IJAE
Vol. 124, n. 2: 125-141, 2019
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Invited reviews – Plenary lecture at the LXXII National Congress of Italian Society of Anatomy and Histology, Parma, September 2018

# Anatomy of the nutritional system

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Abstract

White and brown adipocytes are contained in an anatomically dissectible structure called adipose organ. White adipocytes form white adipose tissue (WAT) brown adipocytes form brown adipose tissue (BAT). They have in common the fact that they manage lipids, but WAT uses them as an energy reserve to be given to the body between meals, while BAT burns them to produce heat. In some areas of the organ WAT and BAT are very distinct and easily recognizable by color, but in others the tissue is mixed. Numerous experimental data suggest that the concomitant presence of WAT and BAT in the adipose organ is due to the fact that they cooperate with each other thanks to their physiological and reversible transdifferentiation property. In the case of chronic cold exposure WAT converts to BAT to expand its thermogenic potential, while in the case of a chronic positive balance, BAT convert to WAT to expand the potential for energy storage. This new plastic property of physiological and reversible genetic remodeling is also present in the breast. In fact, our experimental data suggest that during pregnancy adipocytes transform into glandular epithelium that produces milk, while in the post-pregnancy period the glandular epithelium is transformed back into fat cells. The adipose organ collaborates with the digestive organs producing hormones that influence the most important of the instinctual behavioral activities: research and food intake, they also collaborate in the absorption and distribution of nutrients (both to the organism and to the offspring) and influence each other mutually for thermogenic activities that influence satiety. It can therefore be concluded that adipose organ and digestive system collaborate in a homeostatic system definable as a nutritional system.

Keywords

Adipose tissues, transdifferentiation, mammary gland, pink adipocytes, nutritional system.

## Adipocyte anatomy

The term adipocyte is commonly used to define a cell rich in cytoplasmic lipids under physiological conditions.

Traditionally two types of adipocytes are described in histology books: white and brown [1, 2]. The color is assigned based on the macroscopic aspect of the tissue that contains them. The anatomy of these cells reveals a substantial difference between white and brown. In fact, white is a spherical cell whose content is mainly formed by a single droplet of triglycerides. This droplet takes up about 90% of the cell volume. The remaining 10% forms a thin cytoplasmic rim that surrounds the lipid drop and contains the squeezed nucleus. Elongated mitochondria thin with short and vari-

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http://www.fupress.com/ijae
DOI: 10.13128/ijae-10774

ously oriented cristae without a specific morphology are observed in the cytoplasm. The other organelles are poorly represented. Numerous pinocytosis vesicles (or caveolae) are present on the cytoplasmic membrane. Externally, the latter is characterized by a typical basal membrane (or external lamina) mainly formed mainly by collagen IV. On the outer surface of the basement membrane are variously intertwined collagen fibrils that form a thin network, visible under a high-resolution scanning electron microscope (HRSEM)[3]. The size of the white adipose cell is very variable especially in relation to the location, the state of nutrition and the age of the subject [4].

The smaller mature adipocytes have a diameter of about 20-30 $\mu$m the larger ones can reach 80-100 $\mu$m in the mouse and about 30% more in humans [5].

Brown adipocytes are much smaller than white adipocytes (about 1/3-1/4) and have a polygonal shape with a rounded and often central core. In the cytoplasm many lipid vacuoles and many large mitochondria, mainly spherical, are observed. The size of the vacuoles and mitochondria is closely related to the functional state of the cell: in very active cells the vacuoles are small and the mitochondria are numerous and large and vice versa for cells that are not highly active. The cytoplasmic membrane is rich in caveolae and on the external side there is a distinct basement membrane [6].

## Anatomy of adipose tissues

White fat cells are organized to form white adipose tissue (WAT) (Fig. 1). WAT is well vascularized and provided with innervation. WAT nerves contain adrenergic and sensory fibers [7, 8].

Brown adipocytes are organized to form brown adipose tissue (BAT) (Fig. 1). BAT is about six times more vascularized than WAT and has a rich innervation [9]. The fibers most represented are noradrenergic, but there are also sensitive fibers. The noradrenergic fibers infiltrate BAT and come into direct contact with the brown fat cells [10].

