Chapter 7
Summary and general discussion
General discussion

Hemodialysis patients have a much lower life expectancy than the general population (1;2). Malnutrition is a strong predictor of morbidity and mortality in this patient population and to date there is no clear understanding of the pathological processes that underlie this phenomenon. Using stable isotope dilution methods, we were able to gain more insight into protein metabolism in the chronic hemodialysis patient.

Chapter 1 describes human protein metabolism and the technical problems of the stable isotope infusion technique. Protein metabolism has been studied now for over 150 years and the methods to specifically measure individual processes in the body have increased substantially. Also, hemodialysis has evolved from a life saving technique to a long-term life sustaining therapy with more than 3400 patients in the Netherlands. Dialysis patients have a worse prognosis and multiple risk factors have been identified. Malnutrition is one of the factors that has been hard to define but which has been measured and discussed extensively. In our studies, we studied protein metabolism during both the non-dialysis and dialysis period under various conditions to investigate the responsiveness of the metabolic system in chronic hemodialysis patients.

Protein metabolism and urea kinetics in hemodialysis patients

Chapter 2 describes a study comparing protein oxidation and urea metabolism, which was measured using $^{13}$C-valine and $^{13}$C-urea as tracers for both systems. It appeared that protein oxidation is underestimated by urea measurements during fasting but not during feeding. Urea is hydrolysed in the intestine during fasting and the resulting ammonia could be used metabolically. The magnitude of this process and its dependence on acute protein intake in HD patients was unknown until now. Urea is the major vehicle for the detoxification and excretion of nitrogen arising from the metabolic transformations and catabolism of amino acids in the body. It has been known for a long time that urea production exceeds urea excretion in healthy control subjects (3;4), which is due to intestinal urea hydrolysis. Jackson et al. (5) suggested that body nitrogen balance is critically dependent on the salvage of urea nitrogen and its net retention within tissue protein and amino nitrogen pools. These authors also state that urea production is relatively constant and that nitrogen balance is mainly achieved via regulation of urea hydrolysis so that urea excretion is not a measure of amino acid oxidation (6). Both El Khoury et al. (7) and our results show a correlation between urea production and amino acid oxidation in hemodialysis patients, although weak, so that the suggestion can not be confirmed. El Khoury et al. (8) showed that about 19% of ingested protein was retained in non-urea pools during
meal intake. Our data are in agreement showing that about 10% of ingested nitrogen was retained in the amino acid pool as shown from the increase in amino acid concentrations in plasma. In the future, this limited data set in hemodialysis patients should be extended and a differentiation should be made between whole body urea synthesis and hepatic urea synthesis. The ammonia entering the portal circulation could also regulate hepatic urea synthesis but this remains to be investigated in dialysis patients.

Protein metabolism in chronic hemodialysis patients
During fasting, there are no major disturbances in protein metabolism in hemodialysis patients. In one of the earliest studies by Berkelhammer et al. (9), amino acid oxidation was increased and synthesis was decreased in hemodialysis patients compared to healthy control subjects. However, food intake was not controlled in this study. In pre-dialysis patients or patients on peritoneal dialysis, two studies could not demonstrate a difference in whole body protein metabolism using $^{13}$C-labelled leucine (10;11). The observed protein energy-malnutrition in the hemodialysis population is thus not caused by a malfunction in protein metabolism but could be due to disturbances in utilisation of ingested protein. In chapter 3, we present data on protein metabolism during fasting and during ingestion of a protein-enriched meal to confirm earlier results during fasting and to investigate possible metabolic differences between stable HD patients and control subjects using a primed-constant infusion of L$[1-^{13}C]$ valine. During fasting, whole body protein balance was significantly less negative in HD patients compared to control subjects. During meal intake, protein balance was similar between HD patients and control subjects. Meal intake increased protein balance significantly in both groups but not differently between the groups. Also, protein oxidation was decreased during fasting in HD patients compared to control subjects but not during meal intake. From this chapter, we conclude that the rate of protein metabolism is lower in HD patients compared to control subjects but the efficiency of protein utilisation is normal in HD patients during a non-dialysis day. This makes it very unlikely that an increased breakdown of protein or an impaired anabolic response to protein ingestion contributes to the observed protein-energy malnutrition in these patients.

