

# Abstract

Dolichol is a membrane lipid, which carries monnosaccharides and glycans for *N*-linked protein glycosylation and glycosylphosphatidylinositol-anchor biosynthesis occurring in endoplasmic reticulum. Its structure is composed of isoprenoid units. Dolichol is present in all tissues and in most of the membrane organelles of eukaryotic cells. Recently some types of congenital disorders of glycosylation have been described as a consequence of dolichol biosynthesis and metabolism defects, which are not detectable by standard methods.

The aim of this diploma thesis was to analyze dolichol content in urine and in different tissues from patients with deficiency in dolichol biosynthesis by mass spectrometry and to study the impact of these defects on energetic metabolism.

Biological material for this study consisted of urine samples from 76 controls with age ranging from 1 months to 81 years, 6 patients with congenital disorders of glycosylation and 43 patients with suspicion of congenital disorder of glycosylation; samples of frontal cortex, liver, muscle and heart tissues from 2 patients with mutation in *NUS1* gene and controls. Urine samples were stored at -20 °C and tissue homogenates were stored in -80 °C until analysis. Lipid fraction after extraction was separated by liquid chromatography. Dolichols were analyzed by tandem mass spectrometry. Peaks of dolichols with 17, 18, 19 and 20 isoprenoid units were detected and the ratio of dolichol 18 and dolichol 19 was calculated. Respiration of fibroblasts was analysed on Oxygraph and Seahorse which was also used to analyze glycolytic function.

In the control group, significant correlation between dolichol 18 and dolichol 19 ratio and age was found in urine. Reference range in urine was evaluated. No differences between genders were detected. Ratio of dolichol 18 and dolichol 19 was significantly increased in urine from patients with mutation in *NUS1* and tissues in comparison with controls. Fibroblast line from these patients showed changes in glycolytic function and respiratory in comparison with controls.

Our results show a novel diagnostics option for patients with rare congenital disorders of glycosylation, who cannot be detected by usual screening methods. (*In Czech*)

*Key words: dolichol, diagnostics, mass spectrometry, congenital disorders of glycosylation*