## Physiology of adipocytes

The main function of white adipocyte is to secrete fatty acids in the intervals between meals [1, 5, 11]. This activity allows the normal functioning of the heart and has been fundamental for the survival of the human species when very long times (up to 4-6 weeks) were needed to get the next meal. The spherical shape guarantees maximum volume in the minimum space and the intrinsic energy of fatty acids is the maximum obtainable from the oxidation of the single molecules. This cell also has endocrine properties as it produces leptin, a hormone capable of influencing behavior by acting on the brain and in particular on the limbic system [12, 13]. Circulating leptin correlates positively with the amount of white fat cells in the body and therefore represents an important signal of the body's energy supply to our brain [14]. When leptinemia is low the brain is activated for a fundamental function for survival: the search for food. This is not enough to guarantee the body because the action of another hormone recently discovered and produced by white adipocytes is required:

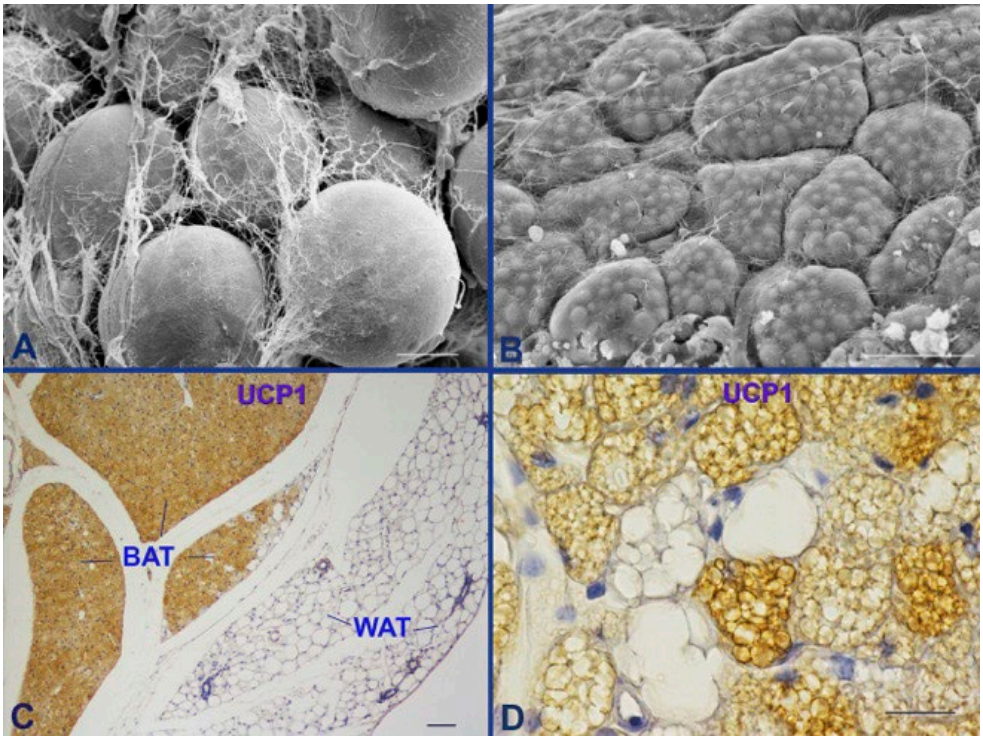


**Fig 1.** Morphology of murine white and brown adipose tissues. A: scanning electron microscopy of subcutaneous white adipose tissue. B: scanning electron microscopy of interscapular brown adipose tissue. C: immunohistochemistry with UCP1 antibodies showing positive brown (BAT) and negative white (WAT) adipose tissues. D: High magnification of UCP1-immunoreactive brown adipose tissue. Bar: 20 mm in A and B; 50 mm in C and D. Reproduced from [2] with permission.

asprosin [15]. The action of this last hormone on the brain is in fact necessary, and in particular on the arcuate nucleus of the hypothalamus to induce the individual to take food [16]. Subjects with mutations in the gene used to produce asprosin are lipodystrophic and therefore have low levels of leptin, but they are not hyperphagic like the majority of subjects with lipodystrophy, on the contrary they are inappetent and ingest very little food. Asprosin, in addition to directly stimulating the brain to take food, stimulates the production of glucose by the liver in order to make the fuel necessary for brain function available to the body. The main stimulus for the production of asprosin is fasting [15].

Subjects lacking functional leptin take large amounts of food from birth and the administration of recombinant leptin in these subjects returns the subject to complete normality. In addition to brain activity, leptin acts in many other organs including the gonads and the endocrine system [13, 17]. Finalistically, it can be hypothesized that the action on these organs prevents procreation to those who do not have energy supplies to guarantee the survival of newborns.

In addition to these two hormones, the white fat cell produces a series of other molecules that intervene in a series of homeostatic functions that mainly concern the glucose metabolism [18, 19]. In particular, adipsin stimulates the production of insulin and adiponectin promotes its peripheral action. A number of other endocrine, paracrine and autocrine factors are also produced by white adipocytes and recently reviewed elsewhere [1].

