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.

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 measurement of protein catabolism 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.

**Introduction**

Chapter 1 describes the background and aims of the studies presented in this thesis. First an overview is given of the metabolic stress response. In reaction to e.g. trauma, infection or surgery, various neuro-hormonal signals lead to the clinical signs and metabolic events that characterize the stress response: tachycardia, tachypnoea, hyperglycaemia, mobilisation of body fat, and increased rates of protein synthesis and breakdown. This response is in essence catabolic, i.e. it will lead to a reduction of total body protein content. Secondly, the different methods in use for the measurement of whole body protein metabolism are discussed. These include either methods involving the measurement of whole body nitrogen excretion rate or its proxies (e.g. urinary urea), or methods applying stable isotopes.

**Methodological aspects of protein catabolism**

a. **Chapter 2: derivatisation of urea samples**

This chapter describes a new derivatisation procedure for the conversion of urea in plasma into 2-methoxypyrimidine. This analytical method enables the analysis of urea enrichments in blood samples using both gas chromatography mass spectrometry (GCMS) and gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS). Also, the required sample volume is reduced to a minimum (less than 500 µl plasma volume), enabling application in young children and neonates.

Numerous other assays exist for the analysis of stable isotope labelled urea enrichments in body fluids by either GCMS or GC-c-IRMS. However, our derivatisation method allows for the application of both chromatography methods, which means that different urea isotopes can be applied within one experiment. Also, given the high accuracy of by GC-c-IRMS measurements, this method is applicable in experiments involving measurements of low tracer enrichments, as is the case in single tracer bolus experiments.

b. **Chapter 3: the \[^{13}C\]urea single bolus protocol**

Chapter 3 describes the development of a new stable isotope protocol for the measurement urea synthesis rate. Any surplus nitrogen resulting from protein ingestion and the balance between
protein degradation and synthesis, is condensed in the urea cycle. Urea synthesis rate is therefore considered a true indicator of protein catabolism.

In the study, a single oral $[^{13}\text{C}]$urea bolus was administered to both fed and fasting piglets. Blood samples were taken for ten hours and urea enrichment analysis was performed by GC-c-IRMS. It was found that urea synthesis rate could be calculated using linear regression analysis of plasma enrichment values, when the sampling protocol is adjusted to the initial passage of the tracer through the central urea compartment. Secondly, valid results could be obtained even when sampling was reduced to hourly samples for the duration of 6 hours. Finally, a fraction of the oral urea tracer bolus was oxidized within 1 h.

In conclusion, this new protocol applying a single, oral bolus of $[^{13}\text{C}]$urea requires minimal blood sampling, and offers the possibility of a minimally invasive method for robust measurement of urea production in fasted and continuously fed animals.

c. Chapter 4: modelling of $[^{15}\text{N}_2]$urea tracer infusions in growing piglets

This chapter 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 $[^{15}\text{N}_2]$urea in 8 fed and 6 fasted piglets.

Calculation of urea synthesis rate during isotopic plateau is based on the assumption of a single, homogenous pool with instant mixing. This assumption is challenged by empirical studies demonstrating two compartments for urea. We compared plateau calculations with single and multiple pool compartmental models for the calculation of urea pool size and total urea rate of appearance. No significant bias between methods was found. Also, total body urea as determined by multiplying urea concentration with total body water (measured with $[^2\text{H}_2]$water) was equal to urea pool size estimated by compartmental modelling. This finding held true for both fasted and fed piglets, indicating no effect of feeding on the urea volume of distribution. These experimental observations support the assumptions underlying single pool calculations.

Nevertheless, some concerns remain regarding the validity of plateau calculations. In the primed, constant rate infusion protocol, a priming dose is administered to speed up attainment of isotopic plateau. However, when a priming dose is administered into a substrate pool with a slow turnover, like that of urea, the priming dose will disappear slowly and determine plasma tracer enrichment during the first hours of infusion. When the size of the priming dose is inappropriate for pool size, this problem is further exacerbated. In our experiments, the size of the urea pool was not assessed prior to tracer administration, and so the priming dose was not adjusted to urea pool size. As a consequence, true isotopic plateau was not achieved in most animals and this problem most probably affects more than just our study.

The concerns regarding the assumptions underlying isotopic plateau calculations are amplified in urea recycling rate measurements. Firstly, it is unknown whether plasma $[^{15}\text{N}_2]$urea enrichment at plateau actually reflects urea recycling in the gut. Secondly, the $[^{15}\text{N}_2]$urea pool is not primed, and so $[^{15}\text{N}_2]$urea plateau enrichment may not be achieved in the short duration of most infusion...
protocols. In our experiment, steady state for $[^{15}\text{N}]\text{urea}$ was not present in most animals at the end of the protocol. Simulations indicated that an infusion of at least 33 h would be needed to obtain a plateau of $[^{15}\text{N}]\text{urea}$ for valid calculation of recycling rates. As a consequence, plateau calculations using actual $[^{15}\text{N}]\text{urea}$ enrichments significantly underestimated urea recycling rate. Finally, urea recycling rate was calculated with little overall accuracy, because of a high coefficient of variation in $[^{15}\text{N}]\text{urea}$ enrichments. The lower accuracy for urea recycling calculations propagates into the error for de novo urea synthesis rate.

