# Preface

Adipose tissue has an important regulatory role in mammalian metabolism by storing and releasing high-energy metabolites according to the body energy needs, whether by storing excess energy in the form of triglycerides (TG), or by breaking down TG into fatty acids (FA) and glycerol. In this book, the authors present topical research in the study of the biology, regulation and health impacts of adipocytes. Topics discussed include the development, differentiation, structure and function of each type of adipose tissue in association with chronic diseases, such as obesity and insulin resistance; mTOR and regulation of adipose tissue metabolism and perfusion; adipose tissue-derived microvesicles and exosomes and the diagnosis of metabolic diseases; early regulation of adipogenesis; calpain-mediated regulation of bone and fat mass and glucose homeostasis in osteoblasts; and the role of estrogen-related receptors in adipocyte differentiation.

Chapter I – In mammals, the white adipose tissue (WAT) and brown adipose tissue (BAT) are distinguished. The adipogenesis is the process of the differentiation of mesenchymal stem cells (MSCs) into mature adipocytes. This process occurs in several stages and involves a complex integration of cytoarchitecture, signaling pathways and transcription regulators, which are considered the crucial determinants of adipocyte outcome. Cell differentiation of brown and white adipocytes is determined by their precursors; white adipocytes are derived from myogenic factor 5 (Myf5) negative preadipocytes, while brown adipocytes are originated from Myf5 positive precursors, which also can give rise to skeletal muscle cells. WAT is principally deposited in two compartments - subcutaneously and visceral. WAT is the predominant type of adipose tissue in adult humans and is distributed throughout the body in subcutaneous regions, surrounding visceral organs and in the face.

Subcutaneous adipose tissue (SCAT) is found in the gluteofemoral regions, back and anterior abdominal wall, while the visceral adipose tissue (VAT) is present mainly in the mesentery and omentum, and drains directly through the portal circulation to the liver. WAT is characterized by adipocytes containing a single lipid droplet, which occupies almost the entire cellular space to form a large vacuole. WAT ranges from white and dark yellow and has a uniform thickness in the newborn, but accumulates in certain regions of the adult body, regulated by hormones. BAT is located in discrete pockets in the paravertebral, supraclavicular and periadrenal regions, found in large quantities in hibernating animals and newborn babies. BAT is structurally distinct from WAT, provides your cells with multiple fat droplets, and has a number of fat vacuoles and several mitochondria. Its color is brown due to the abundant vascularity and numerous mitochondria, which do generate energy faster than WAT. The view on the roll of both SCAT and VAT has changed, from being solely a passive energy store, to an important endocrine organ. Adipose tissue is known to express and secrete a variety of signaling molecules, collectively termed adipokines and cytokines. These peptides have important autocrine/paracrine roles in regulating systemic lipid and glucose metabolism through endocrine/systemic actions in the brain, liver and muscle. In obesity, an imbalance between pro-inflammatory and anti-inflammatory adipokine secretion may serve as a pathogenic link between obesity and current chronic diseases. At this way, obese subjects presented higher expression of pro-inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, resistin and C-reactive protein (CRP), which are strongly correlated with insulin resistance and higher risk of cardiovascular disease. Otherwise, in obese subjects the expression of adiponectin is lower. Also, recent literature point for the key role of leptin as a regulatory peptide of energy expenditure, through central mechanisms. Higher concentrations of plasma leptin represent a surrogate of central leptin resistance, which contribute to the obesity state. Different from WAT, BAT is involved in energy dissipation rather than storage. Briefly, its action consists by the mitochondrial release of chemical energy in the form of heat, which is mediated by uncoupling protein 1 (UCP-1). Recent evidence indicates that BAT function is affected in obesity, considering that its activity is negatively correlated with age and body mass index, and women display higher activity compared to men. Thus, this chapter aims to discuss the development, differentiation, structure and function of each type of adipose tissue in association with the current context of chronic diseases, such as obesity and insulin resistance.

Chapter II – Many cell types release exosomes and microvesicles (EMVs), i.e. membrane vesicles with a size range form 0.05-5μm, containing receptors, bioactive proteins, molecular mediators and nucleic acids (DNA, mRNA, microRNA) for cell-to-cell communication. Three main types of EMVs exist, (i) microvesicles that bud directly from the plasma membrane, (ii) exosomes that are derived from multivesicular bodies (MVB) within the cell and (iii) apoptotic bodies released from cells undergoing cell death by apoptosis. Evidence is accumulating for the orchestration of complex biological processes by EMVs. Elevated levels of EMVs have been demonstrated in plasma, liquor and other body fluids of patients suffering from a wide range of human diseases of the elderly including vascular and metabolic diseases, chronic inflammation, autoimmune diseases, neurodegenerative disorders and cancer. EMVs may stimulate target cells directly by surface bound ligands for target cell receptors or by transfer of signaling components, such as transmembrane surface receptors, glycosylphosphatidylinositol (GPI)-anchored proteins, soluble polypeptides, microRNA, bioactive lipids and even mitochondria, into the target cells in cell type-specific fashion. The use of EMVs as diagnostic markers in clinical medicine is becoming increasingly attractive for many, including metabolic diseases. New preparation techniques emerge together with improved purification of the heterogenous vesicle populations, which differ in size, density and molecular composition. In addition, the recent revolution in mass-spectrometry and other enabling technologies, such as micro-flow cytometry, provide the analytical tools to quantify EMVs and to resolve the proteome, lipidome, DNA/mRNA profiles and microRNA signatures. These tools are prerequisite .for future characterization and validation of EMVs in body fluids as novel diagnostic biomarkers as well as surrogate markers for therapy monitoring and outcome prediction in metabolic diseases. In particular, it has to be studied (i) whether EMVs are released from adipose tissue cells, such as adipocytes, macrophages and vascular endothelial cells, and (ii) which molecular constituent components are most informative to generate actionable health information, as well as (iii) what is the most appropriate methodology, and (iv) body compartment for rapid, reliable and low cost in vitro analysis of EMVs and their components.

