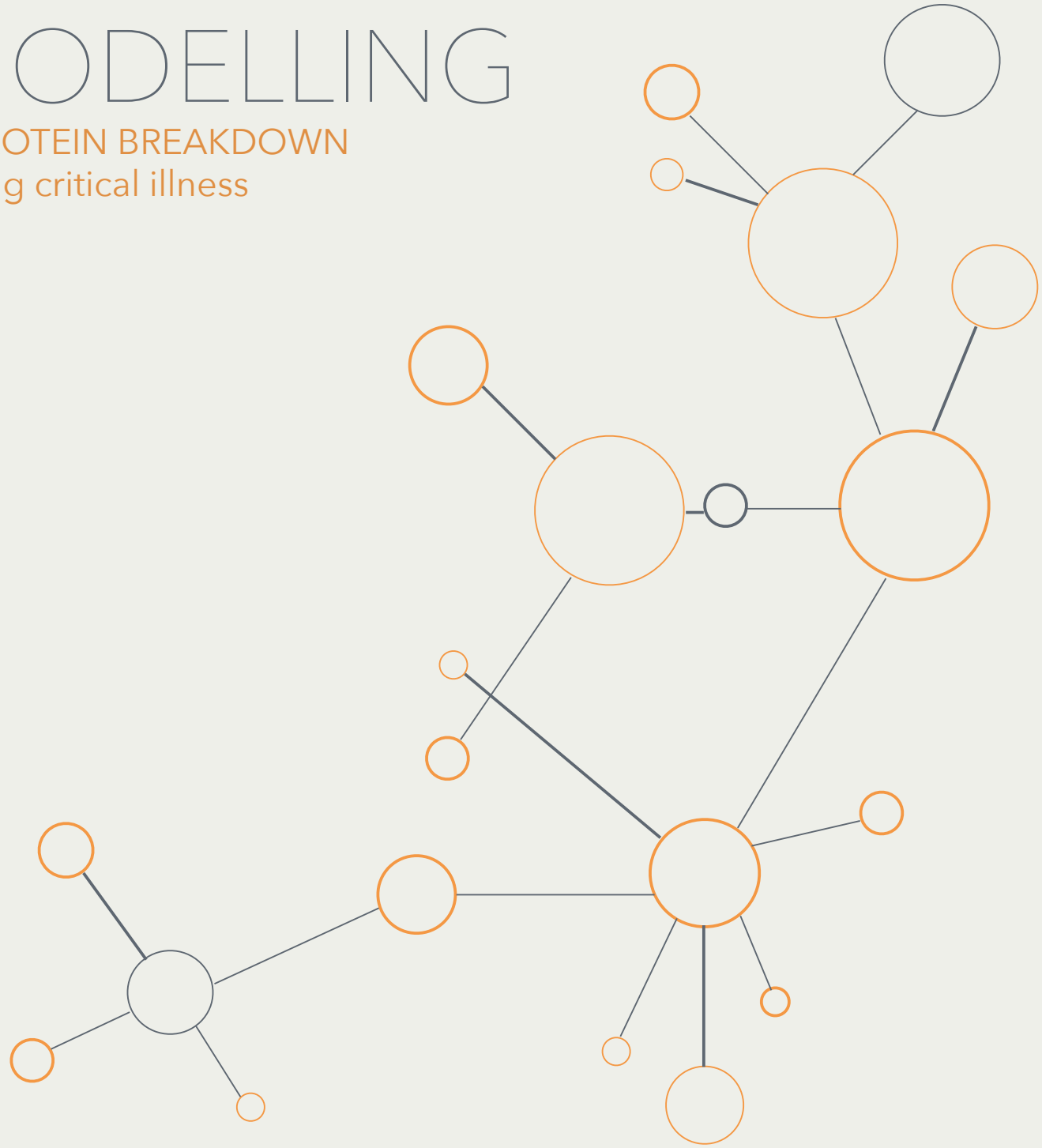
MODELLING

of PROTEIN BREAKDOWN during critical illness



Master's Thesis, Biomedical Engineering & Informatics
June 3, 2015

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Preface

The thesis was performed by group 15gr1072 in the time period from the 1st of February 2015 to the 3rd of June 2015. The project is performed as the master's thesis within Biomedical Engineering and Informatics at Aalborg University.

Reading Guide

Source references in the report will be listed according to the Harvard method, with given [Surname of author, Publication year] in the text. All references are collected in the bibliography at the end of the project and listed alphabetically.

If no reference is given for a figure or table in the report, then these have been created by the project group. Tables and figures are numbered according to their occurence in the chapter in question, e.g. the first figure in chapter 2 will have the reference number 2.1. Any abbreviations used in the report are defined at first occurrence and placed in brackets.

Mette Evald

Kasper Aarup Houlberg

Resumé

Tab af muskelmasse er et problem for kritisk syge patienter indlagt påintensivafdelinger, da dette kan have alvorlige konsekvenser for kritisk syge patienters helbred pålængere sigt. Muskeltabet efterlader patienterne i en svækket tilstand, som medvirker til forlænget sygdomsophold og forøget mortalitet efter udskrivelse fra intensivafdelingen. Dette tab af muskelmasse skyldes særligt en hypermetabolsk respons påden kritiske sygdom. Protein ernæring har vist sig at være et vigtigt element til at mindske tabet af skeletal muskelmasse, dog uden at kunne forhindre protein tab fuldstændigt. Estimation af nitrogen balance er over en lang periode blevet anvendt til at estimere protein tab for patienter. Denne metode er dog blot et estimat og kan ikke tage højde for al nedbrydelse af proteiner i kroppen under kritisk sygdom. Da der ikke findes metoder til at estimere det enkelte individs muskelmassetab over en indlæggelsesperiode påen intensiv afdeling, har målet med dette projekt været:

1) At forståfysiologien, der ligger til grund for det metabolske stress, som kritisk syge patienter oplever og dennes effekt påprotein nedbrydelse.
2) At indsamle klinisk data, der kan repræsentere den fysiologiske stress respons og heriblandt muskel proteolyse, som er at finde ved kritisk sygdom.
3) At anvende den tilegnede viden og data til at definere en model, til repræsentation af metabolsk stress over indlæggelsen for den kritisk syge patient.

Stress responsen ved kritisk sygdom kan typisk indeles i en hypometabolsk 'ebb', hypermetabolsk 'flow' fase, og endelig en rekonvalescens fase. I flow fasen, defineret ved et forøget energiforbrug, vil tab af muskelmasse forekomme, for at bidrage til energiforbruget. Protein fra muskler anvendes i en gluconeogenetisk process, hvor protein, lactat og glycerol omdannes til glucose, der vil frigives til blodcirculationen og optages i kroppens celler for at danne energi.

Data for intensivpatienter blev indhentet den kliniske database MIMIC II. Fra denne blev 123 patienter, med i alt 134 indlæggelsesforløb, ekstraheret. Visse stress parametre kunne ekstraheres fra MIMIC II, men data om stress parametre som eksempelvis cortisol kunne ikke indhentes. Patienternes energiforbrug (REE) blev estimeret med prediktionsligninger påbaggrund af ekstraherede patientspecifikke parametre. Grundet det begrænsede antal parametre der kunne ekstraheres fra databasen, blev målet med den opstillede model justeret.

En fysiologisk kompartment model blev opstillet med formålet at beskrive anvendelsen af amino syrer fra proteinnedbrydelse til gluconeogenese i den kritisk syge patient.

Fremadrettet arbejde bør ligge i indsamling af data til temporal analyse af metabolsk stress og muskelnedbrydelse for kritisk syge patienter. Målinger af stress hormoner, særligt cortisol, i kombination med mål for protein nedbrydelse, ville være af stor værdi for videre arbejde med modellering af metabolsk stress.

Contents

Chapter 1

Introduction

In earlier years, the discharge of a patient from an intensive care unit (ICU) was viewed as a successful ending to a course of disease. The patient was brought back from the brink of critical illness and was now a survivor. However, spending time in an ICU as a critically ill patient may result in long-term consequences, which eventually may become the cause of post-discharge mortality. The focus of critical care medicine has therefore shifted from short-term to long-term outcomes. Patients must be treated for survival in a time-span reaching beyond the time spent within the ICU ward. This requires actions against the critical illness, but also a minimization of sequelae related to the ICU stay [Wischmeyer, 2013], [Vincent and Norrenberg, 2009], [Lee and Fan, 2012].

Critically ill patients are known to suffer from muscle protein breakdown (proteolysis), leaving patients in a weakened state referred to as ICU acquired weakness (ICUAW). This is one of many potential sequelae of critical illness, which may show consequences both during ICU stay and after discharge. Consequences of ICUAW may be increased morbidity in terms of prolonged mechanical ventilation and prolonged hospital stay. Long-term consequences may appear as loss of lean body mass and lack of physical activity, resulting in weakness and immobilization. Patients may never return to previous levels of physical abilities, and studies have shown poorer quality-of-life scores for prior ICU patients due to degradation of physical function. Muscle wasting is therefore considered one of the most devastating consequences of critical illness [Weijs and Wischmeyer, 2013], [Wischmeyer, 2013], [Puthucheary et al., 2010], [Preiser et al., 2014].

Proteolysis is an attribution to the metabolic abnormalities experienced by critically ill patients. The energy demands of patients increase during critical illness, forcing the body to alternate between metabolic pathways of energy production depending on the availability of energy substrates. First-choice glucose reserves are quickly depleted (within 24 hours), requiring an utilization of other energy substrates, such as muscle protein, to maintain energy production. Proteins from skeletal muscle are also degraded for protein synthesis, providing new proteins to be applied in inflammatory and immunological processes. These catabolic factors combined with patient inactivity during ICU stay may synergistically accelerate skeletal muscle wasting [Preiser et al., 2014], [Berg et al., 2006], [Biolo, 2013].

Severe muscle wasting from proteolysis requires retaliatory actions, in the form of protein administration, to minimize this sequelae of critical illness. Studies by Shaw et. al. have shown that body protein catabolism continues, even though protein was administered to sepsis and trauma patients through parenteral nutrition. Protein administration did, however, have a tissue sparing effect by promoting protein synthesis [Shaw et al., 1987], [Shaw and Wolfe, 1989].

A patient's nitrogen balance has traditionally been used to reflect the difference between rate of protein breakdown and protein synthesis. From this balance, the minimum protein

administration can be derived as the lowest rate of nitrogen loss through urea formation. However, the body's utilization of skeletal muscle protein for protein synthesis, due to the stress condition of patients, is not reflected in the nitrogen balance. Therefore, a greater protein administration in stress conditions may be required to reflect this protein loss from skeletal muscle. Knowledge about muscle turnover, in regards to adaptation during critical illness, is however limited [Biolo, 2013], [Preiser et al., 2014], [Puthucheary et al., 2010].

Estimation of muscle proteolysis in adaptation to critical illness could be pursued from a modelling approach. A model of specific human physiology may present a picture of physiological behaviour, potentially varying over time in relation to inter- and intra-patient variability [Chase et al., 2011]. Modelling physiological structures and processes affected by stress parameters may provide a picture of the physiological behaviour of critically ill patients and the interconnectivity between stress-related parameters. Presuming a connection between muscle proteolysis rate and the stress condition of ICU patients, a physiological picture of muscle turnover may be formed from modelling stress parameters and proteolysis interconnectivity.

1.1 Research Objectives

Methods to determine the individual magnitude of muscle wasting through the process of critical illness are still unavailable. Muscle proteolysis may be estimated in relation to stress parameter values, varying over time, in a physiological model. The model must represent relevant physiological structures and their behaviour in relation to muscle proteolysis and stress parameter development (e.g. energy expenditure). Model behaviour may be stratified from clinical data or literature related to relevant model structures and parameter kinetics. Research objectives are therefore:

- Understand the underlying physiology of stress related to critical illness and the interconnections with muscle protein breakdown.
- Gather clinical data relevant to physiologic consequences of critical illness and subsequent muscle proteolysis.
- Apply acquired knowledge of physiology and clinical data to define model structures and parameter kinetics relating to muscle proteolysis and stress conditions of the critically ill patient.

Part I

Physiological Background

Chapter 2

Metabolism of the Human Body

Keeping the human organism alive requires energy, which may be obtained through the progression of different metabolic pathways. During critical illness, the chosen pathways of energy production are altered to support increased energy demands. The current chapter will provide an introduction to the human metabolism in terms of substrate utilization and product outcome by central metabolic pathways. This physiological knowledge will provide a foundation for understanding the activated metabolic pathways and substrate appearances during critical illness presented in the subsequent chapter.

2.1 General Metabolic Concepts

For the human body to maintain homoeostasis, i.e. a state of internal equilibrium, energy is required. Energy is generated and utilized through a series of chemical reactions collectively referred to as a persons **metabolism**. Metabolic reaction pathways create a balance between breaking down substrates and building these up or storing these, which is demonstrated by Figure 2.1 [Martini and Nath, 2009].

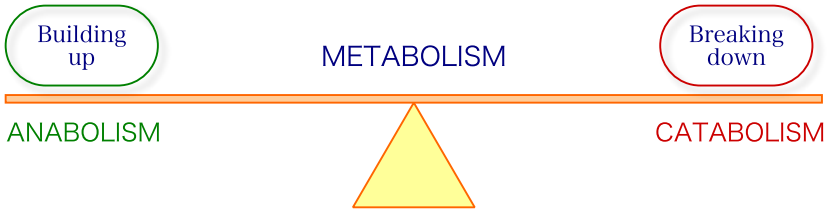

Figure 2.1: *Indexation of pathways in cellular metabolism, inspired by [Khan Academy, 2013].*

A metabolic pathway may be classified as either **catabolic** or **anabolic**. During catabolism, organic molecules are broken down to release cellular energy for adenosine triphosphate (ATP) synthesis, cf. Equation 2.1. Anabolic processes, cf. Equation 2.2, apply generated ATP and other precursors for synthesis of new organic molecules and other cellular functions [Berg et al., 2006].

$$\text{Substrate(carbohydrate, fat)} \xrightarrow{catabolism} \text{CO}_2 + \text{H}_2\text{O} + \text{energy} \tag{2.1}$$

$$\text{Energy} + \text{simple precursors} \xrightarrow{anabolism} \text{complex molecules} \tag{2.2}$$

Generation of energy can be divided into three catabolic stages, depicted in Figure 2.2. At stage I larger substrates from foodstuffs or cell reserves are hydrolyzed into smaller molecules such as fatty acids, glucose, and amino acids. This stage is strictly preparatory and does not yield any useful energy. At stage II some of these smaller molecules are broken

down even further to the acetyl unit of acetyl CoA for final mitochondrial processing when oxygen is present. ATP is generated by the catabolic processes performed at this stage. However, this amount of ATP is small compared to the output obtained from the third stage. At stage III the acetyl unit enters the citric acid cycle (TCA) within the mitochondria. Here, acetyl units are oxidized to CO_2, transferring four pairs of electrons for each acetyl unit to NAD^+ and FAD. These electrons are used for reduction of molecular O_2 to H_2O through oxidative phosphorylation, releasing a large amount of free energy for ATP synthesis [Berg et al., 2006]. Acetyl CoA enters the aerobic pathway consisting of the TCA and the electron-transport chain performing oxidative phosphorylation, where processing yields an amount of 28 ATP.

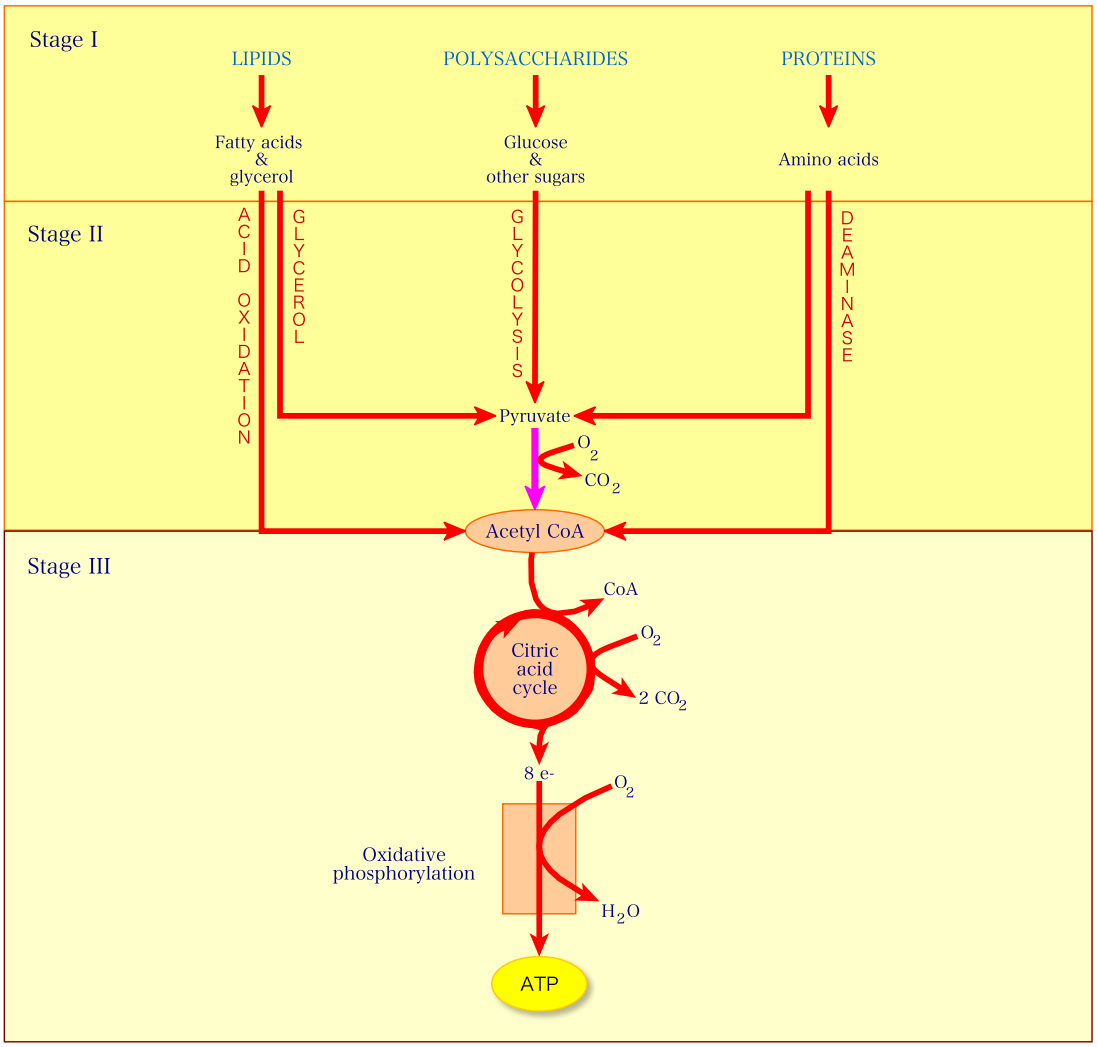

Figure 2.2: *Subdivision of catabolic processes, edited from [Berg et al., 2006].*

Glucose molecules are most often broken down to generate ATP, succeeded by fatty acids. Amino acids are usually conserved in the pool of available nutrients, since these are more often needed to synthesize new cell compounds. Amino acids may, however, be catabolized as a "last-ditch" energy source in situations of critical illness or starvation [Martini and Nath, 2009]. In the following sections, the catabolic processes of stage II depicted in Figure 2.2 will be described in more detail.

