Summary

In our modern society, obesity and obesity-related illnesses (e.g., type 2 diabetes and cardiovascular disease) have reached grand proportions; therefore, the need for new therapeutic strategies has increased proportionally. The pathological condition of insulin resistance is a significant factor that leads to the development of type 2 diabetes. However, with a myriad of factors contributing to insulin resistance, the underlying processes are poorly understood, urging the need for further biological investigations.

A particular interest in metabolic research has been the connection between insulin and white adipose tissue (WAT) expansion in obesity. In a state of positive energy balance, WAT can expand by increasing the size of adipocytes (hypertrophy) or by recruiting more adipocytes from precursors (hyperplasia or adipogenesis) to store excess energy. The majority of obese individuals suffer from metabolic disease and develop insulin resistance due to having excessive amounts of hypertrophic and dysfunctional WAT. However, other individuals who gain weight predominantly through WAT hyperplasia appear capable of maintaining insulin sensitivity and metabolic health. Therefore, recent research efforts have placed a high focus on studying the mechanisms of adipose tissue development and identifying adipocyte precursor cells (APCs). Insulin is a vital hormone, which plays a fundamental role in WAT metabolism and adipocyte function. In line with this, the work presented in this thesis was aimed to investigate whether targeting the insulin receptors on the cell surface of WAT precursors can induce hyperplastic or "healthy" adipose tissue expansion.

The first part of this work focused on the isolation of APCs from mouse WAT depots and the characterization of these cells in culture. However, to achieve this, high quantity and purity of WAT cells are required. Therefore, we describe several methods to enrich the mix of isolated stromal vascular fraction (SVF) cells for adipogenic APCs or non-adipogenic cells. These methods include fluorescence-activated cell sorting (FACS) and a newly developed column-free immunomagnetic bead separation technique. Purified cell fractions were used to establish WAT precursor cultures, which were assayed for gene expression and lipid incorporation. During the *in vitro* pharmacological assessment of these cultures, isolated cells were stimulated with insulin receptor ligands, and we discovered that APCs represent a highly adipogenic and insulin-sensitive cell population within WAT.

Following *in vitro* characterization, we used continuous insulin delivery through osmotic minipumps combined with chemical labeling and lineage tracing to investigate insulin's action on WAT precursors *in vivo*. Using 5-Ethynyl-2′-deoxyuridine (EdU) labeling of WAT precursors, we assessed *in vivo* cell proliferation under insulin treatment. Next, we analyzed harvested WAT depots from genetic reporter mice by histology to explore the origin of adipocyte precursors under insulin treatment. While assessing insulin-induced cell proliferation did not lead to a definite conclusion, lineage tracing in genetic reporter mice revealed that insulin could drive adipocyte formation from perivascular precursors in healthy, lean mice.

Altogether, the results of this thesis show that APCs in mouse WAT are very insulin-sensitive and indicate that insulin plays an essential role in preadipocyte differentiation. Provided that future studies of human WAT are comparable to our results, the findings presented here could serve as the foundation for novel strategies designed to promote healthy adipose tissue expansion and thus combat obesity and its comorbidities.