The Adipose Organ

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Abstract

White and brown adipocytes form tissues (WAT and BAT, respectively) contained in a dissectible organ formed by subcutaneous and visceral depots. WAT and BAT have almost opposite roles in partitioning energy between two fundamental needs for survivals: metabolism and thermogenesis. All organs in mammals are composed by different tissues acting with different physiology to reach a common finalistic purpose. The plasticity of adipocytes, i.e., their reversible physiologic transdifferentiation ability, offer an explanation to their common membership to adipose organ, but imply a new physiologic ability for mature cells: the physiologic reversible transdifferentiation property. This conversion ability of mature adipocytes is supported also by the plasticity of mammary glands during pregnancy, lactation, and postlactation periods when white adipocytes convert reversibly to milk-secreting glandular cells (pink adipocytes). During chronic positive energy balance, the adipose organ undergoes a whitening phenomenon with hypertrophic adipocytes. Hypertrophic adipocytes show several alterations of their organelles including those able to activate the inflammasome system. Stressed adipocytes die leaving conspicuous debris that must be removed by macrophages. This last cell surrounds debris and form crown-like structures (CLS) responsible for a chronic low-grade inflammation that link obesity to T2 diabetes. The plasticity of adipocytes could be used to reverse the phenomenon.

Keywords

Adipocytes · WAT · BAT · Pink adipocytes · Obesity · T2 diabetes · Hypertrophic adipocytes

Adipocytes of White Adipose Tissue of Obese Animals and Humans Are Hypertrophic

Adipocytes are large spherical cells allowing to store high levels of energy in minimum space. A total of 90% of their volume is formed by a single lipid droplet contained into the cytoplasm (unilocular adipocytes). The nucleus is squeezed at periphery and the thin cytoplasmic rim contains all normal organelles found in other cell types. Quite specific for adipocytes are numerous pinocytotic vesicles on the cell membrane and a distinct external lamina on its outer side. A variable amount of collagen fibrils is also present on the interstitial side of external lamina. Mitochondria are thin and elongated with sparse and randomly oriented cristae (Cinti 2017).Their energy, under the chemical form of triglycerides, is essential for survival in the intervals between meals that can be prolonged up to several weeks if the number of adipocytes in the organism is sufficient. In order to guarantee the maximal energy reserve, adipocytes are able to increase their size (hypertrophy) and number (hyperplasia) during positive energy balance periods (Faust and Miller 1981). In genetically obese mice and humans, the size of adipocytes can be seven (subcutaneous in mice, 1.6 in humans) or six (visceral in mice, 2.6 in humans) times larger

than lean controls; thus, the large adipocytes can become gigantic in obese mice and humans (Camastra et al. 2017; Murano et al. 2008).

Unilocular adipocytes are also called white adipocytes because they form a white tissue (WAT, yellow in humans) that is supplied by nerves (mainly unmyelinated noradrenergic fibers) and vessels.

White adipocytes secrete a series of adipokines (leptin, adiponectin, adipsin, resistin, etc.) with many direct endocrine properties playing important roles also in the regulation of animal behavior mainly regarding food search and intake and glucose and lipid metabolism (see Giralt et al. 2015 for recent review of this topic).

Hypertrophic Obese Adipocytes Are Stressed

Electron microscope analyses revealed that hypertrophic adipocytes in genetically as well as in diet-induced obese mice have several abnormalities in their organelles. Mitochondria become smaller and reduced in number, with some hypertrophic irregular mitochondrion. Golgi complex become hypertrophic, rough endoplasmic reticulum dilates, and often glycogen cumuli are present. Some dense small crystals resembling calcium aggregates have been found at the lipid droplet surface where the proteins (e.g., perilipin1) regulating lipolysis are usually located. Some hypertrophic adipocytes resulted so rich in calcium crystals that were positive for the calcium-specific von Kossa histochemistry reaction at light microscopy (Giordano et al. 2013). In some obese adipocytes, cholesterol crystals were described in line with the well-known positive correlation between size of adipocytes and their cholesterol content. Obese adipocytes with signs of cytoplasmic degeneration and lipid extrusion were also found with both transmission electron microscopy and high-resolution scanning microscopy. Macrophages close to these altered obese adipocytes were frequently found. These techniques also revealed an increased amount of collagen associated to the external surface of the external lamina.

Quantitative analyses revealed that all the above-described alterations (with the exception of calcium crystals) in obese adipocytes were more represented in visceral than in subcutaneous fat.

Hypertrophic Obese Adipocytes Die

Specific features denominated crown-like-structures (CLS) are frequently found in the adipose tissue of obese animals and humans (about 30 times more frequent in obese than lean fat) (Fig. 1).Each single CLS is formed by active (MAC2 immunoreactive) macrophages surrounding debris of death adipocytes (Cinti et al. 2005). The debris is mainly composed by a gigantic free lipid droplet liberated in the interstitial space by the dead adipocyte. More than 90% of all MAC2 positive macrophages formed CLS. Electron microscope revealed that the lipid droplet is surrounded by active macrophages in direct contact with the surface of the lipid droplet. In most cases, the side of cytoplasm in contact with the lipid droplet resulted

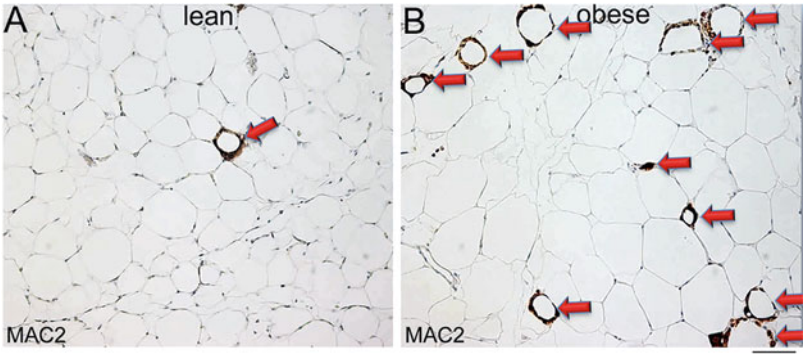

Fig. 1 Immunostaining of active macrophages (MAC2) in visceral WAT of lean (**a**) and obese (**b**) mice. Note the presence of crown-like structures (CLS, arrows) apparently surrounding adipocytes. CLS density is about 30 times higher in obese fat. Bar = 100 μm in both panels (From Cinti et al. 2005)

filled by reabsorbed lipids. On the opposite side (toward the interstitium), an irregular basal membrane is often observed; thus, the macrophages layer result in the space between the basal membrane and the lipid droplets (Fig. 2). Importantly in most CLS no cytoplasm of adipocytes is visible between the lipid droplet and the basal membrane, allowing to speculate that this part of adipocytes was completely lost probably due to a degenerative phenomenon. In line with this hypothesis, residual cytoplasmic debris were found into phagosomes inside the cytoplasm of macrophages. Furthermore, electron microscope revealed all morphologic transformative steps between normal adipocytes and degenerating adipocytes. These last types of cell were often surrounded by macrophages and lipid droplets extruding or just extruded from degenerating adipocytes surrounded by cytoplasmic projections of macrophages or inside their cytoplasm were also observed. Perilipin1 (Plin1) is an adipocyte-specific protein localized at lipid surface of metabolically active adipocytes. Immunohistochemistry with anti-Plin1 antibodies revealed absence of signal from CLS supporting the death of adipocytes (Fig. 3).These observations offered an explanation to the cause of the well-known chronic low-grade inflammation due to the macrophage infiltration of obese adipose tissues.