Chapter III – White adipose tissue is a central organ in energy storage and homeostasis of the whole organism. The study of adipose differentiation has been highly facilitated by cell culture models, such as primary adipocyte cultures,the cell lines *ob/ob*, and the sister fibroblastic 3T3-L1 and 3T3-F442A clones. These two clones have helped to advance our knowledge on the

adipose differentiation program following stimulation with adipogenic serum proteins or growth hormone, and the metabolic regulation in terminally differentiated adipocytes. The 3T3-F442A cells can also differentiate into fat pads *in vivo*, therefore responding to physiological signals in the body. The authors' studies in adipogenesis have been carried out mainly using the 3T3-F442A cells, which have high efficiency to differentiate into adipocytes. Thus, this cell line is suitable to study adipose differentiation, molecules that regulate adipogenesis, and the expression of genes involved very early in this pathway. Recently, the authors demonstrated that staurosporine (St) induces adipose differentiation in serum-free medium, or in the absence of adipogenic proteins. Dexamethasone (Dex) combined with St enhances adipose differentiation of 3T3-F442A cells. This model offers the following advantages: i) St/Dex promote a maximum differentiation within 4 h without any other adipogenic stimuli (incubation with adipogenic serum requires approximately 48 h for the cells to undergo adipogenesis). ii) St induces two well-defined stages for adipogenesis before clonal expansion. The first stage consists of 4 h of induction during which St induces progenitor cells to differentiate, and the second stage consists of a subsequent 44 h of stabilization, during which adipogenesis continues in the absence of the inducer but it can still be reversed by anti-adipogenic substances or cytokines. These two stages permit the identification and analysis of early gene regulation during adipogenesis. Adipose differentiation is regulated by a complex cascade of transcriptional factors. C/EBPβ (gene: *cebpb*) activity precedes the expression of *pparg2* and *cebpa* by several hours, raising the possibility that other genes might be expressed during such time regulating the expression of *pparg2* and *cebpa*. The transcription factors Sterol Responsive Element Binding Proteins (SREBPs) function as the central hubs in lipid metabolism, and are encoded by the *srebf1* and *srebf2* genes. *Srebf1* encodes two proteins, SREBP1a and SREBP1c. The authors found that expression of *srebf1a* is required for the 3T3-F442A cells to undergo adipogenesis, since its knockdown inhibited the adipogenic program blocking the expression of genes encoding PPARγ2, C/EBPα, SREBP1c, and FABP4. SREBP1a activation is upstream of the three essential adipogenic transcription factors. Kinetic analysis during adipogenesis illustrated that the order of expression of adipogenic genes was the following: *cebpb*, *srebf1a*, *pparg2*, *cebpa*, *srebp1c* and *fabp4*.

Chapter IV – The receptor for parathyroid hormone (PTH) and PTH-related peptide (PTH1R) belongs to the class II G protein-coupled receptor (GPCR) superfamily. The calpain small subunit encoded by the gene *Capns1* is the second protein and the first enzyme identified by a yeast two-hybrid

screen using the intracellular C-terminal tail of the rat PTH1R. To investigate a role for the calpain small subunit in cells of the osteoblast lineage *in vivo*, the authors generated osteoblast-specific *Capns1* knockout mice and characterized their bone phenotype. Mutant mice exhibited severe osteoporosis mainly due to reduced osteoblast number and function. Molecular mechanisms by which calpain modulates cell proliferation of the osteoblast lineage were further examined *in vitro*. Moreover, the authors utilized the mutant mice as a disease model of osteoporosis accompanied with impaired bone resorptive function, and demonstrated that the mutant mice on a high fat diet develop excess weight gain and glucose intolerance presumably through a mechanism mediated by reduced serum uncarboxylated osteocalcin levels. The authors suggested a possible clinical impact of the authors' basic research finding.

Chapter V – Estrogen-related receptors (ERRs) regulate key developmental, physiological, and pathological processes by controlling gene networks. Notably, ERRs have been recognized as global regulators of cellular energy metabolism, including oxidative phosphorylation, heat production, and fat transport. Three ERR subtypes (ERRα, β, and γ) control unique biological functions in a tissue-specific manner in response to a variety of physiological stressors. In addition, recent studies have revealed that ERRα and ERRγ, which are induced during adipocyte differentiation, promote white adipocyte differentiation from preadipocytes and mesenchymal derived cells. These findings indicate the effects of ERRs on lipid metabolism, suggesting that strategies targeting ERRs may be useful for treating metabolic diseases.