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ENERGY EXPENDITURE AND SUBSTRATE UTILIZATION DURING CRITICAL ILLNESS

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Energy expenditure and substrate utilization during critical illness
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It's just as if a man were wounded with an arrow thickly smeared with poison.

His friends & companions, kinsmen & relatives would provide him with a surgeon, and the man would say, 'I won't have this arrow removed until I know whether the man who wounded me was a noble warrior, a priest, a merchant, or a worker.' He would say, 'I won't have this arrow removed until I know the given name & clan name of the man who wounded me... until I know whether he was tall, medium, or short... until I know whether he was dark, ruddy-brown, or golden-colored... until I know his home village, town, or city... until I know whether the bow with which I was wounded was a long bow or a crossbow... until I know whether the bowstring with which I was wounded was fiber, bamboo threads, sinew, hemp, or bark... until I know whether the shaft with which I was wounded was wild or cultivated... until I know whether the feathers of the shaft with which I was wounded were those of a vulture, a stork, a hawk, a peacock, or another bird... until I know whether the shaft with which I was wounded was bound with the sinew of an ox, a water buffalo, a langur, or a monkey.' He would say, 'I won't have this arrow removed until I know whether the shaft with which I was wounded was that of a common arrow, a curved arrow, a barbed, a calf-toothed, or an oleander arrow.'

The man would die and those things would still remain unknown to him.

"The parable of the poisoned arrow". From the Shorter Exhortation to Māluṅkya Cūḷa Māluṅkyovāda Sutta (MN 63)

ABSTRACT

<u>Background</u> Critical illness leads to major alterations in metabolism. The net result is a state where catabolism predominates over anabolism. The associated loss of lean body mass is significant and potentially harmful. It is commonly held that providing calories and protein from nutrition may attenuate this response. Despite significant research efforts, an optimal dose for these therapies remains to be defined.

The metabolic rate of ICU patients is readily measured by indirect calorimetry. Questions regarding the accuracy of modern instruments in the setting of mechanical ventilation have been an impediment to wider application of this technique. The physiological effects of common nutritional interventions on protein balance are difficult to assess in clinical practice but can be quantified using stable isotope tracers.

<u>Aims</u> The studies of this thesis had two main aims: to validate techniques for gas exchange measurements in ICU, and to describe the effects of energy and/or amino acid supplementation on protein kinetics. In studies I and II we evaluated the measurement properties of three new instruments for indirect calorimetry in mechanically ventilated ICU patients against a clinical gold standard (Deltatrac). Study III investigated the effects of a supplemental amino acid infusion on whole-body protein balance in critically ill patients. In study IV, we quantified whole-body protein kinetics after 24 hours of full-dose or half-dose enteral nutrition in a randomized cross-over study design.

<u>Results</u> In study I, we performed 48 measurements with the evaluated instruments and reference method in sequence. Mean resting energy expenditure (REE) was similar between Quark RMR and Deltatrac ($p = 0.17$). Mean REE from CCM Express was 64% higher than Deltatrac ($p < 0.001$). In study II we conducted 48 simultaneous measurements with the evaluated instruments and Deltatrac. Compared to Deltatrac, both Quark RMR and E-sCOVX overestimated REE with similar bias and 95% limits of agreement. In study III, a 24-hour intravenous amino acid infusion resulted in a positive protein balance during the study period ($p = 0.0016$) without increasing amino acid oxidation ($p = 0.147$). In study IV, whole-body protein kinetics could be determined in six patients during half-dose and full-dose enteral nutrition. An improvement in protein balance was observed during full-dose nutrition ($p = 0.044$).

<u>Conclusions</u> Measured energy expenditure is variable between instruments for indirect calorimetry. Apart from one device, agreement compared to the reference method was better than what is commonly accepted for other monitoring technologies in critical care. The studies of protein kinetics indicate that an increase in energy or amino acid delivery improves whole-body protein balance in critically ill patients. Indirect calorimetry and tracer techniques are promising methods to further our understanding of alterations in energy metabolism and substrate utilization during critical illness. In turn, this knowledge may assist in the development of clinical trials with patient-centered outcomes.

LIST OF SCIENTIFIC PAPERS

I. **Indirect calorimetry in mechanically ventilated patients. A systematic comparison of three instruments.**
Sundström M, Tjäder I, Rooyackers O, Wernerman J
Clin Nutr. 2013 Feb;32(1):118-21

II. **Measuring energy expenditure in the intensive care unit: a comparison of indirect calorimetry by E-sCOVX and Quark RMR with Deltatrac II in mechanically ventilated critically ill patients.**
Sundström Rehal M, Fiskaare E, Tjäder I, Norberg Å, Rooyackers O, Wernerman J
Crit Care. 2016 Mar 5;20:54.

III. **A supplemental intravenous amino acid infusion sustains a positive protein balance for 24 hours in critically ill patients.**
Sundström Rehal M, Liebau F, Tjäder I, Norberg Å, Rooyackers O, Wernerman J
Crit Care. 2017 Dec 6;21(1):298

IV. **Whole-body protein kinetics in critically ill patients during 50 or 100% energy provision by enteral nutrition: A randomized cross-over study.**
Sundström Rehal M, Liebau F, Wernerman J, Rooyackers O
Manuscript, submitted for publication.

CONTENTS

LIST OF ABBREVIATIONS

AA	Amino acid
ABW	Adjusted body weight
AKI	Acute kidney injury
ALI	Acute lung injury
BMI	Body mass index
BW	Body weight
CRRT	Continuous renal replacement therapy
CV	Coefficient of variation
E_A	Arterial enrichment of tracer
EE	Energy expenditure
E_i	Enrichment of infusate
EN	Enteral nutrition
$FeCO_2$	Expired fraction of carbon dioxide
FeO_2	Expired fraction of oxygen
FFA	Free fatty acid
FiO_2	Fraction of inspired oxygen
HBE	Harris-Benedict equation
HME	Heat and moisture exchange
i	Infusion rate of tracer
I.V.	Intravenous
ICU	Intensive care unit
LoA	Limits of agreement
PE	Percentage error
Phe	Phenylalanine
PN	Parenteral nutrition
Ra	Rate of appearance
RCT	Randomized controlled trial
Rd	Rate of disappearance
REE	Resting energy expenditure
RQ	Respiratory quotient

SAPS	Simplified acute physiology score
SD	Standard deviation
SOFA	Sequential organ failure assessment
TEE	Total energy expenditure
TPN	Total parenteral nutrition
Tyr	Tyrosine
U_N	Urinary nitrogen
VCO_2	Carbon dioxide production
v_e	Expired volume
v_i	Inspired volume
VO_2	Oxygen consumption

1 INTRODUCTION

There is no universal definition of critical illness. The only common feature of patients admitted to an intensive care unit (ICU) is that they are considered too sick to safely reside in another part of the hospital, and that there is curative or palliative treatment to offer. Although the origins of intensive care medicine are often traced to centralization of simple respiratory support during the polio epidemics of the 1950s, the evolution of medical technology for monitoring and life support now permits patients to stay alive well beyond what was thought possible only a few generations ago [1]. Physiological changes during critical illness depend on a complex interplay of patient factors and medical interventions. It is difficult to determine what is optimal, or even normal, under these conditions. This holds especially true for the metabolic alterations in ICU patients.

Although much work has been done to develop our knowledge in this field, many issues remain to be explored. A central problem to monitoring metabolism in intensive care is that the clinical parameters of interest are often unavailable to conventional observation tools. This invariably results in a black box approach to patient care. The purpose of this thesis is to improve our understanding of alterations in energy and protein metabolism in the critically ill, both regarding how these changes can be monitored and the response to nutritional therapies. The studies included evaluate current techniques to monitor energy expenditure and investigations of substrate utilization in vivo. To put these research projects into context, the following section will briefly describe the biology of energy metabolism in humans, applications of indirect calorimetry in intensive care, and the major metabolic changes associated with critical illness.

2 BACKGROUND

2.1 ENERGY METABOLISM

2.1.1 Bioenergetics

Energy, roughly defined, is a quantitative property transferred between objects to perform work or generate heat. Entropy is a measurement of the dispersion of energy within a system, i.e. the number of states that the system can take on. In chemistry, energy is transferred by re-arranging bonds between atoms to more energetically favorable states, increasing entropy. The basis of biological life is to create and maintain a stable internal environment (=homeostasis) by generating mechanical energy and heat from chemical energy in compartmentalized reactions. Order is achieved by increasing disorder of the environment in exchange reactions, what Erwin Schrödinger described as "feeding on negative entropy" [2].

The energy requirements of humans are almost exclusively met from the oxidation of glucose, fat and amino acids by molecular oxygen. In order to effectively utilize the energy liberated in these reactions, they are catalyzed in several enzymatic steps through the TCA cycle, extracting energy through the formation of reducing agents (NADH and $FADH_2$) [3]. These generate an electrochemical gradient of protons over the mitochondrial inner membrane that powers the formation of ATP from ADP by a process called oxidative phosphorylation. ATP has several benefits as an intermediary for energy transfer: it is stable in an aqueous solution at physiologic pH but only requires water as a reactant in enzymatic hydrolysis to ADP + $[PO_4]^{3-}$ [4]. Hydrolysis of the phosphoanhydride bond is very energetically favorable, with a Gibbs free energy of -50-70 kJ/mol [5]. From this figure it is evident that ATP is not suitable for storage of energy, as human metabolism consumes around 75 kg (150 moles) of ATP/day. Whole-body ATP content is approximately 0.2 moles, giving an average molecule of ATP a turnover rate of ~750 times per day [6]. To safeguard a continuous energy supply on a cellular level for regeneration of ATP, humans have developed several complementary metabolic pathways to ensure constant availability of energy substrates for oxidative phosphorylation: mainly glucose, lipids, amino acids and ketones.

2.1.2 Energy requirements and methods for estimating substrate oxidation

The global energy requirements of humans are commonly expressed as

TEE = BEE + AEE + DEE

Where TEE: Total energy expenditure; BEE: Basal energy expenditure; AEE: Activity-induced energy expenditure; DEE: Diet-induced energy expenditure [7].

BEE represents the minimum demands of bodily maintenance functions: sustaining electrochemical gradients across cell membranes, nerve conduction, synthetic and regenerative functions, mechanical work of the cardiorespiratory system etc. [8]. As this is an idealized state, resting energy expenditure (REE) is often used to describe EE in a resting postabsorptive individual. Of note, REE in ICU patients generally refers to a resting state

during continuous feeding. As there is a strong correlation between REE and metabolically active cell mass [9], it can be estimated from characteristics such as age, sex and anthropometric variables [10].

The gold standard for measuring energy expenditure (EE) is by direct calorimetry. The first and second laws of thermodynamics state that the energy of a closed system is constant and that entropy will always increase over time. Accordingly, the transfer of chemical energy to mechanical work will eventually result in all potential and kinetic energy dissipating into heat (=maximal entropy). In direct calorimetry all heat radiating from a body is measured and quantified as EE in joules or calories per unit of time [11].

Due to the constraints of isolating a subject from the environment to accurately measure heat dissipation, alternative methods have to a large extent superseded direct calorimetry in research and clinical practice. The theoretical basis is to measure the end-products and reactants of metabolism, and then calculate the corresponding energy liberated by complete oxidation/combustion from stoichiometric equations. This can be accomplished by ingestion and elimination of doubly labeled water ($^2H_2{}^{18}O$) or by measuring O_2 consumption and CO_2 production from respiratory gas exchange [12]. As the doubly labeled water technique has limited utility in critically ill patients due to rapid changes in total body water and substrate utilization, only the second method, indirect calorimetry, will be discussed further within this thesis.

2.1.3 Indirect calorimetry: theoretical considerations

The basis of indirect calorimetry is measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2) from respiration. The energy liberated as heat and the volume of CO_2 released from consumption of one liter of O_2 during complete combustion of common energy substrates can be measured by burning in a bomb calorimeter. Standard values for glucose, fat and protein are provided in Table 1 [13].

Using these known values as constants, J.B. Weir derived a formula for calculating EE from gas exchange measurements in 1949 which is still widely used [14]:

$$EE = 3.941 * VO_2 + 1.106 * VCO_2 - 2.17 * U_N$$

Where EE: Heat output (kcal) per unit of time; VO_2: O_2 consumed (L); VCO_2: CO_2 produced (L); U_N: Urinary nitrogen (g). This formula is often expressed as EE = Kcal/24 hours, VO_2/VCO_2 in ml/min and U_N in g/24 hours:

$$EE = 5.68 * VO_2 + 1.59 * VCO_2 - 2.17\, U_N$$

This formula also requires quantification of urinary nitrogen (U_N) loss, although omitting this variable will only have a minor effect on the net result within physiological ranges of U_N. There is good agreement between EE measured by direct and indirect calorimetry in healthy subjects [15].

Table 1. Energy content of macronutrients.

	Energy equivalent of 1L* O_2 (kcal)	Energy from oxidation of 1 gram of substrate (kcal)	Respiratory quotient (VCO_2/VO_2)
Glucose	4.97	3.73	1.00
Fat	4.68	9.42	0.71
Protein (forming urea)	4.64	4.80	0.83

Adapted from Frayn K.N. Metabolic Regulation. A Human Perspective (Wiley-Blackwell publishing 2010). Different values can be found in other literature depending on the type of carbohydrate, fat or protein oxidized.

*Standard temperature pressure dry.

This derivation of EE from gas exchange measurements requires several assumptions regarding substrate utilization:

1. The contribution of anaerobic glycolysis to EE is quantitatively insignificant. This is valid under resting conditions, in the absence of regional hypoperfusion and provided that oxygen delivery is greater than VO_2.
2. The primary metabolic fate of reactants (energy substrates and O_2) is complete oxidation to CO_2 and water. This assumption is conditional, as several metabolic pathways (glycogenesis and lipogenesis) will sequester carbon atoms in the body without oxidizing them.
3. Alternative substrates (ketone bodies, alcohols) do not provide a significant contribution to EE.
4. The volatile carbon pool (HCO_3^- and CO_2) is in a steady state, i.e. VCO_2 reflects metabolic CO_2 production.
5. Urinary nitrogen excretion is constant over time and accurately reflects protein oxidation during gas exchange measurements.

Although all of these conditions are unlikely to be met in an ICU patient, the resulting error when applied during stable clinical circumstances is likely to be small. Intermediary steps not accounted for by the Weir equation (i.e. gluconeogenesis, glycogenesis or lipogenesis) will not affect the calculation of liberated energy over longer time frames as this depends on the net stoichiometry of all reactions, but may alter short-term appearance/disappearance of carbon and will influence net ATP generation from substrates [11, 16].

2.1.4 Indirect calorimetry in the ICU setting

The interest for indirect calorimetry in the intensive care setting grew during the 1980s, as is evident from publications around this time evaluating metabolic monitors in mechanically ventilated patients [17-20]. The Deltatrac Metabolic Monitor (Datex-Ohmeda, Helsinki, Finland) has been validated in vivo by Takala and Tissot [21, 22]. It uses a mixing chamber technology which circumvents the potential difficulties of matching spirometry curves to measured gas concentrations. Due to its satisfactory performance characteristics in validation studies and the robust design, it has been considered the most reliable device on the market [23].

After production of Deltatrac was discontinued, several manufacturers have been trying to replace it with a new generation of instruments. A common characteristic is the use of "breath-by-breath" technology. Gas is continuously sampled in the proximity of the endotracheal tube to analyze the concentrations of CO_2 and O_2 during inspiration and expiration. Gas flow is measured separately by a spirometer. The differences in gas concentrations over the respiratory cycle are matched to the corresponding breath and multiplied by flow to calculate VO_2 and VCO_2. As standard care has shifted towards lighter sedation and spontaneous breathing, the demands on software algorithms performing these calculations have increased. Potential benefits of breath-by-breath devices are the relative ease of performing measurements, the possibility for compact instrument design and rapid data acquisition. However, instruments marketed for use in the ICU require evaluation under conditions that resemble current clinical practice. For historical reasons the Deltatrac has been used as a reference device and clinical "gold standard". Results from validation studies comparing breath-by-breath devices to Deltatrac have been mixed. McLellan evaluated the M-COVX (Datex-Ohmeda, Helsinki, Finland) using sequential measurements [24]. They found a small mean difference (=bias) but wider 95% limits of agreement (LoA) for VO_2 and VCO_2 (±56 and ±36 ml/min respectively). In another study of the M-COVX using simultaneous measurements Singer and co-workers reported even greater divergence between individual measurements (LoA for VO_2 ±87 ml/min) [25]. Similar variations in LoA and bias have been observed in other validation studies comparing new instruments to Deltatrac during mechanical ventilation [26-28]. The variable agreement raises concerns about their application in clinical practice and research.

2.2 ALTERATIONS IN ENERGY METABOLISM DURING CRITICAL ILLNESS

In health, energy homeostasis is regulated by an integration of biological signals in the hypothalamus. These mechanisms efficiently match energy intake to expenditure by influencing hunger and satiety, level of physical activity and metabolic rate at the cellular level [29-31]. When intake exceeds metabolic demands, nutrients are stored as glycogen and triglycerides from the actions of insulin. During starvation endogenous energy stores are mobilized by gluconeogenesis and lipolysis from the actions of counterregulatory hormones such as glucagon, adrenaline, noradrenaline, cortisol and growth hormone (GH) [13]. Protein degradation will indirectly contribute to energy metabolism from amino acid oxidation, but

inevitably results in a loss of functional tissues as there is no repository for amino acids in the body.

Patients admitted to the ICU have often been exposed to some form of insult (tissue trauma, infection, ischemia-reperfusion etc.) which leads to inflammation. Inflammatory mediators in conjunction with the neurohormonal response to multiple physiological stressors such as pain, hypotension, hypovolemia and hypoxia trigger the metabolic alterations commonly described as "the metabolic stress response" [32]. This is characterized by:

- Upregulation of major catabolic pathways for glucose, lipids and protein.
- An increased turnover of energy substrates and protein.
- Altered utilization of macronutrients.
- A decreased sensitivity to the anabolic actions of insulin.
- Differential regional capacity for aerobic metabolism [33].
- Impaired behavioral signals for volitional nutrient intake [34, 35].

These changes were rudimentarily described almost a century ago by Sir David Cuthbertson as the "flow" response to traumatic injury [36]. Our current understanding of this phenomenon is mainly based on small physiological studies in animal models or patients of variable age, co-morbid conditions, acute pathologies and duration of hospitalization. In addition, these findings have been published over the course of several decades, and treatments that may attenuate the metabolic response (surgical methods, sedation, nutritional practices, physiotherapy etc.) will be very different depending on the setting in which a study was performed. Any general statements should therefore be viewed as abstractions with a high degree of uncertainty regarding the implications for individual patients in a modern ICU setting. With this caveat, the following sections will briefly summarize the alterations in energy and protein metabolism during critical illness.

2.2.1 Glucose metabolism

Hyperglycemia is common in ICU patients [37]. Plasma glucose may be raised either from increased endogenous production or impaired uptake. Both mechanisms contribute to the alterations seen during critical illness, resulting in a higher glucose turnover rate.

The main driver behind hyperglycemia in critical illness is an increase in gluconeogenesis [38, 39]. This is mediated by the actions of cytokines and counterregulatory hormones [40]. Plasma concentrations of cortisol, GH, catecholamines and glucagon are all increased during critical illness [41-44]. In contrast to healthy volunteers [45, 46], multiple observations indicate that the increased rate of gluconeogenesis in critical illness is only partially attenuated by parenteral glucose infusions [47-51]. It may be suppressed by the administration of high doses of exogenous insulin [49, 51-54].

