Chapter 1

General introduction

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The aim of this thesis is to optimize identification of undernutrition, and explore the impact of the arginine/asymmetric dimethylarginine (ADMA) ratio and nutrition during surgery, in patients with cardiovascular failure. The first part describes results from a prospective cohort study of patients undergoing cardiac surgery in which different undernutrition screening and assessment methods were investigated. The second part of this thesis includes an observational study about the arginine/ADMA ratio in patients with shock and a randomized trial in patients undergoing coronary artery bypass grafting (CABG) in whom nutrition was given during the operation.

Undernutrition identification in cardiac surgery

Screening

Disease-related undernutrition has been defined as ‘a state of nutrition in which primarily a deficiency of energy and/or protein causes measurable adverse effects on body composition, function, and clinical outcome’. In cardiac surgery, disease-related undernutrition is an often overlooked preoperative co-morbid condition [1] while 10-25% of patients undergoing cardiac surgery being or becoming undernourished [2-4]. Undernutrition prior to cardiac surgery increases the risk for postoperative infectious and non-infectious complications, a prolonged length of intensive care unit and hospital stay and impaired wound healing [2,3,5,6]. Therefore, patients suffering from undernutrition prior to cardiac surgery have to be identified and treated (6-8).

Identification of undernourished patients begins with nutritional screening (Figure 1) for which several quick and easy tools are available [7]. Without use of these tools, 50% of undernourished patients remain unidentified [8]. The Malnutrition Universal Screening Tool (MUST) [9] and Short Nutritional Assessment Questionnaire (SNAQ) [10] are frequently used to identify undernourished patients. Both tools include an item about unintended weight loss (UWL) in the months before surgery, and an item about nutritional intake. In contrast to the MUST, the SNAQ does not include the item low body mass index (BMI). In cardiac surgery, the accuracy in detecting undernutrition was 59% for the MUST and 19% for the SNAQ meaning that 41% of cardiac surgery patients are misclassified as wellnourished when screened by the MUST and 81% when screened by the SNAQ [11]. These unrecognized undernourished patients do not receive nutritional therapy. When the items advanced age and female gender were added to the MUST, the accuracy increased to 74%, still suboptimal. Therefore, more research on this topic is required to increase the number of correctly screened undernourished patients undergoing cardiac surgery.

Assessment

After a patient is scored at high risk for undernutrition by a screening tool, referral to the dietitian takes place (Figure 1) [7]. The dietitian further assesses nutritional status by
performing several measurements such as assessment of body composition, UWL, dietary intake, nutrient losses, energy requirement, blood markers, and functional indicators [12,13]. If the patient is identified as undernourished or at high risk to become undernourished, a dietary treatment is prescribed and evaluated.

![Diagram of the process of identifying undernutrition.](image)

**Figure 1** Process of identifying undernutrition.

FFM, fat free mass; MUST, Malnutrition Universal Screening Tool; SNAQ, Short Nutritional Assessment Questionnaire.

The parameter low BMI and UWL have been applied extensively to assess undernutrition in cardiac surgical patients [2]. However, BMI does not provide exact information on the two body mass components; fat free mass (FFM; kg) and fat mass (FM; kg). Previous research in cardiac surgical patients suggested that specifically the low FFM-part of a low BMI is responsible for the increased occurrence of adverse outcome [3]. FFM mainly represents muscle mass which functions as an important source of amino acids for protein synthesis and gluconeogenesis in times of stress and starvation. Therefore,
patients with a low FFM may have insufficient reserves to respond adequately to operative stress resulting in less capacity to recover from surgery. Indeed, when patients were classified by BMI or UWL, half of the patients with a low FFM index (FFMI; kg/m²) were misclassified as wellnourised [3]. Besides a low BMI, a high BMI, present in 30% of patients undergoing cardiac surgery, is reported as independent predictor of wound and respiratory tract infections, mortality and a prolonged length of hospital stay [5,6,14]. Most likely the high FM-part of a high BMI is responsible for the increased occurrence of adverse outcome. It is unknown, however, what the effect of both a low FFM and high FM, so-called sarcopenic obesity (SO), is on clinical outcome after cardiac surgery. In general, loss of muscle mass (i.e. FFM) occurring in disease-related undernutrition will result in decreased muscle strength which can be reflected by worse functioning [15]. Therefore, muscle function measured by handgrip strength (HGS; kg) is advised to use as indicator of nutritional status and postoperative complications [15]. In cardiac surgical patients, it is unknown whether SO, and its separated components FFM and FM, are related to muscle function in this population.

