THE ULTRASTRUCTURE OF THE WHITE SUBCUTANEOUS ADIPOSE TISSUE

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In order to know the ultrastructural particularities of the adipose tissues, especially when very accurate in situ location of some molecules of interest (mRNA, proteins) must be identified in their associations with specific infrastructures during adipogenesis correlated with the fat type body location, the electron microscopic investigation is the best way to get it. We analysed the subcutaneous adipose tissue at the high resolution. Maturing lipoblasts exhibited a large lipid inclusion and additional few smaller fat inclusion in way to fuse to create an huge lipid droplet pushing the nucleus and the main cytoplasmic organites (mitochondria, endoplasmic reticulum etc.) to the cell periphery (signet-ring appearance) characteristic to the adipocytes. Often, blood capillaries are located in close vicinity of adipocytes. Traffiking infrastructures (coated pits, coated vesicles) and a continuous basal lamina at the periphery of adipocytes can be detected.

Key words: white subcutaneous adipose tissue; lipoblast; adipocyte; ultrastructure.

INTRODUCTION

There are two classes or types of fat-storing tissue: (1) white fat or unilocular adipose tissue and (2) brown fat or multilocular adipose tissue. Brown tissue is developed and well represented in the fetus and is metabolized to ensure heat during neonatal time period. Also in very limited amount, multilocular (brown fat tissue) can still persist during adult life, it is gradually lost during childhood.

Conversely, unilocular adipose tissue (white fat) is less represented during childhood, but may increase very much during adult life. Unilocular and multilocular storing fat cells are developed from embryonic mesenchyme. Mesenchymal cell precursors appear as spindle cells termed lipoblasts[1,2].

Electron microscopic investigations offer the possibility to get pertinent data about the ultrastructural particularities of the adipose tissues concerning the dynamics of infrastructures during adipogenesis, correlated with the fat type body location etc. When high resolution needs, the TEM is the elected tool. There is no replacement for the electron microscope when very accurate location of some molecules of interest (mRNA, proteins) must be identified in their associations with specific infrastructures[3]. Here we analysed the white subcutaneous adipose tissue at the ultrastructural level.

MATERIAL AND METHODS

Small fragments of mouse skin were surgically excised and prefixed in 4% Glutaraldehyde in sodium cacodylate buffer on ice (+4^{o}C) and postfixed in 2% osmium tetroxide. After dehydration in an ethanol series, biological material was embedded in Glycid ether 100 (Epon 812 equivalent).

After polymerization, ultrathin sections were cut using a diamond knife and contrasted by Reynolds method for routine transmission electron microscopy (TEM)[4]. Ultrastructural investigations were performed using a transmission electron microscope operated at 80 kV.

RESULTS AND DISCUSSION

An overview through a small portion of the subcutaneous adipose tissue shows unilocular adipocytes which exhibit large lipid droplets. The huge lipid inclusion pushed the rest of cytoplasm, including the mitochondrion, at the periphery of the cell (Fig. 1). Nuclei of adipocytes are eccentric located (*signet-ring* appearance).

They contain prevalently euchromatin, and are nucleolated.

Cytoplasm is rich in mitochondria and smooth endoplasmic reticulum is visible. Moreover, traffiking membranous infrastructures (coated pits and coated vesicles are detectable). A continuous basement membrane can be seen at the periphery of the adipocyte (Fig. 2, detalied in Fig. 3).

