator of urea nitrogen recycling. They found that lysine enrichment continued to rise up to 30-36 h. In other studies in infants using urea enrichment at plateau, urea recycling was reported to be only between 0 and 4.7% of urea rate of appearance (Ra) when [$^{15}$N$_2$]urea was given as a single bolus or in a short-term protocol of intermittent doses. However, in one study where [$^{15}$N$_2$]urea was administered continuously to infants for 24 h, recycling accounted for 9.3% of urea Ra. The latter study, the data from Millward et al., and the findings of our study in piglets suggest that calculation of urea recycling rate from [$^{15}$N$_2$]urea plateau enrichments in primed constant rate infusions with [$^{15}$N$_2$]urea may seriously underestimate urea recycling rate, unless the tracer is given for sufficient time (at least 24 h) to achieve true plateau.

Finally, the urea recycling rate was calculated with less overall accuracy than urea rate of appearance, because [$^{15}$N$_2$]urea enrichments were much lower than [$^{15}$N$_2$]urea enrichments and thus had a higher coefficient of variation. The lower accuracy for urea recycling calculations propagates into the error for de novo urea synthesis rate.

We conclude that for the [$^{15}$N$_2$]urea model the use of compartmental modelling or the use of simulation analysis can improve accuracy and precision of total urea production and recycling rates as compared to the widely used estimations from urea plateau enrichments. The results suggest that plateau calculations applied to plasma urea enrichments during a primed, constant [$^{15}$N$_2$]urea infusion underestimate gut urea recycling rate in piglets by ~50%, and affects accuracy for total urea production.

f. Single bolus protocols

The first studies into urea synthesis measurements were single bolus experiments. In single bolus protocols, the accuracy of urea synthesis rate calculations hinges on the exact determination of the urea pool size. This in turn depends on two variables: the correct interpretation of the plasma disappearance curve and the exact dose of the administered tracer bolus.

The mathematical interpretation of the decay curve of plasma enrichment is generally deemed rather complex and is therefore seen as an objection to the application of the single bolus method. Previous authors have found that the multi-exponential decay curve following a single
urea bolus, is best described by a two pool system\textsuperscript{10, 165} and might require multi-compartmental modelling.\textsuperscript{194} We compared multi-compartment modelling of the $[^{13}\text{C}]$urea decay curve following a single oral bolus with less complex, stochastic calculations (Chapter 3). This approach seems reasonable, as previous studies applying two-compartment analyses have found rapid exchange of urea between both urea pools.\textsuperscript{165, 196, chapter 4} Matthews and Downey, for instance, reported that the first exponential decay term of the bi-exponential enrichment decay curve contributed less than 3\% to the total plasma area under the curve following an intravenous urea tracer bolus\textsuperscript{196} and consequently suggested that the precision of the urea production measurement depended primarily on the second slope and intercept. In our study (Chapter 3), single pool regression calculations compared well with multi-compartmental calculations, when initial data points were discarded (as suggested by Matthews and Downey). Post-hoc simulations corroborated this approach, even when sampling was truncated at 6 h. This suggests that an oral tracer urea protocol in growing mammals (including young children) may be limited in time (e.g. sampling at baseline and hourly between 2 and 6 h) and sample number (i.e. 5 blood samples).

Despite the fact that the analysis of the enrichment decay curve can be simplified to regression calculations with a limited number of samples, the validity of any single oral tracer method is compromised by the underlying assumption of immediate and complete absorption of the oral tracer dose. As mentioned before, the exact tracer dose is pivotal in single bolus method calculations. In our experiments, there was a small, yet significant increase in plasma $[^{13}\text{C}]$bicarbonate enrichment, indicating that some of the administered $[^{13}\text{C}]$urea tracer was split in the initial phase of the experiment (Chapter 3). Additional analyses revealed that the possible overestimation of urea synthesis rate was limited. In one study wherein the tracer was administered intravenously and orally on separate occasions, Jackson reported good agreement between both tracer administration protocols.\textsuperscript{194} No excessive hydrolysis of tracer was reported in any of the subjects. Other studies applying oral doses of $[^{15}\text{N}_2]$urea however found evidence of tracer hydrolysis in a number of individuals.\textsuperscript{72, 195} In these individuals, gastrointestinal hydrolysis of the doubly labelled urea tracer led to an abnormally high rate of excretion of $[^{15}\text{N}_2]$urea and consequently, to spurious urea kinetics calculations. For this reason, gastric colonisation with H. pylori should be ruled out prior to application of oral urea tracers.\textsuperscript{290} Despite this, hydrolysis of urea in either the upper or lower gastrointestinal tract by other intestinal microflora with urease activity cannot fully be excluded.

