In last few years the role of vitamin D_3 has been studied extensively. Vitamin D_3 is one of the most popular supplements nowadays. Low vitamin D levels have been linked to defects in bone mineralization, chronic autoimmune and infectious diseases, cardiovascular disease, diabetes, multiple sclerosis, rheumatoid arthritis, tuberculosis, and other malignant tumors. Vitamin D is an important component of the endocrine system that controls calcium homeostasis and bone mineralization. Vitamin D affects the regulation of genes expression that are involved in processes of differentiation, activation, and proliferation many types of cells. Hence vitamin D_3 bioactivity is only possible after two hydroxylations taking place in liver and kidneys. First metabolite (calcidiol - $25(OH)D_3$) is created in the liver through 23-hydroxylase and the second metabolite (calcitriol - $1,25(OH)_2D_3$) is generated upon 1α-hydroxylase. However, both metabolites may be activated through 24-hydroxylase (CYP24A1) action. $1,25(OH)_2D_3$ itself was noted to negatively regulating CYP27B1 and inducing CYP24A1 enzyme to generate $1,24,25(OH)_3$-vitamin D_3, a first step in the catabolism of $1,25(OH)_2D_3$. In our study we want to include VDBP (vitamin D binding protein) and VDR (vitamin D receptor).

To benefit from the effects of vitamin D on human cells, it is necessary to check whether higher doses of its metabolites increase the level of produced VDBP (that is responsible for transport) and VDR (that is responsible for further signal transduction). As we only know the effect, there is a need to find the cause of low levels of vitamin D, VDR, VDBP. Results may be useful in the future to develop a potential way that allows individuals to reap the full benefits of vitamin D supplementation or even its metabolites with no risk of cytotoxic effects on liver cells.

Vitamin D_3 status measurement result depends on total 25(OH)D concentrations – meaning the sum of $25(OH)D_3$ and $25(OH)D_2$ concentrations as standardized method collected by epidemiological and clinical studies, as well as clinical trials. Different agencies and societies recommend varies of concentrations of 25(OH)D that would be considered as vitamin D deficiency, insufficiency, sufficiency, and toxicity. However, there is no safe dose of $1,25(OH)_2D_3$ included in the official recommendations due to its micromolar on nanomolar concentrations and short half-life in the circulation. It is possible that instead of measuring the level of the metabolite, the genes expression and protein abundance of the enzymes responsible for the metabolism of the vitamin could be analyzed and correlated with the low/high level of the metabolite.

Is the low level of vitamin D tested at the physician's order associated with low levels of the transport protein, receptor, and above all, enzymes, and its metabolites? Will taking a high dose of vitamin D for a short period of time increase the expression of genes involved in its metabolic pathway? Can a vitamin D metabolite be toxic to liver cells, and if so - in what doses? What doses of vitamin D intake allow to achieve a concentration of $1,25(OH)_2D_3$ at a level that performs cytotoxic activity? Although the project we propose will not help answer all these fascinating questions, we believe that it would be a great contribution to widen knowledge about vitamin D metabolism. The project combines issues related to molecular biology as well as biochemistry and will be a great asset for further projects strictly related to nutrigenomics and medical biochemistry.

In the project we involved:

(1) Identification of gene expression levels of CYP27B1, CYP24A1, VDR, VDBP under the influence of $1,25(OH)_2D_3$ in Hep G2 cell line by Real Time PCR.
(2) Identification of protein abundance of CYP27B1, CYP24A1, VDR, VDBP under the influence of $1,25(OH)_2D_3$ in Hep G2 cell line by Western Blot analysis.
(3) Identification of (a) cell proliferation, differentiation, growth and (b) cytotoxic effects based on doses of $1,25(OH)_2D_3$ on Hep G2 by BrdU and WST-1 assays.

The research model is the Hep G2 cell line.