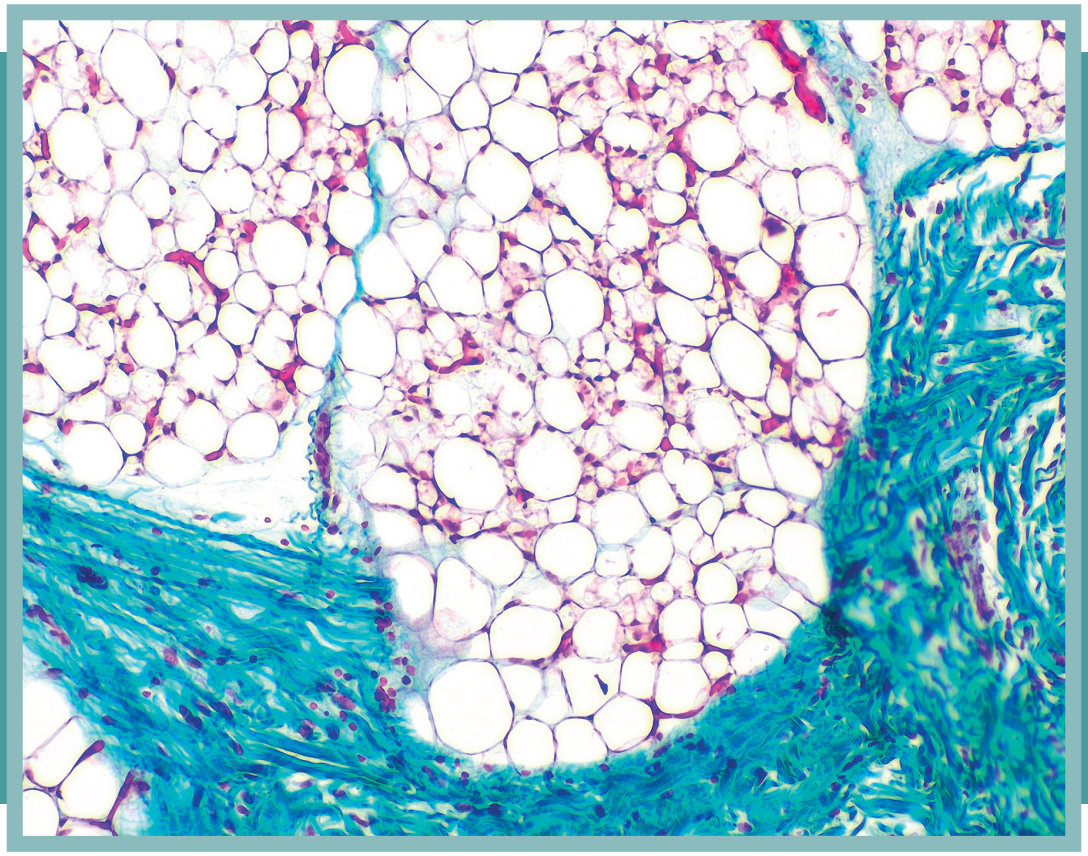
Institute of Pediatrics
Faculty of Medicine University of Debrecen

Dr. habil. Tamás Röszer

Histopathology of Obesity



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Preface

If you find honey, eat only what you need.
Proverbs 25:16

Obesity is defined as an excessive expansion of the adipose tissue in response to positive energy balance. This condition is associated with profound metabolic changes in the adipocytes, with immune cell invasion and inflammation causing multi-organ diseases. Due to its physiology, human is prone to develop obesity, and this trait was an adaptive success of the hominid evolution. Obesity however triggers a set of metabolic- and immune diseases, such as insulin resistance, renal and vascular diseases, fatty liver, and diabetes, making it one of the most serious public health challenges of the 21st century. It is alarming that the prevalence of overweight doubled among children and tripled in teenagers between 1980 and 2000. In 2010, approximately 6.7% of preschool-aged children were overweight or obese worldwide, and this number is predicted to rise further, forecasting a non-precedential prevalence of obesity by the end of this decade. Albeit adipose tissue pathology is the origin of all obesity-associated diseases, histology textbooks provide only a brief overview of the adipose tissue, most likely due to its relatively simple morphology. Anatomy atlases similarly omit the representation of adipose tissue depots. In certain instances, the cellular composition, fat catabolizing and thermogenic potential of specific human fat depots is still unexplored. However, the diagnostic and prognostic values of histological and molecular biology analysis of adipose tissue biopsies, as well as fat removed during esthetic surgeries is foreseen to increase in the clinical practice. It is hence important to introduce obesity research in the training of biomedical and medical professionals. This lecture book has been written to support a lecture series on the normal morphology and histopathology of the adipose tissue, or as it is often called, the adipose organ.

Debrecen, 1 July 2023 *Dr. habil. Tamás Röszer*

How to use this book

This lecture book contains two parts: a summary of key aspects of adipocyte biology and adipose tissue morphology in a classical textbook format, and a histology atlas that may be used as a guide in histopathology practice. Thus, it may be a suitable resource not only in a lecture room setting, but also at the side of the microscope in a histopathology laboratory. Content level of this book is adjusted to meet the needs of medical students in fourth to fifth year of studies, and postgraduate students enrolled in a doctoral program in medical biology or its related fields.

Acknowledgements

The manuscript has been reviewed by Dr. Vissarion Arionas Efthymiou, Harvard Medical School, Boston, USA. His valuable comments are appreciated. The Author is a fellow of the Bolyai Scholarship of the Hungarian Academy of Sciences. Work in the Author's laboratory is supported by a grant from the Hungarian Research Fund (OTKA NKFI #142939). Histology images have been acquired at the Author's laboratories at Ulm University, Germany and at University of Debrecen, Hungary. The use of human histology and *in vivo* imaging records has been approved by local and national ethical committees (#RKEB.6467-2023, #6149-2022, #8/2023/EÜIG).

Part One

ADIPOSE ORGAN PATHOLOGY

1. The adipose organ

Adipose tissue (*tela adiposa* in Latin, *Fettgewebe* in German histology literature) is a multifunctional organ that, beyond its traditional role as a lipid storage site, is pivotal in metabolic and endocrine physiology* [1-3]. The adipose tissue converts androgens into estrogens through aromatase enzyme activity [4] and produces various adipokines and hormones – such as leptin, adiponectin, omentin, interleukin-6 – that control body weight and appetite [5]. The adipose tissue also produces antimicrobial cathelicidin peptide [6, 7], and releases cytokines into the blood stream affecting inflammatory status, bone density, and hematopoiesis [8].

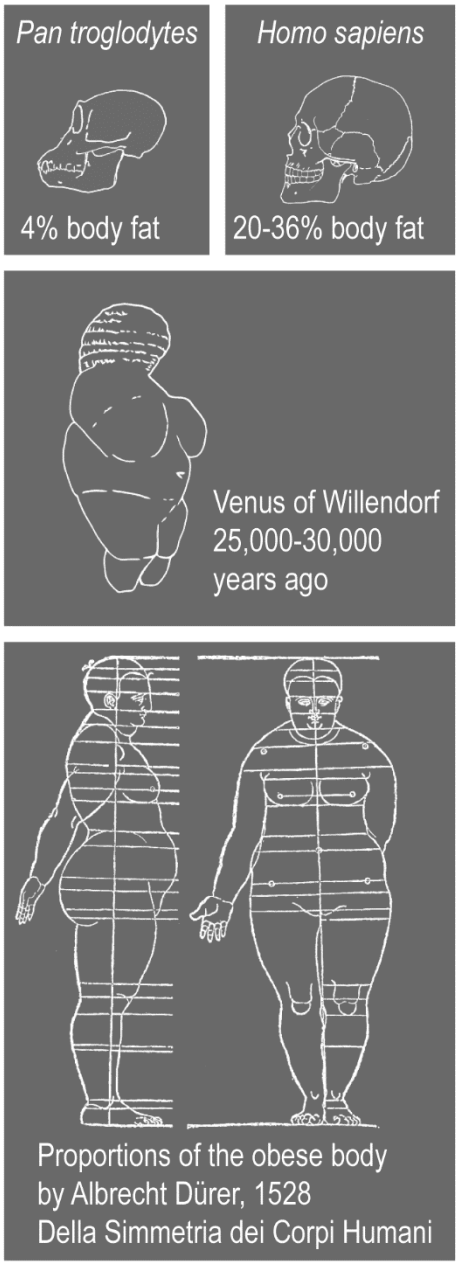

Figure 1. Evolution of adiposity

Hominid evolution also has favored the development of fat depots, as fat is an excellent energy reserve and thermal insulator. Hence, body adiposity of an adult human exceeds the body adiposity of non-human primates (Figure 1). This is plausibly a result of increased chromatin accessibility of adipogenic genes and a muted transcriptional activity of thermogenic genes in fat cells [9, 10]. The human body hence develops fat depots to store energy and

* Sections 1-7 contain an edited version of two publications: Röszer, T. (2021) Co-evolution of breast milk lipid signaling and thermogenic adipose tissue. *Biomolecules*, 2021, 11(11), 1705., and Röszer, T. (2021) Adipose tissue immunometabolism and apoptotic cell clearance. *Cells* 2021, *10*, 2288. These articles have been published under an open access Creative Common CC BY license, and the original articles have been cited here.

maintain core body temperature. Excess development of adipose tissue however leads to obesity which can trigger various chronic incurable diseases, such as coronary heart disease, non-alcoholic fatty liver disease, renal and retinal vascular complications, insulin resistance and type 2 diabetes [11-13]. Worryingly, the incidence of obesity is increasingly prevalent among children: global pediatric obesity incidence raised by 30% globally in the last two decades and increased tenfold in the last four decades [11, 14]. Consequences of pediatric obesity is projected to affect about 58% of the world's adult population by 2030 [12, 13, 15, 16]. Obesity-related diseases develop in children with obesity, and having obesity as a child increases the likelihood of having obesity as an adult and suffer from obesity-related health complications [17, 18].

2. Lipid storing and thermogenic adipose tissues

The adipose tissue is a modified connective tissue and is built up from adipocytes (fat cells), and cells of the so-called stromal vascular fraction. The latter contains precursors of adipocytes (preadipocytes, adipoblasts), endothelial cells of the capillary bed of the tissue, mesenchymal stem cells, and immune cells. Traditionally, two types of adipose tissue are recognized, based on their adipocyte morphology: the fat-storing white adipose tissue (WAT) and the fat-oxidizing/heat-producing brown adipose tissue (BAT) [19, 20]. It is more plausible to denote WAT as depot fat or storage fat (as it was initially described in German histology literature mid-19th-century, as *Speicherfett* or *Reservefett*). In turn, BAT – and all other forms of heat producing fat depots –, may be denoted as thermogenic adipose tissues (Figure 2).

WAT develops in all vertebrates, and invertebrates are also capable of storing neutral lipids in some organs – for instance, the midintestinal gland or hepatopancreas is a major lipid storage site in mollusks and crustaceans, and insects have "fat bodies" adjacent to the dorsal

surface of the intestine [21-23]. In humans, WAT is an energy storage site and functions also as a passive element of the locomotory system [24]. The major sites of fat storage are the abdominal subcutaneous fat and the omental fat, and both can expand with overnutrition and cause obesity. Other WAT depots are relatively stable, such as the fat pads in the eye sockets or in the palmar surface of the hands or the plantar surface of the feet. In these cases, the fat pads function to reduce mechanical stress and to support the work of the muscles [24]. Another relevant WAT depot is the female breast, which increases in size after puberty and particularly during pregnancy [25]. The fat accumulated in the breasts supports the breast gland during lactation and, importantly, provides the necessary fat for milk production [26].

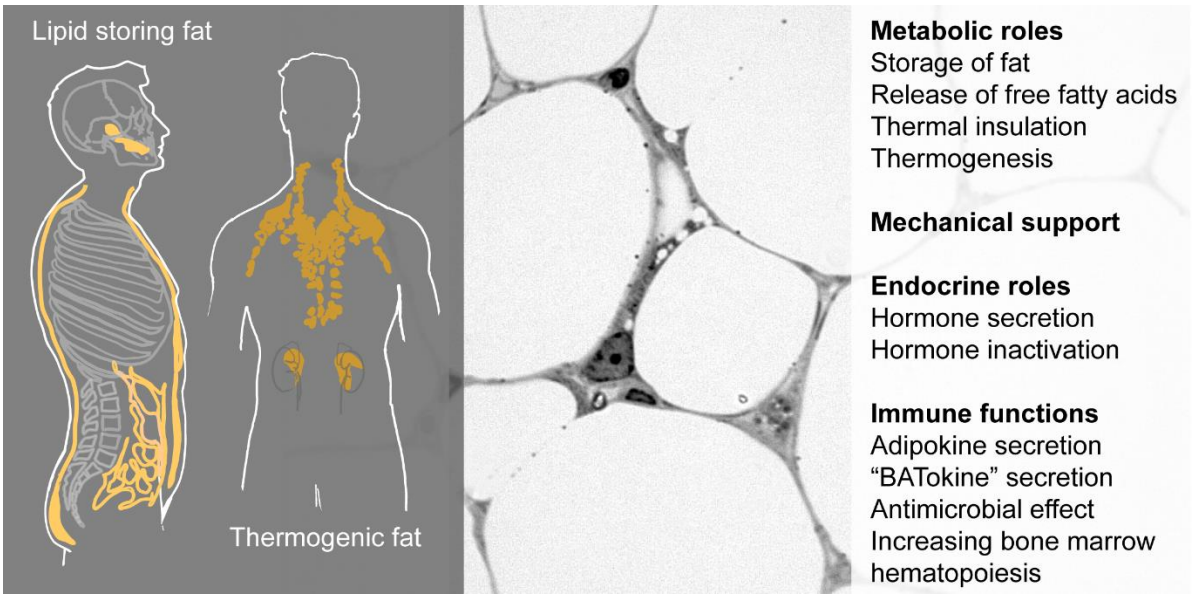

Figure 2. Key physiological functions of the adipose tissue

All homeotherms depend on the maintenance of their core body temperature for survival, and thermogenic fat has evolved in mammals to generate heat by increasing energy expenditure through actively oxidizing fatty acids and uncoupling mitochondrial respiration. The thermogenic activity of BAT is mediated by uncoupling protein 1 (UCP1), also termed thermogenin, which uncouples respiration from energy (ATP) generation.

BAT was first described in the interscapular region of the hibernant marmot *(Marmota alpinus)* as early as 1551 and was denoted as "the hibernating gland" in the late 19th century [27]. In this sense, the brown adipose depot is among the longest studied metabolic organs. In rodents, BAT develops before birth, and has a metabolic role across the entire lifespan by supporting non-shivering thermogenesis [28-30]. UCP1-expressing, thermogenic fat cells may support core body temperature in recovery from hibernation in some mammals [27, 31], and a cold environment also induces thermogenic fat expansion in humans [32]. Interestingly, several mammalian species that have adapted to a habitual cold environment lack UCP1 expression [33] or, alternatively, non-shivering heat generation occurs in the skeletal muscle without the need for thermogenic fat [34]. Similarly, human populations living in arctic environments do not develop excess thermogenic adipose tissue [35]. Thermogenic adipocytes are, hence, not the sole contributors to non-shivering thermogenesis, and the expansion of white adipocyte depots preserves core body temperature by offering an effective thermal insulation layer [27, 36]. Moreover, thermogenesis in adipocytes is possible in UCP1-independent mechanisms [37].

The ontogeny of adipocytes (fat cells) is heterogenous: in mice, thermogenic adipocytes of the interscapular BAT share progeny with the skeletal muscle, and brown adipocytes mostly develop from myogenic factor 5 (Myf5)-positive progenitors (Figure 3). Descendants of Myf5^{+} progenitors are scarce in WAT depots [38], and thermogenic adipocytes within the WAT develop independently from the Myf5^{+} lineage [38]. Fat storing adipocytes develop from the paraxial mesoderm, and it is also possible to generate thermogenic adipocytes from the same progenitors. The fate of adipocyte precursors (adipoblasts and preadipocytes) largely depends on endocrine and immune signaling cues. There is recent evidence showing that thermogenic fat cells can also develop from ectodermal progenitors, specifically from stem cells of the neural crest [39, 40].

Recently, a new addition to the adipose tissue terminology has been introduced to describe another form of thermogenic fat: “brite” (brown-in-white) adipose tissue, which is also known as induced brown adipose tissue or diffuse brown adipose tissue [29, 41]. Brite adipose tissue was initially observed in the subcutaneous adipose tissue of rodents exposed to cold and consists of both brown and white adipocytes. Further thermogenic adipocytes are the “beige” adipocytes. They represent a transitional form between white and brown adipocytes. Beige adipocytes may develop without a cold stress and are prevalent in some subcutaneous fat depots in the young [42, 43] and in the adult mouse [44, 45]. Brown and beige adipocytes however have distinct ontogeny in the mouse [46], although they share some functional traits [42]. It has recently been suggested that adult humans have beige rather than brown adipocytes [47, 48].

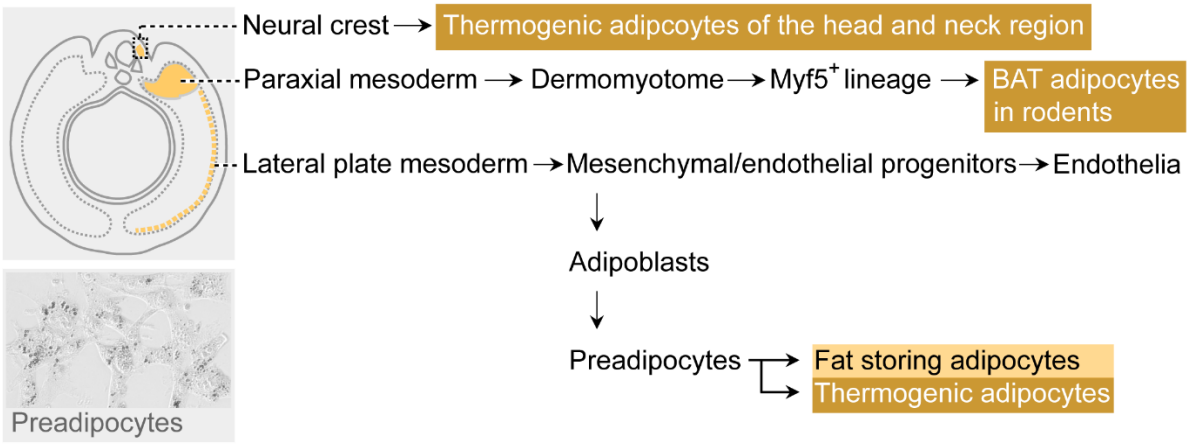

Figure 3. Adipocyte ontogeny

Thermogenic fat depots surround the kidney and arteries of vital organs throughout lifespan [49-51], and electron microscopy data show that the subcutaneous adipose tissue in the human newborn contains two types of cells: one type is large and has one large lipid droplet and small mitochondria, and the other is small and has several lipid droplets and contains large and numerous mitochondria [52]. The subcutaneous adipose tissue of newborns express UCP1 and other gene products associated with brown adipocytes [42, 43, 52-56]. Similarly, the subcutaneous adipose tissue of the newborn mouse expresses gene products of mitobiogenesis

and thermogenic fat differentiation [42]. In human infants, the level of UCP1 declines after 6 months of age and is sensitive to the type and duration of breastfeeding [43, 56].

Thermogenic – brown or beige – fat cells in the subcutaneous fat depot are present in children, and thermogenic potential is reactivated at puberty, concomitant with an increase in muscle mass [57]. During postnatal development, however, the majority of fat depots undergo a pronounced transformation that is usually accompanied by the loss of UCP1 [58].

Adipocytes in the human subcutaneous adipose tissue express UCP1 not only in infancy, but also in adulthood, and its level is positively correlated with that of *PRDM16* (PRD1-BF1-RIZ1 homologous domain containing 16), a transcriptional regulator and known inducer of thermogenic fat development [59]. The adult subcutaneous adipose tissue fat cell contains a much smaller number of mitochondria than equivalent newborn fat cells [55].

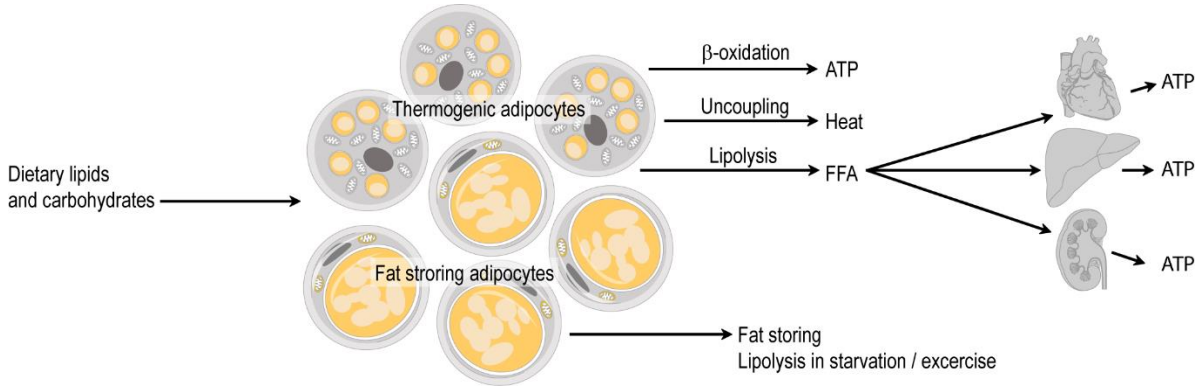

Figure 4. Metabolic traits of the thermogenic and the fat storing adipocytes
Modified from [2].

3. Adipocyte metabolism

The fat storing adipocytes – which predominate in the adult body – do not generally oxidize their fat stores. ATP synthesis from the stored neutral lipids occurs only under periods of starvation and in response to exercise when the fat cells hydrolyze the stored neutral lipids and

produce free fatty acids (FFAs) for the use by other metabolically active cells (Figure 4.). By contrast, the newborn mammal, does not experience starvation as long as breastfeeding is provided [60]. Moreover, hormonal signals of lipolysis and uncoupling (i.e., β-adrenergic activation) are provided in the newborn [61]. Accordingly, the newborn adipose tissue is capable of actively oxidizing fatty acids to produce ATP and generate heat by mitochondrial uncoupling. The human newborn has subcutaneous fat depots that contain both fat storing "white", and fat oxidizing "brown" or "beige" adipocytes. This combination of fat storing ability and potential for lipolysis and burning-off lipids fulfils the dual metabolic role of the infant fat (Figure 4). However, the relative number of mitochondria in these two cell types is different, allowing a changing degree of fat storage or fat catabolism. Obesity develops when fat catabolism is inhibited, and lipogenesis is favored.