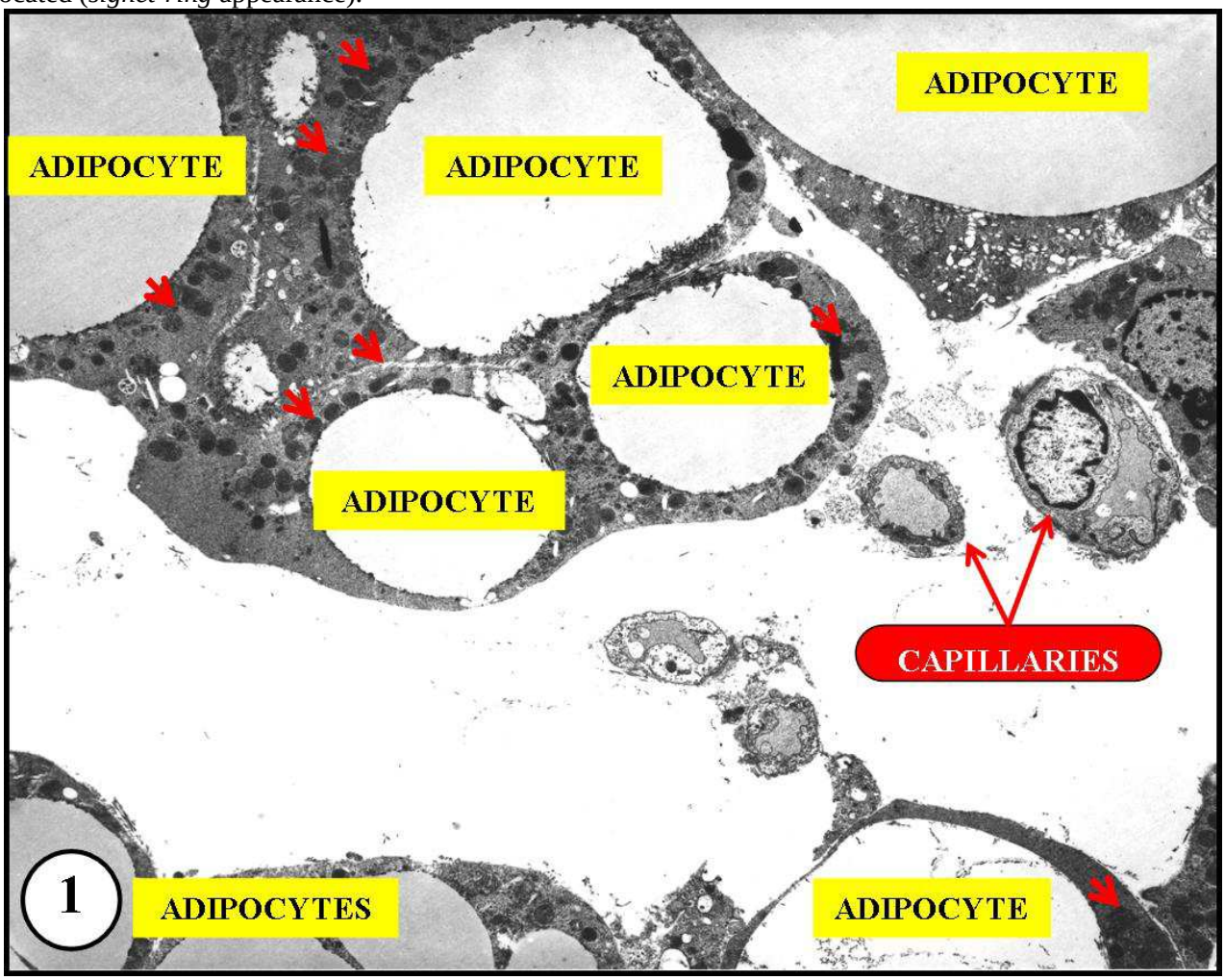

Fig. 1. Subcutaneous adipose tissue. Unilocular adipocytes exhibit large lipid droplets. A lot of mitochondria (red head arrows) can be seen inside of cytoplasm pushed at the periphery by the huge lipid inclusion.

Extracellular matrix is represented by a loose fibrous stroma of collagen and reticular fibres (Fig. 4). Sometimes, short profiles of rough endoplasmic reticulum can be detected (Fig. 5). Each maturing lipoblast exhibits few lipid droplets able to fuse each other to form unilocular adipocytes. In order to ensure a rapid and permanent traffik of lipids (accumulation and discharged), adipose tissue exhibit a lot of blood capillaries in close vicinity of adipose cells (Fig. 1 and Fig. 6). All above mentioned figures (with slight modifications) are reproduced from Mirancea and Mirancea (2010) with written permission of the publisher[5]. Adipogenesis – the process of preadipocyte differentiation into adipocytes – is initiated by commitment of multipotent mesenchymal cells to develop adipoblast lineage. Once committed to the adipogenic lineage, multipotent mesenchymal cells are not longer able to transform into osteoblasts, myocytes or chondrocytes.

