

Adipose Organ Development and Remodeling

This is the peer reviewed version of the following article:

Original

Adipose Organ Development and Remodeling / Cinti, Saverio. - In: COMPREHENSIVE PHYSIOLOGY. - ISSN 2040-4603. - ELETTRONICO. - 8:4(2018), pp. 1357-1431-1431. [10.1002/cphy.c170042]

Availability:

This version is available at: 11566/266552 since: 2022-05-31T08:52:54Z

Publisher:

Published

DOI:10.1002/cphy.c170042

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

Adipose Organ Development and Remodeling

Saverio Cinti^{*1}

ABSTRACT

During the last decades, research on adipose tissues has spread in parallel with the extension of obesity. Several observations converged on the idea that adipose tissues are organized in a large organ with endocrine and plastic properties. Two parenchymal components: white (WATs) and brown adipose tissues (BATs) are contained in subcutaneous and visceral compartments. Although both have endocrine properties, their function differs: WAT store lipids to allow intervals between meals, BAT burns lipids for thermogenesis. In spite of these opposite functions, they share the ability for reciprocal reversible transdifferentiation to tackle special physiologic needs. Thus, chronic need for thermogenesis induces browning and chronic positive energy balance induce whitening. Lineage tracing and data from explant studies strongly suggest other remodeling properties of this organ. During pregnancy and lactation breast WAT transdifferentiates into milk-secreting glands, composed by cells with abundant cytoplasmic lipids (pink adipocytes) and in the postlactation period pink adipocytes transdifferentiate back into WAT and BAT. The plastic properties of mature adipocytes are supported also by a liposecretion process *in vitro* where adult cell in culture transdifferentiate to differentiated fibroblast-like elements able to give rise to different phenotypes (rainbow adipocytes). In addition, the inflammasome system is activated in stressed adipocytes from obese adipose tissue. These adipocytes die and debris are reabsorbed by macrophages inducing a chronic low-grade inflammation, potentially contributing to insulin resistance and T2 diabetes. Thus, the plastic properties of this organ could open new therapeutic perspectives in the obesity-related metabolic disease and in breast pathologies. © 2018 American Physiological Society. *Compr Physiol* vol_number: page_range, year.

Didactic Synopsis

Major teaching points

- Adipocytes are lipid rich cells.
- White adipocytes, organized in white adipose tissue (WAT), store energy allowing intervals between meals.
- Brown adipocytes, organized in brown adipose tissue (BAT), burn lipids for thermogenesis.
- Both WAT and BAT are contained in a dissectible organ.
- The adipose organ shows a similar composition in all mammals, including humans.
- The prevalent tissue is WAT, but BAT is present in several depots.
- The adipose organ is provided with dense vascular and nerve supply especially in BAT.
- WAT and BAT are interconvertible tissues to satisfy specific physiologic requirements: whitening to allow energy storing when the energy balance is chronically positive and browning when thermogenesis is chronically required.
- Remodeling is mainly due to plasticity of parenchymal noradrenergic nerve fibers and hormonal factors.
- During pregnancy-lactation white adipocytes convert reversibly to alveolar cells (pink adipocytes).

- The plastic properties of adipose organ allow energy repartition among three vital needs: metabolism, thermogenesis, and lactation.

Introduction

Obesity and T2 diabetes are linked diseases and the term diabetes has been coined (129). Diabetes has a series of cardiovascular and neoplastic consequences representing major health problems and one of the primary cause of death for humans not only in industrialized countries but also in developing areas of the world (531,971). The inevitable continuous civilization process of humans has enhanced exponentially the two main causes underlining this pathology: low-cost food availability and reduction of physical activity with inevitable positive energy balance outcome.

Therapeutic approaches attempted in the last decades was oriented mainly to drugs with anorectic effects with the goal of calorie intake reduction. This approach has failed mainly because of important secondary effects such as depression and

^{*}Correspondence to cinti@univpm.it

¹Professor of Human Anatomy, Director Center of Obesity, University of Ancona (Politecnica delle Marche), Ancona, Italy

Published online, month year (comprehensivephysiology.com)

DOI: 10.1002/cphy.c170042

Copyright © American Physiological Society.

suicide. This was quite expected considering the importance of food intake rewording effect especially in the main range of age affected by diabetes (i.e., 50-70 years old) (256, 333, 1018). One therapeutic procedure able to obtain long-term effects is bariatric surgery (844), but this is not possible for most people.

Basic science studies are of paramount importance to avoid surgery by discovering the cause and prospecting innovative therapeutic approaches (187).

Adipose tissues anatomy, physiology, and pathology are central for understanding diabetes but have been neglected by scientists for many years. In the last 50 years, many scientific achievements were performed in this field including the discovery that adipose tissues are organized to form a large organ with impressive remodeling capacities. These remodeling properties could represent a valid objective for future diabetes therapeutic approach. This comprehensive review outlines some of the basic aspect of remodeling properties of adipose organ.

Adipose Organ Anatomy

Histology

White adipocyte

For historical reasons, the definition of a cell as an adipocyte is simply related to the conspicuous amount of lipids in the cytoplasm of the cell, without any reference to its function (153, 622, 848).

White adipocytes are capable of storing and releasing highly energetic molecules known as fatty acids. Their morphology is ideal to obtain the highest concentration of molecules used to perform biological energy in a minimum space. As a matter of fact, the best geometrical shape for this achievement is the spherical shape and adipocytes are spherical cells that assume this shape at very early stages of development. Their anatomy is quite simple because about 90% of their volume is occupied by a single droplet of triglycerides (lipid droplet) (Fig. 1). The lipid droplet is contained into the cytoplasm but it is not bounded by a plasma membrane, as are other organelles. At the interface with cytoplasm, the lipid droplet presents a dense line, visible by electron microscopy (Fig. 1), due to the presence of proteins playing important roles in the physiology of fatty acids traffic and metabolism (38, 74, 356, 821). The most studied of these protein is perilipin 1, a lipid droplet coat protein that protect the lipid droplet from lipolysis. It is quite specific for adipocytes and lipolytic enzymes require its phosphorylation to act on triglycerides (821). Fsp27 is another of these lipid droplet proteins and has recently been shown to be essential for unilocular arrangement of cytoplasmic lipids in white adipocytes (352, 1016).

Mitochondria of adipocytes are thin elongated and form a branched network well visible by confocal microscopy techniques (240). Electron microscopy shows their short and randomly oriented cristae. The endoplasmic reticulum is

composed of elongated linear cisternae in the rough part (RER) and small cisternae in the smooth part (SER). This last is often in contact with the lipid surface, suggesting functional relationships (Fig. 1).

The Golgi complex is usually small and located in the perinuclear area. A series of microvesicles of various sizes are often diffuse in the thin rim of cytoplasm.

Several pinocytotic vesicles are present at the cell membrane. On the outer side of the cell membrane, a distinct external lamina is always well visible by electron microscopy (153, 622, 848). Its molecular composition is similar to that of other mesenchymal derived cells, such as muscle cells. Immunohistochemistry data showed the presence of collagen IV, laminin, and heparan sulfate in correspondence of the external lamina in mature human subcutaneous adipocytes (691). In this work, we did not detected immunostaining for fibronectin in line with *in vitro* data showing a strong decrease of fibronectin synthesis during adipocyte development, but other authors have found fibronectin in adult adipocytes contained in bovine intermuscular areas (619).

On the outer side of external lamina fibrillary collagen is well visible by both transmission and high-resolution scanning microscopy (336, 550, 618, 674, 792).

The nucleus is crescent shaped squeezed by the lipid droplet at periphery of the cell both in mature and in developing adipocytes even from very early stages of their development.

The size of mature adipocytes is variable and depends on the technique used for the study. Data from fresh fixed fat are about 20% to 30% larger (169, 282) than those usually reported from paraffin embedded tissue. In adult lean small mammals, the largest adipocytes are usually located in the abdominal subcutaneous and epididymal visceral fat with sizes ranging from 50 to 70 μ m in diameter.

The smallest white adipocytes are usually found in other visceral depots or in intraorgan locations (bone marrow, parathyroid gland, parotid gland, heart, thymus, gastrointestinal tract, skeletal muscles, and lymph nodes). Their size is variable but roughly it can be considered around 2/3 of that of subcutaneous white adipocytes (165).

White adipose tissue

White adipocytes are organized to form a tissue called white adipose tissue (WAT) (Fig. 2) because its color is white in small mammals but in humans, it appears yellow (169). The tissue is composed of several cell types, including vascular cells (endothelium, pericytes, muscle cells, and adventitial cells) and nerve cells (Schwann cells, perineural cells, and neurons) (166). Fibroblasts and immune cells such as macrophages, eosinophils, lymphocytes, and mast cells are the most common interstitial cells found in WAT. Furthermore, a variable amount of adipocyte precursors is usually present in the pericapillary space (552, 683, 878).

All these elements have peculiar ultrastructural features and can be easily distinguished by electron microscope

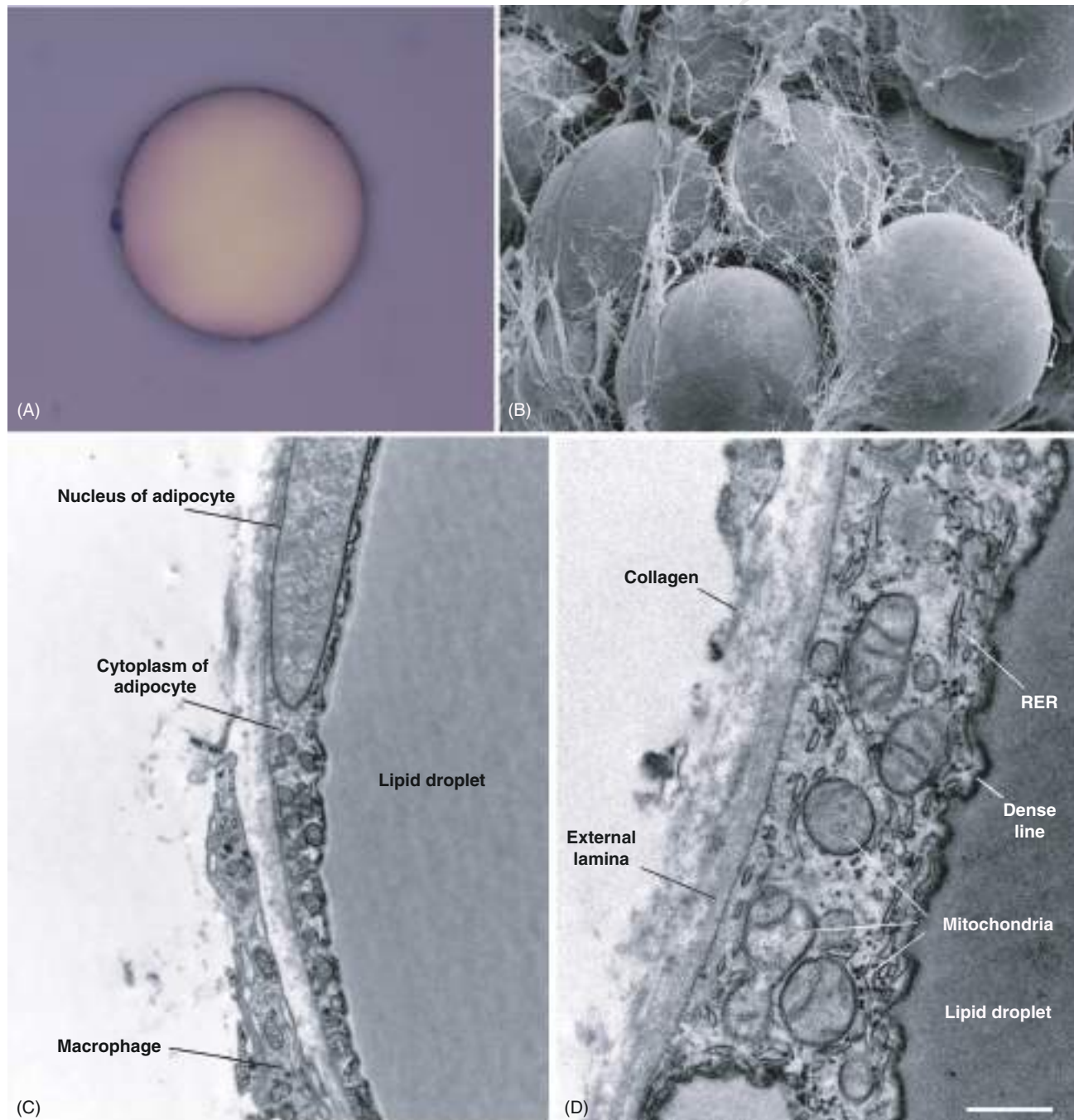


Figure 1 Light microscopy (A) and electron microscopy morphology of isolated human adipocyte (A), murine white adipose tissue (B, scanning electron microscopy), human subcutaneous adipocytes (C and D, transmission electron microscopy). Bar: in A, 15 μm ; in B, 20 μm ; in C, 1.2 μm ; and in D, 0.3 μm . B adapted, with permission, from [167].

analyses (171, 180, 424, 622). Fibroblasts are spindle shaped and characterized by abundant dilated RER. They are responsible for extracellular matrix formation including collagen fibrils. Collagen VI seems to be highly represented in human fat (676, 797). Macrophages are irregularly shaped with elongated sinuous, thin cytoplasmic projections, abundant primary and secondary lysosomes, and well-developed Golgi complex. Eosinophils show classic granules with a discoid

crystal in an equatorial position. Lymphocytes have a very high nucleus/cytoplasmic ratio with characteristic nuclear heterochromatin. Mast cells are roundish, enriched with large cytoplasmic dense and structured granules. All five of these cell types lack an external membrane, which is instead a distinctive feature of adipocytes and adipocyte precursors (281, 622). This last cell is also characterized by high nucleus/cytoplasmic ratio and poorly differentiated

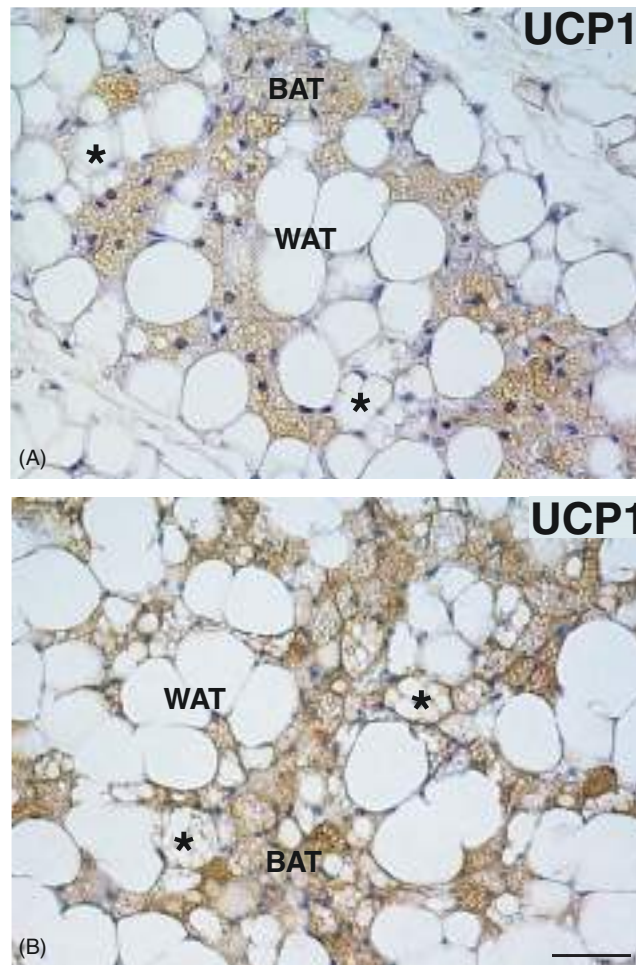


Figure 2 UCP1 immunohistochemistry of mixed (white and brown adipose tissues) areas of murine (A) and human (B) adipose organ. Bar: in A, 35 μm ; and in B, 50 μm .

aspects both in the cytoplasm and nucleus (rich in euchromatin). Small lipid droplets and glycogen granules are often present (see also origin of adipocytes paragraph). Vascular and nerve cells in addition to their specific cellular ultrastructural features present topographic relationships, intrinsic to their anatomical organization, allowing even an easier identification by electron microscope (171).

WAT has a dense vascular and nerve supply, formed by unmyelinated noradrenergic nerve fibers and myelinated sensitive nerves (42,43,45,46,169). Many fibers are present in the perivascular areas but rare small noradrenergic fibers can also be found in the parenchyma (i.e., in contact with adipocytes) (332,335).

Brown adipocyte

Brown adipocytes are polygonal cells smaller than white adipocytes (between half and one third). Their anatomy is quite different from that of white adipocytes. The nucleus is regularly round and usually located in the central part of the

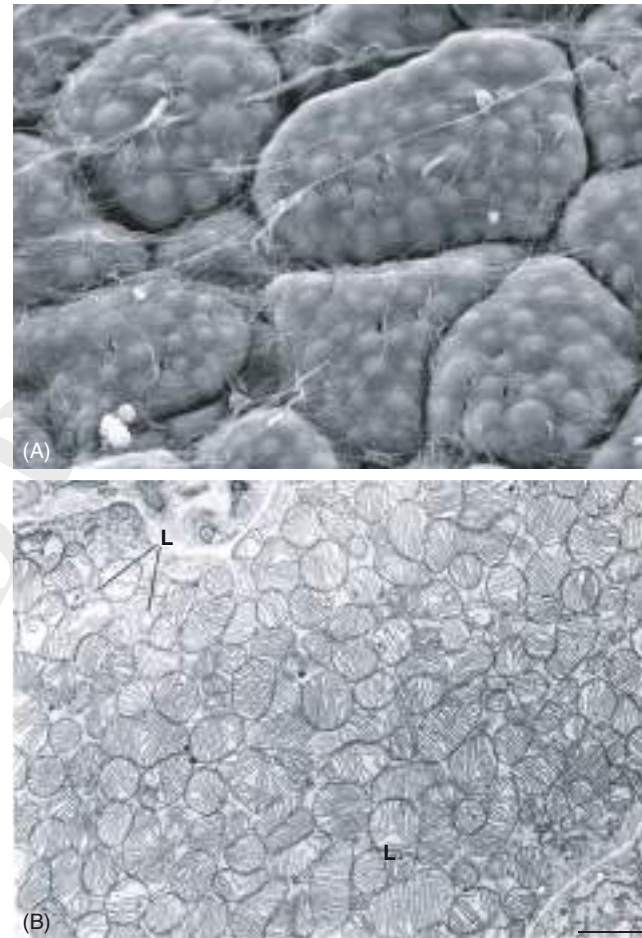


Figure 3 Scanning (A) and Transmission (B) Electron microscopy of murine brown adipose tissue. Bar: in A, 6.0 μm ; and in B, 1.0 μm . A adapted, with permission, from (167).

cell. Cytoplasmic lipids are organized in several small vacuoles (Fig. 2) (169,282,872). In addition, in brown adipocytes, the lipid vacuoles are limited by a membrane-free dense line containing the antilipolysis protein perilipin 1 (74,356). Among the other lipid associated proteins perilipin 5 seems to be more specific of brown adipocytes and other highly oxidative tissues (462). Mitochondria are large, numerous, and packed with lamellar cristae (Fig. 3) and contain a protein called UCP1 that is uniquely found in this cell type and widely considered marker of metabolically active brown adipocytes (110,306,735). Other organelles are similar to those described for white adipocytes, including the external lamina on the outer side of the plasma membrane. Dense granules similar to those found in endocrine cells are often visible in several areas of their cytoplasm (37,169).

Brown adipose tissue

Brown adipocytes are organized to form brown adipose tissue (BAT) (Figs. 2 and 3). The brown color is due to the high density of mitochondria and vascular network. Each

brown adipocyte is in contact with three or more capillaries (624). In some instance, a single adipocyte can surround the whole circumference of the capillary wall with its cytoplasmic projections outlining the paramount importance of brown adipocytes-vessels functional relationships (154,169). As a matter of fact, the capillary network density of BAT is five to six times that found in WAT (624). BAT is also highly innervated (Fig. 4A). Both myelinated and unmyeli-

nated nerves are present in BAT (47,111,229,332). Parenchymal nerve fibers run among adipocytes and confocal and electron microscopy reveal synaptoid contacts between parenchymal nerve varicosities and brown adipocytes (Fig. 4B). Synaptoid varicosities contain empty vesicles and a minority of dense core granules (624,647). Immunohistochemistry data support their noradrenergic content although, in some specific anatomical sites (mediastinum) vesicular

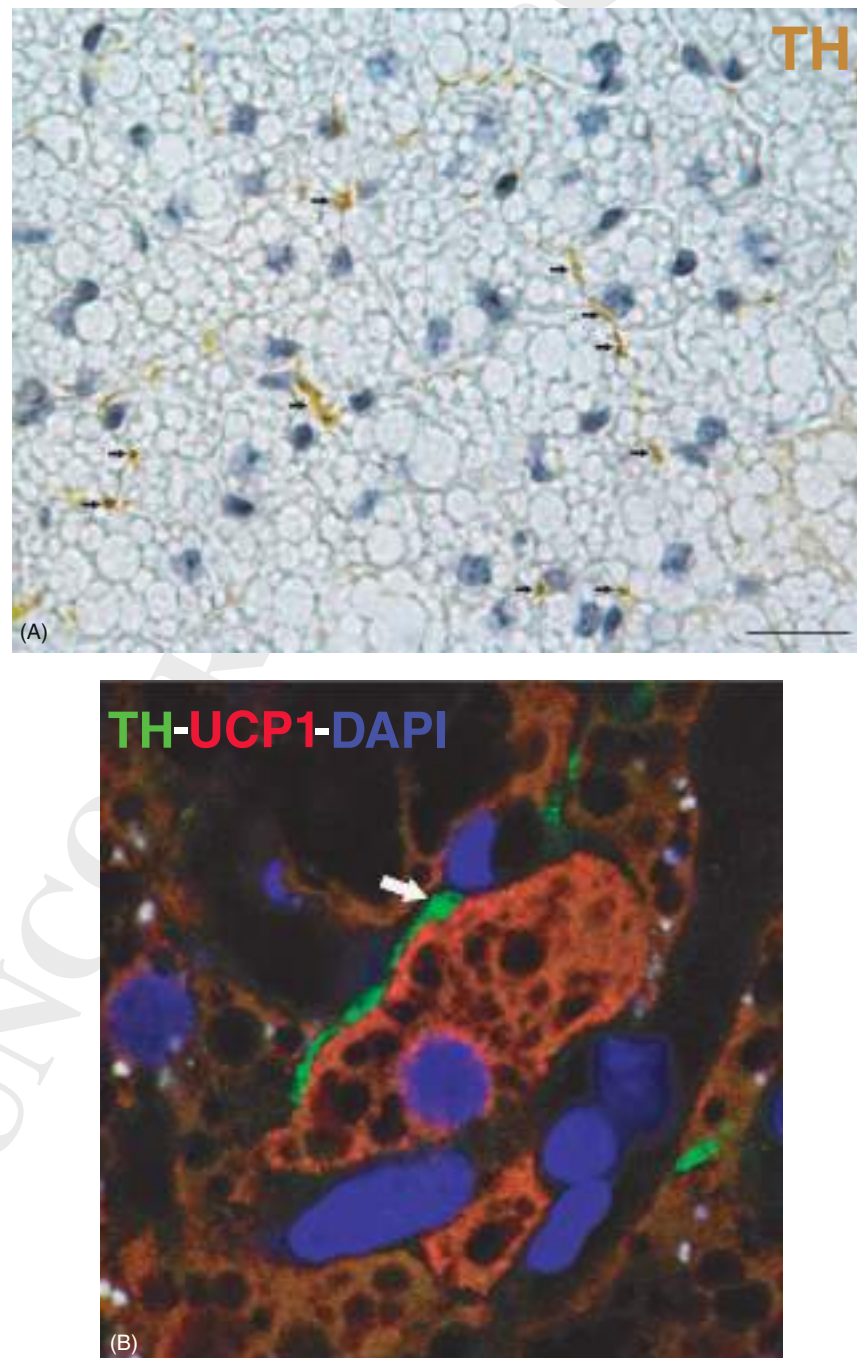


Figure 4 TH Immunohistochemistry (noradrenergic fibers) in murine (A) and human (B) brown adipose tissue. In B (confocal), a double staining UCP1/TH is shown. Bar: in A, 15 μ m; and in B, 6.5 μ m. B adapted, with permission, from (851).

acetylcholine transporter (VACHT) immunoreactivity support the coexistence of cholinergic parenchymal fibers (331).

Gross Anatomy

In anatomy, most of the available definitions of “organ” converge on the followings: a dissectible structure containing at least two different tissues responsible for different functions converging toward a single finalistic purpose. For example, the stomach, that is widely recognized as an organ, is composed by several tissues among which: epithelial glandular cells in the mucosa and muscular layers in its walls. Their functions are different: production of gastric juice (epithelium) and peristalsis (muscles), but both contribute to the final purpose of digestion.

The adipose organ is a dissectible structure

We showed that most of adipose tissues present in the body of mice of both genders and at different ages are contained into a large dissectible structure: the adipose organ (Fig. 5) (153-155, 157-159).

This large organ in lean animals weight about 15% to 20% of the total body weight and is composed by two tissues: WAT and BAT. A total of 60% to 70% of the organ is localized in the subcutaneous compartment where it forms two main depots at the root of the limbs. Thus, anterior and posterior subcutaneous depots (ASC and PSC, respectively) can be easily delineated. ASC is a complex depot located mainly in the dorsal part of the body in interscapular and subscapular

area with cervical and axillary extensions. The subscapular, cervical, and axillary extension should be considered as intermuscular fat, thus not strictly belonging to the subcutaneous compartment. PSC is a band of fat starting in the dorsal area of the trunk and running in the inguinal region to join the contralateral symmetric part at pubic level where it is also continuous with gluteal fat (153-156, 158, 159).

Visceral depots are located in the trunk and surround aorta and its main branches: subclavian, carotid, intercostal, mesenteric, renal, and pelvic arteries. Omental fat is the only depot not in continuity with aorta or one of its branches.

We denoted a single visceral fat depot composed by perirenal, periovarian, parametrial, and perivesical parts as abdomino-pelvic (15, 159).