Insulin-mediated glucose uptake, primarily in skeletal muscle, is impaired during critical illness [53-55]. In contrast, data from animal models of sepsis indicates that non-insulin mediated glucose uptake may be increased in macrophage-rich tissues [53, 55]. The mechanisms of insulin

resistance during critical illness are multifactorial and influenced both by endocrine signals and inflammatory mediators [56]. Plasma insulin levels are increased compared to healthy controls but the ratio of insulin/glucagon is reduced [41, 49, 54, 57].

Whole-body glucose oxidation may not be impaired [39, 48]. However, the higher turnover rate during critical illness necessitates an increase in non-oxidative glucose disposal. This may be facilitated by substrate cycling where three-carbon metabolites of glycolysis and gluconeogenic amino acids are exported from peripheral tissues and reconstituted to glucose by the liver [48, 58].

The net effect of these alterations is to increase the availability of glucose to tissues with insulin-independent uptake. This is potentially an adaptive response, to improve energy delivery toward organ functions vital to short-term survival [59, 60].

2.2.2 Lipid metabolism

Fat stores are an important source of energy during critical illness. Studies of free fatty acid (FFA) kinetics in sepsis, burns, trauma and experimental models of systemic inflammation consistently demonstrate an elevated rate of appearance of FFAs and glycerol, indicating an increased rate of lipolysis [58, 61-64]. This appears to be mediated by the actions of catecholamines and proinflammatory cytokines [65, 66]. Adipose tissue of septic patients is resistant to the actions of insulin [67].

The rate of endogenous FFA mobilization may exceed energy expenditure and likely provides the majority of energy requirements in a fasted state [64, 68]. Kinetic studies show diverging results regarding the potential of exogenous nutrients to suppress lipolysis: it may be attenuated or persist despite supplementation of calories [48, 61, 62, 69]. Body composition studies demonstrate a loss of adipose tissue over time in patients with energy provision lower than expenditure [70, 71]. De novo lipogenesis in critically ill patients may also be induced by high exogenous glucose loads [50].

The preferential oxidation of lipids or glucose appears to depend on the relative availability of exogenous substrates [50, 68, 72]. Studies using indirect calorimetry to calculate substrate oxidation rates have observed low respiratory quotient (RQ) values indicative of predominant lipid oxidation [73], but this is not a ubiquitous finding [74]. Mitochondrial alterations may also influence the utilization of lipids as a fuel source. Acquired carnitine deficiency can impair transport of FFAs across the mitochondrial membrane [75], and the expression of enzymes for beta oxidation are depressed in skeletal muscle of critically ill patients [76].

2.2.3 Energy expenditure during critical illness

There is poor agreement between predictive equations and measured REE in critically ill patients, indicating a greater inter-subject variability of metabolic rate compared to a healthy population [77-81]. It has long been held that critical illness is a hypermetabolic state, i.e. that the basal metabolic rate is higher than that predicted by simple population characteristics [82, 83].

Physiological factors such as fever, increased substrate cycling, and synthetic functions associated with the host response to stress and inflammation provide a theoretical basis for this generalization. Energy expenditure is also influenced by common ICU therapies [84, 85].

The average metabolic rate reported in studies of ICU patients is variable. A number of small studies measuring REE by indirect calorimetry in the context of sepsis, multi-organ failure, and trauma have observed a metabolic rate >30 kcal/kg/day [74, 86-90], or a more moderate increase between 25-30 kcal/kg/day [91-95]. Other studies found an average REE between 20-25 kcal/kg/day [96-99]. The largest observational study of REE in a modern ICU setting by Zusman and colleagues found a mean REE of 24 kcal/kg/day in a sample of 1440 patients [81]. Studies that report correlations between morbidity or mortality and energy expenditure indicate that sicker patients and non-survivors have a lower REE [86, 90, 93, 100], but this is not a universal observation [89]. Interpretations of these findings are limited by potential survivorship bias and confounders such as age and physical activity.

2.2.4 Energy balance during critical illness

In health, energy balance is when caloric intake equals total energy expenditure over time. As a critically ill patient may have significant endogenous substrate mobilization even during the provision of exogenous nutrients, it is difficult to determine when energy balance occurs in an ICU setting.

The importance of this concept during critical illness is a subject of ongoing contention. Several observational studies have reported positive associations between accumulated energy deficits and mortality, nosocomial infections and other adverse outcomes [101-104]. Only a small proportion of the centers in these studies calculated energy balance from indirect calorimetry. A recent single-center observational study of nearly 1200 mechanically ventilated patient identified a U-shaped relationship between % intake/measured REE and mortality, with the lowest odds ratio at 70% [100]. The authors interpret this as a signal of harm from both under- and overfeeding. Causal mechanisms for these observed relationships have not been clearly elucidated, although findings of some studies point toward the importance of protein intake and the attenuation of protein catabolism by non-protein calorie intake [105]. An inverse correlation between nutritional delivery and mortality could also be an epiphenomenon to illness severity. Gastrointestinal dysfunction is an independent predictor of poor outcome, and feeding intolerance may reflect the degree of multiorgan failure in ICU patients [106].

During the last decade, several large multicenter randomized controlled trials (RCTs) have been conducted to investigate the relationship between energy delivery and clinical outcomes in ICU patients. The inclusion criteria, therapeutic interventions, control groups and outcome measures of the largest trials to date are summarized in Table 2 [107-111]. The main differences are the underlying hypotheses (that an exogenous energy deficit is either harmful or beneficial) and the route of energy delivery (enteral or parenteral). Although there are potentially important physiological differences between enteral nutrition (EN) and parenteral

nutrition (PN) with regard to the endocrine response, nutrient uptake and gastrointestinal mucosal and immune function [112], two recent multicenter RCTs investigating early exclusive use of EN or PN in ICU patients did not find any differences in mortality, infectious complications or ICU length of stay [113, 114]. The results from the trials listed below may therefore be considered from a perspective of energy provision rather than route of delivery.

Table 2. Summary of study characteristics in five multi-center randomized controlled trials investigating energy delivery in critically ill patients.

	Patients (N)	Population	Intervention	Control	Primary outcome
Casaer et al [107]	4640	Adult ICU patients with NRS ≥3	Late initiation of PN (day 8) to meet energy target	Early initiation of PN (day 3) to meet energy target	Time to ICU discharge
Rice et al [108]	1000	Adult ICU patients with ALI & mechanical ventilation	Trophic EN day 1-6	EN to meet energy target from day 1	Ventilator-free days to day 28
Doig et al [109]	1372	Adult ICU patients with short term contraindication to EN	PN to meet energy target after inclusion	Standard care	Death by day 60
Arabi et al [110]	894	ICU patients with enteral feeding <48 hours from admission	Permissive underfeeding (40-60% of energy target with protein supplementation)	Standard care (70-100% of energy target)	90 day all-cause mortality
Chapman et al [111]	3957	Adult mechanically ventilated ICU patients initiating EN	Energy-dense (1.5 kcal/ml) EN formula with volume target	Standard (1 kcal/ml) EN formula with volume target	90 day all-cause mortality

ALI: Acute lung injury; EN: Enteral nutrition; ICU: Intensive care unit; NRS: Nutrition risk score; PN: Parenteral nutrition.

None of the trials found a benefit in mortality or major morbidity to any of these strategies. The only trial that detected harmful effects (a higher rate of infections and longer ICU stay) from early increased energy delivery used supplemental parenteral nutrition with high glucose content in combination with tight glycemic control with insulin, mainly in elective cardiothoracic surgical patients [107]. The trials that included a long-term follow-up did not identify any difference in physical function between intervention and control groups [115, 116].

Only a few clinical trials have investigated the utility of matching intake to measured energy expenditure during critical illness, with mixed findings [117-119]. Heidegger and colleagues reported a lower rate of hospital-acquired infections in patients receiving supplemental parenteral nutrition to reach 100% of their energy target from day four in ICU [118]. Of note, this result did not remain statistically significant when the event rate during the entire study period was analyzed, and only 65% of patients had nutritional targets set by indirect calorimetry. Singer and co-workers randomized 130 patients to a tight-calorie control group using daily REE measurements or standard care. The intervention group received significantly more daily calories, primarily as parenteral nutrition. There was no difference in the primary outcome of hospital mortality using intention-to-treat analysis. The recently published EAT-ICU trial also assessed individualized nutrition in mechanically ventilated ICU patients, using daily indirect calorimetry and nitrogen balance to guide therapy in the intervention group. 203 patients were recruited with a sample size determined from the least relevant change in the primary outcome of physical quality of life six months after randomization. Despite achieving significant separation in energy and protein balance between groups, no differences in primary or secondary outcome measures were observed.

In summary, there is insufficient evidence to support a clear signal of benefit or harm over a broad range of energy provision in a general population of critically ill patients.

2.3 PROTEIN METABOLISM

2.3.1 Muscle protein regulation in health

Proteins are the macromolecules performing mechanical work in cells and serve a multitude of other vital functions [6]. The synthesis of proteins from amino acids by transcription of DNA is a fundamental step in the regulation of cellular function. As all proteins have other primary functions than as a fuel source for oxidative metabolism, there are no deposits of stored amino acids in the human body comparable to glycogen and adipose tissue for glucose and fat. To ensure availability of amino acids for synthetic functions in all organs, these are in a constant state of turnover from breakdown and synthesis of proteins. The two main pathways for protein degradation are the ubiquitin-proteasome system and autophagy [120]. These also protect against the accumulation of dysfunctional or damaged proteins which threaten cell function [121]. Most of the recycling occurs within cells, but amino acids are also exported to the bloodstream. After uptake by other organs they have three possible metabolic fates: integration into new proteins, modifications of functional groups into other amino acids, or catabolism. As dietary amino acids contribute to the whole-body pool a net oxidation rate

roughly equal to intake (~1 g/kg/day) is necessary for homeostasis. The turnover rate of body protein is approximately fourfold that of daily nutritional requirements [13].

Skeletal muscle contains 50-75% of total body protein [122]. It is therefore a major potential source of free amino acids. Skeletal muscle protein balance is regulated by a complex interplay of anabolic and catabolic signals (insulin, IGF-1, cortisol), inflammatory mediators, amino acid availability, and loading conditions. These factors interact through intracellular second messengers regulating gene transcription and proteolysis. The protein complex mammalian target of rapamycin complex-1 (mTORC-1) is the main site of integration for anabolic signals and sensing of nutritional status [123]. Bed rest and caloric restriction have been demonstrated to induce a negative protein balance in healthy volunteers, while resistance training, increased protein intake, branched-chain amino acids and insulin can improve protein balance and promote skeletal muscle anabolism [124-128].

2.3.2 The implications of critical illness on muscle structure and function

The loss of lean body mass that accompanies severe acute illness has been appreciated for over a century. As improvements in health care result in higher rates of survival after critical illness, the burden of long-term functional disability and muscle weakness in survivors has generated increasing interest [129]. ICU-acquired weakness is common and correlates with severity of illness. The cause is multifactorial; impaired nerve conduction and injury, mitochondrial dysfunction, inflammatory changes in muscle architecture, altered membrane ion conduction and increased proteolysis have all been implicated as contributing factors [33, 130].

Studies investigating whole-body protein kinetics in various states of critical illness have observed an increased rate of synthesis coupled with a larger increase in breakdown, resulting in a net negative balance [131-133]. These changes are heterogeneously distributed between different organ systems. Synthesis appears to be upregulated in the splanchnic, hepatic, reticuloendothelial and innate immune system [134]. Skeletal muscle catabolism provides the main source of amino acids for the increased demands of the acute phase response. Biopsies from skeletal muscle in sepsis show normal synthetic function, increased breakdown and reduced protein content [135-138]. This is consistent with lower limb arteriovenous balance studies in sepsis and burns where leg muscle amino acid efflux is markedly increased [137, 139]. Radiological body composition studies during critical illness also reveals a loss of around 0.5-1% of skeletal muscle mass per day [70, 71, 140]. The loss of muscle protein appears to be most pronounced in the early phase of critical illness [138]. Dos Santos and colleagues recently conducted a long-term follow up of these alterations in 11 patients requiring mechanical ventilation for >7 days. Loss of muscle mass persisted at six months in the majority after hospital discharge, but with normalization of proteolytic activity and mitochondrial content [141].

Although there are many potential factors confounding the association between muscle loss and functional status, it has been hypothesized that interventions to ameliorate catabolism

may reduce physical disability and improve quality of life in ICU survivors [142]. In healthy subjects there are three main ways to stimulate skeletal muscle anabolism: hormonal therapy, exercise, and nutrition. GH can reduce muscle wasting in ICU patients but the interest for anabolic hormones has been tempered by trials demonstrating excess mortality from the administration of GH during critical illness [143, 144]. Promoting muscle use by physical therapy or passive electrical stimulation may also alter the proteolytic response [145]. The following section will review the effects of nutritional interventions, in particular protein supplementation, on protein catabolism and muscle loss.

2.3.3 The effects of calorie and protein supplementation on protein catabolism in critical illness

The physiological effects of protein supplementation in ICU patients have mainly been investigated on a whole-body level. The most widely used technique to assess changes in body protein content is nitrogen balance, which is performed by measuring nitrogen losses in relation to intake. Several studies published up to 2012 investigating different levels of protein intake are summarized in a review by Hoffer and Bistrian, indicating that increasing daily protein intake up to 2-2.5 g/kg/day improves nitrogen balance [146]. Despite its widespread application in critical care research the nitrogen balance technique has several limitations in this setting. Accurate quantification of nitrogen losses is challenging, and the long-term steady state conditions required for this method to be valid are often violated [147, 148]. Whole-body protein kinetics can also be assessed by isotope tracer techniques that require a shorter period of stable measurement conditions (discussed further in Chapter 5). Tracer investigations in critically ill patients have also demonstrated an improvement in whole-body protein balance from increased provision of exogenous calories and/or protein [149-151].

However, changes in whole-body protein balance are not necessarily reflected in preservation of skeletal muscle mass. A recent systematic review of the association between nutrition and muscle loss during critical illness highlighted that existing studies are small, include a heterogenous group of patients, use different methods to assess muscle loss and have large variations in macronutrient dose delivery [152]. One observational study of ICU patients found a positive correlation between protein intake and muscle loss estimated by change in rectus femoris cross-sectional area over time [138]. This was a post-hoc finding and susceptible to the multiple comparisons problem. Muscle biopsies from a study nested within a large RCT of early vs late parenteral nutrition did not show a correlation between myofiber density and macronutrient deficit [153]. One pilot RCT of increased protein and energy delivery to ICU patients found a statistically significant difference in quadriceps muscle layer thickness from higher protein (1.2 vs 0.75 g/kg/day) and energy (21 vs 18 kcal/kg/day) intake [154]. This did not translate to improvements in functional outcomes. Ferrie and colleagues found similar improvements in muscle mass in patients receiving 1.1 vs 0.9 g/kg/day of protein during ICU stay but no difference in handgrip strength [155]. Other small-intermediate size RCTs investigating the effects of increased protein and energy intake on functional disability have not found any benefit to higher macronutrient delivery [119, 156]. This is consistent with long-

term follow-ups of functional outcomes in patients from large multi-center RCTs investigating higher vs lower energy provision, where mortality and morbidity were the primary outcome measures [115, 116].

2.3.4 Protein supplementation and ICU outcomes

Several observational studies have found an inverse correlation between protein intake during ICU stay and mortality [100, 102, 157, 158]. Low skeletal muscle mass on admission is also associated with an increased risk of death in ICU [159]. A possible interpretation of these findings is that a reduced availability of amino acids may impair recovery. Despite a physiological rationale for exogenous protein delivery during critical illness, there is limited evidence to support a causal relationship with mortality and organ dysfunction in the ICU. Recent iterations of nutritional guidelines recommend protein dosing between 1.2-2 g/kg/day based on very low quality of evidence [160, 161]. Currently there are no published clinical trials of higher vs lower protein targets in ICU patients adequately powered for mortality. Doig and co-workers have conducted the largest RCT of amino acid supplementation in ICU patients to date [162]. In 474 patients randomized to amino acid supplementation up to 100 grams/day or standard care, the investigators did not find any difference in duration of renal dysfunction or 90-day mortality. A post-hoc analysis of the trial identified a lower risk of death associated with amino acid supplementation in patients with normal renal function at baseline, but this result should only be interpreted as hypothesis-generating [163]. Other clinical trials of protein supplementation previously referenced have not detected any differences in organ dysfunction or death as found in observational studies. It has also been postulated that an increased systemic availability of amino acids may adversely affect outcomes through inhibition of autophagy [164]. While this hypothesis is supported by some preclinical observations and surrogate outcome measures, the relevance of these findings is still unclear.

2.4 TIME COURSE OF METABOLIC ALTERATIONS AND PATIENT HETEROGENEITY

The "ebb" and "flow" responses to injury described by Cuthbertson provide a conceptual framework for changes in metabolic derangements over time. The most recent iteration of the European clinical practice guidelines for ICU nutrition have adopted this terminology but divide the "flow" phase into an acute and post-acute period [160]. The post-acute phase is assumed to either resolve in clinical improvement or a state of persistent catabolism (Fig. 1). Beyond avoiding early overfeeding, the authors make no specific recommendations regarding nutritional support based on the trajectories described.

However, changes in metabolism over time could hypothetically have implications for nutritional and metabolic therapies. The physiological alterations during the prolonged post-acute phase is an evolving area of research and remain to be fully characterized. A recent consensus statement from an expert group on ICU nutrition proposes that stratification by clinical "phenotypes" or metabolic markers may provide a basis for individualized care in the future [165]. This section will briefly explore our current knowledge in this expanding field.
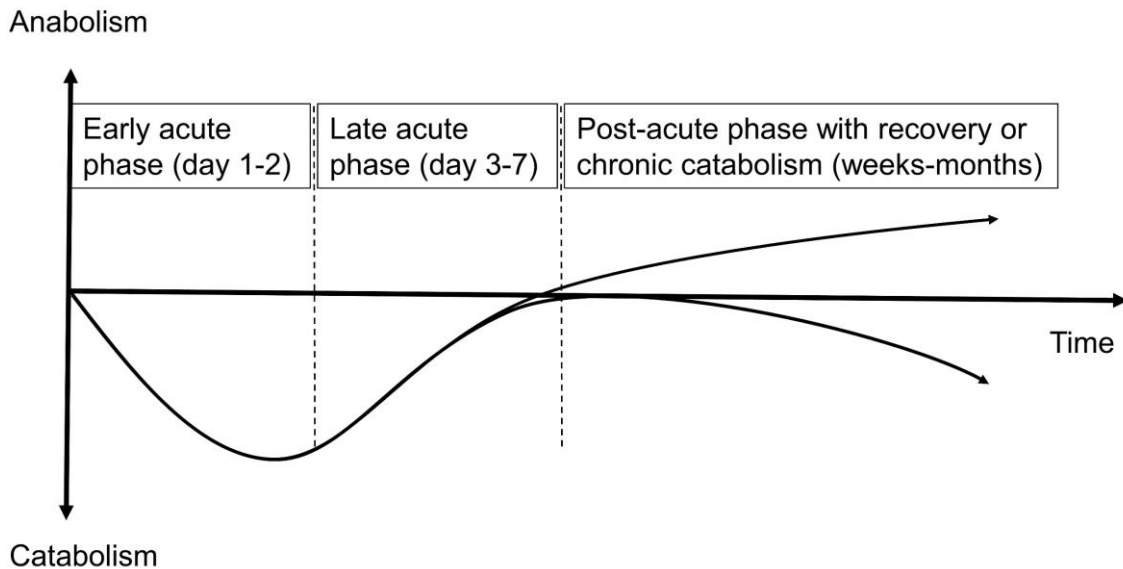
Figure 1. A conceptual model of temporal changes in the metabolic response to critical illness.
Adapted from ESPEN guideline on clinical nutrition in the intensive care unit [160].