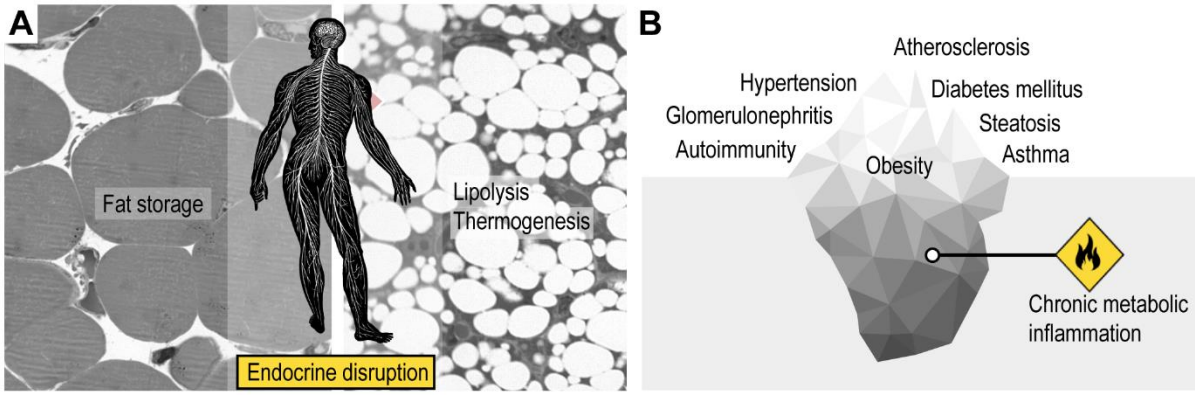

Figure 5. Models of obesity development
(**A**) Endocrine disruption of energy balance and "adipocyte browning"
(**B**) The iceberg model of metabolic inflammation

One model of obesity development considers altered balance of lipogenesis and lipolysis as a central player in fat storage. In this model altered endocrine regulation of appetite and energy expenditure – so-called endocrine disruption – leads to positive energy balance and excessive fat storage (Figure 5A).

This setting can be induced by obesogenic diets, environmental endocrine disruptors or by inherited endocrine diseases. Reverting the positive energy balance may be facilitated by inducing thermogenic potential in the fat storing adipocytes. This mechanism is often termed as “adipocyte browning”, referring to the transformation of fat storing “white” adipocytes into heat producing “brown” or “beige” adipocytes. Adipocyte browning requires the stimulation of nuclear encoded genes of mitobiogenesis (the generation of new mitochondria) and mitochondrial uncoupling [62, 63].

4. Mitochondria: indispensable organelles for fat catabolism

At the dawn of physiological chemistry in the middle 19th century, the German chemist Justus von Liebig (1803–1873) postulated that a negative correlation exists between the rate of respiration and obesity [64], albeit at the time neither the structure and molecular weight of lipids nor the biochemistry of fat oxidation were known [65].

Fat catabolism is indeed dependent on cellular respiration – that is, on the number and integrity of mitochondria. Mitochondria catalyze the β-oxidation of fatty acids, generate ATP from oxidative phosphorylation and heat from uncoupled oxidative phosphorylation. Carbohydrates can be used for ATP production without mitochondria owing to glycolysis; however, lipid breakdown requires mitochondria, and in some extent, peroxisomes. Neutral lipids and fatty acids are stored in the lipid droplet of adipocytes, and lipolysis occurs in the lipid droplet itself, but further processing of the released fatty acids requires mitochondria (Figure 6). When fat oxidation is intensive in fat cells, mitochondria are closely associated with lipid droplets [66]. Not only lipids, but also the creatine futile cycle may serve as a substrate for heat production in mitochondria [37, 67]. Not surprisingly, thermogenic fat cells are rich in mitochondria, and actively mobilize fuels for energy and heat generation.

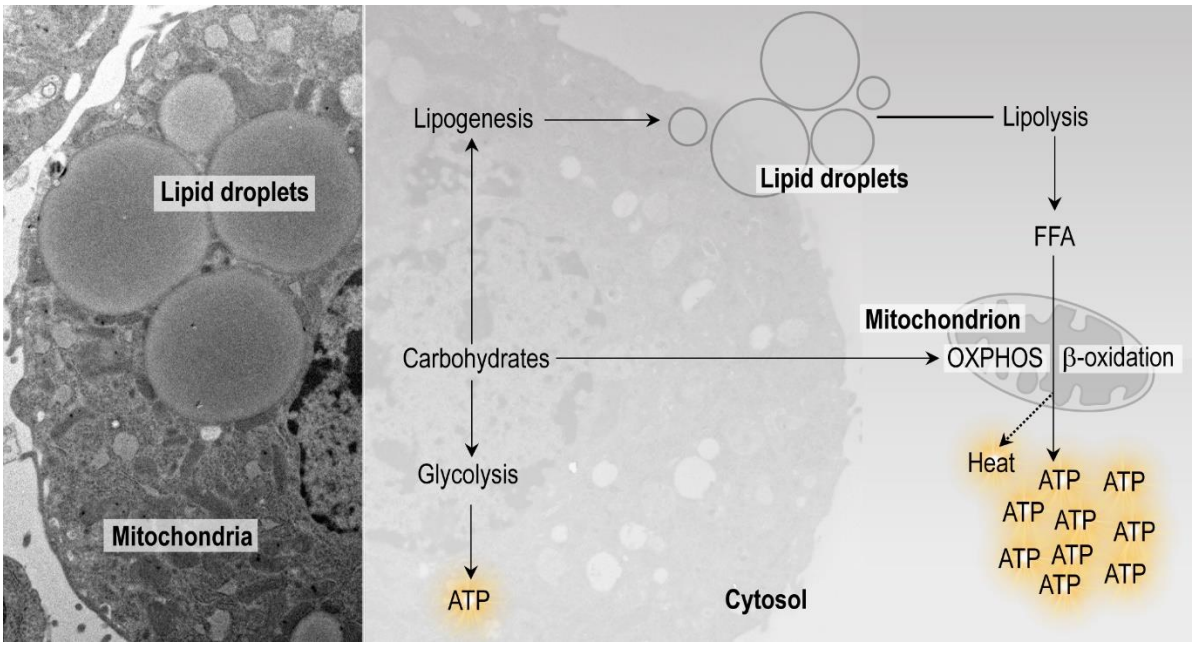

Figure 6. Scheme of energy production from stored fat
OXPHOS: oxidative phosphorylation, dotted line indicates mitochondrial uncoupling

Due to the active lipolysis and fatty acid oxidation in adipocytes, the newborn fat reserves may be rapidly depleted if there is no sufficient supply from feeding. In a human infant, the heat-generating fat exhausts its reserves within 36–48 hours [68, 69] and, accordingly, infections that cause diarrhea or excessive vomiting can lead to the loss of fat depots.

5. Dual metabolic roles of the newborn adipose tissue: fatty acid oxidation and lipogenesis

A 4-month-old infant has 26–33% body fat volume, accounting for 45–50% of the total body weight [61]. Overall, 12% of the total body fat can be found in the adipose tissue in a human newborn versus 3.3% in a normal adult [61]. The human newborn has large subcutaneous fat depots, and the omental fat – a large visceral fat depot in the adult – is lacking. Some visceral fat depots are, however, already established in the newborn, including mesenteric fat, the fatty capsule of the kidney and fat depots around the large arteries [70]. The human infant also has

specific subcutaneous fat pads, such as the buccal fat pad, which assists in suction during breastfeeding [71]. Other large fat pads are found at the ischiorectal fossa and in the eye sockets; also, the palms have fibrous fat pads, and a fat pad can be found in the axillary region associated with the axillary fascia, which unites the fascia of the pectoral muscle, the latissimus dorsi and the brachial muscles [70].

Infant fat is richer in adipocytes than adult fat: the estimated number of adipocytes in a human infant at 6 months of age is 130,000/kg body weight versus 45,000/kg body weight in an adult [61]. While the degree of adiposity seems to be prominent, oxidative metabolism is greater in infant fat than in adult fat, allowing the rapid utilization of energy stored in fat. Indeed, the human newborn subcutaneous fat depot utilizes almost 4,000 μmol O_2/kg tissue, versus ~1,000 μmol O_2/kg in the adult subcutaneous fat depot [61]. Fatty acid mobilization is oxygen-dependent in the newborn adipose tissue [54] and mitochondrial enzyme levels are also 2–4-fold higher in the newborn subcutaneous fat cells than in their adult counterparts [53].

The fatty acid oxidizing activity of the infant adipose tissue is necessary to metabolize the lipid-rich breast milk (or lipid-rich formula milk). Fatty acids are the preferred metabolic fuels of energy-demanding organs in the infant, such as the heart, the renal cortex and the small intestine, and their oxidation in the liver remains active throughout the breastfeeding period [72]. In general, the ratio between fatty acid oxidation and glycolysis with respect to systemic metabolic homeostasis decreases considerably with age [61].

In addition to being a key energy source after birth, fatty acids are also fuels of heat production in the infant [72]. The newborn must maintain body temperature, and fatty acid oxidation in the uncoupled mitochondria of adipocytes provides thermogenic capacity to the fat depots. Hypoxia is known to inhibit heat production in the newborn, underlying the importance of mitochondrial respiration for heat generation [61]. Cold exposure in the human infant increases peak oxygen uptake (VO_2) and increases also the levels of free fatty acids and glycerol

in the circulation, whereas hypoxia diminishes fatty acid mobilization and lipolysis in response to β-adrenergic stimulation [68]. These data indicate that mitochondrial respiration produces heat through lipolysis and fatty acid oxidation.

6. Adipose tissue inflammation and obesity-associated diseases

Inflammation is a key factor leading to obesity-associated diseases. Chronic adipose tissue inflammation – often termed as metabolic inflammation – is considered as a root of obesity-associated metabolic and immune diseases (Figure 5B). As a visual framework, the iceberg model depicts metabolic inflammation as a core of an iceberg built from obesity-associated diseases. However, innate immune signaling is essential for the proper differentiation of adipocytes in the early postnatal life [43, 73, 74], and determines whether adipocytes accumulate fat or generate heat and energy [75, 76]. Physiologically, heat production is a major task of the newborn adipose tissue, and when this is compromised, excessive fat accumulation – i.e., obesity – occurs. The adipose tissue hosts various immune cells, such as T-cells, B-cells, natural killer cells, adipose tissue macrophages and mast cells [77] (Figure 7). These cells determine the cytokine environment in the adipose tissue and may ignite inflammation [3].

Adipose tissue macrophages (ATMs) colonize the developing adipose tissue at birth, and they are necessary to stimulate heat production from fat [43, 78, 79]. Similarly, innate immune signaling in adipocytes – through RNA recognition receptors and type I interferon (IFN-I) response – is essential for heat production in the early postnatal life [43, 73-75]. Major signals that promote thermogenic fat development are mitochondria-derived RNA species [75]. Albeit these signals sustain healthy fat metabolism [77, 80], when released in excess they may cause mitochondrial damage, chronic adipose tissue inflammation, obesity [13, 77], insulin

resistance, and diabetes [81]. This is due to the similarity of mitochondrial RNA species to virus RNA molecules, leading to robust anti-viral immune activation in the adipose tissue.

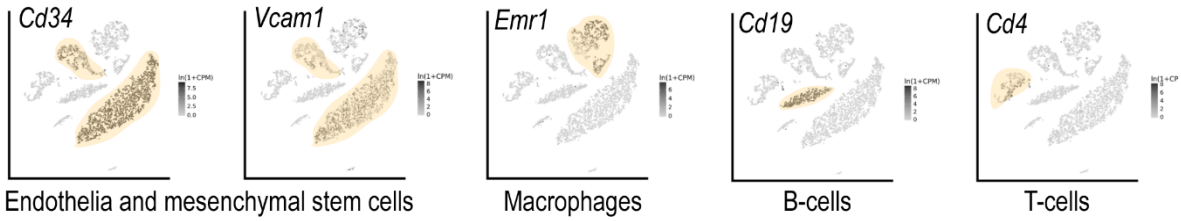

Figure 7. Immune cells in the adipose tissue stroma
Single cell sequencing of mouse adipose tissue stroma, using fluorescence assisted cell sorting. Marker gene expression levels for endothelial and mesenchymal stem cells, as well as various immune cells have been retrieved from Tabula Muris [82].

ATMs are resident immune cells of the adipose tissue, and are responsible for the development of metabolic inflammation, insulin resistance, adipose tissue fibrosis, and immune disorders associated with obesity, such as diabetes and self-immunity [83-89]. ATMs were first identified in the fat depots of obese mice in the 1960s, however their presence in human adipose tissue and the central role of ATMs in obesity-associated immune pathologies remained unnoticed until the 2000s [90-93].

ATMs appear in the adipose tissues of all mammalian species tested – rodents, ruminants, carnivora and primates [43, 94]. Adipocyte-ATM interactions have evolved in parallel with the emergence of the adipose tissue in vertebrates, suggested by the presence of ATMs in amphibia [79]. Research on ATM biology has mostly been conducted in the setting of obesity, since adipose tissue hypertrophy is associated with a significant increase in ATM number [89]. Prevalence of ATMs in the obese adipose tissue increases because of monocyte infiltration and local proliferation of ATMs [79, 95, 96]. Under homeostatic conditions ATMs develop from progenitors of the fetal hematopoiesis and replenish by proliferation or from circulating blood monocytes [79, 97, 98].

Fat-overloaded (hypertrophic) adipocytes release chemotactic and pro-inflammatory signals, which increase monocyte development in the bone marrow, promote monocyte and macrophage chemotaxis toward the obese fat depots, and eventually increase pro-inflammatory ATM activation [8, 90]. ATMs engulf lipid-overloaded and apoptotic fat cells, by forming multinucleated syncytia, so-called crown-like structures around the dying fat cells [83-89, 96, 98, 99]. Albeit apoptotic cell uptake promotes anti-inflammatory macrophage activation in most tissues, removal of apoptotic adipocytes triggers a pro-inflammatory ATM activation [3]. Since ATMs are situated within a complex adipose tissue immune cell niche, built up by mast cells, T cells and B cells, a pro-inflammatory ATM activation may initiate a cascade of intercellular signaling events, leading to an uncontrolled inflammation [3].

7. Adipocyte proliferation and apoptosis

Number of adipocytes, which build up the adipose tissues, is stable over a prolonged period, and the mature adipocytes are relatively resistant to apoptosis [100-102]. Albeit adipocyte apoptosis appears during development and remodeling of the adipose tissue, and the adipokine C1q/TNF-related protein 9 (CTRP9) increases the uptake of apoptotic cells by macrophages [103], adipocyte number is relatively constant during infancy and childhood [104].

The lipid content of the adipose tissue increases along postnatal development due to the expansion of the cell volume of adipocytes [105], and a notable increase of adipocyte number appears at the onset of puberty [104]. In infancy, adipocytes undergo differentiation and activate lipolysis in response to insulin and β-adrenergic stimulation [106, 107]. Two adipocyte populations can be identified later, in puberty and in adulthood: small, still maturing cells, and larger, lipid-laden mature adipocytes [105, 108]. The number of mature adipocytes increases in severe obesity, and newly generated adipocytes also appear. An increase in

adipocyte number is termed adipocyte hyperplasia, whereas an increment in cell volume due to lipid accumulation is termed adipocyte hypertrophy [101].

Adipocyte differentiation is associated with an increase in the expression of survival factors, such as the BCL2 apoptosis regulator and the baculoviral inhibitor of apoptosis repeat-containing 2 [100]. WAT adipocytes are more resistant to apoptosis than BAT adipocytes [102]. Adipocyte apoptosis in WAT becomes prevalent under pathological conditions – for instance, lipodystrophy is associated with adipocyte apoptosis and necrosis [109]. Also, the antiviral cytokine interferon alpha (IFNα) triggers adipocyte apoptosis both *in vitro* and *in vivo* in mice [110], and patients treated with HIV-1 protease inhibitors develop lipodystrophy due to adipocyte apoptosis [111]. In congenital generalized lipodystrophy, both WAT and BAT mass is lost due to adipocyte apoptosis and necrosis [109].

Hypertrophic adipocytes undergo apoptosis or secondary necrosis in obese adipose tissue. Obesity is associated with meta-inflammation, which is a possible trigger of adipocyte death. TNFα, which is abundantly expressed in obese adipose tissue [89, 112], induces both white and brown adipocyte apoptosis [102, 113]. Human abdominal subcutaneous preadipocytes are more resistant to TNFα–induced apoptosis than are omental preadipocytes, and the distinct WAT depots contain at least two different adipocyte populations based on their resistance to TNFα-induced apoptosis [114].

Lipid overload in hypertrophic adipocytes can lead to the “spillover” of lipids into the cytosol, leading to so-called lipotoxicity and, ultimately, apoptosis [115]. Failure of fatty acid oxidation and oxidative phosphorylation initiates the mitochondrial pathway of apoptosis in adipocytes [116], and also triggers inflammatory cell death, termed pyroptosis [116]. Impaired lipolysis and hypertrophy are hence powerful triggers of adipocyte apoptosis [117]. Lipotoxicity also triggers apoptosis of adipose tissue stem cells [118]. Adipocyte cell death is

an inflammation-generating process and is a prelude to a sequence of events leading to obesity-associated metabolic diseases [119].

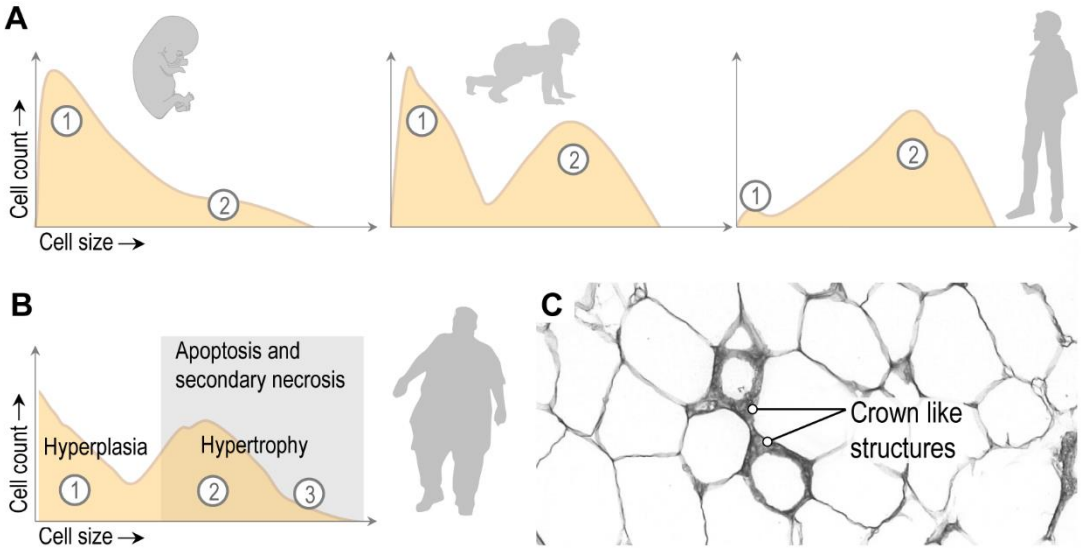

Figure 8. Adipocyte size in normal development and in obesity
(**A**) Differentiating adipocytes (cell population ①) are abundant after birth. In infancy, the prevalence of mature adipocytes (cell population ②) increases and, in adulthood, the lipid-storing mature adipocytes build-up the adipose tissue. (**B**) The number of differentiating and hypertrophic adipocytes (cell population ③) increases dramatically in obesity. Adipocyte hypertrophy is associated with apoptosis and secondary necrosis. Graphs modified from [3, 98, 107]. (**C**) Crown like structures in obese murine adipose tissue. Crown like structures are formed by multinucleated ATMs (labeled with immunostaining of the F4/80 antigen) around dying adipocytes.

In the setting of severe obesity, adipocyte apoptosis is prevalent and adipose tissue macrophages (ATMs) accumulate in the adipose tissue and form so-called crown-like structures around the dying adipocytes [93, 120]. Because the hypertrophic, lipid-overloaded adipocytes are larger than the ATMs, the ATMs fuse with each other to form a syncytial structure – a "giant", multinucleated cell – that firmly encapsulates the dying adipocytes [90, 121]. ATMs engulf cellular debris, lipid droplets, modified lipids, damage-associated molecules, apoptotic bodies, and fragments of adipocytes that are generated by secondary necrosis [122].

Unlike other tissues, the removal of these apoptotic cell contents triggers inflammation [90, 104, 123], and there is therefore a need for anti-inflammatory signals – including endocrine

signals and Th2 cytokines – to equip ATMs with the ability to safely process adipocyte remnants [98, 124]. Adipocyte death is hence an igniting event, which leads to pro-inflammatory ATM activation and adipose tissue inflammation, triggering further adipocyte apoptosis in a vicious cycle. A pro-inflammatory ATM phenotype is metabolically damaging, as it releases inflammatory mediators into the blood circulation, impedes insulin signaling, triggers meta-inflammation, initiates invasion of the adipose tissue with immune cells, and impairs the endocrine control of appetite and energy balance [8, 89]. Inflammation also attracts monocytes and granulocytes to the adipose tissue, and these immune cells may also undergo apoptosis and their remnants also need to be cleared from the tissue to mitigate inflammation [119].

How macrophages respond to apoptotic adipocytes may depend on the immunological context in which adipocyte death occurs, the signals provided by the apoptotic cells [125], and additional immune signals such as complement and cytokines [126]. Under homeostatic conditions, when apoptotic adipocytes are scarce and Th2 cytokines are expressed in the WAT, the patrolling ATMs clear the dying adipocytes and adopt an anti-inflammatory (so-called M2-like) activation state [127]. It is plausible that the first wave of apoptosis of adipocytes during the development of obesity is well controlled by the ATMs, and they can maintain an M2-like activation state [128]. However, the prevalence of damage-associated molecules and pro-inflammatory lipid species in the dying adipocytes can switch the function of ATMs towards the release of inflammatory cytokines and reactive oxygen species. Moreover, the capacity of ATMs to clear apoptotic cells may be exhausted in obesity [129], and the apoptotic or necrotic cell debris aggravates inflammation.

Inflammatory ATM traits are considered metabolically harmful [89]. However, inflammatory signaling is necessary for adipose tissue development, and inflammation coordinates energy distribution between tissues [130]. Paradoxically, when the response to pro-

inflammatory cytokines is impaired, “inflammation resistance” develops and the risk for obesity increases [130].

Type I interferon signaling, interleukin-6 (IL-6), and signal transducer and activator of transcription 3 (STAT3) signaling are required for healthy adipose tissue development and can be even protective against obesity-induced metabolic deterioration [43, 73, 131-135]. After birth, ATMs may create a local inflammatory milieu, which may sustain the heat production and lipid oxidation capacity of the adipocytes [43]. It is conceivable that preadipocyte or adipocyte apoptosis in the developing WAT induces ATM responses, which contribute to the maintenance of a local inflammatory signaling niche. Moreover, apoptotic cell-derived signals may promote WAT development, as suggested by the increased expression of adiponectin receptor 1 in response to conditioned medium of apoptotic cells [103]. ATMs are scarce in BAT [79, 136], and the impact and mechanisms of apoptotic cell clearance in the BAT remain unexplored.

Signals that allow apoptotic cells to control macrophage behavior are complex, and involve diverse molecules and metabolites released by the apoptotic cells themselves. Apoptotic cells seem to actively elaborate cell metabolites, which serve as “find-me” signals, and display molecular patterns on the cell surfaces which serve as “eat-me” signals for the macrophages [125]. After ingestion of the apoptotic cells, cellular contents are lysed and processed in the phagosomes of the macrophages, ultimately producing signal molecules that control macrophage functions [137]. The study of the metabolites that shape immune response towards apoptotic cells is a new field of immunometabolism, recently termed as “efferotabolism” (efferocytosis-associated metabolism) [138].