Death of Adipocytes Is due to Hypertrophy and Not Linked to the Obesity Per Se

In order to verify if hypertrophy per se is sufficient to induce CLS, the adipose tissues of mice lacking hormone sensitive lipase was studied. This enzyme is important to allow lipolysis in adipocytes; thus, it absence induce adipocytes hypertrophy. Adult $HSL^{-}/^{-}$mice are lean, but their adipose tissues resulted rich of CLS (same density as in obesity) with all the morphologic and immunohistochemical characteristics of CLS found in obese fat (Cinti et al. 2005). Thus, hypertrophy of adipocytes is sufficient to induce a histopathology very similar to that found in obese adipose tissue. In line with

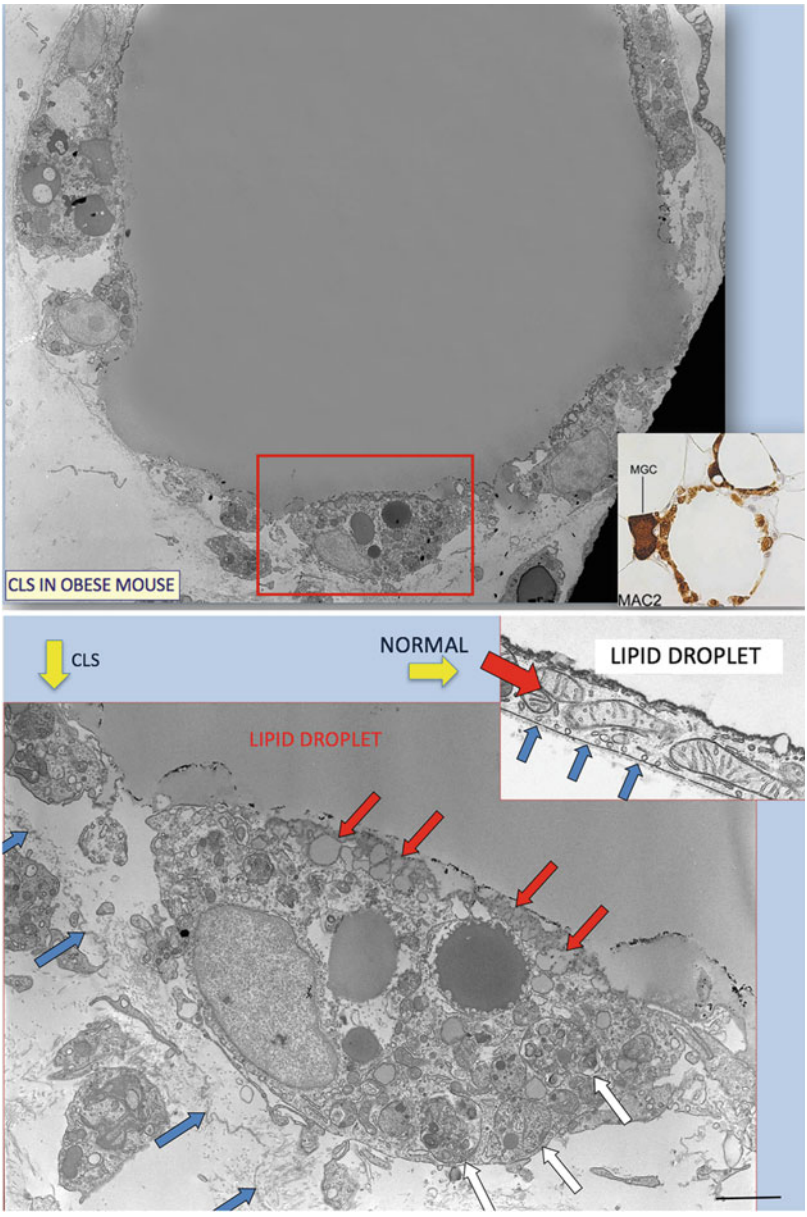

Fig. 2 Electron microscopy of a CLS. Note the classic ultrastructure of macrophages surround in the lipid droplet (upper panel). MAC2 immunohistochemistry of a similar CLS is shown in the small frame of upper panel. The red framed area of upper panel is shown in lower panel. The normal ultrastructure is shown of small frame in lower panel. Note that the macrophage is located between the lipid droplet and the basal membrane (blue arrows). The cytoplasm of adipocyte is not visible in CLS (compare with small frame), and residual structures of degenerating cytoplasm are visible inside phagosomes into macrophage (white arrows). Reabsorption lipid vacuoles are visible on the side facing the residual lipid droplet (red arrows). Bar = 5 μm in upper panel and 1.7 μm in lower panel (From Cinti et al. 2005)

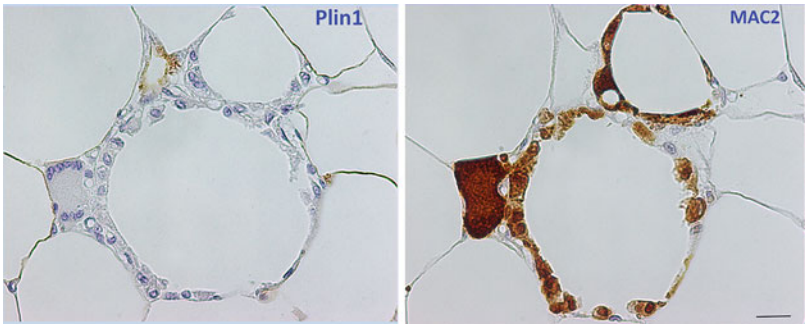

Fig. 3 Serial sections of a CLS in obese mouse visceral fat. Perilipin1 (Plin1) immunostaining is present only in adipocytes surrounding the CLS. Most macrophages of CLS are MAC2 immunoreactive, including giant multinucleated cells. Bar = 28 μm in both panels (From Cinti et al. 2005)

the hypothesis that CLS are important to induce insulin resistance and T2 diabetes is the old notion that there is a positive correlation between size of adipocytes and insulin resistance. Furthermore, it is interesting that most obese persons and animal models with hyperplastic obesity (increased number of small adipocytes) do not develop a metabolic phenotype. In line with these data, a higher number of CLS are present in lean and obese patients with larger adipocytes (Cinti et al. 2005).

