Chapter 9

Discussion

In this thesis several aspects of the metabolic disorder PKU were highlighted. The two main topics concerned the effects of the protein substitute on the body of PKU patients and the mechanism underlying the brain damage in PKU that causes mental retardation.

THE EFFECTS OF THE PROTEIN SUBSTITUTE ON THE BODY

We do not know the different effects of the protein substitutes used nowadays on the body. Previous studies suggested that infants treated for PKU with a protein substitute showed slight growth retardation. The first question asked was "What is the effect of the amount of protein substitute on growth of length and head circumference in PKU infants?" This question was addressed in **chapter 2,** where protein intake (from intact protein and from the protein substitute) was correlated with growth of height and head circumference. This retrospective study showed that protein intake does not correlate with height growth in PKU infants. Growth of head circumference was found to correlate with the intake of natural protein but not with the intake of the protein substitute. Growth of head circumference is an important parameter as it relates to brain growth that could affect psychomotor development. The results of the study could mean that the protein substitutes that were used by the patients in this study were still not optimal in composition as compared to natural protein. It could be that the amount of certain amino acids is not optimal or that the amount of certain minerals or essential fatty acids that constitute natural protein was insufficient. In **chapter 3** we asked whether there is a difference in growth of length and head circumference comparing different populations, using different amounts of protein and protein substitutes. We compared a cohort of Dutch PKU infants with a cohort of American PKU infants (region Los Angeles). The relationship between growth and dietary data was studied with multiple regression analysis. We found that the protein intake in the American population was significantly higher but notwithstanding this, growth rate was comparable in the two populations. So, a mean total protein intake clearly above RDA over the first 3 years of age is not important as the only factor for optimal height growth in PKU infants. Further research to investigate the effect of other nutrients than protein on growth of PKU patients is necessary.

The way in which PKU patients metabolize the protein substitute is largely unknown. In this respect we asked the question "Is protein metabolism in PKU patients comparable to protein metabolism in the general population?" In **chapter 4** we studied protein metabolism in adult PKU patients and in adult healthy volunteers. We used the stable isotope L-[1-^{13}C]-valine and an isotopic model using valine intake, labelled valine infusion, and isotopic enrichment in plasma and expired air to calculate protein turnover during the fasting state and during the continuously fed state. No differences in protein turnover were observed between PKU adults and healthy volunteers. Thus, the protein metabolism in PKU patients is comparable to protein metabolism in the general population. From this study it can be concluded that adult PKU patients do not need a protein intake above the recommended daily allowance. In daily practice, food intake is not continuous but rather in

boluses. We do not know how to divide the boluses of amino acids over the day. To assess this problem, we performed a second study, using stable isotopes, which is presented in **chapter 5**. The main question was "What is the effect on protein metabolism of one large portion of amino acids compared to a small portion?" From this study we concluded that the relative contributions of the processes participating in protein turnover are not different in patients consuming either a large or a small bolus of amino acids.

The current treatment of PKU with a protein restricted diet and a protein substitute that contains micronutrients, vitamins and all amino acids but Phe, has proven its effectiveness. The clinical condition of PKU patients has improved unequivocally. Mental retardation is prevented when the diet is started early in life and this is an enormous gain for the PKU patient and his environment.[1] The diet is a relatively accessible treatment for patients that are diagnosed with PKU. In the Western world PKU diets can be installed for almost all patients and continued life-long in most European countries. In other countries where newborn screening has started, the treatment is developing. As all treatments, the current treatment of PKU has its disadvantages. The diet is hard to comply with: the amino acids are impalatable and the diet is socially impairing as patients cannot eat regular meals or go out for diner without preparation. Moreover, the protein substitute does not have the optimal composition. Growth is still not optimal in PKU patients and besides, deficiencies of vitamins, minerals and essential fatty acids could lead amongst others to problems with bone mineralization and neurological problems.[2-10] Treatment could be optimized in various ways. For example, the composition of the protein substitute could be further improved. This should be done in close collaboration between physicians, dieticians, food specialists, patients and manufacturers of protein substitutes. Second, the distribution of the protein substitute over the day may be altered, especially for adults and older children. The diet of young infants is easily controlled by their parents but for older children and adults, compliance with the diet would be largely improved by taking the protein substitute less frequently. When adult patients could take the protein substitute only once a day, compliance and thereby outcome probably will improve.