The adipose organ is mixed: Composed by two different tissues (WAT and BAT)

The relative amount of WAT and BAT of the organ is variable: depending on age, gender, strain, and by environmental and nutritional conditions (90, 177, 328, 364, 535, 537, 586, 614, 736, 793, 950).

Quantitative analyses showed that the adipose organ of adult female Sv129 and C57BL/6 (B6) mice maintained at 28°C for 10 days have a relevant percentage of its parenchyma composed of multilocular adipocytes: that is, about 55% in Sv129 and about 30% in B6 mice (614, 950). About 90% and 50% of these multilocular adipocytes resulted UCP1 immunoreactive in Sv129 and B6 mice, respectively.

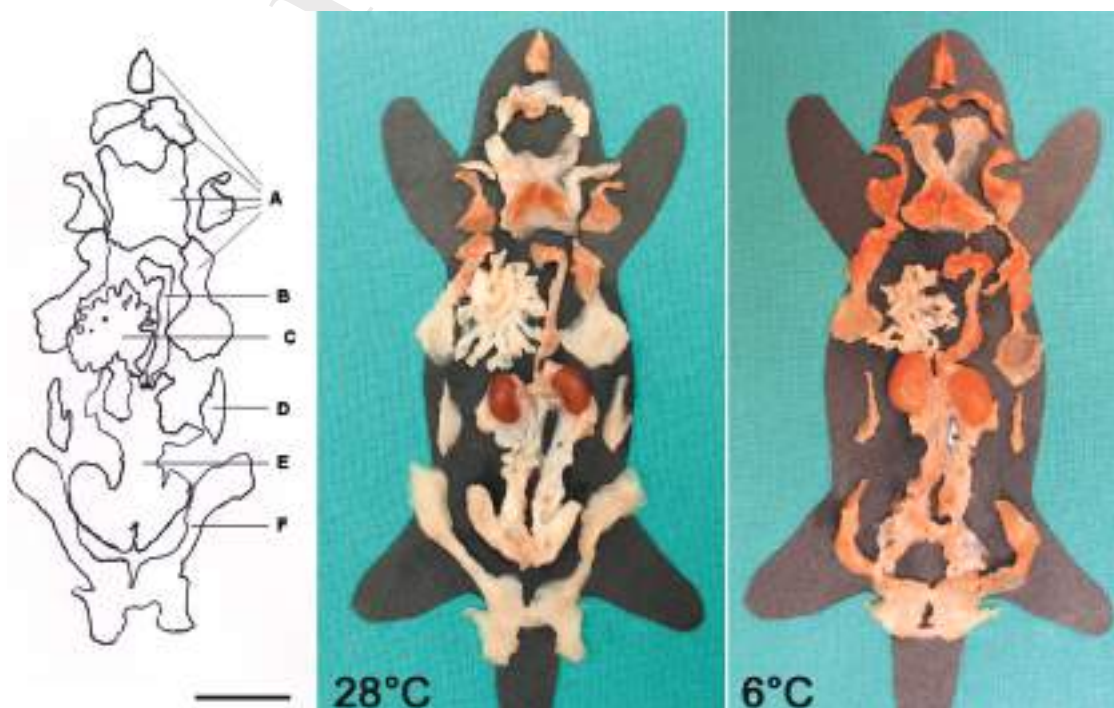


Figure 5 Murine adipose organ gross anatomy of warm and cold acclimated Sv129 adult mice. (A and F) Subcutaneous depots B to E visceral depots. Bar: 1 cm. Adapted, with permission, from (614).

Thus, parenchyma of the adipose organ of adult mice maintained at a temperature near thermoneutrality is mixed: composed by white (unilocular UCP1 negative), brown (multilocular UCP1 positive), and multilocular UCP1 negative adipocytes (ML/UCP1-). Of note, this last type of adipocyte has a range of morphology: from very similar to the brown to very similar to white adipocytes. The variable morphology visible at light microscopic level is due to variation in size of the cells and type of multilocularity. White adipocytes are larger than brown adipocytes and the largest ML/UCP1-cells approached the size of white adipocytes (Fig. 2). Their cytoplasmic lipids are organized to form a predominant central vacuole surrounded by several smaller vacuoles. We denoted this cell type as paucilocular cell (36). The smallest ML/UCP1- cells show a size similar to that of brown adipocytes and cytoplasmic lipids in form of regular small lipid vacuoles similar to those of brown adipocytes. A range of intermediate forms between the largest and smallest ML/UCP1- cells is always visible. Mitochondria of brown adipocytes exhibit a typical morphology: roundish, large, and packed with laminar cristae (181,283,623,794). ML/UCP1-adipocytes show mitochondria with a range of morphology from the typical brown mitochondria to the nontypical morphology of white mitochondria (i.e., mitochondria of white adipocytes: elongated with few, randomly oriented cristae) (Figs. 1 and 3). Of note, the more the ML/UCP1- adipocytes approach the morphology of brown adipocytes, not only the morphology of the mitochondria approached that of typical brown mitochondria, but also their density in the cytoplasm increase (36,428). Thus, all these morphologic and immunohistochemistry data support the idea that ML/UCP1-adipocytes are intermediate forms between white and brown adipocytes.

The distribution of brown adipocytes in the different areas of the adipose organ is similar in the two strains: in both the largest number of brown adipocytes was found in the ASC: about 80% in Sv129 mice and 65% in B6 mice. The high density of brown adipocytes in this region account for the brown color of several parts of ASC. The remaining brown adipocytes are located in the periaortic mediastinal fat and in the perirenal fat of both strains but also in perigonadal fat of Sv129 mice.

White adipocytes are mainly present in PSC and abdominal visceral depots: mesenteric, omental, perivesical, and retroperitoneal fat, thus the color of these areas is mainly white.

ML/UCP1-adipocytes with all the morphologic range are present in all transitional areas at the boundaries between WAT and BAT.

Vascular and nerve supply

The adipose organ is supplied by nerves and vessels usually reaching the organ in peduncles that can be easily isolated and dissected (169). We found vascular-nervous peduncles at the peripheral extremity of lateral wings of the interscapular fat

of ASC, at the dorsal extremity of PSC, at the middle of the inguinal part of PSC. Isolated single nerves reach the organ in the ventral interscapular part of ASC and the lower part of the retroperitoneal fat. Many other diffuse small vascular-nervous peduncles and isolated nerves reach other areas of the organ.

Immunohistochemistry and ultrastructural studies of nerves in the adipose organ showed that it is provided with myelinated and unmyelinated nerves both in WAT and BAT areas (42,43,45-47,296,335,340,826). Myelinated are larger than unmyelinated nerves. Myelinated nerves are immunoreactive for Calcitonin-Gene-Related-Protein (CGRP) and Substance P (SP) both considered markers of sensitive nerves. The unmyelinated small nerves are immunoreactive for Tyrosine Hydroxylase (TH), considered a marker of noradrenergic nerves. In the vascular wall, Neuronal Peptide Y (NPY) in BAT areas have been identified (111). We found VACHT immunoreactivity (marker of parasympathetic fibers) in parenchymal nerves of BAT exclusively in mediastinal area (331).

Unitary finalistic purpose

Thus, the anatomical requirements to classify this structure as an organ seems to be fully satisfied but the cooperation between WAT and BAT to a common finalist role remains to be elucidated. In the next section, dynamic data of the organ (remodeling) offer an explanation for this functional aspect.

Development of WAT and BAT

The adipose organ is mixed, but many areas of ASC are formed by pure BAT and some areas of visceral depots, such as epididymal fat, are composed by pure WAT.

Most of the historical work on the development of BAT and WAT refer to these two anatomical sites to describe the developmental aspects of these two different tissues.

WAT

The murine fetal epididymal WAT anlage is formed by a classic loose mesenchymal tissue without any specific characteristics. At postnatal days 4 to 6 the morphology changes into a very characteristic tissue formed by vasculo-adipocytic islets well delimited by fibroblast-like cells forming a clear boundary from the rest of the tissue that remain loose and poorly differentiated (Fig. 6) (305,916). Instead, inside the islets large capillaries are surrounded by numerous pericytes and white adipocyte precursors at various stages of differentiation. White precursors are never found outside the islets strongly suggesting that the environment inside the islets is necessary for the precursors development. Apart from the cellular composition aforementioned described, one of the main difference between the inside and outside interstitial space is in the matrix composition: the collagen fibrils density is very high inside and loose outside.

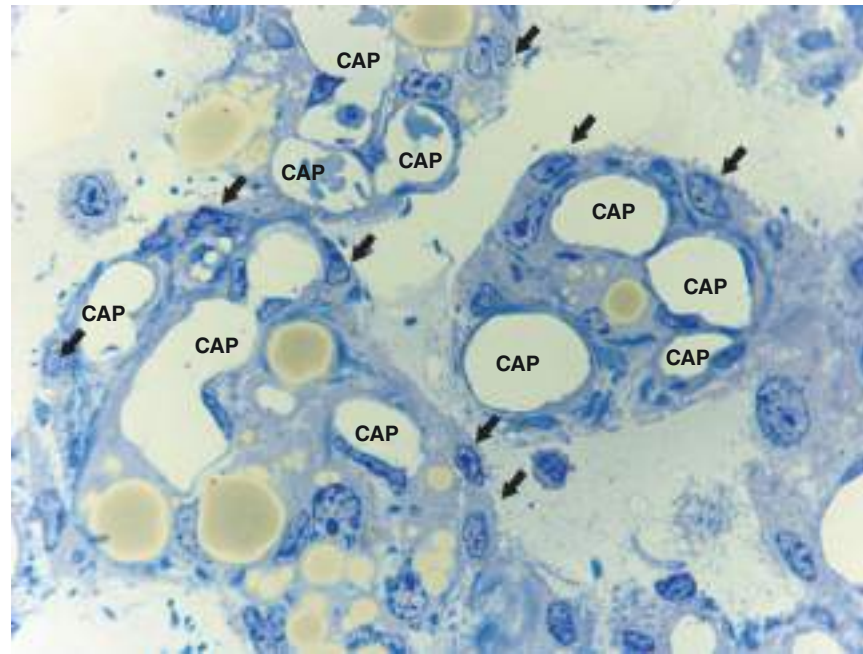


Figure 6 Vasculo-adipocytic islets of epididymal white adipose tissue from a newborn rat. Light microscopy. Bar: 7.0 μ m. Adapted, with permission, from (169).

Thus, the islets result formed by a dense network of large capillaries rich in pericytes and immersed in a dense collagen stroma in which several steps of adipocyte development can be observed (Fig. 6). Together with these cell types, mast cells and macrophages are often present. Adipocyte precursor morphology is well established after many years of both *in vivo* (171, 622, 848) and *in vitro* studies (171, 172, 629, 847, 939). At early stages of differentiation, poorly differentiated cells, always in tight connection with capillary wall and surrounded by a distinct external lamina show high nucleus/cytoplasm ratio, few nontypical elongated mitochondria, glycogen granules, and few lipid droplets (Fig. 7A). Usually lipid droplets tend to coalesce quickly and most of the precursors assume a unilocular aspect with crescent-shaped nucleus even at early stage of differentiation when the cell is still small (5–10 μ m in diameter) (Fig. 7B).

These white precursors show nuclear immunoreactivity for pRb (381) (a tumor suppressor protein playing also an important role in cell cycle and differentiation) (385, 528), -C/EBP α , -C/EBP β , and PPAR γ 2 proteins and cytoplasmic immunoreactivity for S-100b (a multifunctional Ca²⁺-binding protein) (173, 916).

The main morphologic aspect of development from early unilocular precursors and mature white adipocytes is the progressive enlargement of lipid droplet until the size typical for the specific fat depot: usually smaller in visceral than in subcutaneous fat (169, 275). This lipid droplet enlargement is accompanied by a progressive reduction in thickness of the cytoplasmic rim with apparent reduction in number and size of mitochondria (169).

Early precursors are very similar to pericytes (171, 622, 848, 916). In murine pericytes, lipid droplets are not found, but glycogen particles are often present. Pericytes are characteristically included in doubling of the basal membrane of capillaries. Some pericytes show an “extruding” morphology: that is, part of the cell included in the doubling of the capillary basal membrane and part of the cell abutting toward the interstitial space (153, 169, 916). These pericytes usually show cytoplasmic glycogen particles and could represent early stages of preadipocytes detaching from the capillary wall. Preadipocytes, at different developmental stages, are usually in contact with capillary walls with those less differentiated usually closest to the capillary wall. Of note, rare endothelial cells are in part in pericytic position (endothelial-pericytic cells) (169, 916).

Pericytes and endothelial cells are joined by gap junctions in the cytoplasmic projections apparently linking these cells (916). The physiologic significance of these joining projections is unknown, but can be found also in models of adipogenesis from tissue explants (916). Some endothelial cells containing glycogen clusters similar to those of pericytes are often found in developing epididymal fat at this stage (916).

BAT

In mice and rats, the first fat anlage, visible by light microscopy, appears around the 15th gestational day in the subscapular area of murine fetuses (169, 644, 834). In this area, dorsal skeletal muscles border a loose mesenchymal tissue characterized by the presence of a network of capillaries. Most

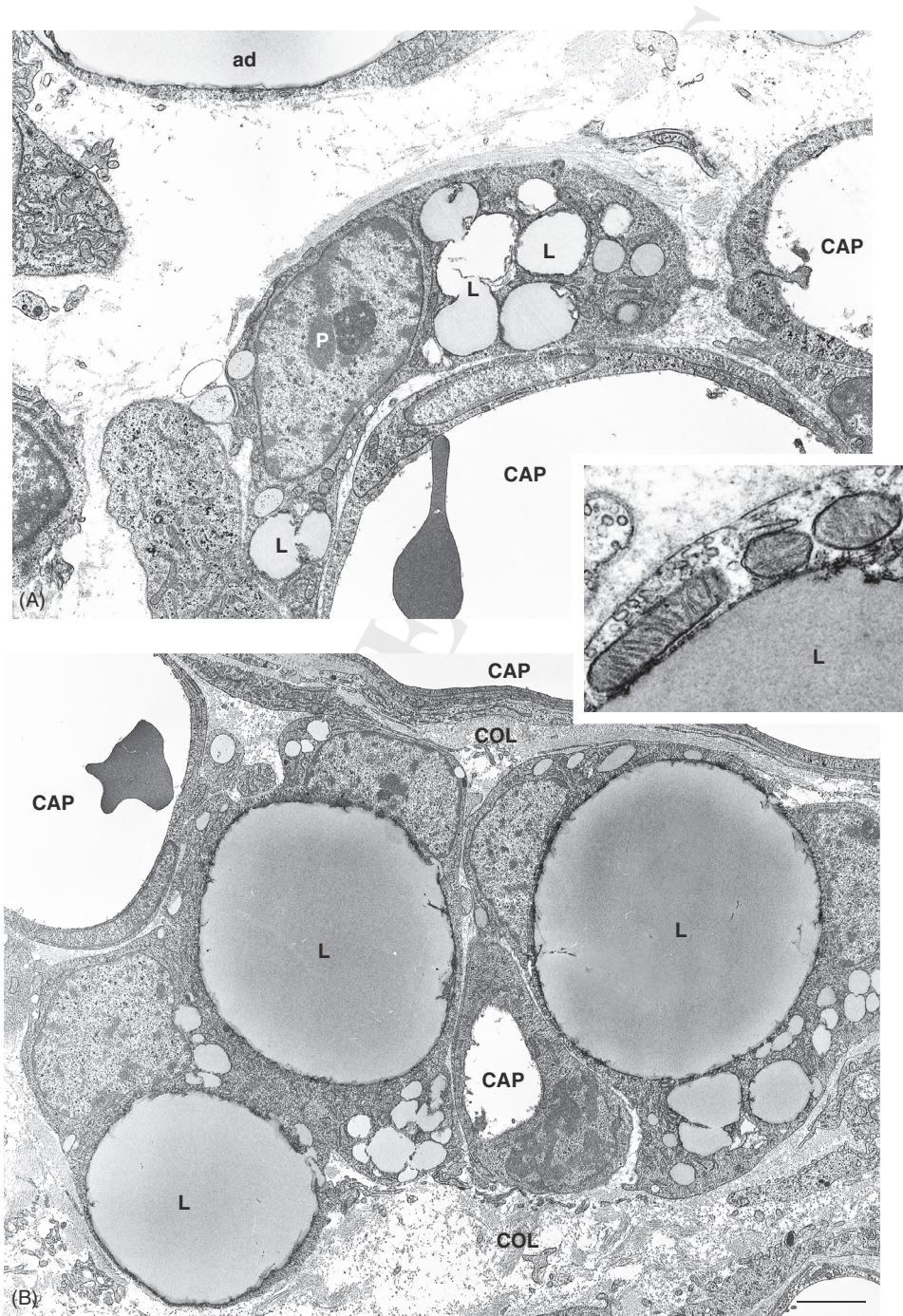


Figure 7 Transmission electron microscopy of vasculo-adipocytic islets shown in Figure 7. Bar: in A, 1.5 μm ; and in B, 3.0 μm , in inset 0.5 μm .

of them contain also well-developed pericytes lining the capillary wall. A few interstitial cells are also present. Thus, at this very early stage of BAT anlage development three cell types are recognizable: endothelial cells, pericytes, and interstitial cells (Fig. 8A). At the 18th to 19th gestational day, the anatomy of the anlage is strikingly changed mainly because the loose tissue is substituted by a parenchymal-like tissue, filling up the meshes delimited by the capillary network (Fig. 8B). The cells

forming this parenchymal-like tissue, often in mitosis, have the classic ultrastructure of brown precursors, with numerous large pretypical mitochondria (similar to those of mature brown adipocytes and often with large dense matrix granules) dispersed in a ribosome and polyribosome-rich cytoplasm (characteristic of poorly developed cells) (281). Large dense matrix granules of pretypical mitochondria have been suggested to be involved in cristae formation (37). 20-30% of

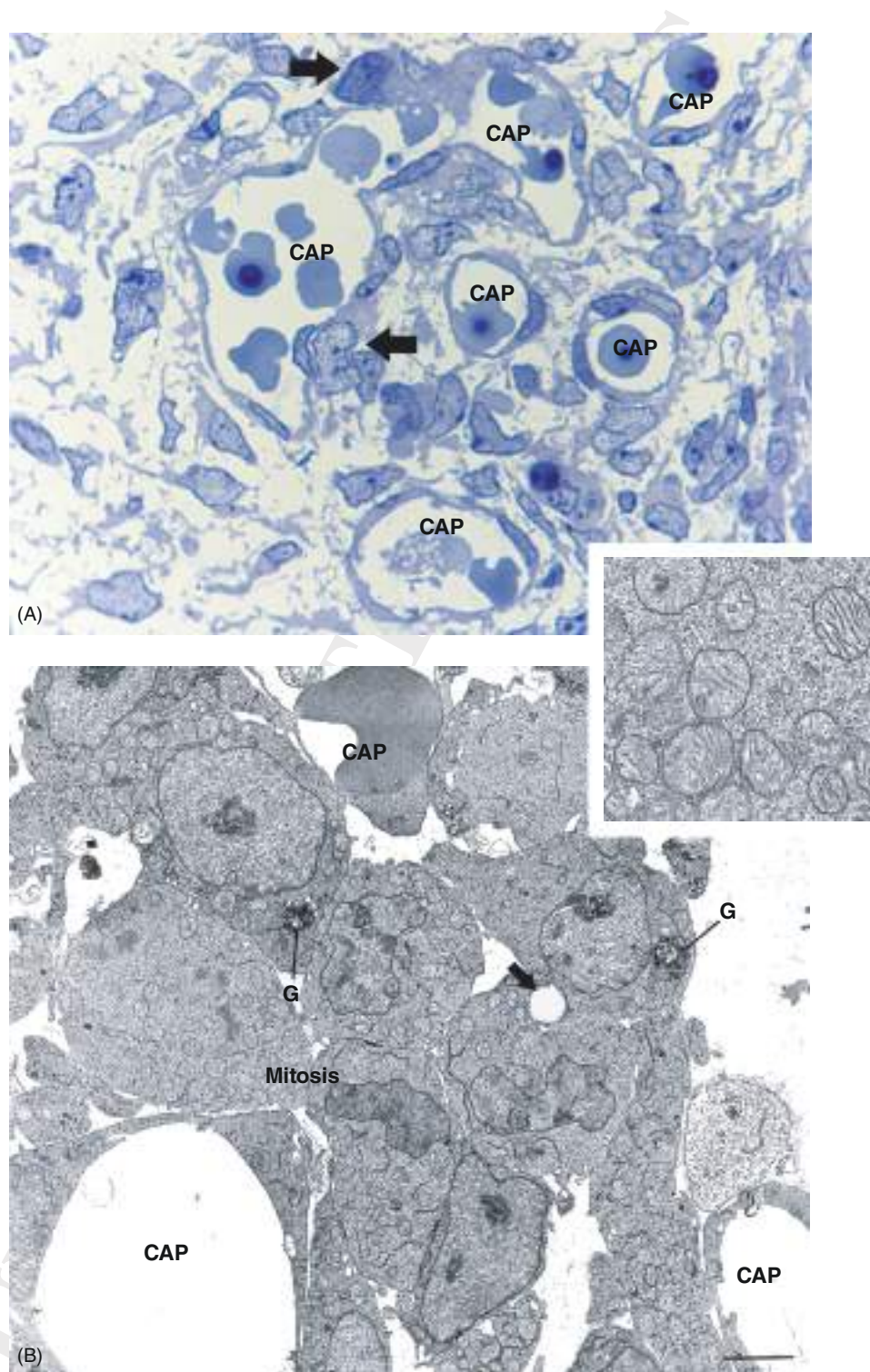


Figure 8 Rat brown adipose tissue anlage at E16 (A, light microscopy) and E18 (B, electron microscopy). Inset: enlargement of electron microscopy showing pretypical mitochondria. Bar: in A, 6 μm ; and in B, 2 μm , in inset 0.6 μm .

these brown precursors show roundish clusters of glycogen and small lipid droplets, indicating adipose differentiation (153, 357, 358, 848) (Fig. 8B).

Immunohistochemistry revealed that brown precursors at this stage of development stain positive for PPAR γ , C-EBP α ,

and β proteins in their nuclei and for UCP1 in their cytoplasm (916). At this developmental stage, brown precursors are not immunoreactive for Retinoblastoma (pRb) (381) or S-100b protein (35, 169, 250), proteins that are hallmarks of early white preadipocytes (see the preceding text) (173, 381, 585).

Many pericytes have an ultrastructure very similar to that of brown precursors, including pretypical mitochondria, and shared with them the nuclear immunoreactivity for the aforementioned transcription factors known to be typical of brown precursors from *in vitro* studies (180, 423, 916). Immunogold staining revealed the presence of UCP1 protein in the mitochondria of brown precursors and pericytes (916). Rare endothelial cells (1%-3%) show large mitochondria similar to those of pericytes and brown precursors (180).

By fetal days 20 to 21, just before birth, the morphology of brown precursors progressively approaches that typical of mature brown adipocytes (169).

Thus, morphology of white precursors is quite different from that of brown precursors and the main differences are in mitochondria (pretypical in brown, not typical in white) and in the lipid droplets (small and numerous in brown and unique in white) reflecting the morphologic differences of adult cells. Furthermore, immunohistochemistry revealed similarities in immunoreactivity for some transcription factors (C/EBP β , α and PPAR γ) in line with *in vitro* studies of adipogenesis (761) and differences for nuclear (pRb), cytoplasmic (S-100b), and mitochondrial (UCP1) proteins (35, 173, 381, 916). In both cases, preadipocytes are easily recognized by their specific features that make them distinct from other cell types (fibroblasts, macrophages, mast cells, lymphocytes, and granulocytes) that can be found in fat during development. The specific morphologic features are: a distinct external lamina

surrounding a poorly differentiated cell with early signs of adipocyte development (37, 171, 357, 622, 848).

These data, in line with many other studies (368, 404, 505, 506, 508, 814), strongly point to the vascular wall as the site of origin for both white and brown adipocyte precursors.

Biogenesis of adipocytes

Old and recent studies seem to converge on the idea that the vascular wall of adipose tissue capillaries could represent the structure from which the committed adipocyte precursor both during adipose organ ontogenesis and in the adult life arise (57, 171, 890). Only two cell types form the wall of capillaries: endothelial cells and pericytes. Both cells can show morphologic evidence of some aspects of adipocytic differentiation (external lamina, glycogen clusters, pre-typical mitochondria), but immunoreactivity for markers of adipocytic commitments are found in pericytes (204, 505-508) and not in endothelial cells, although some rare endothelial cells can contain morphologic aspects (glycogen clusters, pretypical mitochondria) (180, 916) or proteins such as Zfp423 (369) (see also Fig. 9 suggesting a possible role of endothelial cells for both white and brown adipocyte precursors).

Lineage tracing is a potent technique to follow the developmental destiny of a specific cell type and it is based on the expression of reporter genes driven by the activation of gene promoters that can not only be cell specific (if a gene is

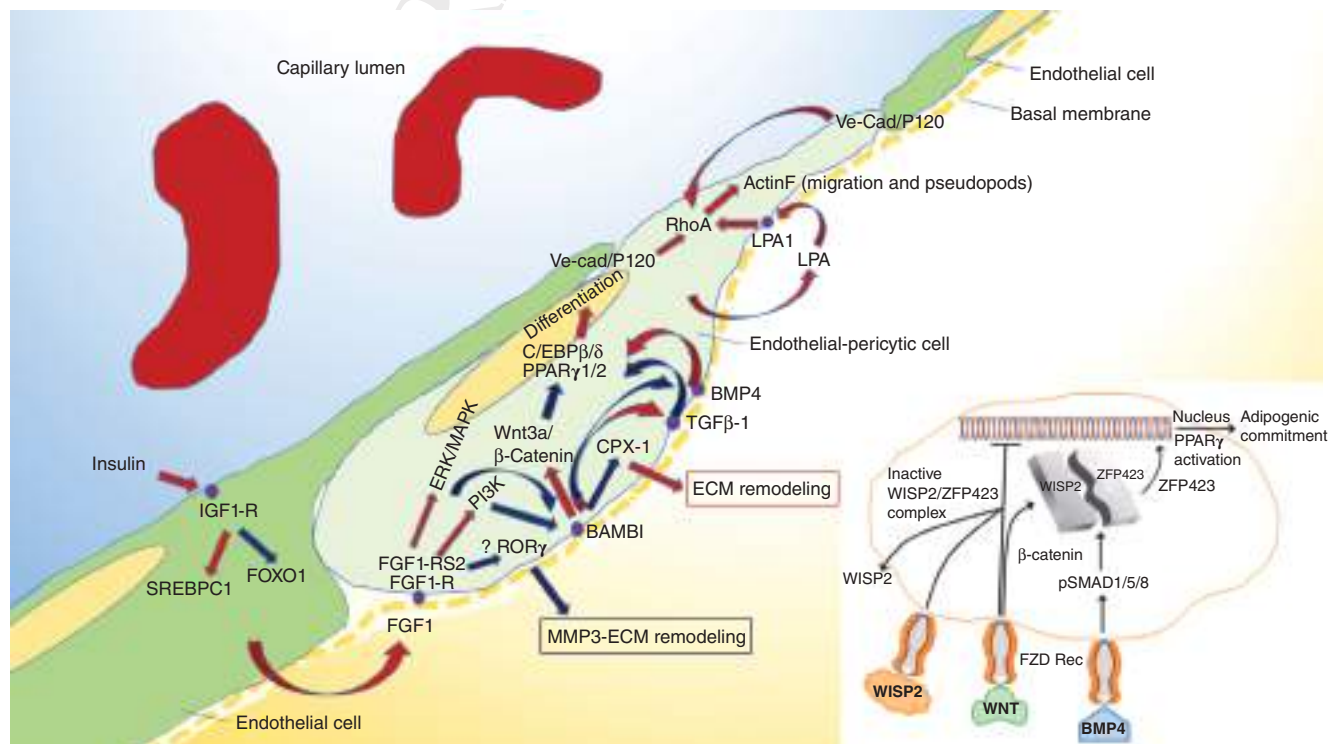


Figure 9 Scheme showing a hypothesis of molecular signaling inducing endothelial-pericyte-preadipocyte conversion. Inset adapted, with permission, from (378).