Finally, single bolus calculations assume immediate and complete absorption of the tracer. When administered as a single intravenous bolus this assumption seems reasonable, but in case the tracer is administered orally, it can only be assumed. This further challenges the validity of short-term protocols applying single oral tracer boluses. It is for this reason that most urea oral tracer protocols use either constant infusions,\textsuperscript{72, 163} repeated tracer doses,\textsuperscript{162, 178, 186, 187, 193, 195, 208-211, 276, 277, 291-294} or resort to long-term urine collections following a single oral bolus.\textsuperscript{194, 281, 283, 295, 296}
Part II (Chapters 5, 6 and 7)

Chapters 5 and 6 describe studies performed in the setting of a tertiary paediatric intensive care unit (PICU). Three groups of patients admitted to the PICU were prospectively followed from admission for a maximum of 7 days. In these critically ill children, we assessed factors associated with energy intake, expenditure and balance (Chapter 5), and the determinants of protein breakdown rate by means of nitrogen excretion measurements (Chapter 6). Finally, Chapter 7 describes a study of a nutritional supplement on urea synthesis rate in healthy men.

a. Methodological considerations

Chapters 5 and 6 describe the results from a longitudinal, prospective study in three major PICU diagnostic groups. Patients were included when admitted for sepsis, trauma or after elective thorax or abdominal surgery. Patients were followed for a total of 7 days, including after discharge from the PICU to the high and medium care ward. Daily indirect calorimetry measurements were performed from study inclusion. In addition, prescription and administration of energy and macronutrients were noted. Finally, daily 24-hour urine collections were obtained, albeit only for the full study duration in the subgroup described in Chapter 6. These measurements enabled us to determine exact daily balances of energy and macronutrients.

The patients described in Chapters 5 & 6 constituted a heterogeneous study population with a wide range in age, weight and illness severity. Also, group composition changed during the course of the study due to patients being discharged from the hospital. Finally, extremely ill patients could not be included because of methodological limitations of indirect calorimetry. Previous studies performing serial measurements of energy expenditure in PICU patients have focused on selected patient populations. In our study, the heterogenicity of the patients studied limited direct comparisons between groups for statistical considerations. Also, patient number per diagnostic subgroup was limited. Finally, disease severity, as assessed by PRISM score, varied significantly between groups and was limited on the whole. This could mean that the catabolic stress response may not have been present in all patients, which may have impeded the detection of its effects. Indeed, the ratio of measured to predicted EE did not differ between diagnostic groups. This result indicates no effect of diagnosis or severity of illness on EE. Nevertheless, and despite the inherent disadvantages of a heterogeneous study population, we think our results are applicable to the majority of PICU patients, as most European PICU’s cater to a wide variety of diagnostic groups and patient ages. Secondly, due to the prolonged and consistent character of measurements, we were able to assess the time course of the metabolic stress response. Energy expenditure measurements were obtained daily in most patients for the duration of the study; in Chapter 5, daily measurements were obtained in 89% of study days. This compares well with previous longitudinal studies, that have reported shorter study periods, limited patient numbers and infrequent measurements. Also, the large number of measurements allowed us to assess multiple possible determinants of protein catabolism, notably nutrition, by means of multivariate analysis. Previous studies had not performed such analyses.
b. Energy expenditure in critically ill children

Numerous studies in critically ill children have reported discrepancies between predicted and measured energy expenditure. This can be attributed partly to the special conditions imposed by treatment in the intensive care setting, such as a temperature-controlled environment, immobility, use of sedatives or muscle relaxants, and mechanical ventilation. On the other hand, the metabolic stress response characteristic of critical illness is assumed to alter energy expenditure (EE) too. For instance, elevated EE has been reported in children with burns, neonatal septicaemia, congenital heart disease, or head injury.

