1. Summary

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid that acts as a ligand for a family of five specific membrane receptors. Although all cell types can synthesize S1P, the concentration of this sphingolipid is relatively high in blood compared to its level in solid tissues. This gradient is a consequence of unique features of blood cells, mainly erythrocytes and platelets, which express very low activity of S1P degrading enzymes. This fact makes them able to produce and release a marked amount of S1P. Another sources of plasma S1P include vascular endothelial cells and hepatocytes. However, the relative contribution of each of this sources to the plasma S1P pool is a matter of debate. In plasma, S1P is mainly bound to HDL (approximately 60%) and albumin (around 30%). The type of carrier affects S1P half-life and receptor availability, and therefore, modulates its function in blood. Activation of S1P receptors cause multiple physiological effects including inhibition of cell apoptosis and stimulation of muscle regeneration after injury. S1P also regulates the process of angiogenesis, influences vascular tone and bone homeostasis, and is crucial for maintaining integrity of the vascular endothelium and for normal lymphocyte egress from lymphoid organs to blood. In addition, S1P was suggested to have cardioprotective and antiatherogenic activities, but also to play a role in cancer development.

To date, metabolism, transport in plasma, and origin of circulating S1P are well described, but the mechanisms responsible for maintaining its homeostasis in blood are still unclear. The following study was designed and carried out in order to better understand these mechanisms.

The aim of the first experiment was to assess the changes of sphingolipid concentration in the plasma across the vascular beds of lungs, liver, and skeletal muscle. The study was conducted on male Wistar rats. In the first group of animals blood samples were taken from femoral vein, right ventricle of the heart, and abdominal aorta, whereas in the second one from left hepatic vein, portal vein, and abdominal aorta. Concentrations of selected sphingolipids were measured in plasma, as well as in fractions of lipoproteins and lipoprotein depleted plasma(LPDP) by means of HPLC. Comparison of the level of sphingolipids in arterial and venous blood allowed evaluation of the direction of changes in their concentration across selected organs.

Total plasma S1P concentration and its level in LPDP in the arterial blood were lower compared to mixed venous blood taken from the right ventricle of the heart. Conversely, total plasma and LPDP-associated S1P level increased across the leg. However, the highest increase in S1P, as well as sphingosine and sphinganine, concentration was observed in all examined plasma fractions during transfer of blood through the hepatic circulation.

The second experiment was carried out on thirteen healthy male subjects that completed an 8-week endurance training program on a rowing ergometer. Before the start of the training, and 3 days after the last training session blood samples were taken from antecubital vein. The aim of this study was to find out whether regular physical activity affects S1P concentration and metabolism in blood.

Endurance training increased erythrocyte and platelet sphingosine kinase activity by 20 and 45%, respectively. In addition, the rate of S1P release from red blood cells was markedly

stimulated. S1P concentration in plasma was increased in response to training. This effect resulted almost entirely from the increase in the level of HDL-bound S1P.

In summary, the concentration of S1P and other sphingolipids in plasma is subjected to dynamic changes during transfer of blood through different vascular beds. These changes, however, affect lipoprotein- and albumin-bound S1P pool to a different extent, with the latter one being much more labile in the circulation. In the pulmonary circulation albumin-bound S1P is intensively degraded, which is in contrast to skeletal muscle and liver, across which S1P release takes place. A marked amount of sphingolipids released in the hepatic circulation makes liver a key organ for maintaining plasma S1P homeostasis. It is also concluded that endurance training selectively increases HDL-bound S1P in the plasma due to enhanced S1P synthesis and release in erythrocytes. Training-induced elevation in HDL-bound S1P level might represent one of the mechanisms underlying beneficial effects of physical activity on the cardiovascular system.