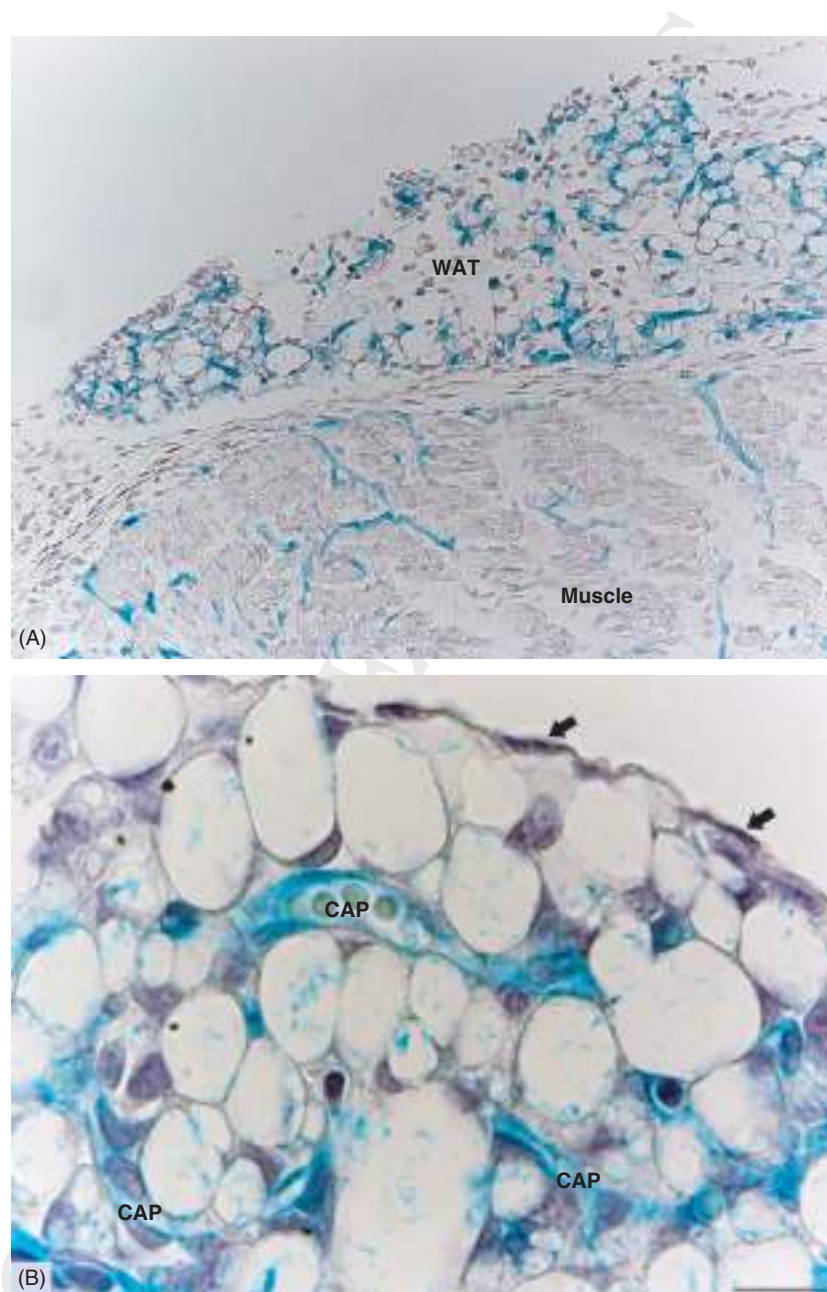


Figure 10 Inguinal adipose tissue from a Ve-Cad/Cre/R26R mouse showing lineage-tracing evidence of endothelial origin of adipocytes. Bar: in A, 330 μ m; and in B, 15 μ m.

expressed uniquely in that cell type), but also temporally specific (if a gene is expressed uniquely during a specific period of time) (854,911).

We used VE-Cadherin-Cre/R26R mice that express the reporter gene (β -galactosidase) only in endothelial cells of all tissues in the body. In the original work Alva et al. (12) demonstrated that in these mice, none of the parenchymal cells of the organs studied (heart, kidney, brain, lung, intestine, retina, uterus, tongue, ovary, testis, salivary gland, mammary gland, adrenal, skeletal muscle, sclera, and choroids, and mesentery) expressed the reporter gene, with the exception of hematopoietic bone marrow stem cells (1021). In our experiments,

both white and brown adipocytes of VE-Cadherin-Cre/R26R mice resulted marked by the reporter gene suggesting an endothelial origin of adipocytes in line with the morphologic data described in previous paragraph (Fig. 10A and B) (305,916).

In line with our data, Shan et al. identified aP2-expressing adipocyte progenitors in SVF and endothelial cells of both WAT and BAT (814). Furthermore, in a recent study, perilipin+/adiponectin+preadipocytes were found to emerge at embryonic day 16.5 in inguinal WAT and proliferated to form clusters strongly interacting with growing adipose vasculature: that is, endothelial-specific *Vegfr2* depletion induced

vascular disruption with interruption of *in vivo* adipogenesis (404).

Capillary networks and single cell suspensions from microvessels of human fat explants give rise to well characterized adipocyte progenitors able to develop into mature adipocytes in a cell-autonomous manner (591).

In line with these data, isolated mature human adipocytes dedifferentiate into endothelial-like cells (125, 694, 697) and endothelial cells can be converted into mesenchymal stem cells which can differentiate into adipocytes, chondrocytes, and osteoblasts (581).

The endothelial origin would imply endothelial-mesenchymal transition (EndMT) that is linked to BMP/TGF β signaling (554, 1007) and absence of the downstream effector myocardin-related transcription factor A seems to induce commitment of progenitors to adipogenesis (578).

Following the observation that interstitial cells, distinct from muscle satellite cells, in muscles have efficient adipogenic potential both *in vivo* and *in vitro* (432, 928), Granneman and colleagues showed that similar cells in abdominal visceral fat have a bipotential possibility to develop into white and brown adipocytes under appropriate stimuli *in vivo* (507).

This cell type has been described as stellate interstitial cells (PGFR α +, CD34+, CD24-, IB4-, and PDGFR β -) provided with multiple thin cytoplasmic processes of about 50 μ m in length located in close proximity to stromal cells, adipocytes, and capillaries in developed WAT. Similar CD34+ cells were described in human and murine subcutaneous fat (573). Their role as brown adipocyte precursors in WAT have been supported by experiments showing that suppression of the PDGFR β + population of stromal cells give rise to a compensatory proliferation of PDGFR β α + cells ending in browning of WAT (214).

Recent data from human patients as well as data from murine experiments seem to support the bone marrow origin of at least a subpopulation of fat adipocytes (205, 323, 772) thus, a link between the hematopoietic multipotent population and endothelial cells with the potential ability to give rise to both white and brown preadipocytes and adipocytes cannot be excluded.

Vascular endothelium and pericytes seems therefore maintain the ability to differentiate into adipocytes, but other experimental data suggest that mesothelial cells can be the progenitors of visceral adipocytes (133), and other data seem to deny any role for endothelial cells (58) thus, the final picture of the origin question still offer the opportunity for future studies.

Molecular Mechanisms

White Adipocytes

Differentiation

Development of a new adipocyte is due to adipogenic induction of adipose stem cells and subsequent activation of differentiation signaling. Studies of white adipogenesis

in vivo and *in vitro* have described a complex differentiation signaling. This transcriptional cascade involves the master regulator peroxisome proliferator-activated receptor γ (PPAR γ), with its isoforms 1 and 2 and its heterodimeric partner RXR (retinoid \times receptor), and three members of the CCAAT/enhancer-binding protein (C/EBP) family—C/EBP δ , C/EBP β , and C/EBP α —that are activated sequentially (276, 421, 509, 757, 908). A recent paper elegantly confirmed the important role of PPAR γ for both WAT and BAT differentiation in all anatomical sites using adiponectin-fat-specific knockout mice (955), but accurate comparison between *in vivo* and *in vitro* molecular mechanisms suggests peculiar aspect for *in vitro* adipogenesis (150).

C/EBP β and C/EBP δ are induced transiently in the early steps and are considered to play key roles during the initiation of the adipogenic program. The two C/EBPs acts in cooperation and mice lacking both C/EBP β and C/EBP δ have a severe lipotrophic phenotype (889). C/EBP β and C/EBP δ activation induces C/EBP α and PPAR γ expression that are responsible for terminal adipocyte differentiation (510). In this complex network, the homeobox gene Hoxc8 suppresses C/EBP β and this activity is very important to induce the white phenotype (327).

Other C/EBP isoforms such as CHOP (transcription factor homologous to CCAAT-enhancer binding protein) and C/EBP γ , seems to suppress adipogenesis, perhaps through heterodimerization or inactivation of C/EBP β (215).

Among other transcription factors required for adipocyte differentiation cAMP regulatory element-binding protein (CREB) seems to play an important role (731) and the E3 ubiquitin ligase murine double minute 2 (Mdm2) seems to be also involved in adipogenesis by promoting cAMP-mediated transcriptional activation of CREB and induction of C/EBP δ expression by facilitating the recruitment of CREB-regulated transcription coactivator (Crtc2) to a cAMP-response element (CRE) in the promoter of *c/ebp δ* (376).

In addition to C/EBPs factors, Krupper-like factors (KLFs) have been shown to activate at least one of the two PPAR γ promoters even if the relative roles of PPAR γ 1 and PPAR γ 2 remain to be elucidated (758).

KLF 4, 5, 6, 9, and 15 have been showed to promote adipose differentiation with different mechanisms but KLF2, 3, and 7 are antiadipogenic factors (32, 603, 655, 836). It has been proposed that appropriate exchange of these factors occur during adipogenesis (655). Many other factors are claimed to have a positive or negative role in this complex network of molecules interacting during adipogenesis are shown in Table 1 (544, 702, 758, 859).

Determination

Determination of stem cells toward adipose lineage is due to extracellular signaling acting on adipose stem cells upstream to the differentiative PPAR γ -CEBPs transcriptional cascade described above. The nature of adipose stem cell *in vivo* is debated but the cell population of vascular walls of adipose

Table 1



AU: Please provide the caption of Table 1.

Transcription factors

BMAL1	(Brain and muscle Arnt-Like protein-1, component of the molecular clock) Induces the expression of several factors involved in lipogenesis with circadian rhythm (452, 827).
EBF1, 2, and 3	(Early β -cell factor 1, 2, and 3). Control of genes of terminal differentiation. Direct action on PPAR γ 1 and C/EBP α promoter (7, 429).
EPAS1	(Endothelial-PAS superfamily, also known as hypoxia-inducible factor 2alpha). Promotes adipogenesis through glucose uptake and lipid synthesis (828).
GATA2 and 3	Repress adipogenesis inhibiting PPAR γ 2 and C/EBPs (905, 906).
KROX20	(Early growth response protein-2). Promotes C/EBP β (64, 141).
LKR α and β	(Liver X receptors). Their role is controversial (326, 417, 765, 812).
SREBP1c/ADD1	Induces PPAR γ . Promotes insulin-mediated lipid synthesis (458, 907).
STAT5a	(Signal transducer and activator of transcription 5). Binding to subunits of the pyruvate dehydrogenase complex (PDC) that converts pyruvate to acetyl-CoA. PDC may modulate STAT5's ability to regulate gene expression by controlling histone or STAT5 acetylation (293, 621, 732).

Transcriptional, translational cofactors, and enzymes

CBP	(CREB-binding protein) histone acetyltransferase (HAT) modifies chromatin directly promoting adipogenesis (989).
COUP-TFII	The orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II (or Nr2f2) repressor of adipogenesis with direct action at the C/EBP α promoter (983).
ETO/MTG8	Corepressor acting on C/EBP β antiadipogenic action (745).
Fbxw7	(Human CDC4) is the substrate recognition component of a specific SCF ubiquitin ligase that targets for degradation C/EBP α (49).
HDACs/Sirtuins	(Histone deacetylases)/(HDAC activity): antiadipogenic action (690, 998).
HRASLS3	(Phospholipase A2 superfamily). Target of PPAR γ /RXR heterodimers. Induces adipogenesis (416).
IRFs	(Interferon regulatory factors). Repressors of adipogenesis (257).
NCoR/SMRT	(Nuclear receptor corepressor)/(Silencing mediator of retinoid and thyroid hormone receptors) antiadipogenic action (1001).
p160 family	Scaffold to recruit chromatin modifier and HAT (543).
p300	HAT modifies chromatin directly promoting adipogenesis (884).
PLZF	Member of the BTB/POZ-ZF [Broad complex, Tramtrack, Bric à brac (BTB) or poxvirus and zinc finger (POZ)-zinc finger] protein family. Repressor of adipogenesis by recruiting nuclear receptor co-repressors (N-CoRs) and histone deacetylases (HDACs) (587).
SRF	(Serum response factor) a MADS-box transcription factor repressor of adipogenesis (587).
Slk40	(serine/threonine kinase 40) repressor of translational control of C/EBP β and δ (1002).
SWI/SNF complexes	(ATP-dependent chromatin remodeling proteins) bind to and serve as co-activators of many nuclear receptors, including estrogen, glucocorticoid, retinoic acid receptor families, and PPAR γ (233).
TAF8	(TATA-binding protein-associated factor-8, basal promoter binding factor) promote adipogenesis (363). Unclear the transcription factor interactive.
TRAP220	(Or PBP PPAR-binding protein) binding partner of PPAR γ promote adipogenesis (324).

Cell cycle-related proteins

CDK4	Activates PPAR γ without its phosphorylation (69). It promotes anabolism by blocking catabolic processes (541).
CDK6	(Cyclin D3-cyclin-dependent kinase-6) complex phosphorylate PPAR γ increasing its transcriptional activity (784).
Cyclin D1	Repress PPAR γ (309).
Cyclin G2	Regulates adipogenesis through PPAR gamma coactivation (5).
E2F1	Induces PPAR γ transcription during clonal expansion (273).
E2F4	Represses PPAR γ expression during terminal adipocyte differentiation (273).

Cell fate coactivators

HIC5	Binding partner of PPAR γ in colonic epithelium. Direct cell fate to epithelial phenotype and drastically drop during adipogenesis (252).
Lats2	(Large tumor suppressor kinase 2) is one of the core kinases of the Hippo pathway and its action ultimately inactivate TAZ and reduces cyclin D1 promoting adipogenesis (13).
TAZ	(Transcriptional coactivator with PDZ-binding motif) interact with PPAR γ to inhibit adipogenesis and allowing osteogenesis (102, 387).
TIP1/3	(Tension-induced/inhibited protein-1/3. TIP1 recruits HAT activity to muscle-specific promoters (myogenesis), TIP3 recruits HAT activity to Ppar γ promoter (adipogenesis) (426).

tissues is a widely accepted niche for adipocyte progenitors (see the biogenesis paragraph for details). Some data support the possibility that endothelial cells of capillaries could give rise to adipocyte precursors both in WATs and in BATs (368,916). The anatomical structure and topographical organization of endothelial cells of capillaries in developing fat imply the need for dynamic cellular adaptation and extracellular remodeling for an eventual endothelial-preadipocyte conversion and development. Molecular signals implicated in adipose determination of stem cells could be in line with the endothelial hypothesis (Fig. 9).

1. MAPK (mitogen-activated protein kinase) family members have been studied for their implication in adipogenesis and it has been shown that ERK1 (extracellular signal-regulated kinase-1) is required in the proliferative phase, but need to be reduced by MAPK phosphatase-1 in the terminal differentiation phase to prevent its inhibitory effect on PPAR γ (85,778). Interestingly MAPK family members promote endothelial adaptations to extracellular signals allowing homing of mesenchymal stem cells to injured tissues (1015). The molecular mechanism allowing stem cell homing include the activation of MAPK family signaling and this activity could be related to the endothelial-mesenchymal transition necessary for preadipocyte development from endothelial cells. As a matter of fact, during murine WAT development endothelial cells with unusual morphology and rare endothelial-pericytic cells have been documented by electron microscopy both in developing murine and human fat anlage (169,916) (Fig. 9).
2. Adipose stem cells produce lysophosphatidic acid (LPA) that has been shown to activate RhoA (RAS homolog gene family member A) (502) and RhoA is also activated in endothelial cells by P120-Catenin, a VE-Chaderin associated kinase (777). RhoA play a role in cell transformation (necessary for the transition from endothelium to endothelial-pericyte cell) and a role for Rho-A in stem cells commitment is well established (577).
3. Furthermore, the FGF1-BAMBI system claimed to be important for adipose determination could play a role in endothelial-pericyte-preadipocyte conversion. It has been shown that microvascular endothelial cells of adipose tissue produce FGF1 (419). FGF1 via an FGF receptor 1/fibroblast growth factor receptor substrate 2(FRS2) activates the MAPK pathway (important for cell remodeling necessary to endothelial-preadipocyte conversion, see the preceding text). FGF-1 signaling also induces inhibition of BAMBI (bone morphogenetic protein and activin membrane bound inhibitor) activity that is a potent modulator of factors influencing adipogenesis such as Wnt and TGF superfamily members (TGF- β and BMPs, see the succeeding text) (547). Thus, a combined action of LPA-FGF1-BMPs-MAPK could play a role in

the endothelium-pericyte-preadipocyte conversion during development (Fig. 9).

Together with the morphological transformation and adaptations during the endothelial-adipose conversion, it is highly probable that an extracellular matrix remodeling is also necessary. The importance of extracellular matrix is supported by data showing that proteases able to remodel extracellular matrix have positive and negative effects on adipogenesis (9, 92, 521, 522, 583) and it has been shown that leptin modulates extracellular matrix molecules and metalloproteinases (126) (Fig. 9). Furthermore, the well-known fetal vasculo-adipocytic islets that give rise to adipose lobules both in visceral and subcutaneous murine and human fat are delimited by polarized fibroblast-like cells that are probably responsible for the intra islets collagen-rich microenvironment necessary for adipogenesis (162, 169, 916). Interestingly, FGF-1 induces the inhibition of BAMBI and consequent activation of carboxypeptidase x-1 (CPX-1) that induces an extracellular matrix remodeling necessary for adipogenesis (461). ROR γ play a negative role on adipogenesis through expression of its target gene matrix metalloproteinase 3 (MMP3) (584). Thus, it could be hypothesized its downregulated by FGF-1 activation.

4. The strong inhibitory stimulus to adipose commitment due to Wnt signaling downstream to FGF-1/BAMBI system could be removed by a combined action of this system and BMP proteins (305, 627).

Thus, ERK/MAPK signaling, LPA, and FGF1 could be key signals to activate the determination of adipose stem cell to preadipocyte, but which signal induce these pathways is not known. Insulin exert a potent effect on adipogenesis (175), probably trough insulin growth factor-1 (IGF1) receptor signaling because preadipocytes express more receptors for IGF-1 than for insulin (849). Insulin induces inactivation of FOXOs (forkhead box proteins) (inhibitors of adipogenesis) trough AKT (serine-threonine protein kinase) and activation of SREBC1 (activator of adipogenesis, see TAB I) through mTOR (mammalian target of rapamycin) (373, 617). Members of Bone Morphogenetic Proteins (BMPs, see also paragraph on endocrine functions) family have been suggested to play a key role in the commitment of adipocyte precursors and in particular BMP7 for brown adipogenesis (922) and BMP2 for white adipogenesis and BM4 for both white and brown adipogenesis (94, 264, 371, 372). Intrinsic production of BMP4 during preadipocytes differentiation would induce adipogenic commitment by the dissociation of an intracellular cytosolic complex formed by ZNF423 (zinc-finger-protein) and WISP2 (WNT1-inducible signaling pathway protein-2). ZNF423 is a transcriptional activator of PPAR γ (367) found in early adipocyte precursors and adipose stem cells (368). Its role in adipocytes seems also to be important to maintain the white phenotype inhibiting the activity of Ebf2 and suppressing Prdm16 activation (818). The ZNF423/WISP2 complex

dissociation would allow ZNF423 nuclear entry and PPAR γ activation (Fig. 9 inset).

Other BMP regulators should play an important role in adipogenesis and several potential inhibitors/activators have been found in adipose organ, such as activin (1006), follistatin (292), Dickkopf (51, 372), Kielin/chordin-like protein (523), and Dm/Gremlin (370, 909).

Another pathway that plays a role in adipogenesis is the ancient hedgehog signaling pathway. In rodent models, it seems that the inhibitory effect of hedgehog proteins act through the antiadipogenetic factor GATA2 (877), in human models data suggest that hedgehog does not interfere with induction but instead with maturation of human adipocytes (294). Recently, it has been reported that ciliary transduced hedgehog signaling regulated the expression of TIMP3, a secreted metalloproteinase inhibitor, that inhibited matrix metalloproteinase 14 to block intramuscular adipogenesis (471).

The transforming-growth factor β (TGF β) superfamily members, including the above reported BMPs and myostatin, regulate the differentiation of several cell types including adipocytes (94).

TGF β proteins activate SMAD-dependent and independent mechanisms and are inhibitory of adipose differentiation mainly through the effects of SMAD3 on C/EBPs (146, 147). Interestingly FAD-104 (factor for adipocyte differentiation 104) a positive regulator of adipogenesis (903), has been shown inhibitory properties on TGF β in cancer cells (350).

Myostatin action on adipogenesis is debated (22, 23, 285, 366, 724), but a recent quantitative study, using label-retaining wild-type and myostatin $-/-$ mice, support its inhibitory effects on adipogenesis (518).

Notch is a transmembrane receptor that translocates to the nucleus after ligands-induced cleavage. In the nucleus, Notch binds to and activates RBP-jk transcription factor. This signaling is required for differentiation of 3T3-L1 preadipocytes (317) and its absence induces the inhibitor of differentiation Dlk1/PREF1 (764). In addition, direct promoting activity on PPAR γ expression has been suggested (25, 813).

PREF1 (Preadipocyte factor 1 or Dlk1/FA1) is a molecular gatekeeper of adipogenesis that acts by preventing adipocyte differentiation. It is a transmembrane protein cleaved by TNF α -converting enzyme to generate a soluble form which interacts with fibronectin and activates integrin signaling though ERK/MAPK to inhibit adipocyte differentiation (414).

Bromodomains containing proteins seems to play an important role in chromatin regulation and transcriptional control of adipogenesis (232).

Using a coculture method of adipocytes/preadipocytes, a proteomic study showed that several inhibitors/enhancer factors could be involved in the neo-adipogenesis phenomenon and Slc27a1(long-chain fatty acid transport protein 1), Vim (Vimentin), Cp (Ceruloplasmin), and Ecm1 (Extracellular matrix protein 1) secreted factors have been suggested as promoters of adipogenesis (130).

AU: Please check this term for correctness.

Table 2



Wnt	miR-8 (453), miR-120, miR-148a (823), miR-210 (822), miR-335, miR200 (822, 823).
MAPK/ERK	miR-375, (822, 852, 1010), miR-143 (269).
KLF	miR-146b (136), miR-448 (852).
PPARs-C/EBPs	miR-27a,b (524), miR-130a, miR-519d (565), miR-138, miR-31, miR-326, miR-155 (852) Insulin signaling miR26b (853, 978), miR-29, miR31, miR-93 (140), miR-103/107, miR-146b (6, 915), miR-143 (436), miR-320, miR-375 (852).
TGF/BMPs	miR-21, miR-199a (852).
CREB	miR-124, miR-132, miR-155 (852).
Cell cycle	let-7, miR-15a, miR17-92 (852, 958), miR-1908 (990)
Cell fate	let-7 (881, 963), Ret-7, miR-30, miR-204 (822, 852), miR-221/222 (845), miR-320 (377).

Many other miRs have been shown to be involved in adipogenesis, but their targets are still unknown. Proadipogenetic activities have been shown for miR-24, miR-107, miR-150, miR-200, miR-335, miR-378 (121, 270, 361, 579, 968, 1017). Antiadipogenetic properties have been shown for miR-326 (891).

AU: Please provide the caption of Table 2.

Furthermore, the hormone leptin produced by adipocytes could also play a role in the preadipocytes recruitment (see paragraph of endocrine properties of adipose organ) in adult animals (see the succeeding paragraph on leptin).

MicroRNA MicroRNAs are a class of small noncoding single-stranded RNAs able to silencing mRNAs and posttranscriptional regulation of gene expression.

A growing body of evidence suggests positive or negative roles for several MicroRNA on critical steps in both aspects of adipogenesis (determination and differentiation), see Table 2 (6, 395, 822, 823, 958, 975). Interestingly, the obesity-associated gene Fto activity affects genes regulating adipogenesis (753, 896, 1014) and data support a functional link between FTO and miRNAs (754).

Brown adipocyte determination and differentiation

The determination and differentiation signaling for brown adipogenesis is less known and studied, but several important molecular signaling specific for brown adipogenesis have been identified.