8. Adipocyte morphology

Morphology of lipid storing and lipid catabolizing adipocytes

The adipose tissue consists of adipocytes or fat cells, adipocyte precursors, mesenchymal cells, immune cells, capillaries, and extracellular matrix (Figure 2.1). Adipocyte morphology reflects the metabolic activities of the adipose tissue. For instance, the cell volume is filled with a large, central lipid droplet in fat storing adipocytes (Figure 2.1). Only a small portion of the cytoplasm is visible, that contains few mitochondria and an elongated nucleus. This morphology is a trait of the unilocular, fat storing or white adipose tissue (Figure 2.1).

In turn, multilocular adipocytes are fat catabolizing (lipolytic), or thermogenic. They have multiple lipid droplets and a larger volume of the cytoplasm (Figure 2.2). This morphology is a trait of the multilocular, often termed as brown adipose tissue. This adipose tissue type has a richer blood supply than its fat storing counterpart. Multilocular adipocytes are often rich in mitochondria (Figure 2.2). The difference between fat storing and fat catabolizing adipocytes is prominent when the ultrastructure of their cytoplasm is compared (Figure 2.3).

A fat storing “white” adipocyte has a very thin cytoplasm surrounding the lipid droplet, and few mitochondria. In turn, a thermogenic “brown” or “beige” adipocyte has a mitochondria-rich cytoplasm, and scattered lipid droplets. Capillaries are also more abundant in the thermogenic adipose tissue, most likely due to the intensive metabolism of the tissue, thus allowing the thermogenic adipocytes to dissipate heat to the blood flow.

Figure 2.1 shows fat storing adipocytes from the inguinal fat depot of an adult mouse, Figure 2.2 thermogenic fat cells from the interscapular brown adipose tissue of an adult mouse. Figure 2.3 compares human fat storing and thermogenic adipocytes. The fat storing adipocyte was obtained from an adult, while the thermogenic adipocytes from an infant [43, 139].

Lipid droplets

The lipid droplets are reservoirs of lipids and are hence in the center of adipocyte metabolism [140]. A lipid droplet is formed by a core of neutral lipids and bordered by a phospholipid monolayer (Figure 2.4). Core of a lipid droplet is synthesized *de novo* in the endoplasmic reticule membrane and eventually released by "budding" from the membrane. When the lipid core is being released, the phospholipid layer of the endoplasmic reticulum membrane covers the droplet surface.

The phospholipid layer is rich in phosphatidylcholine and phosphatidylethanolamine. Lipid droplets store lipids and provide energy and heat by degrading the stored lipid and provide substrates for membrane synthesis. Many of the enzymes that synthesize phospholipids, triacylglycerols, and their intermediates, as well as lipases and lipolytic regulators, localize to the lipid droplet surfaces. In the developing adipocytes lipid droplets are scattered in the cytoplasm. Size of the lipid droplets may vary from a few nanometers to the micrometers range. Small lipid droplets tend to fuse with each other during adipocyte maturation, eventually leading to the formation of a central lipid droplet. Size of the lipid droplets increases with obesity, and in turn, they shrink in response to lipolytic signals. Figure 2.4 shows lipid droplets of human adipocytes, Figure 2.5 mouse adipocytes. Figure 2.5 also shows the process of lipid droplet expansion by triacylglycerol esterification ("lipid storage") and by lipid droplet fusion.

It is not only adipocytes that can generate lipid droplets. Hepatocytes and muscle cells also synthesize lipid droplets. In many invertebrates the key metabolic organ, the so-called hepatopancreas or midintestinal gland, also builds lipid stores by forming lipid droplets. Figure 2.6 shows examples of hepatopancreas cells from crab *(Carcinus meanas)*, snail *(Helix aspersa)* and edible blue mussel *(Mytilus edulis)*. The hepatopancreas or midintestinal gland is the organ of digestion and intermediate metabolism, hence it functions as a lipid storage site [21]. It also

generates immune cells, and it appears as an immune-metabolic organ. Moreover, its cells also engulf and digest food particles, indicated by abundance of phagosomes within the cells (Figure 2.6). In adipocytes of mammals, similar phagosomes may appear, however their function is to recycle damaged cell organelles, mainly mitochondria.

Mitochondria

Mitochondria are required for energy production – by mean of oxidative phosphorylation and beta oxidation –, and heat generation – by mean of mitochondrial uncoupling – in adipocytes. Preadipocytes and thermogenic adipocytes are especially rich in mitochondria and a substantial amount of their cytoplasm is occupied by mitochondria (Figure 2.5A, 2.7A). Lipid droplets are surrounded by mitochondria during lipolysis and thermogenesis, allowing access of mitochondria to fatty acids released from lipid droplets (Figure 2.7 B,C).

Endosomes, multivesicular bodies and extracellular vesicles

Adipocytes take up certain materials by pinocytosis, forming endosomes. Among others, small nucleotides, dextrane particles may be engulfed by adipocytes (Figure 2.8A-C). Endosomes may form so-called multivesicular bodies (Figure 2.8D). These membrane structures encapsulate small membrane vesicles with cargo containing cytoplasmic molecules (Figure 2.8C). The multivesicular bodies may fuse with the cell membrane, allowing the release of small membrane vesicles with various cargo. These membrane vesicles are forms of extracellular vesicles (Figure 2.8C,E). Their cargo may function as trigger of inflammation or may convey information to other cells. Signaling through micro-RNA content of the adipocyte-derived extracellular vesicles is an intensive area of research today [141, 142].

Lysosomes and autophagosomes

Such as any other eukaryotic cell, adipocytes have lysosomes, that serve breakdown of cellular components (Figure 2.9). Autophagosomes are dedicated to the removal and digestion of unwanted cell organelles. Removal of damaged mitochondria – mitophagy – is a form of autophagy and is relevant in the maintenance of adipocyte functions [143]. Adipocyte differentiation and metabolic adaptation requires mitophagy [43, 143]. When mitophagy is compromised, damaged mitochondrial contents accumulate in the cells, inducing inflammasome activation and inflammation, e.g., interferon response [144-146].

Inflammasomes

Adipocytes contain inflammasomes, that are cytosolic multiprotein oligomers [147]. Inflammasomes are being activated in response to pathogenic stimuli and for damage associated molecular patterns (e.g., efflux of mitochondrial content into the cytosol) and initiate caspase-1 activation. This leads to the secretion of inflammatory mediators (interleukin 1β and interleukin 18) and may also evoke inflammatory cell death (so-called pyroptosis). In the setting of obesity, pyroptosis of adipocytes leads to a chronic inflammation and deteriorates insulin sensitivity [147, 148].

Peroxisomes

Adipocytes require peroxisomes for the breakdown of long chain fatty acids into medium chain fatty acids. These fatty acids are eventually undergoing beta oxidation in the adipocyte mitochondria to generate energy or heat (in the case of uncoupled mitochondria of the

thermogenic adipocytes). Peroxisomes are required not only for lipid catabolism, but also for synthesis of lipid mediators, such as plasmalogens. Peroxisome biogenesis is stimulated by cold exposure, and both adipogenic and thermogenic transcriptional regulators control peroxisome generation in adipocytes [149].

9. Morphology of the stromal vascular cells

In addition to mature adipocytes, the adipose tissue is formed by adipocyte precursors, mesenchymal cells, immune cells, and capillaries (Figure 2.10). Capillaries are formed by single layer of endothelial cells, often surrounded by mesenchymal cells and immune cells.

Mesenchymal cells lack lipid droplets and are rich in mitochondria. These cells are termed as adipose-derived stem cells (ADCs) and have medical impact in regenerative medicine. (i.e., they are used as progenitor cells to repair tissue damage) [150]. Preadipocytes contain varying number of lipid droplets. Immune cells of the adipose tissue are mastocytes, lymphocytes, innate lymphoid cells, and macrophages [78]. Most prevalent immune cells in the adipose tissue stroma are macrophages (adipose tissue macrophages, ATMs). ATMs show morphological hallmarks of phagocytosis: large endocytic vesicles and phagosomes are characteristic traits of these cells (Figure 2.10). ATMs also grow extensive filopodia and are prone for migration. Activation state of ATMs is key to determining fibrosis, inflammation, bone marrow myelopoiesis and immune cell invasion of the obese adipose tissue [77].

10. Extracellular matrix

Stromal vascular cells and adipocytes are unified into a tissue by adhesion molecules and extracellular matrix molecules (Figure 2.11 – 2.13). Major components of the extracellular

matrix are collagen I, IV, V and VI. In mouse adipose tissue collagen type VI α3 (COL6A3) is the most prevalent collagen. Since adipocytes are filled with lipid droplets, a mechanical stress evolves with increasing fat storage. This mechanical stress can be decreased by a collagen network, that forms a basement membrane-like capsule (similar to an "exoskeleton") around adipocytes [151] (Figure 2.11, 2.12). Adhesion of adipocytes to the extracellular matrix serves as a survival signal.

Collagens are produced by the adipocytes (Figure 2.3, 2.11), although the preadipocytes, endothelial cells and the stem cells can also produce collagens. Developing collagen fibrils often can be seen in electron microscopy images of adipose tissue (Figure 2.3). Depending on the fat depot, elastic fibers also appear, providing elastic mechanical properties to the tissue (Figure 2.13). In obesity, extracellular matrix may be deposited in excess, that leads to fibrosis. Adipose tissue fibrosis reduces the ability of adipocytes to expand and store excess lipids, eventually causing adipocyte death and the ectopic accumulation of lipids. This event triggers inflammation and may lead to insulin resistance [152]. Remodeling of the adipose tissue allows to reshape fat depots, and hence allows age-related changes to appear in adipose tissue distribution.

11. Histology of various fat depots

The laboratory mouse is the most frequently used model of obesity. Typical fat storing depot – that is resistant to transformation into thermogenic fat – is the epididymal adipose tissue (Figure 2.14A). It appears only in males and surrounds the epididymis and testicles. It is a visceral fat depot, and often used in animal studies to model human visceral obesity. This depot is built up from uniform, unilocular "white" adipocytes (Figure 2.14A). In human, visceral obesity is

associated with the expansion of unilocular white adipose tissue, as illustrated by a histology record of an abdominoplasty sample shown in Figure 2.14B.

The interscapular fat depot is a typical brown adipose tissue depot with multilocular adipocytes in rodents (Figure 2.15). It is adjacent to dorsal subcutaneous fat depots, that show unilocular appearance (Figure 2.15). The thermogenic fat cells also contain glycogen, that can be detected by periodic acid Schiff staining (Figure 2.15). In addition to these thermogenic fat depots, the mesenteric fat and the fatty capsule of the kidneys also contain thermogenic adipocytes. These cells are often termed as beige adipocytes (Figure 2.16). In the human body, thermogenic fat cells appear around large arteries, in the kidney capsule, in the fat pads around the thymus and the mesentery [46]. In infancy and childhood, there are thermogenic fat cells within the subcutaneous fat depots as well [56].

Unilocular adipocytes of “white” fat depots do not express UCP1, and these fat depots expand in response to high caloric diet or high fat intake. In turn, the interscapular fat depot in mouse is rich in UCP1 expressing, thermogenic fat cells. Some of the subcutaneous fat depots in mouse also express UCP1 after birth or in response to cold stress (Figure 2.17) [42, 153].

Adipose tissue plays a mechanical supporting function, by forming fat pads around muscles, nerves, vessels, and internal organs. This is illustrated by a set of organ histology records shown in Figure 2.18.

12. Histopathology diagnosis in the adipose organ

Clinical diagnosis of obesity is based on physical examination and on an increased body mass index (BMI), or its derived measures such as BMI z-score or BMI standard deviation score (BMI-SDS). However, especially in pediatric practice, when an early diagnosis of obesity susceptibility is key, BMI does not reflect adiposity accurately. BMI-based assessment hence

may not allow parents and physicians to diagnose pre-obesity or low-grade obesity. This may be overcome by measuring body composition and determine body fat content, or with various anthropometric measures, such as the adipo-muscular ratio or the skinfold tests (Figure 9). These tests are however much less convenient than calculating BMI. However, an increased BMI may indicate increased muscle mass, rather an increased body adiposity.

Measuring skinfold thickness may help to estimate the thickness of the subcutaneous fat layer, and this may reflect the actual adiposity. There are variants of the skinfold thickness test. For instance, it may be measured on the arms and on the thigs, giving the so-called brachio-femoral adipose muscular ratio (Figure 9). Accurate measurement of body fat content is certainly the best technique to determine whether the patient has a healthy adipose tissue mass. It also helps to determine the amount of pathogenic fat mass, and set a goal for weight loss with diet, exercise, and/or pharmacological and surgical interventions.

However, histopathology is a “golden standard” when it comes to the assessment of adipose tissue composition, adipocyte volume, lipid droplet size, metabolic state of adipocytes, adipocyte proliferation or apoptosis, fibrosis, and inflammatory status. With increasing prevalence of obesity-associated diseases and given that being obese is determined early in life, there is a demand of adipose tissue-based early diagnosis and prognosis of obesity. This requires adipose tissue analysis with histology and molecular biology techniques.

Adipose tissue analysis also has an impact in public health screening, as it provides information on the prevalence of obesity and obesity-predisposing conditions in the population. For instance, approximately 10% of male infants undergo elective surgery due to undescended testis or inguinal hernia, allowing obtaining fat specimens and estimating obesity status of their age group.

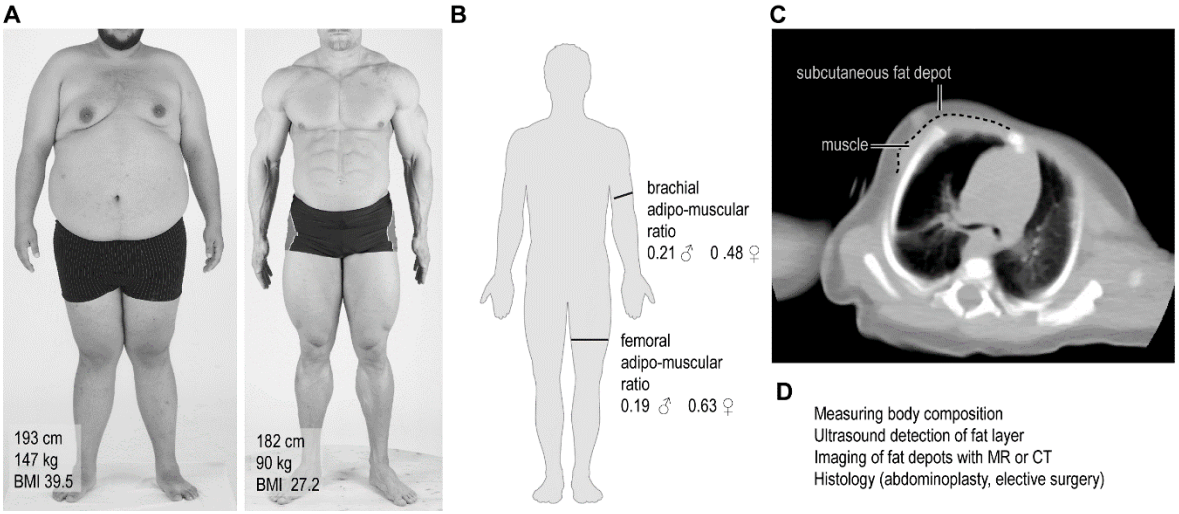

Figure 9. Examples of obesity assessments in clinical practice
(**A**) Example of a misleading interpretation of BMI. *Left:* A young male suffering from morbid obesity (BMI 39.5). *Right:* A young male with increased skeletal muscle muss due to resistance training (BMI 27.2). His BMI may be misinterpreted as indicative of overweight. Images are courtesy of "Human photo references and textures" (3D.sk) image repository. (**B**) Scheme summarizing a two-points assessment of adipo-muscular ratio with reference values for men and women. (**C**) CT imaging of the subcutaneous fat depot at level of the chest. (**D**) Further possible assessments of obesity status.

Adipose tissue is however yet not collected by targeted diagnostic procedure at clinical practice. Exception may be esthetic fat removal and bariatric surgery, that may require a histopathology assessment of the removed adipose tissue. Malignant tumors of the adipose tissue are rare; hence differential diagnosis using histopathology analysis of the adipose tissue has a relatively minor relevance in pathology [154].

For instance, in our practice, adipose tissue samples are analyzed from biopsy samples and materials obtained from elective surgery. This serves research purposes, and still not used in diagnostic procedures. Autopsy protocols do not include the analysis of fat unless specific cues justify such analysis, e.g., adipocyte tumors. Interestingly, a handbook on perinatal pathology from the 1950s states the lack of adipocytes in the infant [155], however this observation may be due to emaciation or cachexia. In perinatal life adipocytes use up their lipid stores rapidly [61], albeit in our own experiences that does not lead to the disappearance of mature adipocytes in tissue sections.

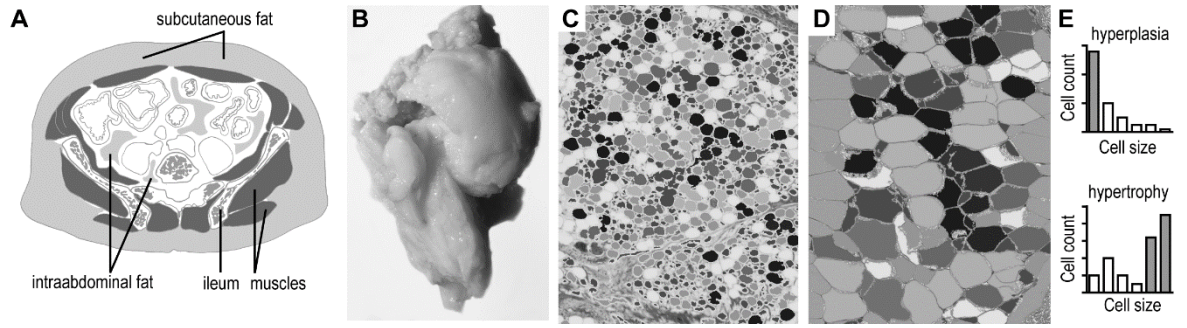

Figure 10. Adipose tissue specimen and morphometry
(**A**) Scheme of the distribution of subcutaneous and intraabdominal fat in obesity. Level of the hip bones. (**B**) Macroscopic image of and abdominal fat pad, removed from a 43-years old male patient during abdominoplasty. From the study [98]. (**C** and **D**) Examples of semi-automated image analysis of human adipose tissue sections. Adipocytes are digitally labeled with various color codes, each corresponding to a specific cell size. (**E**) Histograms with size distribution are obtained with this analysis to define the presence of hyperplasia or hypertrophy.

Histopathological observation of the adipose tissue in the context of obesity is intended to determine adipocyte size, hyperplasia, hypertrophy, inflammation, and fibrosis (Figure 10). These may be used to predict a worsening of obesity-associated diseases. In differential diagnosis of adipose tumors, adipocyte morphology, the presence of preadipocytes and non-differentiated cells, degree of vascularization and the structure of the extracellular matrix are relevant.

In brief, there are seven major forms of benign adipose tumors: lipoma, lipoblastoma/lipoblastomatosis, angiolipoma, myolipoma of soft tissue, chondroid lipoma, spindle cell/pleomorphic lipoma, and hibernoma. The latter is characterized by thermogenic potential, hence resembling brown adipose tissue, that historically was termed as “hibernation gland”. Malignant adipose tumors are so-called liposarcomas and have three types: well-differentiated liposarcoma/dedifferentiated liposarcoma, myxoid/round cell liposarcoma, and pleomorphic liposarcoma. As adipose tissue-based diagnostic procedures may reach clinical routine, it is plausible that such malignancies will be detected when fat specimens are collected for histopathology.

Routine histology diagnosis employs hematoxylin and eosin (or equivalent such as iron hematoxylin and Chromotrope 2R) staining. This allows to detect adipocytes, infiltrating immune cells, and contours of collagen fibers. Histomorphometry to measure adipocyte size, diameter, perimeter may be employed. Fibrosis however requires specific staining of collagens and elastic fibers. Goldner trichrome, Masson trichrome, azocarmine-aniline blue, picrosirius red staining can be used to detect collagens. Elastic fibers can be detected with orcein staining. More specific labeling of extracellular matrix components is also possible with immunohistochemistry, albeit this does not have an established diagnostic value. Immune cells may be analyzed with flow cytometry and immunohistochemistry.

Histology analysis can be combined with molecular diagnostics, such as gene expression analysis. In differential diagnostics of adipose tumors and in predicting obesity progression such analysis is proven useful. Examples of adipose tissue-targeted diagnostic procedures are provided below.

Lipoma

Lipomas are benign adipocyte neoplasms. They contain mature, unilocular adipocytes, resembling a normal adipose tissue morphology. However, there are fibrous areas with blood vessels and may contain proliferating adipocytes [156]. Lipomas may be surgically removed, and to exclude malignancy (i.e., liposarcoma) must be processed for histopathology analysis. Use of lipomas for research purpose is hence limited since the entire lipoma is processed for histology analysis and eventually stored by the pathology department of the respective hospital.

Hibernoma

Hibernomas are extremely rare, benign adipocyte neoplasms. They are formed by multilocular and UCP1 expressing adipocyte and are vascularized. They have some morphological variants,

such as typical, lipoma-like, myxoid, and spindle cell variants. The lipoma-like subtype contains unilocular adipocytes as well. Such as the thermogenic “brown” fat cells, hibernoma cells contain cytochrome pigments.

Well-differentiated liposarcoma

Common lipomas and their variants must be distinguished from liposarcomas, which are malignant neoplasms. Imaging with CT or MR identifies tumor location. Histological hallmarks are the presence of proliferating mature adipocytes and preadipocytes. Nuclear morphology and the presence of mitotic nuclei in adipocytes is a key feature in histology finding. With fluorescence *in situ* hybridization, gene expression analysis or with immunohistochemistry the overexpression of gene products, so-called MDM2 und CDK4 can be detected in this tumor. Indeed, MDM2 is a marker gene of this tumor type. MDM2 blocks tumor suppressor p53, while CDK4 is necessary for cell cycle control.

Multi-organ effects of obesity

Multi-organ effects of obesity often appear in pathology diagnosis. Some of the most common ones are illustrated in Figures 2.19 – 2.21. Figure 2.19 shows fatty liver disease or non-alcoholic fatty liver disease (or metabolic fatty liver disease), also termed as liver steatosis. Abundance of lipid droplets in liver cells is a hallmark of obesity-induced liver disease. Lipid may be labeled with oil red O colorant, that dissolves in the lipid droplets, allowing their visualization. Oil red O staining may be performed on frozen sections (cryostat sections), or on agarose embedded vibratome sections. On paraffin embedded tissue specimens however, such staining cannot be performed, since solvents used during tissue processing eliminate lipid content.