Induced Death of Adipocytes in FAT-ATTAC Transgenic Mice Give Rise to CLS

In order to verify if CLS truly represent sites of dead adipocytes, the well-characterized model of mice in which specific death of adipocytes (apoptosis by caspase 8 activation) can be induced by dimerization of the transgenic construct (FAT ATTAC model)has been studied. The time course study of morphologic effects on two different fat depots of dimerizer injection showed an acute response (first days) characterized by infiltration of adipose tissues by granulocytes and lymphocytes followed immediately by MAC2 not-immunoreactive (negative) macrophages infiltration. MAC2 is an index of phagocytosis activation. The next step at day 4–5 after injection showed areas of Plin1 negative adipocytes (i.e., dead adipocytes) surrounded by Plin1 immunoreactive adipocytes (i.e., metabolically active alive adipocytes). In the next day macrophages invaded the areas of apoptotic adipocytes and number of MAC2 immunoreactive adipocytes increased progressively. After 10–15 days after injection the vast majority of macrophages resulted MAC2 positive and all dead adipocytes formed typical CLS. Of note, the acute inflammatory cells disappeared after the first postinjection days. Electron microscopy revealed the same morphologic alterations of adipocytes described above in obese hypertrophic adipocytes including all phases of progressive organelles damage till the evidence for frank degeneration of adipocytes (Murano et al. 2013).

Thus, this time-course study demonstrated that CLS are indeed sites of dead adipocytes surrounded by active reabsorbing macrophages.

Hypertrophic Adipocytes Die by Pyroptosis

Short after the hypothesis of death of adipocytes (Cinti et al. 2005), an elegant study by Spalding et al. showed that human adipocytes die after a lifespan of about 10 years, thus confirming that adipocytes die in physiologic conditions (Spalding et al. 2008). In this work, it was also shown that in obese fat the rate of death is not changed, but due to the higher number of adipocytes the dead adipocytes were indeed higher than in fat of lean persons. The mechanism inducing death of adipocytes was unknown, but morphologic data suggested a stressed status of hypertrophic adipocytes described above. Furthermore, stressed hypertrophic adipocytes contain calcium and cholesterol crystals. These crystals are known inducers of the NLRP3 inflammasome system activation that produce active caspase 1. Caspase 1 is responsible for IL18 and IL1β activation that induces death of the cells. This type of cell death is called pyroptosis. Interestingly NPRP3 has been shown to be activated in obese fat with local production of inflammatory IL18 and IL1β activation. In order to prove that stressed adipocytes had activated, the NLRP3 system several markers (including NLRP3, ASC, TNPIX, and caspase1) have been checked both as gene expression in the tissue and as protein expression inside the cytoplasm of hypertrophic adipocytes (Giordano et al. 2013). Immunohistochemistry for caspase1 resulted in an unusual staining of cytoplasm of adipocytes, i.e., the staining was not uniform as resulted for other proteins such as, for example, Plin1 (Murano et al. 2013) or Leptin (Cinti et al. 1997) or S-100b (Barbatelli et al. 1993) but formed small spherical structures recalling the spherical shape of NLRP3 protein complex. Furthermore, caspase 1 immunostaining is absent in the tissues of the FAT ATTAC model of fat-specific apoptosis described above.

Macrophages and Multinucleated Giant Cells Reabsorb Debris of Dead Adipocytes and Stimulate Adipogenesis

The size of hypertrophic obese adipocytes that die by pyroptosis is gigantic in proportion to that of normal macrophages infiltrating obese fat. Because residual debris of dead adipocytes are close to the same gigantic size, it is not surprising that macrophages form syncytia similar to those formed in foreign body reactions. The size of CLS in obese fat is variable; the largest approach that of surrounding hypertrophic adipocytes the smallest are almost exclusively composed by macrophages with the central residual lipid droplet barely visible. These data allow to think that CLS are sites of reabsorption of debris derived from hypertrophic adipocytes death. In line with this hypothesis, a time course study of fat inflammation in visceral fat of HFD treated mice showed that CLS number progressively increase in this fat depot of these animals in parallel with the increase of fat and of the size of adipocytes

(Strissel et al. 2007). At week 16 of HFD, about 70% of epididymal depot was occupied by CLS and after 4 further weeks of HFD the % of tissue occupied by CLS dropped to less than 20% with a parallel decrease in weight of the depot. These data suggest a renewal of the tissue and recently Lee et al. (2013) identified CLS an adipogenic niche. In particular they found that clusters of proliferating PDGR-α-marked preadipocytes where in close connection with CLS. Furthermore, they showed that CLS-macrophages produce osteopontin and a subpopulation of PDGR-α-marked preadipocytes express CD44 that is the receptor for osteopontin.

Further data supporting the reabsorbitive-clearing role of CLS macrophages have been recently produced by Haka et al. (2016) that showed an extracellular digestive activity of CLS macrophages denominated exocytosis.

In Humans, Cyst-Like Structures Are Gigantic CLS

Hypertrophy of adipocytes seems to be the prerequisite for CLS formation. In a case series study of 28 obese patients undergoing bariatric surgery, two patients with extreme hypertrophy of adipocytes have been described (Camastra et al. 2017). Their adipocytes resulted about 30% larger than any other obese patient studied. Only in these two cases rare gigantic CLS denominated cyst-like structures (CyLS) were found (Fig. 4). Their anatomical composition was similar to classic CLS, i.e., they were composed by CD68 immunoreactive macrophages surrounding a lipid-like structure Plin1 negative. In some CyLS, macrophages formed syncytial structures. Their size was approximately ten times that of the largest CLS found in those as well in all other patients of this case series. This size excludes the possibility that CyLS represent debris of single death adipocyte and the hypothesis shown in Fig. 5 was proposed: the very thin rim of cytoplasm, pushed by the expanding lipid droplet, is damaged and imaging a confluence of enormous lipid droplets extruded by ruptured adipocytes it can be supposed the formation of very large oil-like lipids free in the interstitium. As a matter of fact, olive oil injections in inguinal fat for vitamin A administration reproduced very similar structures. Gigantic lipid droplets represent gigantic debris requiring reabsorption as those smaller deriving from death of single adipocytes; thus, it is not surprising to see CD68 immunoreactive macrophages (also syncytial) surrounding the lipid structure.

Visceral Adipocytes Have a Lower Critical Death Size

Since the original clinical observations, it became evident that accumulation of fat in humans is different in males and postmenopausal females (abdominal or central body forming apple shape) and premenopausal females (gluteo-femoral or lower body forming pear shape). This different distribution has important health consequences because the metabolic associated disorders affect only the apple shaped obesity (central or visceral obesity) implying important differences in the fat site distribution (Bjorntorp 1997). As a matter of fact, fat localize at two compartments of the body:

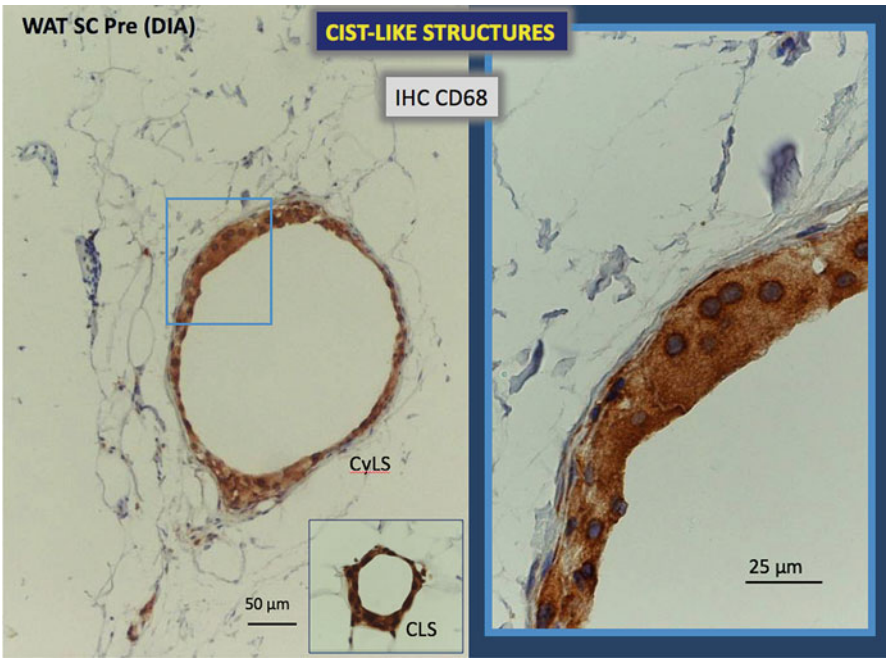

Fig. 4 CD68 immunostaining of macrophages forming CLS and cyst-like structures (CyLS: giant CLS) in subcutaneous fat of obese diabetic patient. Note the difference in size between CLS and CyLS (same enlargement in the left panel). The blue framed area is enlarged in the right panel showing the multinucleated giant macrophage. Bars as indicated (From Camastra et al. 2017)

subcutaneous and visceral. The first is contained in the space between skin and muscle superficial fascia, and the second inside the trunk.

In order to understand why visceral fat accumulation is more dangerous for health than subcutaneous fat, the two depots in two different models (ob/ob and db/db) of genetic murine obesity have been studied. The size of adipocytes resulted increased six to seven times both in subcutaneous and in visceral fat, but subcutaneous adipocytes were larger than visceral adipocytes. The CLS density in both compartments was higher in visceral fat. This was unexpected result because of the well-established positive correlation between size of adipocytes and number of infiltrating macrophages. These data suggested a lower critical death size (CDS, size triggering death) for visceral adipocytes (Cinti 2009).

Fat Inflammation Causes Insulin Resistance

The prevalence of visceral inflammation is relevant because it has been shown a temporal association between fat inflammation and insurgence of insulin resistance. The molecular mechanism linking macrophage infiltration of obese fat and insulin resistance is incompletely known, but several cytokines and factors produced

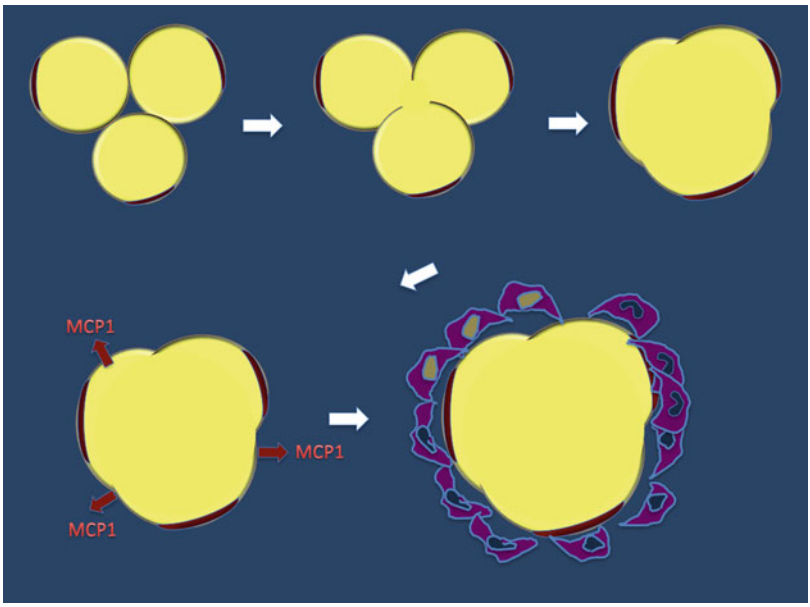

Fig. 5 Hypothesis for CyLS formation: Very large adipocytes with breakage of their very thin cytoplasmic rim coalesce forming very large lipid droplets remnants ready to be reabsorbed by macrophages attracted by secreted chemoattractant (From Camastra et al. 2017)

by macrophages could play an important role. Since the discovery of a direct link between TNFα hyper production in obese fat and insulin resistance, a causal link between obesity and T2 diabetes was carefully searched for (Hotamisligil 2006). TNFα direct interference with insulin receptor normal signaling was well established, but subsequent other works suggested that several other molecules could also play a role. In 2003 two independent laboratories evidenced the great role for a low grade chronic inflammation mainly due to macrophage infiltration of obese fat (Weisberg et al. 2003; Xu et al. 2003). In these papers, the coincidence between macrophages infiltration and appearance of insulin resistance was established. Furthermore, most of the cytokines with potential role in insulin resistance were found to be present in the stroma-vascular fraction of the tissue implying that its source would be different from that of mature adipocytes as previously thought and macrophages were indicated as the most probable source. Furthermore, stressed hypertrophic adipocytes can cause insulin resistance before their death and obese adipocytes reduce their production of adipsin that exert a positive role on pancreatic β-cells (Lo et al. 2014).

In synthesis, the sequence of events in WAT induced by a chronic positive energy balance possibly linking obesity to type2 diabetes could be (Fig. 6):

1. Hypertrophy of adipocytes
2. Stress of adipocytes with reduced secretion of adipsin, adiponectin, and increased secretion of chemo attractants (mainly MCP1)

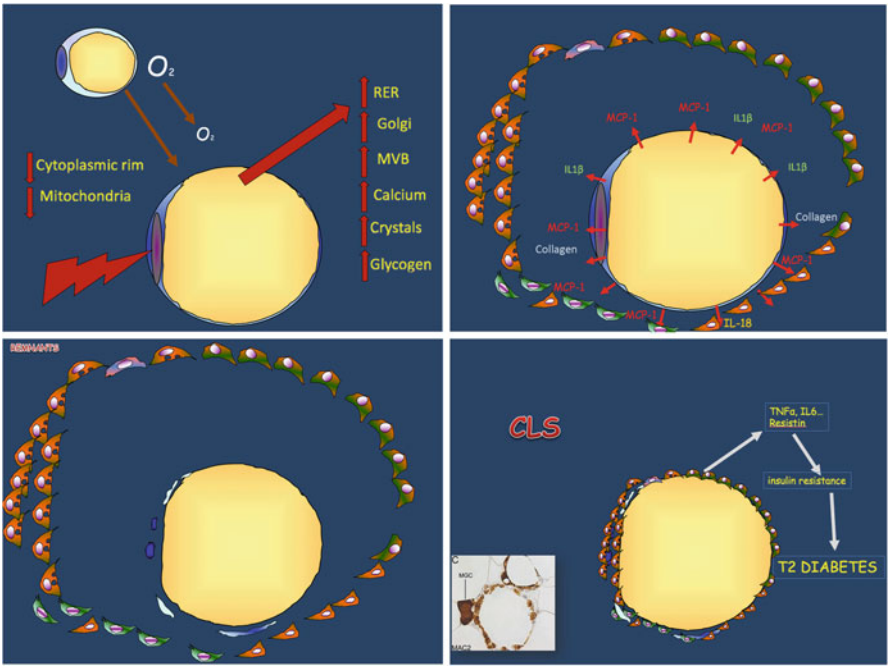

Fig. 6 Summary of events possibly linking hypertrophy and death of adipocytes to T2 diabetes