This process is developed under the control of positive and negative regulators (hormonal and nutritional stimuli, etc.).

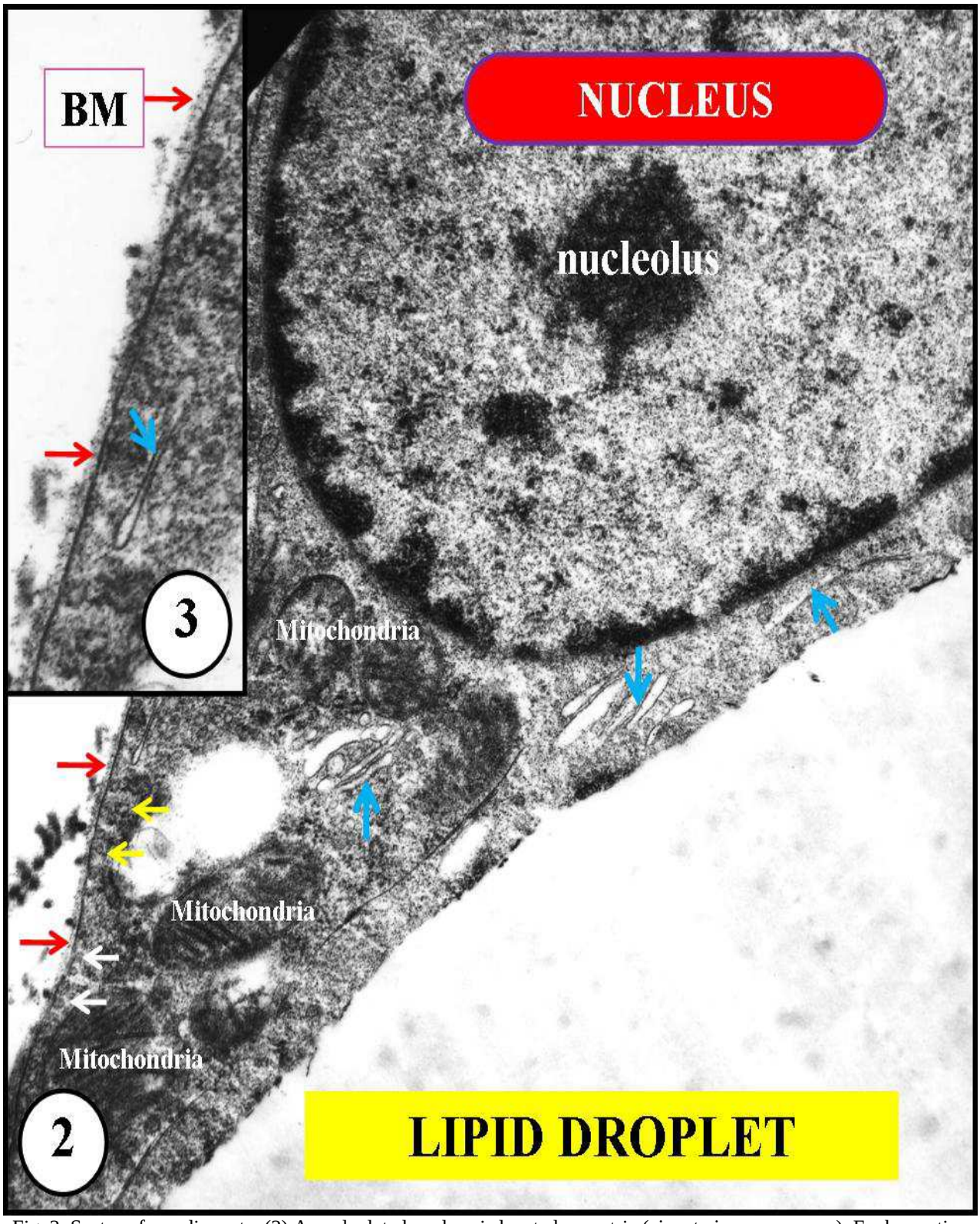

Fig. 2. Sector of an adipocyte. (2) A nucleolated nucleus is located eccentric (*signet-ring* appearance). Euchromatin is prevalent; heterochromatin is mostly attached to the inner membrane of the nuclear envelope. Cytoplasm is rich in mitochondria. Blue arrows mark smooth endoplasmic reticulum. A continuous basement membrane surrounds the adipocyte (red arrows). At the peripheral cytoplasm, coated pits (white arrows) as wel as coated vesicles (yellow arows) can be detected. (3) Detail for the basement membrane (BM). Blue arrow marks a short smooth endoplasmic reticulum profile.