Skeletal muscles may be infiltrated by macrophages in obesity, as shown in Figure 2.20. Ectopic lipid accumulation may appear in both skeletal muscle and in cardiac muscle, as

illustrated in Figure 2.20. Kidneys may also be affected by obesity (Figure 2.21), leading to glomerulonephritis, lipid accumulation in the renal medulla, and eventually, renal failure. Main histology features include of the thickening of glomerular membrane, so-called ballooning of the affected glomerular capillary regions, expansion of the mesangial matrix, and excessive apoptosis and cell proliferation in the glomeruli. Morbid obesity may also cause lipid droplet accumulation in the nephron epithelium.

13. Pillars of obesitology

The below list of literature shows how the principal concepts of adipose tissue biology and obesity have evolved. Titles span from the 19th century "physiological chemistry" concept of metabolism to the mainstream ideas of obesity-associated pathologies today. It is not only a tribute to those who have laid down the framework of obesity research, but also a useful resource to recognize the exponential growth in the prevalence and severity of obesity from the early 1900s to the 2020s.

- von Liebig J (1842) Origin of fat in domesticated animals. in Animal Chemistry: or Organic Chemistry in Its Application to Physiology and Pathology. Justus Liebig. Edited from the author's manuscript by William Gregory with additions, notes, and corrections by Gregory and John W. Webster. 1842, John Owen: Cambridge.

First conceptualization of the causal link between physical inactivity and obesity.

- Ziegler E (1902) Lehrbuch der Allgemeinen Pathologie und der Pathologischen Anatomie. Verlag von Gustav Fisher, Jena

First mention of terms like "adiposity" and *"adipoblast".*

- Leghn A (1905) Dissimilation (Physiologische Chemie), Göschen'sche Verlagshandlung Leipzig

Concept of energy metabolism and dissimilation of nutrients.

- Ingalls AM, Dickie MM, Snell GD (1950) *Obese*, a new mutation in the house mouse. *J Hered* **41**(12): 317-318.

The first animal model of genetically-determined obesity.

- Smith CA (1953) The physiology of the newborn infant. Charles C Thomas Publisher, Springfield, Illinois

A comprehensive summary of lipid and carbohydrate metabolism in the early life.

- Kinsell LW (1962) Adipose tissue as an organ. Charles C Thomas Publisher, Springfield, Illinois

A summary of key concepts of adipose tissue biology. Introducing the term "adipose organ".

- Hummel KP, Dickie MM, Coleman DL (1966) *Diabetes*, a new mutation in the mouse. *Science* **153**(3740): 1127-1128.

The first animal model of genetically inherited insulin resistant – obesity-associated – diabates.

- Hausberger FX (1966) Pathological changes in adipose tissue of obese mice. *The Anatomical Record* **154**(3): 651-660.

First record of adipose tissue macrophages.

- Góth E (1969) Pathophysiologie und Klinik der Fettsucht. Academic Press Budapest
- Jeanrenaud B, Hepp D (1970) Adipose tissue: regulation and metabolic functions. Georg Thieme Verlag Stuttgart, Academic Press New York, London
- Mancini M, Lewis B, Contaldo F (1979) Medical complications of obesity. Academic Press London, New York, Sydney, San Francisco
- Sinclair JC (1978) Temperature regulation and energy metabolism in the newborn. Monographs in Neonatology. Grune & Stratton, New York, San Francisco, London

- Bouchard C, Bray GA (1996) Regulation of body weight. John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore

The above handbooks document concepts of their times on the understanding of clinical outcomes of obesity.

- Ostenhof-Hoffmann O (1987) Intermediary metabolism. Van Nostrand Reinhold Company, New York

A collection of reprinted articles from the 19th and early 20th century, focused on metabolism.

- Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **68** (5): 879-87.

Discovery of lipid-sensing nuclear receptors and the role of peroxisome proliferator activated receptors in beta oxidation.

- Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF A novel serum protein similar to C1q, produced exclusively in adipocytes (1995). *J Biol Chem* **270**: 26746-49.

Discovery of adiponectin.

- Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**: 543-546.

Discovery of leptin.

- Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* **117**: 175-184.
- Weisberg SP, et al., (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**: 1796-1808.

The above articles described adipose tissue inflammation in obesity and the role of adipose tissue macrophages.

Part Two

HISTOLOGY ATLAS OF THE ADIPOSE ORGAN

2.1.
Adipocyte morphology and ultrastructure

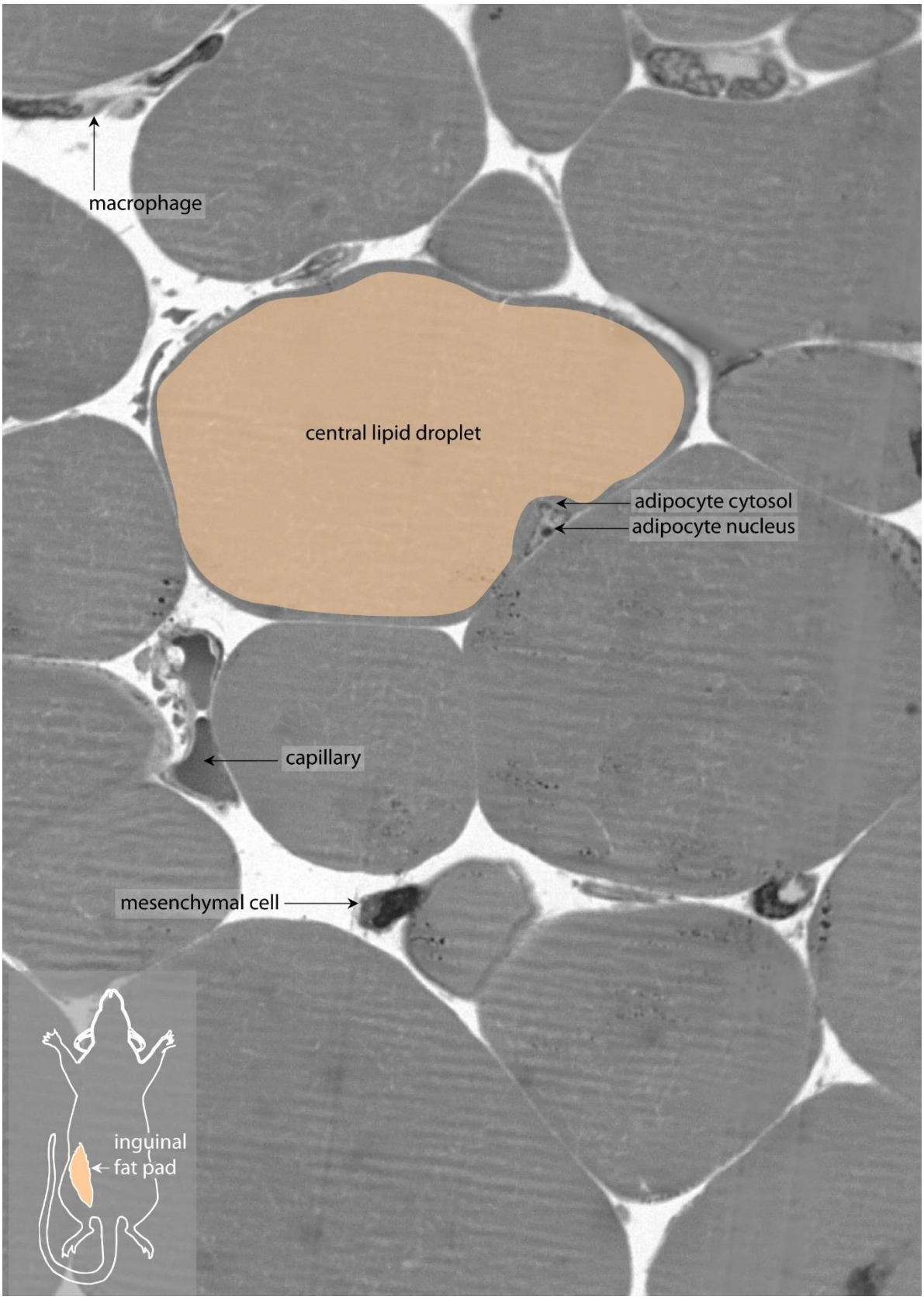

Figure 2.1. Unilocular ("white" or "storage") adipose tissue in adult mouse
Semi-thin section with toluidine blue staining.

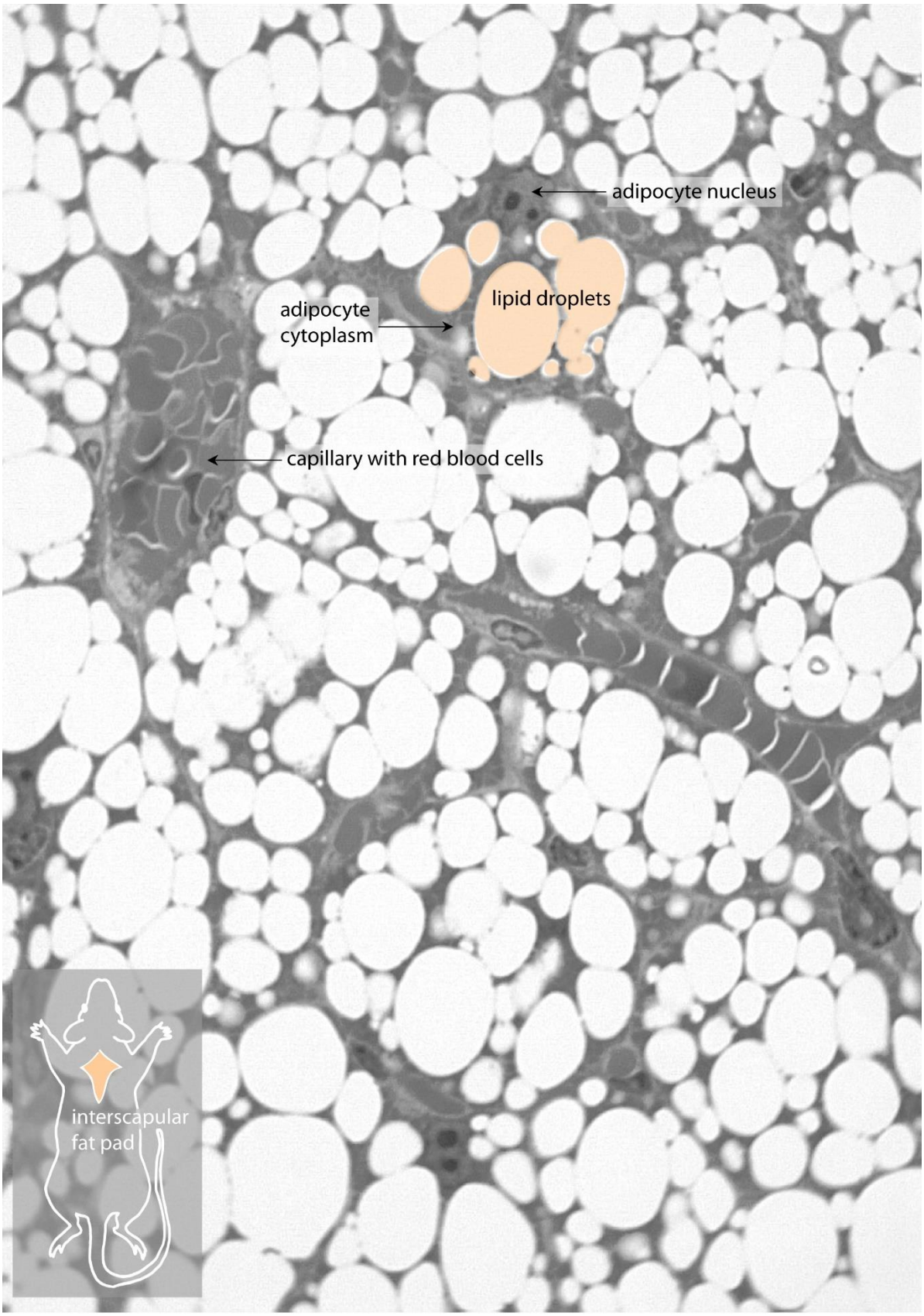

Figure 2.2. Multilocular (“brown) adipose tissue in adult mouse
Semi-thin section with toluidine blue staining.

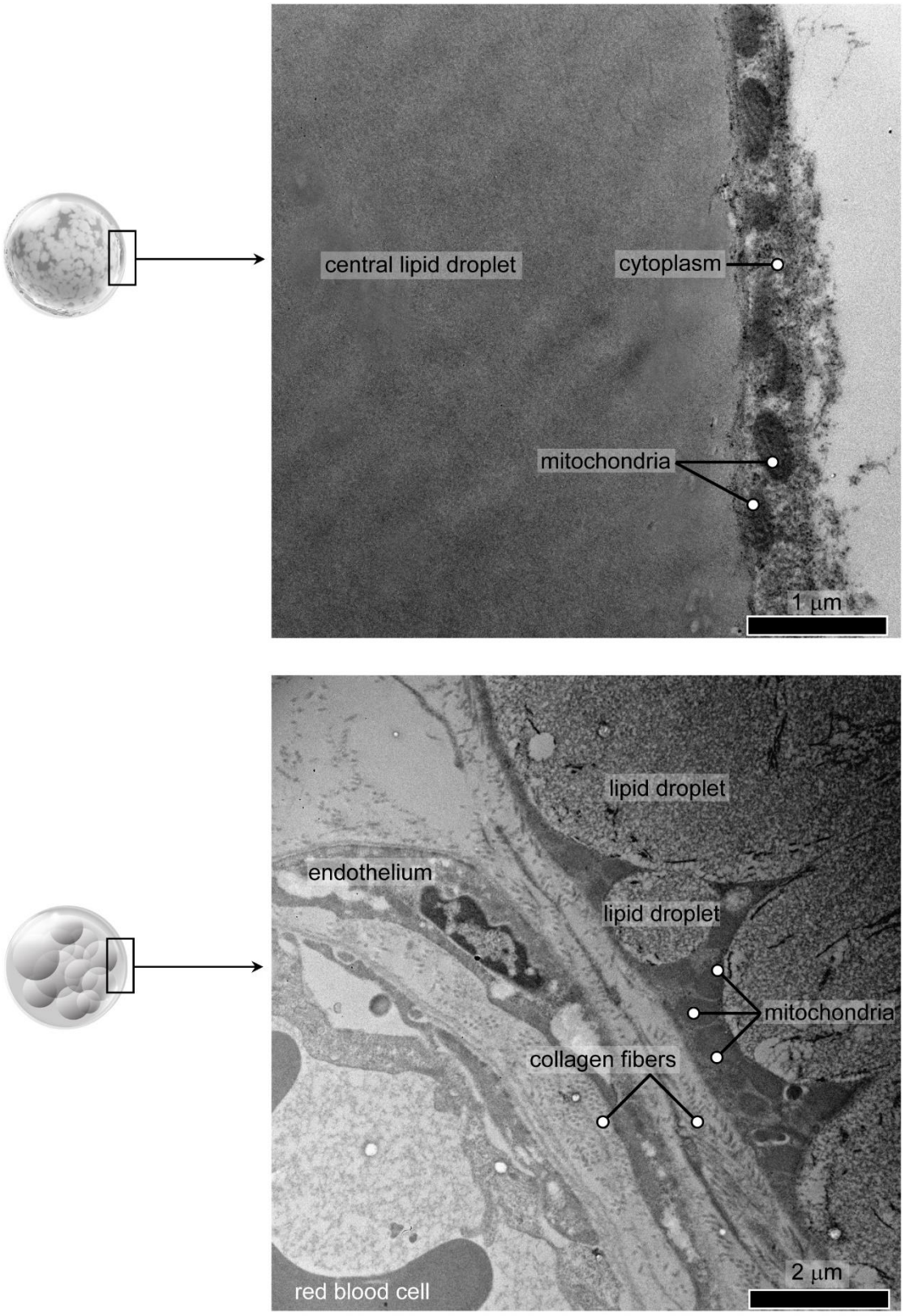

Figure 2.3. Ultrastructure of unilocular and multilocular adipocytes

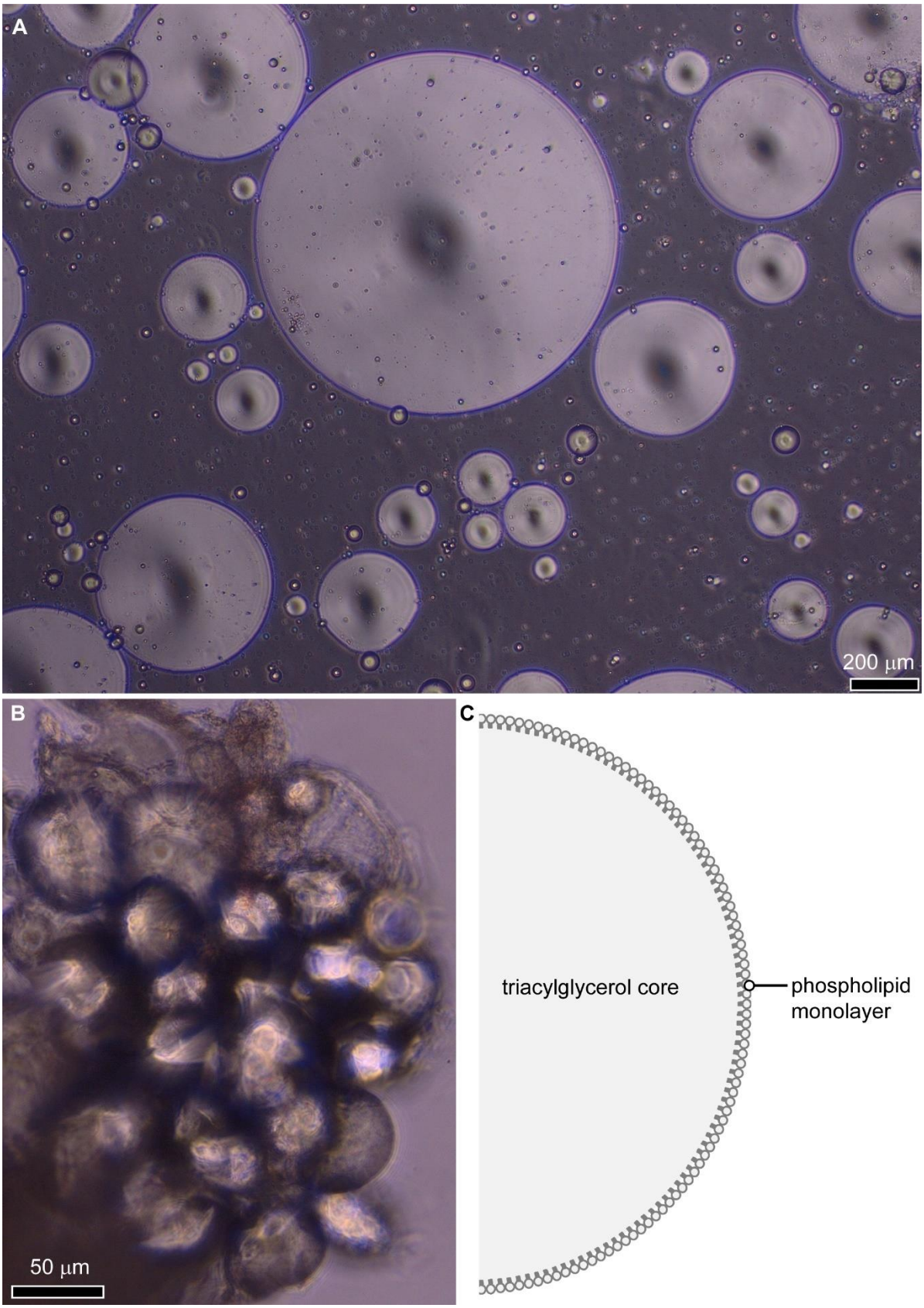

Figure 2.4. The lipid droplet
(**A**) Free lipid droplets of human adipocytes, dispersed in water. (**B**) Macroscopic image of human adipocytes. (**C**) Simplified scheme of a lipid droplet.

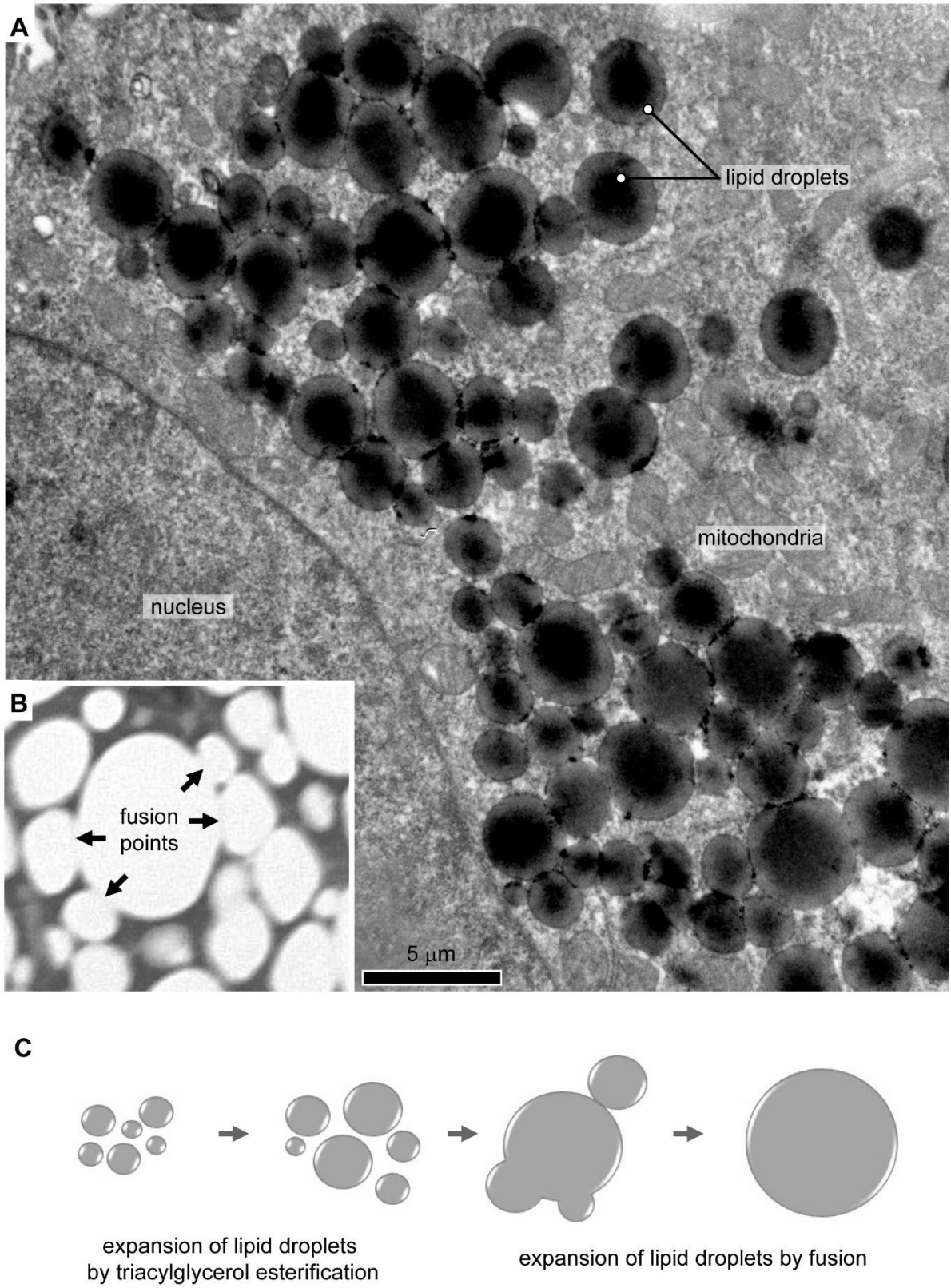

Figure 2.5. Expansion of lipid droplets
(**A**) Ultrastructure of an *in vitro* cultured adipocyte. (**B**) Fusing lipid droplets in a thermogenic adipocyte. Semi-thin section with toluidine blue staining. (**C**) Scheme summarizing mechanisms of lipid droplet expansion.