3. Death of adipocytes by pyroptosis (with lower critical death size in visceral fat)
4. Formation of gigantic debris (mainly represented by residual free lipid droplets)
5. Formation of CLS or eventually (very hypertrophic cases) CyLS
6. Reabsorption of debris by macrophage phagocytic activity in CLS (by exocytosis, eventually with syncytia formation)
7. Macrophage secretion of cytokines with toxic effects on insulin receptor signaling (including TNFα, resistin, Il-6, and iNOS)
8. Insulin resistance
9. T2 diabetes

Weight Loss Induces CLS Density Reduction with Amelioration of Metabolic Parameters

Several histopathologic data from fat of patients with obesity treated by bariatric surgery that lost significant amount of fat, showed a reduction in size of adipocytes with a reduction of CLS number. In parallel with these inflammatory parameters reduction, an improvement of metabolic parameters was shown. Interestingly, in spite of a drastic reduction of fat inflammation a persistence of an obesity signature or

incomplete restoring of pancreatic β-cells glucose sensitivity was detected in these patients (Camastra et al. 2017; Cancello et al. 2005, 2013).

Adipose Tissue Is Organized in Subcutaneous and Visceral Depots

Together with WAT, all adult mammals (humans included) have variable amounts of brown adipose tissue (BAT). BAT is formed by polygonal cells smaller than white adipocytes (about 1/3) with central roundish nucleus and several cytoplasmic small lipid droplets (multilocular adipocytes) (Fig. 7). Brown adipocytes have numerous large mitochondria containing a protein that is uniquely found in this cell type and that is responsible for its function: thermogenesis (Cannon and Nedergaard 2004; Ricquier 2017). When mammals are exposed to cold (temperature under thermoneutrality, i.e., under the temperature not requiring thermogenesis), the sympathetic nervous system (SNS) induces noradrenalin (NE) secretion at the terminal end of sympathetic nerve fibers that directly reach brown adipose tissue (BAT). NE activates cAMP signaling through the specific β3 adrenoceptor. The signaling ends with activation of protein kinase A and subsequent activation of lipases and neo-synthesis of UCP1. This protein is located in the inner mitochondrial membrane (cristae) and acts as protonophore, thus nullifying the proton gradient derived from the beta oxidation of fatty acids and transforming all the energy liberated by the oxidative process into heat. Thus, BAT is composed by cells completely different in anatomy and functions from those of WAT, but we call both these cells adipocytes only for historical reasons essentially due to their old descriptions as lipid loaded cells well before the discoveries of their functions.

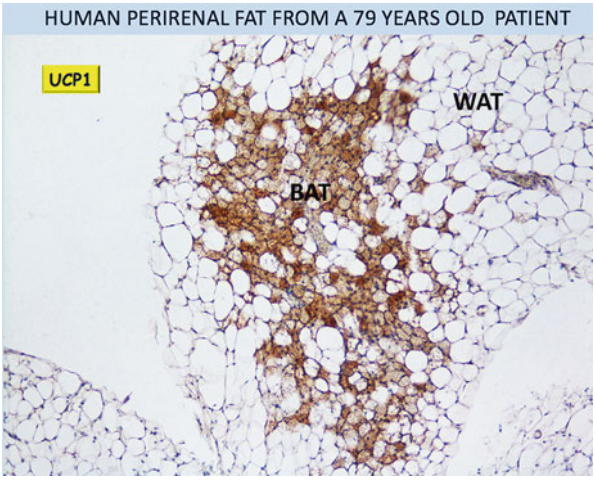

Fig. 7 UCP1 immunoreactive brown adipose tissue (BAT) mixed to white adipose tissue (WAT) in perirenal fat of a 79-year-old man (From Cinti 2017)

Adipose Organ of Mice and Humans Is Mixed and Contains also BAT

In spite of their different morphology and physiology, WAT and BAT are contained together in a dissectible organ composed by subcutaneous and visceral depots in all adult mammals (Fig. 8).

In small mammals (mice, rats, ferrets), 60–70% of this organ is located in the subcutaneous and intermuscular compartment, i.e., between skin and skeletal muscles fasciae, with some projections in deeper intermuscular areas. Two main subcutaneous depots are dissectible in these small mammals: anterior and posterior subcutaneous depots (Cinti 1999, 2000, 2001a, b, 2002, 2005). The anterior subcutaneous depot (ASC) is more complex than the posterior (PSC). ASC is mainly dorsal and located in interscapular area with several symmetrical projections (lateral, cervical, axillary, and subscapular). Each of these parts is mixed: formed by BAT and WAT, i.e., within these areas pure BAT lobules are located near pure WAT lobules with mixed tissue at the boundaries. Thus, ASC is mixed with pure WAT near pure BAT and near areas composed by the two cell types. PSC is mainly WAT and located symmetrically in the inguinal area with dorso-lumbar and gluteal extensions. In the inguinal area is always visible a lymph node. Small mixed depots are present in the

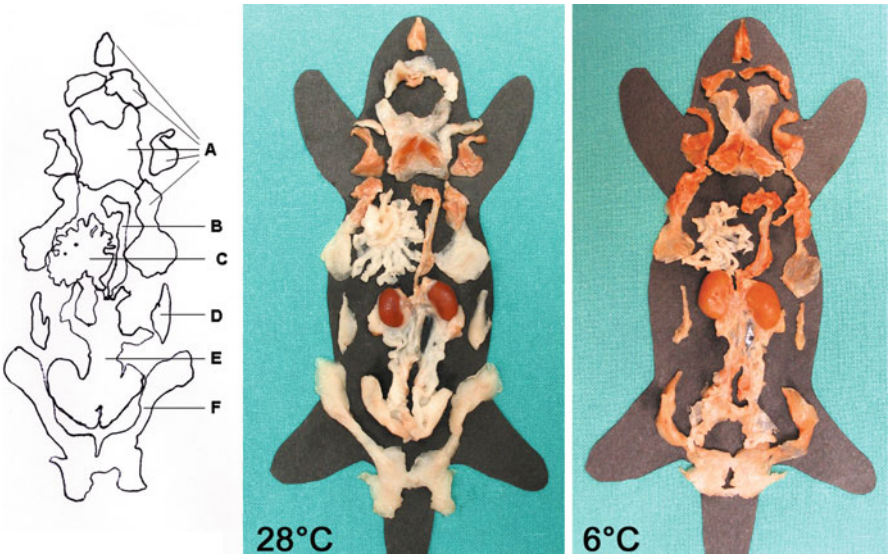

Fig. 8 Gross anatomy of Adipose Organ of adult female Sv129 mice maintained at 28 °C (left) and 6 °C (right) for 10 days. *A* = anterior subcutaneous depot (formed by interscapular, subscapular, axillo-thoracic, and cervical parts), *B* = mediastinal-periaortic visceral depot, *C* = mesenteric visceral depot, *D* = retroperitoneal visceral depot, *E* = abdomino-pelvic visceral depot (formed by perirenal, periovarian, parametrial, and perivesical parts), *F* = posterior subcutaneous depot (formed by dorso-lumbar, inguinal, and gluteal parts) (From Murano et al. 2005)

