ALS is an incurable, chronic neurodegenerative disease characterised by a selective motoneurons death in the motor cortex, brainstem and spinal cord that control any action of the muscles, for which no effective cure has yet been found. Phenotypic characteristic of this disease include loss of muscle tone, paresis, muscle atrophy, and spasticity. Treatment with a drug called riluzole only extends life by about 3 months. Research has shown that the causes of ALS should also be sought outside the nervous system, including skeletal muscles. A therapy that may bring benefits in the course of this disease by improving the length and quality of life of patients is water therapy, which in the presented project will also be called swim training. **The main goal of the study is to determine molecular mechanism of the protective effect of swim training on skeletal muscle atrophy and progresses of the ALS disease.**

In a recently published study, we showed that swim training in mice with ALS prolongs their life, reduces oxidative stress and improves muscle bioenergy. We have also shown that swim training reverses negative changes in structures formed by mitochondrial and the endoplasmic reticulum membranes (MAM / MERCS) in skeletal muscle in ALS mice. The collapse of these structures is associated with the development of the disease. We also found that training in water reduced weight loss and muscle mass, and decreased protein expression of the insulin / AKT signaling pathway, which is responsible for proteolysis of muscle proteins. It should be emphasized, however, that swim training in the described studies in mice suffering from ALS was administered even before the first symptoms of the disease appeared. In this project, we would like to see if it will have similar effects when it is introduced in ALS mice later, i.e. after the appearance of disease symptoms. In our opinion, this is important as it reflects the real situation of people suffering from ALS in a more realistic way.

Therefore, we want to check whether training in water in sick mice: 1) extends their life, 2) improves the motor function of these animals, and above all 3) reduces muscle atrophy, the development of which contributes to the death of ALS patients. Searching for the molecular mechanism responsible for the potential protective effect of training, we want to check whether the reduction of muscle wasting in ALS mice will be accompanied by changes in the expression of proteins responsible for the proteolysis of muscle proteins and proteins of mitochondrial signaling pathways, i.e. mitophagy processes, mitochondrial dynamics, PGC1-α and AMPK related to the phenomenon of muscle atrophy. We also want to define the role of changes in the intensity of oxidative stress and mitochondrial bioenergetics in this phenomenon. Our intention is also to investigate whether swim training will reduce inflammation, oxidative stress in the mouse brains and suppress the transformation of the microglia phenotype from M2, which is neuroprotective in M1, known as toxic. At the same time, we want to identify the nature of changes in the level of vitamin D and its metabolites with the progression of the disease. We want to perform this part of the experiments in the ALS mouse model, i.e. B6SJLTg (SOD1-G93A) 1Gur / J mice transgenic mice expressing the mutated human SOD1 G93A gene (hSOD1G93A) and in literrmates mice, which will be used as a control group. Swim training will begin after the first symptoms of the disease appear, as determined by clinical score tests and a locomotor test (Rotarod). In the project, we will use standard molecular biology methods, and the visualization will be based on research using electron microscopy. We will conduct research on cell culture to explain the relationship between the atrophy process and individual signaling pathways mentioned above, and the phenomenon of modification of MAMs / MERCs structures. The aim of our research will also be to investigate whether cells containing the mutated human SOD1 gene export outside the cells extracellular vesicles (EVs) rich in ferritin-bound iron, and to determine how toxic these EVs are to other cells. Describing these phenomena will help understand the mechanisms underlying both muscle atrophy in ALS and the protective effect of water training on the course of this disease. At the same time, these studies will allow to determine whether swim training will be able to become part of the so-called hybrid therapy involving the simultaneous reduction of muscle cell atrophy (the role of training) and the treatment of this disease.