2.1.1 The Breakdown of Glucose Through Glycolysis

Glucose is a very important metabolic fuel, serving as the primary energy source for the brain and as a source of energy for cells throughout the whole body. This fuel presents itself to the body through food intake or from glycogen reserves located predominantly in the liver and skeletal muscles. The initial steps to generate energy from glucose take place in the **glycolysis** process where one glucose molecule is catabolized to two pyruvate molecules, giving a net production of two ATP molecules [Berg et al., 2006], [Martini and Nath, 2009]:

$$\text{Glucose} + 2\ \text{NAD} + 2\ \text{ADP} + 2\ \text{P}_i \xrightarrow{glycolysis} 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} \quad (2.3)$$

Firstly, glucose enters cells of the body by means of glucose transporters. There are different types of glucose transporters, each having a distinct role; $\text{GLUT}_{1,3}$ are responsible for the basal glucose uptake, i.e. these transporters continuously flux glucose into cells at a constant rate. GLUT_2 is present in the liver and pancreatic β cells and transports glucose into these cells at a significant rate only when glucose levels are high in the blood. $\text{GLUT}_{1,2,3}$ are all independent of insulin. GLUT_4 transports glucose into muscle and fat cells, especially in the presence of insulin, promoting the uptake of glucose. The GLUT_4 transporter is therefore insulin dependent [Berg et al., 2006].

After glucose has entered a cell, the glycolysis process can proceed in three stages, cf. Figure 2.3. In stage I) glucose is converted into fructose 1,6 bisphosphate through an initial phosphorylation to trap the glucose molecule inside the cell and thereafter a second phosphorylation to ready the fructose molecule for separation. Each phosphorylation costs the cell one ATP molecule. In stage II) fructose 1,6 bisphosphate is cleaved into two three-carbon units to be applied for the final ATP harvest in stage III. Dihydroxyacetone phosphate is not on the direct pathway of glycolysis like glyceraldehyde 3-phosphate, however these compounds are readily interconverted. Hereby, the dihydroxyacetone phosphate molecule can be converted for further processing, why stage III in Figure 2.3 happens twice (x2). Energy is extracted intermediately in stage III when the two carbon units each are converted into a pyruvic acid molecule. Two ATP molecules are generated for each three-carbon molecule, providing a total net sum of two ATP molecules from the glycolysis process [Berg et al., 2006], [Martini and Nath, 2009].

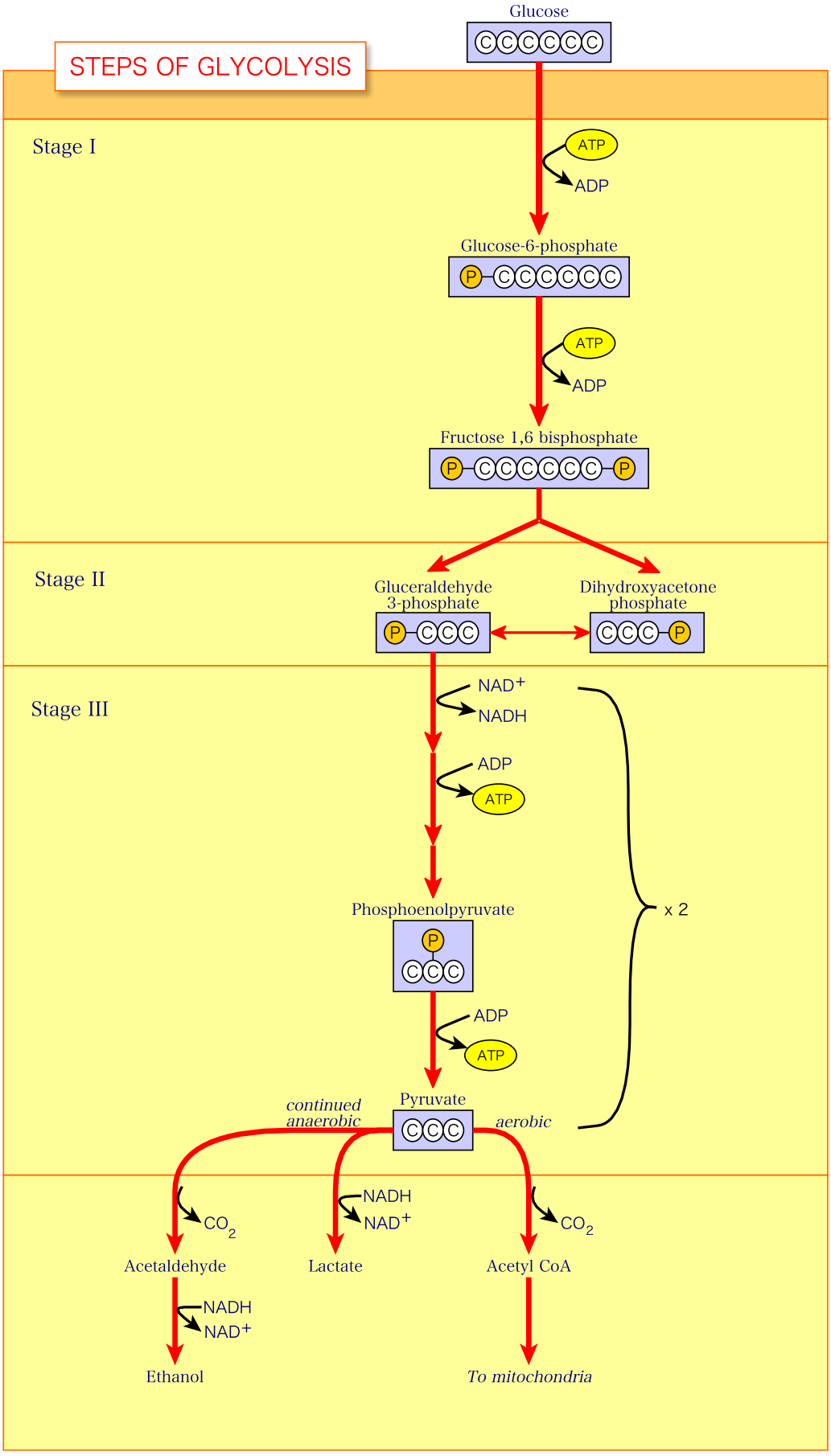

Figure 2.3: *Subdivision of the glycolysis process, inspired by [Berg et al., 2006] and [Martini and Nath, 2009].*

In Figure 2.3 the diverse fates of the generated pyruvate molecules are depicted in the final box. The fates of pyruvate depend on the availability of oxygen; whether oxygen is present (aerobic) or if oxygen is lacking (anaerobic). If oxygen is available to the cell mitochondria, much more energy may be harvested from the synthesized pyruvate molecules. During aerobic conditions, pyruvate may be transported into mitochondria and thereafter oxidatively decarboxylated to form acetyl CoA, cf. Equation 2.4. This chemical reaction is irreversible and links the glycolysis process to the TCA cycle in Figure 2.2 [Berg et al., 2006].

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoA} \xrightarrow{\textit{pyruvate dehydrogenase complex}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} \quad (2.4)$$

When oxygen is unavailable to the cell mitochondria, pyruvate must instead convert to other cell products to keep glycolysis running. Under anaerobic conditions, alcoholic and lactic acid fermentations take place:

$$\text{Glucose} + 2\ \text{P}_i + 2\ \text{H}^+ + 2\ \text{ADP} \rightarrow\ 2\ \text{ethanol}\ + 2\ \text{CO}_2 + 2\ \text{ATP} + 2\ \text{H}_2\text{O} \quad (2.5)$$

$$\text{Glucose} + 2\ \text{P}_i + 2\ \text{ADP} \rightarrow\ 2\ \text{lactate}\ + 2\ \text{ATP} + 2\ \text{H}_2\text{O} \quad (2.6)$$

NADH is reoxidized to NAD^+ through these processes, even though NADH and NAD^+ are not present in the equations above due to a lack of net oxidation-reduction. Regenerated NAD^+ sustains the continued process of glycolysis, cf. equation 2.3, and lactic acid and ethanol are the bi-products of the fermentations.
Lactate is produced through glycolysis in skeletal muscles, brain, erythrocytes etc., but the product is a dead end in metabolism. Lactate must be converted into pyruvate before it can be metabolised, which can be done in well-oxygenated cells. E.g. during strenuous exercise, skeletal muscles produce lactate through the anaerobic path of glycolysis and transport this out of the muscle cells to metabolize in other tissues such as the liver and kidneys. Hereby, the lactate metabolising burden is shifted to other organs than skeletal muscle cells, which lack oxygen during strenuous exercise [Berg et al., 2006].

2.1.2 The Breakdown of Lipids

Lipids, such as triacylglycerols, are an important energy reserve to the body. Carbohydrates are firstly applied for energy production, but glucose reserves are depleted within 24 hours, where-after lipid catabolism can take over to provide the required energy of the body for several weeks. Lipid reserves are difficult to access and many lipids are processed within the mitochondria, which depends on oxygen, why carbohydrates are applied first for energy production. Lipids are stored in adipose tissue and may be broken down through lipolysis to form fatty acids and glycerol. In Figure 2.4 the fates of these lipolysis products are illustrated [Berg et al., 2006], [Martini and Nath, 2009].

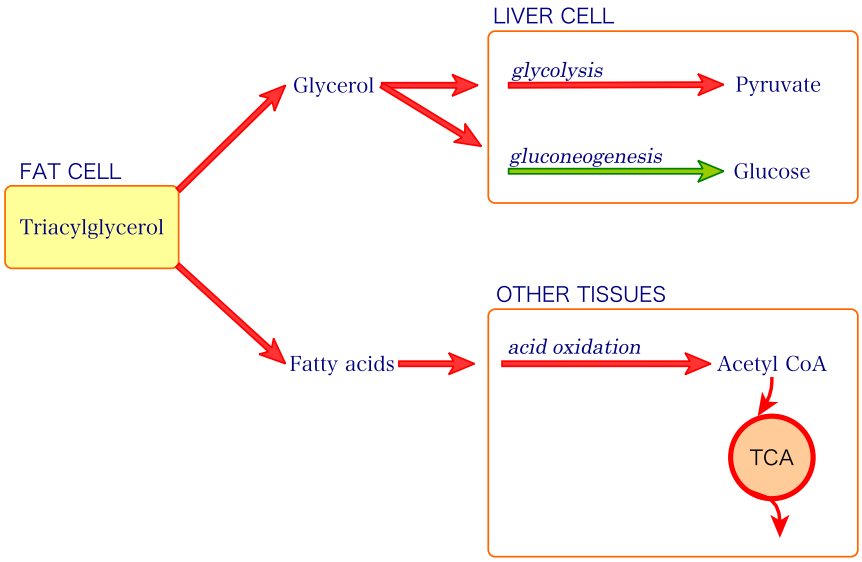

Figure 2.4: *The fates of lipolysis products, from [Berg et al., 2006].*

Fatty acids are transported to other tissues by an albumin carrier and thereafter oxidized in a series of steps, yielding carbon chains that enter the TCA cycle as acetyl-CoA. Substantial energy is gained from catabolizing a 18-carbon fatty acid, exactly 144 ATP molecules, but this energy cannot be generated quickly like that from glucose catabolism. Glycerol is transported to the liver for oxidation into dihydroxyacetone phosphate. As seen in Figure 2.3, this molecule may be converted into pyruvate through glycolysis or may be turned into glucose through gluconeogenesis described in later sections [Berg et al., 2006].

2.1.3 The Breakdown of Body Proteins

The final substrate, responding to the metabolic demands of the body, is protein. Proteins are a construction of amino acids, where different combinations of the same 21 amino acids give the protein its varying form, function, and structure. Ten of the amino acids are essential to the body, implying that these must be provided exogenously for the body to function properly.

Proteins are primarily degraded and resynthesized in response to the bodies changing metabolic demands. The constant degradation of proteins into free amino acids provides building blocks for synthesizing new proteins that will e.g. activate or shut down a signalling metabolic pathway. The degradation of proteins consists partly of a ***transamination*** process, performed by cells in many different tissues, where there is an exchange of functional groups between an amino acid and a ketoacid. An example of a transamination process is depicted in Figure 2.5, where the amino group (NH_3) is removed from alanine and attached to α-ketoglutarate. This converts α-ketoglutarate into glutamate, which may leave the mitochondria for protein synthesis elsewhere. The original alanine amino acid is converted to a ketoacid applied in the TCA cycle for ATP production [Martini and Nath, 2009], [World Health Organisation, 2007].

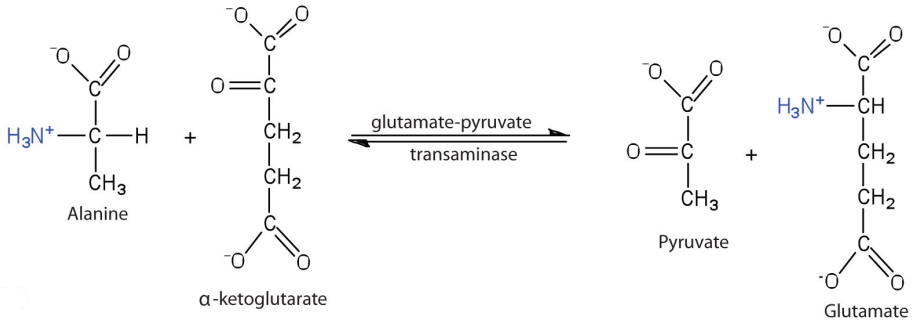

Figure 2.5: *Transamination of alanine into glutamine [Larsen, 2015].*

Secondarily, amino acids may be catabolized by the cell mitochondria in order to generate ATP. The catabolic process on amino acids is in this case termed ***deamination***, depicted in Figure 2.6. In this example, deamination removes the amino group and a hydrogen ion from glutamate, converting glutamate to a ketoacid for mitochondrial processing. A bi-product of the deamination process is an ammonium ion (NH_4+), which is highly toxic for cells. Therefore, deamination primarily occurs in the liver where an enzyme uses the ammonium ion to synthesize urea in the urea cycle. Urea is a harmless water-soluble compound found in urine.

Deamination of proteins will occur if there is an excess of amino acids not required for biosynthesis. These amino acids cannot be stored like glucose as glycogen and fatty acids as triacylglycerols, but must be converted into a metabolic intermediate. If glucose and lipid energy resources are scarce, like during critical illness, then amino acids are applied for ATP production. Extensive deamination threatens homoeostasis by applying proteins for ATP production instead of structural and functional purposes in the cell, why deamination of amino acids is the body's last energy resource when other energy sources are unavailable. [Martini and Nath, 2009].

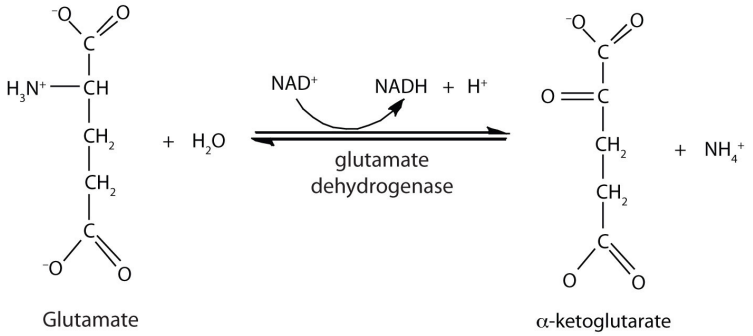

Figure 2.6: *Deamination of glutamate [Larsen, 2015].*

So, the breakdown of protein molecules creates a pool of free amino acids applied primarily for protein synthesis and secondarily to be degraded to carbon skeletons applied as a metabolic intermediate for regulatory purposes or oxidation in the TCA cycle. An illustration of amino acid flow in the human body is seen in Figure 2.7 and Figure 2.7 presents some of the different amino acids applied for certain processes.