In our study (Chapter 5), energy expenditure was not significantly different from predicted values: the ratio of measured EE to predicted EE was 1.0, although wide variations were noted in individual patients. This finding may seem contradictory to the concept of the catabolic stress response. On the other hand, and contrary to adults, hypermetabolism may not be a defining character of the stress response in critically ill children. Firstly, only studies in children suffering from burns have consistently reported elevated levels of energy expenditure. Secondly, various articles have stated that hypermetabolism (defined as a measured to predicted EE ratio > 1.1) is not observed in critically ill children, whereas others have reported hypermetabolic states. Comparison of these studies is hampered by the application of different predictive equations, such as those of Schofield, Harris-Benedict and Talbot. More importantly, the larger studies reporting hypermetabolism in non-burn critically ill patients were cross-sectional by design and measured EE once patients had been admitted to the PICU for some time. Studies assessing EE by serial or continuous measurements immediately following PICU admission tend to find normometabolism or hypometabolism (defined as a measured to predicted EE ratio < 0.9). Because of the acute character of diagnostic groups in our study, calorimetry measurements immediately following admission were not always possible. Nevertheless, 88% of EE measurements were started within 36 hours of inclusion. Hence, the immediate effect of critical illness or injury on EE could not be assessed. It should be realised, though, that the instability of clinical condition, high FiO₂ and air leaks along endotracheal tube often precludes reliable measurements in the initial period.

Finally, in our study, there was no development of measured EE over time, neither overall nor in the subgroups (Chapter 5). Several studies have assessed development of EE over several days following PICU admission or surgery and only one, in postoperative infants, reported an increase in energy expenditure over time.

c. Prescription and administration of nutrition in PICU patients

In the longitudinal study of critically ill children admitted to the PICU, we found that during the first week following admission, patients were underfed in 60% of days and overfed in approximately 30% (Chapter 5). This is of importance, as adequate nutrition is associated with physiologic stability and outcome in critically ill children, whereas both malnutrition and overfeeding are associated with poor outcome.
Previous studies have highlighted the discrepancy between actual energy expenditure and predicted energy requirements when predictive equations are applied. Using predictive equations for nutritional prescription could thus contribute to inadequacies in nutritional intake. In adult intensive care unit patients, inadequacies in nutrition have been ascribed to discrepancies between prescribed and actually administered energy, with interventional and diagnostic procedures limiting energy administration significantly. One study in critically ill children attributed inadequacies in energy intake mainly to fluid restrictions in the cardiac patients. In our study, these factors contributed less to feeding inadequacies, as administered energy more or less matched prescribed energy and no cardiac patients were enrolled.

Applying multivariate analyses to assess the adequacy of nutritional support and the development of nutritional requirements over time, we found that the use of parenteral feeding was the single most important factor determining the amount of energy administered (Chapter 5). Also, as sepsis patients received parenteral nutrition more frequently, energy intake in these patients matched requirements earlier than in other diagnostic groups, yet at the expense of a high frequency of overfeeding in this group.

These results should not so much be interpreted as an incentive to administer parenteral nutrition indiscriminately. Firstly, enteral nutrition has multiple advantages over parenteral nutrition. Secondly, parenteral nutrition may be associated with overfeeding whereas this will not reverse tissue catabolism. A more sound interpretation would be a caveat for the sole dependence on enteral nutrition, notably in patients discharged to the high and medium care wards. It was these patients that were at particular risk of undernutrition.

d. Nitrogen excretion in critically ill children

Increased nitrogen excretion is the hallmark trait of the catabolic stress response. It was first described following skeletal trauma and has since been reported in severe injury, sepsis and burns. In children, however, most studies of protein catabolism have focused on patients with burn injuries or (premature) neonates. In paediatric burn patients energy expenditure is increased and the increased nitrogen excretion rate is related to the degree of hypermetabolism. In other diagnostic groups of critically ill children, the factors determining protein catabolism are less well known, mainly because very few studies have performed stable isotope measurements in this patient population. Only one stable isotope study has reported a relation between whole body protein metabolism and illness severity (as assessed by PIM-score) and body temperature, whereas two other studies found no such association. Of the many studies assessing urea excretion rates or, like ours, nitrogen excretion rates, only two have reported a relation between nitrogen excretion and illness severity.