limbs. Visceral depots are contained into the trunk: thorax and abdomen. In thorax, fat occupies the mediastinal area with projections following all main aorta branches. In abdomen, a unitary structure denominated abdominopelvic depot surrounds abdominal aorta and its main branches forming perirenal, mesenteric, retroperitoneal, periovarian, parametrial, and perivesical fat in female mice. In male mice, abdominal fat mainly forms the perirenal and retroperitoneal fat. Pelvic fat in male mice forms the perivesical and epididymal depots. Visceral fat is also present in omentum that is very small in mice. Mediastinal and perirenal fat are the richest in BAT and a gradient BAT-WAT is present from aorta to its peripheral branches, i.e., all described depots are close (surrounding) to aorta and its main branches. The fat closest to aorta is usually BAT and a gradual transition toward WAT is found following the peripheral part of the branches. Epididymal fat and omentum (both far away from aorta) are always pure WAT. This anatomy is finalistically easy to explain: heat produced by BAT is quickly transferred to the close aorta and its main branches in order to diffuse thermogenesis to all organisms.

In humans, the anatomy of this organ is quite similar to that described above for small mammals.

Major differences are: (1) subcutaneous compartment is more diffuse and continuous also in the limbs, (2) the interscapular area is less developed, (3) most tissue is formed by WAT, (4) BAT is restricted mainly in peri subclavian and perirenal areas, and (5) BAT is rarely pure (usually lobules of BAT are mixed with WAT). In a case series of about 45 adult patients biopsied in the peri subclavian-peri carotid area, UCP1 immunoreactive BAT in about 1/3 of them have been described (Zingaretti et al. 2009). Furthermore, positron emission tomography with deoxy-fluoro-glucose (PET) showed active uptake signal in the same area, which increased in intensity after cold exposure or in patients with pheochromocytoma (a benign tumor od adrenal gland secreting high levels of catechol amines in the blood) (Saito et al. 2009).

Thus, the human adipose organ is mixed and its BAT component can be activated in adult humans (Cypess et al. 2009; van MarkenLichtenbelt et al. 2009; Virtanen et al. 2009). A recent paper showed that a new β3AR agonist drug (mirabegron) approved for hyperactive bladder is able to activate BAT as visualized by PET in young lean voluntary patients (Cypess et al. 2015).

BAT Is an Antimetabolic Syndrome Tissue

The presence of BAT in adult humans is of clinical interest because the energy dissipated by BAT thermogenesis could help to treat patients with obesity (Cypess and Kahn 2010). It has been shown that lack of BAT activity favors obesity and T2 diabetes in small mammals (Bachman et al. 2002; Lowell et al. 1993) and treatment of obese animals with β3AR agonists rescue obesity and related disorders (Ghorbani and Himms-Hagen 1997, 1998). Furthermore, BAT activity improves insulin sensitivity (Seale et al. 2011), lipid metabolism (Bartelt et al. 2011), atherosclerosis (Berbee et al. 2015), and increase longevity (Ortega-Molina and Serrano 2013). It

has also been shown that cold exposure of adult patients improves the insulin sensitivity (Chondronikola et al. 2014).

WAT Can Be Converted into BAT

A major question deriving from the anatomy of adipose organ is related to the finalistic common purpose of WAT and BAT. By definition an organ must be dissectible, containing at least two different tissues, and these tissues should cooperate for a unitary finalistic purpose. Thus, the stomach (widely accepted as an organ) is dissectible and formed by different tissues such as mucosae and smooth muscles. These tissues cooperate with different functions to the unitary finalistic purpose of digestion: mucosae by gastric juice production and smooth muscle by peristalsis. In order to search for the unitary finalistic purpose of WAT and BAT in adipose organ, the dynamic changes of this organ during several physiologic stimuli such as cold exposure and pregnancy have been studied. After just 10 days of cold exposure, the color of the organ changes dramatically (Fig. 8) from mainly white (mice maintained at 28 °C) to mainly brown (mice maintained at 6 °C) both in B6 and Sv129 adult mice. Detailed quantitative measurements of adipose organs in these experiments showed that after cold exposure in both strains the total number of adipocytes was unchanged, of course, as expected, the number of brown adipocytes increased but surprisingly the number of white adipocytes decreased exactly of a number of cells equivalent to the increased number of brown adipocytes (Fig. 9) (Vitali et al. 2012). These data suggested a conversion of WAT to BAT in line with other experiments performed since 2000 when a direct conversion of white into brown adipocytes in old rats treated with β3AR agonists was showed (Granneman et al. 2005; Himms-Hagen et al. 2000) (Figs. 10 and 11).

The ideal technique to demonstrate this conversion is lineage tracing where cell tagged by the cell specific activation of a gene induces irreversible production of β-galactosidase (β-Gal or other reporter gene) that persists in the converted cell independently by the new phenotype and gene expression. β-Gal expression can be visualized at light microscopy level by histochemistry (X-Gal reaction) as a blue cytoplasm adding to the genetic precision the cell specific morphology precision. With this powerful technique, Christian Wolfrum group demonstrated the reciprocal conversion of WAT-BAT in 2013 (Rosenwald et al. 2013).

With the same lineage tracing technique, it was also demonstrated that white and brown adipocytes derive from the same stem cell reinforcing the concept of possible interconversion (Tran et al. 2012).

Browning can be obtained mainly by cold exposure, administration of β3ARs agonist drugs, and physical exercise (Bostrom et al. 2012; De Matteis et al. 2013; Frontini and Cinti 2010).