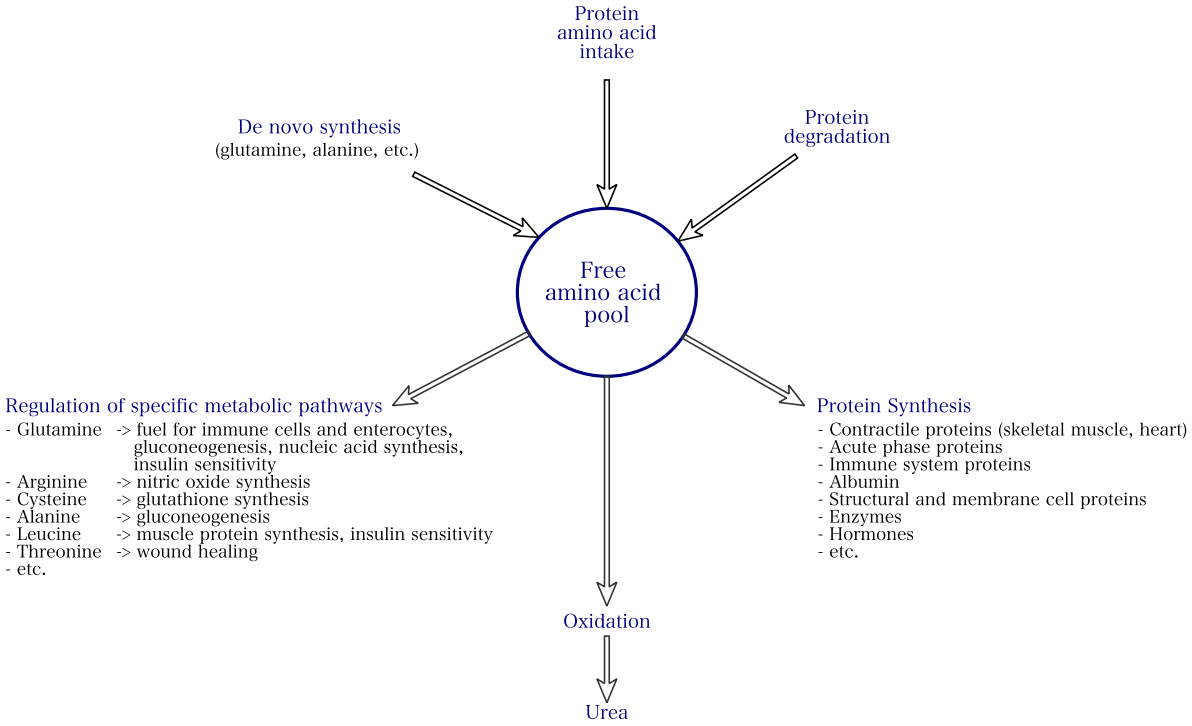

Figure 2.7: *Protein supplement to the amino acid pool and the fates of different amino acids (oxidation, biosynthesis, and regulatory purposes) [Biolo, 2013].*

2.1.4 Synthesis of Glucose Through Gluconeogenesis

Glucose is such an important metabolic fuel for the brain and other body organisms, that glucose molecules must be added to the system through diet or synthesized in the body continuously. Irreversible steps in the glycolysis process (phosphorylation and pyruvate kinase) makes it impossible to form glucose by performing glycolysis in reverse. To synthesize glucose, another process involving a different set of regulatory enzymes must be executed. The **gluconeogenic** pathway converts pyruvate into glucose through a series of chemical steps, most of which are common to glycolysis except for those bypassing the irreversible reactions of glycolysis:

$$\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \xrightarrow{\textit{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + \text{P}_i + 2\text{H}^+ \quad (2.7)$$

$$\text{Oxaloacetate} + \text{GTP} \xrightarrow{\textit{phosphoenolpyruvate carboxykinase}} \text{phosphoenolpyruvate} + \text{GDP} + \text{CO}_2 \quad (2.8)$$

$$\text{Fructose 1,6-bisphosphate} + \text{H}_2\text{O} \rightarrow \text{fructose 6-phosphate} + \text{P}_i \quad (2.9)$$

$$\text{Glucose 6-phosphate} + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{P}_i \quad (2.10)$$

The liver and kidneys are the only two organs in the human body, which possess glucose-6-phosphatase to hydrolyse gluconeogenic precursors into free glucose through the gluconeogenic pathway. Substrates for gluconeogenesis are non-carbohydrate precursors ,such as lactate, amino acids, and glycerol, which enter the gluconeogenic pathway at different entry points. Fatty acids and many amino acids cannot be applied in gluconeogenesis because their catabolic pathways produce acetyl CoA, which is an irreversible fate of pyruvate, mentioned in section 2.1.1 [Gerich et al., 2001],[Martini and Nath, 2009].

Glycerol enters the gluconeogenic pathway as dihydroxyacetone phosphate after an initial product conversion. This product is part of the glycolysis pathway in stage II of Figure 2.3, and is converted into glucose by means of the gluconeogenic enzymes.
Lactate enters the pathway after an initial conversion to pyruvate, illustrated in Figure 2.8, and is hydrolysed into glucose in the liver or kidneys. Lactate delivered by skeletal muscle may be converted to glucose in the liver and thereafter brought back to the muscle for ATP synthesis - this constitutes the cori-cycle [Berg et al., 2006].
Amino acids are primarily degraded within the liver, providing carbon skeletons for oxidation, glucose synthesis, or fatty acid synthesis. Glucogenic amino acids, like alanine and glutamine, provide carbon skeletons for the gluconeogenic process. Ketogenic amino acids cannot be converted to glucose, but their carbon skeletons may enter the TCA cycle after conversion to acetyl CoA through ketogenesis. In some instances, amino acids are degraded in other tissues, like skeletal muscle. The release of NH_{4+} from protein deamination must be transported out of these tissues and into the liver to be excreted through the urea cycle. The peripheral transport of nitrogen to the liver is illustrated in Figure 2.9 [Berg et al., 2006].

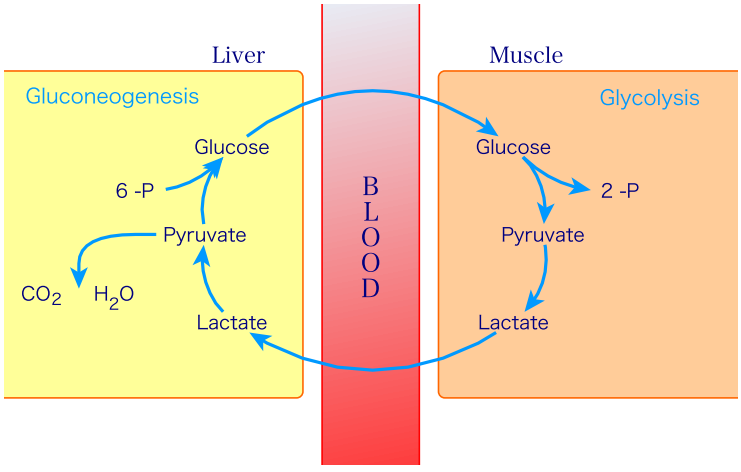

Figure 2.8: *The Cori-cycle [Berg et al., 2006]*

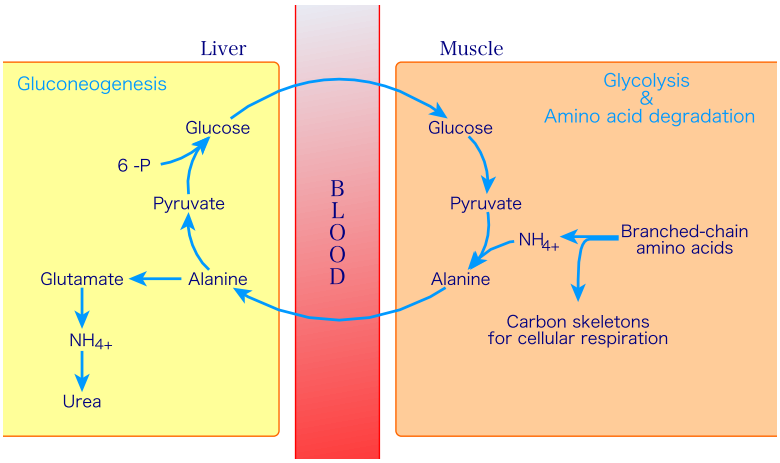

Figure 2.9: *The alanine-glucose cycle. Degradation of amino acids within peripheral tissues and the subsequent transport of nitrogen out of tissue by alanine [Berg et al., 2006].*

In the post-absorptive state, gluconeogenesis is responsible for 55% of all glucose released into the circulation. Glycogenolysis contributes the remaining part of total glucose production. It has been approximated, that the kidney produces 40% of the gluconeogenic substrate and the liver the remaining 60 %. The kidney does not posses glycogen stores like the liver, why glucose released from the kidneys is considered a product solely produced from gluconeogenesis [Gerich et al., 2001].
Gluconeogenesis is especially important during longer periods of fasting or starvation. The human body has direct glycogen reserves to fulfil only one day of whole-body glucose requirement, why it is important to generate glucose from other non-carbohydrates. Gluconeogenesis takes over endogenous glucose production, when glycogen reserves are becoming exhausted [Berg et al., 2006].

Chapter 3

Stress Response of the Critically Ill Patient

Finding an unequivocal definition of critical illness is not an easy task. Studies may describe their patients as critically ill from various severity scores or from their own arbitrary definition of the term, e.g. patients are critically ill if these are burned, septic, or trauma patients [Genton and Pichard, 2011].
Even though the definition of critical illness is somewhat vague, several studies have attempted to describe the general pattern of response to critical illness [Frayn, 1986], [Preiser et al., 2014]. The current chapter aims to describe this temporal pattern of stress experienced by critically ill patients, with focus on stress parameters related to muscle proteolysis. Knowledge of stress parameters and stimulated metabolic pathways will be applied in a subsequent modelling process.

3.1 Phases of Critical Illness

If a person becomes critically ill, the preliminary medical care will center around the repair of injuries or fight against infection in the case of trauma and sepsis, respectively. However, speaking of a patient's stress response to critical illness, focuses more exactly on the general changes in metabolism throughout the patient's disease process. It is well-known that the metabolic response to critical illness changes in a generally predictable way, stimulated by controlling hormonal factors. The sequential changes have been categorized within so-called stress phases depicted in Figure 3.1.

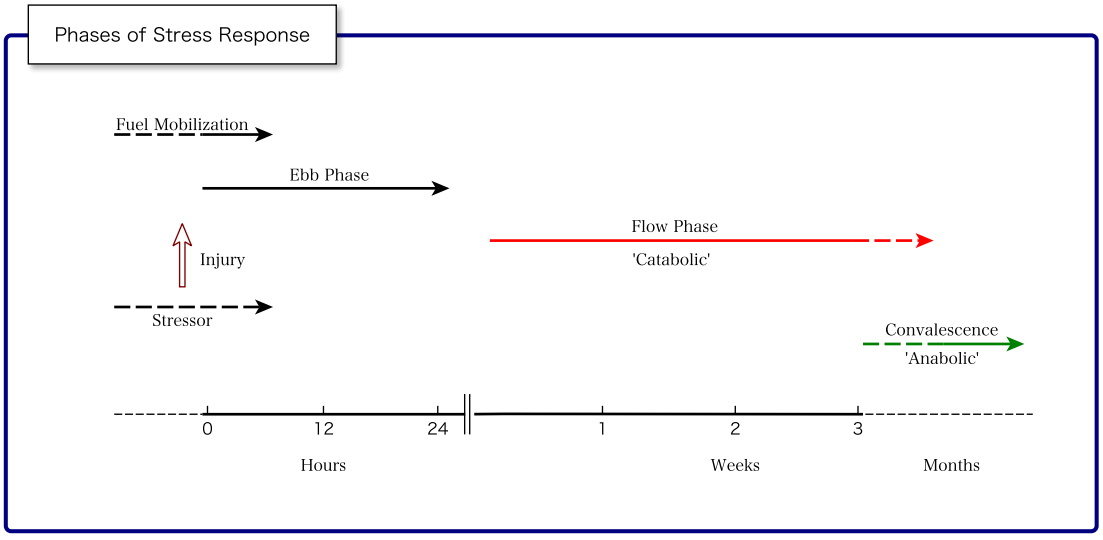

Figure 3.1: *Metabolic response to injury, categorized into an ebb, flow, and convalescence phase. The figure only provides a representative time-view on each phase. The duration may well vary depending on the individual patient disease process. Redrawn from [Frayn, 1986].*

Figure 3.1 indicates that the stress response may begin even before the injury itself has occurred. It is possible for the body to sense approaching danger, which activates the

hypothalamic defence area, leading to the initiation of the ebb phase.

3.2 The Ebb Phase

The ebb phase is short, lasting typically around 12-24 hours depending on the severity of illness. It is characterized by a rapid mobilization of fuels, such as glucose and fat, due to the activated physiological "fight or flight" response to stress. In the classical interpretation of fight-or-flight, the body is prepared by this response for sudden, intense physical activity in order to handle a presented crisis. Mobilized fuel will dissipate into the physical activity, giving the response a fleeting performance time. However, during critical illness, mobilized resources in the ebb phase do not dissipate due to certain restraint mechanisms [Frayn, 1986], [Vermes and Beishuizen, 2001]. The following sections describe the hormonal components secreted in the ebb phase and their metabolic effects.

3.2.1 The Initial Hormonal Response to Illness

The ebb phase begins after an initial stressor has been signalled to the central nervous system (CNS), cf. Figure 3.2. A stressor is defined as any condition that threatens homoeostasis, i.e. this could be hypovolaemia activating baroreceptors or nociceptors detecting pain. The body issues a response to the stressor by activating the hypothalamic-pituitary-adrenal (HPA) axis. This primary stimulation causes a sequence of hormone secretions; a release of corticotropic hormone (CRH) from the hypothalamus, stimulating a further release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH causes the final part of the HPA-axis to secrete epinephrine (adrenalin). Levels of epinephrine in the ebb phase are well above those required to produce metabolic changes, i.e. epinephrine enhances the mobilization of metabolic fuels with levels above 0.5 nmol/l and inhibits insulin secretion from the pancreas at levels above 2.2 nmol/l. Secondary hormonal responses in the ebb phase include the secretion of glucagon by the pancreas and secretion of cortisol from ACTH stimulation on the adrenal cortex. [Frayn, 1986], [Preiser et al., 2014], [Martini and Nath, 2009].

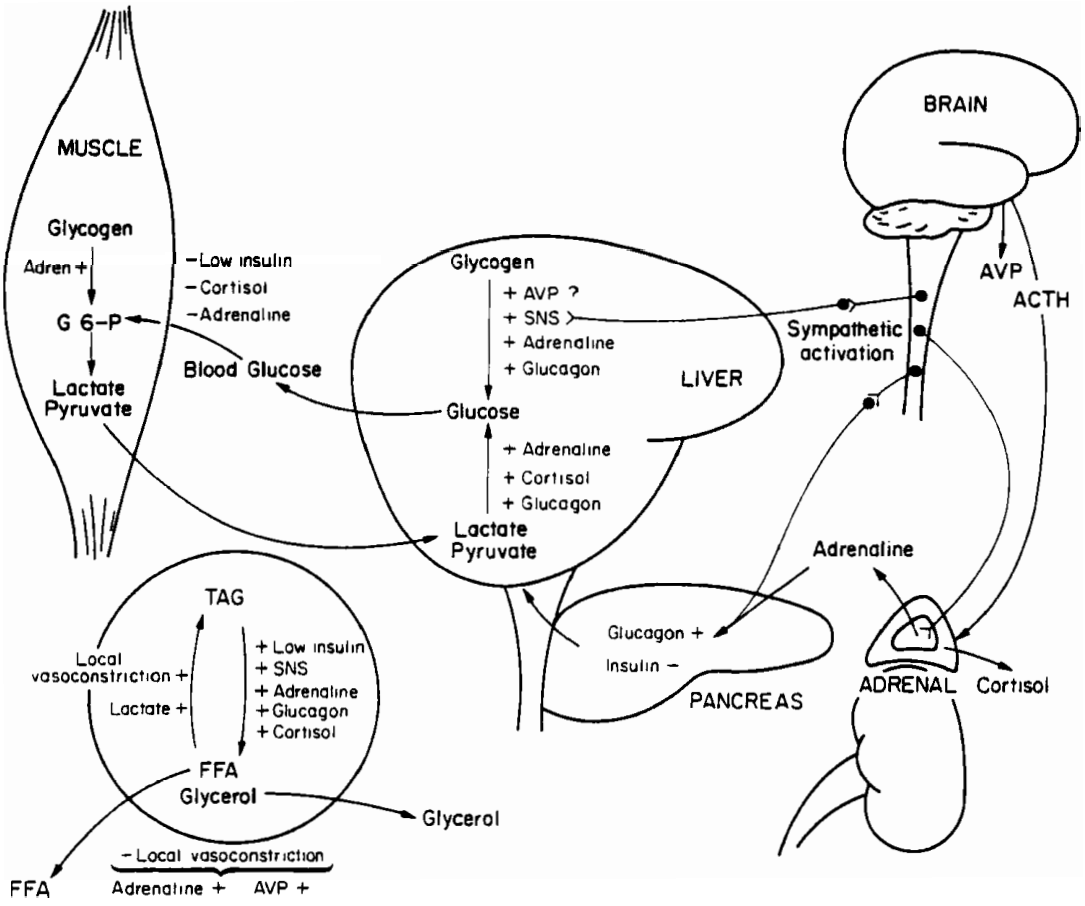

Figure 3.2: *Control of metabolism in the ebb phase [Frayn, 1986].*

3.2.2 Metabolic Effects of Hormones in the Ebb Phase

Epinephrine, glucagon, and cortisol released in the ebb phase stimulate the breakdown of glycogen in the liver and skeletal muscle. Epinephrine stimulates the breakdown of glycogen in muscle and to some degree in the liver. The liver is usually more responsive to glucagon, released by the pancreas in situations of low blood sugar [Berg et al., 2006]. However, epinephrine seems to be the major controlling factor in stimulating glucose breakdown, since an observed hyperglycaemic state post-injury is closely related to plasma adrenaline concentrations. The secretion of glucagon responds slowly to injury and plasma glucagon levels are normal right after stressor onset, why the hyperglycemic state is assumed independent of glucagon during this phase of illness. Plasma cortisol levels are in the ebb phase elevated compared to control subjects, but in this phase it is more concerned with maintenance rather than the initiating factor of the stress response [Frayn, 1986], [Vermes and Beishuizen, 2001], [Mizock, 2001].

Fuels are mobilized extensively during the first hours of injury. Hepatic glucose production, through glycogenolysis in the ebb phase, elevates the plasma glucose concentration, causing the critically ill patient to experience hyperglycaemia. Muscle glucogenolysis contributes only to hyperglycemia through the release of pyruvate and lactate, to be transformed into glucose in the liver. In normal individuals, there is a tight regulation of blood glucose concentrations, controlled by hormonal, neural, and hepatic autoregulatory mechanisms. In a hormonal perspective, insulin is secreted rapidly in response to hyperglycaemia, lowering glucose levels by enhancing glucose uptake and synthesis of glycogen and suppress-

ing hepatic glucose production (glycogenolysis and gluconeogenesis) [Mizock, 2001]. In the critically ill patient, hyperglycaemia seems to withstand after glycogen reserves are largely depleted. This is recognized partly as consequence of further hepatic glucose production from the gluconeogenic pathway, but is also linked to decreased peripheral utilization of glucose. Impaired utilization contributes to insulin being metabolically ineffective. Glucose oxidations is therefore inhibited, when insulin-mediated glucose uptake is inhibited, causing a diminution of metabolic rate during the initial phase of critical illness [Frayn, 1986].

Figure 3.2 also demonstrates FFAs and glycerol substrates in the blood stream, resulting from lipolysis in adipose tissue. Lipolysis is primarily activated by the release of epinephrine, and this catabolic process continues unaffected when the insulin secretion from the pancreas is impaired. However, epinephrine limits the availability of albumin through local vasoconstriction, transporting FFA out of adipose cells into circulation [Frayn, 1986].

3.3 The Flow Phase

If the patient has not expired in the initial ebb phase, then the patient merges into a more prolonged stage defined as the flow phase of the stress response. This phase often corresponds to when the patient has been stabilized and transferred to an ICU.
The flow phase is characterized by an increased metabolic rate and increased catabolism of substrates from different body tissues to withstand these energy demands. The flow phase is not set within a specific time-frame, and it is not defined by a certain intensity. These factors may vary depending on the severity of illness. In an uncomplicated patient scenario, the stress response will peak around 7-10 days after injury and thereafter gradually subsides into a convalescence phase, cf. Figure 3.1 [Frayn, 1986]. The flow phase is, like the ebb phase, characterized by a hormonal effect on metabolism, described in the following.