Recent years have seen an increasing focus on describing characteristics of patients who fail to recover from critical illness. This group carries a disproportionate burden in terms of morbidity, mortality and ICU resource allocation [166]. Several definitions or subtypes of "chronic critical illness" have been proposed based on duration of organ support [167], performance of outcome prediction models over time [166, 168], or concurrent features of chronic inflammation, adaptive immune dysfunction and muscle catabolism (Persistent Inflammation Immunosuppression Catabolism Syndrome or PIICS) [169]. Trends in C-Reactive Protein and Urea/Creatinine ratio have been investigated as markers to identify patients with persisting catabolism and muscle loss [170, 171].

Of interest, a recent observational study of muscle protein kinetics in 20 ICU patients found that muscle protein balance normalized over time from an increase in protein synthesis after 30-40 days of ICU stay [172]. This result stands in contrast to notions of persistent catabolism in patients with a prolonged course of critical illness. Another investigation of muscle mass assessed by rectus femoris ultrasound found a progressive loss of skeletal muscle during the first 14 days in 17 ICU patients [173]. Indices of catabolism (urinary nitrogen loss and 3-methylhistidine) remained elevated despite reductions in CRP. The rate of change in muscle depth appears to decrease over time. The small number of patients and heterogeneity in these studies illustrates the challenge to delineate general characteristics or "phenotypes" of patients with prolonged ICU stay.

2.5 SUMMARY

Catabolism and muscle wasting are well-recognized problems in ICU patients. Protein supplementation and avoiding long-term energy deficits may ameliorate these detrimental effects. Clinical trials have thus far failed to demonstrate improved outcomes from standard or individualized levels of macronutrient intake in a general ICU population, raising the question if strict energy or protein balance is relevant to recovery. An optimal strategy for

nutritional support over a prolonged course of ICU stay and convalescence remains to be defined. Physiological investigations are important in developing hypotheses for future intervention studies.

A better understanding of macronutrient utilization during critical illness may be obtained from available techniques in metabolic research. Analysis of substrate kinetics using stable isotope tracers provides additional information about the physiological impact of nutritional interventions. Changes in energy metabolism over time can be assessed using indirect calorimetry, but the technical challenges of accurate measurements in an ICU setting require validations of new equipment before use in research or clinical practice.

3 AIMS

The overall aims of this thesis were to investigate the measurement properties of instruments used to determine energy metabolism in ICU patients, and to better characterize the utilization of exogenous macronutrients during critical illness. The aims of individual studies are listed below:

- To quantify agreement in gas exchange measurements by indirect calorimetry in mechanically ventilated ICU patients between commercially available instruments and a reference device (studies I and II).
- To assess if a supplemental infusion of intravenous amino acids sustains an improvement in whole-body protein balance in critically ill patients over 24 hours (study III).
- To investigate if the provision of full-dose compared to half-dose enteral nutrition, using energy targets determined by indirect calorimetry, results in an improved whole-body protein balance in critically ill patients with established enteral feeding (study IV).
- To monitor the effects of a higher provision of amino acids or protein on amino acid oxidation, plasma amino acid concentrations and serum urea levels in critically ill patients (studies III and IV).

4 STUDIES OVERVIEW

4.1 DESIGN AND EXPERIMENTAL PROTOCOLS

In study I, we compared measurements of resting energy expenditure in mechanically ventilated ICU patients between Deltatrac metabolic monitor (Datex-Ohmeda, Helsinki, Finland) and two other instruments: CCM Express (Medgraphics Corp, St Paul, Minneapolis, USA) and Quark RMR (Cosmed, Rome, Italy). The protocol was adapted from a previous study of indirect calorimetry in spontaneously breathing healthy subjects [174]. Measurements were performed in sequence with all three instruments during a 10-20 minute period under resting conditions with no changes in the rate of nutrition provided. The order was determined by simple randomization from drawing sealed notes. At the end of the sequence a fourth measurement was performed with the first device used. The average values from measurements 1 and 4 was used for results to compensate for changes in metabolic rate and clinical conditions over time.

In study II, gas exchange measurements (VO_2 and VCO_2) were performed with three different instruments in mechanically ventilated ICU patients. Comparisons of agreement were made between a reference method (Deltatrac II metabolic monitor) and two commercially available devices designed for use in an ICU setting: Quark RMR and E-sCOVX (GE Healthcare, Helsinki, Finland). All instruments were connected to the ventilator circuit and measurements performed simultaneously with Deltatrac and one other study device at a time for 20 minutes after a mandatory stabilization period. The order of comparisons was determined by simple randomization.

In study III, the effects of a 24-hour supplemental amino acid infusion (Glavamin, Fresenius-Kabi) on whole-body protein balance was investigated in critically ill patients. A previous study by our research group found that a 3-hour amino acid infusion improved whole-body protein balance in critically ill patients, without increasing amino acid oxidation [149]. The goal of the present study was to investigate if this response could be sustained for a longer period. The primary outcome was change in whole-body protein balance over time, assessed with a stable isotope tracer method. Secondary outcomes measures were changes in plasma amino acid concentrations, amino acid oxidation rate and serum urea concentration.

In study IV, the effect of full-dose versus half-dose EN on whole-body protein balance was investigated in critically ill patients with established enteral feeding and calorie delivery close to measured energy expenditure. Patients served as their own controls in a cross-over design and were randomized to an initial allocation of 50 or 100% rate of ongoing EN for 24 hours. At the end of this period whole-body protein balance was assessed using a stable isotope tracer protocol similar to that in study III. The rate of EN was then changed to the alternate treatment allocation, and a new measurement of protein kinetics performed at the end of the second 24-hour period. Secondary outcome measures included plasma amino acid profile, indices of increased amino acid oxidation as in study III and splanchnic extraction of phenylalanine.

Additional details on the experimental protocols can be found in the published papers and manuscript.

Connections of indirect calorimeters to the ventilator circuit in <u>studies I and II</u> are described in <u>Fig. 2 and 3</u>. Schematic illustrations of the protocols for nutrition and stable isotope tracers in <u>studies III and IV</u> are depicted in <u>Fig. 4 and 5</u>.
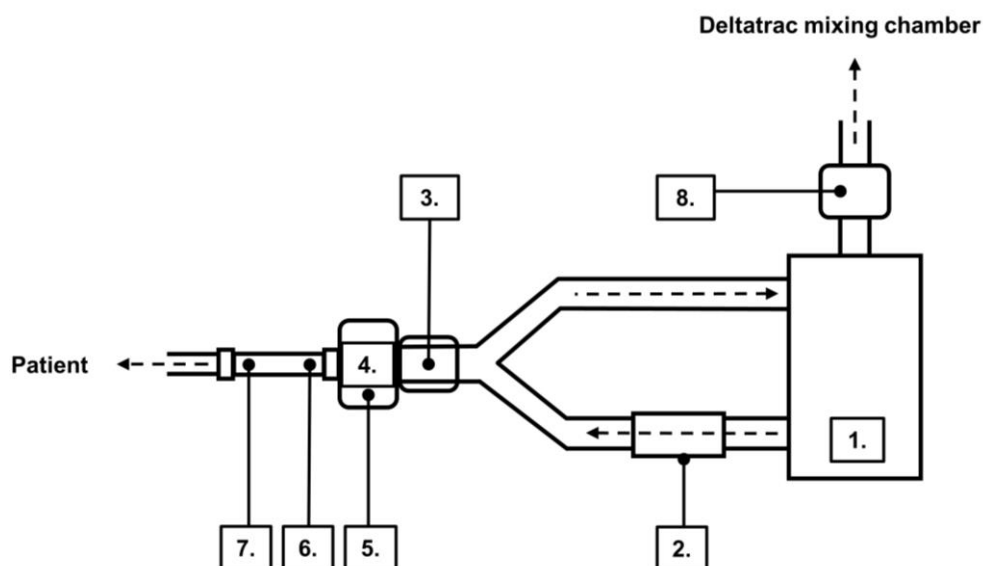

Figure 2. Connection points of Quark RMR, CCM Express and Deltatrac to ventilator circuit in study I. Only one instrument connected at a time during the study protocol. 1. Dräger Evita XL ventilator; 2. Fraction of inspired O_2 sampling to Deltatrac; 3. Mainstream capnography; 4. Heat and moisture exchange filter; 5. Quark RMR gas sampling line; 6. CCM Express pneumotach flowmeter; 7. CCM Express gas sampling line; 8. Quark RMR turbine flowmeter.
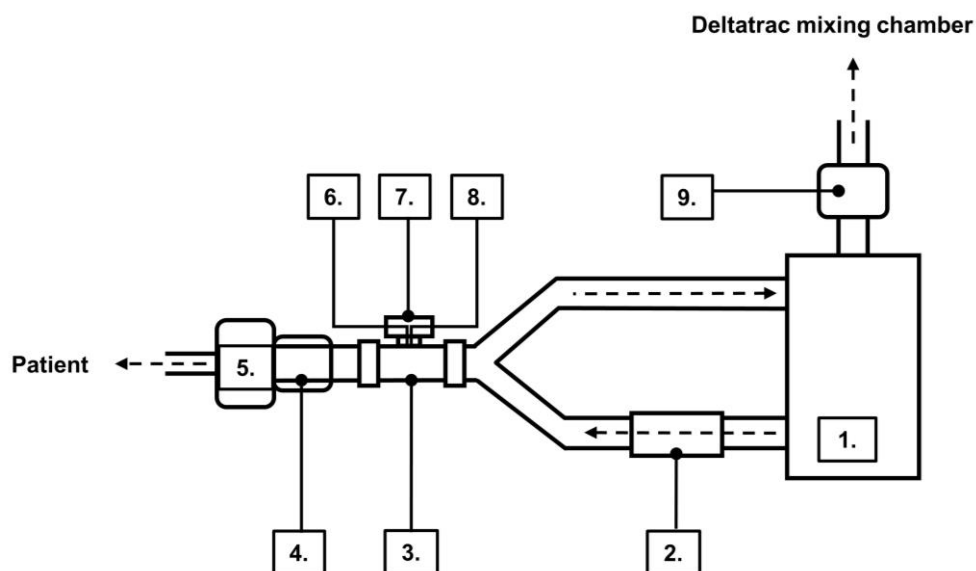

Figure 3. Connection of Quark RMR, E-sCOVX and Deltatrac II to ventilator circuit in study II. All instruments connected simultaneously during the study protocol. 1. Dräger Evita XL ventilator; 2. Fraction of inspired O_2 sampling to Deltatrac; 3. E-sCOVX pneumotach flowmeter; 4. Mainstream capnography; 5. Heat and moisture exchange filter; 6. E-sCOVX gas sampling line; 7. Three-way stopcock; 8. Quark RMR gas sampling line; 9. Quark RMR turbine flowmeter.
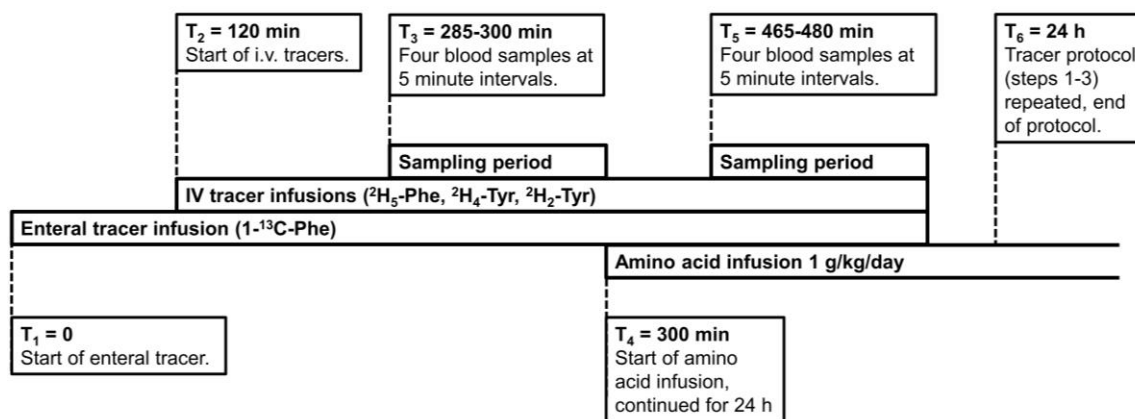
Figure 4. Study protocol of study III (not to scale). IV: intravenous; Phe: Phenylalanine; T: Time; Tyr: Tyrosine
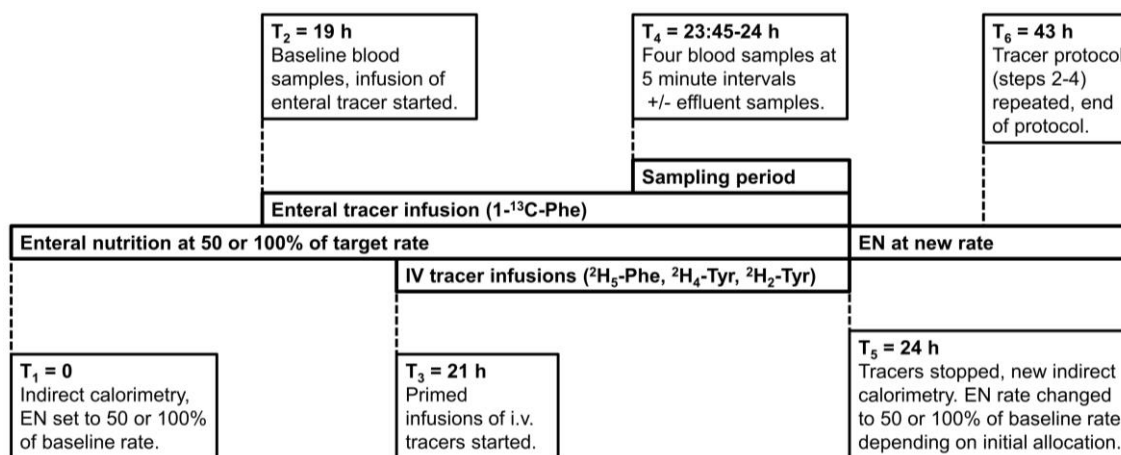

Figure 5. Study protocol of study IV (not to scale). EN: Enteral nutrition; IV: intravenous; Phe: Phenylalanine; T: Time; Tyr: Tyrosine.

4.2 SUBJECTS AND SETTING

All studies were performed in the ICU of Karolinska University Hospital Huddinge, a tertiary referral hospital in the greater Stockholm area. The hospital has a large emergency department and is a regional center of excellence for major upper abdominal surgery, abdominal solid organ transplantation and hematopoietic stem cell transplantation. The ICU is a mixed surgical-medical unit with a 12-bed capacity and a mean 30-day crude mortality of 20% [175]. The unit occasionally admits pediatric patients but is not a dedicated pediatric ICU. Extracorporeal membrane oxygenation, neurosurgery and cardiothoracic surgery are not available on-site.

In studies I and II, adult (≥18 years of age) ICU patients with invasive mechanical ventilation and a constant rate of parenteral and/or enteral nutrition were eligible for inclusion. Exclusion criteria were i) anatomical air leaks (bronchopleural fistulas, pneumothorax etc.) or gas leaks in the ventilator circuit of >10% of minute volume, ii) FiO_2 of ≤0.5 (study I) or ≤0.6 (study II), iii) a respiratory rate of >35 (study II) and iv) lack of informed consent.

In <u>study III</u>, adult ICU patients admitted to the unit during the recruitment period were screened for inclusion. The rate of enteral or parenteral nutrition therapy was not to be altered during the study period. Exclusion criteria were i) renal replacement therapy, ii) ongoing resuscitation for circulatory instability, iii) patient unlikely to complete protocol due to planned investigation/procedures/transfers etc., iv) patient in hospital for >2 weeks prior to screening for inclusion, v) lack of arterial line for blood sampling and vi) no informed consent.

In <u>study IV</u>, adult ICU patients with invasive mechanical ventilation ($FiO_2 \leq 0.6$) and established enteral feeding (enteral calories ≥80% of energy target as determined by indirect calorimetry) could be included. Exclusion criteria were i) if extubation, withdrawal of life support, interruptions in enteral feeding or patient transfer was expected during the study period, ii) lack of central venous or arterial catheters for blood sampling, iii) volume resuscitation with crystalloid or blood products during the measurement periods and iv) lack of informed consent.

The characteristics of patients included in <u>Studies I-IV</u> are listed in <u>Table 3</u>.

Table 3. Patient characteristics studies I-IV.

	Study I	Study II	Study III	Study IV
Number of patients (N)	24	22	12	6
Sex (male/female)	15/9	17/5	8/4	5/1
Age	61 (36-79)	61 (18-83)	65 (47 – 74)	58 (28 – 73)
BMI (kg/m²)	25.5 (18.3-43.1)	25.3 (16.5-51.5)	27.9 (22 – 38.9)	27.1 (19.5 – 40.7)
SAPS III	61(43-77)	67 (38-100)	52 (34 – 68)	83 (45 – 108)

Values are given as median (range). BMI: Body mass index; SAPS: Simplified acute physiology score.

4.3   ETHICAL CONSIDERATIONS

All studies were approved by the regional ethics committee (Regionala etikprövningsnämnden i Stockholm) and conducted in accordance with the principles stated in the World Medical Association Declaration of Helsinki [176]. Critically ill patients are in many ways a "vulnerable group": they experience the psychological burden of being in a life-threatening situation and often suffer from cognitive impairments both from acute illness and sedative drugs. Verbal communication may also be impaired by mechanical ventilation. All

these factors compromise their ability to make informed decisions and communicate their intentions about health care choices or participation in clinical research. Performing clinical studies in ICU patients requires a balance between patient autonomy, the risks of participation and the potential benefits that may be gained for the individual patient or vulnerable group as a whole. In all four studies informed consent was primarily sought from the patient, and in the case where the patient was not able to provide consent, the nearest relative. If consent was provided by proxy, direct consent was sought as soon as possible. Patients or their relatives were informed both orally and in writing about the study aims, procedures, potential risks and benefits, funding sources and that consent could be withdrawn at any time.

In studies I and II there were no potential benefits for the individual patient from participation. For ICU patients as a group, the clinical validation of measurement instruments is important as the results from these devices will influence treatment decisions in routine care. Although there are no demonstrated benefits to measuring energy expenditure during critical illness, this practice is recommended by the major professional societies for ICU nutrition in North America and Europe [160, 161]. Indirect calorimetry was also routine clinical practice at the study site. The risks to patient were considered low, as measurements are non-invasive and only require connection of instruments to the ventilator circuit. Patients dependent on high mean airway pressures to avoid alveolar collapse could potentially be harmed by temporary disconnection from the ventilator, but as high FiO_2 was an exclusion criterion this generally precluded participation of patients with severe acute respiratory distress syndrome.

For study I, the Quark RMR and CCM Express devices were provided by the manufacturers for the duration of the study period. In study II, GE Healthcare provided an E-sCOVX unit for study purposes. The Quark RMR device in study II was owned by the study site. A data sharing agreement was drafted with GE Healthcare and a non-disclosure agreement signed by the primary author and study sponsor regarding proprietary technical details of the instrument. The commercial interests involved provided no financial support or remuneration for the studies and did not have any mandate over study design, data analysis, drafting of the manuscripts or the decision to submit for publication.

In studies III and IV patients were exposed to clinical interventions that deviate from routine care at the study site: a 24-hour period of intravenous amino acids and low-dose ("hypocaloric") EN, respectively. Neither of these interventions have any proven positive or negative effects on patient-centered outcomes [110, 162]. Both the higher amino acid dose from a supplemental infusion and hypocaloric EN fall within limits of what is considered standard practice in other regions [161]. The stable isotope tracers infused for measurements of protein kinetics are not associated with any known health risks. Both study protocols require additional blood samples, but these were drawn from catheters already sited for clinical purposes. The total volume of blood drawn for study purposes was 40 (study III) and 60 ml (study IV).