Body composition is often determined by bioelectrical impedance which calculates FFM and FM from measurements of body water [16]. Another variable that can be derived with bioelectrical impedance, the phase angle (PA; °) might also offer a clinically applicable technique to identify undernutrition [13]. The PA reflects the ratio between reactance - the resistive effect produced by cell membranes, i.e. cell membrane capacity - and resistance - the restriction to the flow of an electric current through the body, primarily related to the amount of water present in tissue [17,18]. In agreement with FFM, the PA includes the assessment of total body water, and therefore reflect body cell mass. In contrast to FFM, the PA also measures cell membrane capacity and therefore also reflects body cell quality. Whether the PA can help to indentify undernourished cardiac surgical patients has to be investigated.

The arginine/ADMA ratio and nutrition during surgery

Arginine

Arginine is a semi-essential amino acid, meaning that under healthy conditions endogenous arginine production is adequate for metabolic needs, however, under stress, when arginine is excessively catabolised by the enzyme arginase, dietary intake of this amino acid is required. Dietary arginine is absorbed in the small intestine and is absorbed from the circulation via system y⁺-carriers of the cationic amino acid transporter (CAT) family into the liver, kidney, and endothelial cells. Besides dietary intake, arginine availability depends on endogenous release via protein degradation, on synthesis from citrulline by the enzymes argininosuccinate synthase and argininosuccinate lyase, and the metabolic pathways. First, arginine can be converted into agmatine. Second, arginine can be metabolized to creatine, an important form of stored energy in (cardiac) muscle tissue.
**Figure 2** Synthesis and metabolism of arginine and ADMA.

ADC, arginine decarboxylase; ADMA, asymmetric dimethylarginine; AGAT, arginine glycine amidinotransferase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; ATS, arginyl-tRNA synthetase; CAT, cationic amino acid transporter; DDAH, dimethylarginine dimethylaminohydrolase; NMMA, N-monomethyl-arginine; NO, nitric oxide; NOS, nitric oxide synthase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; PRMT, protein arginine methyltransferase; SDMA, symmetric dimethylarginine.

The third pathway of arginine is its conversion into urea and ornithine by the enzyme arginase. Two isoforms of arginase are known: type I metabolizes arginine in the cytosol whereas type II is active in the mitochondria. Ornithine can be converted into polyamines, proline and glutamate. The last metabolic pathway is the conversion of arginine into nitric oxide (NO) and citrulline facilitated by nitric oxide synthase (NOS). NO can subsequently diffuse into the vascular smooth muscle layer resulting in cGMP-mediated relaxation and vasodilation. Three different isoforms of NOS are known: neuronal NOS (nNOS or NOS1), endothelial NOS (eNOS or NOS3), and inducible NOS (iNOS or NOS2). In general, nNOS and eNOS are considered constitutive enzymes whereas iNOS is highly regulated by cytokines.
Chapter 1

NO is a prominent compound in the heart and its vasculature and plays an intriguing role in the physiology of this organ [19]. NO is the most important endothelial vasodilator, and is involved in down regulation of cellular adhesion molecules, inhibition of platelet aggregation, vascular proliferation, and angiogenesis. In cardiomyocytes, the actions of NO are more complex as it can induce different, and sometimes opposing effects on cardiac functioning such as triggering apoptosis and improving left ventricular function.

Besides arginine’s involvement in cardiovascular function, the amino acid plays a role in the regulation of inflammation and immunity as it directly or indirectly stimulates proliferation of immune cells [20]. As immune enhancing nutrient, arginine is excessively metabolized by the enzymes arginase and NOS. Arginase metabolizes arginine via ornithine to polyamines and proline thereby playing a key role in cell proliferation and wound healing. NO enhances immunity at cellular level by increasing proliferation of lymphocytes and monocytes, enhancing T-helper cell formation, activating macrophage cytotoxicity, reinforcing natural killer cells, increasing phagocytosis, and enhancing cytokine production [21].