Thus, the answer to the major question could simply be: the unitary finalistic purpose is repartition of food-derived energy into functions for survival (short-term homeostasis) – thermogenesis (BAT) and metabolism (WAT) – but in special occasions such as chronic cold exposure WAT converts to BAT to respond to the

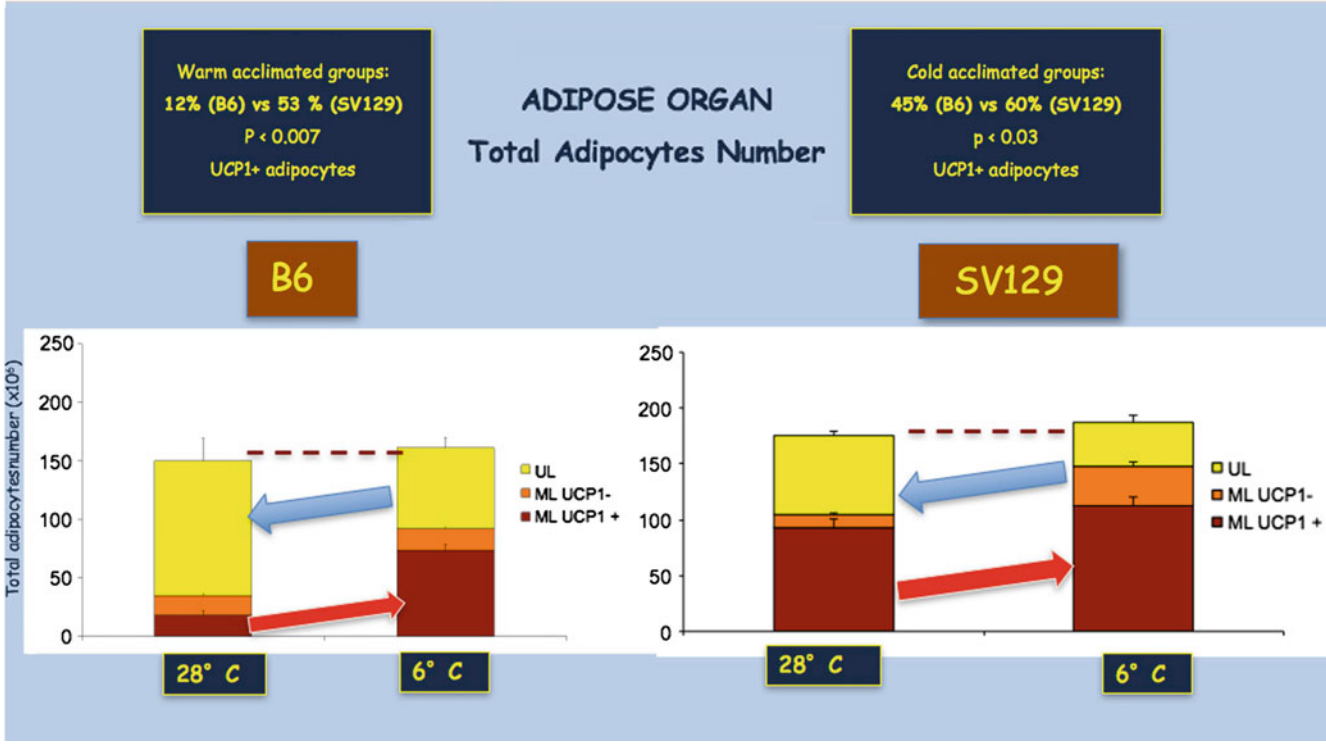

Fig. 9 Quantitative assessment of adipocytes number in the adipose organ of adult female Sv129 and C57/BL6J mice maintained at 28 °C and 6 °C for 10 days. After cold acclimation, in absence of change in total number, brown adipocytes increase of a number equivalent to that of white adipocytes decrease (From Vitali et al. 2012)

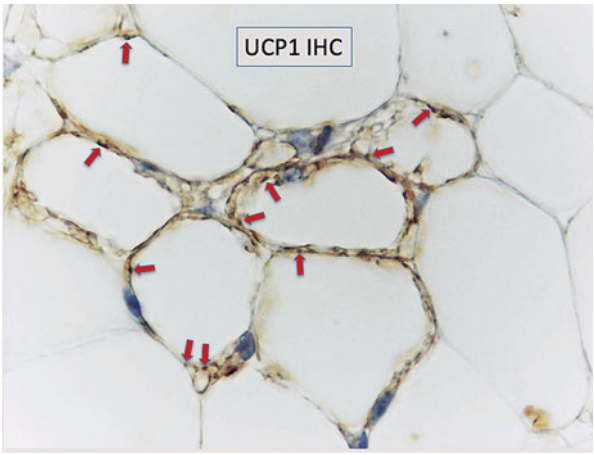

Fig. 10 UCP1 immunoreactive paucilocular adipocytes found in anterior subcutaneous WAT of cold exposed C57/BL6J adult female mouse. Note the immunoreactivity at periphery of the cells, roundish small immunoreactive structures with size and shape of mitochondria (arrows) are visible, suggesting an early stage of white to brown transdifferentiation

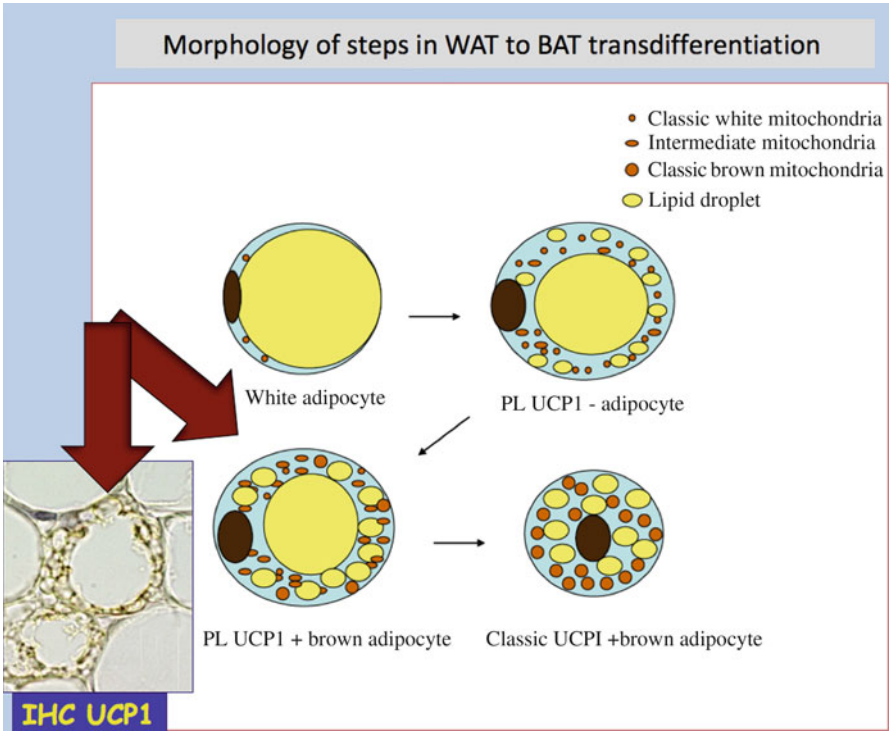

Fig. 11 Proposed morphology of steps during white to brown transdifferentiation. The UCP1 immunoreactive paucilocular step is shown also in the original immunohistochemistry analysis (arrows) (From Barbatelli et al. 2010)

extra need for thermogenesis. On the other hand, in the case of chronic positive energy balance, the organ cannot refuse the precious energy of extra food because of lack of any guarantee for future food availability.