5 METHODS

5.1 INDIRECT CALORIMETRY

5.1.1 Indirect calorimetry in mechanically ventilated patients

Performing indirect calorimetry in mechanically ventilated ICU patients is complicated by the common occurrence of high airway pressures, humidity, circuit leaks, high or variable respiratory rates and supplemental oxygen. Certain restrictions imposed on the conditions of measurements are essential to maintain accuracy, mainly a fraction of inspired oxygen (FiO_2) ≤ 0.6. As determining the difference in oxygen content between inspired and expired gas requires a highly accurate measurement of bidirectional flow, the so-called Haldane transformation is commonly applied. Assuming that there is no exchange of N_2 in the lungs and that the CO_2 content of ambient air is negligible, then:

$$(1 - FiO_2) * v_i = (1 - FeO_2 - FeCO_2) * v_e$$

Where v_i: inspired volume; v_e: expired volume; FeO_2: expired fraction of O_2; $FeCO_2$: expired fraction of CO_2. As:

$$VO_2 = FiO_2 * v_i - FeO_2 * v_e$$

V_i from the first equation can be substituted into the second equation and rearranged further:

$$VO_2 = \frac{((FiO_2 - FeO_2) - (FiO_2 * FeCO_2))}{(1 - FiO_2)} * v_e$$

This equation only requires measurement of expiratory volumes, but as FiO_2 is in the denominator it behaves non-linearly at higher fractions. A similar equation can also be derived for VCO_2.

5.1.2 Deltatrac metabolic monitor

The Deltatrac metabolic monitor measures respiratory gas exchange with a mixing chamber design. It can be used both for non-intubated patients with a canopy and during invasive mechanical ventilation. O_2 and CO_2 concentrations are measured with a differential paramagnetic and infrared sensor respectively. It does not measure flow of inhaled/exhaled gas directly, instead deriving this from other parameters. The principles for deriving VO_2 and VCO_2 are illustrated in Fig. 6. FiO_2 is sampled in the inspiratory limb of the ventilator, and all expiratory gas from the patient is channeled into a 4-liter mixing chamber where FeO_2 and $FeCO_2$ are measured. Gas samples pass through specialized tubing where the partial pressure of water vapor equilibrates to that in ambient air. The gas in the mixing chamber is drawn into a separate chamber by the Venturi effect and diluted by a constant flow of room air (Q), usually 40 L/min in the adult configuration. The concentration of CO_2 in the diluted gas

mixture (FCO_2) is measured downstream. VCO_2 is calculated from the product of FCO_2 and Q. RQ, the quotient of VCO_2 and VO_2, can be calculated from gas fractions alone using the Haldane transformation. VO_2 is then derived from VCO_2 and RQ.

A benefit of this design is that flow measurements and the synchronization of gas concentrations and volumes of each breath are not required. As gas concentrations in the mixing chamber are averaged every 30-60 seconds, the effects of temporary irregular breathing patterns like coughing are dampened.

In study I, the original Deltatrac metabolic monitor was used. For study II, a Deltatrac II unit was used due to technical difficulties with the first device. The measurement principles for these devices are identical but the latter version could export output data in digital format.
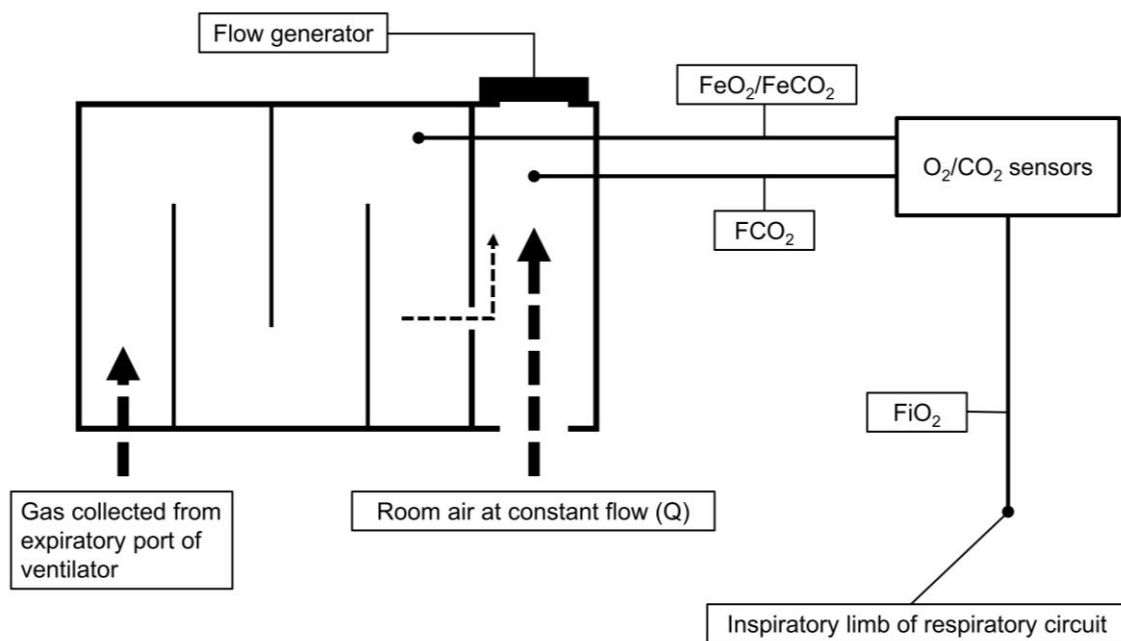

Figure 6. A schematic illustration of the Deltatrac I/II principles of measurement. FCO_2: Fraction of CO_2 in expired gas diluted by constant flow generator; $FeCO_2$: Fraction of expired CO_2; FeO_2: Fraction of expired O_2; FiO_2: Fraction of inspired O_2.

5.1.3 Breath-by-breath instruments

Most modern instruments for indirect calorimetry use so-called "breath-by-breath" technology. O_2/CO_2 concentrations are measured continuously over the respiratory cycle close to the patient-ventilator connection, and gas flow is measured by some form of spirometry. As there is a variable delay from transit time in the sampling lines and distortion of gas concentration waveforms due to fluctuations in airway pressure, the signal has to be processed by software algorithms and synchronized to the corresponding flow measurement of the breath it was sampled from (Fig. 7). VO_2 and VCO_2 then calculated for each breath by integration of the flow and gas signal using the Haldane transformation.
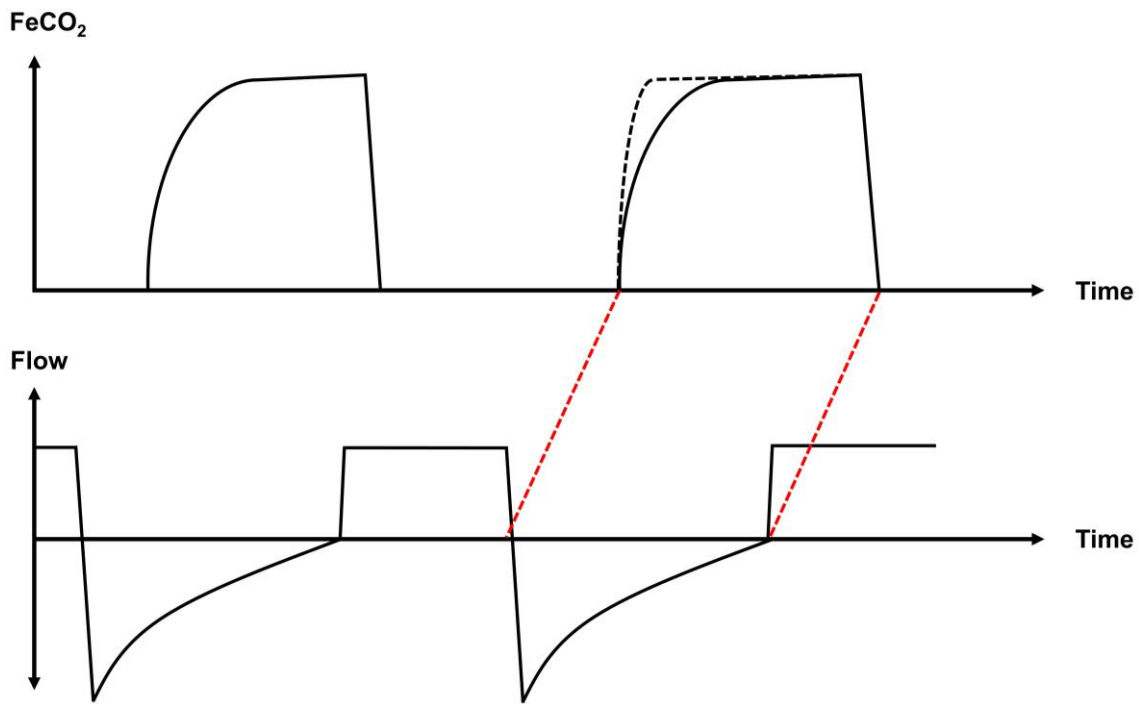
Figure 7. Synchronization of gas sampling and volumetric measurements in breath-by-breath indirect calorimetry. The gas waveform is matched to its corresponding flow measurement, then reconstructed to compensate for variations in sample flow and rise time of sensors. FeCO$_2$: Fraction of expired CO$_2$. Adapted from Figure 3 in Takala, GE Gas Exchange Appliguide, DOC1305573.

5.1.3.1   Quark RMR

Quark RMR uses a paramagnetic O$_2$ sensor and infrared CO$_2$ sensor. Gas is sampled from an adapter proximal to the ventilator Y-piece or by connecting the sampling line to the heat and moisture exchange (HME) filter. Flow is measured at the expiratory port of the ventilator by a turbine flowmeter. The instrument uses software compensation to discount the effect of any bias flow in the ventilator circuit which must be set manually by the operator.

5.1.3.2   CCM Express

CCM Express is a breath-by-breath instrument for indirect calorimetry. Both flow and gas concentration signals are transduced from an adapter attached to the patient's artificial airway. Gas flow is measured by a Pitot-tube pneumotachograph. O$_2$ and CO$_2$ concentrations are measured by a galvanic fuel cell and infrared sensor respectively.

5.1.3.3   E-sCOVX

E-sCOVX also measures gas exchange on a breath-by-breath basis. The instrument is connected to the ventilator circuit between the HME filter and Y-piece of the ventilator tubing. The adapter includes gas sampling ports and a pneumotachograph which measures flow from the pressure drop across a turbulent flow resistor. It contains a paramagnetic O$_2$ sensor and an infrared CO$_2$ sensor.

5.1.4 Calibration

In measurement technology, accuracy represents the conformity of a measured quantity to the actual (true) value. Precision is the ability of an instrument to reproduce that quantity at an identical input. As sensors frequently have non-linear properties and may experience decay over time, maintaining accuracy and precision requires frequent calibration. The output of a sensor is tested over a range of clinically relevant inputs, and sensitivity/zero drift detected can be compensated in signal processing [177].

The instruments used in study I and II were calibrated according to the manufacturer's recommendations. Two-point gas calibration was performed in all devices before each study period. Flow calibration is not required for E-sCOVX. The Quark RMR and CCM Express flowmeters were calibrated daily with a 3-liter syringe. Prior to both studies the flow generator of the respective Deltatrac module used was calibrated by ethanol burning. For study II, a full factory calibration of Deltatrac II was performed by engineers at GE Healthcare in Helsinki, Finland.

5.1.5 Data collection from metabolic monitors

In study I, average values of REE, VO_2, VCO_2 and RQ were recorded from the display of each device at the end of the study period. For CCM Express and Quark RMR, ≥ 10 minutes with a variability of $<10\%$ in REE was required. For Deltatrac this was assessed by visual inspection of the long trend as the monitor does not provide an index of variability. Deltatrac measurements were conducted for ≥ 20 minutes.

In study II, digital storage of raw data from all devices was possible. VO_2 and VCO_2 was calculated from data collected during the 20-minute study period. Measurement artifacts from coughing or asynchronous breaths were excluded according to pre-defined criteria available in the methods section of the published paper.

5.2 TRACER METHODOLOGY

5.2.1 Theoretical background

A "tracer" is a molecule that is functionally indistinguishable from its most abundant naturally occurring counterpart ("tracee") but has structural properties which allows its differentiation from the tracee (Fig. 8) [178]. Tracers are created by labeling a molecule with one or more atoms containing a different number of neutrons in the nucleus than the most abundant naturally occurring form, or isotope. Isotopes can be stable or in a state of decay, i.e. radioactive. Tracers can be administered to study their metabolic fate in the human body, for example their incorporation into macromolecules or metabolites. Another common use of tracers in metabolic research is to measure the dilution of tracer by the tracee in the bloodstream. From this data the mass transfer (=flux) of tracee entering and leaving the circulation can be modeled.
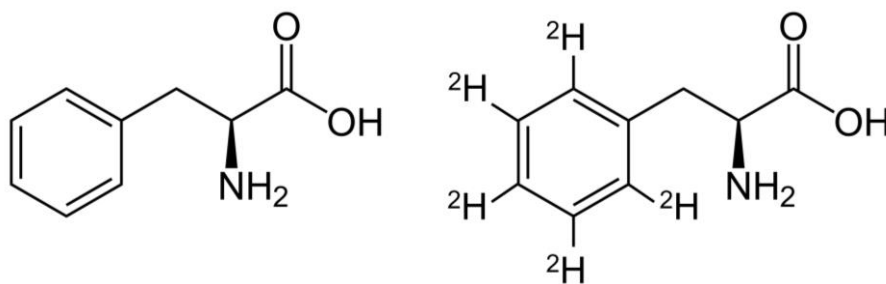
Figure 8. Molecular structure of a tracee (L-phenylalanine) and corresponding tracer (L-^{2}H$_5$-phenylalanine). Original image from Wikimedia Commons.

The true distribution of a substance within the human body is difficult to determine, as it is inhomogeneous and may be regulated both by active and passive processes. Detecting the compound of interest is also limited by practical constraints. For example, sampling is most readily performed from the bloodstream. Due to these limitations the kinetics and distribution of a substance are commonly approximated by mathematical modelling. Theoretical divisions of the body between which transfer of a substance may occur are represented as compartments or "pools", in which the substance is assumed to be uniformly distributed [178].

With regard to amino acids, the simplest representation of the human body is by considering it as two metabolic pools: a free amino acid pool (mostly corresponding to plasma volume) and body protein. This is illustrated in Fig. 9. Using this model with a tracer dilution technique, the fluxes of specific amino acids between these compartments can be estimated. The assumptions required for inference about whole-body protein kinetics are described in detail below. As this method does not quantify the size of respective pools, it can be referred to as "single pool" or non-compartmental modelling [178].
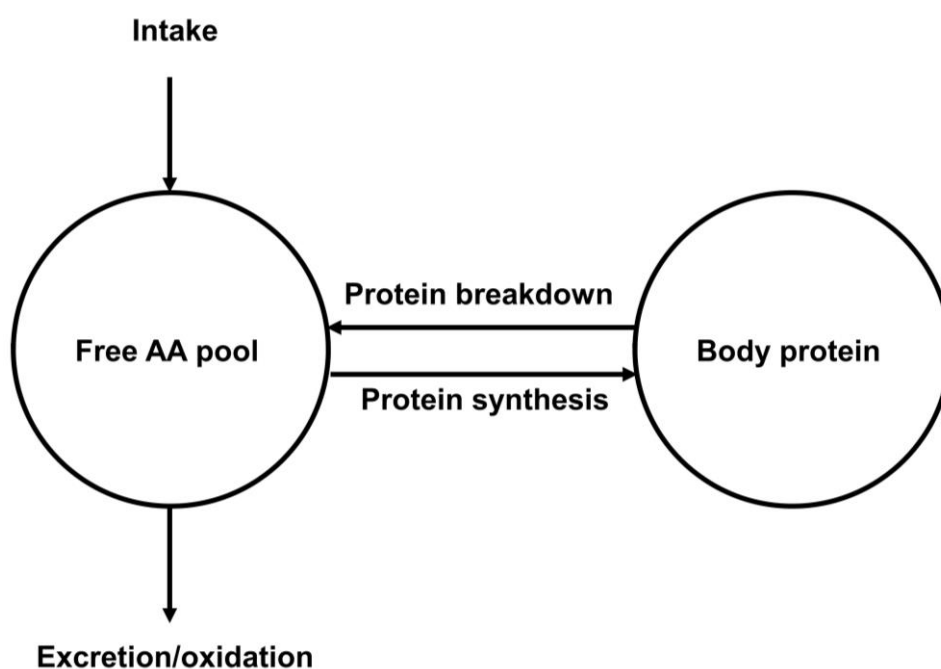

Figure 9. A single pool model for whole-body amino acid kinetics. AA: Amino acid. Image adapted from Wagenmakers [179].

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Amino acids entering the free amino acid pool (Rate of appearance, Ra) will have three possible origins:

i) enteral/parenteral nutrition
ii) breakdown of body proteins
iii) de novo synthesis of amino acids

Using an essential amino acid as tracer will give an accurate representation of protein breakdown as there is no contribution of tracee synthesis to Ra. The most common amino acid tracers in use are L-1-^{13}C-leucine and L-^{2}H$_5$-phenylalanine [179]. Amino acids leaving the circulation (Rate of disappearance, Rd) have five fates:

i) intracellular accumulation
ii) incorporation into body proteins
iii) conversion by modification of functional groups
iv) elimination by oxidation and nitrogen excretion
v) loss from the central compartment via bodily fluids

When a tracer is infused into the central compartment its rate of removal will be proportional to the tracer/tracee ratio, as uptake does not discriminate between tracer and tracee. Eventually the tracer/tracee ratio will plateau at "isotopic equilibrium". At this point the enrichment of tracer (% tracer/(tracer + tracee)) is constant and Ra will be proportional to the tracer infusion rate. This is illustrated in Fig. 10. Expressed in its simplest form, Ra can be calculated as [178]:

$$Ra = \frac{Infusion\ rate\ of\ tracer}{Tracer\ enrichment}$$

Although the single-pool model described here is used to quantify several metabolic fluxes of amino acids on a whole-body level, Ra is the only value that is truly measured.
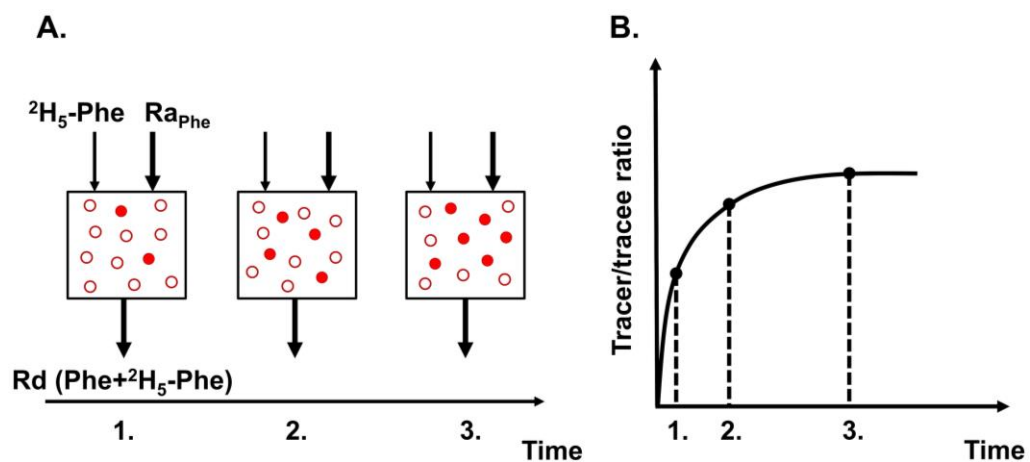

Figure 10. Schematic illustration of isotopic equilibrium in plasma (single pool model) during a constant tracer infusion. A) As uptake (Rd) does not discriminate between tracer and tracee, more tracer will be retained over time until isotopic steady state is achieved. B) Tracer/tracee ratio as a function of time. Sampling at timepoints 1-3 from panel A are marked on the x-axis. Phe: Phenylalanine; Ra: Rate of appearance; Rd: Rate of disappearance. Adapted from Fig. 3.2 in Wolfe and Chinkes, "Isotope Tracers in Metabolic Research".