**ADMA**
There are two compounds that can inhibit NOS, N-monomethyl-arginine (NMMA) and ADMA, which both reduce NO synthesis by competing with arginine for NOS binding [22]. However, NMMA concentrations in plasma are much lower compared with plasma ADMA concentrations. NMMA is formed when protein-incorporated arginine is methylated by the enzymes protein arginine methyltransferases (PRMT)-1 or PRMT-2 (Figure 2). PRMT-1 can subsequently methylate NMMA resulting in the formation of ADMA whereas PRMT-2 can methylate NMMA into symmetric dimethylarginine (SDMA). After proteolysis, the methylated arginines are released as unbound forms in the cytosol where NMMA and ADMA are able to inhibit NOS. In contrast to ADMA, SDMA is not able to inhibit NOS. All methylated arginines are released from the cell into the circulation via CAT from which they can be taken up by other cells that use the same transporters. CAT also facilitates the transport of arginine across the cell membrane. All methylated arginine metabolites are considered to interfere with NO synthesis indirectly because the methylated arginine analogues compete with arginine for transport via CAT. However, because SDMA lacks NOS inhibitory activity and only small amounts of NMMA are found in the plasma, ADMA is considered to be the major inhibitor of NO synthesis. ADMA is excreted into the urine for ~20%. The major part of the NOS inhibitor is cleared by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) resulting in the formation of citrulline and dimethylamine. Both the expression and the activity of DDAH seem to be regulated by NO through feedback mechanisms [23-27].

Since ADMA has been shown to inhibit NO synthesis its role in cardiovascular (dys)function has been investigated extensively. Elevated ADMA levels have been found in a variety of cardiovascular diseases. Moreover, ADMA is indicated to have prognostic
capacities for disease progression and mortality in heart failure patients [28-30], in critically ill patients [31], and in the community [32]. Furthermore, ADMA infusion has been shown to impair relaxation of coronary arteries, induce myocardial remodelling, deteriorate cardiac function, and cause myocardial ischaemia [33-38]. Together with low arginine levels (as induced by arginase infusion), ADMA infusion further deteriorated stroke volume and cardiac output in rats [36]. Nevertheless, NOS inhibitors have been proposed as a treatment for the overproduction of NO in sepsis and cardiogenic shock. The underlying hypothesis is that the increased production of NO by iNOS in shock contributes to hypotension and multiple organ dysfunction. However, results of several randomized trials investigating NOS inhibitors are conflicting. In cardiogenic shock patients, NOS inhibition resulted in a modest increase in mean arterial pressure [39] and reduced mortality rate [40]. In contrast, in a large clinical trial, another NOS inhibitor increased the mortality rate of septic shock patients [41]. Mortality and adverse events in the treatment group were associated with cardiovascular death and haemodynamic dysfunction, including heart failure and decreased cardiac output. In addition, administration of NOS inhibitors reduced coronary flow and induced local ischaemia in endotoxin-treated rat hearts [42]. These negative effects of the NOS inhibitors can probably be explained by microvascular pathology due to inhibition of eNOS, which can ultimately result in myocardial dysfunction. Therefore, non-selective inhibition of NOS is not recommended in the critically ill patient.

Overall, many studies have shown that ADMA can induce detrimental effects. These unfavourable actions are primarily the result of diminished NO availability, resulting in disturbed vasodilatation and anti-thrombotic and anti-apoptotic actions that might finally induce cardiac dysfunction (Figure 3). Furthermore, ADMA is able to uncouple NOS after which NOS becomes a source of superoxide radicals instead of producing NO. Indeed, ADMA infusion has been shown to increase superoxide production [43]. The increase in production of reactive oxygen species after NOS uncoupling can inhibit DDAH activity [43] and can lead to oxidation of cellular components in cardiomyocytes, such as proteins critical for excitation-contraction coupling [44]. It can also lead to increased susceptibility of cardiomyocytes to cell death, which can finally lead to cardiac dysfunction.