The main function of brown adipocyte is to produce heat. The stimulus able to induce thermogenesis is the exposure to temperatures below the thermoneutrality. The latter is variable among species: for the naked human and for the rat about 28°C, for the mouse about 34°C [9, 20-22]. Exposure to the "cold" activates the adrenergic system that causes the parenchymal fibers of the BAT to secrete noradrenaline in the neuro-adipose synaptic buttons. Norepinephrine activates specific ß3 receptors that activate protein kinase A (PKA) via cyclic AMP. This is followed by three fundamental events: 1-release of fatty acids, 2-synthesis of the uncoupling protein1 (UCP1), 3-mitochondriogenesis [11]. The release of fatty acids causes activation of the mitochondrial UCP1 and activation of their mitochondrial oxidation, the other two activities are in direct relation and consequent to the thermogenic request. The oxidized molecules in the respiratory chain induce the formation of a proton gradient between the two mitochondrial compartments separated by the inner membrane. Normally the gradient is exploited by the ATPase which uses the proton flow to form ATP. In the brown adipocyte the UCP1, which is a protonophore, defeats the gradient and all that remains of the energy intrinsic to the molecules of oxidized fatty acids is the heat produced by oxidation as an inevitable secondary effect. Since the number of oxidized molecules is very high and the mitochondria that burn them are numerous, large and rich in cristae, the heat produced is functionally relevant [23]. BAT is able to allow the survival of mammals in areas of the planet where the environmental temperature is often below the thermoneutrality. If we consider that the body temperature must remain at 37 ° C and that the temperature in the Earth, where humans live, varies from +50 to -70 we can easily understand the need to have above all efficient thermogenic systems.

## The Adipose Organ

Anatomical dissections have shown that most of the body's fatty tissues can be removed as a single structure from the body of small mammals (Fig. 2). The unitary structure, called Adipose Organ, has a specific shape that is maintained in different ages and sexes [4, 6, 24-27]. It is composed of parts arranged in the subcutaneous compartment and intra-trunk or visceral parts. In mice the subcutaneous part is particularly developed in correspondence of the roots of the limbs. The visceral part is arranged mainly around the aorta and its main collateral vessels. The continuity between the parts is achieved at the level of the upper opening of the thorax and in correspondence with the inferior narrow of the pelvis.

Macroscopically the color of the organ is brown in the parts containing BAT and white in the parts containing WAT. BAT prevails in the anterior subcutaneous depot at the interscapular, subscapular, deep cervical and axillary parts. In the visceral intra-trunk part BAT is prevalent in the periaortic areas. Throughout the rest of the organ, WAT prevails.

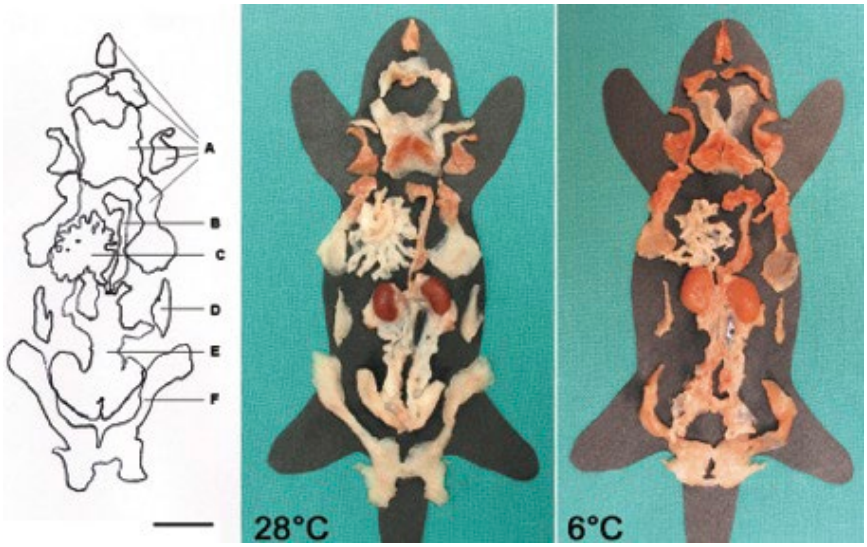


**Fig 2.** The adipose organ of adult Sv129 female mice kept at 28°C or 6°C for 10 days. The organ was dissected with the aid of a surgical microscope and each depot was placed on a mouse template indicating its original anatomical position. Kidneys and ovaries were dissected together with the depots. The organ is made up of two subcutaneous depots: A=anterior (deep cervical, superficial cervical, interscapular, subscapular, axillo-thoracic) and F= posterior (dorso-lumbar, inguinal, gluteal). Several visceral depots are also visible: B= mediastinal, C= mesenteric, D= retroperitoneal and E= abdomino-pelvic (perirenal, periovarian, parametrial, perivesical). Reproduced from [36] with permission. Bar: 18 mm.

The microscopic anatomy revealed that many white areas are actually mixed and morphometric studies have shown that in animals kept at 28°C (close to thermo-neutrality) in the strain Sv129 about 60% of the organ is formed by BAT while this percentage in the C57/BL6 drops to 20%.