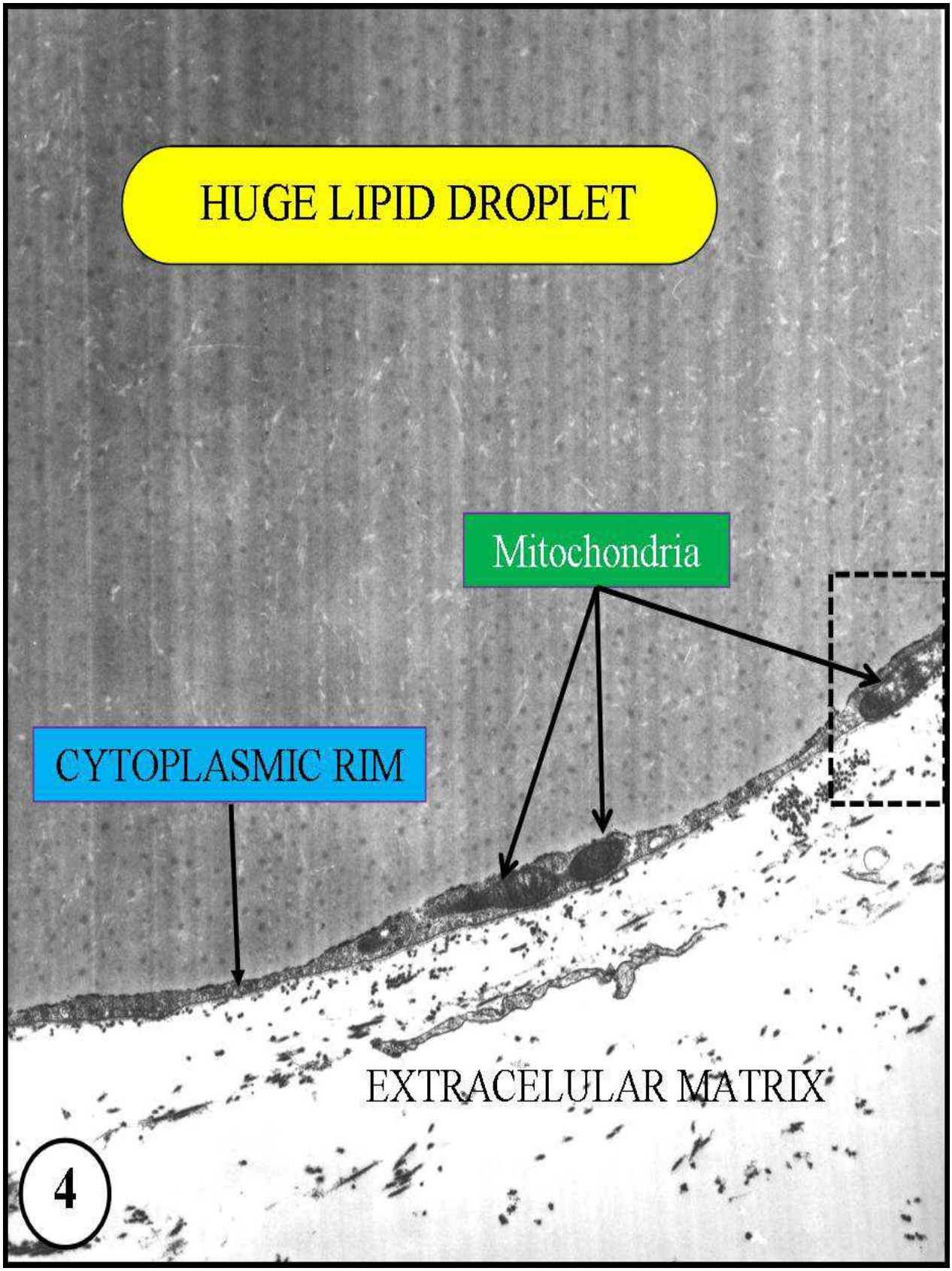

Fig. 4. A sector of an adipocyte. A huge lipid droplet pushes the cytoplasm which appears as a rim attached to the plasma membrane. Few mitochondria can be seen inside of the cytoplasm.

