At least 380 million people worldwide have diabetes. By 2035 this will rise to 592 million [IDF; *International Diabetes Federation*, 2013]. At least 90% of the cases are type 2 diabetes (DM2) (with concomitant insulin resistance). Diabetes often leads to the death from diabetes-related complications, mainly related to a cardiovascular system. Intriguingly, extreme human longevity seen in centenarians is associated with a low degree of insulin resistance. This could suggest that exceptionally long-living people are insulin sensitive throughout their lifespan and are genetically protected from an age-related decline of insulin signaling (this pathway is involved in lifespan regulation). Moreover, there is still continuing debate on whether aging processes promote insulin resistance and diabetes, or the occurrence of insulin function problems may promote and accelerate aging. Additionally, it has not yet been established if chronic inflammation promotes insulin resistance and increased risk of age-related diseases (including cancer and DM2) or if development of metabolic diseases and disturbances in insulin signaling action activate local adipose tissue as well as systemic inflammation (there is strong correlation between obesity, insulin resistance, metabolic syndrome and inflammation). Furthermore, it is known that excess visceral fat accumulation may lead to numerous serious health complications, including hypertension, ischemic heart disease, stroke, diabetes with insulin resistance and cancer. Thus, a reduction in body fat leads to enhanced insulin sensitivity, decreased risk of age-related diseases, healthy aging, and longevity. However, some recent findings are somewhat contradictory with this presumed general perception. Namely, it turned out that two kinds of long-lived dwarf mice: GH-resistant GHRKO and GH-deficient (Ames mice, *df/df*) mice have higher fat content than their normal (N) siblings, yet have longer and healthier lifespans than their normal controls. These mutants also have lower insulin levels, decreased or normal glucose levels, and - more importantly - are hypersensitive to injected insulin in comparison to N littermates. Intriguingly, visceral fat removal (VFR) experiments performed with GHRKO, *df/df* and N mice indicate that surgical removal of visceral fat only improves insulin sensitivity in N mice without any beneficial effects in the above-mentioned dwarf mice. Unexpectedly, the surgical intervention in long-living *df/df* and GHRKO mice had detrimental effects on insulin sensitivity, glucose tolerance, and the level of fasting glucose. This may indicate that higher fat accumulation in these dwarf mice does not have a negative impact on insulin signaling and longevity. Based on the data presented above, the long-term objective of our research is to determine the mechanisms that cause a beneficial alteration of physiology and cellular function of adipose tissue, which can lead to the improvement of insulin signaling, healthy aging and longevity. The project proposal is innovative because the direct involvement of GH in altered functions of adipose tissue under the circumstances of severe suppression of somatotropic signaling, using an unique animal experimental model Ames dwarf mice, has not so far been analyzed. Moreover, it is not known if the different inflammatory grade in *df/df* mice (the adipose tissues from long-living animals were reported to have decreased levels of pro-inflammatory TNFα and IL-6 cytokines) is regulated by a different cellular mechanism of action in adipocytes or a decreased infiltration of white visceral and subcutaneous adipose tissue (WAT) by inflammatory macrophages due to the suppression of GH action.

To test all hypotheses presented above, we will subject GH-deficient Ames dwarf mice and normal (N) controls to: (i) GH replacement therapy to stimulate the GH and IGF-1 axis, or (ii) to low dosage Streptozotocin (STZ) treatment to induce insulin resistance and high glucose concentrations without alteration of GH levels. Additionally, differences in cellular composition (*i.e.* macrophages, pre-adipocytes, adipocytes, *etc*.) of adipose tissue depots from *df/df* and N mice will be determined, followed by subsequent analysis of gene expression and secretory cytokine activity of various cell types extracted from white visceral and subcutaneous adipose tissue (WAT) depots. The differential populations of macrophages and adipose-derived stem cells (ADSC) will be determined in WAT from *df/df* and N mice using flow cytometry and immunohistochemistry. The gene expression will be analyzed in whole adipose tissue homogenate and sorted cell populations from adipose tissue by examining extracted RNA by real-time PCR and PCR Arrays, and DNA analysis (using sequencing method) to determine which gene expression alterations are specific to adipocytes rather than to infiltration of inflammatory macrophages in WAT. Pre-adipocytes will be co-cultured with macrophages to determine how their interaction will alter gene expression signature as well as the pro- and anti-inflammatory statuses of pre-adipocytes after differentiation into adipocytes. Additionally, analysis of lipid peroxidation (LPO) (index of oxidative stress level) will be performed in the examined depots of WAT.

Summing up, better understanding of the relationships between insulin signaling and aging could improve our knowledge in the fields of gerontology with geriatrics, endocrinology, diabetology and physiology, and additionally may improve the effectiveness of prevention and treatment (including gene therapy) of age-related diseases.