In our study, we found that variations in nitrogen excretion rate could for 38% be attributed to clinical characteristics related to illness severity, whereas nutritional variables explained a meagre 4% of variance in nitrogen excretion (Chapter 6). The paediatric risk of mortality (PRISM) score on admission, a quantitative measure of illness severity in PICU patients (with higher scores for more
severely ill children), showed a positive correlation with nitrogen excretion rate. This confirms the classic concept that nitrogen excretion is proportionate to severity of illness and is in line with two previous studies in PICU patients,⁴⁰, ²²³ whereas other have been inconclusive.⁸, ²⁴⁷

Furthermore, nitrogen excretion correlated with oxygen consumption (VO₂) and this suggests increased nitrogen losses are related to hypermetabolism. Whereas protein breakdown rate is related to the degree of hypermetabolism in paediatric burn patients,²³⁵ studies performing indirect calorimetry and protein catabolism measurements in other diagnostic groups of critically ill children have not reported a similar association.⁷, ⁸, ²⁴⁰ It should be noted that in our study the ratio of measured EE to predicted EE was not an independent predictor of nitrogen excretion rate. Furthermore, one could reason that, despite expressing nitrogen excretion per unit body surface area, the correlation between VO₂ and nitrogen excretion could be attributed to a scaling relation between VO₂ and body mass.

Having undergone surgery was associated with decreased nitrogen excretion in our study. This is counterintuitive and the extent of nitrogen loss following surgery has been shown to correlate with the Surgery Severity Score, a proxy for the severity of surgical stress.⁴⁰ One explanation for the findings may be that surgery in our patients, most of whom underwent elective surgery, did not provoke a metabolic stress response to the same extent as in the non-surgical patients. Previous studies have found that the metabolic response to elective surgery may be limited, as assessed by stable isotope measurements of whole body protein metabolism²⁴⁸ and indirect calorimetry.²⁴⁹ Another explanation may be that following surgery significant non-urinary nitrogen losses remained unnoticed in our study. Indeed, one study has noted that non-urinary losses in surgical infants may amounts to significant amounts.⁴⁰

Finally, the analysis of nutritional variables found that only 4% of variance of urinary nitrogen excretion could be attributed to nutritional factors. From studies in adults, it is known that aggressive nutritional support does not prevent body protein loss during severe catabolic illness.¹⁴⁷, ¹⁵⁰ The low explained variance of our model may well be an expression of this limited effect of nutrition in critically ill patients. Nevertheless, caloric intake and lipids, albeit if administered in adequate quantities, were found to have protein sparing effects, in accordance with previous studies in critically ill children.⁸, ²²⁵, ²⁵²-²⁵⁴

e. OKG supplementation and urea synthesis rate

Chapter 7 describes a study into the effect of L-ornithine alphaketoglutarate (OKG) on urea synthesis rate in healthy men. OKG is a nutritional supplement that is applied in both chronically²⁵⁵, ²⁵⁶ and acutely malnourished patients.²⁵⁷-²⁶¹ It has been shown to improve nitrogen retention in burn,²⁵⁷-²⁶⁰ trauma²⁶¹ and surgery patients.⁸⁵, ⁸⁶, ²⁶⁵-²⁶⁹

Nitrogen retention can be achieved either by increased protein synthesis, a decreased rate of proteolysis or both. Studies into the effect of OKG supplementation, have reported maintenance of protein synthesis capacity following OKG as compared to placebo.⁸⁵, ⁸⁶, ²⁶³, ²⁶⁴ Other clinical studies have performed nitrogen balance analyses, and have therefore not revealed the
mechanism responsible for the nitrogen retention ascribed to OKG. For this reason, we studied the effect of OKG on protein breakdown.

