

# **Chapter 1**

## **General Introduction**



## General introduction

Copper and copper containing compounds have been associated with maintenance of health and the treatment of diseases for a very long time: as early as 400 BC, copper was used for medical reasons, such as disinfecting<sup>1,2</sup>. However, it took until the nineteenth century, until its presence in plants and animals was well-recognised<sup>3</sup>. Only in 1926, the evidence was presented that copper is an essential trace element in animal nutrition<sup>4</sup>. The essentiality of copper for humans was first shown during the 1960s in malnourished children in Peru<sup>5</sup>.

In the course of time, copper appeared to be involved in many biochemical processes, e.g. the formation of haemoglobin<sup>6</sup> and the biosynthesis of elastin and collagen<sup>7-9</sup>. Copper metabolism plays a central role in these biochemical processes. Disturbance of or disorders in the copper metabolism may have serious consequences, such as liver cirrhosis and necrosis or death, as is evident from Wilson and Menkes disease<sup>10</sup>.

Copper metabolism actually starts with the copper entering the mammal through the alimentary tract<sup>11,12</sup>. After the digestion of the food, the absorption of copper probably occurs primarily in the small intestine. This process can be disturbed by other nutritional factors such as high intakes of zinc, iron, or ascorbic acid decreasing copper absorption<sup>13-16</sup>. Some adaptation of absorption relative to need takes place<sup>17, 18</sup>. After the absorption has taken place, copper enters the interstitial fluid and blood plasma. The process of copper distribution can be divided into three phases, i.e. transport of copper to the hepatocytes of the liver and to a lesser extent to the kidneys, uptake of copper into the hepatocytes followed by incorporation of copper into several enzymes (e.g. ceruloplasmin and Cu-Zn superoxide dismutase), and transport of copper to and distribution over the other tissues<sup>17, 19</sup>. Finally, most of the copper has to find its way back to the liver. How this happens is unclear. Probably, ceruloplasmin, transcuprein and albumin are involved in this process. The homeostasis of copper is primarily maintained by biliary excretion via the faeces; little is excreted via the urine<sup>20</sup>.

In order to be able to maintain copper homeostasis, the amount of copper that is excreted must be compensated for by the absorption of copper from the food or vice versa. The amount that should be compensated for differs during the various stages of life. In the adult stage, compensation for endogenous loss of copper is needed, which can be expressed as the requirement for maintenance. During growth, reproduction and lactation, more copper is

needed to supply these processes, implying an additional need for copper in the form of a certain requirement for 'production'.

The copper requirements of different animal species, including mouse, rat and human, have been published by the National Research Council (NRC) in the form of minimal requirements or allowances<sup>21</sup>. However, the requirements given for the mouse are not based on thorough research and, therefore, the reliability can be questioned.

Based on similarities with the rat and research of Reeves *et al.*<sup>22</sup>, Mulhern and Koller<sup>23</sup>, Knapka *et al.*<sup>24</sup> and Hurley and Theriault Bell<sup>25</sup>, the NRC estimates the mouse's copper requirement for growth and maintenance to be 6 ppm of Cu and for pregnancy and lactation to be 8 ppm of Cu<sup>21</sup>. However, none of these experiments had the intention to make an estimation of the mouse's copper requirement during the various stages of its life. Reeves *et al.*<sup>22</sup> limited their research to adult male mice, using only biochemical parameters with sustainment of maximum serum copper and serum ceruloplasmin activity as criterion. No zootechnical parameters, such as reproductive performance, were studied in order to arrive at an estimation. Mulhern and Koller<sup>23</sup> followed mice from birth till 8 weeks of age, examining the influence of copper status on the immune response. Knapka *et al.*<sup>24</sup> formulated an open formula diet and examined whether differences in the results of biological research occurred when this open formula was fed instead of a closed formula diet. Copper concentrations in both the open formula as well as the closed formula diet were considerably higher than the estimated requirement. The experiment of Hurley and Theriault Bell<sup>25</sup> was designed to examine genetic influence on the effects of a dietary manganese deficiency during prenatal development. This study did not have the purpose to propose a copper requirement or allowance.

It may be obvious that more thorough research is needed in order to come to a proper and reliable estimation of the mouse's copper requirement. Therefore, an experiment was designed to study both biochemical and zootechnical parameters over several generations of mice in order to get more information about the copper requirement during the various stages of life (chapter 2). Main criteria for proposing a copper allowance for the mouse are the reproductive outcome, growth performance and sustainment of maximum plasma and hepatic copper concentrations and of plasma ceruloplasmin.