3.3.1 Hormonal Response in the Flow Phase

The ebb phase is characterized by high levels of counter-regulatory hormones, such as catecholmines (epinephrine and norepinephrine), cortisol, glucagon, and growth hormone (GH). In the flow phase, many of these hormone concentrations rapidly return to normal values, cf. Figure 3.3.

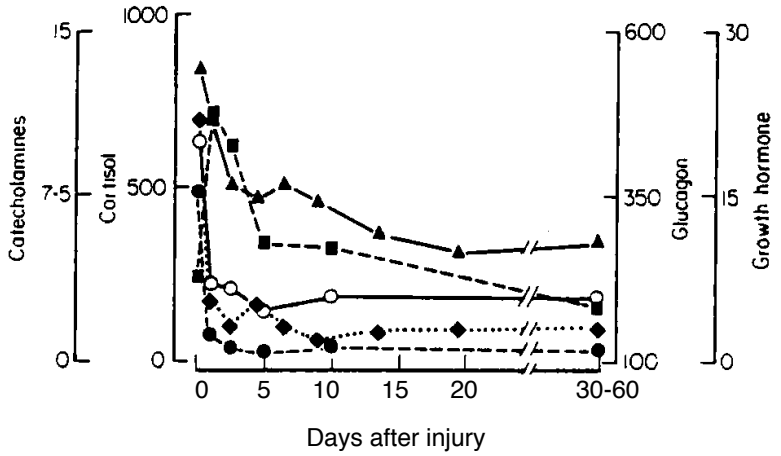

Figure 3.3: *Responses of counter-regulatory hormones during injury;* ♦ *growthhormone,* ■ *glucagon,* ▲ *cortisol,* • *adrenaline,* ○ *noradrenaline. Results were taken from patients suffering from musculoskeletal injuries [Frayn, 1986].*

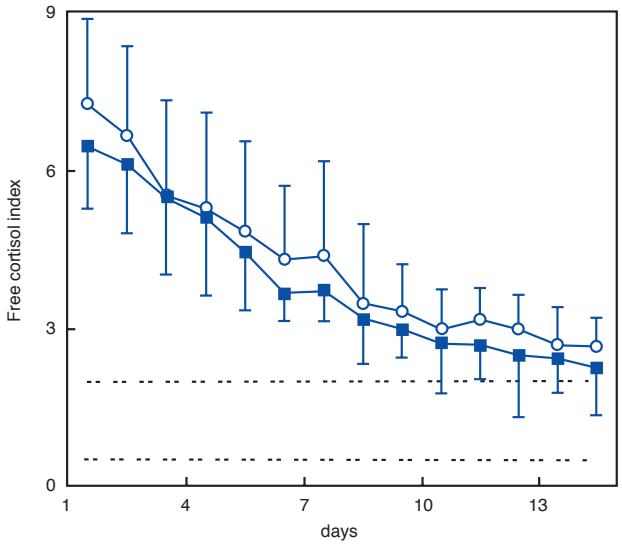

Figure 3.4: *Free cortisol index over time in patients with septic shock (■) and trauma (○) [Vermes and Beishuizen, 2001]. The broken lines in the bottom of the graph illustrates the reference range of normal subject. Free cortisol index is an expression of plasma cortisol related/cortisol-binding globulin (CBG), the transporter for cortisol in the blood.*

At the peak of catabolism, around 7-10 days after injury, many of the hormone concentrations will have reached baseline levels with the exception of cortisol. An example of catabolic peak is seen in Figure 3.5, and has also been mimicked in [Monk et al., 1996] for other trauma patients. The cortisol slope in the above Figure 3.3 is decreasing, with a similar tendency seen in Figure 3.4 of the free cortisol index. Plasma cortisol levels may, however, be moderately elevated at the peak of catabolism compared to other counter-regulatory hormones. During critical illness, the immune system responds to damaged and pathogen-invaded cells by massively producing pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6. The latter is shown to stimulate adrenocortical cells to release glucocorticoids (cortisol) [Vermes and Beishuizen, 2001], [Frayn, 1986].

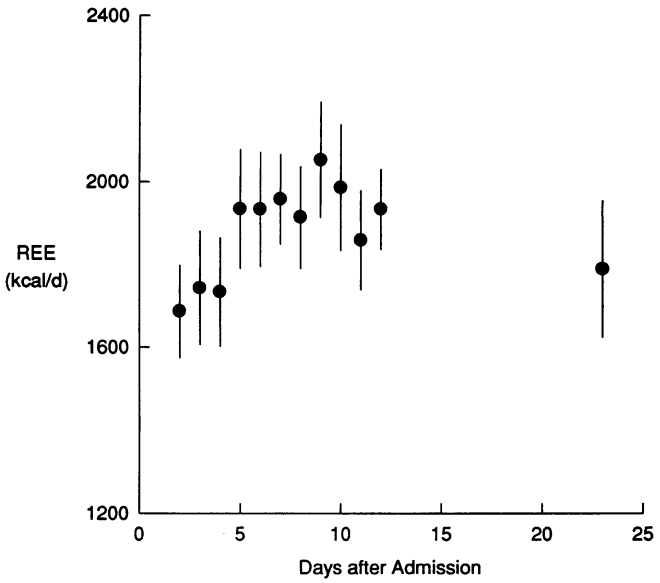

Figure 3.5: *Measurements of daily resting energy expenditure (REE) by indirect calorimetry, from eight ICU patients with peritonitis secondary to perforation of an abdominal viscus [Plank et al., 1998]. Results are plotted as daily mean + SEM, with maximum reached around day 9 after admission to the ICU. The original figure included REE predicted from Harris-Benedict equation, but these have been removed for simplicity.*

In contrast to the presented counter-regulatory hormones, insulin is not depressed during the flow phase. Plasma insulin concentrations consistently rise in the days following ICU admission, cf. Figure 3.6, peaking about the same time as the catabolic peak in Figure 3.5. The disappearance of adrenergic hormones from plasma removes the restraint on the pancreas to secrete insulin in response to hyperglycaemia. A case of hyperinsulinemia occurs, may occur because secreted insulin concentrations are in a period of time inappropriately high in relation to the hyperglycemic state [Frayn, 1986].

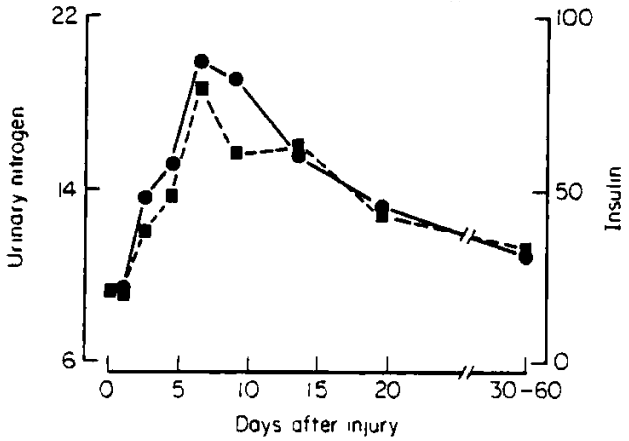

Figure 3.6: *Displayed is the development in urinary nitrogen excretion (•) and plasma insulin concentrations (■) for patients suffering from musculoskeletal injuries [Frayn, 1986].*

3.3.2 Metabolic Effects of Hormones in the Flow Phase

Cortisol is the driving catabolic factor within the flow phase, stimulating the lipolysis of triaglycerides and the breakdown of protein from skeletal muscle [Frayn, 1986]. Several studies have correlated an increase in plasma cortisol concentrations with increased

appearance rates of different amino acids in the blood stream [Simmons et al., 1984], [Brillon et al., 1995]. Amino acids, released by stimulated muscle proteolysis, are provided for hepatic gluconeogenesis and protein synthesis to support inflammatory and immunological responses, cf. Figure 3.7. One type of degraded amino acid is glutamine, which is stored as a free amino acid in skeletal muscle. Glutamine is released in great amounts from muscle to support the rapidly dividing cells of the immune system and gluconeogenesis in the liver, causing a quick depletion of this substrate during critical illness [Biolo, 2013].

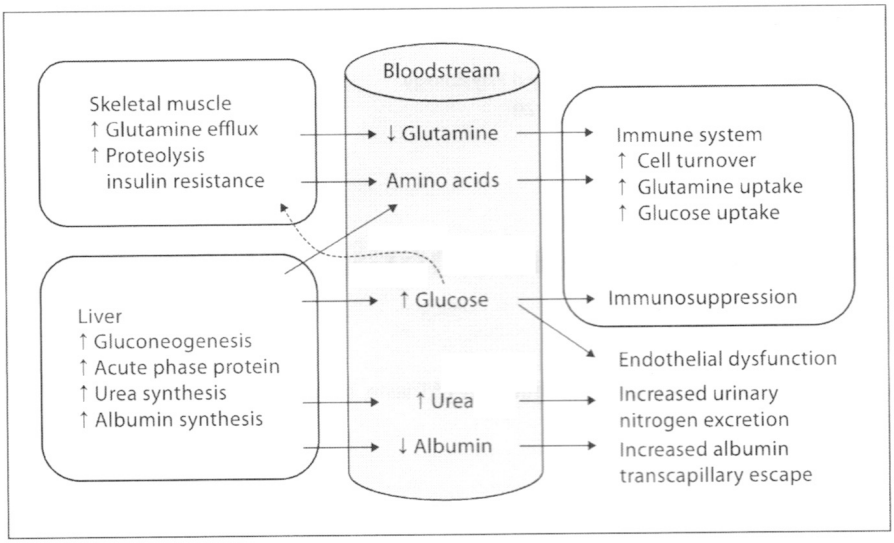

Figure 3.7: *The figure shows the effect sites and utilizations of e.g. glutamine, other amino acids and glucose during critical illness [Biolo, 2013].*

A suggestive marker of protein breakdown is the urinary nitrogen excretion measure, seen in the previous Figure 3.6. The urinary nitrogen excretion does, however, only provide an estimation of oxidized proteins and not whole-body protein turnover [Biolo, 2013].
A connection between glucocorticoids and increased urinary nitrogen excretion was demonstrated by [Wolthers et al., 1997], where eight male subjects were administered glucocorticoids for four days and blood and urine samples were taken over a smaller time period to estimate urea excretion and nitrogen balance. Glucocorticoid administration increased hepatic nitrogen clearance and hence utilization of amino acids in this study.

Accelerated muscle wasting, indicated from an increasing urinary nitrogen excretion balance, causes weakness in critically ill patients. Increased morbidity and mortality has been correlated with muscle loss and experienced physical weakness, why protein synthesis must be favoured to improve outcome. Normally, insulin acts as anabolic factor, stimulating protein synthesis in the excess of plasma amino acids. However, protein turnover is resistant to the anabolic effect of insulin seen as the urinary nitrogen excretion curve follows that of the insulin curve in Figure 3.6 [Frayn, 1986], [Biolo, 2013].

An increase in energy expenditure up to 50 % compared to normal resting energy expenditure has been seen for critically ill patients. An example of the heightened energy expenditure during the flow phase can be seen in Figure 3.8. This increase may be accredited to an excessive uptake of glucose. Following the glucose uptake, an increased rate of glycolysis may incur lactic acidemia [Wolfe and Martini, 2000], [Chioléro et al., 1997].

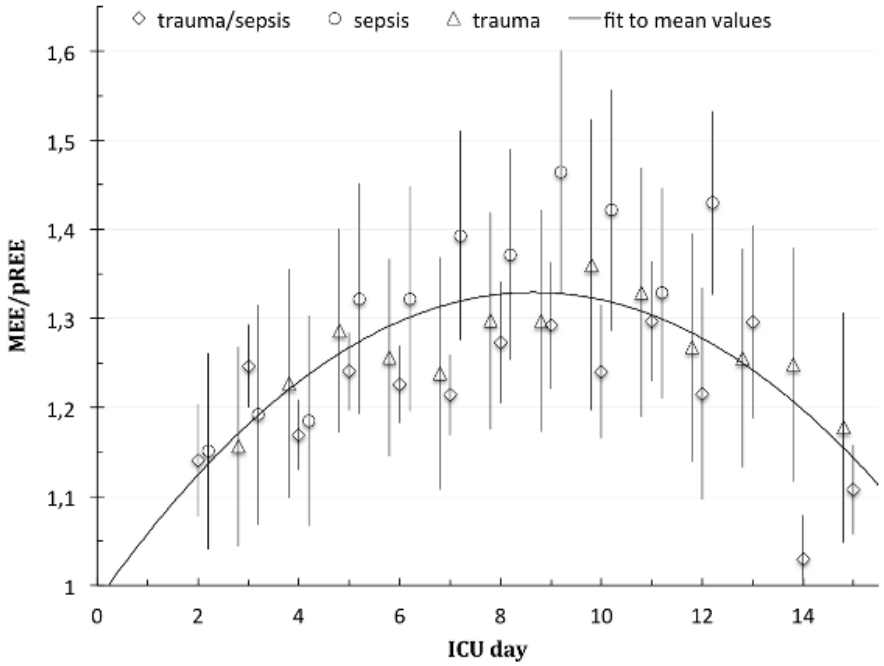

Figure 3.8: *The figure shows a fit of data on the ratio of energy expenditure measured by indirect calorimetry (MEE) and predicted energy expenditure (pREE) over the course of ICU stay. A distinct increase in energy expenditure can be seen when entering the flow phase [Pielmeier et al., 2014].*

Part II

Model of Muscle Proteolysis during Critical Illness

Chapter 4

Clinical Data Acquisition

To estimate unknown parameters in a physiological model of muscle proteolysis during critical illness, clinical data is required for parameter estimation and general conceptualization of a model. Certain data parameters were desired, being indicators of metabolic stress and/or protein loss. Efforts of clinical data retrieval will be described in the present chapter, followed by an analysis of the extracted clinical parameters in the subsequent chapter.

4.1 Desired Physiologic Parameters for Modelling

To be able to describe the stress response of critically ill patients in a physiologic model, clinical data is required, relating to indicative parameters of stress. Knowledge of physiological parameters related to stress from chapter 3 provided a list of desired clinical parameters to be retrieved for the model, see Table 4.1. Information regarding patient demographics is also desired. Physiological models, incorporating knowledge of the human physiology and patient-specific data, may capture the physiological status of the individual patients [Chase et al., 2011].

Patient Demographics
Age
Gender
Admission weight
Height
Diabetes diagnosis

Time-varying chart-data
Blood glucose
Blood lactate
Exogenous insulin
Cortisol
Interleukin-6
Parenteral nutrition
Enteral nutrition
Protein intake
Urine urea nitrogen (UUN)
Blood urea nitrogen (BUN)
White blood cell count (WBC)
Temperature
Energy expenditure
Daily Weight
Minute volume

Table 4.1: *Patient demographic parameters and charted stress-related parameters warranted for modelling.*

4.2 Data Selection Criteria

In addition to the criteria of extracting specific parameters listed in Table 4.1, some data selection criteria were established to homogenize a dataset of critically ill patients for

physiological modelling. A patient group constituting all types of ICU patients is by nature very heterogeneous. Such a patient group will introduce a large spectrum of diseases and injuries, some affecting metabolism more than others. Two of the most prominent patient groups within ICUs are patients with sepsis and trauma. Both sepsis and trauma patients show metabolic characteristics of the ebb and flow phase of critical illness described in chapter 3 [Frayn, 1986]. Selection criteria were identified to filter patients into more specific groups of sepsis and trauma patients, described in the following:

- **Patients must be diagnosed with trauma or sepsis.**
  Due to the large heterogeneity of patients admitted to ICUs, diagnostic inclusion criteria were required to homogenize the patient group considered for modelling. Diagnosis of either trauma or sepsis was specified, seeing as these patients have markedly different metabolism than healthy persons [Shaw et al., 1987], [Shaw and Wolfe, 1989].

- **Patients must be older than 18 years when admitted to the ICU.**
  Critically ill children are sorted from the patient group in applied clinical data, since metabolic demands of the children may be different to those of adults.

- **Patients' course of disease, right after admittance to an ICU, must span at least 10 consecutive days.**
  One of the goals of the physiologic model is to describe muscle proteolysis through the time-course of critical illness. Therefore, only patients with ICU admissions long enough for indications of stress-induced hypermetabolism, cf. section 3.3, are included in the clinical data. A minimum of 10 consecutive ICU days was chosen due to the possibility of including the catabolic peak seen in Figure 3.5 of chapter 3.

- **Patients must have a charted admittance weight and height.**
  Height and weight values are often used when estimating patient metabolic rate from predictive energy expenditure equations [Frankenfield and Ashcraft, 2011], [Walker and Heuberger, 2009]. Height is not expected to change noticeably over the time-course of critical illness, whereas body weight may fluctuate more, especially due to common oedema appearances throughout ICU stay [Walker and Heuberger, 2009]. As oedema-induced weight changes are expected not to incur metabolic changes and seeing as it may be difficult to detect oedema from clinical weight data, a "baseline" weight must exist in applied clinical data.

- **Patients cannot be diagnosed with liver or kidney disease or failure.**
  Patients with unstable liver and/or kidney function was excluded from the applied clinical dataset, due to unpredictable metabolic responses entailed by these organ dysfunctions. Excluding critically ill patients with liver and kidney dysfunctions will make it possible to assume normal functionality of these organs, simplifying model identification.

4.3 Clinical Data Acquisition

To acquire clinical data for physiologic modelling, a source for data acquisition must be chosen. Attempts to collect the above-specified clinical data from patient journals at a clinical site (Aalborg University Hospital) failed due to new regulatory affairs taking effect in March 2015. Instead, the Multi-parameter Intelligent Monitoring in Intensive Care II (MIMIC II) database at PhysioNet was chosen for data extraction, since this database includes general clinical parameters measured for critically ill patients [Saeed et al., 2011], [Goldberger et al., 2000]. Authorization to access MIMIC II was granted the 25th of March 2015, and the database was specified as version 2.6 at the time of data retrieval with 32.536 registered ICU subjects.

Data from MIMIC II was provided in tab-delimited text files, divided into different aspects of ICU admittance for each patient exemplified in Table 4.2. All data available for each critically ill patient was contained in 26 separate .txt files.