5.2.2 Modeling whole-body protein kinetics using a L-ring-^{2}H$_5$-phenylalanine tracer

Studies III-IV model whole-body protein kinetics using a non-compartmental stable isotope tracer dilution method with L-ring-^{2}H$_5$-phenylalanine (Phe). The calculations of amino acid fluxes, required assumptions and limitations of this model are discussed below. These are covered more extensively in the literature [178, 180, 181]. For a stable isotope tracer,

$$Ra = i * \left(\frac{E_i}{E_A}\right) - i$$

Where i: infusion rate of tracer; E_i: enrichment of infusate; E_A: arterial enrichment of tracer.

As the amount of stable isotope tracer required for a kinetic study will have a significant impact on Ra, the second term in this equation corrects for the infusion rate of tracer. Under conditions of enteral and/or parenteral nutrition provided at a constant rate:

$$Phe\ from\ protein\ breakdown\ (endogenous\ Ra) = Ra\ Phe - dietary\ Phe$$

The contribution of parenteral amino acids to dietary Phe is straightforward as they are administered directly into the bloodstream. Enteral amino acids are subject to variable first-pass uptake by the splanchnic organs [149, 150, 182]. The splanchnic extraction fraction can be quantified by infusing an enteral tracer. In studies III and IV 1-^{13}C-phenylalanine was used for this purpose:

$$Splanchnic\ extraction\ fraction = 1 - \frac{\dfrac{E_{A\,[13C]Phe}}{i_{[13C]Phe}}}{\dfrac{E_{A\,[2H5]Phe}}{i_{[2H5]Phe}}}$$

Assumption 1: Endogenous Ra of phenylalanine reflects the relative phenylalanine content in proteins, i.e. whole-body protein breakdown. Data from healthy volunteers with multiple concurrent tracers and determinations of nitrogen balance supports this [183].

Assumption 2: Rd equals Ra at physiological steady state. While this is true, steady state conditions in protein turnover are impossible to verify as changes in Rd will not affect tracer dilution (relative rate of tracer and tracee removal is identical). All kinetic parameters derived from Rd are therefore associated with a higher degree of uncertainty than Ra.

$$Rd = Protein\ synthesis + amino\ acid\ oxidation$$

Assumption 3: The rate of amino acids lost to sweat or urine is negligible compared to other metabolic fluxes. This was not assessed within the context of studies III-IV. In study IV Rd was adjusted for amino acids lost to dialysis effluent.

The first and rate-limiting step of phenylalanine oxidation is the irreversible conversion to tyrosine by hydroxylation. This reaction mainly takes place in the liver [184]. The conversion of phenylalanine to tyrosine (Q_{pt}) can be estimated by simultaneously measuring the enrichment

of ring-$^2H_4$-tyrosine (the metabolite of ring-$^2H_5$-phenylalanine) and whole-body Ra of tyrosine from an infusion of $^2H_2$-tyrosine:

$$Q_{pt} = Ra_{[2H2]Tyr} * \frac{E_{A\ [2H4]Tyr}}{E_{A\ [2H5]Phe}} * \frac{Ra_{Phe}}{i_{Phe} + Ra_{Phe}}$$

<u>Assumption 4</u>: The rate of phenylalanine hydroxylation is proportional to its oxidation rate. As tyrosine may have other metabolic fates this will lead to an underestimation of protein oxidation. Phe oxidation estimated from Q_{pt} was in agreement with oxidation determined by the incorporation of 1-^{13}C-Phe into exhaled CO_2 (a more direct measurement of oxidative metabolism) in healthy volunteers [185]. Absolute values of protein oxidation are variable depending on the tracer used [151].

<u>Assumption 5</u>: The proportion of Rd$_{Phe}$ not converted to tyrosine is used for protein synthesis. The model does not consider the possible of intracellular accumulation of free phenylalanine, which would result in an overestimation of protein synthesis.

Whole-body balance of phenylalanine, and by inference protein balance, is calculated as:

$$Balance_{Phe} = Synthesis\ (Rd - Q_{pt}) - Breakdown\ (Ra - dietary\ Phe)$$

As balance is calculated from two fluxes that are an order of magnitude larger than the result, small errors in the determination of synthesis or breakdown will yield larger errors in net balance.

5.2.3 Determining tracer enrichment

Enrichment can be described in terms of tracer to tracee ratio (TTR, a dimensionless number), atom percentage excess (APE, %) or molar percentage excess (MPE, %) [186]. APE represents the excess of isotopically labeled atoms in a sample and MPE the excess of labeled molecules. APE and MPE are derived from TTR as

$$MPE = \frac{TTR}{(1 + TTR)} * 100$$

Detection of the relative quantities of tracer and tracee in a sample requires highly accurate measurements of molecular mass. This is performed by gas chromatography-mass spectrometry (GCMS). An extensive description of this technique can be found in the reference which this section is based on [178].

The principle of mass spectrometry is to measure the molecular weight of a sample by fragmenting it into component ions. Blood samples are initially centrifuged to plasma and frozen at -80°C waiting analysis. Amino acids in a sample are treated with chemical derivatization agent (MTBSTFA, or N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide) to decrease polarity and make the compounds more volatile. The sample is then injected into the gas chromatograph, vaporized, and passed through a solid phase column by a carrier gas.

This process separates different compounds in the sample and allows them to pass into the mass spectrometer in order, thereby facilitating detection.

Compounds introduced into the MS device are fragmented to ions with a specific mass/charge ratio (*m/z*), and passed through a magnetic field towards a detector plate. The dispersion pattern of the sample will depend on the *m/z* of a compound, providing a "fingerprint" for a particular molecule.

Tracer samples in <u>studies III and IV</u> were analyzed by an Agilent N5973 GCMS (Agilent, Kista, Sweden), *m/z* 336 and 341 for phenylalanine and 466, 468 and 470 for tyrosine.

5.2.4 Other analytical methods

Plasma amino acid profiles were analyzed using high-performance liquid chromatography (HPLC) [187] Alliance HPLC System (Waters Corporation, Milford, MA, USA). Serum urea was analyzed using Urea kit on Indiko analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

5.3 STATISTICAL ANALYSIS

5.3.1 Inferential statistics

Statistical significance tests are used to determine the probability (p-value) of an observation, given that the test hypothesis and all assumptions underlying the test are valid [188]. It is common practice in medical statistics to select an upper limit of "significance" for a p-value a priori (<0.05). Findings with significant p-values are often considered as not attributable to chance [189]. This is an inherently flawed interpretation and the utility in standard reporting of p-values is contested [190]. Furthermore, statistical tests require assumptions about the population from which a sample is derived, and the conditionality of these assumptions (such as the underlying probability distribution) are difficult to ascertain in smaller samples. As all studies included in this thesis (particularly <u>III and IV</u>) contain small groups of patients, reported results from significance testing should be interpreted with caution. This is of even greater importance for secondary outcomes due to multiple comparisons. The statistical tests used in <u>studies I-IV</u> are listed in <u>Table 4</u>.

In <u>study I</u>, no a priori sample size calculation was performed.

In <u>study II</u>, a target of N=50 observations was chosen to determine 95% limits of agreement within ±2 sample standard deviations (SD) from Student's t-distribution.

In <u>study III</u>, a target of N=10 observations was chosen based on previous data[149] to detect a change from negative to net zero protein balance with $\alpha = 0.05$ and $\beta = 0.2$.

In <u>study IV</u>, a target of N=12 observations was chosen based on previous data[151] to detect a change from negative to net zero protein balance with $\alpha = 0.05$ and $\beta = 0.2$, assuming N=2 protocol violations.

All descriptive data and results normalized to body weight (BW) are adjusted using the same formula as applied in local clinical nutrition practice:

$$BW_{adjusted}(kg) = BW_{ideal}\,(height\,(cm) - 100) + (BW_{admission} - BW_{ideal}) * 0.33$$

If admission BW was less than ideal BW, admission BW is used instead of adjusted BW.

In the published paper to study III, results from protein kinetics were normalized to admission BW. To allow better comparisons of results with study IV this data is presented in the thesis as normalized to adjusted BW. One-way ANOVA of adjusted values were calculated applying a Greenhouse-Geisser correction (equal variances not assumed). In addition, administered doses of amino acids are expressed as protein substrate, using a factor of 0.83 to correct for hydration of free amino acids [191].

Statistical tests and calculations in studies I and II were performed in Excel 2010 and 2013 (Microsoft corporation, Redmond, WA, USA) respectively. Tests in study III were performed in SPSS Statistics version 24 (IBM, Armonk, NY, USA) and Prism version 7 (GraphPad Software Inc, La Jolla, CA, USA). Tests in study IV were performed in Prism version 8.3 (GraphPad Software Inc, San Diego, USA).

Table 4. Statistical methods in studies I-IV.

	Descriptive statistics	Inferential statistics	Other methods
Study I	Median/mean (range), mean ± standard deviation	Two-tailed Student's t-test for paired samples	Bland-Altman plots. Acceptable limits of agreement not defined a priori.
Study II	Mean ± standard deviation	Significance testing not presented in paper.	Bland-Altman plots. Acceptable limits of agreement not defined a priori.
Study III	Median (range)	One-way analysis of variance (ANOVA) for comparison of multiple paired samples. Two-tailed Student's t-test or Wilcoxon signed-rank test for paired samples as appropriate.	Sphericity of multiple paired samples assessed by Mauchly's test of sphericity. Greenhouse-Geisser correction applied as appropriate. Bonferroni correction applied for post-hoc testing.
Study IV	Mean (range)	Two-tailed Student's t-test for paired samples	

5.3.2 Bland-Altman analysis

Bland-Altman plots were used to assess agreement between methods in <u>studies I and II</u> [192]. The 95% limits of agreement were calculated as

$$\bar{d} \pm \left(t_{\alpha\, 0.05, d.f.n-1}\right) * s_d$$

using Student's t-distribution, where s_d: sample standard deviation of difference between methods and $\bar{d}$: mean difference between methods. Percentage error (PE), an expression of the range of discrepancy between paired measurements in relation to the mean of the measured quantity, is given as

$$PE = \left(t_{\alpha\, 0.05, d.f.n-1}\right) * s_d / \bar{x}$$

where $\bar{x}$: mean of both methods.

The original description of the Bland-Altman plot only considered independent observations, and alternative approaches using both independent and repeated measurements have been described [193, 194]. As multiple observations of individual patients in <u>studies I and II</u> were only performed on separate days we have considered them as independent for the purposes of assessing agreement between methods.

6 MAIN RESULTS AND DISCUSSION

This section summarizes the main findings of studies I-IV. Full results are reported in the individual papers and manuscript.

6.1 STUDIES I AND II

6.1.1 Results

In study I, we performed 48 sequential measurements with all three instruments in 24 patients. Recruitment was prematurely terminated due to a lack of calibration gas for CCM Express. Mean values of energy expenditure and gas exchange measurements are reported in Table 5. There was no statistically significant difference in mean REE between Quark RMR and Deltatrac. CCM Express measured higher REE values in all cases, corresponding to a mean REE 64% higher than Deltatrac. Mean RQ was also different between all three instruments, with Deltatrac registering the lowest RQ values on average. Graphical comparisons with Bland-Altman plots between Deltatrac and the evaluated devices are presented in Fig. 11. Bias and 95% limits of agreement are listed in Table 6. Percentage error of REE (~2*SD of difference/mean of both methods) was ±31% between Deltatrac and CCM Express, and ±22% between Deltatrac and Quark RMR.

In study II, 48 simultaneous measurements in 22 patients with Deltatrac II and both evaluated instruments were included for analysis. Both Quark RMR and E-sCOVX measured higher mean VO_2 and VCO_2 than Deltatrac II, corresponding to a mean difference in REE of 10% (Table 7). The agreement between instruments is illustrated with Bland-Altman plots in Fig. 12. Bias, 95% limits of agreement and percentage errors are listed in Table 8.

To compare indirect calorimetry with predictive equations, an exploratory analysis of individual patients from studies I and II was performed post-hoc. Mean measured energy expenditure by Deltatrac was higher (1806 kcal/day) compared to estimations of energy expenditure used in local clinical practice (1486 kcal/day) or the Harris-Benedict equation[10] (1613 kcal/day). There was a weak correlation between measured and estimated EE (Fig. 13), with measured EE ranging between 16-35 kcal/kg/day.

Table 5. Mean gas exchange parameters of instruments in study I.

	REE	VO_2	VCO_2	RQ
Deltatrac	1749 ±57	261 ±8	193 ±6	0.74 ±0.01
Quark RMR	1788 ±72	259 ±11	211 ±9	0.81 ±0.01
CCM Express	2876 ±96	408 ±14	352 ±11	0.87 ±0.01

REE: Resting energy expenditure (kcal/day); RQ: Respiratory quotient; VO_2: Oxygen consumption (ml/min); VCO_2: Carbon dioxide production (ml/min).

All values presented as mean ± standard error of the mean.

Table 6. Comparisons of mean difference in gas exchange parameters between instruments in study I.

	Bias*	95% limits of agreement**	Percentage error[†]
Quark-Deltatrac VO_2	-2 (-11 - +6)	-60 - +56	±22%
Quark-Deltatrac VCO_2	+19 (+11 - +26)	-35 - +70	±27%
Quark-Deltatrac REE	+39 (-17 - +96)	-350 - +429	±22%
CCM Express-Deltatrac VO_2	+147 (+131 - +162)	+40 - +254	±32%
CCM Express-Deltatrac VCO_2	+159 (+147 - +172)	+73 - +245	±32%
CCM Express-Deltatrac REE	+1127 (+1022 - +1231)	+404 - +1849	±31%

LoA: Limits of agreement; REE: Resting energy expenditure (kcal/day); VO2: Oxygen consumption (ml/min); VCO2: Carbon dioxide production (ml/min).

*Mean difference (95% confidence interval).

** 95% limits of agreement = bias ± 2 standard deviations of differences between methods.

† Percentage error = ±2 standard deviations of difference between methods/mean of both methods.
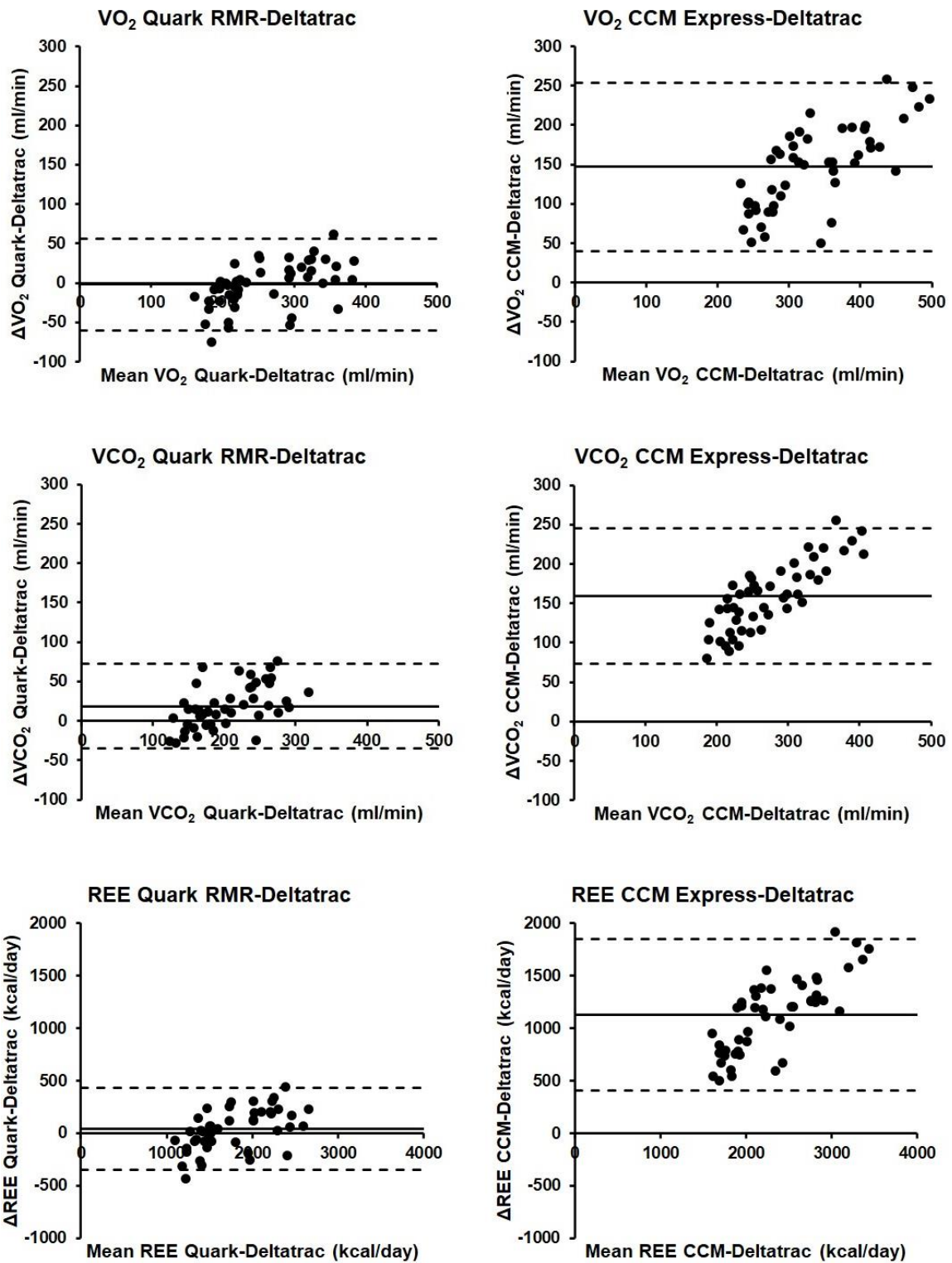
Figure 11. Bland-Altman plots of VO$_2$, VCO$_2$ and REE comparing Quark RMR-Deltatrac and CCM Express-Deltatrac in study I. REE: Resting energy expenditure (kcal/day); VO$_2$: Oxygen consumption (ml/min); VCO$_2$: Carbon dioxide production (ml/min). Solid line: mean difference; Dashed lines: 95% limits of agreement of difference.

Table 7. Mean gas exchange parameters of instruments in study II.

	REE	VO$_2$	VCO$_2$	RQ
Deltatrac*	1986 ±56/2041 ±59	289 ±8/296 ±9	218 ±6/226 ±6	0.76 ±0.01/0.77 ±0.01
Quark RMR	2191 ±65	314 ±10	258 ±7	0.83 ±0.01
E-sCOVX	2255 ±63	323 ±9	263 ±7	0.82 ±0.01

REE: Resting energy expenditure (kcal/day); RQ: Respiratory quotient; VO$_2$: Oxygen consumption (ml/min); VCO$_2$: Carbon dioxide production (ml/min).

All values presented as mean ± standard error of the mean.

*Mean values from Quark/E-sCOVX comparison.

Table 8. Comparisons of mean difference in gas exchange parameters between instruments in study II.

	Bias*	95% LoA**	Percentage error[†]
Quark-Deltatrac VO$_2$	+25 (+14 - +36)	-49 - +98	±24%
Quark-Deltatrac VCO$_2$	+40 (+33 - +47)	-11 - +90	±21%
Quark-Deltatrac REE	+205 (+138 - +272)	-261 - +671	±22%
E-sCOVX-Deltatrac VO$_2$	+27 (+17 - +38)	-44 - +99	±23%
E-sCOVX-Deltatrac VCO$_2$	+37 (+31 - +44)	-8 - +83	±19%
E-sCOVX-Deltatrac REE	+215 (+148 - +281)	-246 - +676	±21%

LoA: Limits of agreement; REE: Resting energy expenditure (kcal/day); VO$_2$: Oxygen consumption (ml/min); VCO$_2$: Carbon dioxide production (ml/min).