In our study in healthy male subjects, a single dose of oral OKG did not affect urea production rate differently than an isonitrogenous placebo (Chapter 7). This is in accordance with the study by Eriksson et al, who administered 4 grams of OKG intravenously in seven healthy volunteers. Despite the immediate metabolic effects of OKG, e.g. the stimulation of insulin and growth hormone secretion, neither the study by Eriksson et al. nor our study found an effect of OKG on urea production rate. The failure to find an effect of OKG on urea synthesis rate can be ascribed partly to the study set up. For instance, participants were responsible for the protein-restricted diet. The varying caloric intake may have obscured possible effects of OKG supplementation on protein metabolism. Also, the number of participants may have been too limited to detect a difference between OKG and placebo. Also, the choice of stable isotope protocol, a single oral tracer protocol of short duration, may not have been the optimal choice to discern changes in urea synthesis rate. On the other hand, OKG failed to change nitrogen excretion rate in our study, both on the first day and following three days of OKG supplementation (Chapter 7). Nevertheless, our study would have benefited from a repeated tracer measurement following the supplementation period.

A second explanation for the result of our study was the good clinical condition of study subjects. The beneficial effects of OKG on protein metabolism and nitrogen economy have all been reported in malnourished elderly or in patients suffering from burns or trauma. The effect of OKG may be limited to conditions of metabolic stress, and for this reason may not have been present in our study. Thirdly and finally, maintenance of protein synthesis capacity may be the sole mechanism responsible for the nitrogen sparing effects of OKG. It is noteworthy, that in one of the few clinical studies to perform stable isotope measurements into the effect of OKG, no evidence of decreased proteolysis was found. Jeevanandam and Petersen found an increased rate of total protein turnover in trauma patients after 4 days of OKG supplementation. This was ascribed to an increased rate of protein synthesis and/or visceral proteolysis as indicators of muscular proteolysis, urinary nitrogen and 3 methyl-histidine excretion, were similar in OKG supplemented and control patients.

**Perspectives for future research**

The studies presented in this thesis focus on measurement, extent, determinants and treatment of protein catabolism in critically ill children.

The first three chapters of this thesis focus on the measurement of urea synthesis rate as a proxy of whole body protein catabolism. The primed, constant rate infusion of urea tracer is the method most commonly applied for this purpose. This method has found widespread application in free-living subjects using continuous infusions or repeated doses of oral tracer. Nevertheless, several methodological concerns are raised in this thesis regarding this tracer infusion protocol, mainly
regarding the measurement of urea recycling. Secondly, a simplified single bolus protocol is presented, that may be particularly suitable for short-term measurements in free-living subjects. Nevertheless, given the assumptions inherent to single oral tracer bolus protocols, the primed, constant rate infusion method is likely to remain the gold standard for the measurement of urea synthesis metabolism.

Future research should address the concerns regarding the validity of urea recycling rate calculations. When the size of the urea body pool is unknown, isotopic priming can be difficult and may affect isotopic plateau values. These concerns are amplified when measuring urea recycling rate. Notably in underfed or critically ill patients in whom the enterohepatic cycle of nitrogen may contribute to maintenance of whole body nitrogen balance, validity of measurements may seriously be compromised.

The second part of this thesis describes three clinical studies. It is shown that underfeeding and overfeeding are common in paediatric ICU patients. Despite ample evidence in adult ICU patients regarding the long-term consequences of malnutrition, very few studies have reported on this subject in critically ill children. Secondly, studies of the metabolic effects of critical illness per se have given conflicting results, with the single exception of studies in children suffering from burns. This is probably due to the wide variety of admission diagnoses and limited numbers of annual admissions in paediatric ICU’s. Very few concerted, prospective studies into the consequences of malnutrition in critically ill children have been performed. Of more value for daily clinical management in PICU’s would be the evaluation of different feeding strategies in critically ill patients in a randomized, controlled and prospective manner. For instance, early postoperative tube feeding, enteral nutrition, nutrition tailored to meet individual requirements by daily EE measurements and various nutritional supplements have yet to be assessed in PICU patients. Given the advances in intensive care medicine achieved in the past decade, nutritional and metabolic interventions hold the key to significant improvements in these patients.