Filename (`.txt`)	**Short description**
`CHARTEVENTS`	Information on all chartings (measurements etc), i.e. data that may have been stored in the patient journal.
`D_PATIENTS`	Details on the specific patient, such as gender and date of birth.
`ICD9`	Listings of ICD-9 diagnostic codes for classification of the patient's diseases and/or injuries over time.
`ICUSTAY_DETAIL`	Information specific to each ICU admittance of a patient, examples are admittance weight, height, ICU admittance, and discharge dates.
`D_CHARTITEMS`	A mapping table of chart IDs and the names of different parameters that can be measured in `CHARTEVENTS`.

Table 4.2: *This table demonstrates some of the 26 files contained in a patient folder downloaded from MIMIC II, which will be used for the subsequent data criteria filtration. All the above-listed file names are appended by the patient ID of the current specific patient, e.g. the file named ICD9 would actually be named ICD9-00164 for a patient with the ID 00164.*

Due to the large amount of data contained within this clinical database (`CHARTEVENTS.txt` can comprise up to 42.000 lines of parameter data), a Python-script was developed to filter MIMIC data based on the desired parameters listed in Table 4.1 and selection criteria from section 4.2. Filtered data was then stored in a local MySQL database, simplifying further data analysis for physiologic modelling. An activity diagram of the data filtration process of MIMIC II data files is depicted in Figure 4.1. The Python-script iterated through each MIMIC-acquired patient folder, each containing 26 `.txt`-files. The data files, used in data filtration, are those listed in Table 4.2. If filtered clinical data for a patient satisfies the applied data selection criteria, then the patient's demographic data, ICD-9 codes, and charted parameter data are stored in the locally created MySQL database.

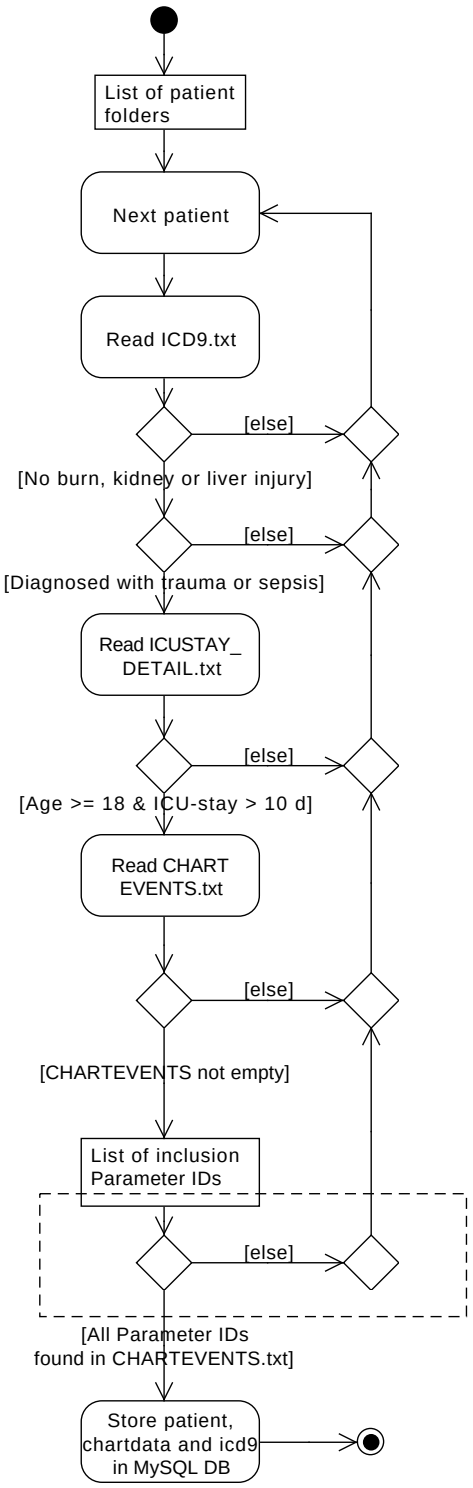

Figure 4.1: *An activity diagram of the data filtration and storage process of MIMIC II acquired clinical data.*

4.4 Final Dataset For Physiologic Modelling

An inspection of filtered data from MIMIC II clearly demonstrated that far from all data parameters listed in Table 4.1 existed for ICU patients in MIMIC II. Some parameters were not even defined in the `D_CHARTITEMS.txt` file, and other parameters existed in dictionary files, but no parameter values were logged for any of the patients fulfilling the data selection criteria. A list of parameters, with existing parameter measurements

for filtered patients from MIMIC II, can be seen in Table 4.3. All of the demographic parameters in Table 4.1 existed in the MIMIC II database.

Existing Time-varying Parameters
Blood glucose
Blood urea nitrogen (BUN)
White blood cell count (WBC)
Temperature
Weight
Minute Volume

Table 4.3: *The table illustrates the existing parameters in MIMIC II for patients fulfilling the data inclusion criteria.*

16,000 patients from the MIMIC II database where filtered by use of the data selection criteria in section 4.2. Of these, 123 patients or 0.77% of the 16,000, with a total of 134 unique patient scenarios, matched the selection criteria and had registered parameter data for the desired modelling parameters shown in Table 4.3. A patient scenario is defined to be an admittance to the ICU. Characteristics of the patient group, representing the filtered dataset, are illustrated in Table 4.4. From Table 4.4 it is clear, that only a small proportion (app. 15%) of the extracted patients have diabetes, almost exclusively in the form of type 2 diabetes. The majority of the extracted ICU patients were male (63.4%).

Total patients	123
Total patient scenarios	134
Mean Age (std)	59.1 (17.8)
Gender	
Male(%)	85 (63.4)
Female(%)	49 (36.6)
Mean Admission weight - kg (std)	81.0 (23.2)
Mean Height - cm	169.9 (18.0)
Diabetes	
Type 1 (%)	1 (0.74)
Type 2 (%)	19 (14.2)
Diagnosis	
Trauma (%)	86 (64.2)
Sepsis (%)	48 (35.8)

Table 4.4: *Patient characteristics. Percentages are of the total number of patient scenarios. std: Standard deviation.*

Chapter 5

Strategy for Model Development

The present chapter provides a description of a general development process of models. The methodology within steps of the development process will be discussed, along with the limitations of developed models. Methodological aspects of the illustrated development process will be applied when deriving a physiological compartment model of muscle proteolysis during critical illness.

5.1 Model Definition

A model is, in its essence, a representation of some sort of reality. In the case of physiological models, these may represent physiological processes (e.g. glycolysis) and/or physiological components (e.g. the liver compartment), describing the internal milieu of the human body to some degree of complexity. A physiological compartment model is a certain type of model, applying differential equations to describe the kinetics of materials within the modelled compartments.
A model design will always be an approximation of the modelled reality, since it is impossible to incorporate all possible components of a reality into one model. In physiological models, a number of assumptions are usually imposed by physical, chemical, and biological processes incorporated in the model [Cobelli and Carson, 2008], [Chase et al., 2011].

5.2 Development of Physiological Models

The development process of a model involves some inter-related steps, identified as model conceptualization, model identification, and model validation. If the methodology within each step is applied appropriately, then a model will be developed to fit its primary purpose [Cobelli and Carson, 2008].

To create a model, it is first important decide the specific goal of the model. Many models have varying goals; they may describe, interpret, predict, or explain the physiological process(-es) in focus. The goal of the developed model in this project was set forth in the research objectives of chapter 1.
With a chosen goal in mind, a model may then be constructed from physiological knowledge and potentially available experimental data, relevant to the model goal. In Figure 5.1 a methodology for model development is depicted, based upon 1) the formulation of a conceptual model, 2) the specification of mathematical expressions for model variables, and 3) solving the model by connecting physiological variables [Cobelli and Carson, 2008].

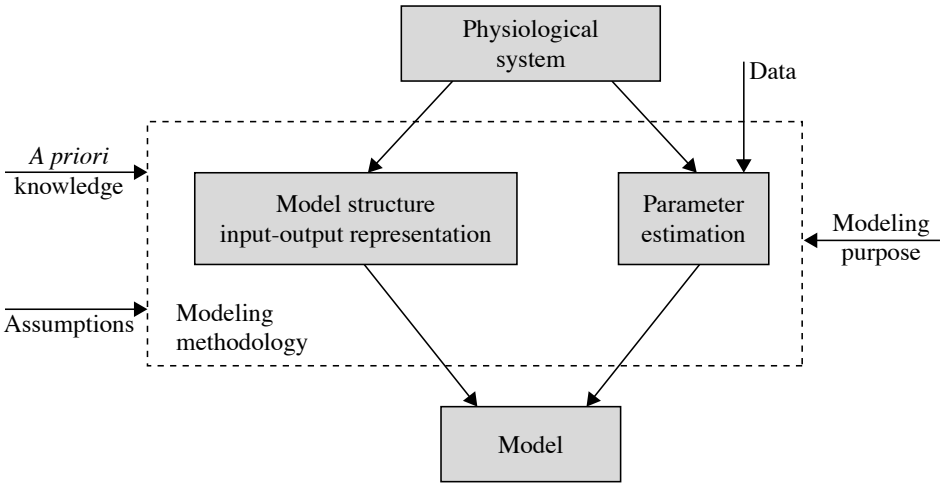

Figure 5.1: *Methodology for creating a model [Cobelli and Carson, 2008].*

The conceptual model describes the physiological process related to the model goal. Model concepts are based upon acquired physiological knowledge, e.g. in this project the model is based upon physiological knowledge from chapter 2 and 3. Deriving the basic concepts of the model may require an aggregation of physical compartments, an abstraction of compartments (e.g. assuming distribution of only certain materials within the compartment), and assuming ideal behaviour/structure to simplify the model (e.g. instantaneous distribution of materials). After conceptualization, mathematical equations may be formed for suggested parameters seen in the conceptual model. Physiological parameters often vary as a function of time, which represents the dynamics that mathematical parameter equations must mimic. Finally, explicit relationships between model parameters must be constituted, representing the solving of a model. The relationships are commonly connected through differential equations [Cobelli and Carson, 2008].

Parameter values or model structures may be known a priori, making it possible to solve and further validate the model. In most cases, however, there is an uncertainty about the model structure or parameter values. In such cases, unknown structures/values must be identified through input/output data seen in Figure 5.2. This often requires a conduction of clinical experiments, where a stimulus relevant the model unknown is applied to a system and the dynamic response is recorded for one or more variables [Cobelli and Carson, 2008]. In view of the current project, literature with isotope tracer infusions of certain materials (e.g. lactate) and their effect on other parameters (e.g. amino acid release) has been reviewed to identify kinetics of certain model parameters between different compartments.

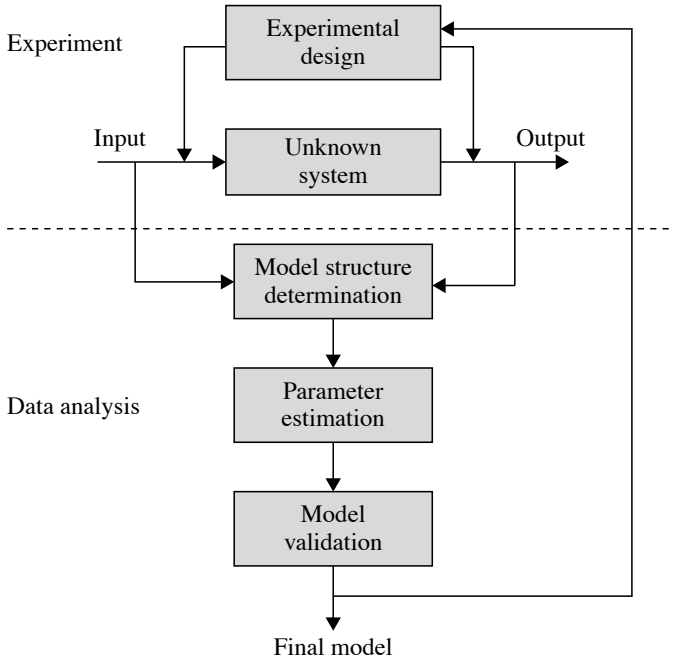

Figure 5.2: *Identification of model structures [Cobelli and Carson, 2008].*

In Figure 5.2 model validation is depicted as the final development step, even though this step is an integrated part of the modelling process overall. The goal of validating a derived model is to investigate whether the model performs well enough in relation to the model goal defined at the beginning of model development. Model behaviour is examined and interpreted, and simulated model outputs may be statistically compared to real-life data [Cobelli and Carson, 2008].

Chapter 6

Data Analysis of Clinical Dataset

The current chapter examines the available clinical data for physiological modelling, depicted by the final filtered dataset from chapter 4. Data parameters have been visualized in a graphical user interface to inspect dynamic changes in parameter values over the time-course of trauma and sepsis patients' ICU admittances.

6.1 Visual Interpretation of MIMIC II Data

Parameter data in the filtered MIMIC II dataset may be visually inspected, to attain an idea of how certain parameter values change dynamically over time. A graphical user interface (GUI) was created in MATLAB for inspection of filtered data in the local MySQL database, an example of the GUI is seen in Figure 6.1. Visual inspection of parameter data was performed for several patients in the filtered dataset, to detect any preliminary tendencies in parameter development over ICU admission time. It was observed that some patients experienced considerable weight losses during their ICU stay, one weight scenario is depicted in Figure 6.1. This correlates with findings by [Plank et al., 1998], where body weight also decreased during a 21-day observation period after onset of sepsis. Furthermore, estimated energy expenditures from patient demographic data, minute volume data, and temperature showed similar dynamic changes over ICU stay between patients in the filtered dataset. A general tendency curve of estimated energy expenditure over admission time was therefore generated for the observed patient group, described in the next section.

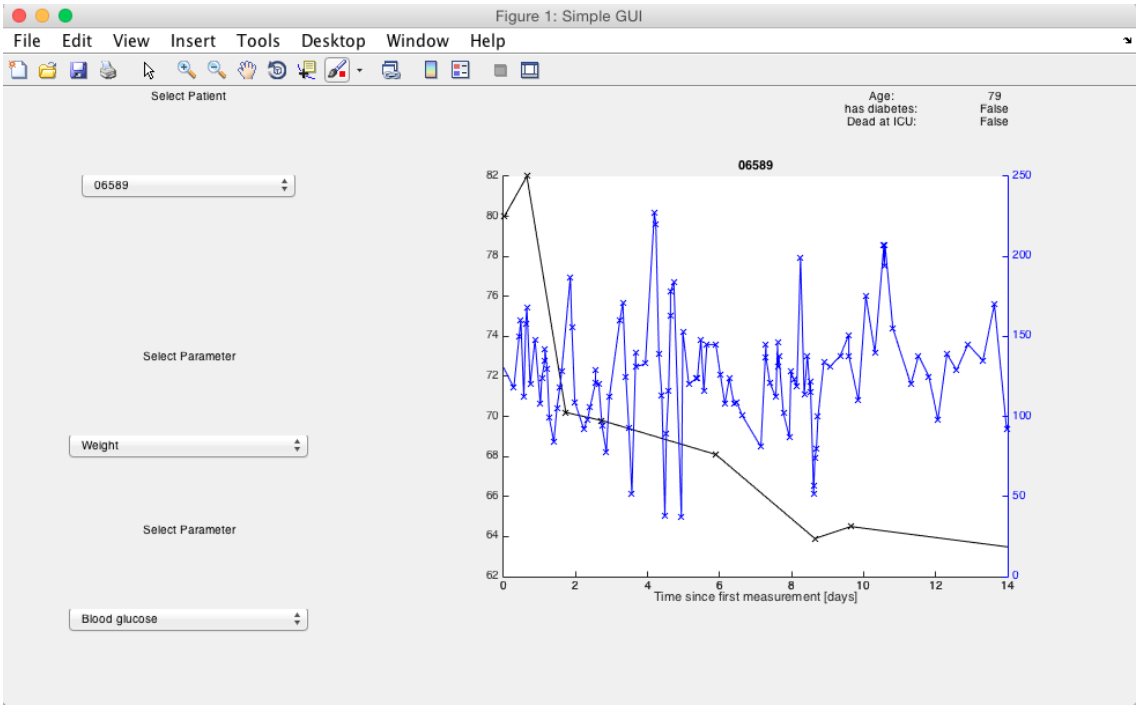

Figure 6.1: *The figure demonstrates a screen shot of the MATLAB GUI, created for visualization of the temporal development of clinical parameters extracted from MIMIC II, see chapter 4. In this specific screen shot, the blue line indicates development of blood glucose concentrations (mg/dl), while the black line shows weight (kg) development over ICU admission time for a patient.*

6.2 Utilization of Data: Estimation of Energy Expenditure

Illustrated by Figure 3.5 in chapter 3, an interesting parameter indicating metabolic stress may be the resting energy expenditure (REE). The standard method for measurement of REE is by indirect calorimetry, which is expensive and cumbersome [Walker and Heuberger, 2009], why it is not surprising that this parameter is absent in the final filtered dataset from MIMIC II. REE may instead be estimated using predictive equations [Frankenfield and Ashcraft, 2011]. The use of predictive equations ensues inaccuracy due to the inherent problem of estimating REE from a specific mathematical formulae for a patient group as heterogeneous as ICU patients [Faisy et al., 2009], [Walker and Heuberger, 2009], [Preiser et al., 2015]. Despite possible inaccuracies, predictive equations will be applied to determine the development of REE for the patient group in the filtered dataset.

A multiplum of equations for estimating energy expenditure have been proposed, both for critically ill patients and healthy persons [Frankenfield and Ashcraft, 2011]. For these purposes, the Penn State Equation (PSE) for the critically ill and the Mifflin-St Jeor (MSJ) equation for the healthy person were used, as these have been found to be the most accurate [Walker and Heuberger, 2009], [Frankenfield and Ashcraft, 2011]. These two equations are written below:

$$MSJ\frac{kcal}{day} = 10 \cdot BM + 6.25 \cdot H + 5yrs^{-1} \cdot A \tag{6.1}$$

$$PSE\frac{kcal}{day} = 0.96 \cdot MSJ + 167 \cdot T_{max} + 31 \cdot V_e - 6212 \tag{6.2}$$

Here BM is body mass in [kg], H is height in [cm], A is age in [years], T_{max} is the maximum temperature in degrees [celsius] for the previous 24 hours, and V_e is the minute ventilation of the patient in [l/min]. A modified version of the PSE is applied to obese, older patients ($age \leq 60$ and $BMI > 30$) [Frankenfield et al., 2012]:

$$PSE_{mod}\frac{kcal}{day} = 0.71 \cdot MSJ + 85 \cdot T_{max} + 64 \cdot V_e - 3085 \tag{6.3}$$

All the necessary parameters for calculating MSJ and PSE exist in filtered data set from MIMIC II. Despite observations of weight loss for the patients in the dataset, an initial admission weight is applied to calculate PSE and MSJ over the time-course of ICU stay. This weight parameter is chosen because oedema might influence the daily weight parameter. A weight loss during ICU stay does not necessarily entail a lower energy expenditure in real-life. However, this will be the picture of the estimated REE, if the dropping weight values are applied in the predictive equations over time. Estimated REE from PSE and MSJ are therefore very much affected by the weight of patients, with the possibility of underestimating expenditure if decreasing weight values are applied in the equations over time [Walker and Heuberger, 2009], [Frankenfield and Ashcraft, 2011].