Therefore, the adipose organ is mixed, i.e. it is formed by WAT and BAT which in some areas are clearly separated but which in some areas form a mixed tissue.

In humans, the study of digitized corpses has allowed us to reconstruct the human adipose organ that has many characteristics similar to those described above.

In particular there are parts located in the subcutaneous and visceral compartments with a histologic organization similar to the murine one. The predominant tissue in adult humans is WAT but areas of metabolically active BAT in the supraclavicular region of the neck have been demonstrated in adult humans. The technologies used for this demonstration were numerous including PET (Positron Emission Tomography) [28-31], MRI (Magnetic Resonance Imaging) [32-34], morphological, immunohistochemical and ultrastructural analysis [35]. In our study of about sixty patients, all patients under the age of 30 had immunoreactive BAT for UCP1, 20-30% of subjects aged 40-50 and rare subjects over 60 years were positive. Most positive subjects had low BMI (lean), but some overweight subjects were also positive. No obese subject was positive in line with data that showed a correlation between the presence of BAT and body weight [28].

## Adipose organ plasticity

The minimum requirement to define an anatomically dissectible structure as an organ is that it is formed by at least two different tissues that cooperate finalistically between them. For example, the stomach is undoubtedly an anatomically dissectible organ in which different tissues cooperate for digestion. To identify the cooperation between WAT and BAT we investigated the organ in different functional conditions and we saw how exposure to cold determines a browning of the adipose organ (Fig. 2). This chromatic variation corresponds from the quantitative point of view to an increase in BAT with a corresponding equivalent reduction in WAT in the absence of any variation in the total number of fat cells that make up the organ [36]. This result was repeated and obtained in two different murine strains [36, 37]. Histological analysis has excluded apoptotic phenomena in line with the fact that the adrenergic stimulus protects the adipose cell from apoptosis. These data therefore favored an interpretation in line with previous experiments that suggested that WAT, subjected to adrenergic stimuli, transdifferentiates in BAT [38-40]. That is, the mature white fat cells, subjected to adrenergic stimulation, would be able to transform directly into brown fat cells [8, 41-45]. Detailed studies of these phenomena have shown that it is possible to demonstrate the presence of all the intermediate stages not only morphological but also of gene and protein expression. On the other hand, the total absence of the adrenergic stimulus induces the inverse phenomenon: the BAT converts to WAT. The ideal technique to definitively demonstrate the transition from one cytotype to another is that of lineage tracing. This technique is based on the fact that it is possible to indelibly mark a cytotype and detect the reporter gene even after the phenotypic conversion. Christian Wolfrum's group in Zurich used this technique to confirm our hypothesis [46, 47].

These data are particularly interesting due to the fact that BAT is essential to prevent obesity [48], T2 diabetes [49-51] and atherosclerosis [52]. In fact, genetically modified mice that cannot activate BAT because they lack the receptors necessary for its activation, while moving like controls and eating the same amount of food as the controls, in a few weeks they become massively obese [48]. Moreover, mice lacking the insulin receptor exclusively in BAT become diabetic [49] and BAT activation is able to prevent atherosclerosis [52] and prolong life [53]. The benefits of BAT activation have also been recently documented for humans [54].

All these data show that one can actually speak of an adipose organ also from a functional point of view, since the cooperation between WAT and BAT seems to be clearly demonstrated by their reciprocal conversion capacity to face particular functional needs of the organ. In particular the WAT-BAT conversion would serve to increase the organ's thermogenic properties in the event of chronic exposure to cold. Conversely, BAT-WAT conversion would serve to increase energy storage capacity in the event of chronic exposure to a positive energy balance. This implies three important consequences: 1-The need to verify whether white and brown fat cells have a common ancestor, 2-The possibility of establishing new therapeutic strategies to combat obesity, diabetes and atherosclerosis and to increase life expectancy stimulating the brown component of the adipose organ 3-Accepting a new cellular property: physiological and reversible transdifferentiation. The latter in fact implies that a mature cell can, physiologically and reversibly, modify its gene program and therefore change its phenotype and, consequently, its function.

## The common ancestor of fat cells

In order to study the origin of adipose cells we observed the ultrastructure of two specific murine adipose depots during development: the epididymal for WAT and the interscapular for BAT. The choice of these two depots was dictated by the fact that in the adult animal the epididymal is exclusively made up of WAT whereas the interscapular by BAT therefore they represent the ideal depots to verify the origin of the white adipocyte and brown adipocyte respectively [1, 5].