Key transcriptional factors The key transcriptional factors for white adipogenesis C/EBPs-PPAR γ are indispensable also for brown adipogenesis, and intense immunostaining for PPAR γ was found in nuclei of fetal brown preadipocytes (916) in line with data showing its dramatic reduction in nuclei of brown adipocytes of adult mice (740). Importantly, C/EBP β , with a complex network of regulators, including CREB, Hoxc8, Plac8, KSR1, and TRB3, plays a

predominant role, instead C/EBP α seems to be dispensable for brown adipogenesis (60, 355, 384, 430, 472, 527, 889).

As for white adipogenesis Wnt signaling plays an important role also for brown adipogenesis and a block of differentiation for brown preadipocytes have been shown (382).

An important role is played by the key activator of brown adipocytes: noradrenaline. *In vitro* and *in vivo* studies support the fact that noradrenaline acts on β 1 adrenoceptors to induce the first steps of determination and differentiation on adipose stem cells probably localized in the vascular wall of fat microvasculature (36, 50, 100). However, mice lacking all β -adrenergic receptors develop a quite normal BAT anlage containing brown precursors with ultrastructural morphology very similar to that typical of wild type mice (169). These precursors develop into adipocytes with morphologic and gene signature more similar to white than brown fat (26, 723), suggesting that noradrenaline induces differentiation but not determination of adipose stem cells. The signaling downstream the β -adrenergic stimulus plays an important role for brown differentiation because the compensatory up-regulation of RI α in mice lacking the PKA regulatory subunit RII β , induces a hypersensitivity of cAMP to PKA and consequent increase of brown phenotype (206). Furthermore, hyperexpression of Foxc2, a factor that controls the expression of RI α , in fat, also induces a brown phenotype (127), but its role has been questioned by experiments showing that forced expression of Foxc2 blocks the adipose conversion of 3T3L1 preadipocytes (218). However, a recent paper confirmed the importance of Foxc2 for the induction of a brown phenotype of adipose tissue (316).

Fetal adipose stem cells are induced to differentiation by insulin (934) and recent data support a role for two pathways involved in brown differentiation: activation of CREB via Ras-ERK1/2 and deactivation of FoxO1 via Akt. Both IRS-1 activated pathways combine to decrease necdin (nuclear protein inhibitor of E2F-mediated PPAR γ 1 activation) levels and consequent brown gene expression (20, 88, 213, 346, 465, 921, 933).

Morphologic-molecular correlations Electron microscopy allows for the distinction between white and brown preadipocytes at very early stages of differentiation both during fetal development or during recruitment into the adult adipose organ (37, 158, 169, 622). The main morphologic differences consist in the quantitative and qualitative characteristics of mitochondria (see biogenesis of adipocytes paragraph). In line with these data, molecular signaling related to mitochondriogenesis and to the brown-specific UCP1-bearing mitochondriogenesis are distinctive features of brown adipogenesis. The proximal and distal promoters of UCP1 contain CREB (c-AMP response-element binding protein) binding sites, further outlining the importance of noradrenergic signaling for differentiation of brown preadipocytes (110, 382). Of note, UCP1 protein is detected by immunogold cytochemistry at very early steps of differentiation (916) and ectopic expression of UCP1 in WAT induces brown mitochondriogenesis

(768) suggesting that the early events of differentiation include the activation of UCP1 promoter. The distal enhancer is necessary for brown adipogenesis and bears binding sites for TRs (Thyroid Receptors), PPARs and RXRs (Retinoid X Receptors). PPAR α is specific for brown differentiation and PPAR γ 2 appears dispensable for brown adipogenesis (474), in line with data suggesting that PPAR γ 2 plays a dominant role for white adipogenesis (582, 1009), with the suppressive role of noradrenaline on PPAR γ 2 (525) and with quantitative data showing the predominance of PPAR γ 1 during brown adipocyte development (740).

At very early stages of fetal adipose organ development, immunohistochemistry showed the differential expression of two proteins: pRb (a tumor suppressor protein playing also an important role in cell cycle and differentiation) and S-100b (a multifunctional Ca²⁺-binding protein) (35, 173, 381) only respectively in nuclei and cytoplasm of white precursors. Interestingly, suppressing the activity of pRb induces brown phenotype in developing preadipocytes and it is inhibited by β 3AR agonists with consequent white to brown transdifferentiation of mature adipocytes (382). On the other hand, S100b is expressed by brown adipocytes during brown to white conversion (35) suggesting the existence of common pathways between differentiation and transdifferentiation.

Master regulators of brown differentiation PGC-1 α (PPAR γ coactivator 1 α) plays a key role for the brown-specific mitochondriogenesis acting on NRF1 (nuclear respiratory factor1) and Tfam (mitochondrial transcription factor A) (384, 708). These factors are regulators of respiratory chain genes and of replication of mitochondrial genome respectively. Of note, PGC-1 α is probably the main target of the inhibitory Wnt signaling (382) and is activated by the adrenergic/CREB induced SIRT3 (member of sirtuin family with protein deacetylase and ADP-ribosylase activity) and by NO (adrenergic-dependent gaseous regulator nitric oxide) (638).

Several other factors drive the brown-specific phenotype inhibiting other phenotypes. Among them PRDM16 (PR domain zinc finger protein 16) through the activity of EHTM1 (euchromatic histone-lysine N-methyltransferase1) and with the cooperation of interactive partner ZFP516 (zinc finger protein 516) plays a dominant role in the inhibition of muscle phenotype (653, 960). Its interaction with C/EBP β is sufficient to induce brown adipogenesis from skin fibroblasts (441). EWS (Ewing sarcoma break-point region1) and YBX1 (Y-box-binding protein 1) also inhibit the muscle phenotype and activate the expression of BMP7 that promotes brown genes (423).

EBF2 (early B-cell factor2) is a transcription factor expressed in embryonic brown preadipocytes and its activity is important to maintain the brown phenotype (960). Of note, ZFP423 is required to maintain the white phenotype suppressing EBF2 (817, 818).

A complex network of nuclear receptors coactivators and corepressors including the p-160 family, RIP-140, ZIP13, and

SRF are also likely to play a role in the differential phenotype between white and brown adipogenesis (313, 382, 758, 762).

MicroRNAs Several microRNAs play a role in brown adipogenesis regulating some of the above reported pathways.

miR-155 is an inhibitor of brown adipogenesis. It has been shown that there exists reciprocal negative regulation between miR-155 and C/EBP β that integrate pro- and antiadipogenic cues, including the autocrine activity of TGF β 1 (antiadipogenic inducer of miR-155). When proadipogenic stimuli prevail the inhibitory effect of C/EBP β on miR-155 allows brown differentiation (139).

Another negative regulator of brown adipogenesis is miR27 that is down regulated by cold exposure. miR-27 inhibits the main actors of browning: PPAR α , PRDM16, CREB, and indirectly PCC1 α (879).

miR-196a suppresses the white-fat gene Hoxc8 (inhibitor of C/EBP β , see the preceding text) allowing the brown phenotype of adipocyte precursors (600).

The cluster 193b-365 is induced by PRDM16 to inhibit the adipogenic repressor Runx1t1 (880).

miR-133 is a PRDM16 repressor and is downregulated by cold exposure allowing the brown phenotype of adipocyte precursors (530, 914, 996).

miR-455 activates AMPK (regulator of ATP production through fatty acids oxidation) promoting the brown adipogenic program and mitochondrial biogenesis. Concomitantly, miR-455 also targets the adipogenic suppressors Runx1t1 and Necdin, initiating adipogenic differentiation (1008).

The cluster miR-106b-93 seems to inhibit brown differentiation acting on PPAR α (975).

Interestingly, most of the above reported developmental browning mechanisms play also a role in adrenergic-induced browning of mature white adipocytes, further supporting similarities between developmental and transdifferentiation molecular mechanisms.

miR-26 family induces brown phenotype genes in HMADS cells (a well-defined model of human cell line derived from subcutaneous WAT) acting with inhibitory effects on the sheddase ADAM17 (447).

miR-378 controls specifically interscapular brown adipocytes expansion targeting Pde1b, a phosphodiesterase that catalyzes the turnover of cAMP and cGMP (673).

Adipose Organ Physiology

The main functions of this organ are thermogenesis and storage of fuel that can be released to the entire organism on demand. BAT is responsible for the first and WAT for the second purpose, but in case of special needs, such as prolonged cold exposure or chronic positive energy balance homeostatic plastic remodeling mechanisms are activated to satisfy these special needs: that is, during chronic cold exposure the BAT component increase (browning) and during chronic positive

energy balance the WAT increase (whitening) allowing a high level of adaptability of the organ to satisfy these two important functions.

Thermogenesis

When the animal is exposed to a temperature below thermoneutrality (threshold temperature below which thermogenesis is induced) BAT, that is, all brown adipocytes contained in the adipose organ, either as compact tissue (interscapular, subscapular, deep cervical, and mediastinal) or as interspersed cells among white adipocytes (axillary, inguinal, perirenal, and perigonadal) produce heat (109, 397). If cold exposure is chronically prolonged (days or more), the browning phenomenon extends the heat production to a progressively larger number of brown adipocytes until a sufficient amount of thermogenesis is reached.

Activated BAT modify its morphology: lipid vacuoles become smaller and mitochondria assume a spherical shape and increase their number and cristae density (169, 397, 737, 967). Parenchymal nerve fibers increase their density (229) and gap junctions between brown adipocytes, responsible for their electric coupling, extend their areas (34). Thus, several morphologic signs mirror the functional activity of BAT and therefore its morphology strongly depends from the level of thermogenesis required by the organism. As a matter of fact, the BAT activity starts when the animal is exposed to a temperature below thermoneutrality and increases progressively in parallel with cold (397).

Molecular mechanisms

The molecular mechanism inducing heat production is essentially due to the activity of the mitochondrial uncoupling protein UCP1 that is activated and newly synthesized in brown adipocytes. When cold is sensed mainly by skin receptors (397) but also by adipocytes (995), nervous afferent stimuli reach the hypothalamus (probably ventromedial nucleus) and the sympathetic nervous system is activated to secrete norepinephrine in all areas of the organ reached by parenchymal nerve fibers (44, 110, 486). β 3ARs of brown adipocytes are the main adrenergic Gs protein coupled receptors responsible for their thermogenic activity. Their activation induces a signaling cascade mediated by adenylyl cyclase activation and cAMP formation. cAMP activates protein kinase A (PKA), that through phosphorylation of a series of target enzymes leads to the final functional effect. In particular, the phosphorylation of the transcription factor CREB (cAMP response element-binding protein) activates the gene expression of UCP1 (110, 114).

PKA also induces the activation of a second MAP kinase pathway, the p38 pathway (115, 194). p38 MAPK has been shown to be an important downstream target of the beta-adrenergic/cAMP/PKA signaling pathway in adipocytes, and one of the functional consequences of this cascade is stimulation of UCP1 gene expression in brown adipocytes. A

recent study also demonstrates that mitochondrial reactive oxygen species, which accumulate in stimulated brown fat cells, enhance UCP1-mediated respiration by promoting the sulfonylation of a cysteine residue in UCP1 itself (145). Furthermore, possible roles for mTORC1 (a nutrient sensor mechanistic target of rapamycin complex 1 which plays a key role in coordinating anabolic and catabolic metabolism at the cellular level) (33) and the liver kinase b1 (Lkb1) has been also suggested. Adipocyte-specific mTORC1 loss in mice completely blocks cold-induced BAT expansion and severely impairs mitochondrial biogenesis (487). Adipocyte-specific Lkb1 loss induced UCP1 activation through C/EBP β recruitment and activation of mTOR (815). β 3ARs activation induces also lipolysis via two PKA mediated processes: activation of hormone-sensitive lipase (HSL) and phosphorylation (deactivation) of perilipin1. Lipolysis results in free fatty acids in the cell that are preferentially directed toward mitochondria where they serve as substrate for thermogenesis and likely are also involved in the regulation of UCP1 activity (110). The β -oxidation of fatty acids results in the pumping out from the mitochondrial matrix of the protons and consequent formation of mitochondrial membrane potential gradient. The presence of the mitochondrial carrier protein UCP1 explains the thermogenic function of the cell: uncoupling the β -oxidation of fatty acids from ATP synthesis, all the heat derived as an inevitable secondary effect of the biochemical reaction serve as principal final product of the cell activity; thermogenesis (89, 735). Other molecular mechanisms could be implied in BAT activation and recently the lipid 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) has been identified as a stimulator of BAT activity (548). Because mitochondria are numerous, large and packed with cristae the final result is that the heat produced is relevant for a physiologic role. Furthermore, the multilocular organization of lipid droplets into brown adipocytes allows a rapid disposal of a large amount of free fatty acids ready for oxidation in mitochondria. It has been calculated that the heat produced by a stimulated brown adipocyte is about 300 times that in average produced by other cell types for their routine biochemical work (628). Brown adipocytes are electrically coupled by gap junctions (802) that increase their size during cold exposure (34). Furthermore, during chronic cold exposure peripheral parenchymal noradrenergic nerve fibers density increase (611) probably with branching mechanism guided by endocrine and paracrine factors (see the succeeding text), and a progressively higher number of white adipocytes is reached by noradrenaline. Recent papers showed that the histone deacetylase 3 (HDAC3) and the nuclear factor I-A (NFIA) are necessary to activate BAT enhancers to ensure thermogenic disposition (265, 400).

White adipocytes are provided with β 3ARs (84, 224) able to respond to noradrenaline with a white-to-brown conversion to increase the total number of thermogenic brown adipocytes (see the succeeding text). Mammals survive in environments from about +50°C to about -60°C, thus the internal temperature 37°C (humans) is closer to the highest than to lowest environmental temperatures denoting the need for important

thermogenic equipment. The adipose organ responds to the thermogenic request with BAT activation and WAT browning.

The Harlequin phenomenon

Acute exposure to cold induces also the so-called Harlequin phenomenon in the BAT areas of adipose organ (170). We showed that acute cold exposure or β 3AR agonist administration induces an intense UCP1 immunostaining or UCP1 mRNA expression in situ of some brown adipocytes in BAT among other that remain completely negative or only weakly immunoreactive, thus the histology results in a tissue formed by polygonal shaped cells differently colored reminding the Harlequin mask (Fig. 11). Interestingly, morphometric analysis of immunogold-stained thin sections showed that UCP1-gold particle density in mitochondria is different among neighboring brown adipocytes with mitochondria of the same size and cristae density. After chronic cold exposure or β 3AR agonist administration the Harlequin attenuates and UCP1 immunostaining result less intense per single cell. Serial sections immunostained with UCP1 and the heat-shock protein (HSP) HO1 showed an intense nuclear positivity for the HSP in the intensely UCP1 stained adipocytes, suggesting that acutely activated brown adipocytes protect themselves from heat-shock promoting neo synthesis or activation of proteins able to inactivate the thermogenic system (170). The necessary thermogenesis would therefore be guaranteed by the alternate activation of brown adipocytes. Chronically activate BAT seems to reduce the amount of heat per cell with a more widespread activation of a large number of adipocytes. This increase could be explained by the branching of parenchymal nerve fibers and expanded activity of coupling gap junctions. Cold exposure induces increased number of brown adipocytes (605, 950) and proliferation of PDGFR α immunoreactive brown precursors in interscapular brown fat by the activation of β 1ARs (506). Thus, the thermogenic capacity of adipose organ in cold exposed animals is also due to development of new brown adipocytes.

WAT browning

Chronic cold exposure requires a progressive increase in number of thermogenic brown adipocytes, thus the white areas of adipose organ turn into brown: this phenomenon is also known as browning of adipose organ and is visually evident in dissected organs of cold acclimated mice (Fig. 5) (153, 155, 157, 159, 166, 220).

Browned areas contain an increased amount of BAT-like tissue that is present in small quantities also in warm acclimated mice (see the aforementioned paragraph on gross anatomy) (614, 950).

The first paper dealing with brown adipocytes in WAT demonstrated a substantial increase of brown adipocytes in the parametrial WAT of cold exposed mice (1000). Then a series of papers showed the remodeling of the tissue toward a BAT-like tissue and appearance of multilocular adipocytes (without

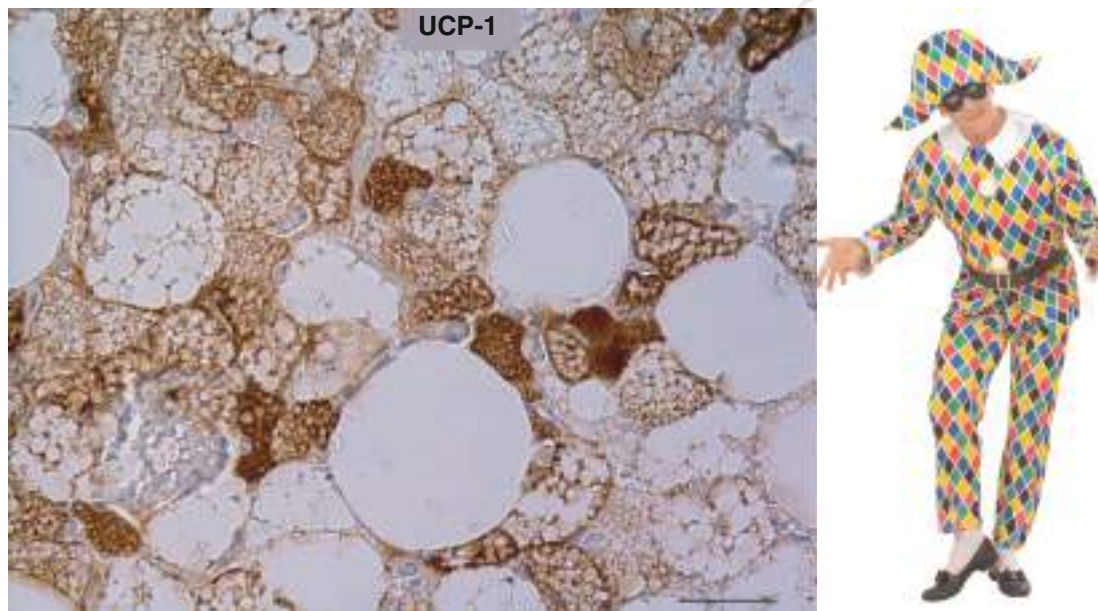


Figure 11 UCP1 immunohistochemistry of acutely activated brown adipose tissue show the heterogeneous immunoreactivity (Harlequin phenomenon). Bar: 35 μ m.

UCP1) after cold exposure in epididymal WAT of rats (535, 536) and in 1991 a reversible browning of inguinal fat of cold exposed mice was shown and the tissue was described as convertible adipose tissue (533, 534).

In 1992, Cousin et al. (202) showed the progressive molecular and morphologic transformation of periovarian white fat of cold exposed rats. In this paper, many molecular and morphologic steps of white-brown conversion were described and the presence of UCP1-immunogold particles in mitochondria of unilocular adipocytes of cold exposed animals were described for the first time. Furthermore, in that paper, the presence of gap junctions (typical junction usually found in interscapular BAT) were also described for the first time between brown adipocytes in browned periovarian WAT.

In a similar experiment in mice, Jimenez et al. (428) showed that the transformation of WAT into BAT-like tissue occur with an intermediate step in which adipocytes become multilocular and express UCP1 mRNA but not the protein. Importantly, the UCP1- multilocular cells appearing in WAT after cold stimulus are quite different in their size, morphology, and UCP1 protein expression from typical brown preadipocytes observed during BAT anlage development. On the other hand, these cells resulted similar to the intermediate forms between white and brown adipocytes found in the boundary areas between WAT and BAT and described in detail earlier (the adipose organ is mixed). Thus, while a convergence of data seemed to support the browning phenomenon and its variability in different depots and in mice with different backgrounds (364), the cellular basis of it remained unknown.

Data mainly from our lab supported the idea for a direct conversion of white to brown adipocytes (153, 158, 163, 164). The retroperitoneal fat of 20 weeks old rat is 100% composed by white adipocytes. We showed that administration of

1 mg/Kg of CL 316,243 (β 3AR agonist) for 7 days induces a striking morphologic transformation of retroperitoneal fat in these old rats. About 17% of the parenchyma was transformed into a multilocular fat and about 8% of the multilocular cells present in this fat expressed UCP1. Bromodeoxyuridine (BrdU) is a substance that is incorporated into the DNA of replicating cells and that subsequently persists in the nuclei of the cells deriving from that replication and can be revealed by immunohistochemistry. BrdU experiments suggested that 95% of the multilocular adipocytes derive from direct conversion of white adipocytes (398). Furthermore, electron microscopy revealed a series of intermediate forms between white and brown adipocytes with a progressive increase in number and a progressive acquirement of typical shape of mitochondria in parallel with the approach to the typical brown cell morphology.

Importantly, some unilocular cell showed a clear thickened cytoplasmic rim with an impressive number of mitochondria exhibiting a morphology that can be considerate intermediate between that showed by white and that of brown mitochondria.

Five years later, Jean Granneman published BrdU data in substantial agreement with an 85% of the multilocular adipocytes possibly derived from a direct conversion of white adipocytes (354). In cold exposed mice, we confirmed the importance of β 3 adrenoceptors (β 3AR), showing the blunted phenomenon in β 3AR-KO mice. Furthermore, we described all aspects (including electron microscopy and immunohistochemistry) of transitional forms between white and brown adipocytes. Paucilocular UCP1 immunoreactive adipocytes were described for the first time (36).

More recently, we performed a detailed quantitative analysis of the whole adipose organ in mice with two different

genetic background (614, 950). Our analyses performed in adult female mice (Sv129 and B6) maintained at 6°C for 10 days, showed that the browning phenomenon is more pronounced in B6 than in Sv129 in line with data obtained also in other's laboratories (364). Our quantitative work showed that the total number of brown adipocytes (defined as multilocular UCP1 immunoreactive adipocytes) increase about four times (compared with warm acclimated mice) in B6 mice (from 12% to 53% of the total number of adipocytes in the adipose organ). In Sv129 mice, the increase is also significant but limited to 0.3 times (from 45% to 60% of the total number of adipocytes in the adipose organ).

In both strains, the prevalent parenchymal cells of adipose organ after cold acclimation are brown adipocytes. White adipocytes represented in fact about only 35% (B6) or 20% (Sv129) of the parenchymal cells of the organ.

The rest of the parenchyma (10%-20%) of adipose organ, in these experimental condition, resulted composed by cells with intermediate phenotype between white and brown adipocytes: that is, multilocular UCP1- (adipocytes with brown-like morphology but lacking the brown molecular marker) and paucilocular UCP1+ adipocytes (adipocytes with white-like morphology expressing the brown molecular marker).

Of note, paucilocular UCP1+ adipocytes are much more frequently found in cold than in warm acclimated mice in line with the idea that browning is caused by a direct white to brown conversion.

Thus, after cold acclimation the main phenomenon in both strains was the increased number of brown adipocytes and the reduction in number of white adipocytes. Of note, in absence of a variation in the total number of adipocytes of adipose organ the increased number of brown adipocytes was equivalent to the reduction in number of white adipocytes. In theory, the disappearance of white adipocytes could be due to apoptotic phenomena, but, cold exposure is antiapoptotic (526, 640) and detailed histologic analyses did not reveal any sign of apoptosis (401, 455) or macrophages infiltration, suggesting a possible direct conversion of white to brown adipocytes in line with previous studies from our and other's laboratories.

Several data seem to converge on the idea that WAT browning is due to two main phenomena: direct progressive conversion of white or white-like adipocytes to brown adipocytes and neo-development of brown adipocytes (also called *de-novo* adipogenesis). These two phenomena are easily distinguished by morphology analyses. As a matter of fact, the direct conversion allows to observe (by electron microscopy and immunohistochemistry) the presence of the whole spectrum of intermediate forms between white and brown adipocytes, including the UCP1+ paucilocular cells (36, 166).

The neo-development allows to observe the whole spectrum of intermediate forms between poorly differentiated cells (progenitors or preadipocytes) and mature adipocytes. Although the precise cell type able to differentiate to a mature

brown adipocyte is not univocally identified (see paragraph on origin of adipocytes), it is widely accepted that early stages of differentiation are easily recognizable by electron microscopy and well reproducible in primary culture since many years (37, 171, 622, 848).

Brown adipocytes at early stages of differentiation are poorly differentiated small cells (diameter around 5-10 μ m) with all the morphologic and immunohistochemistry features described above (see paragraph on origin of adipocytes) (153, 169). These UCP1 immunoreactive small and poorly differentiated cells are well distinguishable from the UCP1 immunoreactive paucilocular cells that are close in size and morphology to mature white adipocytes (36).


Thus, morphometric ultrastructural analyses of WAT in cold exposed mice are able to distinguish between the two cellular components giving rise to the browning phenomenon and several data excluded any significant contribution of *de novo* adipogenesis, at least in the inguinal fat ((36, 202, 354, 398, 428, 950).

Furthermore, data *in vivo* (36, 506) and *in vitro* (100, 101) seem to suggest that the proliferation of brown adipocyte precursors is β 1AR dependent, but only β 3AR and not β 1AR agonist administration is able to induce WAT browning in inguinal WAT.

In line with these data, we observed a significant increase of preadipocytes (*de novo* adipogenesis) after β 1AR agonist administration without a WAT-browning and a significant increase of paucilocular UCP1+ adipocytes (direct WAT-to-BAT conversion) after β 3AR agonist administration with WAT-browning in inguinal WAT of adult mice (36). Thus, β 3ARs seems to play a pivotal role to mediate the browning through a direct white to brown conversion in line with data supporting that β 3AR ablation blunt the browning phenomenon (36, 428, 882).

All these data seem to converge on the idea that browning of adipose organ of cold acclimated or treated with β 3AR agonists rodents is mainly due to the plasticity of adipocytes.

The presence of β 3ARs in mature white adipocytes could therefore serve as key player of their plasticity as shown by the almost absence of browning in WAT of cold exposed β 3AR knockout mice. The appearance of paucilocular UCP1+ adipocytes with mixed mitochondrioma during WAT browning could represent a morphologic marker of white-brown conversion. These data are fully confirmed also by recent experiments of Granneman group (506, 507).

WAT browning is evident in both subcutaneous and visceral compartments, but epididymal, omentum, and retroperitoneum seems the visceral WAT more resistant to BAT conversion and we found delipidated  without clear signs of brown conversion in these depots of rats and mice (153, 169).