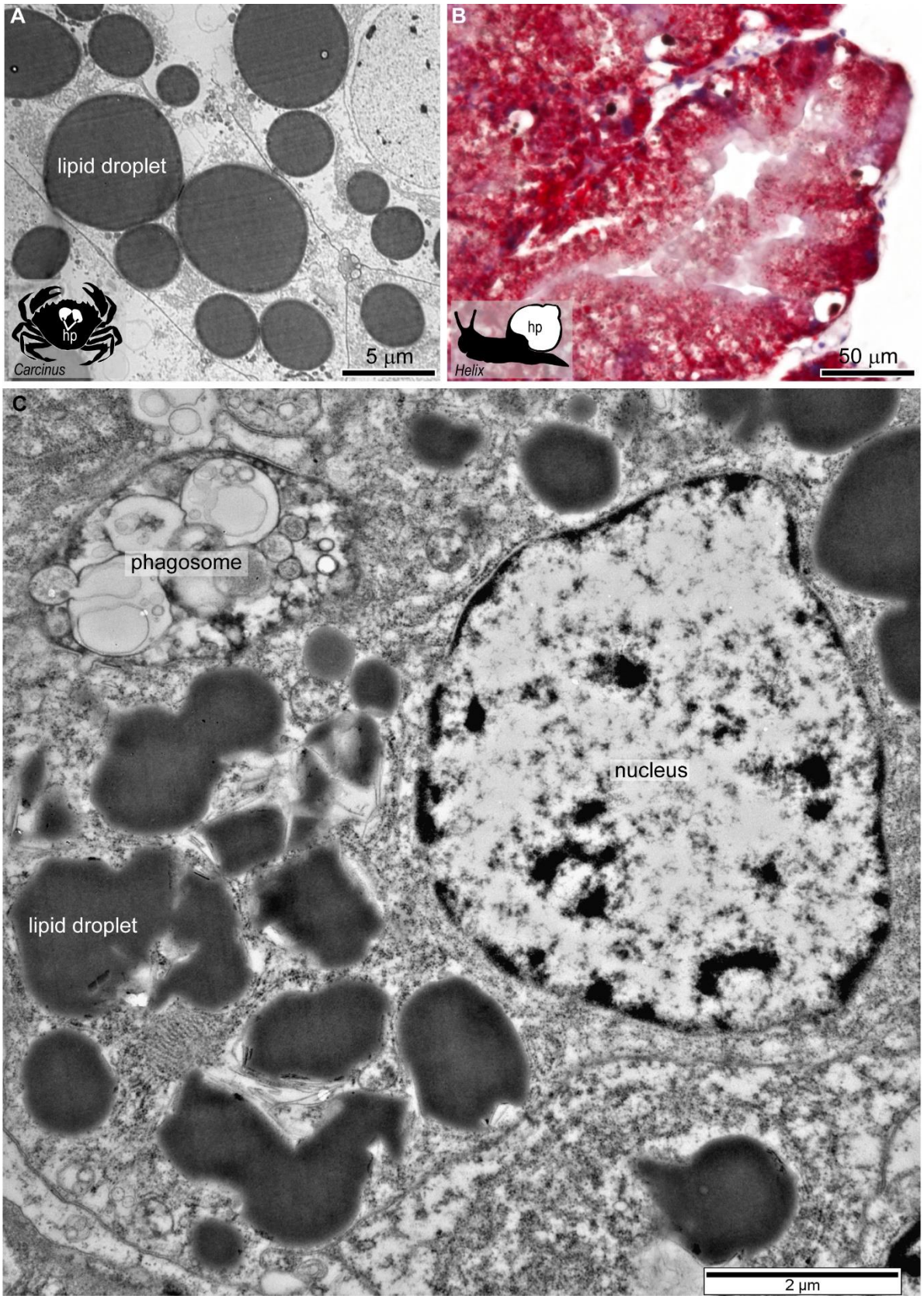

Figure 2.6. lipid droplets: comparative anatomy
Transmission electron microscopy images of midintestinal gland (hepatopancreas) cells of the European green crab *(Carcinus meanas)*, edible garden snail *(Helix aspersa)* and edible blue mussel *(Mytilus edulis)*.

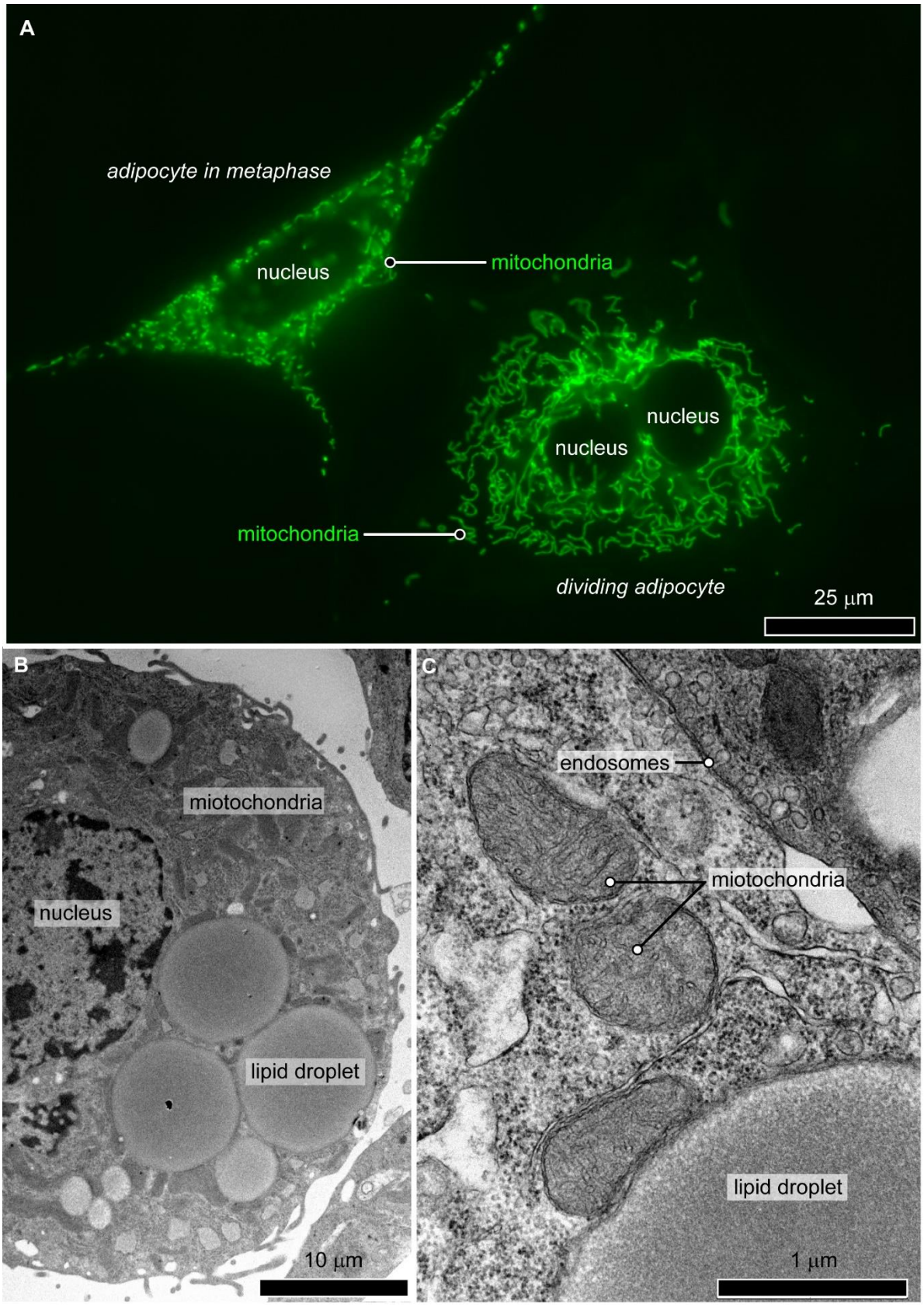

Figure 2.7. Adipocyte mitochondria
(**A**) MitoTracker Green labeling of mitochondria of *in vitro* cultured mouse adipocytes. (**B** and **C**) Transmission electron microscopy images of mouse preadipocytes.

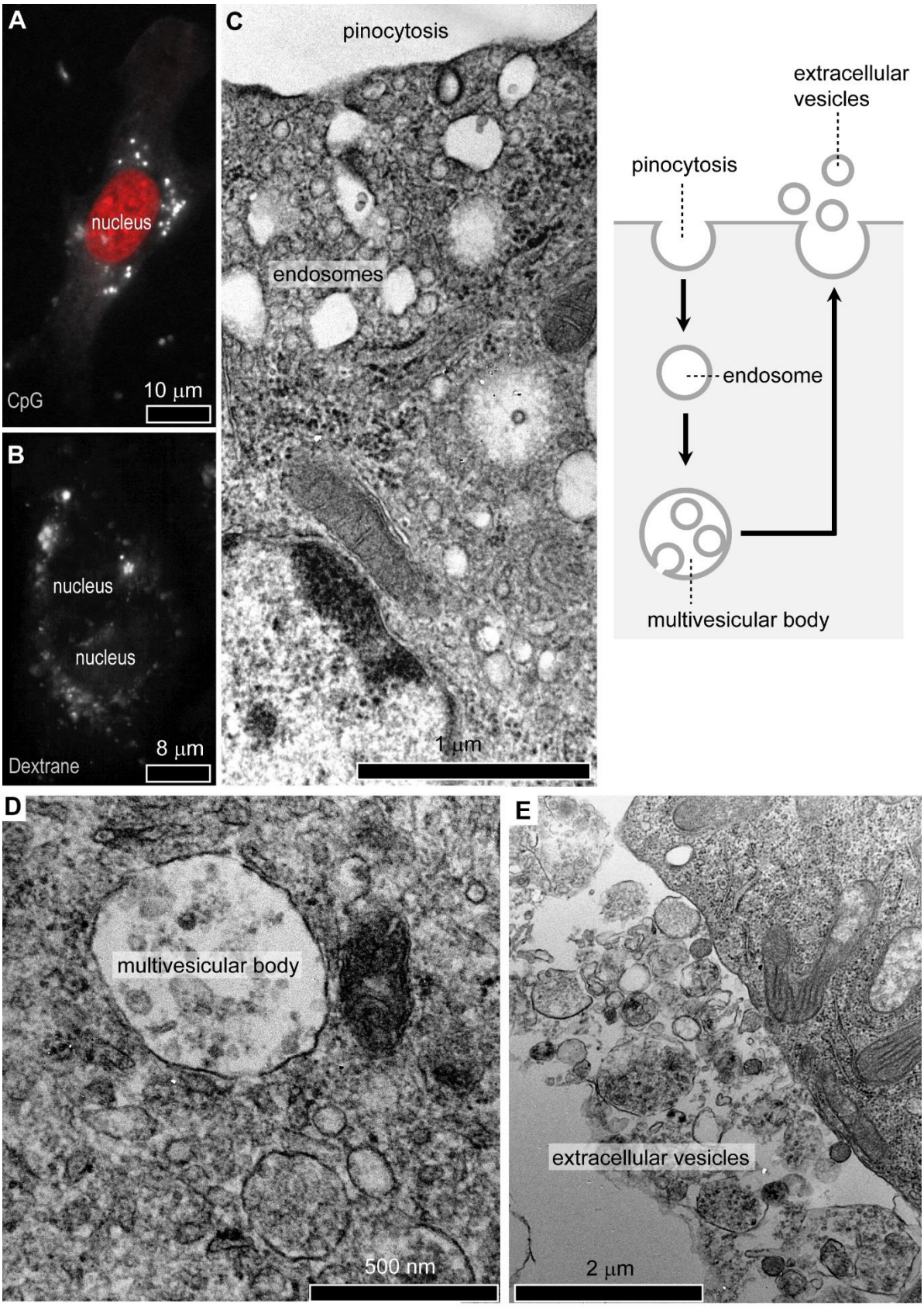

Figure 2.8. Endosomes and extracellular vesicles in adipocytes
(**A**,**B**) Fluorescently labeled cytosine–guanine dinucleotides (CpG) and dextrane uptake by mouse adipocytes. (**C**) Scheme of endosome trafficking. (**D**,**E**) Transmission electron microscopy of multivesicular bodies and extracellular vesicles. Mouse inguinal adipocytes.

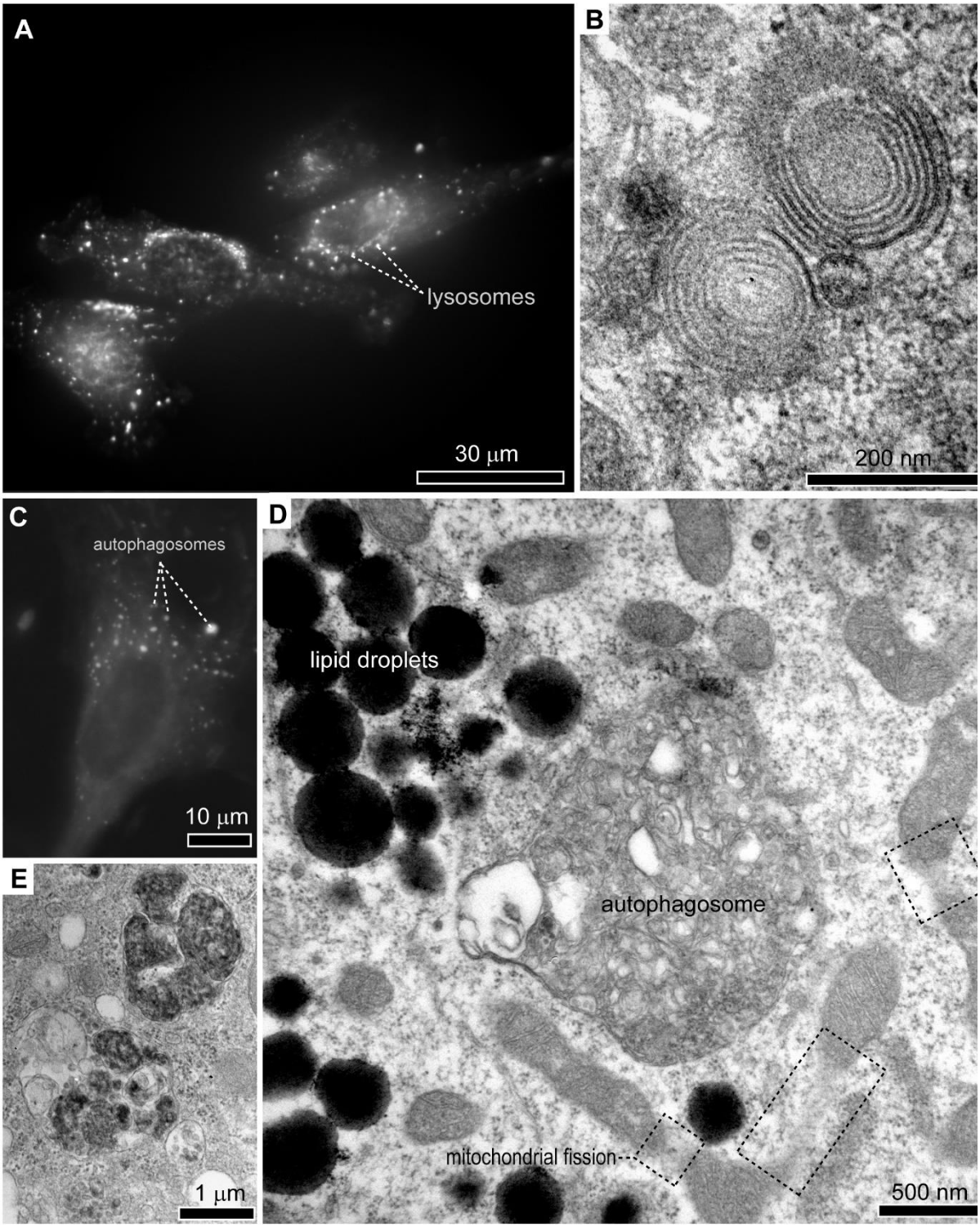

Figure 2.9. Lysosomes and autophagosomes of adipocytes
(**A**) Fluorescent labeling of lysosomes with LysoBright. (**B**) Transmission electron microscopy of lysosomes. (**C**) Fluorescent labeling of autophagosomes. (**D**,**E**) Autophagosomes of mouse adipocytes containing remnants of various cell organelles. Mitophagy is often associated with mitochondrial fission. Dotted frames label mitochondria in fission.

2.2.
Ultrastructure of stromal vascular cells

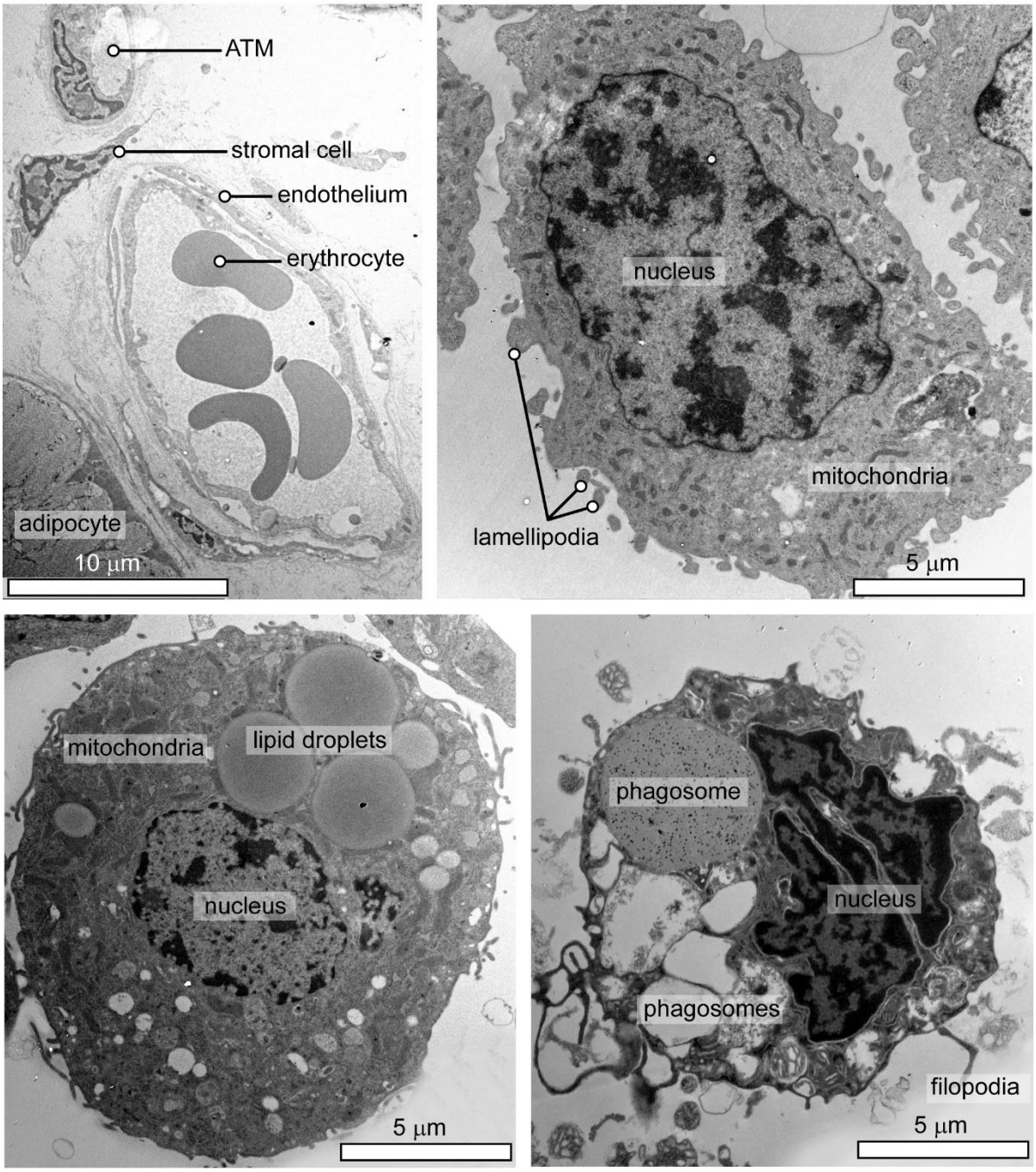

Figure 2.10. Transmission electron microscopy of stromal vascular cells
From left to right: Perivascular niche of stromal cells in mouse. Mesenchymal stem cell. Preadipocyte. Adipose tissue macrophage.