Pink Adipocytes Integrate the Transdifferentiation Triangle of Adipose Organ

This theory explains the mixed anatomy and the need of cooperation between WAT and BAT, but implies a new basic property of mature cell: the physiologic ability of reversible transdifferentiation. In search for new examples of this property, it has been shown that subcutaneous WAT of female mice is able to reversibly convert to milk-secreting epithelial glands during pregnancy and lactation. It is well known that the alveolar component of mammary glands develops during pregnancy and progressively substitutes for the mammary WAT. Early alveoli (day 17–18 of pregnancy) are diffusely formed by glandular cells with cytoplasm occupied by large lipid vacuoles (Masso-Welch et al. 2000; Richert et al. 2000; Smorlesi et al. 2012). Thus, parenchymal cells of adipose organ during pregnancy fulfil the definition of adipocytes that were denominated pink adipocytes because the color of the organ during pregnancy is pink (Giordano et al. 2014). Lineage tracing and explant experiments showed the reversible white to pink transdifferentiation (De Matteis et al. 2009; Morroni et al. 2004). Recently it has also been showed that pink adipocytes (milk secreting glandular cells), in the postlactation period, not only convert to white adipocytes but also to UCP1 immunoreactive brown adipocytes in the dorsal area of ASC (Giordano et al. 2017). Molecular mechanisms underlying this triangle of transdifferentiation (Fig. 12) are under investigation, but comparative microarrays analyses between cleared fat pad (mammary fat after surgical removal of ductal tree) and contralateral normal glands at various period of pregnancy showed that osteopontin secreted by the ductal cells and the master transcription factor Elf5 could play key roles in the white to pink transdifferentiation (Prokesch et al. 2014).

Thus, physiologic reversible transdifferentiation of adipocytes could be the specific property explaining why cells with different anatomy and physiology are mixed to form a well-defined organ. White, brown, and pink adipocytes in the adipose organ respond not only to the need of partitioning energy between thermogenesis (browning) or metabolism (whitening) for short-term homeostasis (survival of the animal), but also for the needs of offspring survival (pinking) for long-term homeostasis.

Browning Can Curb Obesity and Related Disorders

A growing body of evidences suggest that browning of adipose organ can be used to treat the metabolic syndrome (Nedergaard et al. 2011). Since several decades it was evident that obese animals treated by β3AR agonists undergo browning of adipose

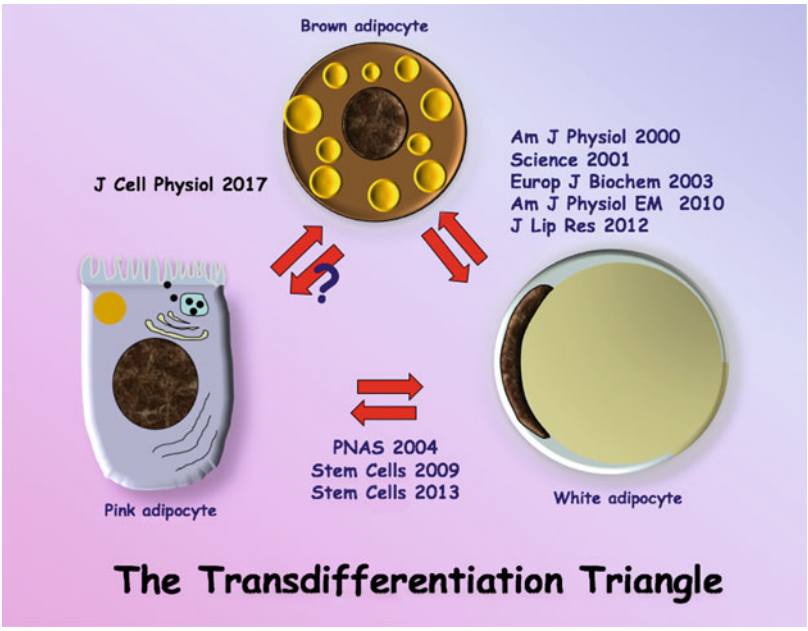

Fig. 12 The transdifferentiation triangle. All published data supporting the physiologic reversible conversions of adipocytes are indicated (From Cinti 2017)

organ and weight loss (Guerra et al. 1998; Tseng et al. 2010). Several data also support positive effects on glucose and lipid metabolism and recently most data have been confirmed in humans (Yoneshiro et al. 2013).

BAT Is Mainly Visceral in Humans

It must be outlined that most BAT in humans is located in visceral fat. PET analyses (Kuji et al. 2008) and biopsy studies (Cinti 2006; Zingaretti et al. 2009) showed that in humans BAT is mainly located in the neck visceral areas (in contact with the visceral and with the neurovascular units) and in the perirenal fat (Cinti 2017).

Visceral BAT Converts into WAT in Obese Mice and Humans

Brown visceral fat convert to WAT with age and weight gain, and this BAT-derived WAT is responsible of most of the adverse effects in obese patients mainly for its low critical death size (Giordano et al. 2016) (see above).

Specific Drugs Able to Convert White Visceral Adipocytes into Brown Adipocytes Are Needed

Thus, specific drugs able to convert visceral WAT to BAT would be very useful not only for their intrinsic potentiality to increase energy expenditure so favoring weight loss, but also because the specific reduction of visceral fat would prevent the adverse effects of visceral fat expansion (Giordano et al. 2016). The general belief that visceral fat is resistant to WAT to BAT transdifferentiation is probably wrong. This idea likely derives from experimental data on visceral fat using epididymal depot as paradigm of visceral tissue. Epididymal fat is indeed resistant to browning, but several other, less studied, visceral depots are much more prone to browning: mediastinal, periaortic, perirenal, periovaric, parametrial, and mesenteric depots showed a high proneness to browning (Vitali et al. 2012).

The Partial WAT-BAT Conversion Could Be Sufficient to Improve the Metabolic State

All data reported above seem to suggest that size of white adipocytes is very important to trigger the adverse metabolic effect of a chronic positive energy balance. The increase in size of adipocytes is particularly dangerous in visceral fat. Pointing to browning of visceral fat could be a strategy to combat the adverse metabolic effects of obesity (Giordano et al. 2016). The most efficient way to obtain browning in small mammals is through the activation of β3ARs. The last generation of β3AR agonists mirabegron activate BAT in humans (Cypess et al. 2015), but its approval only for bladder hyperactivity allows to suspect that clinical trials for the treatment of obesity failed or showed adverse collateral effects. Considering that the first steps of WAT to BAT conversion is the reduction in size of white adipocytes (Fig. 11) and considering the importance of cell size for adipocytes, it could be concluded that a mild activator of β3ARs could realize healthy cell size reduction avoiding collateral adverse effects. The pharmacologic research should point to this direction or to all other emerging alternative mechanisms of browning (also mild browning) not involving this receptor such as natriuretic peptides, BAIBA, irisin, FGF21 (for a recent review see Giordano et al. 2016).

Conclusions

A deep knowledge of anatomy and physiology of adipose organ is very important to understand the physiopathology of obesity and its related disorders. Furthermore, the extraordinary plasticity capacities of this organ offer possible innovative therapeutic strategies that may overcome these pathologies and include unexpected fields of

applications such as those involving the developmental properties of mammary gland.

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