The increased plasma ADMA levels seen in several diseases of the heart and its vasculature might be explained by either increased intracellular production and/or decreased excretion of ADMA from the plasma. However, PRMT-1 expression was unchanged in the hearts of dogs with congestive heart failure [45]. Altered expression of CAT, which transports ADMA across the cell membrane, is also doubtful since both reductions and increases in CAT expression have been found in heart failure patients [46,47]. Another explanation might be found in high ADMA levels resulting from impaired renal function, which is often seen in heart failure patients. However, in patients with coronary artery disease, ADMA correlated negatively with glomerular filtration rate (eGFR,
used as indicator for renal function) [48], whereas in patients with acute myocardial infarction, a relationship between ADMA and eGFR was not present [49]. Finally, increased ADMA levels can be the result of reduced DDAH activity and/or expression. Since ADMA catabolism is mainly regulated by DDAH, it can be hypothesized that a reduction in DDAH induction is the main contributing factor to elevated ADMA levels in cardiac dysfunction [33]. Indeed, complete malfunction of DDAH can lead to a daily increase in plasma ADMA concentrations of ~5 mmol/L.

**Figure 3** Asymmetric dimethylarginine as a predictor of prognosis in patients with heart failure.

ADMA, asymmetric dimethylarginine; NOS, nitric oxide synthase; HF, heart failure; ROS, reactive oxygen species.

**Arginine/ADMA ratio**

Since ADMA inhibits NO production by competing with arginine for NOS binding, the net amount of NO production might be indicated by the ratio between substrate and inhibitor: the arginine/ADMA ratio. For example, when ADMA levels are high, NO...
General introduction

synergy might still be possible when arginine levels are sufficiently high to dislocate ADMA and serve as a substrate for NOS. Therefore, the detrimental effects of ADMA might be inhibited by increasing the concentration of arginine or the concentration of its precursors, citrulline and glutamine. This would increase the arginine/ADMA ratio and might reverse the competitive inhibition of NOS by ADMA. In the heart, increasing arginine might be beneficial by elevation of NO which is essential for optimal cardiovascular perfusion. Especially, because arginine decreases and ADMA increases under conditions of stress like in critically ill patients and after surgery. In these conditions, the catabolised arginine is no longer available to NOS, hence subsequent NO synthesis is diminished. As a result, the actions of NO on the heart and its vasculature can be disturbed, and reactive oxygen species might be produced, which have a negative impact on cardiac functioning [44].

Although the concentration of arginine in endothelial cells is higher than necessary to saturate NOS, it has been shown that increased extracellular arginine can be taken up by endothelial cells and can contribute to NO production, a phenomenon called 'the arginine paradox' [50]. However, the effect of arginine supplementation is complicated as studies have shown both positive and negative results in critically ill patients [51,52]. Arginine supplementation seems beneficial and safe under conditions of cardiovascular dysfunction without notable inflammation. In inflammatory states arginine supplementation is complex as an arginine-induced excess in NO production by iNOS might be deleterious because it may lead to detrimental vasodilatation and to increased formation of ROS leading to cellular damage. On the other hand, an increase in NO facilitated by eNOS is of vital importance as it mediates microvascular vasodilatation. Probably, the arginine/ADMA ratio might play a role as NO availability needs to be perfectly balanced in order to guarantee proper cardiac contraction and vascular dynamics. For that reason, instead of arginine supplementation solely, nutrition containing arginine is a safe way that might increase arginine levels and concomitant arginine/ADMA ratio.