2.3.
The extracellular matrix

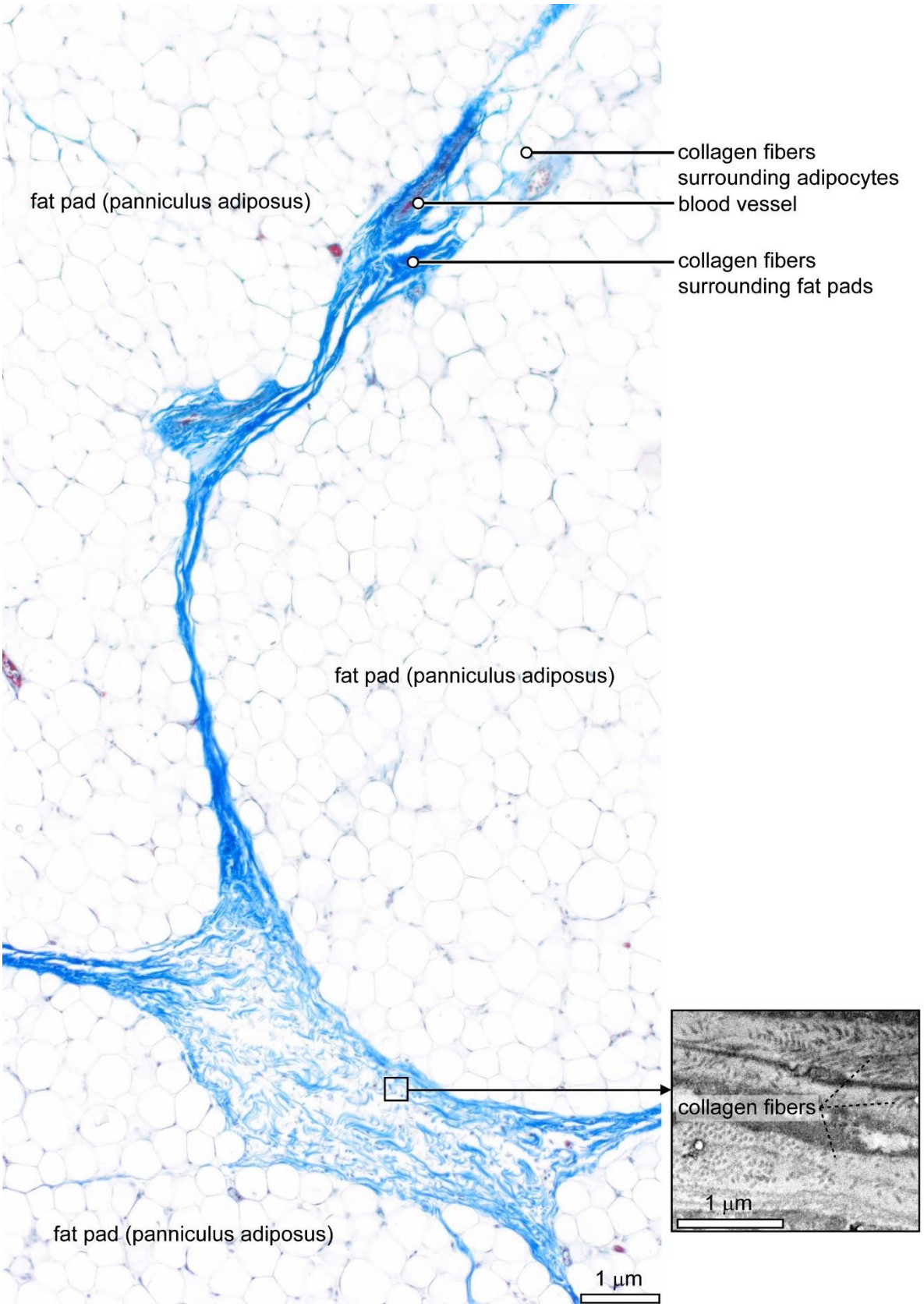

Figure 2.11. Collagen fibers of the human abdominal subcutaneous fat depot
Staining with Masson trichrome. Inlet shows electron micrograph of collagen fibers.

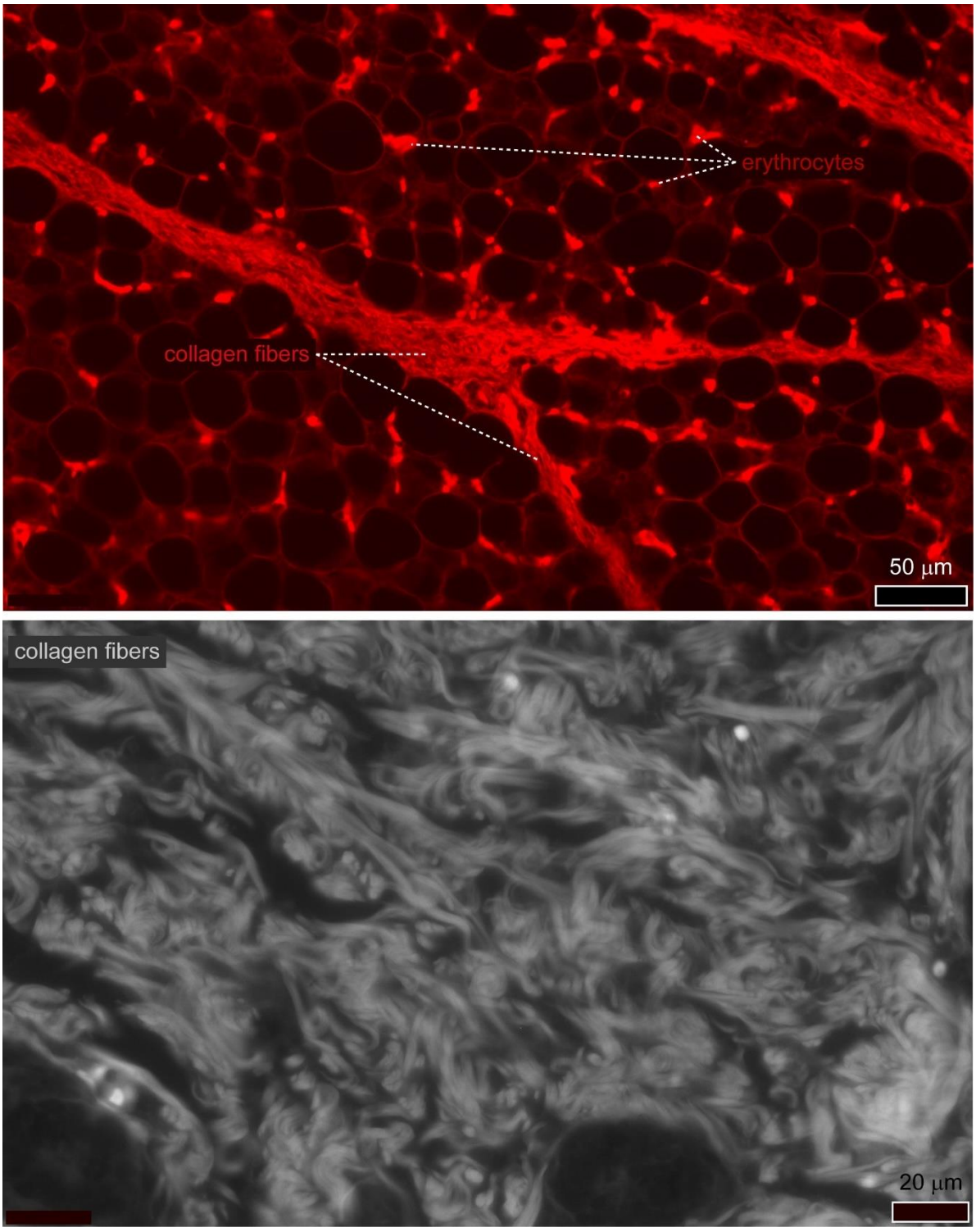

Figure 2.12. Collagen fibers in the adipose tissue
Fluorescent microscopy images showing the “exoskeleton”-like collagen network of mouse subcutaneous fat depot.

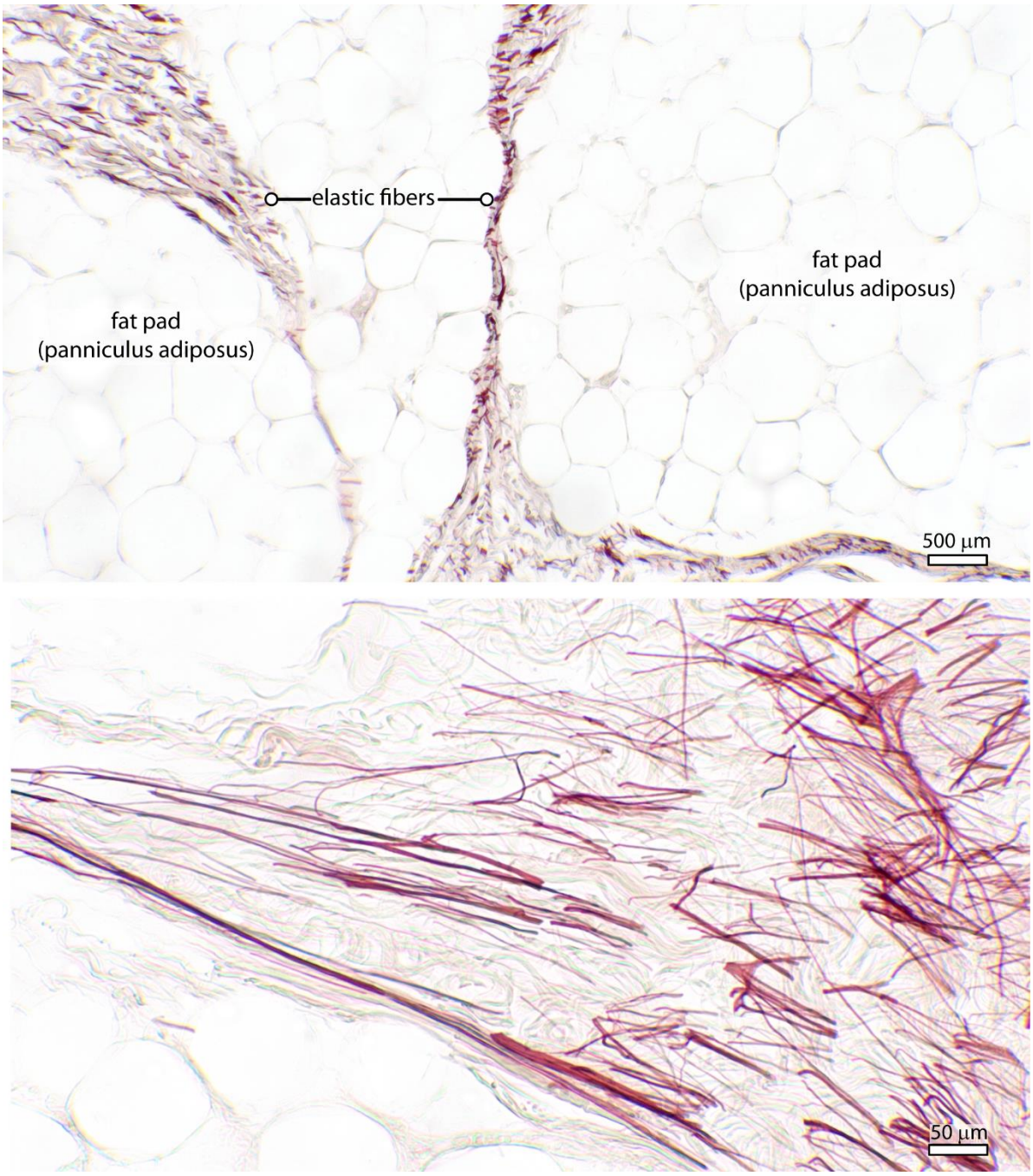

Figure 2.13. Elastic fibers in the adipose tissue
Human abdominal subcutaneous fat depot, with orcein staining.

2.4.
Fat depots in various anatomical sites

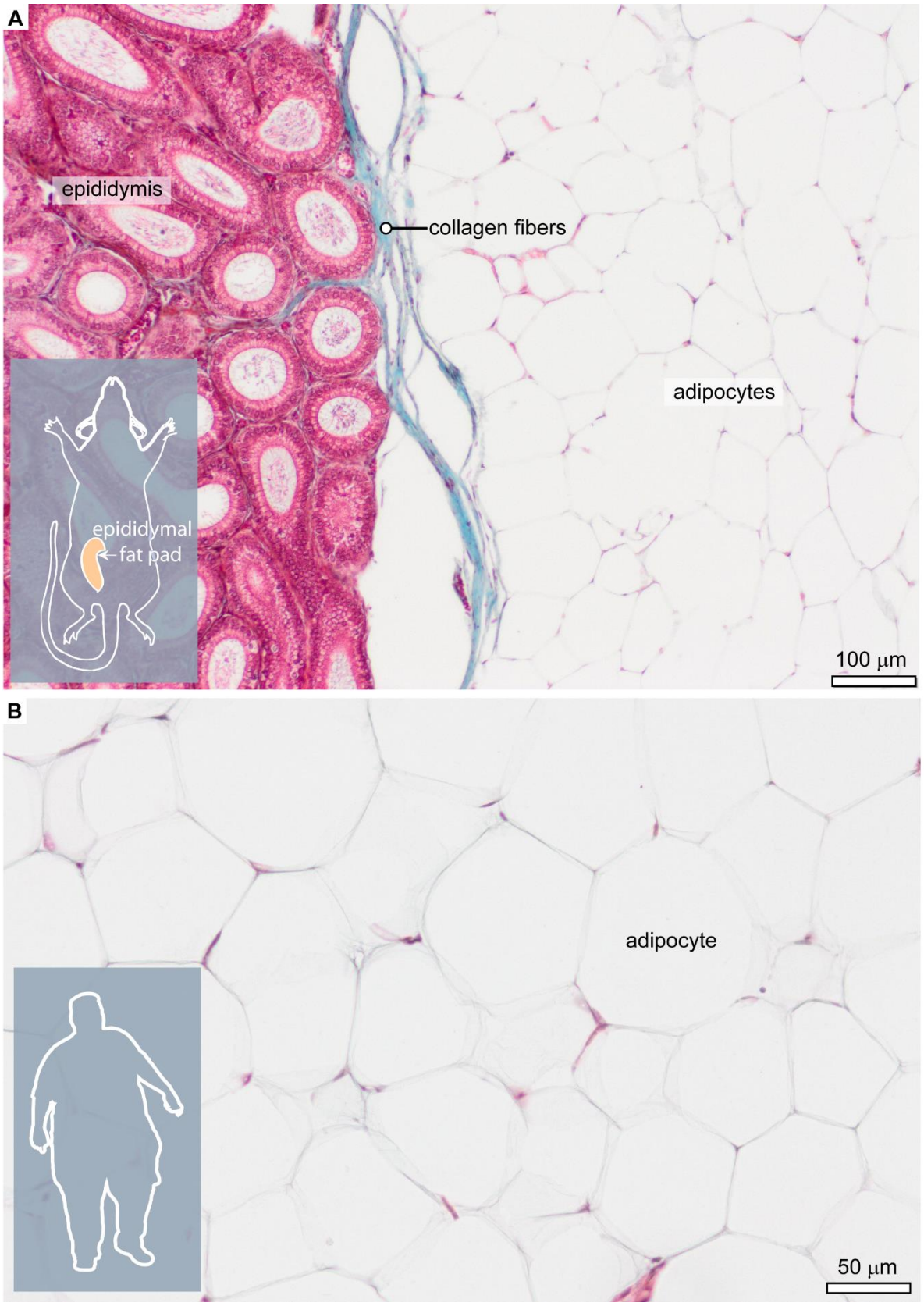

Figure 2.14. White adipocytes forming storage fat (*"Speicherfett"*)
Top: mouse epididymal fat depot. *Bottom:* abdominal fat from a morbid obese patient. Goldner trichrome staining.

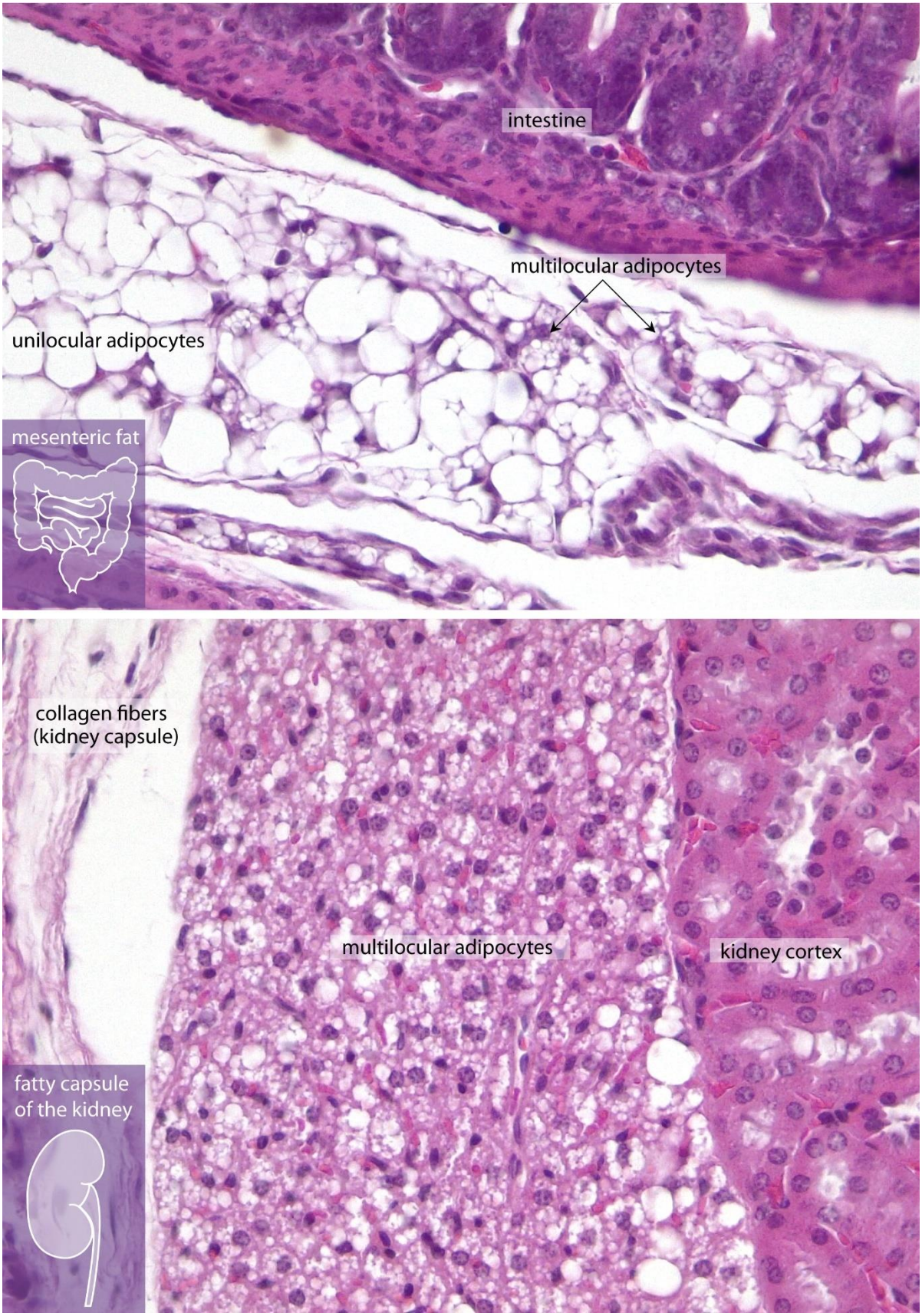

Figure 2.15. Multilocular “beige” and “brown” adipocytes in mouse
Hematoxylin and eosin staining of the mesenteric and perirenal fat depots of an adult mouse.

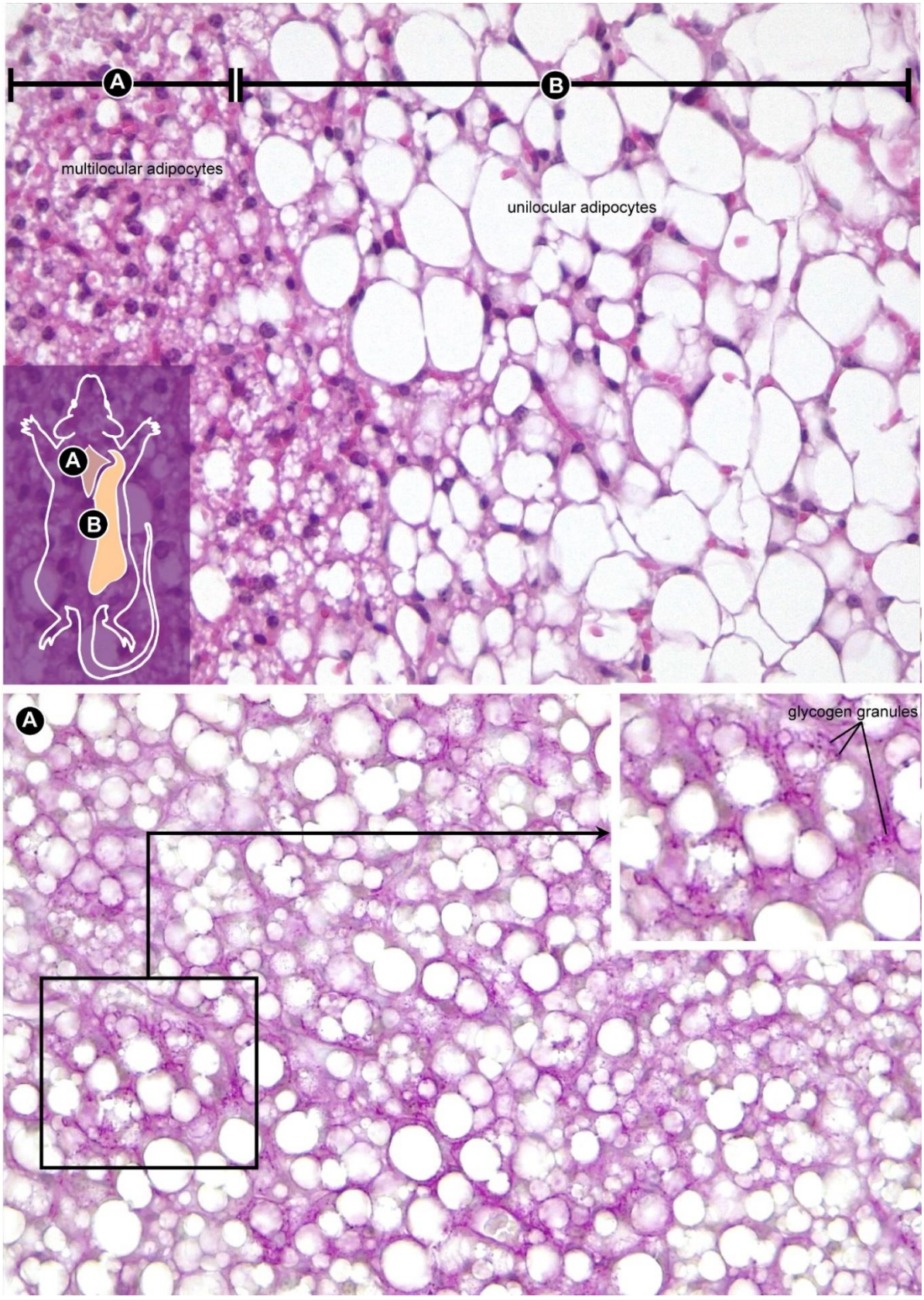

Figure 2.16. Multilocular "brown" adipocytes in mouse
Region "A" labels interscapular brown fat depot. Region "B" labels subcutaneous white adipose tissue of the back. *Top:* Hematoxylin and eosin staining. *Bottom:* periodic acid-Schiff staining

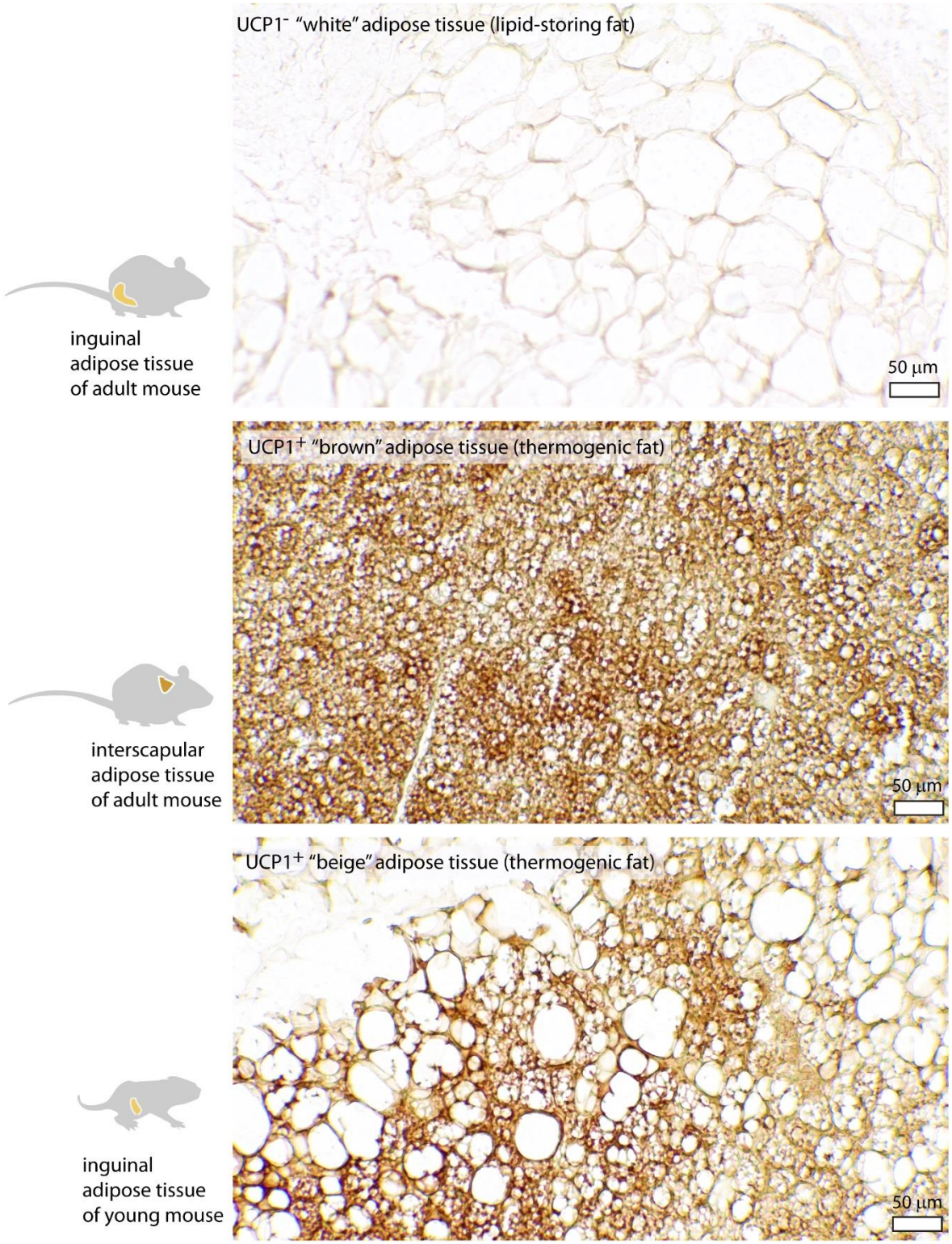

Figure 2.17. Thermogenic fat cells in mouse
Top: Epididymal fat depot of an adult mouse. *Middle:* Interscapular brown fat depot of an adult mouse. *Bottom:* inguinal fat depot of a suckling mouse. Immunohistochemistry against uncoupling protein 1 (UCP1), also called as "thermogenin".