However, recent improvements of biotechnologies suggest that the appropriate technique able to demonstrate the direct white to brown conversion seems to be the lineage tracing experiments (854, 911).

Using this technique, Rosenwald et al. showed that cold-induced formation of UCP1+ multilocular adipocytes in WAT

AU: Please check the sentence for meaning.

of mice is reversed to adipocytes with the morphology and gene expression pattern of white adipocytes within 5 weeks of warm adaptation. Moreover, these white-typical adipocytes can convert into UCP1+ multilocular adipocytes on additional cold stimulation (763).

Wang et al. using a different mouse model with a similar lineage tracing technology outlined that cold induced WAT browning is also due to *de novo* adipogenesis, but essentially confirming an important component of direct conversion (959).

A further confirmation come from the recent work that elegantly demonstrated that PDGFR β + vascular mural cells (i.e., preadipocytes) do not significantly contribute to the initial cold induced browning of WAT, but only after prolonged cold exposure (949). However, a muscle-like origin for cells responsible of WAT-browning have also been proposed (539) and recently confirmed using a variety of Cre inducible mouse line (56).

Molecular mechanisms of WAT browning

White adipocytes are provided with β 3ARs that increase in quantity after cold exposure (84, 224). The molecular signaling following activation of this receptor seems to inhibit a series of inhibitors of transcription factors important to drive the activation of thermogenic genes and therefore the brown phenotype (mainly PRDM16/PGC-1 α and C/EBP β with a series of complementary activators), thus the net result is a white to brown conversion of adipocyte (333). Thus, β 3AR activated miR196a inhibits HOXC8/HDAC3 (Homeobox protein Hox-C8/Histone deacetylase 3) (112, 600) and the β 3AR activated cAMP inhibits CK2 (casein kinase2) (832). β 3AR signaling also inhibits MEF2C (myocyte-specific enhancer factor 2C) that activate miR133a/b (inhibitors of PRDM16) (914) (530). In addition, several other inhibitors are directly inactivated by the β 3AR signaling such as 4EBP1 (924), SMAD3 (985), Rb/p107 (272, 381, 383), RIP140 (149, 463), and H3K27me3 (672).

A recent work supports also a role for mTORC1 in the β 3AR signaling for browning (529). Furthermore, the LC3-dependent autophagy mechanism, that seems to be important for maintenance of the white phenotype and that is under the control of mineralocorticoid (MR) receptor activity (15), can be inhibited by the β 3AR signaling through the TASK1 potassium channel (Twik-related acid-sensitive K+ channel) activity (692).

Together with the inhibition of inhibitors of brown phenotype β 3AR signaling induce the synthesis of FGF21 (Fibroblast Growth Factor 21) (291, 947) that reinforce the browning phenomenon by an autocrine mechanism through FGF21R/ β -Kloto receptor (342). A recent paper supports a role for FGF-21 also in the activation of immune-mediated WAT-browning (see the succeeding text: immune-browning paragraph) (412). Furthermore, β 3AR signaling activates also signals that directly act on positive browning factors PGC-1 α such as COX2 (555, 942). In line with these data, the presence

of inhibitors that, if removed, allow conversion of myoblast to brown adipocytes has been recently proposed (954).

Thus, the white adipocyte could be considered as a masked brown adipocyte that reveal its true face after noradrenergic activation of its β 3ARs and removal of molecular inhibitors (Fig. 12), but a recent paper seems to support a dispensable role for β 3ARs in browning of FVB/N mice (221).

Which other data support the idea that a white adipocyte is a masked brown adipocyte?

Some authors believe that a subpopulation of “white-like” adipocytes are in some way more prone to convert directly to “brown-like” adipocytes (often called brite or beige, see the succeeding text), but morphologic and immunohistochemistry *in vivo* experiments comparing the inguinal WAT after fasting, refeeding, and cold exposure showed that most unilocular adipocytes (i.e., with the anatomy of white adipocytes) in this depot, able to undergo the slimming process (i.e., changing their morphology into a typical delipidated cell type when the animal is fasted) and able to refill with lipids their cytoplasm if the animal is refeeded (thus, showing the classic white adipocyte physiology properties) are also able to convert to thermogenic multilocular UCP1 immunoreactive (thus, true brown) adipocytes (36, 168, 606).

Some visceral areas of murine adipose organ are less prone to browning phenomenon, but this assumption largely derive from the data obtained with epididymal visceral fat (easy to sample and therefore widely used), but many visceral depots (also in humans) are composed by BAT or convertible WAT (all areas of adipose organ surrounding aorta and its main branches: subclavian, carotid, intercostal, renal, and mesenteric) (212, 950). Thus, the anatomical site *per se* do not define the proneness of WAT to BAT conversion and the *in vitro* experiments claiming the cell autonomous properties of adipocytes should consider the level of genetic “influence” made on that specific depot by the parenchymal nerve fibers activity in that specific experimental model.

Browning and noradrenergic parenchymal nerve fibers plasticity

A strong positive correlation between browning and density of noradrenergic parenchymal nerve fibers (NE fibers) has been described (950) (Table 3).

ASC: In Sv 129 mice, the density of parenchymal tyroxine-hydroxylase (TH) immunoreactive nerve fibers (NE-D) increase four times in this depot (mainly composed by: interscapular, subscapular, cervical, and axillar-thoracic fat) in cold acclimated mice (C-mice) versus warm acclimated mice (W-mice) (from ≈ 12 to ≈ 45 fibers/100 adipocytes). Of note, this depot is composed mainly by brown adipocytes (UCP1+ multilocular cells, $\approx 80\%$) in W-mice and cold acclimation increased only the number of multilocular UCP1-adipocytes. Of course, brown adipocytes were more intensely UCP1 immunostained and presented smaller lipid vacuoles in all areas studied of cold acclimated mice.

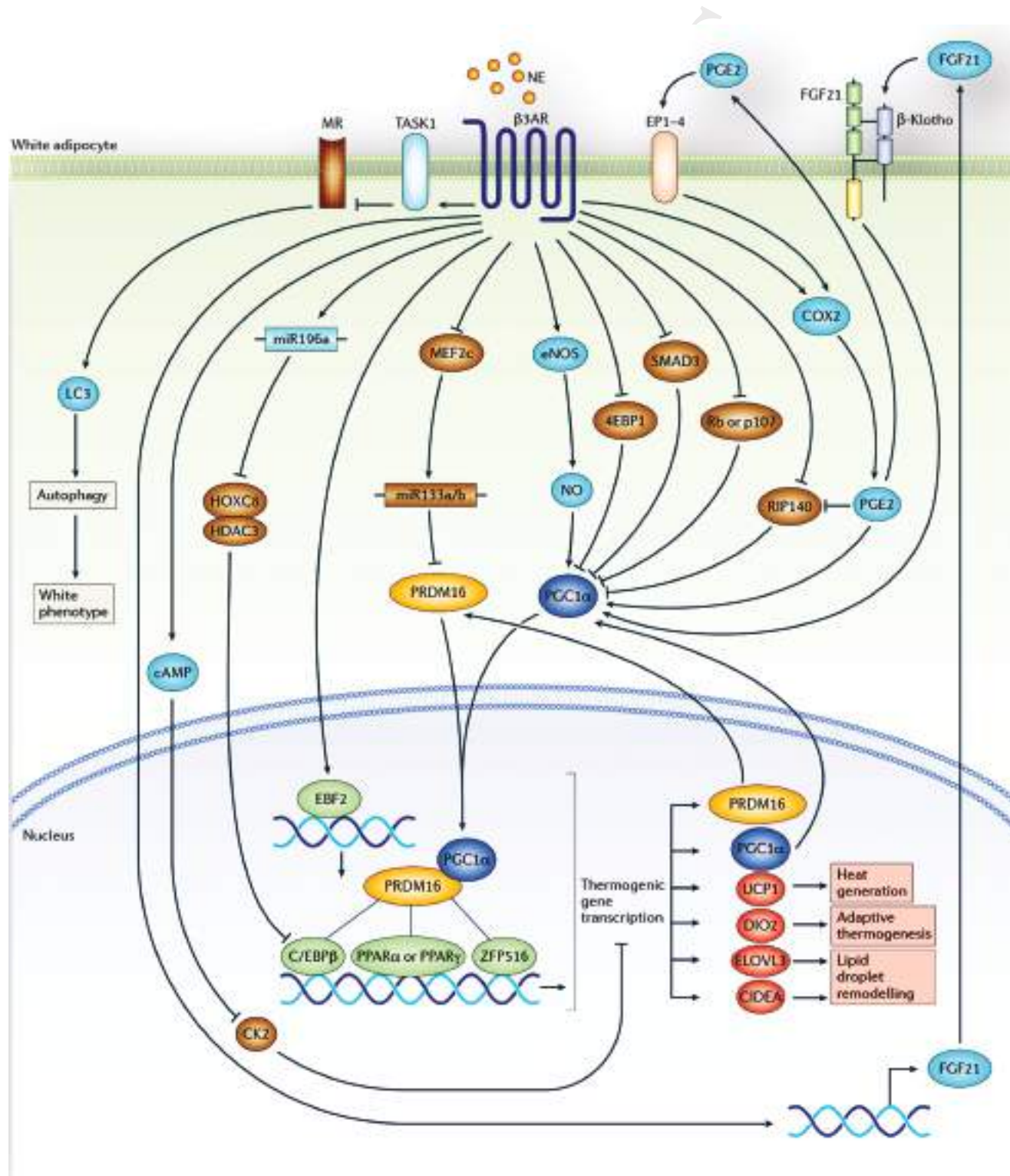


Figure 12 Potential β_3 -adrenoceptor-dependent molecular mechanisms driving white-to-brown adipocyte transdifferentiation. All brown-colored molecules are inhibitors of brown phenotype and are inhibited by activated β_3 -adrenoceptor signaling. Adapted, with permission, from [333].

Table 3 Adipose Organ Composition and Parenchymal Nerve Fibers Density in C57BL/6J and Sv129 Mice; Adapted, with Permission, from (948)

	Anterior subcutaneous	Posterior subcutaneous	Mediastinal	Mesenteric	Retroperitoneal	Abdominopelvic
C57BL/6J						
No. unilocular adipocytes						
28°C	56 ± 8 ^{###}	14 ± 1	6 ± 3 ^{##}	12 ± 1	2 ± 1	27 ± 2
6°C	25 ± 5 ^{*##}	16 ± 3	0.6 ± 0.3	10 ± 1 [#]	2 ± 0.2	15 ± 4 [*]
No. UCP1-negative multilocular adipocytes						
28°C	8 ± 0.3 [#]	1 ± 0.2	0.2 ± 0.08 [#]	0.7 ± 0.4	0	5 ± 0.8 [#]
6°C	6 ± 1 ^{##}	2 ± 0.4 [*]	3 ± 0.1 [#]	1 ± 0.3 [#]	0.4 ± 0.1	11 ± 0.8
No. UCP1-positive multilocular adipocytes						
28°C	12 ± 4 ^{###}	0	8 ± 3	0	0	0.7 ± 0.4
6°C	54 ± 4 ^{*###}	0.6 ± 0.2 ^{##}	7 ± 1	0.003 ± 0.002	0.001 ± 0.0002	7 ± 2 ^{*#}
TH-positive fiber density						
28°C	11 ± 2	2 ± 0.8	34 ± 0.7 ^{###}	6 ± 2 ^{###}	12 ± 1	16 ± 1
6°C	61 ± 5 ^{*##}	6 ± 2 ^{##}	50 ± 9	9 ± 2 ^{###}	7 ± 2	31 ± 3 [*]
SV129						
No. unilocular adipocytes						
28°C	16 ± 3	19 ± 0.2	0.1 ± 0.1 [#]	10 ± 0.0	2 ± 0.5	22 ± 1
6°C	9 ± 2 [*]	16 ± 1 [*]	0	4 ± 2 ^{**}	2 ± 0.6	9 ± 1 ^{***}
No. UCP1-negative multilocular adipocytes						
28°C	2 ± 0.3	0.9 ± 0.5	0.05 ± 0.03	0	0.1 ± 0.1	9 ± 2
6°C	11 ± 3 [*]	4 ± 1 [*]	0.1 ± 0.04	10 ± 2 ^{**}	0.1 ± 0	7 ± 1 [#]
No. UCP1-positive multilocular adipocytes						
28°C	73 ± 6	0	5 ± 1	0	0.006 ± 0.006	15 ± 3
6°C	73 ± 5	6 ± 1 ^{***}	9 ± 2	0.2 ± 0.03 [*]	0.4 ± 0.1 ^{**}	25 ± 3 [*]
TH-positive fiber density						
28°C	12 ± 7	1 ± 0.2	78 ± 2	1 ± 0.1	0.2 ± 0.1	16 ± 2
6°C	45 ± 8 [*]	12 ± 2 ^{**}	72 ± 26	20 ± 2 ^{**}	2 ± 0.9	38 ± 0.8 ^{**}

Data of obesity-resistant Sv129 mice from a previous work (26) are provided for comparison. Number of adipocytes × 10⁶ TH-positive fiber/100 adipocytes.

Indicates statistically significant difference between temperature conditions (same depot, same strain). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

#Indicates statistically significant difference between strains (same depot, same temperature condition). #*P* < 0.05; ##*P* < 0.01; ###*P* < 0.001.

Total number of adipocytes contained in the adipose organ of C57BL/6J and Sv129 results unchanged. *P* = 0.25 in 28°C mice; *P* = 0.18 in 6°C mice.

In B6 mice, NE-D in ASC increased about six times (from ≈11 to ≈60 fibers/100 adipocytes). Thus, the NE-D in Sv129 and B6 W-mice was quite similar but in B6 mice, this depot was composed mainly by white adipocytes (≈75%). Cold adaptation induced an increase of brown adipocytes number of about five times (B6), thus the ASC of two strains have striking differences both in unstimulated and stimulated situations, but in both cold induced a relevant increase of parenchymal noradrenergic nerve fibers density accompanied by a remodeling of the tissue with increased signs of BAT activity and increased number of multilocular adipocytes (Sv129) and brown adipocytes (B6).

PSC (composed by dorso-lumbar, inguinal, and gluteal parts) is mainly formed by white fat in both strains in W-mice the NE-D is sparse (1-2 fibers/100 adipocytes). Cold induced 12 times increase of NE-D in Sv129 and 3 times increase in B6 mice accompanied again by WAT remodeling with increase of brown adipocytes (from 0 to 6 × 10⁶) in Sv129 and of multilocular UCP1- adipocytes (from 0 to 0.6 × 10⁶) in B6 mice.

Mediastinal depot is highly innervated. In W-mice, the NE-D reach the maximal levels (about 75/100 and 35/100 adipocytes in Sv129 and B6, respectively). In line with these high NE-D, the % of brown adipocytes is the highest in both

strains (near 100% and 75%, respectively). In C-mice, the NE-D did not change in Sv129 (72–78 fibers/100 adipocytes) and increased in B6 (from 34 to 50 fibers/100 adipocytes) and an increase of only multilocular UCP1- (from 0.2 to 3×10^6) was observed in B6 mice. In Sv129 mice, the number of brown adipocytes increased from 5 to 9×10^6 in absence of an increase of NE-D.

Mesenteric depot showed low NE-D in both strains (1 fiber/100 and 6 fibers/100 adipocytes in warm Sv129 and B6 respectively). In postcold mice, the increase of NE-D was 0.3 times in B6 with a slight increase in brown adipocytes (from 0 to 0.003×10^6), but in Sv129 mice, the increase of NE-D was near twenty times. In these last mice, the remodeling of WAT was also striking and the % of unilocular white adipocytes that were near 100% in W-mice reduced to about 10%, the rest was formed by multilocular UCP1- adipocytes.

Retroperitoneal depot is poorly innervated in both strains, NE-D increased after cold but remodeling was very modest.

Abdomino-pelvic depot (composed by perirenal, periovarian, parametrial, and perivesical fat) is quite well innervated mainly in the perirenal area. NE-D is 16 fibers/100 adipocytes in both strains and increased in both about two times in C-mice with an important remodeling of adipocyte type composition: from about 50% of multilocular adipocytes (50% UCP1+) to about 80% (80% UCP1+) in Sv129 and from about 20% of multilocular adipocytes (10% UCP1+) to about 50% (40% UCP1+).

In synthesis in both strains cold acclimation induced increased density of parenchymal NE fibers in almost all fat depots studied (in the whole organ the increase was 1.75 times in Sv129 and 2.3 times in B6) with an almost regular parallel increase in number of multilocular and brown adipocytes. Moreover, unilocular white adipocytes reduced their number of an amount equivalent to the increase of multilocular and brown adipocytes. Of note, remodeling of fat included also an increased density of capillaries network that was not quantified.

Thus, the different browning propensity of different fat depots in the two strains seems to be mainly linked to their noradrenergic parenchymal innervation and to the strain specific ability to increase the density of noradrenergic parenchymal fibers. The most reactive subcutaneous depot was the PSC in Sv129 and the ASC in B6 mice.

All visceral fat depots were highly reactive in both strains, but the mesenteric retroperitoneal and perivesical resulted more plastic in Sv129 (10, 11, 212, 364, 476, 611, 950).

Immunebrowning

Data from our and other's laboratory seems to converge on the idea that plasticity of peripheral nerve fibers of SNS is of pivotal importance for the WAT browning phenomenon. As a matter of fact, the density of noradrenergic parenchymal fibers (TH immunoreactive) in WAT increase in parallel with browning and a positive correlation between number of brown

adipocytes and density of noradrenergic parenchymal nerve fibers have been found (see previous paragraph) (611). Thus, even the less innervated part of the adipose organ, that is, the most "white" parts mainly (but not exclusively) composed by white adipocytes, recruit parenchymal noradrenergic nerve fibers during WAT browning and the newly browned areas are always densely innervated. Furthermore, the importance of parenchymal nerve fibers is outlined by the observation that surgical denervation blunts the browning phenomenon even in the most reactive part of the organ such as the inguinal depot of Sv129 mice (a very reactive strain) (506).

Nevertheless, other mechanisms seem to parallel the nerve plasticity and interstitial cells owing to the innate immune cell system seems to play a major role (960).

Cold exposure induces recruitment of eosinophils and of M2 macrophages in subcutaneous WAT (972).

Eosinophils are granulocytes with an important functional role in allergy and in parasitism state; they are sparse in blood of people in western developed countries but abundant in people in less developed countries where parasitism is frequent and metabolic syndrome rare (448). In WAT of lean mice, they are quite abundant and represent about 4% to 5% of SVF cells (972). Eosinophils are the major source of IL-4 that is responsible for activation of M2 macrophages. It has been claimed that IL-4 induces increase of TH (the rate limiting enzyme necessary for all catecholamine synthesis) in M2 macrophages and this is accompanied with a WAT browning dependent from all these components of the T2 immune cell system (632, 712). Of note, this immune-system mediated mechanism of WAT browning is absent in interscapular BAT and seems to be restricted mainly to subcutaneous fat. The elegant studies demonstrating the immune-mediated browning of subcutaneous fat showed that this phenomenon is metabolically relevant inducing increase of energy expenditure and ameliorating metabolic dysfunctions in models of obesity (503).

IL-33 is a cytokine able to activate ILC2s that are lymphocytes able to regulate several types of immune responses. ILC2s are present in WAT and their secretion of IL-5 and IL-13 sustaining eosinophils and M2 macrophages promote glucose homeostasis (379, 589, 595, 648). It has been shown that administration of IL-33 results in subcutaneous WAT browning through recruitment and activation of the cytokine cascade network involving the ILC2s/eosinophils/M2 system (96). Activated ILC2s produce IL-13 and activate eosinophils to produce IL-4. Both cytokines are able to directly recruit PDGFR α + adipocyte precursors (expressing the IL-4R α necessary to respond to both IL-4 and IL-13) developing into adipocytes with brown phenotype, thus contributing to the WAT browning phenomenon (503). Interestingly, this cytokine-mediated mechanism of PDGFR α + adipocyte precursors recruitment plays a role also during physiologic postnatal development of brown committed adipocytes in subcutaneous fat (503).

The physiologic source of IL-33 contributing to the browning phenomenon is not well established, but it has been

shown that human white adipocytes and preadipocytes *in vitro* and *in vivo* are able to produce this cytokine and are provided with its receptors (970).

Furthermore, ILC2s have been identified also in human WAT and a decreased activity has been shown in WAT of obese mice and humans and it has been shown also that endogenous IL-33 is necessary to limit spontaneous obesity and white adipocyte hypertrophy (96) in line with data showing a protective role of IL-33 in obesity and related disorders (591, 662). These Authors also demonstrated that ILC2s are also able to sustain a WAT browning independently from the activation of eosinophils and M2 macrophages. This direct WAT browning activity seems to be due the opioid-like methionine-enkephalin (MetEnk) peptides production by ILC2s lymphocytes acting on specific opioid receptor δ 1 present in subcutaneous white adipocytes (96).

Surprisingly, recent data obtained in mice with different genetic background in six different independent laboratories seems to deny any role for M2 macrophages in catecholamine secretion and WAT browning. Using mice lacking TH in peripheral tissues (TH Δ PER), Fischer et al. (2017) showed that irradiated mice with reconstituted myeloid population from TH Δ PER or WT mice had identical thermogenic metabolism. Furthermore, immunohistochemistry and HPLC experiments failed to reveal any significant amount of TH and catecholamine in macrophages (290).

These conflicting results are difficult to understand (726) and for sure need further studies, but a very recent result seems to support a functional link between macrophages and nerves in BAT activity, thus a role for macrophages and other cell type of innate immunity in the WAT browning phenomenon cannot be definitively excluded so far (969).

Purinergic browning

Browning mechanisms alternative to the classic noradrenergic/ β 3AR pathway are strongly hoped for future therapies of obesity and related disorders in view to the fact that β 3AR agonists produced in the past are effective for small mammals but not for humans (496, 935). The last generation β 3AR agonist mirabegron is able to activate human BAT (212) but it has been approved for clinical use only for overactive bladder allowing to suspect that clinical trials for obesity failed.

In this contest, it is interesting to note that mice lacking all beta-adrenergic receptors (β -less mice) are able to reconstitute their BAT (transformed into a WAT-like in these mice) (26) under a chronic subordinate stress stimulus (723). In this experimental condition, sympathetic parenchymal nerve fibers density increase without a testable contribution of adrenergic signaling to browning. The immunoreactivity of nerve fibers for VNUT (vesicular nucleotide transporter, which is required for ATP storage in secretory vesicles) (391), together with its upregulation in stressed mice, suggest a purinergic signaling in these experimental conditions. Of note, VNUT immunoreactive nerve are present also in human BAT

(723). These data are in line with the observation that adenosine activates human and murine brown adipocytes at low nanomolar concentrations acting on A2A receptors and that A2A agonists induce browning of white adipocytes. Importantly, the WAT browning induced by A2A agonists reduces fat mass and improve glucose tolerance in diet-induced obese mice (345).

Beige, brite, or brown?

This newly formed BAT-like tissue has been also denominated as beige or brite (brown-like in white) or inducible brown fat (196, 686, 973). These denominations have been proposed mainly to underline the unusual presence of brown adipocytes in predominantly white fat depots and to emphasize the molecular differences between the “classic” interscapular BAT and the WAT transformed into a BAT-like tissue by, mainly, adrenergic activators.

The widespread idea that interscapular BAT is indeed the anatomical site of “classic BAT” derive from its anatomical structure (a compact tissue composed almost exclusively by UCP1 immunoreactive brown adipocytes) and several other aspects:

1. This is the site of the first anatomical descriptions (920),
2. The vast majority of BAT studies are performed in this tissue,
3. It is present in most of the small mammals used in experimental protocols (700),
4. It persists in older animals (605, 793),
5. It expresses quite specific ontogenetic markers: En1 (engrailed 1), Myf5 (myogenic factor 5), and PAX7 (paired box7) (808, 835), and
6. It expresses a marker gene (Zic1) that is not expressed by browned WAT (220).

In my opinion, none of the aforementioned reasons allows distinguishing brown adipocytes found in interscapular area from those found in other regions in the adipose organ. Historical anatomic and physiologic studies teach us that a cell and its appropriate denomination should consider mainly its anatomy and function. Until now, no data contrast with the fact that multilocular UCP1+ adipocytes have thermogenic properties wherever their anatomical location would be in the adipose organ. There is no doubt that adipocytes with intermediate morphology between white and brown exist and that their number increases during browning (see detailed description in previous paragraphs). Thus, it is not surprising that gene expression analyses comparing interscapular BAT (where brown adipocytes form a quite compact tissue that is almost uniform in its morphology), with browned white

fat (where brown adipocytes are only a percentage of the mixed tissue), show quite relevant gene expression differences. Furthermore, together with white and brown adipocytes these browned white fats usually contain also all the range of adipocytes with intermediate morphology between white and brown phenotype offering an explanation to intermediate and variable gene expressions. In this context, it is interesting and in line with the above considerations, that during brown differentiation *in vitro* of PDGFR α + adipocyte precursors from inguinal fat the so called “beige/brite” gene expression markers (Tnfrsf9, Klhl13, and Tmem26) are downregulated (503), suggesting that these markers work better for the identification of adipocytes that are not yet fully differentiated, that is, for example: adipocytes in the intermediate steps of browning differentiation.

In B6 mice, the browning phenomenon is quite consistent in ASC where cold acclimation induces five times increase in the number of brown adipocytes (compared to warm acclimated). Thus, the newly formed brown adipocytes own to the same anatomically well-defined depot of adipose organ including the “classic” interscapular BAT and its white periphery is a site of a striking WAT browning. The newly formed brown adipocytes in this area that surround the more central part of the interscapular BAT seems quite identical to classic brown adipocytes including their positional marker Myf5 lineage (783).

In conclusion, a specific cell type that can be described as beige/brite, to date is not precisely defined and I think that considering all together the most reasonable conclusion should be to restrict the definition of beige/brite adipocytes to those with a paucilocular morphology, that is, those that are in the intermediate step of differentiation between white and brown adipocytes. Moreover, this cell type when stained by UCP1 antibodies in immunohistochemistry stain beige (851) (Fig. 13).