We conclude that for the $[^{15}\text{N}]\text{urea}$ 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 $[^{15}\text{N}_2]\text{urea}$ infusion underestimate gut urea recycling rate in piglets by ~50%, and affects accuracy for total urea production.

**Clinical aspects of the metabolic stress response**

a. **Chapter 5: energy intake and balance in critically ill children**

This chapter evaluates the nutritional support in 46 children during critical illness and recovery during the first week of paediatric intensive care unit (PICU) admission. Patients suffering from sepsis, or patients admitted following trauma or major surgery were studied. In this longitudinal study, daily energy expenditure (EE) measurements by indirect calorimetry were performed. This allowed us to obtain accurate daily balances of energy and macronutrients.

In our study, patients were underfed on 60% of days and overfed on 28% of days. Daily measured EE was stable and, on a group level, not significantly different from predicted EE values over the course of the first seven days following PICU admission. This finding contradicts the common assumption of an acute “ebb” phase in EE, followed by a phase of increased EE. In individual patients, the ratio of predicted and actual EE showed wide variations. In order to prescribe amounts of nutrition, at least one measurement of EE should therefore be performed following PICU admission.

Analysis of nutritional prescription and actually administered calories, revealed that caloric intake increased significantly during the course of admission and independently of diagnostic category. Patients receiving parenteral nutrition (either exclusively or in combination with enteral nutrition) achieved adequate energy intake to EE ratios faster than patients dependent on enteral intake only. However the former patients were more likely to be overfed, whereas the latter, commonly on the high and medium care wards, were most at risk of being underfed. These results place into perspective the recent focus on the advantages of enteral nutrition in nutritional management.
b. Chapter 6: determinants of nitrogen excretion in PICU patients

Increased nitrogen excretion is the hallmark trait of the catabolic stress response. In children most studies of protein catabolism have focused on patients with burn injuries or (premature) neonates. Chapter 6 assesses factors determining nitrogen excretion in a subset of the critically ill patients described in Chapter 5. We analysed daily energy and substrate balances combined with 24-h nitrogen excretion rates from 33 patients.

Variations in nitrogen excretion rate could for 38% be attributed to clinical characteristics related to illness severity. 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. Secondly, nitrogen excretion correlated with oxygen consumption (VO₂) and surgical status, i.e. having undergone surgery was associated with a decreased nitrogen excretion. This may be explained by the fact 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. Finally, analysis of nutritional variables revealed that only 4% of variance of urinary nitrogen excretion could be attributed to nutritional factors.

In conclusion, we found that in a mixed population of critically ill children admitted to the PICU, nitrogen excretion was only to a very limited extent influenced by nutrition. On the other hand, parameters associated with disease severity explained 38% of the variance in protein catabolism. The degree of protein catabolism was proportionate to PRISM score and VO₂. In addition, surgical intervention was associated with a decreased nitrogen excretion rate.

c. Chapter 7: OKG supplementation in healthy male subjects

A study into the effect of L-ornithine alphaketoglutarate (OKG) is described in Chapter 7. OKG is a nutritional supplement that is applied in both chronically and acutely malnourished patients and that is known to improve nitrogen retention.

We studied the effect of OKG on urea synthesis rate in healthy men using the single, oral [¹³C]urea tracer protocol described in Chapter 3. Subjects were kept on a restricted protein diet and received either OKG or an isonitrogenous placebo. OKG was found to affect neither urea synthesis rate nor nitrogen retention.

The failure to find an effect of OKG can partly be ascribed to the study set up. As participants were responsible for their diet, varying caloric intake may have obscured possible effects of OKG. Also, the number of participants may have been too limited. Finally, the tracer protocol of short duration may not have been the optimal choice to discern changes in urea synthesis rate. 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.

In conclusion, following oral supplementation of OKG, no immediate effect on urea production rate in healthy male study subjects could be demonstrated. Also, OKG supplementation did not increase nitrogen retention. The previously reported positive effect on nitrogen economy and anticatabolic effect of OKG supplementation may only be present in critically ill patients or following a prolonged period of supplementation.

**In conclusion**

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. Several methodological concerns are raised concerning 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.

The second part of this thesis describes three clinical studies. It is shown that underfeeding and overfeeding are common in paediatric ICU patients. In the same population, factors determining nitrogen excretion rates were assessed. Finally, we studied the effect of OKG on urea production rate in healthy males in a randomized blinded placebo controlled fashion.

**Perspectives for future research**

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.

Very few studies have reported on the long-term consequences of malnutrition in critically ill children. Secondly, studies of the metabolic effects of critical illness in children have given conflicting results. The evaluation of different feeding strategies in critically ill patients in a randomised, controlled and prospective manner would be great value for daily clinical management in PICU’s. 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.