The epididymal depot, before birth, appears as constituted by a mesenchymal tissue without specific characteristics. A few days after birth the tissue is organized to form specific, well-organized structures: the vasculo-adipocytic islands (Fig. 3). The name derives from the fact that these structures are well delimited by fibroblast-like elements that separate them from a loose connective matrix and contain numerous capillary vessels surrounded by fat cells in various stages of development. Intra insular tissue also contains numerous and dense collagen fibrils and to other cellular elements such as fibroblasts and mast cells. No adipocyte is found outside the islands, so they undoubtedly represent the niches of tissue where the progenitor cells give rise to the development of the preadipocytes destined then to form the adipocytes [55]. The vascular origin of the fat cells has been formulated for a long time and in particular the pericytes of the capillaries have been indicated by many researchers as a possible source for the preadipocytes [56, 57], but which was the cell that gave rise to the pericytes was not known.

The presence of a high number of capillaries in a tissue in the initial phase of differentiation, in itself underlines the possible instrumental value of these blood vessels, well represented not so much for the nutritional needs of the tissue but, probably, to guarantee the source of the precursors of the adipocytes. We therefore studied the ultrastructure of these capillaries for a long time, which proved to be fruitful because, in addition to the abundant pericytes, we also identified cells in a somewhat anomalous position that we have termed endothelium-pericytes. These rare elements (about 1/100 endothelial cells) were in fact positioned in the capillary wall in such a way as to place a part of the cell in an endothelial position and partly in a pericytic position. The endothelial position was demonstrated by contact with the lumen and by the typical tight flute beak junction with the contiguous endothelial cell. The pericytic position was demonstrated by the location of about half of the cell itself outside of that of an endothelial cell of the capillary (Fig. 4). Between it and the basement membrane of the capillary itself, that is to say in a classical pericytic position [55].

These ultrastructural data were suggestive of an endothelial origin of the pericyte, and given the unanimous consensus on the fact that the pericyte differentiate into adipocyte, it could be hypothesized that the endothelial cell represents the progenitor of the white adipose cell. To test this hypothesis, we used the lineage tracing technique. To mark the endothelial cells exclusively we used Ve-Cad-Cre/LoxP double transgenic mice already created and well characterized by the laboratory of Maria Luisa Iruela-Arispe of the Department of Molecular, Cell and Developmental Biology and Molecular Biology Institute, UCLA, Los Angeles, California [58]. These animals express the gene reporter (ß-Gal) only in endothelial cells and in any cytotypes derived from them. Our data confirmed that the gene reporter was expressed only in endothelial cells and showed that all fat cells in various stages of differentiation and

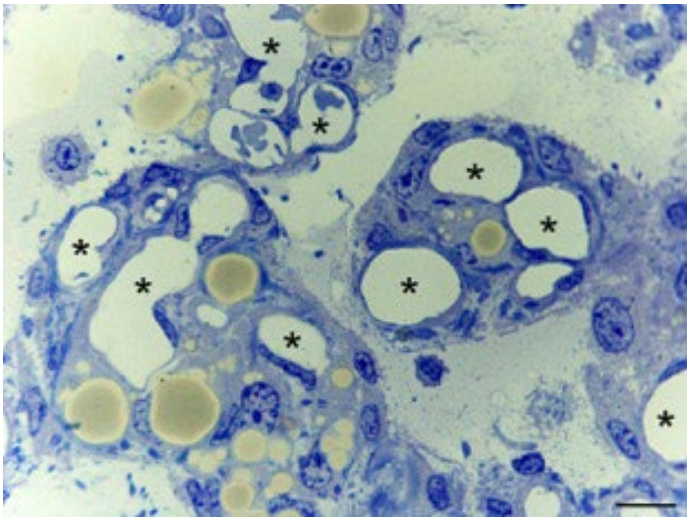

**Fig 3.** Vasculo-adipocytic islands in epididymal fat of newborn mouse. Structural units, delimited by fibroblast-like cells, functioning as anlage for white adipose tissue development. Note the numerous large capillaries that correspond to niches for adipose cell precursors development. Adipocytes at various stage of development are marked by cytoplasmic lipid droplets (yellow), note their anatomical localization only into the islands. Bar: 10 mm.

in various subcutaneous and visceral deposits were marked (Fig. 5). The X-Gal reaction that is used to highlight ß-Gal can also be exploited by electron microscopy [59]. In fact, the reaction product is not only visible in optical microscopy in the form of a cytoplasmic green-blue color but also as well-visible electron-dense crystals in electron microscopy. Electron microscopy confirmed the presence of reporter gene crystals in endothelial cells, fat cells and in all intermediate forms [55].

Interscapular BAT develops in a well-circumscribed area of the adipose organ. In the murine term fetus this area is characterized by the presence of brown preadipocytes. In Ve-Cad-Cre/LoxP mice this area was strongly marked by the reporter gene not only in endothelial cells but also in all brown preadipocytes. The differentiation level of brown preadipocytes allowed the specific identification of these cells as they are marked by the specific protein UCP1.