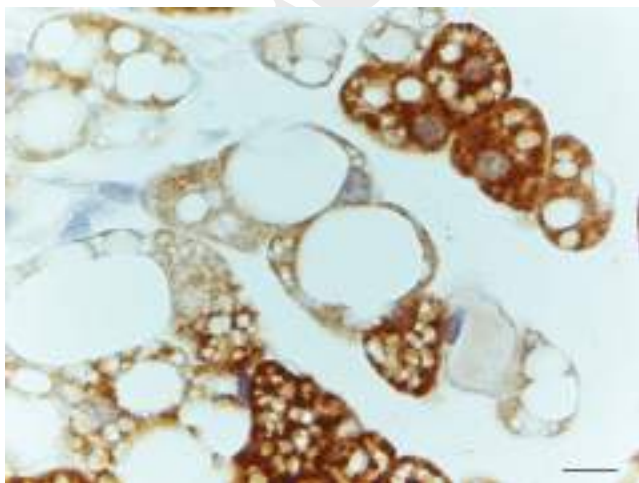


Figure 13 White adipocytes converting to brown adipocytes show a paucilocular morphology and a weak UCP1 immunoreactivity [Beige adipocytes]. Bar: 9.0 μ m. Adapted, with permission, from (851).

Furthermore, the concept of adipose organ as detailed in this and in previous reviews imply the mixture of WAT and BAT as a basic conceptual aspect of the anatomy and physiology of this organ: the mutual reversible conversion of the two tissues as a reservoir of energy partitioning toward thermogenesis (browning) during chronic cold exposure or toward storing (whitening) in case of chronic positive energy balance.

The healthy properties of BAT and browning

Whatever should be the correct denomination of newly formed multilocular UCP1+ thermogenic adipocytes data are widely converging on their healthy properties both in small mammals and in humans (627). Several experimental data showed that removal of BAT or its β ARs result in obesity and its related disorders (26, 186, 545, 809). On the other hand, BAT activation and browning of adipose organ are highly effective to combat obesity and its related disorders including T2 diabetes and atherosclerosis: that is, the metabolic syndrome (625). Thus, it is not surprising that BAT activation and browning are also efficient in prolonging the lifespan (659, 660).

In this view, several studies have been made to find molecular targets for therapeutic strategies alternative to the physiologic adrenergic signaling that could interact with the cardiovascular system.

Among those described in the molecular mechanisms of WAT browning (see the preceding text), recent data seem to support a role for intestinal microbiota.

Microbiota and WAT browning

Since the seminal work of Nicholson et al. (634), it became evident a relationship between microbiota and host metabolic interactions and in 2006 Turnbaugh et al. and Ley et al. (516, 926) showed that intestinal microbiota of obese animals has peculiar characteristics and germ free animals transfected with this characteristic microbiota increase their fat mass suggesting a link between microbiota and adipose organ physiology.

Chevalier et al. (142) recently found a relationship between thermogenesis and intestinal microbiota composition. Cold exposed mice showed a major shift in proportions, especially in the ratio Firmicutes/Bacteroidetes where Firmicutes abundance increased over Bacteroidetes with a reduction of Verrucomicrobia phylum. Transfecting this microbiota from cold exposed to germ-free mice induces fat loss and improvement of glucose and insulin metabolism. Subcutaneous and visceral fat of these animals show smaller adipocytes and WAT browning.

Furthermore, Suárez-Zamorano et al. (874) showed similar results of WAT browning and amelioration of obesity by alteration of microbiota through antibiotics administration.

The mechanisms for this microbiota-induced WAT browning are unknown but several hypotheses have been proposed. Gut microbiota are able to modify the bile acids pool

(738, 739) and brown adipocytes bear the bile acids receptor TGR5 that can be activated also in humans with increase of energy expenditure (99). Furthermore, activated intestinal TGR5 mediates synthesis and secretion of the intestinal incretin GLP-1 (glucagon-like peptide 1) that, together with its glucose-dependent insulintropic properties, can be responsible for a central stimulus of thermogenesis (48, 898). Finally, the short chain fatty acids (SCFAs) are the main product of gut microbiota fermentation and SCFAs can directly activate BAT and WAT browning or exert indirect influence through a stimulus for GLP-1 production by intestinal L cells (449, 774, 901).

Physical exercise and WAT browning

Physical exercise induces size reduction and mitochondrial biogenesis in adipocytes of WAT (203, 863). This well-known and relatively old observation can now be viewed as an early step of WAT browning and several healthy consequences of physical exercise (82) can be attributed to this phenomenon. The mechanisms involved include the activity of several actors. Central nervous system acts with a direct increase of activity and with expansion of its sympathetic branch.

SNS

Mimicking cold exposure physical exercise induces increase of parenchymal nerve fibers density in the adipose organ (227). Interestingly, the same amount of physical exercise induces more browning in animals maintained in an enriched environment. In these animals, the increased BDNF hypothalamic production seems to be responsible for the enhanced browning (113).

Several cytokines or myokines produced by active skeletal muscles have been suggested to play a role in WAT browning (782, 864).

IRISIN

Bostrom et al. showed that transgenic muscles engineered to mimic trained muscles upregulated the gene expression of fibronectin type III domain containing 5 (FNDC5), which after cleavage is secreted into the blood stream as irisin (86, 504). Data suggest that irisin is also produced by both subcutaneous and visceral WAT (743). The browning role of irisin is controversial (8, 262, 263, 646, 722) and recent data suggest that another physiologic target for this hormone could be the locomotor apparatus itself (188-191).

Natriuretic peptides

Natriuretic peptides are hormones produced by heart with the main purpose to maintain homeostasis with regard to blood volume, blood pressure, and salt balance (492, 604, 786). The adipose organ influences the activity of these peptides by clearance receptors. On the other hand, natriuretic peptides

acting on functional receptors increase cyclic GMP levels to activate cGMP-dependent protein kinase and activation of p38MAPK, thus their activity could be synergic with that of classic β -adrenergic receptor stimulation (192). Bordicchia et al. showed that natriuretic peptides promote browning of human white adipocytes and browning in treated mice (83, 193).

IL-6

IL-6 is produced by skeletal muscles and other organs (including fat). Its production increase during exercise (678-680) and in its overexpression in mice increase BAT activity (549). Recently a WAT browning effect of IL-6 has also been proposed (1).

BAIBA

Roberts et al., using a metabolomics approach, showed that exercised skeletal muscles secrete the myokine β -aminoisobutyric acid (BAIBA) (742). BAIBA induces WAT browning and β -oxidation in hepatocytes both *in vitro* and *in vivo*. Furthermore, this myokine induces a brown adipose-like phenotype in human pluripotent stem cells, and improves glucose homeostasis in mice. Interestingly, in a large human cohort study (Community-based Framingham Heart Study), plasma BAIBA concentrations resulted increased with physical exercise and inversely associated with metabolic risk factors (742).

Metnrl

Meteorin-like (Metnrl) is a circulating factor that is induced in muscle after exercise and in WAT after cold exposure (720). Increasing serum levels of Metnrl stimulates WAT browning. Metnrl stimulates an eosinophil-dependent increase in IL-4 expression and promotes alternative activation of adipose tissue macrophages, which are required for the increased expression of the thermogenic gene programs in fat (see also immunebrowning paragraph).

Finally, explanted subcutaneous fat of trained mice into sedentary animals improved their glucose tolerance and glucose uptake in muscle suggesting the possibility that trained subcutaneous fat could release adipokines that could reinforce the healthy WAT browning phenomenon (864).

Other Nonthermogenic Functions

Physical exercise induces WAT browning, but some data support an activation of BAT without an increase of its thermogenic gene expression (227). These data suggest that BAT could exert other functions and the increased expression and membrane localization of MCT-1 (proton-linked monocarboxylate transporter) strongly suggest a role of BAT in trained animals in the lactate metabolism (227). Interestingly lactate induces FGF21 and both play an important role in

WAT browning (427). It has been suggested that browning induced by lactate and other catabolites such as the ketone body β -hydroxybutyrate could represent an adaptive mechanism to alleviate redox pressure (122, 862). Adiponectin (see endocrine paragraph) is a circulating hormone with anti-atherosclerotic properties that is produced by both WAT and BAT, and the BAT production is not linked to its sympathetic activation (707).

A direct influence on cardiovascular system is also exerted by adipose organ expression of clearance receptor of atrial natriuretic peptide (NPr-C). Fasting induces a dramatic suppression of NPr-C gene expression in both WAT and BAT that appears to be accompanied by an increased biological activity of ANP (785). Thus, the natriuresis and diuresis and reduction of blood pressure induced by fasting might result from a reduced expression of NPr-C in adipose organ.

Some data also suggest that browning support an anabolic influence on bone system probably through the production of insulin-like growth factor binding protein 2 (IGFBP2) (714).

Metabolism

The WAT specific main function of adipose organ is to allow survival in the intervals between meals (760, 860).

For millions of years, until about 100 years ago in western countries, it was necessary to spend a lot of time and energy to find food for survival and the presence of a large energy storage depot in WAT of adipose organ was essential to guarantee survivals of humans and other mammals. The metabolic needs of the cells can count on an energy reserve in the WAT component of this organ able to guarantee up to several weeks' survival without any need for new fuel intake. Old experimental evidence shows lean persons surviving to up to 45 days of absolute fasting (454). But adipose organ also provides a strong endocrine stimulus to induce the search for additional source of energy: leptin (1013) (see also next paragraph). White adipocytes have the ideal shape for their physiology: spherical (maximal volume in minimal space) and are able to transform their morphology from spherical cells into elongated small fibroblast-like cells (slimmed cells) when the organism requires fuel (such as during prolonged fasting periods). The morphologic physiologic changes of adipocytes are accompanied by different phases of synthesis and secretion of leptin that is produced in positive relationship to the adipocyte cell size (540, 856). This hormone hematic concentration is proportional to the WAT in the adipose organ and when the energy stored in the organism tend to be dangerously low, then the leptin low level in blood assumes the role of strong stimulus for food search and intake by activation of various sites of limbic system in central nervous system provided with specific leptin receptors (201, 225). Thus, the physiologic fasting period between two meals have two main consequences: the use of stored lipids for metabolic needs and the stimulus for the brain to guarantee a behavior for food search and intake and consequent survival. The stimulus strength will depend on the fasting period and amount of WAT (249).

On the other hand, the organ cannot refuse the request of fuel storage such as during chronic positive energy balance periods and whitening of the organ (BAT to WAT conversion) together with white adipocyte hypertrophy and hyperplasia is one of the mechanisms that help in building up new parenchymal cells with lipid-storage capacities (26, 177, 280).

Thus, the reciprocal physiologic and reversible ability of conversion between WAT and BAT is an important intrinsic physiologic property of this organ (163, 164). Of note, the lipolysis due to fasting seems to be orchestrated by a sympathetic nervous system activity similar to that operating during cold exposure, but the cellular effects on WAT diverge strikingly (334). As a matter of fact, cold activated white adipocytes convert into brown adipocytes, but fasting activated white adipocytes transform into slimmed cells (158, 166). The relevant difference in the hormonal environment in the two conditions could play a role in the different biological effect on adipocytes. In particular insulin and natriuretic peptides could be important actors because insulin low levels in fasting could account for a major lipolytic effect of noradrenaline (17, 488, 491) and the inhibition of natriuretic clearance receptor expression without any reduction of the functional receptor in the adipose organ in fasting conditions could account for a reinforcement of the noradrenaline-induced lipolysis with consequent slimming effect on white adipocytes (83, 192, 239).

On the other hand, the noradrenergic pathway inducing BAT thermogenesis and browning is not only due to noradrenaline. It has been recently shown that cold is more efficient in BAT activation (as revealed by PET analysis) than direct noradrenaline administration to humans (209). Furthermore, beta less animals (i.e., mice lacking all types of β ARs) can be induced to thermogenesis and browning (723) (see also purinergic browning paragraph).

Endocrine properties

The adipose organ produces and secrete a large number of factors with hormonal, autocrine and paracrine properties, and cumulatively called adipokines (Fig. 14).

Proteomic studies showed that adipose organ produces about 600 different adipokines (512). Most of them are produced by white adipocytes with differences between subcutaneous or visceral depots, but some are produced by brown adipocytes (138, 278, 343, 440, 456, 918). Here a short description of the most studied adipokines is reported because several are directly involved in adipose organ remodeling or remodeling *per se* can influence their production and functional role.

Leptin It is a 16-KDa protein with structural homology to cytokines, produced mainly by subcutaneous white adipocytes (301, 302, 1013). Its production is in relationship with size and number of white adipocytes in the organ (300, 610). It is produced with a circadian rhythm with apex in the late evening-midnight, in relationship to food intake

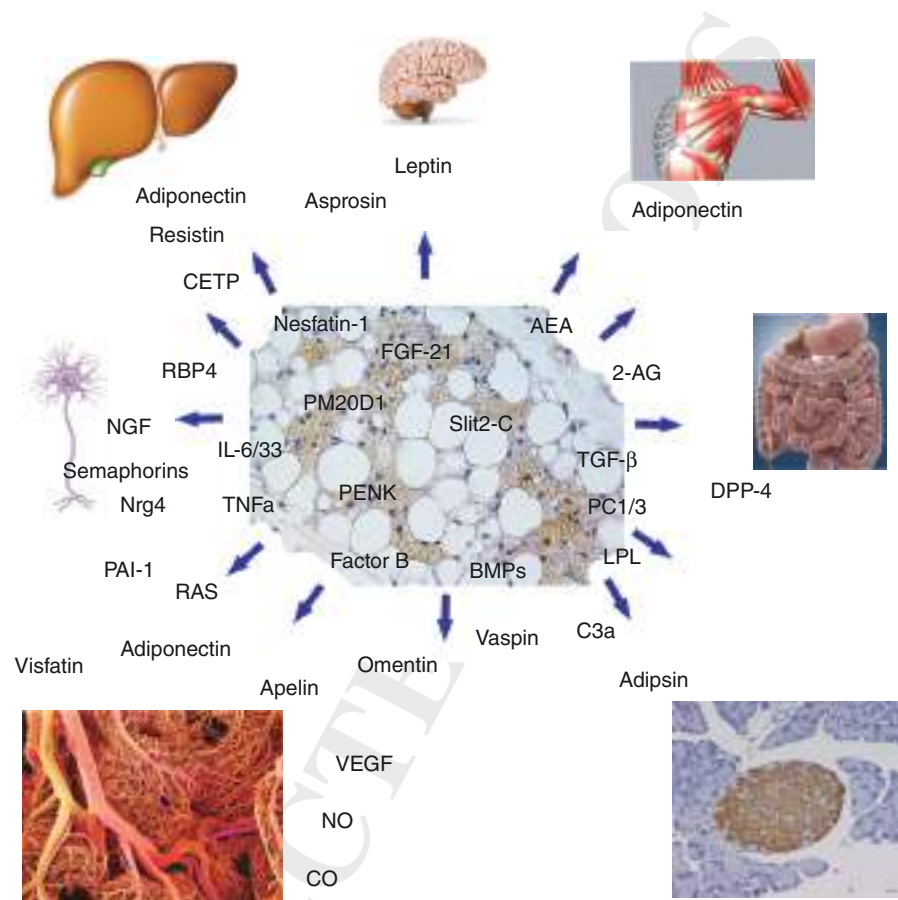


Figure 14 Graphical summary of the most important hormone-like molecules secreted by adipose organ.

timing. The intracellular localization of leptin has never been established definitively (315).

Leptin secretion is also dependent from several factors: such as insulin, glucocorticoids, $\text{TNF}\alpha$, estrogens, $\text{C/EBP}\alpha$ that are positive inducers and $\beta 3\text{AR}$ activity, androgen, free fatty acids, and $\text{PPAR}\gamma$ agonists that are negative stimuli (564, 675).

Its principal functional receptor (Ob-RL member of the superfamily of cytokine receptors) is found mainly in the brain (hypothalamus and limbic system) where leptin exert its positive action on anorexigenic neurons and sympathetic neurons, but the most important behavioral effect is due to its low plasma level that induce a strong stimulus for food search and decrease in energy expenditure (249, 892). Mice and humans lacking leptin or its receptor are massively obese and their behavior denotes an irresistible need for food search and intake (184, 596, 649, 695). Most of the obese persons are leptin resistant (557), but rare genetic obesity due to lack of leptin production can be restored by recombinant leptin administration (277).

Leptin also regulates several neuroendocrine activities mainly acting on CRH, TRH, and GnRH hypothalamic neurons (394, 456, 494).

Leptin replacement during fasting prevents starvation-induced changes in the hypothalamic-pituitary-gonadal and

pituitary-thyroid axes in healthy men (131). Several peripheral tissues express the leptin Ob-RL including: WAT, gonads, lung, placenta, adrenal medulla, liver, pancreas beta cells, jejunum, skeletal muscles, heart, cartilage, and blood mononuclear cells (226, 951). It has been calculated that the total amount of peripheral Ob-R account for less than 10% of the total and the physiologic role of these receptors remain to be elucidated although the old notion that female fertility is linked to a minimum amount of stored WAT (303) could be in relationships with the gonadal expression of Ob-RL and leptin replacement therapy can restore gonadotropin pulsatility in women with hypothalamic amenorrhea (965), but Kawwass et al. recently critically reviewed this topic (450).

Several other endocrine effects include regulation of immune function (mainly associated with malnutrition), hematopoiesis (proliferation and differentiation of hematopoietic cells), angiogenesis (91, 542, 564, 675). The effects of leptin on bones is quite controversial. Leptin-deficient ob/ob mice have increased bone mass, despite hypercortisolemia and hypogonadism (185, 253, 689, 927).

Data suggest that Leptin-responsive neurons in the VMH the ventral medial hypothalamus (VMH) are involved in leptin's effect on bone mass (885). Indeed, mice with defective SNS activity have high bone mass and are resistant to the antiosteogenic effects of leptin, whereas transgenic

overexpression of leptin in osteoblasts has no effect on bone mass. These data suggest that leptin decreases bone mass indirectly via activation of the SNS (185). However, a recent paper describes a leptin osteogenic direct effect on mouse long bone (689) in line with data supporting osteoblast proliferation and mineralization (347,899). Therefore, leptin has several different endocrine functions in addition to its effects on energy homeostasis.

BAT activation and browning reduce the leptin plasma levels (with pushing effect to food search and intake) in line with the need to maintain an equilibrium between BAT and WAT in the adipose organ (707). Of note, classic brown adipocytes do not produce leptin (177), but intermediate forms during whitening of brown adipocytes may produce leptin in line with data suggesting a reciprocal regulation of leptin and UCP1 genes in adipocytes (107).

Leptin is also produced by salivary glands (228) and stomach (27,174,178). An inhibitory activity of leptin on the orexigenic ghrelin (hormone also produced by stomach) has been shown (443), thus a possible role of leptin in the physiologic mechanism of interruption of food intake cannot be excluded.

Leptin receptors have been shown also to be present in preadipocytes and leptin induces adipogenesis *in vitro* (553,952). Interestingly db/db mice (lacking ObR) is the only model of genetic obesity in which adipose tissue is only hypertrophic (433), thus leptin (that is secreted in positive relationship with the size of adipocytes) can be involved in the signaling ending with the stimulus for neo synthesis or development of fat cells (66,386).

In this contest the mammalian target of rapamycin Complex 1 (mTORC1) (790,791) and its effector, ribosomal protein S6 kinase 1 (S6K1) seem to play an important role because mice lacking S6K1 have normal adipocyte terminal differentiation but altered adipogenesis (119). Furthermore, mice with an adipose-specific deletion of Raptor (regulatory-associated protein of mTORC1), which is required for normal mTORC1 activity, have a similar phenotype to that of the S6K1 knockout mice (696). A recent paper confirms the importance of mTOR as a critical regulator of adipogenesis (816).

Adiponectin It is an approximately 30-KDa protein exclusively produced by adipocytes and is abundant in the plasma (315,410,556,798). It is more expressed in subcutaneous than in visceral fat both in white and brown adipocytes (420,945,1012).

The adiponectin production seems to follow an opposite rule respect that of leptin: there is a strong negative correlation between plasma concentration of adiponectin and fat mass (410). Adiponectin circulates in serum as a range of multimers from trimers to high-molecular-weight (HMW) dodecamers (39). The HMW adiponectin seems to be largely the most important form accounting for most of its peripheral effects with the exclusion of CNS where low-molecular-weight trimers and hexamers are prevalent. This hormone improves whole-body insulin sensitivity probably

acting on AMP activated kinase (AMPK) of muscle cells by a signaling through the adiponectin receptor 1 (AdipoR1) (195,665,803,961,962,986,987). Another important role on glucose metabolism seems to be the suppression of hepatic glucose output through the AMPK activation (195,988). An orally active adiponectin receptor agonist has been shown to improve insulin sensitivity and lifespan in genetically diabetic obese mice (656).

Within the vascular wall, adiponectin exerts its anti-atherosclerotic activity by inhibiting monocyte adhesion, macrophage transformation to foam cells and decreases proliferation of migrating smooth muscle cells in response to growth factors (571,1011).

In addition, adiponectin stimulates nitric oxide production in endothelial cells and promote angiogenesis (135). These effects are mediated via increased phosphorylation of the insulin receptor, activation of AMPK, and modulation of the nuclear factor κ B pathway (132,244).

Adiponectin receptors are also present in the central nervous system and low level of adiponectin are present in the cerebrospinal fluid and here adiponectin (low-molecular-weight) stimulates appetite and reduce energy expenditure acting on AMPK (481). AdipoR1 and 2 colocalize in hypothalamus with leptin Ob-RL receptors and have been suggested that the central actions of leptin and adiponectin have reciprocal functions to provide a homeostatic mechanism to maintain fat levels thought the regulation of appetite and energy expenditure (709).

Resistin It is an approximately 12-KDa polypeptide produced mainly by white adipocytes with a 15 times higher expression in visceral than in subcutaneous fat (31,869). Resistin seems to play a role in glucose homeostasis of mice probably mediated by a mechanism involving the activation of SOCS3 (suppressor of cytokine signaling 3), an inhibitor of insulin signaling and by decreased activity of AMPK and reduced expression of gluconeogenic enzymes in the liver. In humans, Resistin is mainly produced by macrophages and obese fat is infiltrated by these cells (see obese adipose organ paragraph). Its physiologic role is still poorly understood even if several evidences point toward its potential link between obesity-inflammation and metabolic diseases (807). Recent data suggest also a role for resistin in the progression of human breast cancers in obesity and *in vitro* studies seems to support a link between resistin and ERM proteins (Ezrin, Radixin, and Moesin family that links F-actin to cell membrane proteins). ERM proteins may have an important role in tumorigenesis, cancer cell invasion, cross-cell signaling, and tumor metastasis, possibly via regulation of adhesion molecules (501).

Asprosin It is a 140 amino-acid secreted polypeptide abundantly expressed by mature white adipocytes of humans and rodents. Circulating levels of asprosin increase in fasting conditions and the hormone acts on the liver stimulating glucose production via cAMP-PKA signaling pathway. In obese humans and mice, plasma levels of asprosin increase in

parallel with insulin and could play a role in the metabolic syndrome because blocking its action a reduction of insulin and glucose hepatic production was observed. Of note, its absence in patients with neonatal progeroid syndrome (partial lipodystrophy that allowed Chopra and colleagues to discover this adipokine) could explain the absence of metabolic syndrome in these patients. Asprosin crosses the blood-brain barrier and directly activates orexigenic neurons. In patient with neonatal progeroid syndrome, its absence causes low appetite and extreme leanness (254,751).

C3, Factor B, and Adipsin These are proteins of the alternate complement system all synthesized and secreted by adipocytes (977). The C3-derived ASP (acylation-stimulating protein) promotes lipoprotein lipase (LPL) activity and triglycerides synthesis and decrease lipolysis and release of fatty acids from adipocytes. ASP also increases glucose transport by translocation of glucose transporters. Both ASP and Adipsin enhance glucose-stimulated insulin secretion from pancreatic β -cells (151,532,976). In particular, C3a, a peptide generated by adipsin, is a potent insulin secretagogue and its C3a receptor is required for the beneficial effects of adipsin. Thus, the adipsin/C3a pathway seems to connect adipocyte function to β -cell physiology, and offer an explanation to the link between lipodystrophy and diabetes (see lipodystrophy paragraph).

PAI-1 Plasminogen activator inhibitor 1 (PAI-1) is a major regulator of the fibrinolytic system that is the natural defense against thrombosis. This serine protease inhibitor is produced also by liver, vessels, and platelets. It is mainly produced by visceral fat and TNF α seems to play a role in its regulation, thus suggesting a link between visceral obesity inflammation and risk of thrombosis (437,846,953).

Apelin It is a bioactive peptide known as the ligand of the G protein-coupled receptor APJ. Originated from a common 77-amino-acid precursor, three active apelin peptides exist under the form of 13, 17, or 36 amino acids. Apelin and APJ mRNA are widely expressed in several tissues of humans and rodents including stomach, heart, skeletal muscle, and WAT. It has functional effects in both the central nervous system and peripheral tissues (124). Apelin has been shown to be involved in the regulation of cardiovascular functions, fluid homeostasis, vessel formation, and cell proliferation. Apelin has been described also as an adipocyte produced and secreted factor, upregulated in obesity. Expression of apelin gene in adipose tissue is increased by insulin and TNF α . Positive as well negative effects on rodent glucose and hepatic metabolism has been described. Higher apelin serum levels have been found in obesity, (124,478).

Apelin receptor antagonist treatment of rats showed diminished hepatic fibrosis (703).

Daily i.p. apelin injection was shown to decrease the triglycerides content in adipose tissue and the weight of different fat depots (393). Plasma triglycerides were also decreased

in both normal and obese apelin-treated mice. The treatment did not affect average food intake but increase circulating levels of adiponectin, reduced leptinemia and increased rectal temperature and O₂ consumption, thus suggesting a browning effect. These data are in line with the observed increased expression of mitochondrial UCP1 in BAT (393) and with the claimed positive effects of browning and negative effects of whitening of adipose organ (897).

Omentin It is produced by nonadipocyte cells in adipose depots, it is mainly found in visceral adipose tissue rather than in subcutaneous adipose tissue (992). Plasma omentin levels are reduced in obesity, insulin resistance, and type 2 diabetes. Omentin has insulin-sensitizing effects and also has been reported to have anti-inflammatory, antiatherogenic, and anticardiovascular disease properties (887).