However, knowing the level of copper required in the mouse's diet does not necessarily mean that this is the exact copper concentration found in commercially available diets. In fact, analyses show that commercially available diets often contain much more copper than is required by the mouse. Copper appears to be involved in the production of free radicals and

reactive oxygen species (ROS), which are very reactive particles, through the Haber-Weiss reaction, which may result in oxidative stress (chapter 3). The hypothesis investigated in chapters 4 and 5 states that copper overload, caused by higher dietary copper concentrations than required, may shorten life span and increases oxidative damage to macromolecules by inducing oxidative stress.

Under normal conditions, a balance exists between the radical generating and the radical-scavenging systems and free radicals and reactive oxygen species are playing an integral part in normal cell physiology<sup>26</sup>. However, they are also capable of damaging biological macromolecules such as DNA and proteins. In the case of oxidative stress, which is the result of an imbalance between the radical-generation and general-scavenging system, more free radicals are being generated than being scavenged, resulting in damage to DNA, proteins, saccharides and lipids<sup>27</sup>. Oxidative stress has been suggested to be associated with accelerating ageing. A number of age-related diseases, such as atherosclerosis and cataractogenesis, and various neurological disorders, such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis, are also associated with oxidative stress<sup>28</sup>. Chapter 3 provides a more detailed review on the role of copper in oxidative stress.

Within the range of possible dietary copper concentrations, a copper overload is one extreme; the extreme at the opposite site is a copper deficiency. Several factors may influence the copper status. One of these is dietary cholesterol concentration. Feeding cholesterol to rabbits and rats may alter the metabolism of copper and may result in decreased liver copper concentrations, though not inevitably in a copper deficiency<sup>29, 30</sup>. A relationship between copper and cholesterol is also indicated by the observation that experimental copper depletion with a copper-deficient diet induced hypercholesterolemia in rats<sup>31-33</sup>. This was the impetus to compare the hepatic copper content of dietary cholesterol resistant (animals showing only a slight response to dietary cholesterol and therefore also called hyporesponders) and dietary cholesterol susceptible (animals showing an enormous increase in plasma and/or liver cholesterol levels and therefore also called hyperresponders) inbred rat and rabbit strains on a diet with or without added cholesterol. Based on literature, it was anticipated that on a cholesterol-rich diet the hyperresponding rat and rabbit inbred strains would have a lower liver copper content and thus would require a higher copper intake than their hyporesponding counterparts. The results of the experiment are described in chapters 6 and 9.

In order to search for possible causative factors that might be involved in these strain-specific differences, we have performed a genetic analysis in both species. The aim of these genetic analyses was to identify the chromosomal regions that may be involved in controlling liver

copper content after a cholesterol-rich diet. Identifying the chromosomal regions may provide clues as to possible candidate genes that may be involved in controlling liver copper content. Quantitative trait locus (QTL) analyses were performed in two sets of recombinant inbred rat strains (derived from SHR/OlaIpcv and BN-Lx/Cub progenitors) and in an F<sub>2</sub>-intercross progeny of a cross between hyporesponding and hyperresponding rats (derived from LEW/OlaHsd and BC/CpbU inbred strains) that had been fed a cholesterol-rich diet (chapters 7 and 8). QTL-analysis was also performed in the F<sub>2</sub>-intercross progeny of a cross between hyporesponding IIIVO/JU and hyperresponding AX/JU rabbits (chapter 10).

An overview of the results found during this PhD project is given in the Conclusions section of this thesis (chapter 11). A short description of the results is reported in the summary; a short description in more plain terms can be found in the ‘Nederlandse samenvatting voor niet-vakgenoten’.

## References

1. Mason, K.E. (1979). A conspectus of research on copper metabolism and requirements of man. *J. Nutr.*, 109, 1079.
2. Blancou, J. (1995). History of disinfection from early times until the end of the 18th century. *Rev. Sci. Tech*, 14 (1), 21-39
3. Prasad, A.S. (1978). *Trace elements and iron in human metabolism*. Plenum: New York.
4. McHargue, J.S. (1926). Further evidence that small quantities of copper, manganese and zinc are factors in the metabolism of animals. *Am. J. Physiol.*, 77, 245-255.
5. Cordano, A., Baertl, J.M., Graham, G.G. (1964). Copper deficiency in infancy. *Pediatrics*, 34, 324-326.
6. Hart, E.B., Steenbock, H., Waddell, J., Elvehjem, C.A. (1928). Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *J. Biol. Chem.*, 77, 797.
7. Hill, C.H. et al. (1968). *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 26, 129.
8. Mason, K.E. (1979). A conspectus of research on copper metabolism and requirements of man. *J. Nutr.*, 109, 1979-2066.
9. O'Dell, B.L., Hardwick, B.C., Reynolds, G., Savage, J.E. (1961). Connective tissue defect in the chick resulting from copper deficiency. *Proc. Soc. Exp. Biol. Med.*, 108, 402.
10. DiDonato, M., Sarkar, B. (1997). Copper transport and its alterations in Menkes and Wilson diseases. *Biochimica et Biophysica Acta*, 1360, 3-16.
11. Linder, M.C. (1991). Nutrition and metabolism of trace elements. In: Linder, M.C. (ed.). *Nutritional biochemistry and metabolism*. 2<sup>nd</sup> ed. New York: Elsevier, p. 215-276
12. Walker, W.R. (1982). The results of a copper bracelet clinical trial and subsequent studies. In: Sorenson, J.R.J. (ed.). *Inflammatory diseases and copper*. Clifton, N.J.: Humana Press, p. 469-478
13. Finley, E.B., Cerklewski, F.L. (1983). Influence of ascorbic acid supplementation on copper status in young adult men. *Am. J. Clin. Nutr.*, 37, 553-556.