It is of interest to express a generalized development curve of estimated REE for critically ill patients, much like the one seen in Figure 3.8 of chapter **??**. The curve is a normalization of the estimated REE with respect to (MSJ):

$$REE_{norm} = \frac{PSE}{MSJ} \tag{6.4}$$

A generalized development curve of the REE_{norm} was found from the 134 patient scenarios in the filtered dataset of MIMIC II. Of these, 36 scenarios were selected due to their continuously charted temperature and minute ventilation measurements during 14 days of ICU stay, with a measurement frequency of at least 1 measurement per day. 20 of the patient scenarios were trauma patients, while 16 were septic patients. REE_{norm} was estimated for each patient scenario, and a mean and standard deviation was found for each of these patient groups per ICU day. The graph of REE_{norm} over time is illustrated in Figure 6.2.

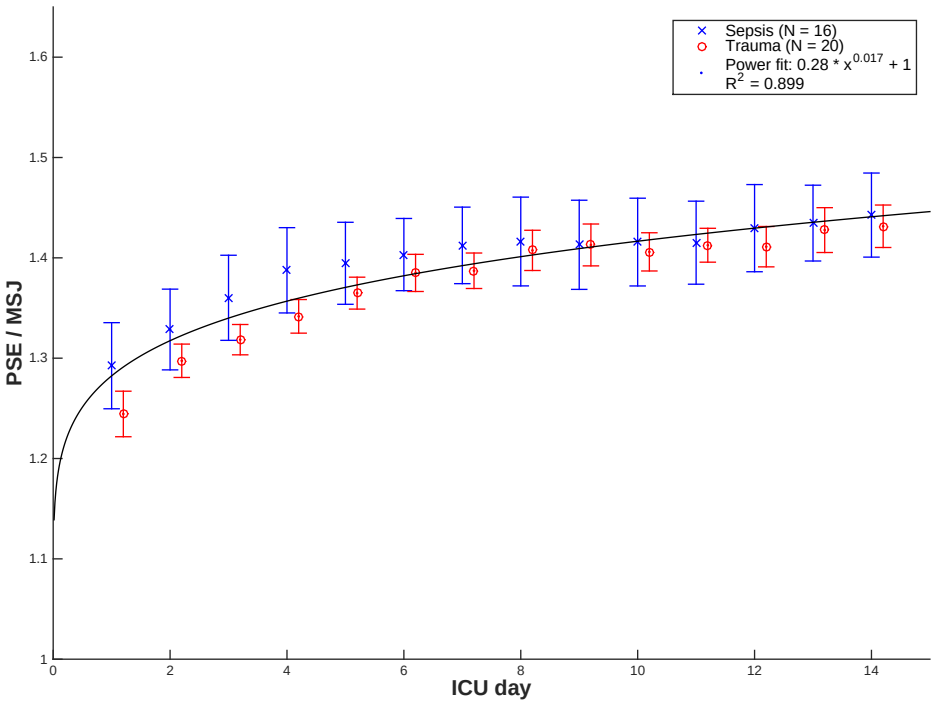

Figure 6.2: *The estimated mean REE, normalized in respect to Mifflin-St Jeor REE estimations of trauma and septic patients. The black curve is a power fit made on the applied* REE_{norm} *data.*

Figure 6.2 indicates that the sepsis and trauma patients in the filtered dataset have an increased energy expenditure, supported by the literature [Plank et al., 1998], [Pielmeier et al., 2014]. A larger deviation from the mean was found for the septic patient group, indicating even higher variations in energy expenditure for this group than trauma patients. This curve may be used prospectively to estimate energy expenditure of critically ill patients over time. In the following chapter this relation will be utilized to include a temporal aspect to the physiologic model of muscle proteolysis during critical illness by trauma and sepsis patients.

For future analysis it would be interesting to also extract nutritional data for the patients, to analyse the energy balance (energy intake - energy expenditure) for the patients. This was done by [Faisy et al., 2009], where 38 mechanically ventilated ICU patients, likewise observed retrospectively over a 14 day ICU period. In this study, the REE was estimated by a different predictive equation than PSE. However, the applied equation in [Faisy et al., 2009] did utilize the same bio-dynamic parameters as PSE (weight, height, minute ventilation, and body temperature). [Faisy et al., 2009] showed no change in the predicted REE over the course of ICU stay, as would have been expected for critically ill patients. The authors speculate that the lack of an initial hypometabolic phase may be that the patients where already in the hypermetabolic 'flow' phase [Faisy et al., 2009]. In Figure 6.3 the results of the use of predictive REE estimation in [Faisy et al., 2009] can be seen.

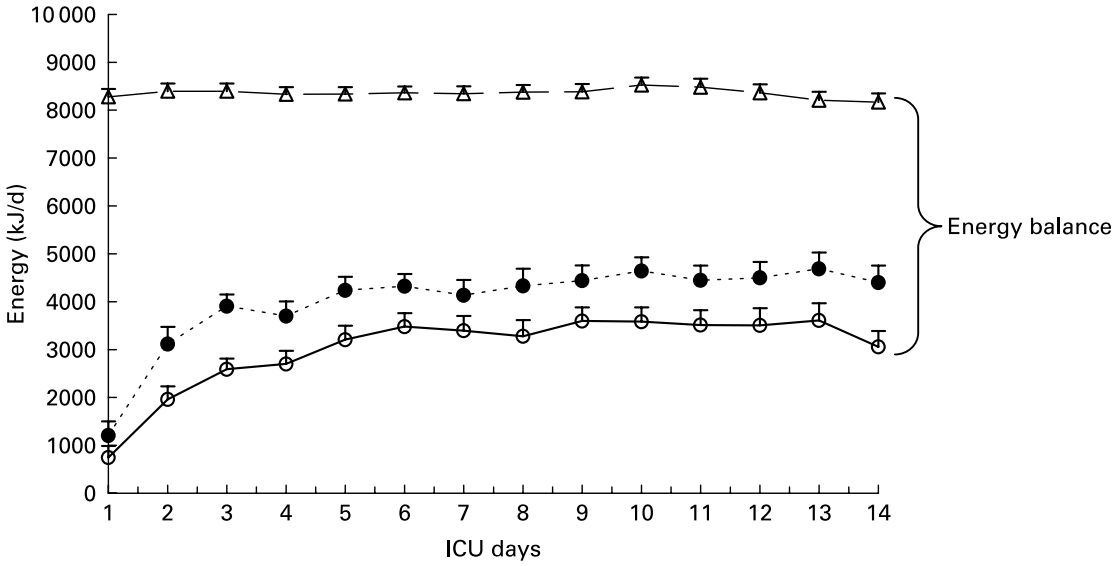

Figure 6.3: *The figure shows results from [Faisy et al., 2009], where open triangles represents predicted REE over the ICU stay for patients. Filled circles indicate prescribed energy, while open circles represent energy delivered. Notice that predicted REE is stable throughout the 14 days of ICU stay.*

In the estimation seen in Figure 6.2 there is an increasing REE during the initial ICU stay (1-3 days). This may be the result of a change from the 'ebb' phase to the 'flow' phase. A decrease in REE at the end of the 14 day ICU period would, however, be expected as a result of entering a convalescence phase. This lack of decrease in REE may be due to the patients never (due to very critical state), or at least not in the chosen 14 day period, reaching the recovery phase.

Chapter 7

Model of Muscle Proteolysis and Critical Illness

In the present chapter, a physiological compartment model is proposed, with the aim of temporally estimating muscle proteolysis in critically ill patients. An initial restriction to the model conceptualization is presented, due to the limitations of the acquired clinical data from MIMIC II.

7.1 Restrictions of Muscle Proteolysis Modelling

Due to the lack of stress parameters in the filtered dataset from MIMIC II, it may prove difficult to model the total muscle breakdown induced by critical illness. A stress marker, such as cortisol, could be valuable in regards to estimating muscle proteolysis over the time-course of critical illness, cf. chapter 3, since this stress parameter has been correlated with amino acid degradation into plasma circulation [Wray et al., 2002], [Weissmann, 1990], [Brillon et al., 1995]. Urinary nitrogen excretion values and protein intake values are required to estimate net protein catabolism by means of nitrogen balance [World Health Organisation, 2007], [Bergstrom et al., 1998].

Protein degradation from skeletal muscle is mainly stimulated for amino acid oxidation, de novo protein synthesis, and gluconeogenesis during critical illness, cf. subsection 2.1.3. The major indicating parameter of amino acid oxidation is the excreted urinary nitrogen, the residual product of catabolised amino acids. Since only blood urea nitrogen values are available in the filtered dataset, protein degradation kinetics due to amino acid oxidation cannot be estimated from the available clinical data, if this parameter is part of the warranted physiological model of muscle proteolysis.
A consistent state of hyperglycaemia is presented by critically ill patients during their disease process, which in the flow phase may be attributed to glucose release from gluconeogenesis. Glycogen reserves will likely be depleted after the ebb phase, making the gluconeogenic pathway the primary provider of endogenous glucose. Blood glucose concentrations in the filtered MIMIC II dataset may then be utilized for parameter estimation of muscle proteolysis kinetics, attributed to gluconeogenesis. A restriction of the physiological model is therefore proposed; the physiological model will focus on the degradation of muscle protein through gluconeogenesis during critical illness.

7.2 Model Overview

Figure 7.1 illustrates a proposed physiological compartmental model of muscle proteolysis contributing to the gluconeogenic metabolic process. The stimulation of this metabolic pathway is accelerated in order to satisfy increased energy demands characterized by the flow phase of critical illness, cf. section 3.3.

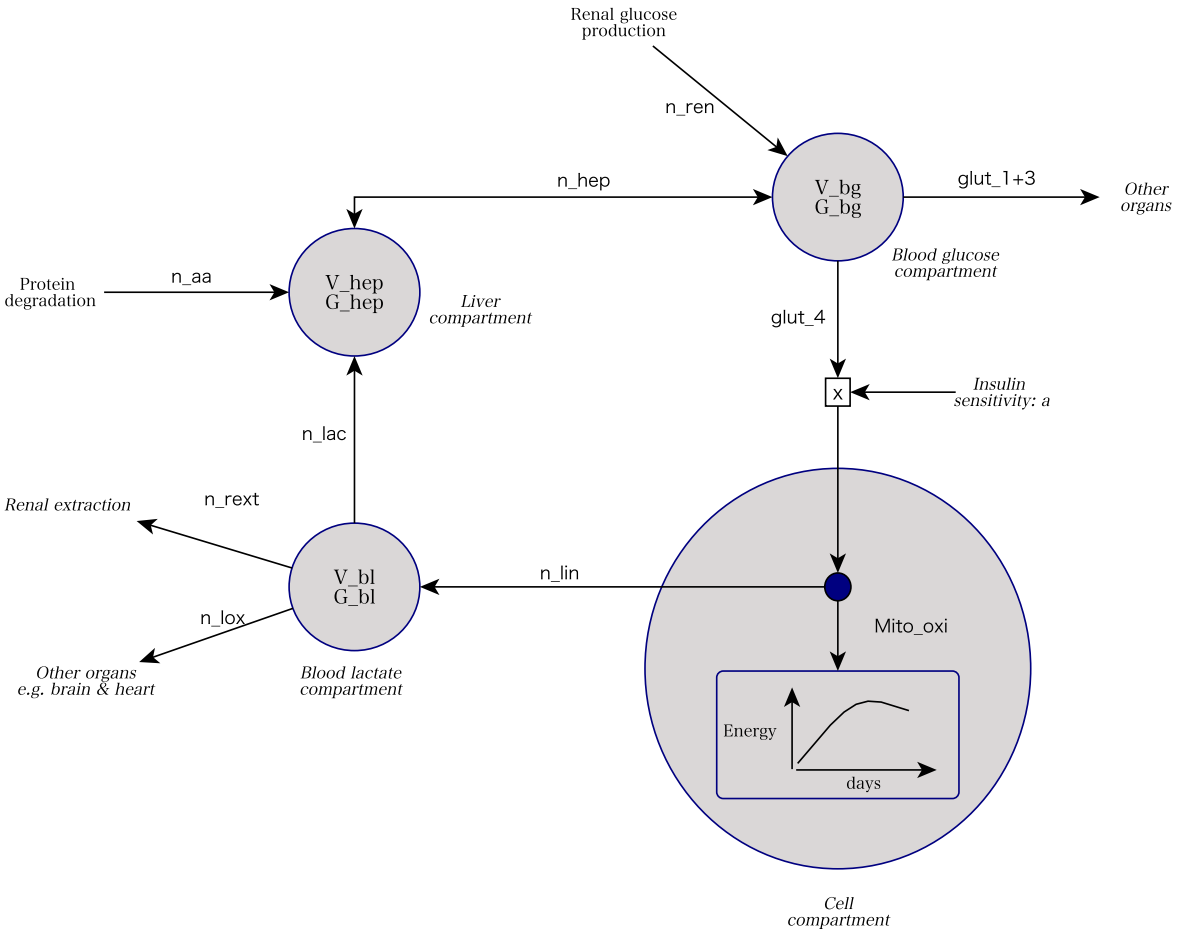

Figure 7.1: *Compartmental structure of the gluconeogenic pathway during critical illness, with muscle proteolysis contributing to glucose production.*

The model draws elements from the Glucosafe model described in [Pielmeier et al., 2010], specifically the definition of a blood glucose compartment as connected to glucose transporters $GLUT_1$, $GLUT_3$ and $GLUT_4$. In the following sections, physiological compartments and fractional transfer rates between compartments will be motivated through literature references.

7.3 Blood Glucose Compartment

The blood glucose compartment represents the concentration of blood glucose distributable to the cells through glucose transporters ($GLUT_{1,3}$ & $GLUT_4$). The compartment is defined by a concentration G_{BG} in mmol/l and a distribution volume of glucose, V_{BG} with the unit of liters. The volume applied in this model is the volume of extracellular fluid (ECV), which is evaluated based on body mass [Arleth et al., 2000]:

$$V_{BG} = BM \cdot 0.19 \frac{l}{kg} \tag{7.1}$$

Application of this volume provides the assumption of an even distribution of glucose throughout the ECV [Arleth et al., 2000]. V_{BG} will be used to describe the change in blood glucose concentration over time in mmol/l/min:

$$\frac{dG_{BG}}{dt} = \frac{(n_{hep} + n_{ren} - glut_{1+3} - glut_4)}{V_{BG}} \tag{7.2}$$

Here n_{hep} and n_{ren} represent the hepatic and renal gluconeogenic contribution of glucose to the blood glucose compartment, both in mmol/kg/min. These rates will be entertained in later sections. glut_{1+3} and glut_4 describe the transfer rates of blood glucose by the combined transport of glucose by the GLUT_1 and GLUT_3 transporters and the insulin-mediated GLUT_4 transporter respectively [Pielmeier et al., 2010], [Arleth et al., 2000]. These also have the unit mmol/kg/min. From the above equation it is clear that the glucose transporters GLUT_1, GLUT_3 and GLUT_4 affect the blood glucose concentration. The GLUT_1 and GLUT_3 transporters are responsible for the basal glucose uptake in numerous areas of the body, whereas GLUT_4 transports glucose to adipose tissue, skeletal muscle, and cardiac muscle. GLUT_4 is dependent on insulin sensitivity, illustrated in Figure 7.1 [Arleth et al., 2000]. $glut_{1+3}$ and $glut_4$ are represented as Michaelis-Menten kinetic equations proposed by [Arleth et al., 2000]:

$$glut_{1+3} = \frac{J_{1+3} \cdot G_{BG}}{K_{mglut13} + G_{BG}} \tag{7.3}$$

$$glut_4 = \alpha \cdot \frac{J_4 \cdot G_{BG}}{K_{mglut4} + G_{BG}} \tag{7.4}$$

In the above equations, $K_{mglut13}$ and K_{mglut4} are the Michaelis-Menten constants for each of the transport processes. These are 1.5 and 5 mmol/l, respectively. J_{1+3} and J_4 are the maximum transport rates for each of these processes and are 0.0093 and 0.0848 mmol/kg/min, respectively. Finally, α represents unitless the insulin effect experienced by the patient. The insulin effect expresses the effectiveness of insulin to facilitate the uptake of glucose in cells from the blood compartment. The insulin effect is generally decreased for critically ill patients [Arleth et al., 2000], [Pielmeier et al., 2010].

7.4 Cell Glucose Utilization

Glucose transported by the GLUT_4 transporter enters the skeletal and adipose tissue target cells. It is assumed that within the skeletal and adipose cells glycolysis transpires immediately, breaking one molecule of glucose down into two pyruvate molecules, cf. chapter 2, as the cell does not store glucose during critical illness. Synthesized pyruvate molecules may then be either 1) utilized by aerobic energy production through the TCA cycle and oxidative phosphorylation in the mitochondria or 2) converted into lactate in an anaerobic process. Reactions 1) and 2) are assumed to occur instantly, hence there can occur no build-up of glucose or pyruvate in the current cells.

Seeing as glucose is not stored in the cells, the determinator of whether glucose is oxidized in the cell or converted to lactate leaving the cell has been decided to be the rate of mitochondrial oxidation. For this purpose, the function between ICU stay days and REE, REE_{norm}, found in section 6.2 is utilized. This leads to the following expression for the rate of mitochondrial oxidation, Mito_{oxi} in mmol/min:

$$Mito_{oxi} = 10^3 \frac{mmol}{mol} \cdot \frac{(REE_{norm}(day) - 1) \cdot MSJ}{7.3 \cdot 10^{-3} \frac{kcal}{molATP} \cdot 24 \frac{h}{day} \cdot 60 \frac{min}{h}} \cdot \frac{1}{34} \frac{Glucose}{ATP} \tag{7.5}$$

Here $REE_{norm}(day)$ is the function of the ratio of estimated REE for critically ill (PSE) over REE for the healthy person with same demographics (MSJ). From this value, 1.0 is subtracted as only the energy requirements above the RMR of the healthy is assumed to be transported by the GLUT_4 transporter to adipose tissue and skeletal muscle cells.

The glucose that is not oxidized in the cells of the adipose tissue and skeletal muscles is converted to lactate. This lactate is transported to the blood. If lactate accumulated in the cells these would become acidic and expire. Because of this, a choice has been made for this model to transfer all glucose not oxidized in the cells to the blood lactate compartment.