These data have therefore identified the endothelial cell of the adipose tissue capillaries as the sole progenitor for both white and brown cytotypes [55, 60]. This data allows us to understand even better the property that these cells have of converting themselves into each other.

## A new example of physiological and reversible transdifferentiation

The physiological and reversible transdifferentiation as a new biological property of mature cells deserved further study and in particular the search for a new example. This example was provided by the mammary gland in the adipose organ. The

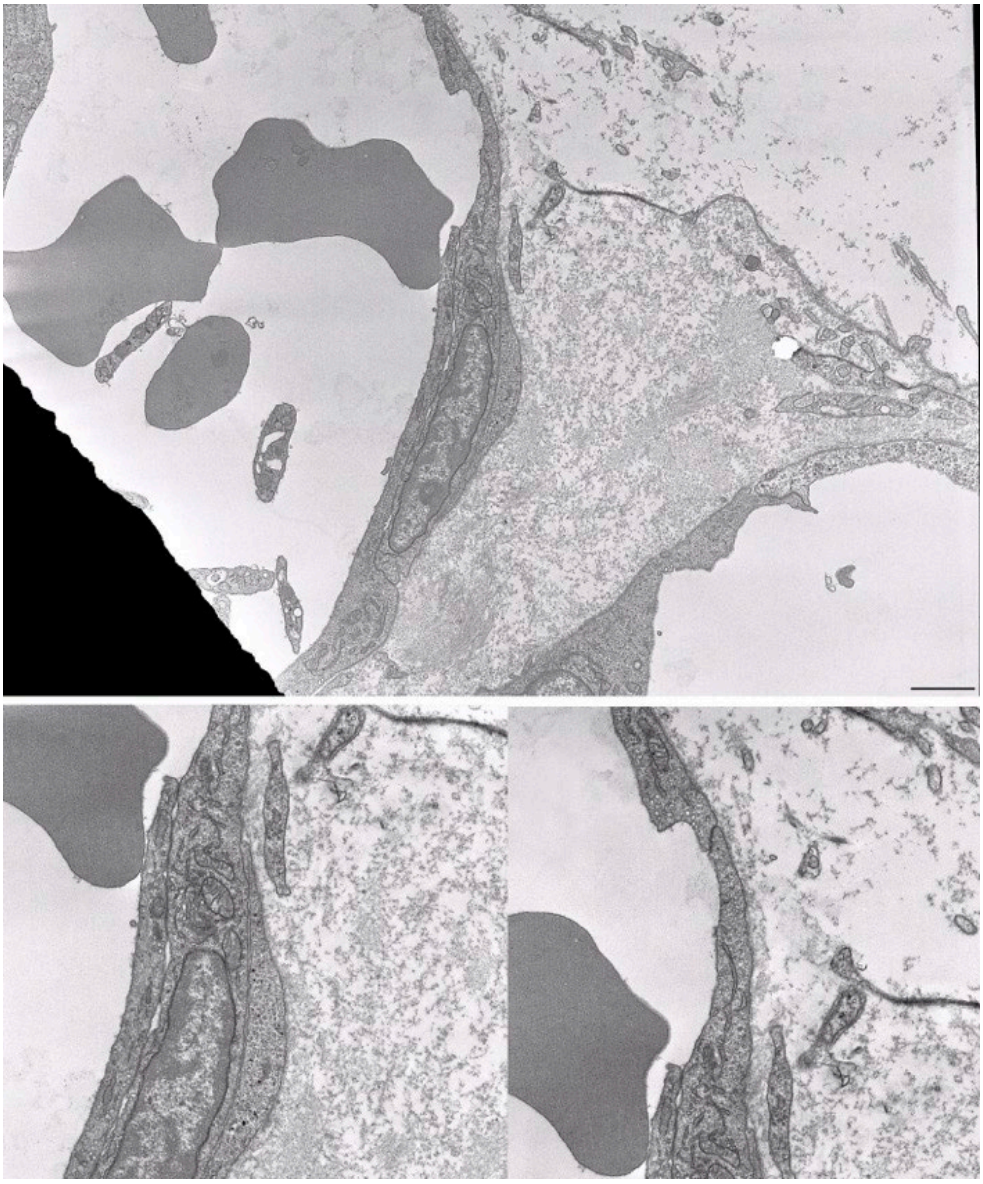

**Fig 4.** Transmission Electron Microscopy of a capillary shown in Fig 3. In the upper panel an endothelial cell is forming a cytoplasmic projection climbing on the adjacent endothelial cell. Note the presence of a cell in endothelial-pericytic position. The pericytic part is enlarged in the bottom left panel, the endothelial part is enlarged in the bottom right panel. Reproduced from [55] with permission. Bar: 1 mm in upper panel, 0.5 mm in bottom panels.