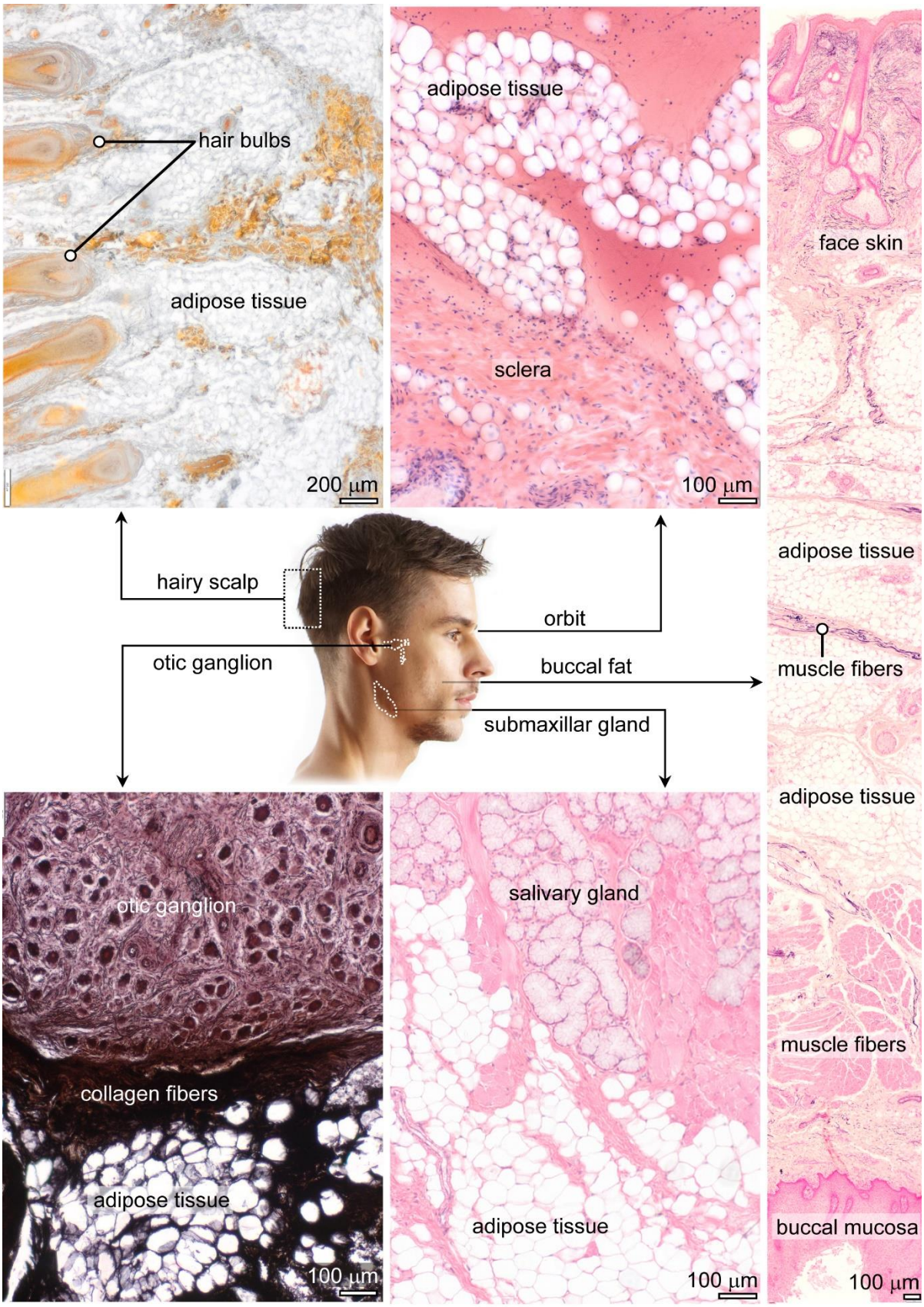

Figure 2.18. Examples of structure fat *"Strukturfett"* in human

2.5.
Multi-organ effects of obesity

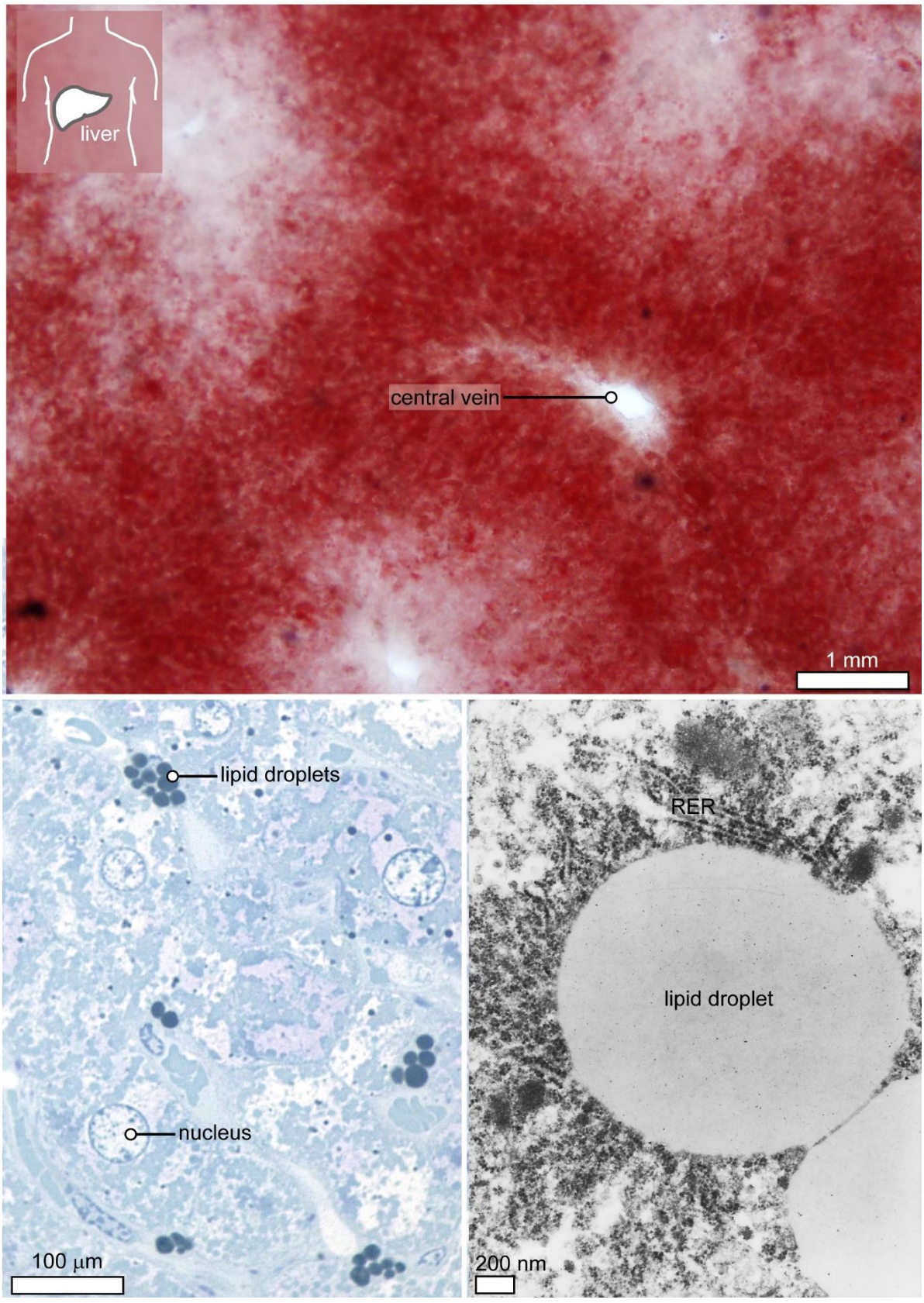

Figure 2.19. Histopathology of non-alcoholic steatohepatitis
Oil red O labeling of lipid droplets in vibratome section of the liver, semi-thin section with osmium-tetroxide labeled lipid droplets, and transmission electron micrograph of a lipid droplet in a steatotic hepatocyte.

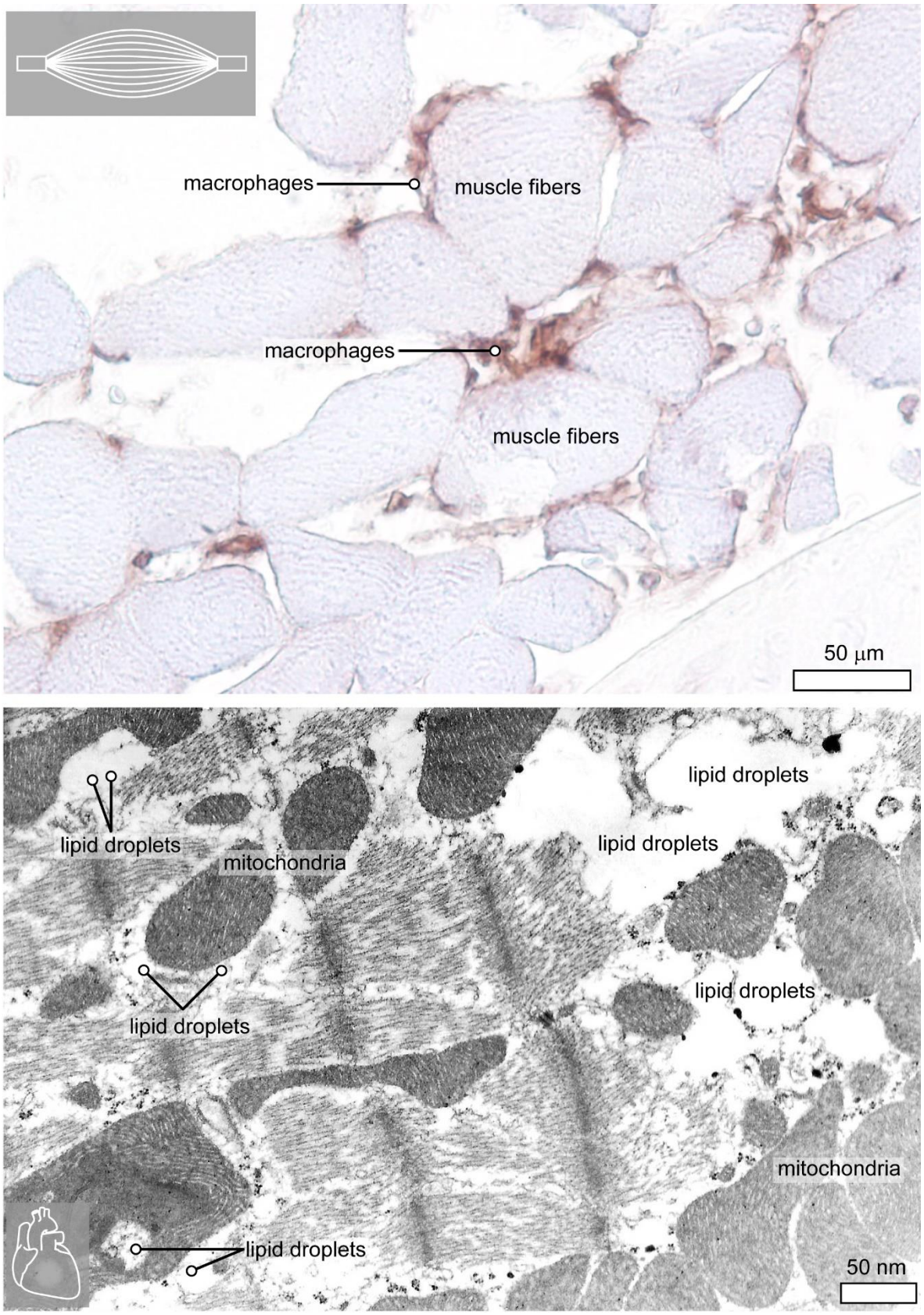

Figure 2.20. Effect of obesity on the skeletal- and cardiac muscle
Top: Macrophage infiltration of the skeletal muscle. *Bottom:* Lipid droplets in cardiomyocytes. Transmission electron microscopy image.

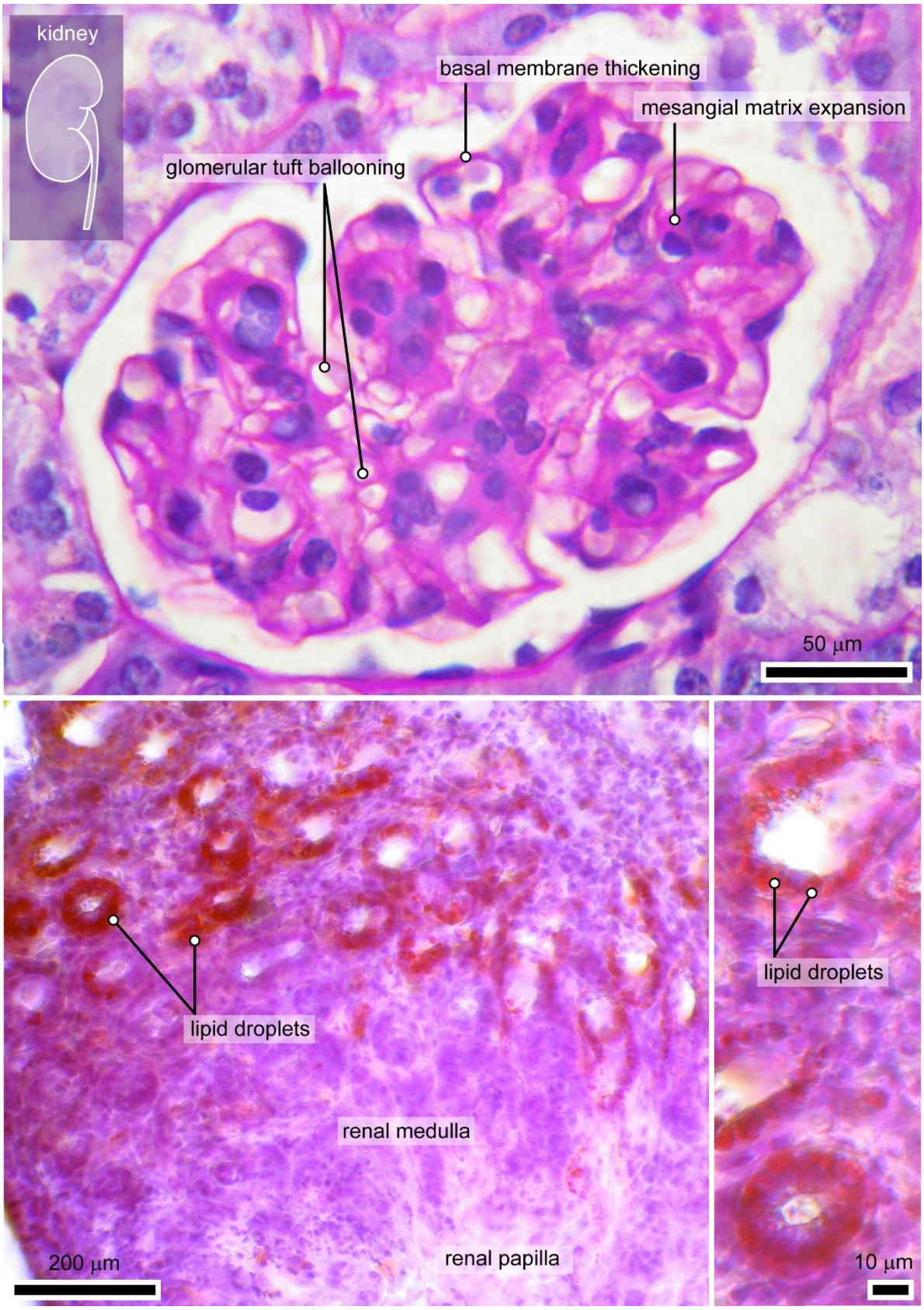

Figure 2.21. Histopathology of glomerulonephritis
Top: enlarged glomerulus with signs of inflammation, mesangial matrix expansion, basement membrane thickening from a genetically obese mouse. Perioidic acid-Schiff staining. *Bottom:* Oil red O labeling of lipid droplets in the renal medulla of the same mouse.

References

1. Rosen, E.D. and B.M. Spiegelman, *What we talk about when we talk about fat.* Cell, 2014. **156**(1-2): p. 20-44.
2. Röszer, T., *Co-Evolution of Breast Milk Lipid Signaling and Thermogenic Adipose Tissue.* Biomolecules, 2021. **11**(11): p. 1705.
3. Röszer, T., *Adipose Tissue Immunometabolism and Apoptotic Cell Clearance.* Cells, 2021. **10**(9): p. 2288.
4. Blouin, K., et al., *Pathways of adipose tissue androgen metabolism in women: depot differences and modulation by adipogenesis.* American Journal of Physiology-Endocrinology and Metabolism, 2009. **296**(2): p. E244-E255.
5. Galic, S., J.S. Oakhill, and G.R. Steinberg, *Adipose tissue as an endocrine organ.* Molecular and Cellular Endocrinology, 2010. **316**(2): p. 129-139.
6. Zhang, L.J., et al., *Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection.* Science, 2015. **347**(6217): p. 67-71.
7. Das, M., et al., *Effect of vitamin D supplementation on cathelicidin, IFN-γ, IL-4 and Th1/Th2 transcription factors in young healthy females.* European Journal of Clinical Nutrition, 2014. **68**(3): p. 338-343.
8. Nagareddy, P.R., et al., *Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity.* Cell Metab, 2014. **19**(5): p. 821-35.
9. Ottaviani, E., D. Malagoli, and C. Franceschi, *The evolution of the adipose tissue: a neglected enigma.* Gen Comp Endocrinol, 2011. **174**(1): p. 1-4.
10. Zihlman, A.L. and D.R. Bolter, *Body composition in Pan paniscus compared with Homo sapiens has implications for changes during human evolution.* Proceedings of the National Academy of Sciences, 2015. **112**(24): p. 7466-7471.
11. Hruby, A. and F.B. Hu, *The Epidemiology of Obesity: A Big Picture.* Pharmacoeconomics, 2015. **33**(7): p. 673-89.
12. WHO, *Global report on diabetes.* 2016: WHO Press, Switzerland.
13. Chobot, A., et al., *Obesity and diabetes—Not only a simple link between two epidemics.* Diabetes/Metabolism Research and Reviews, 2018: p. e3042.
14. Nishtar, S., P. Gluckman, and T. Armstrong, *Ending childhood obesity: a time for action.* Lancet, 2016. **387**(10021): p. 825-7.
15. Geserick, M., et al., *Acceleration of BMI in Early Childhood and Risk of Sustained Obesity.* The New England Journal of Medicine, 2018. **379**(14): p. 1303-1312.
16. Landgraf, K., et al., *Evidence of early alterations in adipose tissue biology and function and its association with obesity-related inflammation and insulin resistance in children.* Diabetes, 2015. **64**(4): p. 1249-1261.
17. Kelsey, M.M., et al., *Age-related consequences of childhood obesity.* Gerontology, 2014. **60**(3): p. 222-228.
18. Hidayat, K., X. Du, and B.-M. Shi, *Body fatness at a young age and risks of eight types of cancer: systematic review and meta-analysis of observational studies.* Obesity Reviews, 2018.
19. Hull, D., *The structure and function of brown adipose tissue.* Br Med Bull, 1966. **22**(1): p. 92-6.
20. Junqueira, L.C., *Adipose Tissue, in: Basic Histology 5th Edition*. 1986, Los Altos, California, USA: Lange Medical Publications 1986.
21. Röszer, T., *The invertebrate midintestinal gland ("hepatopancreas") is an evolutionary forerunner in the integration of immunity and metabolism.* Cell Tissue Res, 2014. **358**(3): p. 685-95.
22. Röszer, T. and E.D. Kiss-Toth, *FMRF-amide is a glucose-lowering hormone in the snail Helix aspersa.* Cell Tissue Res, 2014. **358**(2): p. 371-83.
23. Arrese, E.L. and J.L. Soulages, *Insect fat body: energy, metabolism, and regulation.* Annu Rev Entomol, 2010. **55**: p. 207-25.
24. Braus, H., *Anatomie des Menschen: ein Lehrbuch für Studierende und Ärzte (Band 1): Bewegungsapparat*. Vol. 3. 1921: Springer, Berlin , Heidelberg.
25. Colleluori, G., et al., *Mammary gland adipocytes in lactation cycle, obesity and breast cancer.* Reviews in Endocrine and Metabolic Disorders, 2021. **22**(2): p. 241-255.
26. Vernon, R.G. and C.M. Pond, *Adaptations of Maternal Adipose Tissue to Lactation.* Journal of Mammary Gland Biology and Neoplasia, 1997. **2**(3): p. 231-241.
27. Bligh, J., *Hibernation*, in *Temperature regulation in mammals and other vertebrates / [by] John Bligh* J. Bigh, Editor. 1973, North-Holland ; American Elsevier: Amsterdam, : New York.
28. Cinti, S., *Adipose Organ Development and Remodeling.* Compr Physiol, 2018. **8**(4): p. 1357-1431.

