SUMMARY

Iron belongs among the trace elements and its role in humans is irreplaceable. Up to 5 g of iron can be found in adult body distributed among different compounds. Iron ions are therefore essential to all cells of our body and its homeostasis is thoroughly controlled.

Iron uptake into the organism is mediated by enterocyte cells in the small intestine, where heme as well as non-heme forms of iron are absorbed. Non-heme iron is absorbed via Dcytb (duodenal cytochrome b), DMT1 (divalent metal transporter 1), ferroportin, hephaestin, and ceruloplasmin molecules. Although these molecules can also participate in non-transferrin-bound iron transport across plasma membranes within the whole organism, mechanisms of this transport are not yet fully elucidated.

The aim of the present work was to contribute to our understanding of molecular mechanisms that are involved in non-transferrin-bound iron transport across the plasma membrane of mammalian cells. Our project was focused on the description of non-transferrin-bound iron transport in human cells *in vitro* and *in vivo* under conditions of iron deficiency or iron overload. Transformed cell lines, that represent the three main types of cells involved in iron homeostasis, and tissue samples of duodenal biopsies were used as experimental models.

The expression of DMT1, Dcytb, ferroportin, hephaestin and ceruloplasmin molecules was tested in human cell lines Caco-2 (colorectal carcinoma), K562 (erythroleukemia) and HEP-G2 (hepatocellular carcinoma). The Caco-2 cell line represents intestinal cells responsible for iron absorption, the K562 cell line represents erythroid, iron-utilizing cells, and the HEP-G2 cell line is a model of hepatocytes that exhibit a high capacity for iron storage. The expression of the mentioned molecules was also tested in tissue samples from patients with iron deficiency anemia (IDA), hereditary hemochromatosis (HHC), alcoholic liver disease (ALD) and healthy controls. The level of expression was tested on both mRNA and protein levels. Moreover, HFE (hemochromatosis gene) and TfR1 (transferrin receptor 1) mRNA levels and serum hepcidin levels were analyzed in patient samples.

In *in vitro* experiments we demonstrated that different iron availability affects the expression of tested molecules in cell-type specific manners. In Caco-2 cells, we detected changes that correspond with suggested mechanisms of cellular regulation of iron transport

via IRP/IRE interactions. Under iron deprivation, we detected increase iron uptake in K562 cells. Although the increase in iron uptake was dependent on protein synthesis, we detected no changes in the protein expression of tested molecules. Therefore, we assume involvement of others, as yet, unidentified molecules participating in non-transferrin-bound iron transport into these cells.

In *in vivo* studies, we demonstrated decreased serum hepcidin levels in all tested groups of patients compared to controls. However, the change was significant only in the ALD group of patients. Nevertheless, the effect of hepcidin levels on tested iron transport molecules in our patients was not confirmed. Increased mRNA levels of DMT1, ferroportin and TfR1 were detected, however, these changes were not confirmed on the level of proteins. This may be due to the relatively small group of patients, which contributed to the high heterogeneity of results. However, some positive correlations among Dcytb, hephaestin, DMT1, ferroportin, and TFR1 mRNA in all groups of patients indicate coordinated regulation of the expression of these genes.

In our study, we contributed to our understanding of the regulatory mechanisms of iron uptake into the organism via duodenal enterocytes using *in vitro* and *in vivo* experiments. Active transport of non-transferrin-bound iron was also detected in non-enterocyte cells, however, it was most likely with the involvement of other suspected molecules.