To monitor treatment, plasma Phe concentrations are measured. However, the exact range of plasma Phe concentration to aim at is unknown, as can be concluded from the different recommendations in different countries.[11-15] Besides, the plasma Phe concentration is a surrogate marker as the goal of PKU treatment is to optimize brain development and psychosocial well-being. In optimizing treatment it is thus not only important to focus on plasma Phe concentrations but also on outcomes such as psychosocial and intellectual testing.[16]

THE MECHANISM UNDERLYING BRAIN DAMAGE IN PKU

The exact mechanism by which PKU causes brain damage leading to mental retardation is not known. With dietary Phe restriction mental retardation can be prevented. Still, cognitive function is not optimal, i.e. problems in executive function, concentration, and speed processing were found.[17;18] In

order to further optimize the treatment of PKU patients it is necessary to elucidate the mechanism that causes brain damage. In this way, a treatment can be developed aiming to counteract this mechanism. The second part of this thesis focussed on the barrier that separates the blood from the brain as this barrier must have an important role in process leading to brain damage in PKU. The blood-brain barrier (BBB) contains different transporters that regulate the transport of substances from blood to brain. One of these transporters is the large neutral amino acid transporter (LAT1) that transports Phe and the other large neutral amino acids (LNAA) from the blood to the brain.[19] In PKU, all LNAA are essential amino acids. Under physiological circumstances LAT1 functions at a highly saturated level and has a high affinity for Phe.[20] In **chapter 6** we questioned whether high plasma Phe concentrations decrease cerebral protein synthesis. We found indeed that cerebral protein synthesis rate was diminished with higher Phe concentrations in plasma. The findings of this study suggest that control of the plasma Phe concentration remains necessary even into adulthood, as protein synthesis in brain is important not only during brain development but also thereafter for memory and behaviour. To confirm that the high plasma Phe concentration acted on cerebral protein synthesis via inhibition of transport of LNAA over the BBB, we asked the following question in **chapter 7**: "Does plasma Phe concentration interfere with BBB transport of other LNAA?" We studied this transport over the BBB using the radio active isotope L-[1-^{11}C] tyrosine and positron emission tomography (PET). Transport of tyrosine was decreased by increased Phe concentrations in plasma.

From the two studies on BBB transport and cerebral protein synthesis we posed the hypothesis that not the high Phe concentration itself but rather the impaired transport of other LNAA into the brain causes mental retardation. This hypothesis is discussed in **chapter 8**, where a diagram is presented with the different factors that could lead to mental retardation in PKU and their basic physiological mechanism that could be the underlying factors. During the past decades, treatment of PKU focused on the relationship between plasma Phe and cognitive abnormalities, which could be largely prevented by dietary Phe restriction. The idea behind the Phe restricted diet was that high plasma Phe concentrations result in high brain Phe concentrations that are thought to cause cognitive dysfunction. By reducing plasma Phe, brain Phe concentration is reduced, and thus cognitive function is more or less normal. Also in research that tried to find the underlying mechanism causing brain damage, the focus has been on cerebral Phe concentration that is increased in PKU patients and PKU mice[21-25] and was considered the toxic agent. A decrease in cerebral Phe concentration was also found to be reached by supplementing PKU patients and PKU mice with high doses of LNAA.[21;24;26] In PKU patients, EEG abnormalities were restored after supplementation with high doses of LNAA. It could be hypothesized that this positive effect of LNAA supplementation is not caused by a decrease in brain Phe but rather by an increase in other LNAA in brain.

From our PET studies and from other PKU studies it can be concluded that the BBB has an important role and high plasma Phe concentrations not only result in high brain Phe concentrations but also in low brain concentrations of other LNAA that could disturb cognitive functioning. The incorporation of LNAA into brain tissue was decreased with increasing plasma Phe concentrations

in PKU patients and in PKU mice.[27-29] A low concentration of LNAA in brain could lead to impaired cerebral protein synthesis that is essential for cognitive function.[30] The decrease in cerebral protein synthesis could thus be of central importance in the cognitive deficits found in PKU. In infants and children disturbed protein synthesis could impair brain growth and development, whereas in adults it could interfere with brain plasticity thereby influencing behavior and cognitive function. Future research should focus on disturbances in cerebral protein synthesis and how this could be influenced.

If the hypothesis holds that low cerebral LNAA are the main pathogenic substrate in PKU, a different treatment strategy with LNAA supplementation could alleviate the burdensome Phe restricted diet for PKU patients. Furthermore, PKU can serve as a reference disease for many other inborn errors of amino acid metabolism. In other diseases of LNAA metabolism, e.g. Maple Syrup Urine Disease, Isovaleric acidemia, Tyrosinemia type II and III. The general assumption is that a high cerebral concentration of a specific LNAA is the toxic agent. However, these patients also show an imbalance in their LNAA profile in plasma resulting in reduced LNAA availability in brain tissue. As for PKU, the main pathological substrate in these diseases may be a cerebral deficiency of other LNAA instead of the presumed toxic effect of the specific LNAA. Thus, patients with other inborn errors of amino acid metabolism could benefit from investigations in PKU patients and mice.

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