*Mean difference (95% confidence interval).

**95% limits of agreement = bias ± 2 standard deviations of differences between methods.

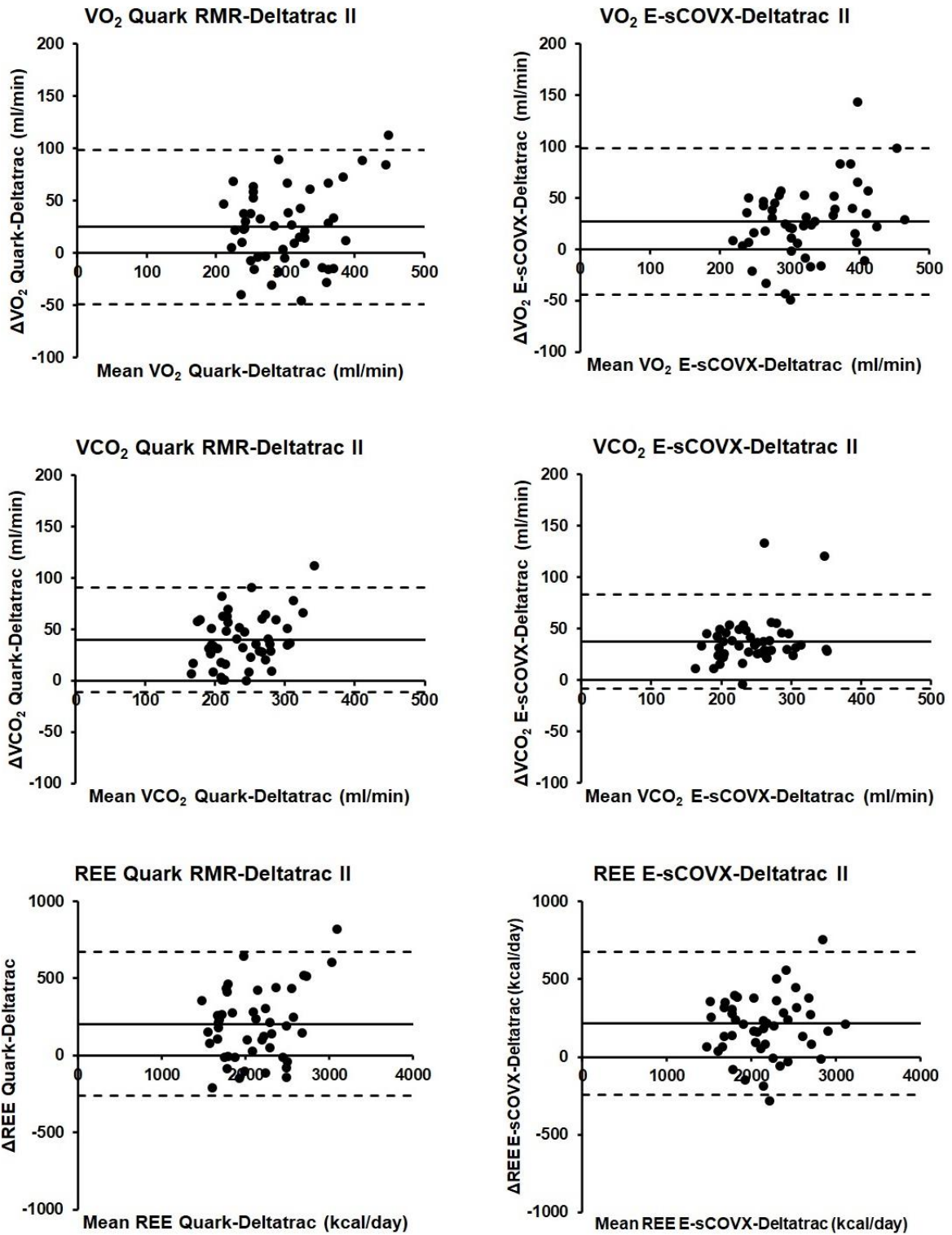†Percentage error = ±2 standard deviations of difference between methods/mean of both methods.

Figure 12. Bland-Altman plots of VO$_2$, VCO$_2$ and REE comparing Quark RMR-Deltatrac II and E-sCOVX-Deltatrac II in study II. REE: Resting energy expenditure (kcal/day); VO$_2$: Oxygen consumption (ml/min); VCO$_2$: Carbon dioxide production (ml/min). Solid line: mean difference; Dashed lines: 95% limits of agreement of difference.
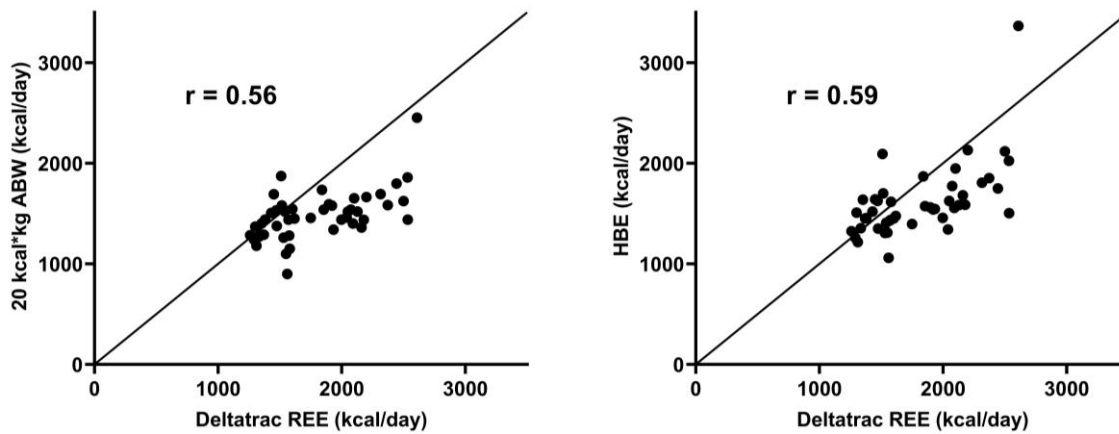
Figure 13. Simple linear correlation between measured REE (Deltatrac) and weight-based estimations of REE (20 kcal/kg ABW/day and the Harris-Benedict equation) from individual patients (N=46) in studies I and II. Mean value of both Deltatrac measurements used from study II. ABW: Adjusted body weight; HBE: Harris-Benedict equation; REE: Resting energy expenditure; r: Pearson product-moment correlation coefficient. Solid line: line of identity.

6.1.2 Discussion

Our results demonstrate a variable agreement between indirect calorimetry with mixing chamber technology (Deltatrac I/II) and breath-by-breath instruments. One device (CCM Express) overestimated REE to a degree that is not acceptable for clinical purposes. The other two instruments had similar performance characteristics for gas exchange measurements compared to Deltatrac. Measured energy expenditure was highly variable and did not correlate well with estimations from anthropometric properties.

The implications of these findings for the use of new devices in an intensive care setting depend on two central questions:

i)   Is the Deltatrac monitor accurately considered a "gold standard" for gas exchange measurements in mechanically ventilated ICU patients?
ii) What level of agreement is considered acceptable in relation to the reference method?

From a diagnostic perspective, a gold standard is the method which will yield a result closest to the true value of the property investigated. An in vitro validation of Deltatrac by Takala using gas injectors and ethanol burning in a model of mechanical ventilation, demonstrated a mean error in VO_2, VCO_2 and RQ of 2-4% over a clinically relevant range of FiO_2 and airway pressures [21]. The authors also performed a comparison of VO_2 in mechanically ventilated patients using the Fick principle, but note that in vivo validation is limited by the absence of an accurate reference method. Tissot and co-workers compared VO_2/VCO_2 measurements by Deltatrac in mechanically ventilated patients in 8-hour measurements with an experimental setup combining a mass spectrometer and pneumotach flowmeter [22]. Limits of agreement are not explicitly stated, but from visual inspection appear to be smaller (around ±17 ml/min for VO_2) than the variability observed in the studies of this thesis.

Despite this superior agreement it is not self-evident that the experimental system used for in vivo validation represents a gold standard comparator. Although mass spectrometry provides highly accurate measurements of gas concentrations, the gas sensors used in commercial instruments also have a high degree of accuracy and is likely not a major source of variable error. The validation setup in the study by Tissot uses a mixing chamber for collection of expired gas [195]. This similarity in design to Deltatrac, and prolonged measurement periods, may explain the high level of agreement observed. The use of mixing chamber technology for gas exchange measurements circumvents the technical challenge of accurate synchronization of breath-by-breath measurements, which can be especially difficult in spontaneously breathing patients. This theoretical advantage in design and the high degree of accuracy observed in in vivo validations are appealing arguments when considering the status of Deltatrac as a reference method in clinical practice. It must also be taken into consideration that the Deltatrac is an aging piece of equipment. Both units in our unit had been in prior use for over 20 years. Although the instruments were serviced and passed a full calibration prior to both studies, deterioration of function over time cannot be ruled out. In particular, the unexplained RQ values outside of normal physiologic range are a cause for concern.

If we accept Deltatrac as a gold standard method, this begets the question to what degree diverging measurements in other devices are acceptable for clinical purposes. A proportional bias over the full range of measurements could be compensated by a calibration factor. Regarding variability, the original article by Bland and Altman proposes that the differences encompassed by the 95% limits of agreement ($\sim \pm 2SD$) should be within a clinically acceptable range [192]. Long-term underfeeding or overfeeding have potentially negative consequences and significant deviations from the true value of EE are undesirable for clinical decision-making. As there is insufficient evidence to determine a dose-response relationship for benefit or harm from energy delivery during critical illness, an acceptable degree of variability is difficult to define a priori.

The criterion proposed by Critchley is commonly applied for method comparisons of cardiac output measurements [196]. It is based on the assumption that any method measuring a constant true value will yield variable results by random error. The magnitude of this error can be described by its normalized probability distribution, or coefficient of variation (CV = SD/mean). The combined CV of two methods, directly proportional to the standard deviation of the differences between methods, is then calculated as the square root of the summed variance of each device (Fig. 14).
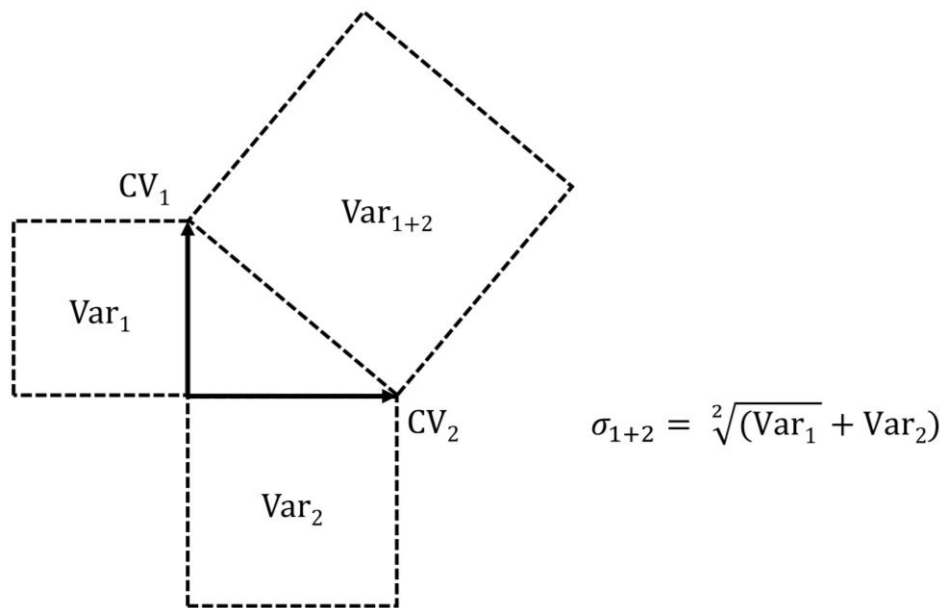
Figure 14. An illustration of a Pythagorean approach to calculating the combined precision of two measurement techniques. CV: Coefficient of variation, Var: Variance; σ: Standard deviation. Adapted from Critchley & Critchley 1999 [196].

Assuming a precision ($\approx 2*CV$) of $\pm20\%$ for the reference method, a percentage error of no more than $\pm28.3\%$ is acceptable if the alternate method is to measure the true value with equal or greater precision. A review by Cecconi elaborates on this idea by stating that the precision of the gold standard may in fact be lower than $\pm20\%$ [197]. Accordingly, they conclude that the a priori acceptable percentage error should be determined by first measuring the CV of the reference method within the context of the same study.

One limitation of this approach when applied to indirect calorimetry is that the output variables (VO_2 and VCO_2) may behave in a non-stochastic fashion depending on the input conditions (respiration patterns, airway pressures, humidity, etc.), resulting in variable non-random, systematic error (=bias) for individual measurements. Due to the black box design of modern monitors the contribution of such factors to observed variability could not be determined within the context of these studies. Post-hoc analysis of raw data from E-sCOVX by engineers at GE Healthcare indicated that the absence of bias flow may interfere with synchronization of gas and flow measurements and offset certain results. Interestingly, Stapel and colleagues performed a similar study comparing E-sCOVX and Deltatrac using ventilators with a bias flow setting [28]. Although concluding that the mean difference (12% of REE) was unacceptable for clinical practice, the 95% limits of agreement were narrower (percentage error $\pm14\%$) than those found in study II.

The 95% limits of agreement of REE for Quark and E-sCOVX compared to Deltatrac expressed as percentage error were $\pm22\%$. This is lower than the upper boundary proposed by Critchley and substantially less than that of minimally invasive cardiac output monitors commonly used in critical care [198]. Our results are consistent with other comparisons of Deltatrac and modern instruments for indirect calorimetry reported in the literature [25-28]. Of

note, results from a recently published evaluation of a new calorimeter calls the reproducibility of estimated agreement into question [199]. In a multicenter study of the Q-NRG device, the investigators report comparisons with Deltatrac from three different study sites. Bias varied between -307 to +23 kcal, and 95% limits of agreement from ±158 to 328 kcal. Several factors may contribute to observed differences: local ventilation practices, attention to detail in minimizing potential sources of measurement error, function and maintenance of the reference device, and random effects from patient sampling. Regardless of the reasons for these discrepancies, the observed variations lower the probability that any new device will consistently outperform those currently on the market with regard to agreement with Deltatrac. It also raises the question for how long a decommissioned instrument can be considered a gold standard. For future developments in indirect calorimetry, it is essential that manufacturers evaluate their products in vivo under simulated conditions close to those in clinical practice. It is also desirable that new devices are systematically compared to other commercially available instruments in the relevant clinical setting.

The mean observed difference between Quark RMR, E-sCOVX and Deltatrac corresponds to a 10% higher REE from breath-by-breath instruments. In study I we found no difference in mean REE between Quark and Deltatrac. However, this result is likely subject to systematic error from bias flow compensation. The presence of ventilator bias flow was manually determined by visual inspection of the flow profile according to the manufacturer's recommendation. During the conduct of study II we were informed that the ventilators in our unit (Dräger Evita XL) did not have a bias flow setting in adult mode, and bias flow compensation was retroactively set to zero. The latter result therefore represents a more accurate estimation of the mean difference between Quark and Deltatrac.

One monitor (CCM Express) gave exceptionally high values of VO_2, VCO_2 and resulting REE. The mean difference in VO_2 and VCO_2 between this device and Deltatrac corresponds to 64% higher REE values. Limits of agreement were also wider (PE = ±32%) than comparisons for other instruments. Another validation study using CCM Express may provide insight into a potential cause for this difference. Graf and colleagues evaluated CCM Express against Deltatrac in critically ill patients, using a protocol similar to that in study II [27]. They found a lower bias (+273 kcal/day) but similar 95% limits of agreement (±532 kcal/day). In this setup the CCM flowmeter was attached distal to the HME filter, whereas in study I it was connected directly to the endotracheal or tracheostomy tube according to instructions from the manufacturer. This could affect synchronization of gas concentrations and flow from the difference in dead space volume proximal to the point of measurement. It may also lead to different conditions in heat and humidity with implications for the output accuracy of gas analyzers. From an engineering perspective, it is crucial that manufacturers take these issues into consideration when designing equipment for use in intensive care.

There are several limitations to study I which we attempted to address in study II. The main disadvantage was the long period required to perform all measurements in sequence. This increases the risk of changing metabolic rate and non-steady state conditions in the volatile

carbon pool between measurements. Second, no consideration was taken to differences in artifact suppression between devices. Third, the protocol necessitates several disconnections of the ventilator circuit between measurements. The introduction of ambient air into the mixing chamber will affect measurements by Deltatrac until steady state conditions are achieved. In study II, a mandatory run-in period after connection was introduced to minimize this source of error. Fourth, both studies allowed for repeated measurements on different days. For the purposes of comparative statistics and Bland-Altman analyses these data points were treated as independent observations, as there is potential for considerable day-to-day variability in the physiologic state of individual ICU patients. Hypothetically, a specific set of patient-ventilator interactions in an individual patient could generate outlier results or stronger agreement in a certain device. If multiple measurements are performed under similar conditions this may be sufficient to offset the sample mean. To account for this a minimum of 20 measurements in unique patients was mandated. Post-hoc analysis of the first measurements performed in subjects shows a similar mean REE for each instrument as the complete data set in both studies.

6.2   STUDIES III AND IV

The common primary aim of studies III and IV was to evaluate the effects of macronutrient delivery on whole-body protein balance. Due to the similarities in methodology the studies are reported together below.

6.2.1   Results study III

12 patients were recruited during the study period. 8 patients completed the full protocol. 3 patients had outcome data for baseline and 3 h, and one patient for baseline and 24 h.

Median length of stay in ICU on study day 1 was 6 (range 3 – 18) days. Energy and protein delivery for individual patients is described in Table 9.

During the 24 h amino acid infusion there was a sustained improvement in protein balance over time, from net negative to positive. Post-hoc testing did not indicate any change in balance between 3 h and 24 h (p = 1.00). Changes in synthesis, breakdown or oxidation rate did not reach statistical significance. Mean values of protein kinetic parameters are listed in Table 10. Protein kinetics for individual patients are illustrated in Fig. 15 and mean balance in Fig. 16.

Table 9. Nutritional therapy in patients with complete data for the primary outcome in study III.

	EN formula	Parenteral nutrition	Energy (baseline)*	Energy (AA infusion)*	Protein (baseline)**	Protein (AA infusion)**
Patient 1	Fresubin 2 kcal HP		24.8	28.7	1.24	2.04
Patient 2	Fresubin 2 kcal HP		17.6	21.5	0.88	1.68
Patient 3	Fresubin 2 kcal HP	Glucose 5%	24.6	28.4	0.92	1.72
Patient 4	-	Olimel 1600	18.0	21.9	0.96	1.76
Patient 6	Nutrison 1 kcal	-	22.7	26.6	0.91	1.71
Patient 10	Fresubin HP Energy	Glucose 5%	31.5	35.4	1.43	2.23
Patient 11	-	Olimel 1070, Glucose 5%	14.6	18.4	0.59	1.39
Patient 12	Fresubin HP Energy		22.2	26.1	1.11	1.91
Mean			22.0	25.9	1.00	1.81

AA: Amino acids; EN: Enteral nutrition; LoS: Length of stay (days).

* Kcal/kg/day.

** Grams/kg/day. Amino acids converted to protein substrate with hydration factor of 0.83.

Table 10. Phenylalanine kinetics, plasma free amino acids and serum urea in study III.