7.5 Blood Lactate Compartment

The blood lactate compartment represents the lactate in the blood. This compartment is defined by a concentration G_{BL} and the volume of the blood V_{BL}. V_{BL} is calculated by [Medscape, 2015]:

$$V_{BL} = avgBV \cdot BM \tag{7.6}$$

$avgBV$ is $0.075\frac{L}{kg}$ for men and $0.065\frac{L}{kg}$ for women [Medscape, 2015]. The change in blood lactate concentration in mmol/l/min is described by:

$$\frac{dG_{BL}}{dt} = \frac{n_{lin} - n_{lac} - n_{rext} - n_{lox}}{V_{BL}} \tag{7.7}$$

Here n_{lin} is the lactate influx due to anaerobic glycolysis described earlier in mmol/min, n_{lac} is the hepatic extraction of lactate in mmol/min, n_{rext} is the renal extraction of lactate in mmol/min and n_{lox} is the rate of lactate oxidized in other organs in mmol/min. As mentioned earlier, the glycolysis of one glucose molecule will yield two pyruvate molecules which, if converted to lactate, will yield two lactate molecules [Martini and Nath, 2009].

$$n_{lin} = (glut_4 - Mito_{oxi}) \cdot 2 \tag{7.8}$$

Should Mito_{oxi} be larger than $glut_4$, this difference is set to zero, as n_{lin} is decided to not assume negative values.

The liver is the main organ for recycling of lactate in the body [Stumvoll et al., 1998]. In a study by [Dietze et al., 1976] hepatic extraction of lactate from arterial blood was found to increase when arterial blood lactate concentrations were increased. Figure 7.2 shows the linear fit of this relation.

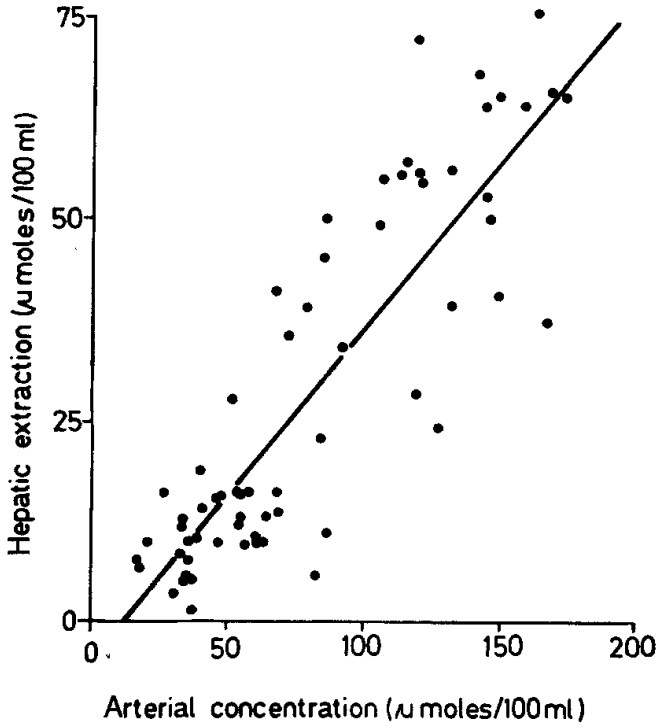

Figure 7.2: *The figure shows a linear fit of the data on calculated hepatic lactate extraction and arterial lactate concentrations for 8 subjects. The linear expression is* $y = 0.43 \cdot x - 5.8$ *and the r-value for the regression was listed as 0.86 [Dietze et al., 1976]*

The fitted relation between hepatic lactate extraction and arterial lactate concentration is utilized in this model. To use this expression it is however necessary to take into account the hepatic blood flow as this affects the hepatic lactate extraction. Hence the hepatic lactate extraction is expressed as:

$$n_{lac} = (-0.058 + 0.43 \cdot G_{BL}) \cdot HBF \tag{7.9}$$

where HBF is the hepatic blood flow in l/min. A different depiction of the hepatic lactate extraction would be to view the process as a first order Michaelis-Menten reaction. In a model of the role of glucose homeostasis by the liver proposed by [König et al., 2012], the kinetics of the lactate transporter from the blood to the hepatic cells were defined as a reversible process by Michaelis-Menten kinetics:

$$v_{[liver],[blood]} = \frac{\frac{V_{max}}{K_m} \cdot (G_{BL} - G_{liver\_lact})}{1 + \frac{G_{BL}}{K_m} + \frac{G_{liver\_lact}}{K_m}} \tag{7.10}$$

where G_{liver_lact} would be the concentration of lactate in the liver cells and K_m and V_{max} are $0.8\frac{mmol}{l}$ and $0.033\frac{mmol}{min \cdot kg}$ respectively [König et al., 2012]. The expression of n_{lac} was however chosen for this model. This was to avoid increasing the complexity of the liver compartment by adding a concentration of lactate to it. We also deemed it undesirable to model a reversible transport of lactate between blood and the liver, seeing as the main purpose of the liver compartment in this model is to function as a site of gluconeogenesis of precursors.

Although the liver is the largest contributor to lactate utilization, the kidneys are important organs for this as well. The kidneys are responsible for the disposal of 30% of an

exogenous load of lactate. Although the renal lactate metabolism is dependent on pH, increasing at acidosis, this is not a factor in this model [Bellomo, 2002]. The renal extraction n_{rext} is hence described by:

$$n_{rext} = 0.3 \cdot G_{BL} \tag{7.11}$$

The final means of outflux of lactate from the blood lactate compartment is for oxidation in other organs, such as the brain and heart. The rate of transfer of lactate to these miscellaneous organs have been assumed to be 0.27. Based on this assumption the lactate oxidation to other organs can be described by:

$$n_{lox} = 0.27 \cdot G_{BL} \tag{7.12}$$

7.6 Hepatic Compartment

The liver compartment represents mainly the gluconeogenic properties of the liver. An equation to estimate the liver volume of caucasian persons in liters was proposed by [Heinemann et al., 1999]:

$$Vol_{liver} = 1.0728 \cdot BSA - 0.3457 \tag{7.13}$$

Where BSA is the body surface area of the person in m^2 [Heinemann et al., 1999]:

$$BSA = BM^{0.425} \cdot H^{0.725} \cdot 0.00784 \tag{7.14}$$

For the purposes of this model it is deemed necessary to determine specifically the volume of the fluid component of the liver, as this would provide more accurate concentrations of glucose in the liver. The fraction of non-hepatocytes in the liver is approximately 0.42 [Kapanen et al., 2005]. This factor may then be used to calculate the volume of the liver for this model:

$$V_{Hep} = 0.42 \cdot Vol_{liver} \tag{7.15}$$

where V_{Hep} is in liters. The liver is able to convert glucogenic precursors (lactate, glycerol and glucogenic amino acids) into glucose. This functionality is of the utmost importance, seeing as patients with trauma and sepsis have an increased proportion of glucose production attributed to gluconeogenesis [Dahn et al., 1995]. An example of this can be seen in Figure 7.3:

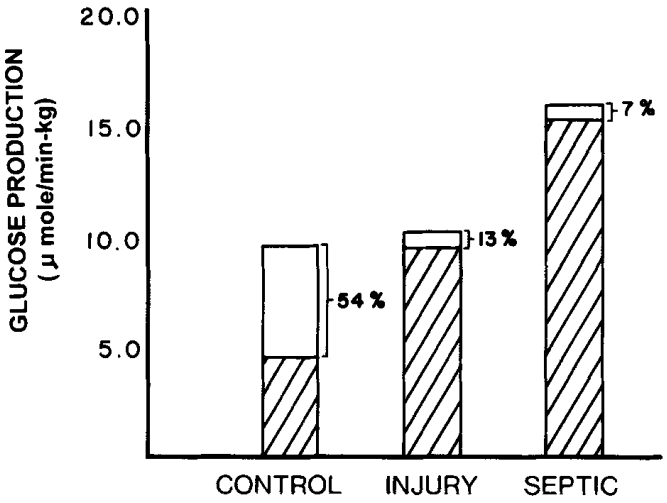

Figure 7.3: *The relative relation between glucose production by gluconeogenesis and glycogenolysis, for groups of 1) healthy individuals, 2) injured patients and 3) septic patients. The crosshatched part of each column represents the contribution of gluconeogenesis to hepatic glucose production. The white part of the column represents contribution of glycolysis [Dahn et al., 1995].*

Figure 7.3 illustrates that the main glucose producing process in the liver during critical illness is gluconeogenesis, supporting the choice of focusing on this hepatic function. Lactate in the liver may be utilized by conversion into pyruvate by lactate dehydrogenase. It may then be either oxidized directly or converted to glucose by gluconeogenesis as part of the Cori cycle [König et al., 2012]. The change in concentration of glucose in the hepatic compartment $\frac{dG_{Hep}}{dt}$ is given by the following differential equation:

$$\frac{dG_{Hep}}{dt} = \frac{\frac{n_{lac}+n_{aa}-n_{hlox}}{2} - n_{hep}}{V_{Hep}} \tag{7.16}$$

where n_{aa} is the amino acid uptake of the liver from protein degradation and n_{hlox} is the oxidation of glucogenic precursors by the liver, as seen in Figure 2.8. Notice the division by two in the expression. This is due to these transfer rates representing in- or outflux of either lactate or amino acids, which requires two mole to one mole of glucose.

Amino acids extracted by the liver may be used for synthesis of other proteins, e.g. acute phase proteins or for gluconeogenesis [Weissmann, 1990]. For the purposes of this model, only the gluconeogenesis will be modeled, as it has not been possible to attain data on the amount of amino acids utilized for protein synthesis.

The gluconeogenesis of precursor substrates increases the amount of glucose in the liver. This glucose is subsequently released into the systemic blood circulation by means of the $GLUT_2$ transporter, normally to retain euglycemia. The rate of transfer of glucose between the liver and blood, is denoted n_{hep} and is expressed by the following reaction proposed by [König et al., 2012]:

$$n_{hep} = \frac{0.25\frac{mmol}{kg \cdot min} \cdot (G_{Hep} - G_{BG})\frac{mmol}{l}}{1 + \frac{G_{BG}}{K_{mHep}} + \frac{G_{Hep}}{K_{mHep}}\frac{mmol}{l}} \tag{7.17}$$

where K_{mHep} is the Michaelis-Menten constant for this $GLUT_2$ transport kinetic, being 42.3 mmol/l determined by [Gould et al., 1991].

Glucose production also occurs in the kidneys, more precisely the renal cortex of the kidneys. The kidneys have been found to approximately constitute 40% of total gluconeogenesis [Bellomo, 2002], [Gerich et al., 2001]. If the liver is assumed to be responsible for the rest, the renal glucose production can be calculated based on the values of n_{hep}:

$$n_{ren} = \frac{40\%}{60\%} \cdot n_{hep} \tag{7.18}$$

To describe n_{aa} one way to do so would be to take into account that cortisol, cf. section 3.3, increases proteolysis. Hence, a relation between blood cortisol and amino acid uptake could be hypothesized. Data from [Dahn et al., 1995] indicates that splanchnic amino acid uptake is increased with increasing levels of blood cortisol. This together with data on Free Cortisol Index (FCI) and Corticosteroid-Binding Globulin (CBG) over time for septic and trauma patients from [Beishuizen et al., 2001] may be applied to hypothesize a relation between splanchnic amino acid uptake and days of ICU stay. See Figure 7.4 and Figure 7.5 for examples of this relation.

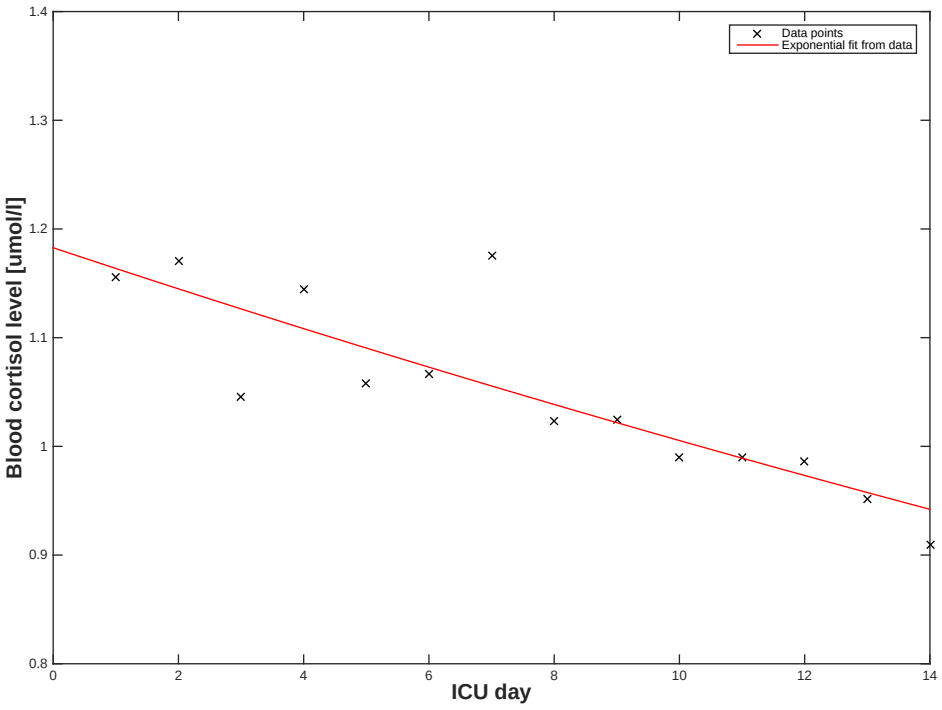

Figure 7.4: *Correlation between ICU day and blood cortisol for patients with trauma or sepsis. The blood cortisol values used (black crosses) are derived from data from [Beishuizen et al., 2001], also seen in Figure 3.4.*

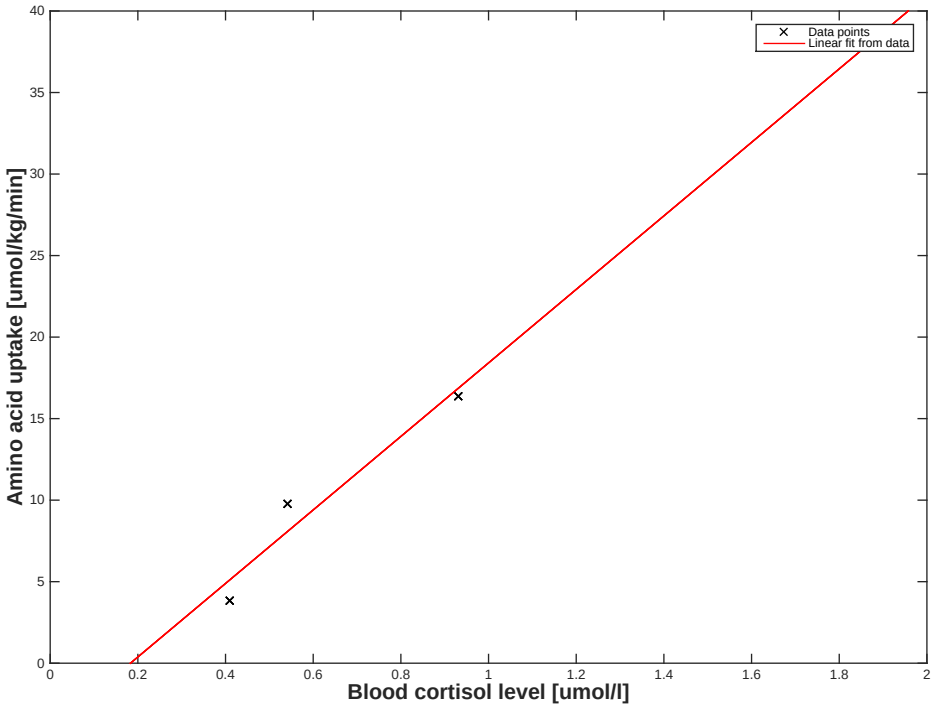

Figure 7.5: *Correlation between blood cortisol and amino acid uptake. The amino acid uptake data (black crosses) are from [Dahn et al., 1995].*

However, as we do not have data for the extracted patients on cortisol, we choose not to apply estimate this relationship for the patients. Instead, parameter estimation is done in chapter 8

Chapter 8

Parameter estimation

Following the identification of model parameters, some parameters may be regarded as unknown. These unknown parameters may not be adequately described by a priori knowledge, e.g. in the literature. Therefore other ways of estimating these parameters may be necessary. For the current project a lack of data relating to amino acid degradation over ICU stay duration has been found. The following chapter will describe the process of estimating this unknown parameter by use of data from the MIMIC II dataset extracted in chapter 4.

8.1 Need for estimation

Although a function of liver amino acid uptake on blood cortisol was proposed in chapter 7, it has not been possible to verify this relation in the literature beyond the data from [Dahn et al., 1995]. Furthermore, seeing as no data on cortisol exists for the patient data collected in this project, the use of this relation would rely on assumptions made for the patient's blood cortisol measurements derived from data from [Beishuizen et al., 2001].