Effects of dialysis on protein metabolism
The dialysis procedure augments protein catabolism (12-15) as shown from urea kinetics. These experiments showed an accelerated rise in urea concentration after the hemodialysis procedure, which was initially interpreted as an increased oxidation of amino acids. However, these data must be interpreted with caution since the urea rebound i.e. the redistribution of urea over the urea dilution volume, was not taken into account, although
it is probably the most important factor causing this accelerated rise in urea concentration after dialysis. During fasting, amino acid oxidation was decreased compared to the pre-dialysis period and protein synthesis was also reduced (16). This resulted in a more negative protein balance. These findings were confirmed by Ikizler et al. (17) who showed that whole body protein breakdown was increased resulting in a similar negative protein balance as shown in the study by Lim et al. (16). We show in chapter 4 that consumption of a protein and energy enriched meal improves the whole body protein balance during dialysis in chronic hemodialysis (CHD) patients to a similar degree as in hemodialysis patients during a non-dialysis day (which is comparable to control subjects). During a non-dialysis day, feeding changed the negative whole body protein balance observed during fasting into a positive protein balance. Dialysis worsened the negative balance during fasting, whereas feeding during dialysis induced a positive balance comparable to the balance during the non-dialysis day with meal intake. Plasma valine concentrations during the studies correlated with whole body protein synthesis and correlated inversely to whole body protein breakdown. We conclude that the consumption of a protein and energy enriched meal by CHD patients while dialysing can strongly improve whole body protein balance, probably due to the increased amino acid concentrations in blood. Our results are confirmed by a recent study by Pupim et al. who found that an infusion of amino acids also increased the amino acid concentrations in blood and in their study, protein balance also became highly positive (18). These studies were indicative that amino acid losses are the most important factor for the more negative protein balance seen during hemodialysis, at least if biocompatible membranes are used.

**Membrane compatibility**

There is evidence that bioincompatible dialysis membranes lead to protein catabolism (19-21). Using arterio-venous amino acid concentration differences and leg blood flow Guttierrez et al. showed that there was more release of amino acids from leg muscle during dialysis using bioincompatible membranes. This was only seen several hours after the dialysis session. This suggests that lack of biocompatibility of the dialysis membrane, which results in low-grade inflammation (22), could be responsible for the more negative protein balance seen during dialysis. Production of cytokines and a low clearance of middle molecules is also seen in these patients (see for review Horl (23)). The mechanism by which activation of complement could result in increased catabolism is not clear. In chapter 5, we investigated whether protein-energy malnutrition could be partly due to incompatibility of the dialyzer by comparing whole body protein metabolism during dialysis with biocompatible and bio-incompatible dialyzers. Protein metabolism parameters during both study protocols were not different and resulted in the same protein balance.
during polysulfone/cellulose-triacetate and cuprophan dialysis. During this short-term study in stable hemodialysis patients with no apparent complications, protein metabolism during dialysis was thus not affected by the compatibility of the dialysis membrane. In a randomised controlled clinical trial, long-term clinical use of these different membranes showed that after one year, dry weight increased in the biocompatible group while this was not the case for the bioincompatible group (24). These differences can thus not be explained by changes in short-term modulation of protein metabolism. We suggest that bioincompatibility could have effects on whole body protein metabolism only during periods of illness and not while patients are stable. This further highlights the importance of monitoring the nutritional status of hemodialysis patients during periods of infection or malaise.

**Dietary energy intake and whole body energy expenditure**

Indirect calorimetry measurements have shown an increase in energy expenditure in hemodialysis patients, which has been used to explain the malnutrition present in many of these patients. This method however, becomes inaccurate when the bicarbonate concentration in plasma changes during the measurement, as is the case in hemodialysis patients during dialysis. We measured bicarbonate production in control subjects and hemodialysis patients before and during dialysis using stable isotopically labelled bicarbonate, which provides an alternative method to calculate energy expenditure. Whole body oxidation in hemodialysis patients was reduced compared to control subjects. During dialysis, this reduction was even more pronounced. Low food intake during medical complications appears a more likely cause of energy malnutrition than increased energy expenditure in chronic hemodialysis patients.
General conclusions

These experiments show that stable hemodialysis patients, when fed during dialysis have no major deficiencies in the nutritional adaptations to the lower than normal intake, and to the losses of amino acids during hemodialysis. The dysbalance between essential and non-essential amino acids as shown in chapter 4 could play a role in the long-term development of protein-energy malnutrition. Further studies should focus on measuring whole body protein metabolism together with the synthetic rate of specific proteins and/or genetic factors (25) and on local protein metabolism such as in liver or muscle tissue. This requires that multiple tracers have to be used, so that the conclusions are not dependent on the assumption that one essential amino acid is representative of all essential amino acids. In future research, it would also be interesting to study the roles of individual amino acids on nutritional status in chronic hemodialysis patients. This could be performed by giving solutions without a single amino acid or by supplying single or multiple amino acids (as for example in a study by Hiroshige et al. (26)). These studies could give more information about the regulatory roles of individual amino acids on parameters of metabolism and nutritional status in hemodialysis patients.

In this manuscript, studies are described investigating protein-energy malnutrition in chronic hemodialysis patients. We can conclude that hemodialysis patients are not more catabolic than control subjects as is widely believed. The hemodialysis patient is more susceptible to infections and periods of malaise from which the patient is possibly less capable of recovering. This hypothesis remains to be investigated.
Reference list