	Baseline	3h AA infusion	24h AA infusion	P-value**
Net balance*	-1.6 (-8.3 – 5.0)	6.8 (-1.7 – 15.3)	8.4 (3.8 – 13.1)	0.0044
Synthesis*	67.9 (61.3 – 74.4)	82.0 (59.5 – 104.5)	71.7 (60.6 – 82.9)	0.28
Breakdown*	69.5 (58.0 – 81.0)	75.2 (49.0 – 101.4)	63.3 (52.2 – 74.4)	0.42
Oxidation*	15.2 (11.7 – 18.8)	19.4 (12.8 – 26.0)	14.2 (11.3 – 17.0)	0.15
Plasma AA (µmol/L)	3064 (2533 – 3596)	3229 (2982 – 3476)	3599 (3186 – 4012)	0.059
Serum urea (mmol/L)	13.8 (6.8 – 20.8)	13.8 (7.0 – 20.6)	15.4 (8.3 – 22.4)	0.053

AA: Amino acids.

* µmol Phenylalanine/kg/h, mean (95% confidence interval).

** One-way ANOVA for repeated measurements, Greenhouse-Geisser correction applied.
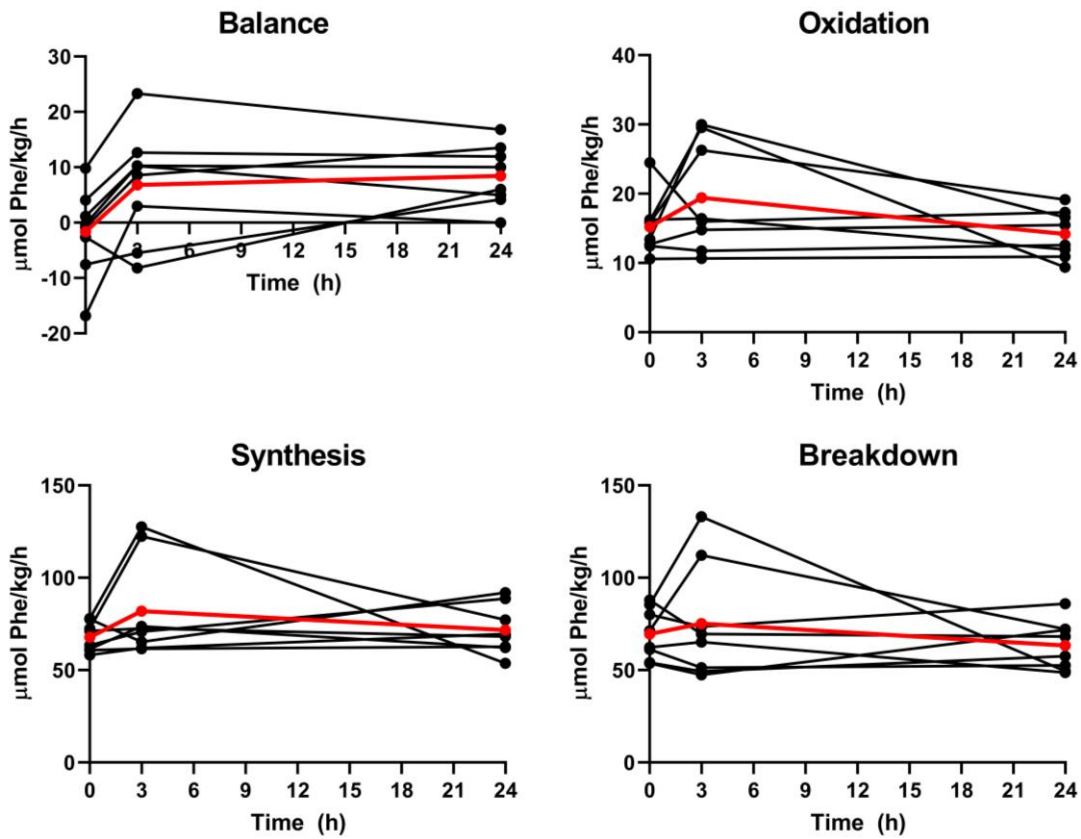
Figure 15. Whole-body phenylalanine balance, synthesis, breakdown and oxidation rates over time in study III. Phe: Phenylalanine. Black lines: individual patients; Red line: mean.
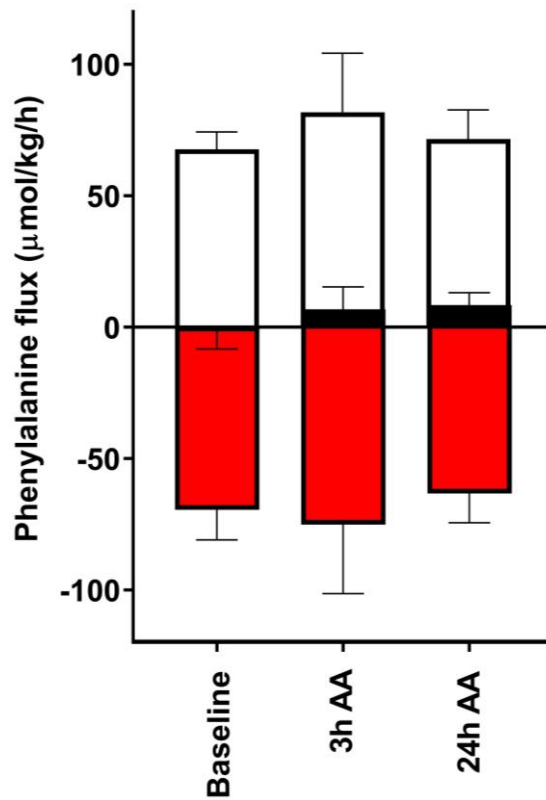

Figure 16. Mean whole-body phenylalanine balance in study III. AA: amino acids. Black bars: net balance; Red bars: breakdown; White bars: synthesis; Whiskers: Upper limit of 95% confidence interval.

6.2.2 Results study IV

Of 12 patients recruited during the study period, 6 were excluded due to protocol violations or clinical circumstances. The nutritional and metabolic characteristics of individual patients are described in Table 11.

Table 11. Individual patient nutritional and metabolic characteristics in study IV.

	LoS study day 1	CRRT	EE* baseline	EE* 50% EN	EE* 100% EN	Kcal 50%	Kcal 100%	Protein** 50%	Protein** 100%
Patient 1	5	Day 1&2	27.8	22.4	Missing value	14.0	26.4	0.80	1.60
Patient 2	21	Day 1&2	29.3	29.3	33.2	15.6	29.3	0.89	1.78
Patient 5	28	No	40.8	31.3	37.8	19.9	39.9	1.00	1.99
Patient 8	30	Day 1	44.6	46.8	Missing value	24.9	44.5	1.38	2.77
Patient 10	9	No	26.3	30.5	34.7	12.8	25.5	0.64	1.28
Patient 11	7	No	29.2	34.5	38.5	17.1	31.2	0.60	1.20
Mean			33.0			16.3	32.2	0.89	1.77

CRRT: Continuous renal replacement therapy; EE: Energy expenditure; EN: Enteral nutrition; LoS: Length of ICU stay.

*Kcal/kg/day

**g/kg/day

During 100% EN we observed an increase in mean protein balance, from -6.1 to 2.9 µmol Phe/kg/h (p = 0.044). There were no significant changes in other kinetic parameters. Mean plasma amino acid concentrations were also higher during full-dose EN, 2632 vs 2173 µmol/L (p = 0.011). Protein kinetics for individual patients during 50% and 100% EN are illustrated in Fig. 17. Mean balance is illustrated in Fig. 18.
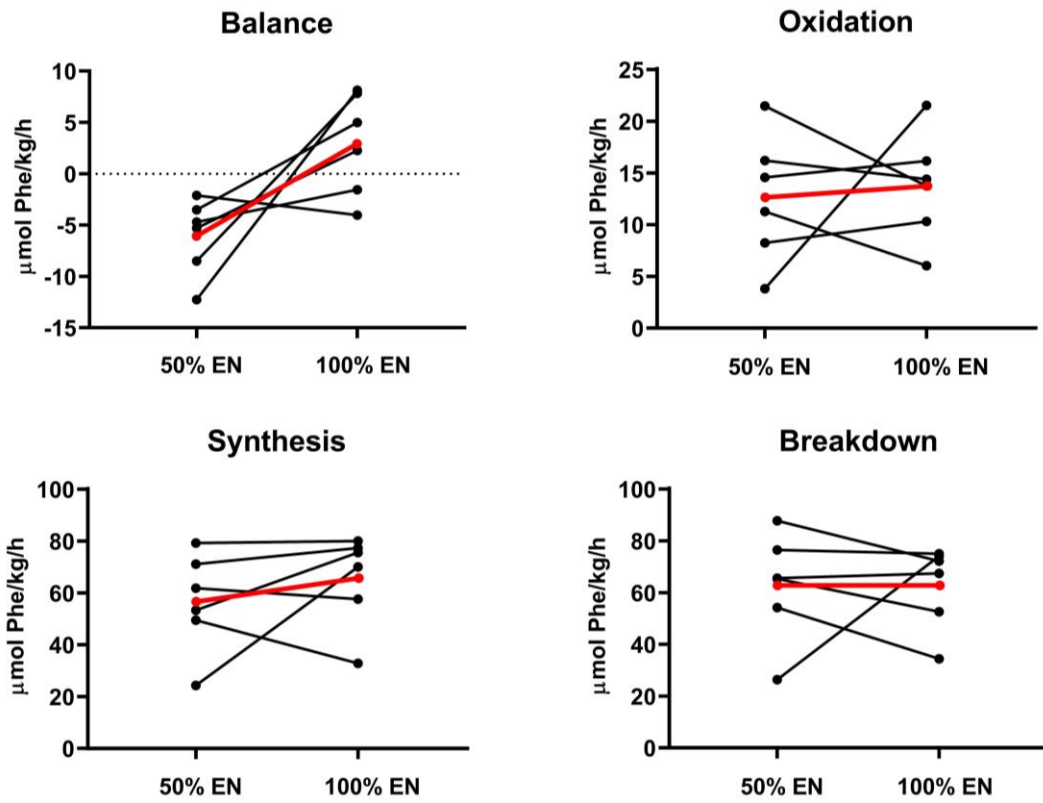
Figure 17. Whole-body phenylalanine kinetics during 50 and 100% EN in study IV. EN: Enteral nutrition; Phe: Phenylalanine. Black lines: individual patients; Red lines: mean.
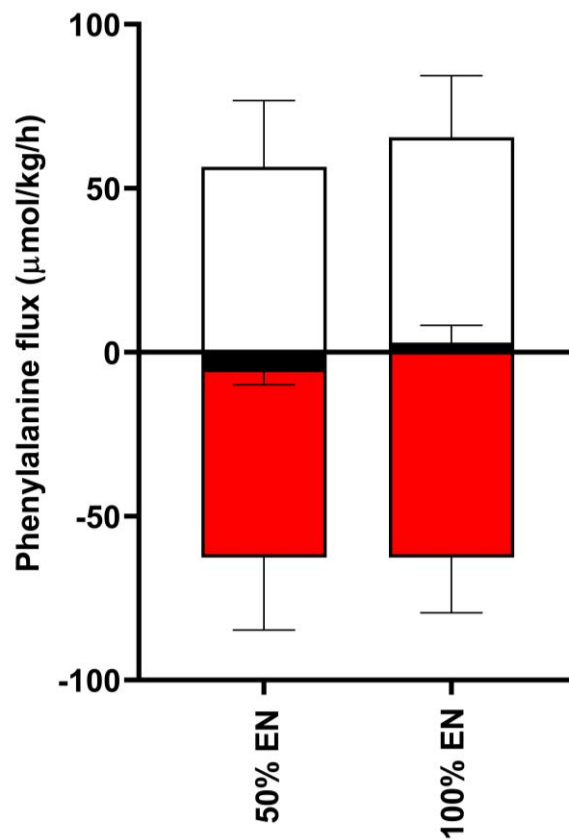

Figure 18. Mean whole-body phenylalanine balance in study IV. EN: Enteral nutrition. Black bars: net balance; Red bars: breakdown; White bars: synthesis; Whiskers: Upper limit of 95% confidence interval.

6.2.3 Discussion

<u>Studies III and IV</u> investigated the effects of two different macronutrient supplementation strategies on whole-body protein balance in critically ill patients. In <u>study III</u> we administered an infusion of parenteral amino acids in addition to standard nutrition over 24 hours. In <u>study IV</u> we provided half- or full-dose EN in relation to energy expenditure for 24 hours in a cross-over design. The similar and diverging aspects of these studies discussed in this section are listed below:

1. *The importance of protein intake for whole-body protein balance*
2. *Is there a clinical benefit to a neutral/positive protein balance?*
3. *Is energy delivery an important determinant of whole-body protein balance?*
4. *Limitations of studies III and IV*
5. *Strengths of studies III and IV*

1. *The importance of protein intake for whole-body protein balance*

A common feature in both studies is the significant increase in protein delivery. Mean protein substrate intake during the high supplementation periods was 1.8 (1.20 – 2.77) g/kg/day. An exploratory analysis of all patients at baseline and after the 24 h intervention periods shows a significant correlation ($R^2 = 0.446$, p <0.001) between protein intake and net whole-body balance (<u>Fig. 19</u>). This dose-response relationship is consistent with previous findings from short-term tracer studies by our research group [200]. In addition, the observations from <u>studies III and IV</u> show that this response is sustained for up to 24 hours, both during enteral feeding and parenteral AA supplementation.
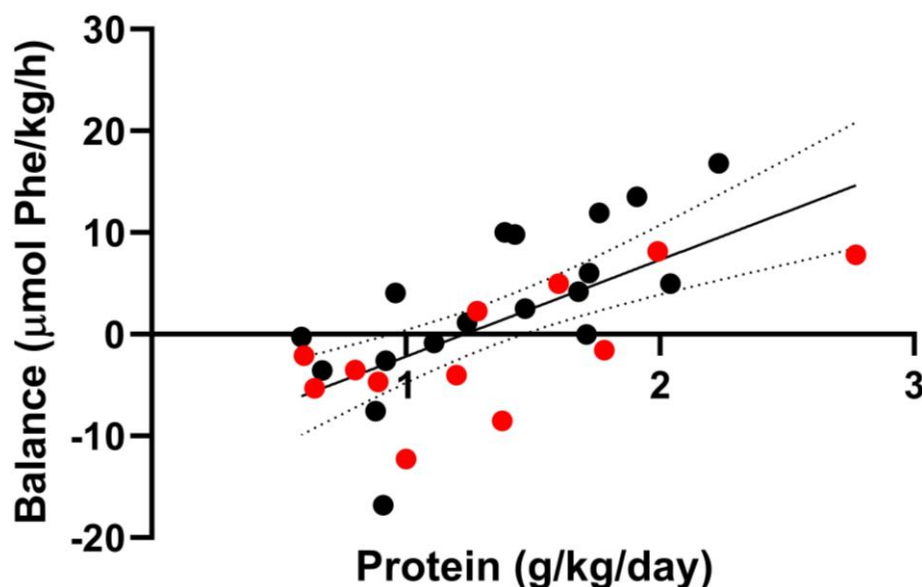
Figure 19. Whole-body protein balance in relation to protein intake in studies III and IV. Values included from baseline and after the 24 h intervention period with supplementation (Glavamin infusion or 100% enteral nutriton), N = 15 patients. Phe: Phenylalanine. Black dots: individual data points study III; Red dots: individual data points study IV; Solid black line: simple linear regression; Dashed lines: 95% confidence interval of regression line.

Although any form of post-hoc analysis needs to be interpreted with caution, two hypotheses may be drawn from linear regression in this data set:

i)   Mean protein intake to achieve a net neutral balance appears to lie somewhere between 1-1.5 g/kg/day. This is roughly equivalent to the recommendations in clinical guidelines, based on nitrogen balance studies and retrospective observational data correlating protein intake to survival [160].

ii) There is no obvious ceiling effect between protein delivery and net balance. Due to the limited number of patients receiving >2 g protein/kg/day, this observation is tenuous and would have to be confirmed in studies designed to investigate a dose-response relationship over a wider range of intakes. Data from small physiological investigations in patients with burns, trauma and sepsis indicate that intakes above 1.4-1.5 g/kg/day have no additional effect on protein synthesis or catabolism [201-203].

2. Is there a clinical benefit to a neutral/positive protein balance?

To be of utility as a surrogate outcome, any improvement in protein balance from an intervention during critical illness needs to

i)   result in the sparing of lean body mass or increased synthesis of proteins relevant to survival or functional outcomes, and

ii) be sustained over time.

An inherent limitation of the tracer dilution method is that it provides no information about the relative contribution of regional protein balance in different tissues towards an observed change in whole-body balance. Phenylalanine balance for individual patients in <u>studies III and IV</u> spanned between -4.0 and 16.8 µmol/kg/h during high protein delivery. Assuming a phenylalanine content of 4% in muscle protein, this would correspond to a change in hydrated lean body mass between -2.0 to 8.3 g/kg/day. It is implausible that any patient would gain significant muscle mass during ICU stay. Therefore, the major impact of supplementation is likely on tissues with higher baseline rates of protein turnover such as the splanchnic organs and immune system. The only study investigating the effects of nutrition on regional protein metabolism in muscle during critical illness is by Leverve from 1984 [204]. Their results demonstrate a lower femoral vein output of amino acids during TPN with a high protein content. Future tracer investigations in ICU patients should attempt to quantify the relative contribution from specific organ pools to the observed difference in whole-body protein kinetics.

The results from <u>studies III and IV</u> indicate that the improved protein balance from increasing protein delivery is sustained for up to 24 hours. We cannot rule out that this response would be attenuated over time by adaptation to higher intake. This phenomenon has been observed when measuring nitrogen balance during critical illness. Larsson found an improved daily protein balance with 0.65 g/kg/day compared to 1.3 g/kg/day in 39 patients with trauma and burns, but by day 8 there was no difference in cumulative balance [203]. The larger and more

recent EAT-ICU trial by Allingstrup and co-workers makes a similar observation [119]. 203 ICU patients were randomized to either standard nutrition or isocaloric energy provision and 1.5 g/kg/day of protein. Calculated balance was more positive in the intervention group on day 1, but the 95% confidence intervals of the mean difference spanned zero on day 3 and beyond. Also, the increase in protein and energy delivery did not result in functional improvement after six months. These findings indicate that critically ill patients with higher protein intake may use an increased proportion of amino acids for oxidative metabolism over time. We did not observe an increase in amino acid oxidation or serum urea, but this could be due to the relatively short observation period and small sample size. The Nephro-Protective trial by Doig and colleagues randomized 474 adult ICU patients to standard care or a daily supplement of i.v. amino acids similar to the dose in study III [162]. They observed a daily increase in serum urea over the first seven days in the intervention group (Supplement 2), which in the absence of difference in the duration of renal dysfunction indicates an increased amino acid oxidation. Amino acid supplementation did not result in the improvement of any tertiary functional outcome measures assessed after 90 days. In summary, further work is required to elucidate the intermediate-long term effects of protein or amino acid supplementation on skeletal muscle mass during critical illness. Physiological investigations that aim to characterize the metabolic changes in tissue protein balance can provide complementary information to clinical trials with patient-centered outcome measures.

3. *Is energy delivery an important determinant of whole-body protein balance?*

The primary aim of study IV was to investigate the difference in whole-body protein balance during full- or half-dose enteral nutrition, at a rate determined by measured energy expenditure. As energy delivery was manipulated by changing the feeding rate, the effects of increased calorie provision cannot be assessed separately from protein. Due to the prevalence of enteral formulas with a high protein/kcal ratio in our unit this also resulted in a large absolute mean difference in protein delivery (0.88 g/kg/day). This stands in contrast to a similar study protocol by Berg and co-workers using TPN in neurosurgical ICU patients, where mean protein delivery at full dose nutrition was 1.07 g/kg/day. Despite a large absolute difference in protein delivery, the observed mean difference in protein balance (7.3 µmol/kg/h) was comparable to that in study IV (9.0 µmol/kg/h). A possible explanation is that the response to increasing protein delivery is non-linear and proportionally greater at lower intakes.