29. Harms, M. and P. Seale, *Brown and beige fat: development, function and therapeutic potential.* Nat Med, 2013. **19**(10): p. 1252-63.
30. Kozak, L.P., *The genetics of brown adipocyte induction in white fat depots.* Front Endocrinol (Lausanne), 2011. **2**: p. 64.
31. Bligh, J., *Engineering models of mammalian thermoregulation*, in *Temperature regulation in mammals and other vertebrates / [by] John Bligh* J. Bigh, Editor. 1973, North-Holland ; American Elsevier: Amsterdam, : New York.
32. Finlin, B.S., et al., *Mast Cells Promote Seasonal White Adipose Beiging in Humans.* Diabetes, 2017. **66**(5): p. 1237-1246.
33. Gaudry, M.J., et al., *Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades.* Science Advances, 2017. **3**(7): p. e1602878.
34. Wright, T., et al., *Skeletal muscle thermogenesis enables aquatic life in the smallest marine mammal.* Science, 2021. **373**(6551): p. 223-225.
35. Young, T.K., *Obesity, Central Fat Patterning, and Their Metabolic Correlates among the Inuit of the Central Canadian Arctic.* Human Biology, 1996. **68**(2): p. 245-263.
36. Stothers, J.K., *Head insulation and heat loss in the newborn.* Archives of disease in childhood, 1981. **56**(7): p. 530-534.
37. Kazak, L., et al., *A Creatine-Driven Substrate Cycle Enhances Energy Expenditure and Thermogenesis in Beige Fat.* Cell, 2015. **163**(3): p. 643-655.
38. Sanchez-Gurmaches, J. and D.A. Guertin, *Adipocyte lineages: tracing back the origins of fat.* Biochimica et biophysica acta, 2014. **1842**(3): p. 340-351.
39. Billon, N., et al., *The generation of adipocytes by the neural crest.* Development, 2007. **134**(12): p. 2283-92.
40. Fu, M., et al., *Neural Crest Cells Differentiate Into Brown Adipocytes and Contribute to Periaortic Arch Adipose Tissue Formation.* Arteriosclerosis, Thrombosis, and Vascular Biology, 2019. **39**(8): p. 1629-1644.
41. Lidell, M.E., et al., *Evidence for two types of brown adipose tissue in humans.* Nature Medicine, 2013. **19**: p. 631.
42. Hoang, A.C., H. Yu, and T. Röszer, *Transcriptional Landscaping Identifies a Beige Adipocyte Depot in the Newborn Mouse.* Cells, 2021. **10**(9): p. 2368.
43. Yu, H., et al., *Breast milk alkylglycerols sustain beige adipocytes through adipose tissue macrophages.* The Journal of Clinical Investigation, 2019. **129**(6): p. 2485-2499.
44. Zhang, F., et al., *An Adipose Tissue Atlas: An Image-Guided Identification of Human-like BAT and Beige Depots in Rodents.* Cell Metab, 2018. **27**(1): p. 252-262.e3.
45. Carobbio, S., B. Rosen, and A. Vidal-Puig, *Adipogenesis: new insights into brown adipose tissue differentiation.* Journal of Molecular Endocrinology, 2013. **51**(3): p. T75-T85.
46. Wu, J., et al., *Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human.* Cell, 2012. **150**(2): p. 366-76.
47. Cannon, B., et al., *Human brown adipose tissue: Classical brown rather than brite/beige?* Experimental Physiology, 2020. **105**(8): p. 1191-1200.
48. Sharp, L.Z., et al., *Human BAT possesses molecular signatures that resemble beige/brite cells.* PLoS One, 2012. **7**(11): p. e49452.
49. Clara, M., *Entwicklungsgeschichte des Menschen*. 1955, Leipzig: Georg Thieme Verlag.
50. Leitner, B.P., et al., *Mapping of human brown adipose tissue in lean and obese young men.* Proceedings of the National Academy of Sciences, 2017. **114**(32): p. 8649-8654.
51. Hu, H.H., et al., *MRI detection of brown adipose tissue with low fat content in newborns with hypothermia.* Magn Reson Imaging, 2014. **32**(2): p. 107-17.
52. Hahn, P. and M. Novak, *Development of brown and white adipose tissue.* Journal of Lipid Research, 1975. **16**(2): p. 79-91.
53. Novak, M. and E. Monkus, *Metabolism of Subcutaneous Adipose Tissue in the Immediate Postnatal Period of Human Newborns. 1. Developmental Changes in Lipolysis and Glycogen Content.* Pediatric Research, 1972. **6**(2): p. 73-80.
54. Novak, M., D. Penn, and E. Monkus, *Regulation of lipolysis in human neonatal adipose tissue. Effects of alteration in carbohydrate metabolism.* Biol Neonate, 1973. **22**(5): p. 451-67.
55. Novak, M., E. Monkus, and V. Pardo, *Human neonatal subcutaneous adipose tissue. Function and ultrastructure.* Biol Neonate, 1971. **19**(4): p. 306-21.
56. Rockstroh, D., et al., *Direct evidence of brown adipocytes in different fat depots in children.* PLOS ONE, 2015. **10**(2): p. e0117841.
57. Gilsanz, V., H.H. Hu, and S. Kajimura, *Relevance of brown adipose tissue in infancy and adolescence.* Pediatric Research, 2013. **73**(1): p. 3-9.

58. Symonds, M.E., et al., *The placenta, maternal diet and adipose tissue development in the newborn.* Annals of Nutrition and Metabolism, 2017. **70**(3): p. 232-235.
59. Lim, J., et al., *Depot-specific UCP1 expression in human white adipose tissue and its association with obesity-related markers.* International Journal of Obesity, 2020. **44**(3): p. 697-706.
60. Whyte, R.K. and H.S. Bayley, *Energy Metabolism of the Newborn Infant*, in *Advances in Nutritional Research*, H.H. Draper, Editor. 1990, Springer US: Boston, MA. p. 79-108.
61. Stave, U., *Perinatal physiology*. 1970, New York, London: Plenum Medical Company.
62. Bartelt, A. and J. Heeren, *Adipose tissue browning and metabolic health.* Nature Reviews Endocrinology, 2014. **10**(1): p. 24-36.
63. Hilton, C., F. Karpe, and K.E. Pinnick, *Role of developmental transcription factors in white, brown and beige adipose tissues.* Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 2015. **1851**(5): p. 686-696.
64. von Liebig, J., *Origin of fat in domesticated anmals*, in *Animal Chemistry: or Organic Chemistry in Its Application to Physiology and Pathology. Justus Liebig. Edited from the author's manuscript by William Gregory with additions, notes, and corrections by Gregory and John W. Webster*. 1842, John Owen: Cambridge.
65. Hoffmann-Ostenhof, O., *Intermediary metabolism* 1987, New York: Van Nostrand Reinhold Co.
66. De Robertis, E.D.P., W.W. Nowinski, and A.F. Saez, *Morphlogy of the mitochondria*, in *Cell Bilogy*. 1965, W. B. Saunders; 4th edition: London.
67. Kazak, L., et al., *Ablation of adipocyte creatine transport impairs thermogenesis and causes diet-induced obesity.* Nature Metabolism, 2019. **1**(3): p. 360-370.
68. Dawkins, M.J.R. and J.W. Scopes, *Non-shivering Thermogenesis and Brown Adipose Tissue in the Human New-born Infant.* Nature, 1965. **206**(4980): p. 201-202.
69. Aherne, W. and D. Hull, *Brown adipose tissue and heat production in the newborn infant.* J Pathol Bacteriol, 1966. **91**(1): p. 223-34.
70. Crelin, E.S., *Anatomy of the newborn: an atlas*. 1969, Philadelphia: Lea & Febiger.
71. Tostevin, P. and H. Ellis, *The buccal pad of fat: A review.* Clinical anatomy (New York, N.Y.), 1995. **8**: p. 403-6.
72. Sinclair, J.C., *Temperature Regulation And Energy Metabolism In The Newborn (Monographs in Neonatalogy)*. 1978: Grune & Stratton, Florence, Kentucky, U.S.A.
73. Babaei, R., et al., *Jak-TGFβ cross-talk links transient adipose tissue inflammation to beige adipogenesis.* Science Signaling, 2018. **11**(527).
74. Sun, K., Z. Gao, and M.G. Kolonin, *Transient inflammatory signaling promotes beige adipogenesis.* Science Signaling, 2018. **11**(527): p. eaat3192.
75. Hoang, A.C., et al., *Mitochondrial RNA stimulates beige adipocyte development in young mice.* Nature Metabolism, 2022.
76. Jing, X., et al., *COVID-19 instigates adipose browning and atrophy through VEGF in small mammals.* Nature Metabolism, 2022. **4**(12): p. 1674-1683.
77. Röszer, T., *M2 Macrophages in the Metabolic Organs and in the Neuroendocrine System*, in *The M2 Macrophage*, T. Röszer, Editor. 2020, Springer International Publishing. p. 171-187.
78. Röszer, T., *Metabolic impact of adipose tissue macrophages in the early postnatal life.* J Leukoc Biol, 2022. **112**(6): p. 1515-1524.
79. Waqas, S.F.H., et al., *Adipose tissue macrophages develop from bone marrow-independent progenitors in Xenopus laevis and mouse.* Journal of Leukocyte Biology, 2017. **102**(3): p. 845-855.
80. Röszer, T., *Editorial overview: Immunomodulation 2020 - nuclear receptors.* Current Opinion in Pharmacology, 2020. **53**.
81. Shoelson, S.E., J. Lee, and A.B. Goldfine, *Inflammation and insulin resistance.* J Clin Invest, 2006. **116**(7): p. 1793-801.
82. Tabula Muris, C., et al., *Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris.* Nature, 2018. **562**(7727): p. 367-372.
83. Epelman, S., K.J. Lavine, and G.J. Randolph, *Origin and functions of tissue macrophages.* Immunity, 2014. **41**(1): p. 21-35.
84. Osborn, O. and J.M. Olefsky, *The cellular and signaling networks linking the immune system and metabolism in disease.* Nat Med, 2012. **18**(3): p. 363-74.
85. Lee, B.C. and J. Lee, *Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance.* Biochim Biophys Acta, 2014. **1842**(3): p. 446-62.
86. Glass, C.K. and J.M. Olefsky, *Inflammation and lipid signaling in the etiology of insulin resistance.* Cell Metab, 2012. **15**(5): p. 635-45.
87. Qiu, Y., et al., *Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat.* Cell, 2014. **157**(6): p. 1292-308.

88. Li, P., et al., *NCoR repression of LXRs restricts macrophage biosynthesis of insulin-sensitizing omega 3 fatty acids.* Cell, 2013. **155**(1): p. 200-14.
89. Boutens, L. and R. Stienstra, *Adipose tissue macrophages: going off track during obesity.* Diabetologia, 2016. **59**(5): p. 879-94.
90. Lumeng, C.N., J.L. Bodzin, and A.R. Saltiel, *Obesity induces a phenotypic switch in adipose tissue macrophage polarization.* J Clin Invest, 2007. **117**(1): p. 175-84.
91. Lumeng, C.N., et al., *Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes.* Diabetes, 2008. **57**(12): p. 3239-46.
92. Weisberg, S.P., et al., *Obesity is associated with macrophage accumulation in adipose tissue.* Journal of Clinical Investigation, 2003. **112**(12): p. 1796-1808.
93. Xu, H., et al., *Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance.* The Journal of Clinical Investigation, 2003. **112**(12): p. 1821-1830.
94. Ampem, G., et al., *Adipose tissue macrophages in non-rodent mammals: a comparative study.* Cell Tissue Res, 2016. **363**(2): p. 461-78.
95. Haase, J., et al., *Local proliferation of macrophages in adipose tissue during obesity-induced inflammation.* Diabetologia, 2014. **57**(3): p. 562-71.
96. Amano, S.U., et al., *Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation.* Cell Metab, 2014. **19**(1): p. 162-71.
97. Röszer, T., *Mechanisms Which Control the Size of M2 Macrophage-Dominated Tissue Macrophage Niches*, in *The M2 Macrophage*, T. Röszer, Editor. 2020, Springer International Publishing. p. 99-111.
98. Waqas, S.F.H., et al., *Neuropeptide FF increases M2 activation and self-renewal of adipose tissue macrophages.* The Journal of Clinical Investigation 2017. **127**(7): p. 2842-2854.
99. Fuentes, L., T. Röszer, and M. Ricote, *Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages.* Mediators Inflamm, 2010. **2010**: p. 219583.
100. Sorisky, A., R. Magun, and A.M. Gagnon, *Adipose cell apoptosis: death in the energy depot.* Int J Obes Relat Metab Disord, 2000. **24 Suppl 4**: p. S3-7.
101. Hirsch, J., I. Faust, and P.R. Johnson. *What s New in Obesity: Current Understanding of Adipose Tissue Morphology*. 1979.
102. Nisoli, E., et al., *White adipocytes are less prone to apoptotic stimuli than brown adipocytes in rodent.* Cell Death & Differentiation, 2006. **13**(12): p. 2154-2156.
103. Song, C.X., et al., *CTRP9 Enhances Efferocytosis in Macrophages via MAPK/Drp1-Mediated Mitochondrial Fission and AdipoR1-Induced Immunometabolism.* J Inflamm Res, 2021. **14**: p. 1007-1017.
104. Kuroda, M. and H. Sakaue, *Adipocyte Death and Chronic Inflammation in Obesity.* J Med Invest, 2017. **64**(3.4): p. 193-196.
105. McLaughlin, T., et al., *Adipose Cell Size and Regional Fat Deposition as Predictors of Metabolic Response to Overfeeding in Insulin-Resistant and Insulin-Sensitive Humans.* Diabetes, 2016. **65**(5): p. 1245-54.
106. Björntorp, P. and L. Sjöström, *The Composition and Metabolism in Vitro of Adipose Tissue Fat Cells of Different Sizes.* European Journal of Clinical Investigation, 1972. **2**(2): p. 78-84.
107. Boulton, T.J., M. Dunlop, and J.M. Court, *Adipocyte growth in the first 2 years of life.* Aust Paediatr J, 1974. **10**(5): p. 301-5.
108. Dauncey, M.J. and D. Gairdner, *Size of adipose cells in infancy.* Archives of disease in childhood, 1975. **50**(4): p. 286-290.
109. Vogel, P., et al., *Pathology of congenital generalized lipodystrophy in Agpat2-/- mice.* Vet Pathol, 2011. **48**(3): p. 642-54.
110. Birk, R.Z. and M. Rubinstein, *IFN-alpha induces apoptosis of adipose tissue cells.* Biochem Biophys Res Commun, 2006. **345**(2): p. 669-74.
111. Domingo, P., et al., *Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy.* AIDS, 1999. **13**(16).
112. Alcalá, M., et al., *Increased inflammation, oxidative stress and mitochondrial respiration in brown adipose tissue from obese mice.* Scientific Reports, 2017. **7**(1): p. 16082.
113. Zhang, H.H., et al., *Dexamethasone inhibits tumor necrosis factor-alpha-induced apoptosis and interleukin-1 beta release in human subcutaneous adipocytes and preadipocytes.* J Clin Endocrinol Metab, 2001. **86**(6): p. 2817-25.
114. Tchkonia, T., et al., *Abundance of two human preadipocyte subtypes with distinct capacities for replication, adipogenesis, and apoptosis varies among fat depots.* Am J Physiol Endocrinol Metab, 2005. **288**(1): p. E267-77.
115. Prieur, X., T. Röszer, and M. Ricote, *Lipotoxicity in macrophages: evidence from diseases associated with the metabolic syndrome.* Biochim Biophys Acta, 2010. **1801**(3): p. 327-37.

116. Qian, S., et al., *BMPR2 promotes fatty acid oxidation and protects white adipocytes from cell death in mice.* Communications Biology, 2020. **3**(1): p. 200.
117. Osuga, J., et al., *Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity.* Proc Natl Acad Sci U S A, 2000. **97**(2): p. 787-92.
118. Guo, W., et al., *Aging results in paradoxical susceptibility of fat cell progenitors to lipotoxicity.* American Journal of Physiology-Endocrinology and Metabolism, 2007. **292**(4): p. E1041-E1051.
119. West, M., *Dead adipocytes and metabolic dysfunction: recent progress.* Curr Opin Endocrinol Diabetes Obes, 2009. **16**(2): p. 178-82.
120. Cinti, S., et al., *Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans.* Journal of Lipid Research, 2005. **46**(11): p. 2347-2355.
121. Braune, J., et al., *Multinucleated Giant Cells in Adipose Tissue Are Specialized in Adipocyte Degradation.* Diabetes, 2021. **70**(2): p. 538-548.
122. Strissel, K.J., et al., *Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications.* Diabetes, 2007. **56**(12): p. 2910-2918.
123. Lindhorst, A., et al., *Adipocyte death triggers a pro-inflammatory response and induces metabolic activation of resident macrophages.* Cell Death & Disease, 2021. **12**(6): p. 579.
124. Dai, L., et al., *Macrophage alternative activation confers protection against lipotoxicity-induced cell death.* Molecular Metabolism, 2017. **6**(10): p. 1186-1197.
125. Ravichandran, K.S., *Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums.* J Exp Med, 2010. **207**(9): p. 1807-17.
126. Benoit, M.E., et al., *Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells.* Journal of immunology (Baltimore, Md. : 1950), 2012. **188**(11): p. 5682-5693.
127. Fischer-Posovszky, P., et al., *Targeted deletion of adipocytes by apoptosis leads to adipose tissue recruitment of alternatively activated M2 macrophages.* Endocrinology, 2011. **152**(8): p. 3074-3081.
128. Shaul, M.E., et al., *Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet--induced obesity in mice.* Diabetes, 2010. **59**(5): p. 1171-81.
129. Luo, B., et al., *The deficiency of macrophage erythropoietin signaling contributes to delayed acute inflammation resolution in diet-induced obese mice.* Biochim Biophys Acta Mol Basis Dis, 2019. **1865**(2): p. 339-349.
130. Wang, H. and J. Ye, *Regulation of energy balance by inflammation: common theme in physiology and pathology.* Reviews in endocrine & metabolic disorders, 2015. **16**(1): p. 47-54.
131. Wieser, V., et al., *Adipose type I interferon signalling protects against metabolic dysfunction.* Gut, 2018. **67**(1): p. 157-165.
132. Derecka, M., et al., *Tyk2 and Stat3 regulate brown adipose tissue differentiation and obesity.* Cell metabolism, 2012. **16**(6): p. 814-824.
133. Kristóf, E., et al., *Interleukin-6 released from differentiating human beige adipocytes improves browning.* Exp Cell Res, 2019. **377**(1-2): p. 47-55.
134. Alsaggar, M., M. Mills, and D. Liu, *Interferon beta overexpression attenuates adipose tissue inflammation and high-fat diet-induced obesity and maintains glucose homeostasis.* Gene therapy, 2017. **24**(1): p. 60-66.
135. Asterholm, I.W., et al., *Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling.* Cell Metabolism, 2014. **20**(1): p. 103-118.
136. Peterson, K.R., D.K. Flaherty, and A.H. Hasty, *Obesity Alters B Cell and Macrophage Populations in Brown Adipose Tissue.* Obesity, 2017. **25**(11): p. 1881-1884.
137. Han, Claudia Z. and Kodi S. Ravichandran, *Metabolic Connections during Apoptotic Cell Engulfment.* Cell, 2011. **147**(7): p. 1442-1445.
138. Yurdagul., A., *Metabolic Consequences of Efferocytosis and Its Impact on Atherosclerosis.* Immunometabolism, 2021. **3**(2): p. e210017.
139. Hoang, A.C., et al., *Mitochondrial RNA stimulates beige adipocyte development in young mice.* Nature Metabolism, 2022. **4**(12): p. 1684-1696.
140. Zhang, C. and P. Liu, *The lipid droplet: A conserved cellular organelle.* Protein & cell, 2017. **8**(11): p. 796-800.
141. Phinney, D.G., et al., *Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs.* 2015. **6**: p. 8472.
142. Abels, E.R. and X.O. Breakefield, *Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake.* Cellular and molecular neurobiology, 2016. **36**(3): p. 301-312.
143. Lu, Y., et al., *Mitophagy is required for brown adipose tissue mitochondrial homeostasis during cold challenge.* Scientific Reports, 2018. **8**(1): p. 8251.

144. Gkirtzimanaki, K., et al., *IFNα Impairs Autophagic Degradation of mtDNA Promoting Autoreactivity of SLE Monocytes in a STING-Dependent Fashion.* Cell reports, 2018. **25**(4): p. 921-933.e5.
145. Youle, R.J., *Mitochondria—Striking a balance between host and endosymbiont.* Science, 2019. **365**(6454): p. eaaw9855.
146. Nakahira, K., et al., *Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome.* Nature immunology, 2011. **12**(3): p. 222-230.
147. Vandanmagsar, B., et al., *The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance.* Nat Med, 2011. **17**(2): p. 179-88.
148. Giordano, A., et al., *Obese adipocytes show ultrastructural features of stressed cells and die of pyroptosis.* J Lipid Res, 2013. **54**(9): p. 2423-36.
149. Kim, S.-H., et al., *MD001, a Novel Peroxisome Proliferator-activated Receptor α/γ Agonist, Improves Glucose and Lipid Metabolism.* Scientific Reports, 2019. **9**(1): p. 1656.
150. Si, Z., et al., *Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies.* Biomedicine & Pharmacotherapy, 2019. **114**: p. 108765.
151. Ruiz-Ojeda, F.J., et al., *Extracellular Matrix Remodeling of Adipose Tissue in Obesity and Metabolic Diseases.* Int J Mol Sci, 2019. **20**(19).
152. Spencer, M., et al., *Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation.* American Journal of Physiology - Endocrinology and Metabolism, 2010. **299**(6): p. E1016-E1027.
153. Feldmann, H.M., et al., *UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality.* Cell Metab, 2009. **9**(2): p. 203-9.
154. Pedeutour, F. and A. Italiano, *Adipose Tumors*, in *Encyclopedia of Cancer*, M. Schwab, Editor. 2011, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 71-75.
155. Potter, E.L., *Pathology of the Fetus and the Newborn*. 1 ed. 1953, Chicago: The Year Book Publishers Inc.
156. Forcucci, J.A., et al., *"Low-Fat" Pseudoangiomatous Spindle Cell Lipoma: A Rare Variant With Loss of 13q14 Region.* Am J Dermatopathol, 2015. **37**(12): p. 920-3.