Nutrition during surgery

Pre- and postoperative nutritional supplementation has been shown to have beneficial effects like less reduced weight loss, less postoperative infections and shorter hospital length of stay [53-57]. In addition, early postoperative feeding has shown to be safe [58,59]. Besides, patients having elective operations can safely drink clear fluids until two hours before surgery as research has shown that preoperative fasting does not result in a reduced risk of aspiration, regurgitation or mortality [60-62]. Moreover, preoperative carbohydrate loading can reduce postoperative insulin resistance and can improve clinical outcome. Therefore, current guidelines advice patients to eat until six hours and to drink two hours before surgery, and to start nutrition as early as possible after surgery [55,63]. Unfortunately, it is still common practice in many hospitals that patients are fastened
from midnight before surgery because of (unnecessary) fear for pulmonary aspiration [63,64]. Furthermore, in both cardiac surgery and other major surgeries, most patients receive only clear fluids during the period prior to surgery through the day after surgery leading to fasting of the patient over a longer period of time. Fasting can induce postoperative thirst, stress, and insulin resistance [61]. Moreover, elective operations are commonly postponed because of priority of an emergency operation which can lengthen fasting time. Glycogen reserves that last only a few hours will deplete with the result that further fasting induces gluconeogenesis. Gluconeogenesis mainly depends on amino acid supply by body protein catabolism. In addition, in times of surgery, there is an extra need for amino acids as they are needed for the operative stress response and tissue repair [65]. Consequently, fasting weakens the catabolic state of the surgical patient which further weakens the patient and can slow down recovery after surgery [66].

The lack of amino acids might have more side effects as amino acids are essential for proper myocardial metabolism and protein synthesis [67,68]. A lack of optimal nutrition might change myocardial substrate utilization which can have adverse effects on myocardial metabolism such as adenosine triphosphate (ATP) production and utilization [69]. Therefore, it can be hypothesized that avoidance of fasting can improve cardiac metabolism. To avoid fasting, nutrition should not only be provided before and after surgery but also during surgery. Supplying nutrition during surgery can be done by the parenteral route, or by the enteral route when fed into the duodenum [70]. Nutrition should be hypocaloric as overfeeding can harm well-nourished patients [71]. To study the effects of nutrition on cardiac cells, patients undergoing surgery for coronary artery bypass grafting (CABG) can be selected as cardiac tissue biopsies can be taken during this procedure. In order to prevent cardioplegic effects on cardiac cells, patients should be selected that can undergo an off-pump (i.e. without cardiopulmonary bypass (CPB)) CABG procedure.

In addition to myocardial metabolism and protein synthesis, amino acids, including the NO-precursor arginine, are essential for proper immune cell responses [72]. When immune cells are activated by inflammatory signals, their demand for amino acids increases rapidly. In turn, inadequate access to amino acids during immune cell activation may deteriorate immune response by inhibiting immune cell division, differentiation, and migration. Surgery provokes a whole body inflammatory response which predisposes surgical patients to the development of infections [73]. In accordance, low plasma levels of arginine [74,75] and other amino acids have been found in surgical patients [76], and pre- and postoperative nutritional supplementation has been found to diminish the inflammatory response (57). In cardiac surgery, CPB and cardioplegic cardiac arrest specifically activate the systemic inflammatory response by contact of blood to the foreign surfaces of the CPB system and by ischemia-reperfusion injury. CABG performed without CPB on the working heart, i.e. off-pump CABG, is known to reduce the systemic inflammatory response as measured by plasma markers of inflammatory cells, but does
not prevent is completely [77]. However, according to our knowledge, the inflammatory response of the heart itself and the effect of nutritional supplementation before and during (cardiac) surgery have never been investigated.

For the above reasons, nutrition during surgery may increase the arginine/ADMA ratio and amino acids in plasma and myocardial tissue which may influence cardiac metabolism and its inflammatory response. When searching the literature, no studies can be found that investigated the effect of nutrition before, during, and after surgery on the heart. Umenai et al. gave parenteral amino acids for six hours starting from two hours before the induction of anesthesia in patients undergoing off-pump CAGB which increased esophageal core temperature, shortened duration of postoperative mechanical ventilation, intensive care stay and hospitalization, and speeded tracheal extubation [78]. McElroy et al. explored the safety of perioperative enteral nutrition in surgical patients but nutrition was still interrupted from the time of transfer to the operating room until postoperative [79]. A few previous studies investigated the effects of nutrition, either given by the parenteral or enteral route, on cardiac function and metabolism, and show favorable results. Randomized controlled trials in humans in which the amino acids arginine [80-82], aspartate [83] and/or glutamate [84] were administered, have shown improved cardiac flow [81,82], cardiac function (measured as plasma troponin T, creatine kinase (CK), and CK-MB) [80,83,84] and/or cardiac metabolism (measured as myocardial acidosis, ATP and lactate in myocardial biopsies) [83,84]. In animal studies, amino acid supplementation minimized cardiomyocytes apoptosis probably by increasing ATP production and myocardial oxygen consumption [85], by reducing myocardial ischemic damage, and by increasing diastolic pressure [86]. Enteral nutrition in cardiac surgical patients repleted cardiomyocytes with nutrients, improved left ventricular end-diastolic volume before surgery [87], improved preoperative host defense, reduced the number of postoperative infections, and preserved renal function [88]. In patients with acute ischemic stroke, enteral nutrition reduced interleukin-6 and increased glutathione levels suggesting it may decrease inflammation and increase antioxidant defense [89]. These aforementioned studies supplied nutrition before and/or after surgery. According to our knowledge, studies in which the effect of uninterrupted perioperative nutrition is investigated do not exist.