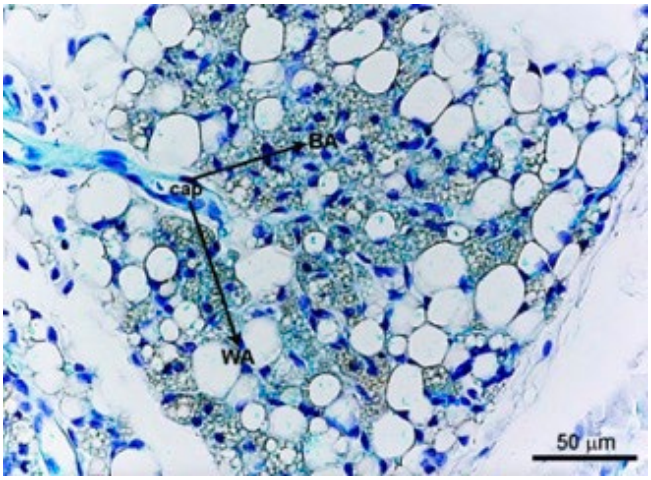


**Fig 5.** Subcutaneous fat from Ve-Cad-Cre/LoxP double transgenic mouse. X-Gal cytochemistry. In these mice, only endothelial cells and white (WA) and brown adipocytes BA) express the gene reporter (ß-Gal: green-blue). Reproduced from [60] with permission. Bar 50 mm.

mammary gland in the virgin is composed of adipose (predominantly white) subcutaneous tissue infiltrated by branched ducts that collect in a single nipple. The infiltration area identifies each individual gland and, in the mouse, the entire subcutaneous is infiltrated at puberty. In the mouse, the ducts refer to five symmetrical nipples, for which ten mammary glands are described. In women, only the thoracic subcutaneous is infiltrated and has two nipples.

During the pregnancy the alveoli appear, that is the adenomeres of the gland that produce the milk. They progressively occupy the organ volume while the adipose component in parallel disappears. At the height of breastfeeding, 90% of the organ is composed of dilated alveoli rich in milk and ducts, while fat cells are rarely visible (Fig. 6). At the end of breastfeeding, within a few days you return to the initial anatomy with disappearance of the alveoli and reappearance of the adipocytes [61].

A series of experiments carried out to evaluate these evident plasticity phenomena occurring in adult organisms allowed us to hypothesize that the basic phenomenon was an adipose-epithelial transdifferentiation in pregnancy and lactation and an epithelial-adipose transdifferentiation in post-lactation. We used aP2-Cre/LoxP mice to study the former and WAP-Cre / LoxP to study the latter. aP2 (adipocyte Protein 2) is a protein that acts as a transporter of fatty acids and is a specific gene for fat cells. Indeed, aP2-Cre/LoxP mice express the gene reporter only in adipose cells and in any cytotypes derived from them, while WAP-Cre/LoxP mice express the gene reporter only in milk-producing epithelial cells. In fact, Wey Acidic Protein (WAP) is a milk protein expressed only by breast alveolar cells during pregnancy and lactation.

These lineage tracing experiments confirmed the experimental hypothesis [62]. Moreover, transplantation experiments have further confirmed. In fact, both the transplantation of pure marked adipose tissue and that of mature, marked isolated mature adipocytes have shown the development of marked glands in pregnancy [63].