Another way to estimate the amino acid degradation is to use our model to identify that blood glucose is affected by gluconeogenesis of amino acids in the liver and the renal cortex. Assuming that observations of high blood glucose concentrations are due to an increased amino acid gluconeogenesis, we may estimate the amino acid uptake from the blood glucose data extracted in chapter 4 by simulation of the model. This is a way of parameter estimation where the goal is to find the expression of the parameter in question, that minimizes the error between model simulation and the data from the actual system. An illustration of this process can be seen in Figure 8.1

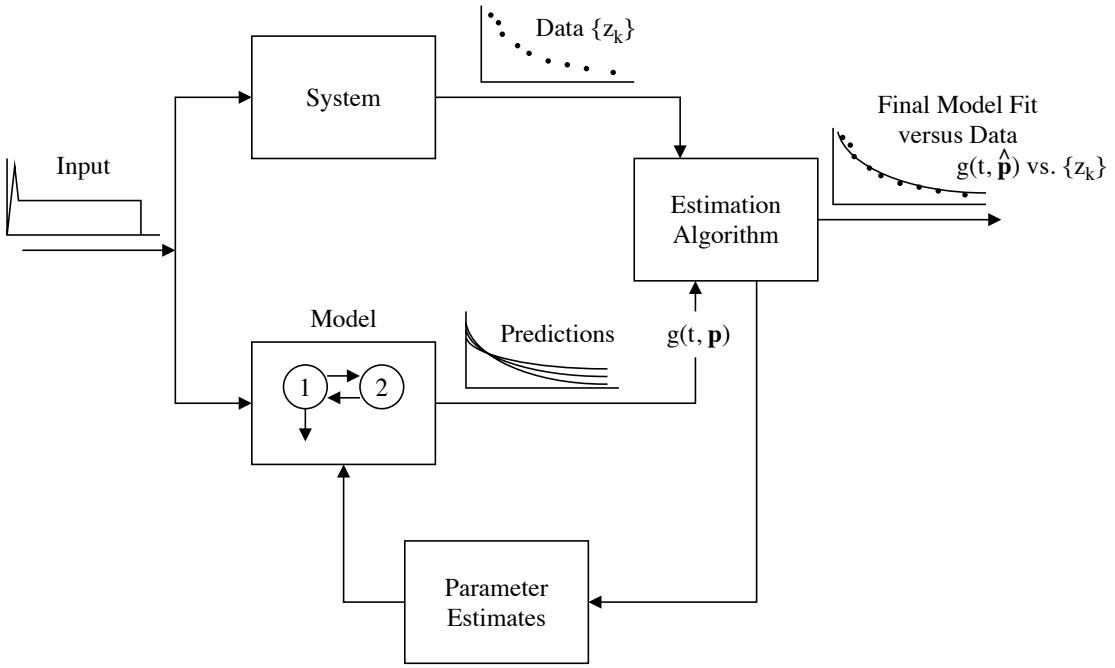

Figure 8.1: *The figure illustrates the fundamental iterative process of parameter estimation [Cobelli and Carson, 2008].*

8.2 Method

As can be seen from Figure 8.1 an estimation algorithm is needed to determine the error of model simulation compared to real system data. For this parameter estimation, A grid search methodology is applied where the parameter in question is step-wise altered and the induced change in model simulation accuracy compared to real system data is minimized. The error measure to minimize was chosen to be the Root Mean Square Error (RMSE):

$$RMSE = \sqrt{\frac{1}{n}\sum_{n=1}^{N} |\hat{Y}_n - Y_n|^2} \tag{8.1}$$

where $\hat{Y}_n$ is predicted values of blood glucose by the model and Y_n are blood glucose values from the patients. $\hat{Y}_n$ and Y_n are synchronized in time. RMSE was used rather than Residual Sum of Squares, as a mean statistic was preferred due to a varying number of blood glucose measurements per day during the ICU stay.

The unknown parameter, the amino acid uptake, was varied from 0 to 40 μmol/kg/min with a stepsize of 0.2. These constraints were chosen as they were deemed to be within physiological limits, based on results on splanchnic amino acid uptake by [Dahn et al., 1995]. This was done for simulation periods of 1 day. The model was simulated with increasing amino acid uptake rates over 1 day intervals where RMSE was calculated. The starting value for the concentration of the blood glucose compartment for each simulation was synchronized with the first true blood glucose level for the patient in that respective time interval. Figure 8.2 shows examples of simulations of the model with various settings on the amino acid uptake.

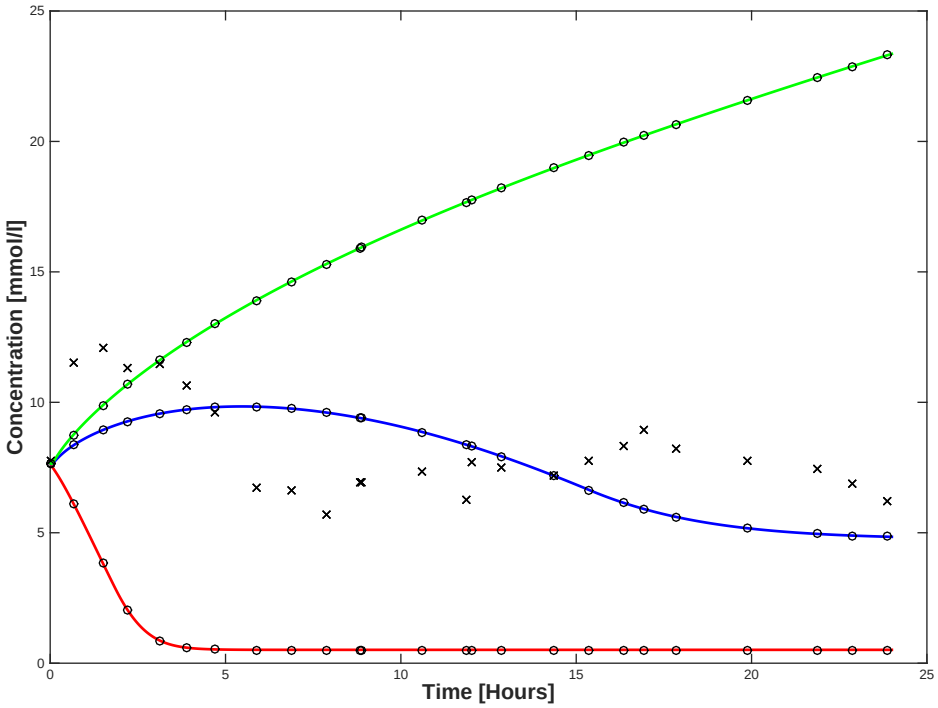

Figure 8.2: *The figure shows a section of the simulations performed, varying the amino acid uptake on the first day of ICU stay. Crosses indicate actual data measurements of blood glucose concentration. The red, blue and green line are simulations of blood glucose concentrations (G_{BG}) with amino acid uptake set to 5, 19.4 and 22 umol/kg/min respectively. The blue line was infact the simulation with the lowest RMSE in this specific grid search.*

Seven patients where chosen for the parameter estimation where four of them were diagnosed with sepsis, while the remaining three were diagnosed with some sort of trauma. The mean age of patients was 58.3 years. Insulin effect, α, for the patients was assumed to be 0.25, as this was deemed representative of the state of critical illness the patients were in. A criteria for inclusion into the parameter estimation was that patients had at least four blood glucose values registered per ICU day, this to increase the validity of the parameter estimation. Moreover blood glucose values had to be present throughout a minimum of 10 consecutive ICU days.

8.3 Results

At the time of writing the parameter estimation has only been done for one patient case. The results can be seen in Figure 8.3

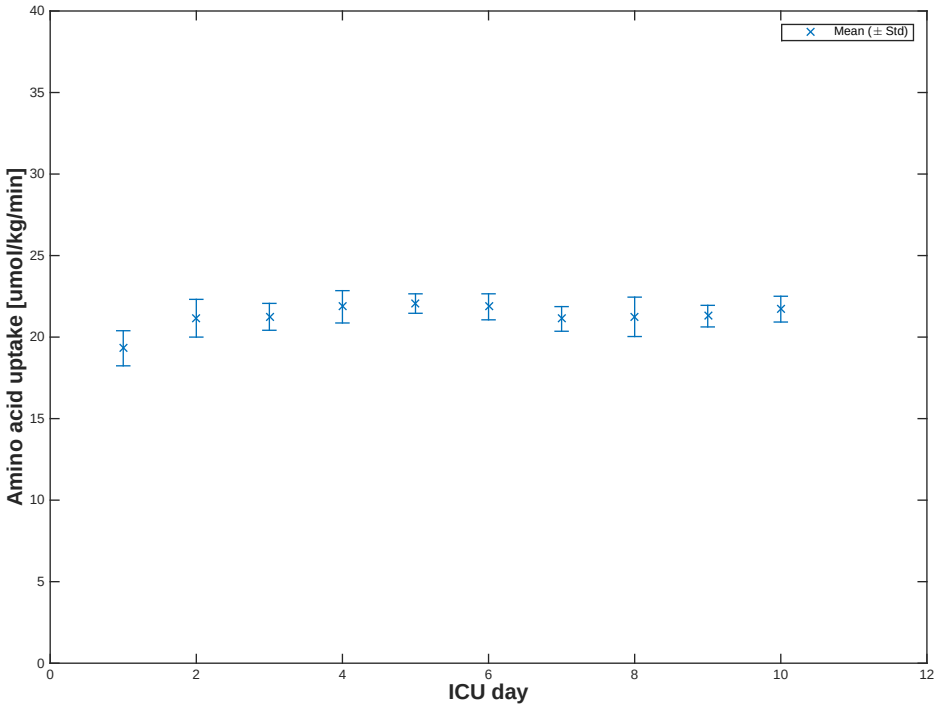

Figure 8.3: *The figure shows the amino acid uptake values that would serve to minimize RMSE of the simulations of the model for the 7 patients.*

The results of this parameter estimation indicates that the amino acid uptake rate minimizing RMSE does not change much, neither between patients or over the course of the 10 ICU days.

Part III

Synthesis

Chapter 9

Discussion

During this project, multiple elements of work was needed to define a model for representing the muscle proteolysis and stress conditions of the critically ill patient. In this discussion, the proposed model and the results of simulations and parameter estimations of this will be reviewed.

9.1 Parameter estimation

In chapter 8 parameter estimation of the amino acid uptake by the liver was performed. It is a problem to make parameter estimation of the gluconeogenesis of amino acids by the liver, when the blood glucose data provided in the MIMIC database cannot be considered "noise-free". In our parameter estimation the insulin effect is kept constant ($\alpha = 0.25$) and the patient is assumed to be fasting. These assumptions were necessary to make, due to the lack of data of data found in the MIMIC II database relating to the specific nutritional intake of the patients or exogenous insulin load. However, these assumptions are most likely big, as ICU patients just as well might be receiving nutrition and insulin therapy, both affecting the blood glucose significantly. Hence when estimating the gluconeogenesis of amino acids, the estimated amino acid degradation may not compare with the true value of system, because the change in BG may have been influenced by changes in insulin therapy or feeding. Without data on these parameters, it is hard to make the case that amino acid degradation is uniquely identifiable in the subsequent parameter estimation.

9.2 Model limitations

A model is only an approximation of reality. In this project the limitations of the proposed model relates mainly to the assumptions related to model identification. Simulations of the proposed model showed situations where the concentration of the blood lactate compartment reached zero. This was due to a decrease in transport of glucose by the GLUT4 transport to a point where all glucose transported to the skeletal muscle and adipose tissue cells was used in oxidative phosphorylation, e.g. going to the mitochondria. This resulted in a non-existant flow of lactate to the blood lactate compartment, eventually resulting in "drainage" of lactate from this compartment. This behaviour does not represent actual system behaviour, as there will always be a certain lactate concentration in the blood (app. 1.2 mmol/l) [König et al., 2012]. This undesirable behaviour is found to be due to the structural choices of the model. An assumption was made that the entire increase in energy expenditure for the patient group was due to increased oxidation of glucose in the skeletal muscle and adipose tissue cells and that REE for the same healthy individual was fully portrayed by the combined GLUT1 and GLUT3 transporter $glut_{1+3}$. In reality however, it may be more appropriate to assign the energy expenditure in the graph in Figure 6.2 to all oxidation of pyruvate followed by utilization in the TCA-cycle and oxidative phosphorylation of the mitochondria. This would entail that multiple of the transfer rates of the model would be bound to the predicted REE over time, as illustrated in Figure 9.1.

In relation to this, it is important to remember that $REE_{norm}(day)$ from Figure 6.2 is based on predictive equations of REE. This leads to inherent inaccuracy of the values of REE. Under optimal conditions, the REE was calculated

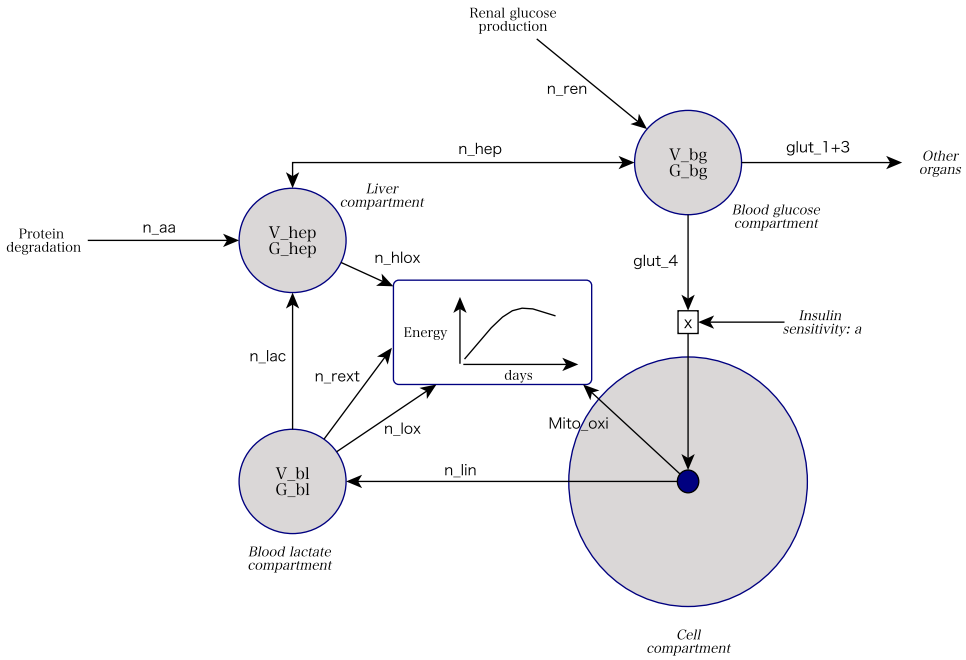

Figure 9.1: *An example of a restructuring of the proposed model for gluconeogenesis of amino acids. Notice that all transfer rates because of oxidation are related to the energy expenditure of the patient.*

The amino acid degradation parameter was estimated with regards to the blood glucose levels of the patients during ICU stay in chapter 8. However, clear limitations were found in this parameter estimation. As no data on feeding of the patients or amount of exogenous insulin administered could be extracted for the patients, see chapter 4, an assumption was made that the patients were fasting and received no exogenous insulin. These assumptions may be far from how the patient scenarios actually transpired and leads to a parameter estimation were amino acid degradation was estimated based on an artificial patient scenario. Without data on these parameters, it is hard to make the case that amino acid degradation is uniquely identifiable in the subsequent parameter estimation.

Developed models will always have limitations to their representation of reality. In some cases, experimental data is unavailable to identify model parameters, providing gaps in the overall model structure, suggesting possible research areas. Available data may not be rich enough to uniquely describe all unknown parameters in a model. This is known as the identifiability problem, where the model is too complex for the available data or the data is insufficient in relation to the concept of the model (e.g. parameters may only be estimated for normal individuals and not patients). All assumptions composed for a model will contribute to parameter inaccuracy and will affect the overall model performance [Chase et al., 2011], [Cobelli and Carson, 2008].

9.3 Future work

It is exceedingly difficult to describe a natural system, without access to relevant experimental data to uniquely identify the processes of the system [Steuer and Junker, 2009]. This was found to be the case of this model of amino acid degradation for use in gluconeogenesis. Although studies do provide some data, there is a need for raw measurement data for individual critically ill patients to meaningfully identify the parameters of the model [Dahn et al., 1995], [Dietze et al., 1976].

As discussed by [Biolo, 2013] it is complex to determine parameters that give indications of optimal protein administration for ICU patients, due to the amount of specific amino acids having differing function in the human body and rates of natural synthesis and degradation. As adequate provision of proteins can lower negative outcomes, it is still of the highest interest to model the protein requirements of ICU patients [Biolo, 2013].

Patient data on urine urea nitrogen and protein intake would make it possible to estimate the nitrogen balance of patients, which would aid in understanding and modeling the protein requirements of patients. However, these data may not prove adequate as increased availability of amino acids may be required under stress conditions, resulting in protein intakes that make the nitrogen balance positive rather than zero [Biolo, 2013].

In chapter 8 the blood glucose data of patients were used to estimate the amino acid uptake of patients during their course of ICU stay. A different and interesting prospect would be to further investigate how cortisol could be applied as a parameter in the model, serving as a factor affecting the amount of muscle proteolysis and amino acid degradation experienced for the patient, cf. section 3.3. As we have found from some studies, cf. chapter 7, one could hypothesize a *cortisol factor* directly influencing general amino acid degradation. As indicated by [Vermes and Beishuizen, 2001], Free Cortisol Index might be an even better measure of the influence of cortisol in the body, as it takes into account the proportion of that is free, not bound by CBG and hence not effective. Future work would need more 'raw' data to support or reject this relation, either through experimental studies or extraction of data from a more extensive clinical database than the one chosen for this project.

Much work has been put in our attempts to gather clinically relevant data for use in this project. An initial plan was to gather clinical data ourselves in collaboration with a hospital. However, due to unexpected new regulatory affairs taking effect during the project period, another plan for data acquisition had to be set in motion. We chose to gather data from the MIMIC II database. However, working extensively with the dataset we found that it did not contain the parameters we were the most interested in attaining (cortisol, interleukin-6, protein intake, UUN, energy expenditure).

It is a complex task to make a model on metabolic processes in the human body. In this project, we have however proposed an initial model to describe an aspect of protein breakdown of the critically ill. Although the model presents some limitations, and that future work will needed to apply more data for further conceptualization and identification , the proposed model is regarded as a proof of concept of how to model one aspect of the uses of muscle proteolysis in periods of stress induced by critical illness.

Chapter 10

Conclusion

Conditions, such as ICU acquired weakness, potentially leads to further morbidity and prolonged hospital stay of critically ill patients. Patients may experience a lowering of quality of life due to decreased physical ability by aqcuired weakness. A hypermetabolic 'flow' phase in the course of critical illness may contribute to an increased degradation of muscle protein reserves by proteolysis. The aim of this project was to examine the metabolic response to critical illness and the effect of this on muscle wasting. Moreover, clinical data supporting the notion of metabolic stress in ICU patients was sought and gathered. Data was gathered from MIMIC II, an intensive care unit research database containing more than 32,000 patient experiences. A list of relevant and desired clinical parameters intended as markers for metabolic stress, along with criteria for selection of patient scenarios, were defined. Only af a few of these stress parameters existed for the extracted patients in MIMIC II. Due to the lack of desired patient data, a physiological model, relating to the role of amino acids in gluconeogenesis during critical illness, was proposed. For this model, amino acid degradation due to gluconeogenesis was estimated by patient blood glucose levels. The lack of data on nutritional load and possible undergoing insulin therapy for the patients made a parameter estimation of amino acid uptake inaccurate.

This physiological model comprises of important physiologic compartments in the scenario of critical illness. However, additional work on the conceptualization and identification of model parameters may be necessary for the model to represent the critically ill patient group. Furthermore, gathering of experimental study data on metabolic stress and muscle proteolysis in critically ill patients is warranted, as current studies do not present "raw" data for use. Use of a "cortisol factor" to describe muscle wasting will likely prove a better marker of protein degradation than blood glucose in a physiological model.

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