Rationale and outline of the thesis
The first part of this thesis is intended to optimize identification of undernutrition in cardiac surgical patients. Current screening tools misclassify a high amount of undernourished cardiac surgery patients as wellnourished. These undernourished patients are at increased risk of worse postoperative outcome and do not receive nutritional therapy. More research on this topic is required to increase the number of correctly indentified undernourished patients. Therefore, Chapter 2 presents the results of our
study in which the Cardiac Surgery-Specific Undernutrition Screening Tool was evaluated as screening instrument of undernutrition in cardiac surgical patients. After a patient is screened to be at risk, referral to a dietitian takes place who performs further diagnostic assessment including FFM measurements by bioimpedance spectroscopy. Besides a low FFM, a high fat mass (FM) have been associated with adverse clinical outcome in cardiac surgical patients. However, the effect of these risk factors present at the same time, i.e. sarcopenic obesity (SO), on outcome and muscle function is unknown. Therefore, Chapter 3 describes the associations between SO and handgrip strength and with adverse clinical outcome after cardiac surgery. Besides body composition, bioelectrical impedance measures the phase angle which is defined as the ratio between reactance and resistance thereby reflecting both cell quantity and quality. To further improve diagnostic assessment, we investigated in Chapter 4 whether the bioelectrical impedance phase angle can help to improve identification of undernutrition and can predict adverse clinical outcome in cardiac surgical patients.

The second part of this thesis is intended to investigate the impact of arginine/ADMA ratio and nutrition during surgery, in patients with cardiovascular failure. As ADMA competes with arginine for NOS binding, the net amount of NO might depend on the proportions of arginine and ADMA. Therefore, the arginine/ADMA ratio might be a better indicator of prognosis compared to ADMA solely in patients with cardiovascular dysfunction. In patients with septic or cardiogenic shock, plasma arginine is low while ADMA levels are high. We hypothesise that this imbalance of arginine and ADMA contributes to the poor organ perfusion seen in patients with shock. Therefore, Chapter 5 describes the association between the arginine/ADMA ratio and markers of circulation, organ function and mortality in shock patients. A high arginine/ADMA ratio and amino acids can be essential for proper cardiovascular perfusion and metabolism. Supplementation of nutrition may increase myocardial and plasma arginine/ADMA ratio and amino acids. To minimize the surgery-induced decreases in myocardial amino acid levels and to avoid fastening, nutrition should be given before, after, but especially during surgery. Therefore, Chapter 6 shows the effect of (par)enteral nutrition before, during, and after cardiac surgery on myocardial arginine/ADMA ratio and amino acids and the associations with myocardial glucose metabolism. (Cardiac) surgery provokes a whole body inflammatory response in order to heal tissue damage, but the inflammatory response in the heart during these procedures has not been described. Furthermore, preoperative and postoperative nutritional supplementation has been found to diminish the inflammatory response but the effects of nutrition during surgery on inflammation has never been investigated. In Chapter 7, we study the myocardial inflammatory response and investigate the effect of nutrition during surgery in patients undergoing off-pump CABG.

Finally, Chapter 8 discusses the results in this thesis by describing main findings, clinical implications and future perspectives, and by making conclusions.
General introduction

References
General introduction


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