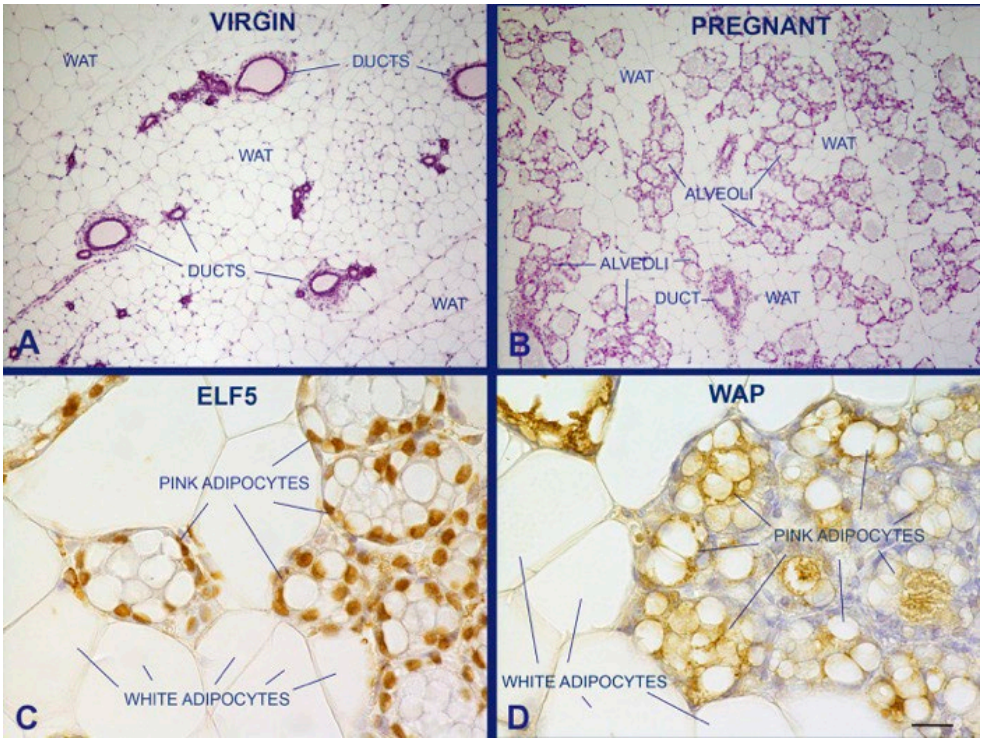


**Fig 6.** Histology of mammary gland of adult female mice. Alveoli composed by pink adipocytes are absent in virgin mice (A) and appear in the second half of pregnancy (B). Pink adipocytes (C and D) are immunoreactive for the master transcription factor of alveologenesis (ELF5) in nuclei (C) and for the milk protein WAP (whey acidic protein) in cytoplasm (D). Reproduced from [2] with permission. Bar: 50 mm in A and B, 12 mm in C and D.

In order to study the molecular mechanisms responsible for this tissue plasticity, we removed the ductal component monolaterally in animals that were subsequently pregnant.

The glands private of the ductal component (cleared fat pad) did not undergo any modification of the adipocytes during pregnancy, while the contralateral glands developed normally as described above. This result was somewhat expected because it is well known that the entire visceral component of the adipose organ does not respond to the hormonal pregnancy stimulus. In order to study the possible paracrine factor responsible for adipose-epithelial transdifferentiation, we performed a comparative analysis using microarrays between the cleared fat pad and the contralateral glands in mice at different stages of pregnancy. The data showed that osteopontin (Spp1) could be a candidate among the factors responsible for adipose-epithelial transdifferentiation [64-66]. It is in fact secreted by the ductal epithelium [67], its presence is necessary for normal alveologenesis [68, 69] and its transgenic overexpression causes alveolar hyperplasia [70]. We also highlighted the expression

of the master regulator of the ELF5 alveologenesis in the nucleus of adipocytes in the transdifferentiation phase, while the "normal" fat cells never expressed this transcription factor.

## The new concept of nutritional system

In the organism of mammals, the organs work together for complex functions dedicated to maintaining the short and long-term homeostasis of the organism itself. In particular, the organs of the digestive system collaborate with the adipose organ for important homeostatic activities: the nutrition of the organism and of the offspring. For these complex functions it is necessary to influence the brain in that part which determines instinctual behavior. Both the adipose organ and the digestive system produce hormones that influence eating behavior. In fact, the organs of digestion produce Ghrelin, $PYY^{3-36}$, insulin, GLP1 which essentially act as orexigen (ghrelin) or anorectics (the others) on the hypothalamic centers [71-76]. The adipose organ produces leptin and asprosin (see above). Therefore, the adipose organ and organs of the digestive tract cooperate in activities related to the research and intake of food.

Furthermore, they produce hormones and substances (FGF21, secretin, bile acids) able to mutually modulate thermogenesis and this last influence, especially in newborns, the rhythm in food intake [77-81].

Finally, several data seem to support the idea that intestinal microbiota influence both gastrointestinal functional activity and adipose organ browning reinforcing the data supporting their functional relationships in this contest [82-92].

Therefore, the whole of the adipose organ and of the digestive system can be considered a system of the organism able to influence the fundamental behavior for survival (research and food intake), the absorption of nutrients (digestive system), the nutrient distribution to the entire organism (adipose organ) and to newborns (adipose organ temporarily transformed into a mammary gland).

Finally, considering all together, my proposal is therefore to add to the usually recognized systems of the human body, such as the nervous, cardiovascular, respiratory, endocrine, excretory, immunological and reproductive systems, a new important system that can be denominated as nutritional system.

## Acknowledgments

I am grateful to all the Collaborators of my laboratory who faithfully and with great dedication have helped me in the last 43 years of work in Ancona and to all external Collaborators. In particular I acknowledge the President and Board of Italian Society of Anatomy and Histology for allowing me to hold, for the fourth time, an opening plenary lecture at our National Congress (Parma in 2018, previous: Messina 1988, Palermo 1998, Verona 2008). Finally, I thank the Members of our Scientific Society who have always inspired and encouraged me to continue in my morphological studies.

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