14. Castillo-Durán, C., Vial, P., Uauy, R. (1988). Trace mineral balance during acute diarrhea in infants. *J. Pediatr.*, *113*, 452-457.
15. Prasad, A.S., Brewer, G.J., Schoemaker, E.B., Rabbani, P. (1978). Hypocupremia induced by zinc therapy. *JAMA*, *1978*, 2166-2168.
16. Williams, D.M. (1983). Copper deficiency in humans. *Semin. Hematol.*, *20*, 118-128.
17. Linder, M.C. (1991). *The biochemistry of copper*. New York: Plenum
18. Turnlund, J.R., Keyes, W.R., Anderson, H.L., Acord, L.L. (1989). Copper absorption and retention in young men at three levels of dietary copper using the stable isotope, <sup>65</sup>Cu. *Am. J. Clin. Nutr.*, *49*, 870-878
19. Harris, E.D. (1991). Copper transport: an overview. *Soc. Exp. Biol. Med.*, 130-140
20. Linder, M.C., Hazegh-Azam, M. (1996). Copper biochemistry and molecular biology. *Am. J. Clin. Nutr.*, *63*, 797S-811S
21. National Research Council (1995). *Nutrient requirements of laboratory animals*, 4<sup>th</sup> edition. Washington, DC.
22. Reeves, P.G., Rossow, K.L., Johnson, L. (1994). Maintenance requirements for copper in adult male mice fed AIN-93M rodent diet. *Nutrition Research*, *14* (8), 1219-1226.
23. Mulhern, S.A., Koller, L.D. (1988). Severe or marginal copper deficiency results in a graded reduction in immune status in mice. *J. Nutr.*, *118*, 1041-1047.
24. Knapka, J.J., Smith, K.P., Judge, F.J. (1974). Effect of an open and closed formula rations on the performance of three strains of laboratory mice. *Laboratory Animal Science*, *24* (3), 480-487.
25. Hurley, L.S., Theriault Bell, L. (1974). Genetic influence on response to dietary manganese deficiency in mice. *J. Nutr.*, *104*, 133-137.
26. McCord, J.M., Fridovich, I. (1969). Superoxide dismutase assays: An enzymatic function for erythrocyte hemocuprein. *J. Biol. Chem.*, *244*, 6049-6050.
27. Gille, G., Sigler, K. (1995). Oxidative stress and living cells. *Folia Microbiol.*, *40* (2), 131-152.
28. Brown, R.H. Jr. (1995). Amyotrophic lateral sclerosis: Recent insights from genetics and transgenic mice. *Cell*, *80*, 687-692.
29. Klevay, L.M. (1988). Dietary cholesterol lowers liver copper in rabbits. *Biol. Trace Elem. Res.*, *16*, 51-57.
30. Abu-el-Zahab, H.S., Abdel-Aal, W.E., Awadallah, R., Mikhail, T.M., Zakaria, K. (1991). The correlation between serum total cholesterol and some trace elements in serum, liver, and heart of rats fed high cholesterol diet. *Nahrung*, *35* (8), 827-834.
31. Al-Othman, A.A., Lei, K.Y. (1990). Alterations in plasma pool size of lipoprotein components and fatty acid composition of high density lipoprotein phospholipids in copper-deficient rats. *FASEB J.*, *4*, A393.
32. Al-Othman, A.A., Rosenstein, F., Lei, K.Y. (1994). Pool size and concentration of plasma cholesterol are increased and tissue copper levels are reduced during early stages of copper deficiency in rats. *J. Nutr.*, *124* (5), 628-635.
33. Carr, T.P., Lei, K.Y. (1990). High-density lipoprotein cholesteryl ester and protein catabolism in hypercholesterolemic rats induced by copper deficiency. *Metabolism*, *39* (5), 518-524.