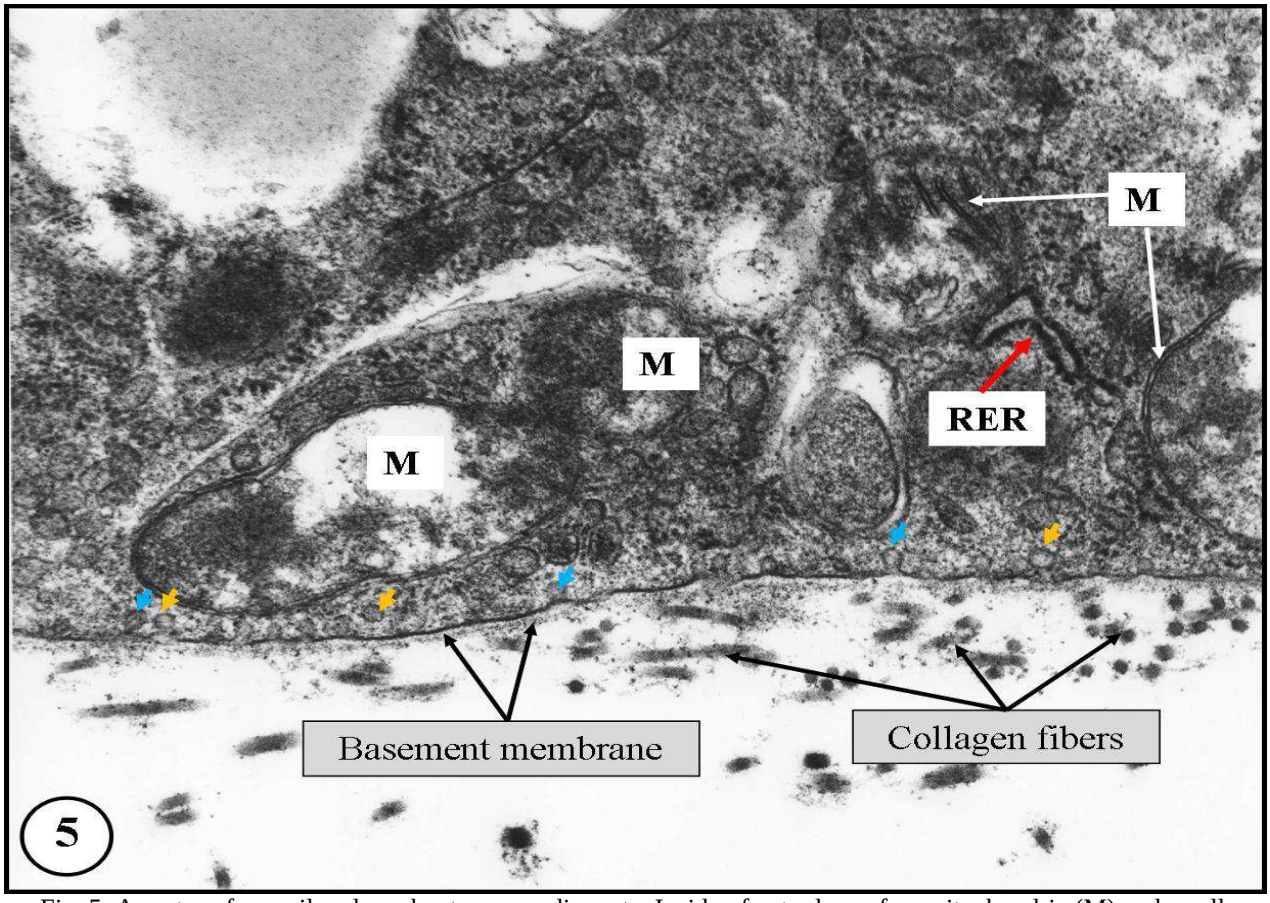

Fig. 5. A sector of an unilocular subcutaneous adipocyte. Inside of cytoplasm, few mitochondria (M) and small profile of the rough endoplasmic reticulum (RER) can be seen. At the periphery, coated pits (blue arrows) and coated vesicles (yellow arrows) are detectable. A continuous basement membrane follows the plasma membrane. Collagen fibres are longitudinally and cross sectioned.

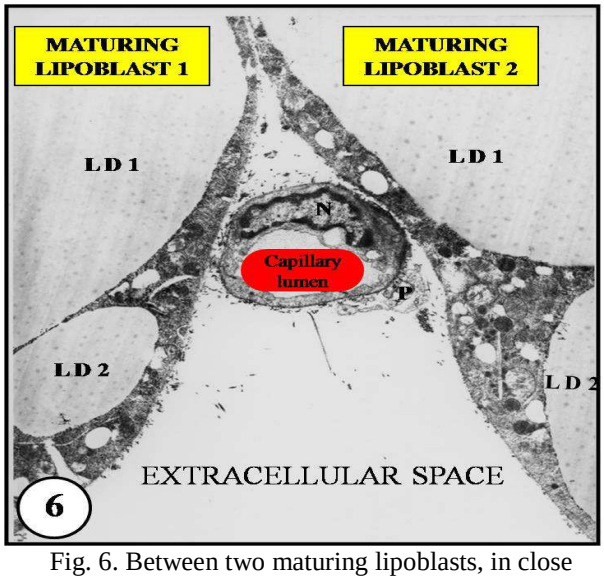

Fig. 6. Between two maturing lipoblasts, in close vicinity, there is a blood capillary. In each maturing lipoblast there is a large lipid droplet (L D 1) and another small lipid droplet (L D 2) in way to fuse to form an unilocular adipocyte. N = endothelial nucleus. P = a pericyte attached to the capillary.

Cross-talk between extracellular signals (as are secreted WNT signaling molecules) and mesenchymal stem cells plays an important role in adipogenesis. Perturbations in body energy balance by limitation of the adipose tissue amount or an excess of adipose tissue (obesity) lead to severe dysfunctions and disease[6,7]. In our days, because of wrong diet, rich in glucose and fat as well as of the adopted abnormal life style, a lot of people are affected by obesity. There are reports which emphasised the correlation between obesity and cancer initiation and progression.

There is a great interest to know more about adipose tissue, adipogenesis and fat metabolism maintaining the body homeostasia. Moreover, when a correlation between expression of specific molecules involved in different patho-physiologic processes and their infrastructural localization is required, the electron microscopic investigations are very useful. In this context, just to mention the immune electron microscopic contribution detecting at the high resolution of some molecules

of interest in different diseases, including cancer[8,9,10]. In response to different factors released by adipocytes, liver synthesizes the C-reactive protein (CRP). It seems to be a correlation between CRP levels, inflammation and cancer[11]. Another important molecules involved in fat metabolism also associated with adipocytes are represented by leptin (stress-related peptide) and its receptor (Ob-Receptor). Leptin m-RNA and protein are expressed by mature adipocytes but so far is still unclear the dynamics (temporal-vectorial expression) of these molecules and their infrastructural distribution.

We anticipate that investigations of the above mentioned molecules (CRP, Leptin, Leptin-receptors) by using transmission electron microscopy as well as In Situ hybridization at the ultrastructural level (ISH-US) correlated with Immuno Electron Microscopy (IEM) in white fat from different body regions will provide new insights concerning the importance of the TEM study applied to adipose tissue.

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