Retinol-binding protein 4 (RBP4) Retinol-binding protein 4 (RBP4) is a protein mainly produced by visceral adipocytes and hepatocytes (467). Its physiologic role is related to binding and transport of retinol, but other roles have been suggested mainly in relationship to its elevated levels in obese and insulin resistant humans and mice. A recent paper, using mice with hepatocyte-specific deletion of RBP4 (LRKO) showed that adipose tissue does not contribute significantly to circulating RBP4 even in presence of an expected increase during diet-induced insulin resistance. The authors conclude that adipocyte RBP4 is not a significant source of circulating RBP4, even in the setting of insulin resistance. Adipocyte RBP4, therefore, may have a more important autocrine or paracrine function that is confined within the adipose tissue compartment. In line with this idea, it has been shown that RBP4 is able to activate adipose tissue antigen-presenting cells (59,597) with consequent activation of innate immunity and promotion of adipose tissue inflammation, thus contributing to the molecular link between adipose tissue inflammation and insulin resistance (351,475,991). Activated BAT also release RBP4 (755) but its functional role is uncertain and should not be related to insulin resistance because it is well known that BAT activation favors insulin sensitization (41).

Vaspin Visceral adipose tissue-derived serpin (Vaspin) is a member of serine protease inhibitor family. Its expression has been found in human adipose tissue, stomach, liver, pancreas as well as in hypothalamus of db/db and B6 mice. Lean human individuals have undetectable vaspin mRNA in fat, whereas its expression increase in overweight and obese individuals especially in visceral fat (78,392). This was not confirmed in all work (500). Administration of recombinant vaspin to obese mice improves glucose tolerance and insulin sensitivity and acutely reduces food intake. In addition, antiapoptotic effects of vaspin have been described in endothelial cells.

The mechanism of action is not known but it has been proposed that vaspin glucose lowering effects are due to its

serpin inhibition of the protease kallikrein 7, which plays a role in the half-life of insulin (389).

Visfatin In 2005, Fukuara et al. (312) proposed a new adipokine mainly secreted by visceral fat with insulin-like properties, but 2 years later, the authors retracted the paper for lack of reproducibility of the hypoglycemic properties and subsequent studies showed that visfatin is identical to pre-B cell colony-enhancing factor (PBEF), a previously described cytokine promoting maturation on early B-lineage precursor cells (781), but the name remain widely used. Visfatin displays intrinsic enzymatic activity as a nicotinamide phosphoribosyltransferase (Nampt), and is now better referred as Visfatin/Nampt/PBEF (749, 750, 752). Some reports deny a specific production by visceral fat (55, 466, 888), but an increased visfatin gene expression in visceral fat has been found in obese patients (667) and participation in inflammatory mechanisms of human visceral fat has been suggested (81). Visfatin is produced also by other organs including liver and skeletal muscles (308, 318, 321, 484), but also macrophages (that infiltrate the fat of obese animals and patients, see obese organ paragraph) are a physiologic source of visfatin (207, 349, 651). Visceral fat is always in tight anatomical relationship to vessels and one of the main activity of visfatin could be related to cells of vascular walls including characteristic macrophages of atherosclerotic plaques (749, 750).

Nesfatin-1 Originally described as a satiety molecule in the hypothalamus (652), it was subsequently found in several peripheral tissues including fat. This peptide derives from a precursor molecule (nucleobinding2: NUCB2) by prohormone convertase that produces three cleavage products: nesfatin 1, 2, and 3. In fat, it is mainly produced by subcutaneous adipose tissue both in humans and mice and its synthesis and secretion increase with adipocyte differentiation. Similarly, to leptin its expression also increases in high fat diet and decrease during fasting (716). Interestingly both in murine and human stomach nesfatin-1 is colocalized with ghrelin in endocrine cells. Thus, the same cell can secrete molecules with opposite effects on food intake (hunger for ghrelin and satiety for nesfatin-1) (868). Several other peripheral tissues also produce nesfatin-1 including pancreatic β -cells, heart, anterior pituitary gland and testis, suggesting its involvement in several homeostatic pathways including a potentiation effect on glucose-induced insulin secretion on pancreatic β -cells (620, 718).

DPP-4 DPP-4 (dipeptidyl peptidase IV) is a protein that is expressed by endothelial cells, salivary gland, prostate, seminal vesicles, endometrium, renal tubules, and small intestine and decidual cells (278, 311, 609). One physiologic role of this enzyme is to degrade incretins and DPP-4 inhibitors are in clinical use as antidiabetic drugs (105). Human adipose tissue was shown to be an additional source of circulating DPP-4. Its serum concentration correlate with adipocyte size,

and visceral fat of obese patients showed a fivefold higher level of protein expression than subcutaneous fat but no difference was found in lean subjects. Its secretion in visceral obese subjects could therefore contribute to development of metabolic syndrome (216, 490, 511, 748).

Cannabinoids Endocannabinoids (ECs) are lipids with autocrine and paracrine actions (668, 838). They are synthesized in the cells on demand from cell membrane phospholipids and immediately released to target their receptors (CB1 and CB2) that are usually localized in the neighbor cells or in the same EC producing cell. The most studied are N-arachidonylethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG). CB1 receptor is mainly expressed in the central nervous system and mainly in the areas dealing with the energy intake. In particular, neurons responding to the main peripheral satiety and orexigenic hormones use cannabinoid as modulators of their activity. Here they function in a retrograde manner: they are produced by postsynaptic cells and act on CB1 in presynaptic terminals to inhibit excitatory or inhibitory neurotransmitter release (654). Endocannabinoid release is immediate without any storage in vesicle allowing a real-time answer to the variable feeding state of the organism. Furthermore, they have an important role as modulators of neurons in the mesolimbic system, thus participating in both the homeostatic and hedonic aspects of food intake (98, 242, 866). It has also been shown an influence of EC on neural circuits involved in the control of energy dissipation (439, 713).

In peripheral tissues, CB1 is expressed mainly in organs, which play important roles in metabolic homeostasis. CB2 is mainly expressed in cells of the immune system (409).

Mature white adipocytes express CB1 and enzymes for the production and degradation of EC (52, 79, 200, 570, 744). In white adipocytes, CB1 activity seems to stimulate the processes of lipogenesis and inhibit lipolysis (241, 839, 944). Most of these data come from *in vitro* studies, but it has been also shown that treatment of baboons with the CB1 antagonist rimonabant induces a weight-loss independent healthy activity on adipose tissues (930).

Brown adipocytes also have functional CB1 receptors, but their functional role remain to be elucidated although some data point to their inhibition of sympathetic inputs to BAT and consequent decrease of thermogenesis and whitening of adipose organ (30, 713, 838).

Very recently, Ruiz de Agua et al. showed that mice lacking CB1 specifically in the adipose organ show a browning of visceral and subcutaneous fat with increase of parenchymal noradrenergic nerve fibers density and a tight anatomical relationship between M2 macrophages and nerve fibers (770).

Growth factors (FGF21, BMPs, TGF β , and GDFs)
FGF21. It is a growth factor without relevant proliferative mitogen capacities (344, 633, 636, 900, 974). It is mainly expressed by liver, but after cold stimulus it is also produced and secreted in the blood by BAT, whereas in this

condition liver production decrease (947). This adipokine has important browning properties both direct on WAT and indirect through increase of sympathetic outflow. It is secreted both in mice and humans also by adipocytes with intermediate phenotype between white and brown (i.e., paucilocular adipocytes also known as beige/brite), thus reinforcing the WAT browning phenomenon induced in WAT after cold exposure (291,402,403,504) (see browning paragraphs). Furthermore, FGF21 induces glucose oxidation in many tissues thus promoting protection against obesity and T2 diabetes (199,344).

BMPs. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor β superfamily. They are morphogens (62,717,925) that play an important role in the development of many tissues including adipose tissues. As morphogens, they act in the site where they are produced. The adipose organ is highly plastic and its development and involution can be required during the whole lifespan of mammals, thus it is not surprising that morphogens play an important role in its development and remodeling plasticity.

BMP2 has been claimed to play a role in white adipocyte differentiation (593,1003) as a matter of fact mice lacking Schnurri-2 (a downstream regulator of the BMP-2 signaling pathway) have a drastic reduction of WAT (431). BMP7 seems to play a key role in brown adipocyte development. In fact, BAT development is impaired in mice lacking BMP-7 (593,710,922). BMP4 activity is not well elucidated because data support its role in white adipocyte development, but its transgenic expression induces WAT browning and protect from diet-induced obesity (710,913). TGF β and activin A inhibit differentiation of both white and brown adipocytes (999), but mice lacking Smad3 (downstream) signaling show WAT browning with all its healthy consequences (985). The activity of BMPs seems to derive from a balance with inhibitors such as TGF β , activin A and Gremlin1 (370). Most BMP signaling is mediated via two receptors: type 1 (BMPR1) and/or type 2 (BMPR2) and downstream activation of the SMAD transcription factors (593,710). Interestingly, specific genotypes of the BMPR isoforms BMPR1A and BMPR2 have been shown to associate with obesity in human (87,134,800). Of note, specific deletion of BMPR1a in Myf5-expressing cells (precursors of classic brown adipocytes) results in a severe paucity of BAT and the compensatory activation of WAT browning (805). Within adipose tissues, BMP7 is probably produced by the adipose tissue niche contained in the stromal vascular cells (593,806). Thus, it is not surprising that the BMPs production seems to play an important role in commitment and determination of adipocyte progenitors (see also origin paragraph). BMP8b is mainly produced by mature brown adipocytes and physiologic stimuli (cold, high-fat diet) increase its expression (966). BMP8b functions locally by enhancing the response of BAT to β 3-adrenergic stimulation. Its expression is gender specific (higher in females) and together with its local activity BMP8b seems to activate BAT also through the sympathetic nervous system. GDF5 (growth differentiation factor5) (399) and BMP9 (485) are

other BMP/TGF β members that seem to induce WAT browning. It has been also shown that BAT is able to produce GDF8 (growth differentiation factor 8 or myostatin) with inhibitory autocrine effects (95,867) and conversely follistatin with positive excitatory autocrine effects (843).

Vasculotrophic factors (VEGF-NO-CO) The adipose organ plasticity regarding browning and nerve-vascular remodeling seems to be strictly connected to the ability of adipocytes to synthesize and secrete vascular and neuro-regulatory factors.

VEGF. The vascular endothelial growth factor (VEGF) family is composed of six secreted glycoproteins: VEGF-A, B, C, D, E, and placental growth factor (PlGF) (664).

VEGF-A (VEGF) is one of the most potent angiogenic factors (635) and is expressed both in white and brown adipocytes *in vivo* and *in vitro* (24,182,592). Our data showed that VEGF production appears to be under the stimulatory control of noradrenaline, mainly through β 3-adrenoceptors, thus allowing the supply of VEGF when functionally required and explains its deficit in genetically obese animals (904).

VEGF is very important for normal embryonic development because experimental deletion of even a single VEGF allele results in abnormal blood vessel development and embryonic lethality by E9.5 in murine models (118,287).

VEGF ligands bind specifically to two receptor tyrosine kinase membrane-bound proteins—VEGFR1 and VEGFR2 (considered primary signaling receptor), which are expressed in most endothelial cells (310).

Antisense knockdown of VEGFR1 does not affect endothelial cell proliferation, migration, and platelet activating factor expression, while knockdown of VEGFR2 severely impairs these processes (54). Furthermore, VEGF seems to have together with paracrine activities on endothelial cells also autocrine properties because induces the master regulator of mitochondrial biogenesis: PGC-1 α , and have direct beneficial effects on brown adipocytes to promote their survival, proliferation, and maintenance of mitochondria (28). In line with these data, cold exposure induces browning and vasculogenesis with upregulation of VEGF in inguinal WAT that is hypoxia independent and VEGFR2 dependent (984).

NO. White and brown adipocytes express different isoforms of nitric oxide synthase (NOS) and thus synthesize and release nitric oxide (NO) via noradrenergic stimulation (266,616,642). NO seems to play a role in the sympathetic induction of BAT vasodilation to match thermogenesis with perfusion, as well as in the proliferation and differentiation of brown adipocytes *in vitro* (341).

The main function of NO seems to be promotion of mitochondria biogenesis and bioenergetics with favorable impact in several chronic diseases including obesity, T2 diabetes (638,931).

CO. We also showed that brown adipocytes express the isoenzymes for the production of heme oxygenase (HO). HO is a ubiquitous microsomal enzyme, which produces a gaseous mediator, carbon monoxide (CO), and plays a crucial role

in maintaining cellular heme homeostasis and hemoprotein levels (559,560).

Interestingly their localization in the adipose organ was found in the cytoplasm and nuclei of brown adipocytes and in vascular walls. In brown adipocytes, the cold exposure upregulated the HO-1 isoform suggesting that the HO system may be involved in brown fat function (337).

Angiotensin. The renin–angiotensin system (RAS) is a well-known system that play a key role in the regulation of blood pressure. Angiotensinogen is cleaved by the enzymes renin and angiotensin-converting enzyme, to form angiotensin II that is the main bioactive peptide of this system. Angiotensin II exerts its physiological actions, primarily via two G-protein coupled receptors: Ang II type 1 (AT1R) and type 2 (AT2R) receptors (801). Both WAT and BAT are sites for the production of the major components of RAS and AT1 and 2Rs (123,267,569). Transgenic mice overexpressing angiotensinogen in adipose organ (driven by aP2) develop hypertension, white adipocyte hypertrophy and insulin resistance (442,567,568).

Neurotrophic factors (NGF, Semaphorins, and Nrg4)

The very important role of parenchymal noradrenergic fibers in the activation of BAT and browning of the organ is described in paragraph on noradrenergic parenchymal nerve fibers plasticity. Two neurotrophic factors (NGF and Sema3) seems to counteract and one (Nrg4) promote the parenchymal innervation of adipose organ.

NGF. The potent neurotrophic factor NGF that promotes the survival and proliferation of neurons (288,515) is produced *in vivo* and *in vitro* also by white and brown adipocytes (624,641,682,855). Its production seems to be in an inverse correlation with BAT functionality, thus suggesting a role restricted to the maintenance of existing innervation (643).

Semaphorins. Murine BAT express Sema3a, a chemorepellent neuronal factor active on both sympathetic and sensory peripheral nerves (470). In rats maintained in thermoneutral conditions, brown adipocytes produce both active isoforms of Sema3a and show a distinct peripheral polarized immunostaining pattern suggesting a role for Sema3a secreted by brown adipocytes in the guidance of axons growth (329,330). In cold-acclimated rats, where parenchymal nerve fibers density is higher, both the expression and the immunostaining of the two active isoforms are reduced.

Thus, Sema3a could play a role in the plastic adjustment of BAT innervation observed in different conditions of functional request.

A recent study confirms the importance of semaphorins system in BAT and showed that M2 macrophages lacking the nuclear transcription regulator Mecp2 (‘methyl-CpG-binding protein 2’) increased the expression of PlexinA4 that might act to repel Sema3a expressing sympathetic axons in BAT and thereby diminish its innervation (969).

Nrg4. Nrg4 (neuregulin 4) is produced and secreted by mature adipocytes *in vitro* and *in vivo* and induces nerve

growth *in vitro* in a dose dependent manner (687,756,957). During cold exposure, murine WAT expresses more Nrg4 than BAT supporting the idea that this adipokine play a key role in the growth of peripheral sympathetic nerve fibers, thus playing a key role of adipose organ browning (148).

Inflammatory cytokines (TNF α , IL6, IL33, IL1B, RANTES, IL-8, SDF-1, MIF, and MCP1) A series of proinflammatory cytokines are also produced by white adipocytes as well as by other cell types (mainly: macrophages, eosinophils and lymphocytes, see also immunebrown paragraph) of WAT.

The most studied is TNF α because of its high expression in obese fat and its property to interfere with insulin signaling by reducing tyrosine phosphorylation on insulin receptor (IR) and insulin receptor substrate 1 (IRS1) on key tissues: skeletal muscles, liver and fat (405,407,858,861,980). Two papers in 2003 (964,979) showed that the increased expression of TNF α in obese fat was mainly due to macrophages (see obesity paragraph), thus the role of TNF α secreted by adipocytes remain to be established. Interestingly *in vitro* studies showed that TNF α is able to increase the secretion of IL-33 by adipocytes and IL-33 could play a role in the modulation of activities of innate immune cells of WAT (970).

Many other cytokines are produced by adipocytes and their roles on different types of leucocytes normally present in the adipose organ are at beginning of their exploration. Most of them (IL-6, IL-1B, RANTES, IL-8, IP-10, SDF-1, MIF, and MCP1) have chemotactic properties (343,446) suggesting a possible physiologic functional interrelationship, but their precise functional role to date is far to be known. It must be remembered that some depots of the adipose organ are particularly rich in leucocytes (omentum and mesenteric fat) suggesting a more specific functional relationship for those depots (796).

Human BAT-like tissue derived from the capillaries of subcutaneous WAT and implanted into mice with diet-induced obesity improved the metabolic syndrome and produced secretory factors: IL-33, proprotein convertase subtilisin/kexin type 1 (PC1/3), proenkephalin (PENK) (591).

MCP1 is a potent chemoattractant that play a key role in macrophages infiltration of obese fat (29,711,721) (see obesity paragraph).

Lipid metabolism (LPL, CETP) LPL is a key regulator of triglycerides deposition in adipocytes. It is synthesized by both visceral and subcutaneous adipocytes and transferred to the luminal surface of endothelial cells by transcytosis. Insulin and glucocorticoids are the physiological stimulators of LPL. This enzyme hydrolyzes the triglyceride in circulating lipoproteins such as chylomicrons and VLDL (very low-density lipoprotein) and produces free fatty acids that are used for metabolic energy or for fat storage (17).

Colesteryl-ester protein transporter (CETP) is mainly produced by visceral adipose tissue in humans (314). This protein promotes the remodeling of plasma lipoproteins,

thus influencing their peripheral metabolism (886). CETP mainly promotes the exchange of cholesterol esters and triglycerides between plasma lipoproteins. It appears to be an important actor of the so-called reverse cholesterol transport from peripheral tissues to the liver and excretion (728, 831, 886, 953).

Zinc- α 2-glycoprotein (ZAG) is a lipid-mobilizing factor expressed by white adipocytes that could play a role in cachexia (63).

Other BAT-adipokines (batokines) M20 domain-containing protein 1 (PM20D1) is a secreted enzyme, highly enriched in brown adipocytes. It catalyzes the synthesis of N-acyl amino acids from free fatty acids and the reverse hydrolytic reaction. N-acyl amino acids directly bind mitochondria and function as endogenous uncouplers of UCP1-independent respiration increasing whole body energy expenditure (538).

The C-terminal fragment of secreted Slit2: Slit2-C promotes thermogenesis activating the PKA signaling pathway (883).

Other endocrine effects (T4/T3, cortisone/cortisol, and androgens/estrogens) T4/T3. BAT produces type II thyroxine 5'-deiodinase (Dio2), which converts T4 (thyroxine) to T3. It is highly induced during BAT activation (837, 956). It has been suggested that BAT-derived T3 is important as a local stimulus for thermogenesis because mice lacking Dio2 suffered hypothermia upon cold exposure despite normal plasma T3 levels (219).

Cortisone/cortisol. Cortisol (corticosterone in rodents) availability and action depend not only upon circulating levels and its ability to bind and activate the glucocorticoid receptor (GR), but also by the activity of two isoenzymes: 11 β -hydroxysteroid dehydrogenase 1 and 2 (11 β -HSD1 and 11 β -HSD2) (598). 11 β -HSD2 inactivates cortisol and it is highly expressed in mineralocorticoid target tissues [salivary gland, kidney, and colon (599)] to prevent the activity of cortisol on the mineralocorticoid receptor (MR).

11 β -HSD1 have the opposite function and is mainly expressed in highly metabolic tissues such as liver, fat, and skeletal muscles. Clinical data from patients with excess of circulating cortisol deriving from adrenal or pituitary tumors with specific defects in the activity of 11 β -HSD1 lacked the classic Cushingoid syndrome: visceral obesity, hypertension, skeletal muscle myopathy, insulin resistance, and T2 diabetes. Gain and loss of functions in total or tissue specific murine models have confirmed that tissue intrinsic 11 β -HSD1 activity is the major determinant of the adverse metabolic manifestations of circulatory cortisol excess (598).

Interestingly, it has been shown that inflammatory cytokines (TNF α) are able to increase the activity of HSD1 (271) thus offering an explanation for a possible vicious circle in visceral fat where the fragility of adipocytes due to scarce expansibility can cause inflammation (see obesity paragraph). In this context, MR antagonism has been shown to protect

mice from the adverse obesogenic and metabolic effects of a high-fat diet via conversion of a substantial amount of visceral and subcutaneous WAT into BAT (15, 692).

Androgens/Estrogens. Another important effect of adipose organ activity on circulating hormones is due to the enzyme cytochrome P450 aromatase (435, 671, 841).

WAT is an important site of aromatase activity that converts androstenedione and testosterone to estrone and estradiol. It has been calculated that about 80% of estradiol in men is produced in extragonadal tissues (551). Estrogen receptors are widely diffused in the organism and their functional role is much wider than that on female sex characteristics and reproductive capabilities (729, 840). In particular, estrogens have important effects on brain neurons plasticity (590), neuroprotection (562), and brain mitochondrial functionality (993). Furthermore, estrogens exert positive effects also in the bioenergetics system of the brain (97, 247) and in virtually all other peripheral organs (459, 729).

Distribution and activity of the two estrogen receptors α and β in adipocytes of the subcutaneous and visceral compartments account for the different sex-related distribution of fat in the adipose organ of women and men (824, 825) (see human adipose organ remodeling paragraph).

In synthesis, many endocrine-paracrine molecules produced by adipose organ play a role in its plasticity: neurotrophic factors and vasculotrophic factors influence the remodeling in nerve and vascular composition of the organ. All growth factors described, cannabinoids and apelin influence directly or indirectly the reciprocal WAT-BAT conversion and many (leptin, resistin, C3, PAI-1, adipsin, visfatin/Nampt/PBEF, Nesfatin-1, and DPP-4) are influenced by the conversion both for the change in parenchymal tissue composition or for the infiltration of inflammatory cells (mainly macrophages). Finally, also the influence of adipose organ on the endocrine system is also highly dependent from its cellular composition.

Lactation: Adipose tissue remodeling to milk producing glands

The plastic properties of adipose organ offer an explanation to its mixed anatomy: that is, in normal conditions energy derived from food intake is channeled toward the two main needs to survive: thermogenesis (BAT) and metabolism (WAT). In particular situations such as chronic cold exposure, it is necessary to increase thermogenesis (and plasticity of the organ allows the browning phenomenon) or increase the energy storing for metabolism such as in positive energy balance situations (and plasticity of the organ allows the whitening phenomenon). Thus, plasticity of the organ allows to adapt the organ function to specific needs. The cellular phenomenon at the base of this plasticity is widely accepted as due, at least in part, to a direct conversion (transdifferentiation) of white adipocytes into brown adipocytes and vice versa (2, 41, 153, 158, 168). This phenomenon implies a

physiologic reversible reprogramming (519) of adult cell genome and deserve further examples.

We found a striking new example of physiologic reversible transdifferentiation in the female adipose organ during pregnancy, lactation, and postlactation periods.

Mammary glands anatomy in adult virgin mice is very simple: branched epithelial ducts infiltrate subcutaneous fat and end in a single nipple. Five bilateral nipples are present in the ventral surface of the mouse skin, the first three collect ducts infiltrating the whole anterior subcutaneous depot and the last two collect ducts infiltrating the whole posterior subcutaneous depot. Thus, the real glandular part of mammary glands: that is, milk-producing alveoli, is not present in virgin adult mice (631, 734). Alveologenesis is a hormone-induced phenomenon of pregnancy and lactation periods (408). During alveologenesis a progressive reduction in number of adipocytes parallel the alveolar development and no or very few residual adipocytes are present in lactating mammary glands (Fig. 15). Following the steps of mammary morphogenesis during pregnancy we noticed three interesting

structural aspects regarding: 1-Transforming adipocytes 2-Early alveoli anatomy 3-Intermediate structures between transforming adipocytes and early alveoli (606).

1-Transforming adipocytes. We noticed that at the apex period of alveolar formation (around day 18th of pregnancy in mice) several adipocytes assume a morphology never observed in other physiologic condition: compartmentalization of lipid droplets, development of organelles in a thickened cytoplasmic rim (stacked rough endoplasmic reticulum, peroxisomes hyperplasia, hypertrophic Golgi complex, mitochondria hypertrophy) and development of cytoplasmic projections. Of note, this unusual anatomy of adipocytes coincided with their atypical immunoreactivity for Perilipin2 (typical of mammary alveolar cells) while maintaining the typical immunoreactivity for Perilipin1 (typical of adipocytes) (705).

