Abstract

The term ‘’oxidative stress’’ was introduced by Sies in 1991 as ‘’a disturbance in the prooxidant (e.g. free radicals) – antioxidant balance in favour of the former, leading to potentional damage. Free radicals are reactive compounds that are naturally produced in the human body. They can exert positive effects (e.g. on the immune system) or negative effects (e.g. lipids, proteins or DNA oxidation). To limit these harmful effects, an organism requires complex protection – the antioxidant system. This system consists of antioxidant enzymes (CAT, GP_x, SOD) and non-enzymatic antioxidants (e.g. vitamin E, vitamin A, vitamin C, GSH and uric acid). An imbalance between free radical production and antioxidant defence leads to an oxidative stress state, which may be involved in aging processes and even in some pathology (e.g. cancer and Parkinson’s disease). Oxidative stress can also be increased under physiological conditions such as physical exercise.

In recent years, in an effort to increase the antioxidant defense and protection of the body from the harmful effects of oxidative stress, there is an increased interest in finding natural sources of antioxidants. Some proteins from certain foods have been shown to have the ability to neutralize reactive oxygen species. Whey protein is a popular nutritional supplement which enhances the antioxidant defense, probably due to its rich cysteine content through intracellular conversion into glutathione. Whey, a by product of cheese manufacturing, was considered for a long time a waste product but in recent years is described as a functional food. In the present study, the potentional beneficial effects of whey protein were examined both *in vitro* and *in vivo*. It was found that sheep whey protein exhibited scavenging activity against free radicals ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ και $OH^{\cdot}$) *in vitro*. Furthermore, it was studied the antioxidant activity of sheep whey protein in muscle cells C2C12 and endothelial cells EA.hy926. It was found that in both cell systems, sheep whey protein increased GSH and TAC levels and decreased ROS, TBARS, protein carbonyls and GSSG levels. Thus, the results indicate that sheep whey protein exhibits strong antioxidant activity both *in vivo* and *in vitro*. The antioxidant activity of sheep whey protein is probably due to the rich cysteine content that is converted intracellularly to glutathione. Finally, it was conducted a study in the C2C12 and EA.hy926 cells to determine the molecular mechanism through which sheep whey protein exerts its antioxidant action. It was examined the effects of sheep whey protein on the transcription factor Nrf2 and on a

number of antioxidant enzymes regulated by this factor. In EA.hy926 cells, it was found that sheep whey protein increased Nrf2, SOD-1, HO-1 and CAT levels as well as the enzymatic activity of CAT, SOD and GST. In C2C12 cells, the Nrf2 levels were not affected while the levels of SOD-1, HO-1, CAT and GCS as well as the enzymatic activity of CAT, SOD and GST were increased. Thus, the results show that the molecular mechanism through which sheep whey protein enhances the antioxidant capacity is cell specific, and it may be either Nrf2-dependent or Nrf2-independent. Finally, it was found that a supplement in a cake form containing sheep whey protein, reduced TBARS levels (a marker of lipid peroxidation) and exhibited anti-inflammatory activity, as it reduced the proinflammatory cytokine IL-6, the acute phase protein CRP and increased the anti-inflammatory protein IL-10 after exhaustive exercise in humans.