Older studies in patients with trauma and sepsis have demonstrated a lower rate of protein catabolism during glucose infusions, but only in comparisons with a fasting basal state [72, 202]. It is possible that a calorie deficit corresponding to 50% of measured EE is of lesser importance for net protein catabolism given an adequate provision of protein substrate. In the largest multicenter trial to date of enteral calorie delivery, the TARGET investigators randomized ICU patients to 100% or 70% of estimated calorie requirements by enteral nutrition. Protein delivery was similar in both groups (1.1 g/kg/day). A six-month follow-up using patient self-assessment did not reveal any difference in mobility or performance of

usual activities [116]. Similar future trials investigating different levels of protein delivery independent of energy intake are highly warranted, and ideally be complemented by tracer studies or assessments of change in lean body mass.

An important aspect to consider regarding energy provision is the duration of critical illness and individual metabolic rate. For patients with a prolonged ICU stay over several weeks or months, it is more likely that an accumulated energy deficit will have negative consequences in terms of body composition and functional disability at discharge. In study IV, two patients had a length of stay ≥4 weeks and a measured REE of ≥40 kcal/kg/day. These subjects also displayed the greatest increase in protein balance during full-dose EN. While it is impossible to draw any general conclusions from two cases, it is a reasonable assumption that similar outliers may benefit from different nutritional regimes than the average ICU patient. As these patients are underrepresented in clinical trials, there is limited evidence to guide therapy. Tracer investigations in conjunction with indirect calorimetry have potential to identify and describe specific metabolic alterations in subgroups of critically ill patients, but also to evaluate the response to treatments. This is an area of great interest for future research in ICU metabolism.

4. *Limitations of studies III and IV*

Both studies in this section have several limitations in common. The main issues are the small sample sizes and heterogeneity of patients enrolled, which limits the external validity and potential for inference from our findings to a general ICU population. The reasons for this are primarily related to the methodology used:

i) Both protocols required a long period (28-48 hours) of relatively stable conditions and access to the patient by research staff. Protocol violations due to external clinical circumstances were therefore frequent, leading to a high dropout rate. This was particularly challenging in study IV. Further enrollment towards the recruitment target was halted due to a restructuring of the local pharmaceutical organization, resulting in a shortage of stable isotope tracers.
ii) There is a high cost of performing tracer studies, both in terms of materials, clinical research staff, laboratory facilities and personnel.
iii) The broad inclusion criteria with regard to age, anthropometric characteristics, acute disease states, comorbidities and duration of ICU stay increase heterogeneity of the patient sample. Despite this, most patients screened were not eligible to participate. Further limiting the scope of studies by narrowing inclusion criteria would likely have made recruitment even more difficult.

Other limitations of our method, such as the uncertain clinical importance of changes in whole-body protein balance, potential adaptations to changes in macronutrient intake over time and the general caveats of the stable isotope tracer dilution technique, have been discussed previously.

There was an imbalance in the ratio of male/female patients in studies III and IV. In study IV, protocol violations were more common in female patients. We could not determine any other reasons for this skewed sampling, beyond the fact that a higher proportion of men (~60%) were admitted to our ICU during the study periods [205]. For future investigations it is important to consider balancing recruitment with regard to sex, as this factor may influence metabolic alterations during critical illness.

Increasing macronutrient delivery has potentially harmful effects that were not considered in our studies. Other research groups have hypothesized that exogenous amino acids may be detrimental to ICU patients, mediated by the inhibition of autophagy [153, 164]. We did not attempt to measure the simultaneous effects of the interventions in studies III and IV on protein kinetics and autophagy regulation. Ideally this will be addressed in future studies.

5. Strengths of studies III and IV

The main strength of studies III and IV lies in the novel application of the stable isotope tracer method in this population. Our research group is the first to use a combination of enteral and intravenous tracers to quantify the contribution of enteral exogenous tracee in critically ill patients. Previous studies have been limited to assessing patients in a fasted state or during TPN, but early enteral feeding has since become standard care in ICU nutrition. Our method enables the quantification of whole-body protein balance, synthesis and breakdown in a modern ICU setting without restrictions in inclusion criteria that would limit external validity.

The studies in this thesis are also among the first to investigate the effect of nutritional interventions on whole-body protein balance, under circumstances resembling current clinical practice in intensive care. Earlier work has generally been limited to the transition from basal to fed state using glucose infusions or intravenous hyperalimentation with TPN [61, 72, 202, 206]. Prior studies by our own research group investigating enterally fed ICU patients have focused on short-term changes in protein kinetics from nutritional interventions [149, 150]. Despite the technical challenges of a longer protocol, studies III and IV demonstrate that it is possible to monitor the effect of macronutrient therapies on protein kinetics in enterally fed ICU patients over the course of 24-48 hours.

In study III we excluded patients with CRRT due to the uncertain loss of tracee over the hemodialysis membrane. In study IV we addressed this limitation by measuring and adjusting for the rate of phenylalanine accumulation in the effluent. As patients with acute kidney injury (AKI) represent a population subset with higher illness severity and increased risk of adverse outcomes, they are of particular interest when attempting to characterize metabolic alterations in critical illness. The successful inclusion of patients with dialysis-dependent AKI represents an advance in the execution of tracer studies in an ICU setting, and this method should be applied in future works.

7 CONCLUSIONS

The main conclusions of this thesis are summarized below:

- The agreement between Deltatrac and instruments using breath-by-breath technology in mechanically ventilated patients is variable. With the exception of one device, next generation indirect calorimeters have performance characteristics more closely aligned to the previous standard in monitoring than what is generally accepted in other fields (studies I and II).

- The supplementation of an amino acid infusion to standard nutritional therapy improved whole-body protein balance in critically ill patients for up to 24 hours. (study III).

- Providing full-dose compared to half-dose enteral nutrition in relation to energy expenditure improved whole-body protein balance in critically ill patients with established enteral feeding. The relative contributions of energy or protein intake to this result cannot be discerned (study IV).

- An increased provision of parenteral or enteral amino acids did not result in increased amino acid oxidation during the study periods (studies III and IV).

- Although not a pre-defined aim, we demonstrate that a stable isotope tracer protocol can be used in heterogenous samples of ICU patients to monitor changes in whole-body protein kinetics from nutritional interventions (study III and IV).

8 FUTURE PERSPECTIVES

The methods evaluated in this thesis provide information about individual physiology that is normally unavailable to the clinician. To be of value for the patient, this information must both be accurate and applicable in clinical decision-making.

Regarding the measurement of energy expenditure, we view the performance characteristics of commercially available indirect calorimeters as acceptable for bedside and research applications. The alternative, using anthropometric formulas in estimating energy targets, has been demonstrated to be highly inaccurate in multiple studies. An exploratory analysis of our own results supports these previous observations.

However, the consequences of over- or underfeeding for patient-centered outcomes are uncertain and not addressed in this thesis. Results from study IV indicate that matching intake to measured energy expenditure may improve whole-body protein balance. Due to the limited sample size and confounding factor of protein delivery, this conclusion should strictly be viewed as hypothesis-generating. Despite observed correlations between macronutrient deficits and mortality during critical illness, major RCTs have not found a clear signal indicating benefit or harm from increasing calorie provision in a general ICU population.

A measured interpretation of these results should acknowledge both the strengths and limitations of large-scale clinical trials with broad inclusion criteria and standardized interventions. Although the average effect of higher or lower energy targets can be estimated with a relatively high degree of certainty from available evidence, this may be insufficient to inform clinical practice in all cases. It is hypothetically plausible that patients with persisting hypermetabolism, poor baseline nutritional status and a prolonged ICU course are at increased risk of harm from an accumulated negative energy balance. Another aspect to consider is the consequence of energy deficits after ICU discharge. In clinical trials of ICU nutrition, patients typically receive the intervention for the duration of ICU stay. The time spent in regular hospital wards generally exceeds that in intensive care, and return to adequate oral intake after liberation from mechanical ventilation is often delayed [35]. Indirect calorimetry may have a role in optimizing nutritional support for patients with a long hospitalization and rehabilitation period after critical illness. These questions remain to be addressed in clinical trials.

For future research, indirect calorimetry is an important tool to further characterize the metabolic alterations during critical illness. As an example, the contribution of endogenous substrates to energy expenditure over time is still unknown but central to the conceptual model of a bi- or multiphasic stress response. Multiple tracer protocols using labeled glucose, glycerol or palmitate in combination with indirect calorimetry could potentially shed light on this matter. Longitudinal trends in energy expenditure during prolonged critical illness and recovery also remain to be described.

The tracer protocols used in this thesis also have promising future applications. Studies III and IV indicate that an increase in exogenous amino acids improves whole-body protein

balance in ICU patients. Although the small sample sizes warrant a cautious interpretation, this general trend is in accord with findings of previous kinetic investigations.

Heterogeneity, however, makes inference more problematic. Both studies have large between-patient variations in body mass, length of stay, admission diagnosis and metabolic rate. Future tracer research in intensive care should attempt to precisely define the study population of interest, narrowing inclusion criteria based on one or more of the characteristics listed above. Biomarkers or metabolomic profiles may potentially be of value to this end [207]. Combining tracer protocols with longitudinal assessments of body composition or muscle biopsies could also provide information relevant to the development of metabolic phenotypes as conceptual models, which can then be used to inform the design of adequately powered clinical trials. The main impediment to these types of in-depth physiological characterizations is the complexity in study design, limiting the number of patients eligible for inclusion and reducing sample sizes due to high costs. This may lower external validity and risks yielding granular information only applicable to individual patients. Cooperation between research groups and a common international research agenda on ICU metabolism is probably necessary to move beyond these issues and towards a potential future of precision medicine.

Another question raised by studies III and IV is the effect of nutritional interventions on regional protein balance, particularly in skeletal muscle. This could be answered by combining whole-body protein kinetics with a lower limb arteriovenous balance technique during different levels of protein substrate delivery. The potential for combined or synergistic effects on skeletal muscle protein balance using amino acid supplementation and exercise is also an intriguing area of research. Currently there is at least one clinical trial investigating the effects of combined in-bed cycling and parenteral amino acids on functional disability and muscle cross-sectional area in ICU patients [208]. Stable isotope tracers in combination with biopsies could provide additional information regarding the physiological effects of these interventions on skeletal muscle, and aid in the interpretation of observed patient-centered outcomes.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Människans fysiologi är en konsekvens av miljontals år av evolution. Mekanismer för hur vi hanterar påfrestningar från omgivningen är centrala för vår överlevnad, och är därför starkt bevarade över artgränserna. Ett sådant fenomen är de omfattande förändringar i ämnesomsättning som inträffar vid svår akut sjukdom. För att hantera vävnadsskada, infektion eller syrebrist på ett ändamålsenligt sätt behöver kroppen öka aktiviteten i flera organ, framför allt immunförsvaret. Denna omställning är en energikrävande process. Kroppen tillhandahåller energi och aminosyror för proteinsyntes genom att bryta ner befintliga reserver av fettväv och protein, ibland med en uttalad förlust av skelettmuskulatur som konsekvens.

För patienter som behöver intensivvård på grund av svikt i vitala funktioner är detta katabola tillstånd ett stort problem. Det är väl dokumenterat att patienter snabbt förlorar muskelmassa under intensivvården. Detta riskerar att bidra till svaghet och funktionsnedsättning, som kan vara bestående under flera års tid. Ett möjligt sätt att minska dessa skadeverkningar är genom särskild hänsyn till patienters näringsintag. Omfattande kliniska prövningar har hittills inte visat någon effekt på överlevnad eller funktionsnivå genom högre eller lägre energitillförsel under intensivvårdsförloppet. Generellt rekommenderas en hög proteintillförsel vid kritisk sjukdom, men det vetenskapliga underlaget för sådana rekommendationer är svagt.

En brist i nutritionsforskning inom intensivvård är att allmänna åtgärder ofta utvärderas utan hänsyn till individens fysiologi. Energibehovet hos svårt sjuka patienter är mycket varierande, men kan uppskattas genom att mäta syrekonsumtion och koldioxidproduktion, så kallad indirekt kalorimetri. Dessa mätningar sker sällan rutinmässigt inom intensivvården. Ett hinder är att kommersiellt tillgänglig utrustning är otillräckligt utvärderad i denna patientgrupp. Det är också ofullständigt kartlagt om energitillförseln i relation till uppmätt förbränning har en proteinsparande effekt, samt hur intensivvårdspatienter hanterar extra tillförsel av protein eller aminosyror på helkroppsnivå. Detta avhandlingsarbete syftar till att förbättra kunskapsläget i metoder för att utvärdera ämnesomsättning vid kritisk sjukdom. Det avser också att stärka vår insyn i de fysiologiska effekterna av energi- och proteintillförsel vid intensivvård.

Huvudfrågeställningen för studie I och II var hur väl det mest beprövade instrumentet för indirekt kalorimetri, Deltatrac (Datex-Ohmeda, Helsingfors, Finland), överensstämde med andra mer moderna instrument vid jämförelser i ventilatorbehandlade intensivvårdspatienter.

I studie I jämfördes Deltatrac med Quark RMR (Cosmed, Rom, Italien) och CCM Express (Medgraphics Corp, St Paul, Minneapolis, USA). Intensivvårdspatienter med invasiv respiratorbehandling och syrgasfraktion ≤0.5 rekryterades till studien. Mätningar av vilande energiförbrukning utfördes i följd med samtliga instrument i slumpmässig ordning. Det genomsnittliga värdet för uppmätt energiförbrukning skiljde sig inte mellan Deltatrac och Quark RMR. CCM Express mätte i genomsnitt cirka 60% högre värden än de andra

instrumenten. Orsaken till skillnaderna mellan instrumenten kunde inte utrönas inom ramen för den aktuella studien.

I studie II jämfördes gasutbytesmätningar mellan Deltatrac och ett nytt instrument för indirekt kalorimetri, E-sCOVX (GE Healthcare, Helsinki, Finland). Då vi använde en uppdaterad metodologi utfördes dessutom en ny jämförelse mellan Quark RMR och Deltatrac. Till skillnad från studie I genomfördes mätningarna med referensmetoden och de nyare instrumenten samtidigt. Större hänsyn togs till att utesluta felkällor och data samlades in elektroniskt för granskning och bearbetning. Resultaten visade att överenstämmelsen med Deltatrac var i stort sett likvärdig för Quark RMR och E-sCOVX. Spridningsmåttet vid jämförelserna var lägre än för andra vedertagna mätmetoder inom intensivvård. Vi kunde inte bestämma varför skillnaderna mellan instrumenten var större vid vissa mätningar, men tillverkarnas eftergranskning av datan gav upphov till hypoteser som bör beaktas vid utveckling av ny teknik.

Studie III och IV tillämpade en så kallad tracer-metod för att undersöka effekten av näringstillförsel på förändringar i proteinomsättning hos intensivvårdspatienter. Isotopmärkta aminosyror ("tracers"), med annorlunda molekylär vikt men i övrigt samma struktur och funktion som de mest vanligt förekommande naturliga motsvarigheterna, gavs intravenöst och via en sond i magsäcken under studiernas gång. Genom upprepad provtagning och analyser av kvoten mellan tracers och vanliga aminosyror i blodbanan kan hastigheten för nedbrytning och syntes av kroppseget protein uppskattas. Utifrån dessa storheter kan netto-balansen beräknas, det vill säga om patienten till större del bygger upp eller bryter ner funktionella proteiner.

Studie III undersökte huruvida vuxna intensivvårdspatienter kan tillgodogöra sig extra aminosyror under en längre tids observation. Efter en baslinjemätning av proteinkinetik gavs en kommersiellt tillgänglig intravenös aminosyrablandning (Glavamin) i 24 timmar. Proteinbalansen bestämdes på nytt efter tre timmars infusionstid samt ett dygn senare. Resultaten visade att patienterna i genomsnitt förbättrade sin proteinbalans, från negativ till positiv, och att förändringen var bestående under hela infusionstiden. Estimerad förbränning av aminosyror ökade inte heller under studietiden. Studien kompletterar en tidigare undersökning av vår forskargrupp som visade en positiv effekt på helkropps proteinbalans med samma intervention i upp till tre timmar.

I studie IV ville vi undersöka om full (>80% av uppmätt energiförbrukning) energitillförsel via sondmat resulterade i en bättre proteinbalans jämfört med om halva dosen administrerades. Patienter med respiratorbehandling och etablerad sondnäring fick ingå i studien. Proteinkinetik bestämdes efter 24 timmar med full dos respektive halv dos av sondmat. Ordningen avgjordes via randomisering och patienterna fick tjäna som sina egna kontroller i en s.k. cross over-design. Vid full dos nutrition var både kalori- och proteintillförseln betydligt högre än vid halv dos. Helkropps proteinbalans förbättrades också, från i genomsnitt netto negativ till positiv. Till skillnad från studie III hade protokollet modifierats för att omfatta patienter med kontinuerlig hemodialys, vilket aldrig tidigare

genomförts i tracer-studier. Den beskrivna metoden är relevant för framtida användning då patienter med akut njurskada utgör en särskilt sårbar grupp av intensivvårdsfall.

Sammanfattningsvis kunde vi visa att flera av den nya generationens instrument för indirekt kalorimetri ger resultat jämförbara med den metod som ofta anses vara mest tillförlitlig i en intensivvårdskontext. Resultaten från tracer-studierna antyder också att intensivvårdspatienter kan tillgodogöra sig tillförd näring för att minska nettoförlusten av kroppseget protein. Både tillförseln av intravenösa och enterala aminosyror, samt kaloriinnehåll i näringen, kan vara av betydelse i detta avseende. Metoderna som använts visar också på möjligheten att undersöka effekten av näringsterapi på proteinkinetik under ett brett spektrum av kliniska omständigheter i en modern intensivvårdsmiljö. Både indirekt kalorimetri och tracerstudier är centrala redskap i framtida arbeten som syftar till en djupare förståelse av metabolism- och näringsfysiologi vid kritisk sjukdom.

10 ACKNOWLEDGEMENTS

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12 ERRATA TO PUBLISHED PAPERS

Study I

P. 119, section 2.1, first paragraph:

"…extracorporeal machine oxygenation…" should read "…extracorporeal _membrane_ oxygenation…".

P. 119, section 2.3.1, first paragraph:

"The air exhaled by the patient…" should read "The _gas_ exhaled by the patient…"

P. 120, section 3.1, first paragraph:

"Repeated readings of REE from the same instrument gave a coefficient of variation for…" should read "Repeated readings of REE from the same instrument gave a _percentage difference between the first and second measurement (mean ±SD)_ for…"

P. 120, Fig. 2:

The solid black dots in both Bland-Altman plots are displaced duplicate values. The circled white dots indicate the correct values.

Study II

P. 6 of 9, Discussion, fifth paragraph:

"…a precision (coefficient of variation (CV)) of 20 %." should read "…a precision (=_2*_coefficient of variation (CV)) of ±20 %." All following references in the text to "precision" should refer to this definition (2*CV).

P. 7 of 9, Discussion, sixth paragraph:

"This was based on observational data derived from a study of supplemental parenteral nutrition…" should read "This was based on _an observation_ derived from a study of supplemental parenteral nutrition…

Study III

P. 3 of 8, Statistics:

"… a recruitment target of n = 10 was determined sufficient to assess the primary endpoint with 80% power…" should read "…a recruitment target of n = 10 was determined sufficient to _detect a change in the primary outcome from negative to net zero_ with 80% power…"

P. 5 of 8, Table 2, column "Completed protocol (n = 8)":

Row "AA intake baseline (g/kg/24 h)": 1.11 (0.59-1.72) should read 1.11 (_0.71_-1.72). Row "AA intake with IV amino acids (g/kg/24 h)": 2.07 (1.56-2.68) should read 2.07 (_1.68_-2.68).