In line with the hypothesis that transforming adipocytes give rise to alveolar cells, and in line with the earlier reported ultrastructural findings in these adipocytes, it has been shown that mice lacking X-box-binding protein 1 (XBP1: a central

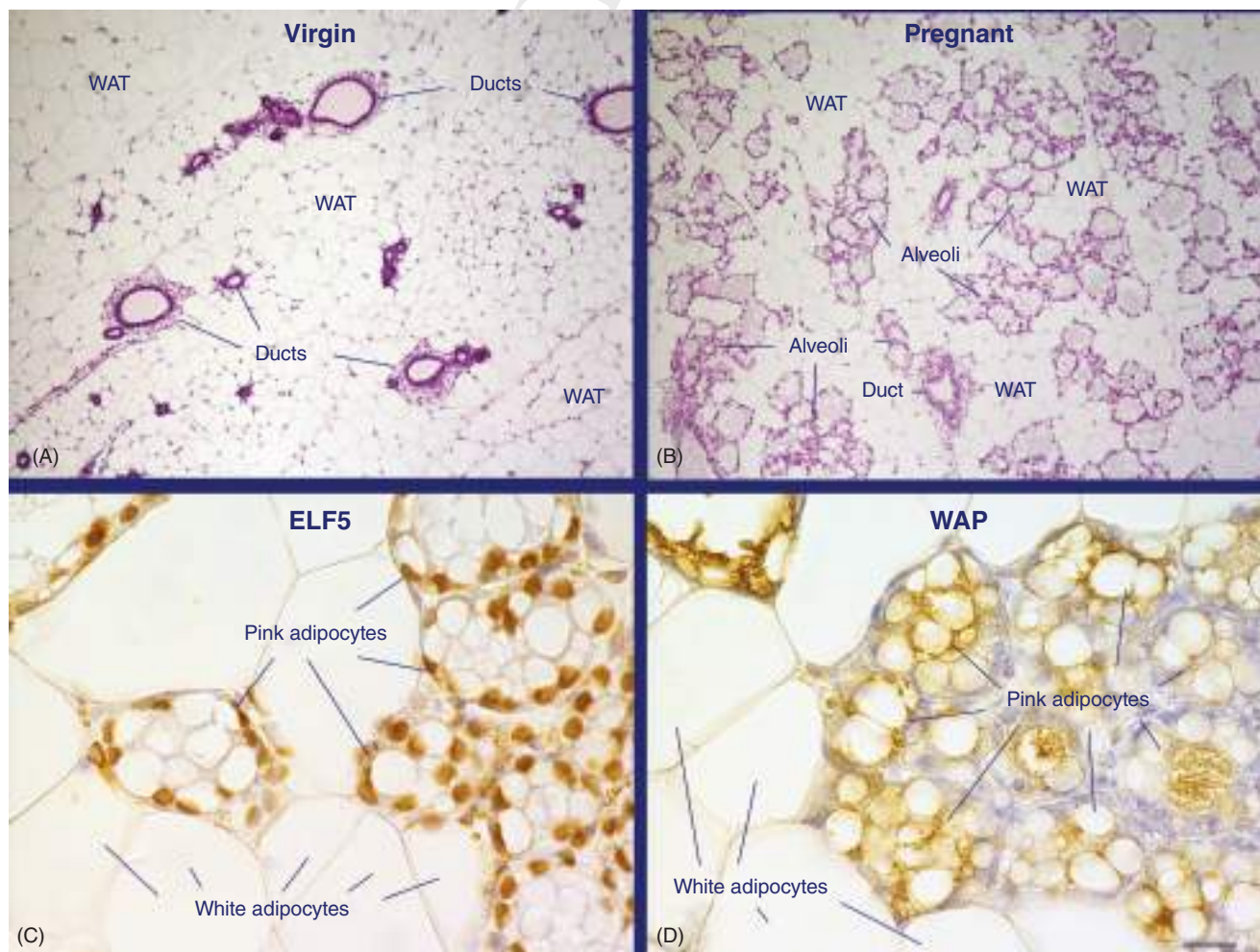


Figure 15 Histology of inguinal adipose tissue of virgin (A) and pregnant (B-D) mice. Epithelial alveolar cells appear only during pregnancy and are immunoreactive for ELF-5 (C) and WAP (D). This milk-producing alveolar cells show large cytoplasmic lipid vacuoles (pink adipocytes). Bar: in A and B, 50 μ m; and in C and D, 12 μ m. Adapted, with permission, from (167).

regulator of endoplasmic reticulum adaptive responses) specifically in fat had no effect on adipocyte formation but a blunted process of alveologenesis during pregnancy with decreased milk production (359). Furthermore, the absence of membrane channel Pxmp2 in peroxisomes of mammary fat altered the normal development of alveoli during pregnancy (941).

Moreover, we showed that together with alveolar cell nuclei only nuclei of some transforming adipocytes (and not adipocytes without signs of transformation) at day 18th of pregnancy resulted immunoreactive for ELF5 (E74-like factor 5, ets domain transcription factor) (705), that is a potent and specific transcription factor that is essential to induce alveologenesis (143,493,650).

2-Early alveoli (around day 18th of pregnancy, that is the time of major alveolar development) anatomy revealed a characteristic impressive abundance of cytoplasmic lipids in the epithelial cells (734). In fact, glandular epithelial cells (forming a well visible lumen, joined by typical epithelial junctions, with apical classic milk-protein secretory granules identical to those secreted in the lumen), immunoreactive in the cytoplasm for milk-typical whey acidic protein (WAP) and presenting ELF-5 immunoreactivity in the nuclei, contained a single cytoplasmic lipid droplet conferring to these cells a morphology more close to that of adipocytes than to any other glandular epithelial cell in the organism (734,851). Our reasoning was that these elements are lipid-rich parenchymal cells of adipose organ, thus, by definition, they are adipocytes (considering that the term adipocyte imply only the abundant cytoplasmic lipids without any physiologic implication). Thus, we called these cells pink adipocytes because the color of the organ during pregnancy is pink (153,333,339). Interestingly, some alveolar structures develop also earlier (between 15th and 17th day of pregnancy) and most of them lack the cytoplasmic lipid droplets described earlier suggesting that these early alveolar structures could derive from ductal stem cells progenitors (851).

3-Intermediate structures with a morphology intermediate between transforming adipocytes and early alveoli are often found in this period of pregnancy (day 18th). They are multinucleated structures with typical features of very early alveoli (with milk-protein granules, nuclear immunoreactivity for ELF-5 and presence of myo-epithelial cells), but with cytoplasmic lipid droplets of the same size of that contained in surrounding adipocytes (606,851).

These data suggest that under the hormonal pregnancy stimulus adipocytes of the mammary gland gradually transform their anatomy, aggregate with other adipocytes and myoepithelial cells to form intermediate structures and finally develop a glandular alveolar anatomy.

On the other hand, at the end of lactating period adipocytes reappear in the gland in parallel with a progressive disappearance of alveolar structures. In mice, the pre-pregnancy anatomy is reconstituted ten days after the end of lactation. We noticed that not all the alveolar epithelial cells underwent an apoptotic process and some of them accumulated

lipid droplets with a progressive development into adipocytes. Of note, about 17% of postlactation developing adipocytes showed cytoplasmic granules similar to those typical of alveolar epithelial cells (606).

Thus, the complete picture suggested by our morphologic and immunohistochemistry analyses was a physiologic and reversible adipo-glandular transdifferentiation guided by pregnancy and lactation hormonal environment. This would strongly confirm the plastic properties of adipose organ, again for an energy partitioning, but this time not for animal survival but for species survival.

Lineage tracing experiments seems to be the ideal technique to demonstrate the direct conversion of a cell into another type of cell (854,911). Double transgenic mice able to express a cell specific and temporally specific reporter gene, that will be expressed thereafter whatever phenotypic conversion will happen, are also commercially available. We used aP2-Cre/R26R mice, that express beta-galactosidase (beta-gal that can be visualized by x-gal histochemistry) only in adipocytes to demonstrate adipo-epithelial conversion. In virgin mice only adipocytes and not ductal epithelial cells resulted x-gal positive. About 60% of alveolar cells were x-gal positive at day 18th of pregnancy, suggesting an important contribution of adipo-epithelial conversion in alveologenesis (606). We then used WAP-Cre/R26R mice that express beta-gal only in milk-producing epithelial cells to demonstrate the epithelial-adipo conversion. Data showed that in virgin mice neither adipocytes and epithelial cells were x-gal positive, during pregnancy only epithelial cells were positive and in the postlactation also adipocytes were positive in the first postlactation day, after 10 days and after 6 months (606). Furthermore, we found in the postlactation mammary glands, 10% to 15% of adipocytes immunoreactive for WAP a protein that is never expressed by adipocytes in virgin mice (705).

Recently, we also showed a gland-BAT (pink-brown) conversion in the postlactation period (338). In the dorsal part of the first three mammary glands interscapular BAT, that is functionally inhibited during pregnancy and lactation (479,919), is in contact with glandular tissue and during pregnancy mammary glands infiltrate the peripheral part of interscapular BAT (Fig. 16). We noticed that in the first days of postlactation period some adipocytes with the ultrastructural features of classic brown adipocytes showed cytoplasmic structures with the classic features of milk-protein granules-containing vacuoles, usually found in milk-producing and secreting mammary alveolar cells. In WAP-Cre/R26R postlactating mice, we found beta-gal stained multilocular cells that resulted also marked by the specific immunostaining with UCP1 antibodies, thus confirming that alveolar epithelial glandular cells convert into thermogenic brown adipocytes in the postlactation period.

To confirm the striking adipo-epithelial (white-pink) conversion phenomenon, we explanted pure mammary fat from Rosa26 tagged mice (with all cells expressing beta-gal) into mammary gland of virgin wild-type mice. During pregnancy, explanted tagged fat gave rise to beta-gal positive alveolar



Figure 16 Dorsal view of anterior mammary glands. Note interscapular brown adipose tissue still visible in mouse in A and not visible in mouse in B. Bar: in A, 1.9 cm; and in B, 2.8 cm. Adapted, with permission, from (169).

structures among beta-gal negative native milk-producing glands of the host (230). Although the tagged glands derived from explanted fat resulted less developed than native glands they showed secretory products at electron microscopy that resulted milk-proteins immunoreactive at immunohistochem-

istry analyses. Furthermore, also explanted isolated mature adipocytes were able to differentiate into mammary glands during pregnancy (230).

Data showing brown to pink transdifferentiation are still lacking to complete the transdifferentiation triangle (Fig. 17).



Figure 17 The remodeling properties of adipocytes in the adipose organ. Adapted, with permission, from (169).

To dissect the molecular mechanisms responsible for the reversible adipo-glandular transdifferentiation we did a series of experiments. In the first experiment, we tested the effects of pregnancy on cleared fat pad. Since decades, it is possible to surgically remove the epithelial part of mammary glands leaving only the fat: cleared fat pad (234). Pregnant mice with cleared fat pad on one side and normal glands in the other side were analyzed by light microscopy that revealed no effects of pregnancy on cleared fat pad and suggesting a role for ductal produced and secreted paracrine factors. Wide gene expression analyses comparing clear fat pad and contralateral wild type glands at several time points of pregnancy showed that osteopontin could be one of the candidate paracrine factor (706). Of note, the osteopontin receptor is a beta3 integrin that is expressed in mammary adipocytes and differentially increased in the normal glands during pregnancy in parallel with the increased expression of osteopontin. Mice lacking osteopontin have impaired alveologenesis during pregnancy (630).

These data on subcutaneous fat-mammary glands strongly reinforce the concept of plasticity of adipose organ to partitionate energy between the short-term homeostasis (thermogenesis-browning and metabolism-whitening) and long-term homeostasis (lactation-pinking).

Interestingly and in line with the above data on adipo-mammary plasticity, very recently Li et al. showed that brown adipocytes can display a mammary basal myoepithelial phenotype (5).

The rainbow adipocyte

Since decades, it is widely accepted that mature adipocytes are able to dedifferentiate *in vitro* (875, 893). Considering the plastic properties of adipose cells observed *in vivo*, we wanted to further analyze the details of the plastic properties of mature adipocytes *in vitro*.

In 1986, Sughiara et al. developed a method to maintain mature adipocytes *in vitro* (876). They solved the problem due to the presence of abundant lipids in the cytoplasm of these cells. Lipids tend to float and push up the cells inducing detachment of cultivated cells from the bottom of culture system, thus interfering with their further maintenance-development. Thus, the solution was to reverse the vials to allow mature adipocytes to survive attached at the top of the vials: ceiling cultures.

This technique allowed to observe a progressive loss of lipids that was interpreted as a dedifferentiation-like phenomenon. The main structural characteristic of mature adipocytes consists in the cytoplasmic lipid droplet that occupy about 90% of the cell, thus the loss of lipid droplet transforms the adipocyte into a fibroblast-like cell similar, at light microscopy level, to poorly differentiated adipose cells (438, 875). Furthermore, delipidated (slimmed) fibroblast-like cells were considered de-differentiated also because they

assumed gene expression profiles typical of mesenchymal stem cells (658, 699).

When adipocytes undergo a lipolysis process *in vivo*, such as during fasting, the morphologic modifications are very characteristic. In the early stages of lipolysis, when the lipid content is still almost intact, adipocytes produce cytoplasmic invaginations conferring a villous-like appearance at the cell surface (120, 169). These invaginations are very characteristic and allow distinguishing a slimmed adipocyte from any other cell type in the adipose tissue (Fig. 18). During lipolysis, the size of the lipid droplet reduces progressively and some smaller lipid droplets become visible at the periphery of the cell. In parallel with the size reduction of the lipid droplet, the cytoplasmic invaginations increase in size and number. Interestingly, the tannic acid method (76, 77) allows to see, by electron microscopy, the steps of this gradual slimming process because fatty acids are visualized as membranous lamellar whorls (LW) structures (75, 153). The LW structures are visualized at the lipid droplet surface, in the cytoplasm and specifically concentrate into the cytoplasmic invaginations (153). Here the LW structures seems to exit from the cells and fatty acids LW structures are visible in the interstitial space where they reach the capillaries (Fig. 19). Thus, this delipidated cell (slimmed) presents a quite characteristic morphology well far away from that typical of poorly differentiated mesenchymal stem cells. This delipidation process *in vivo* do not lead to a poorly differentiated phenotype and we observed that also *in vitro* delipidated mature adipocytes have a well differentiated phenotype, at electron microscopy level, well different from that typical for mesenchymal stem cells in spite of their expression of stem cell genes and in spite of their properties of multilineage differentiation (248, 286, 451, 572, 694, 698, 699, 820). Thus, we studied in details this spontaneous delipidation process of mature adipocytes *in vitro* and observed that it is completely different from that happening *in vivo* under fasting conditions. As a matter of fact, we showed ultrastructural (Fig. 20) and time lapse data (Fig. 21) supporting a process of liposecretion that allow a direct conversion of the mature adipocyte into a well differentiated postsecretion cell that we denominated rainbow adipocyte for its multilineage differentiation properties (575, 576). These data support the idea that also *in vitro* mature adipocytes are able of transdifferentiative capacities and maintain a unique plastic phenotype.

Pathologic Remodeling

Positive energy balance, aging and genetic alteration of adipocytes molecular signaling induce a peculiar remodeling of adipose organ with important unhealthy consequences. Some aspects of whitening remodeling are particularly important and offer an explanation to old clinical observations that can be viewed to date under a wider perspective including the plastic properties of adipose organ.

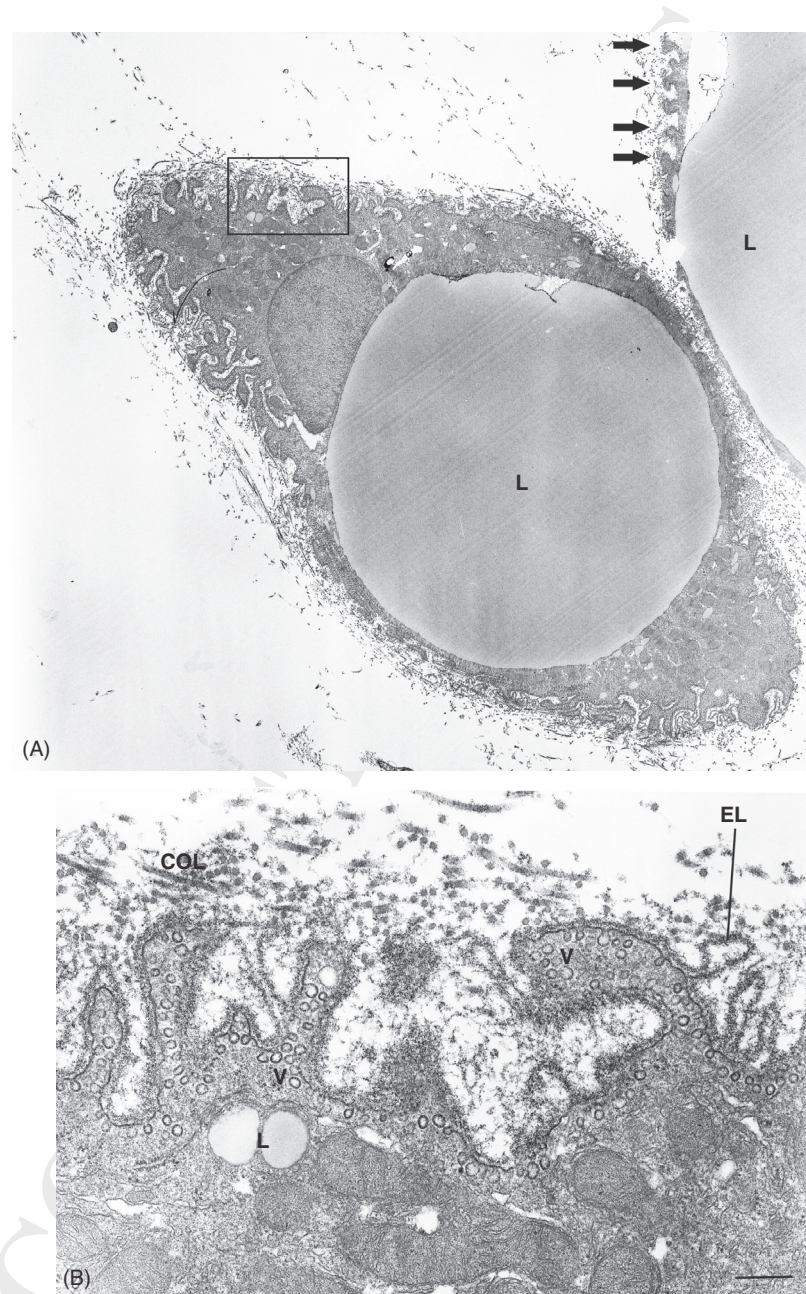


Figure 18 Electron microscopy of slimming adipocytes. Bar: in A, 1.6 μm ; and in B, 0.2 μm .

The obese adipose organ

Hypertrophy and hyperplasia of adipocytes

In obese animals and humans, WAT of adipose organ is expanded due to an increased number of cells (hyperplasia) and to enlargement of single cell size (hypertrophy) (279, 280, 433, 574). The fat expansion in humans can reach 60% to 70% of total body weight (386, 704). Furthermore, most BAT in the adipose organ is transformed into a WAT-like tissue (whitening) especially in genetically obese mice (177).

In rare cases, the obese adipose organ is mainly or exclusively hyperplastic or hypertrophic.

Mice lacking the functional receptor of leptin (db/db) are massively obese and their WAT is exclusively hypertrophic (158, 433), suggesting a role for leptin in adipogenesis (see paragraph on origin of adipocytes).

Mice with “human-like” WAT (i.e., with high $\alpha 2/\beta$ -AR balance in adipocytes) after high-fat diet (HFD) are obese with only WAT hyperplasia (932).

In all other known conditions of murine obesity, WAT is characterized by both hypertrophy and hyperplasia of

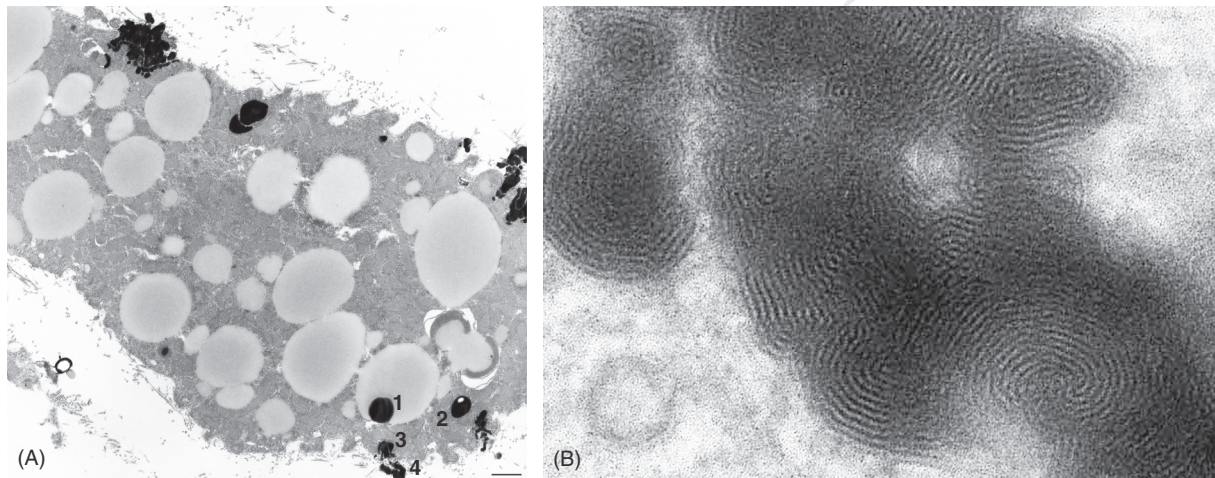


Figure 19 Morphologic aspects of lipolysis in slimming adipocytes. Electron microscopy (osmium-tannic acid cytochemistry). Bar: in A, 1.0 μm ; and in B, 40 nm.

adipocytes (279, 468, 469) and the chronic positive energy balance can influence both neo-adipogenesis as well as expansion of mature adipocytes and the different reaction of subcutaneous versus visceral fat can produce relevant clinical differences (683).

It has been proposed that an altered mechanism of preadipocytes development (involving the BMPs system, see paragraph on origin of adipocytes) could be the cause for ectopic (visceral) fat accumulation with important clinical consequences (850).

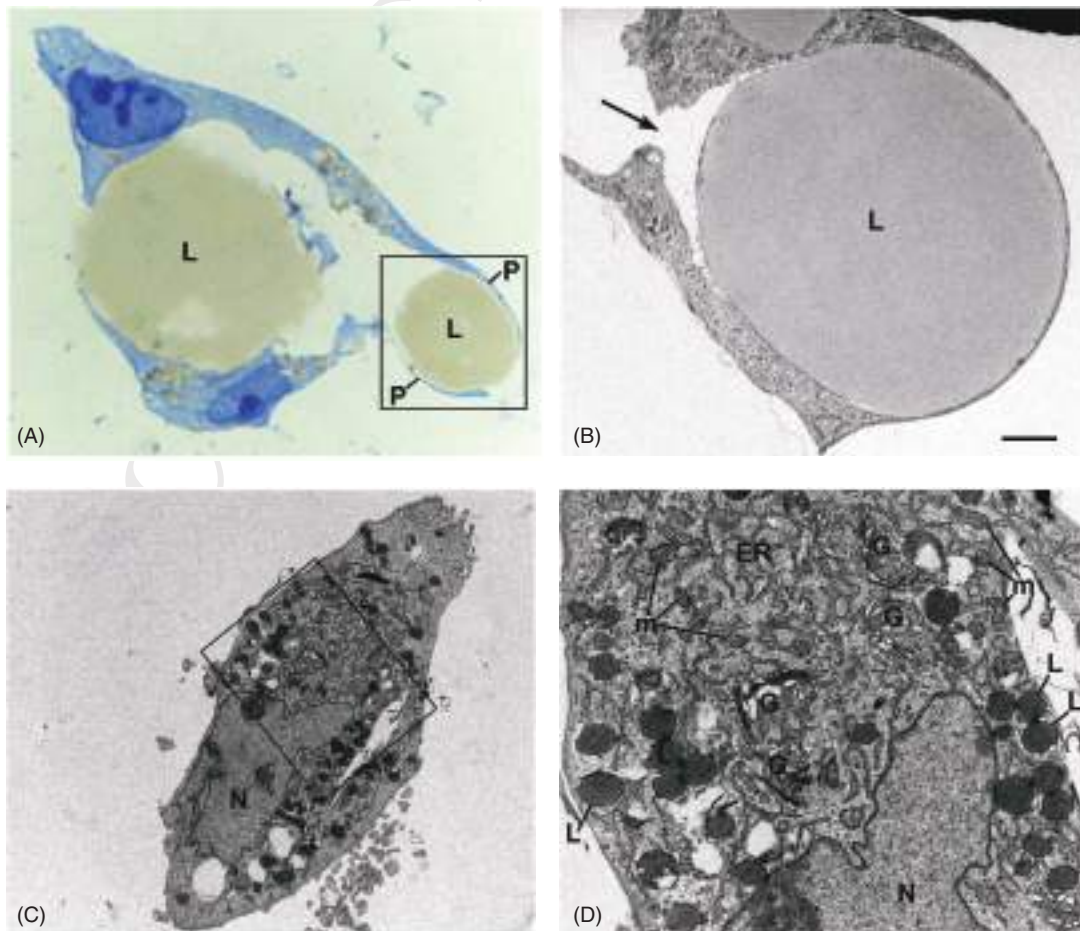


Figure 20 Critical steps of liposecretion process of mature adipocytes cultivated in primary culture (see also video: Fig. 21). Bar: in A, 2.5 μm ; in B, 1.0 μm ; in C, 1.5 μm ; and in D, 0.5 μm . Adapted, with permission, from (576).



Figure 21 Still of time-lapse video reproducing a record of about 70 h of mature adipocytes in primary culture (see also Fig. 20; see video in 576). Adapted, with permission, from (576).

Thus, the cytological reaction of adipocytes to the positive energy balance as well as the site of reaction (subcutaneous or visceral) assume a clinical relevance mainly in relation to the adverse consequences of obesity.

In humans, the most important disorders related to an excess of adipose tissue accumulation (diabetes, cardiovascular diseases, hypertension) are correlated to the WHR ratio that is an index of visceral adipose tissue accumulation (central), suggesting an adverse potential of visceral adipose tissue in comparison with subcutaneous adipose tissue (67, 71). Importantly, it has been claimed that up to 85% of population variance in central abdominal fat is attributable to genetic influences (117).

The discovery that the $\text{TNF}\alpha$ is an adipose secreted factor with potential negative influence on insulin receptor physiology (407) and that can mediate brown adipocytes apoptosis (637), caused a wide interest among scientists in search for a link between obesity and related disorders (mainly T2 diabetes). The well-known positive correlation between size of adipocytes and insulin resistance (870) opened the new perspective that expanded fat with enlarged adipocytes causes increased circulating levels of $\text{TNF}\alpha$ and consequent insulin resistance and subsequent T2 diabetes.

Chronic low-grade inflammation In 2003, two independent groups discovered that the obese WAT is infiltrated by macrophages creating a chronic low-grade inflammation (964, 979). Furthermore, they found a positive correlation between the size of adipocytes and number of macrophages infiltrating fat. It is well known that WAT can be artificially divided in two fractions by a method based on collagenase incubation and subsequent mild centrifugation. This method allows to separate the stroma-vascular fraction (SVF) from the floating fraction (FF) (72). FF contains the isolated mature adipocytes; SVF contains the rest of WAT including endothelial cells, adipocyte precursors, and inflammatory cells (171, 172).

Most of the cytokines produced by obese WAT (mainly: $\text{TNF}\alpha$, IL-6, and i-NOS) were found in the SVF suggesting that macrophages and not hypertrophic adipocytes per se are the source of molecules with adverse effects on insulin receptor and that are able to cause, in long run, T2 diabetes (964, 979).

Death of adipocytes The cause of macrophages infiltration and establishment of a chronic low-grade inflammation is debated (93, 284, 444, 657, 795). We found that the overwhelming preponderance (>90%) of MAC2 immunoreactive (in active state of phagocytosis) macrophages infiltrating WAT of obese mice and humans are organized to form specific histopathologic figures denominated crown-like structures (CLS) (179) (Fig. 22). These structures are formed by CD206 immunoreactive (M2) macrophages surrounding an adipocyte-like structure. Frequently, giant multinucleated

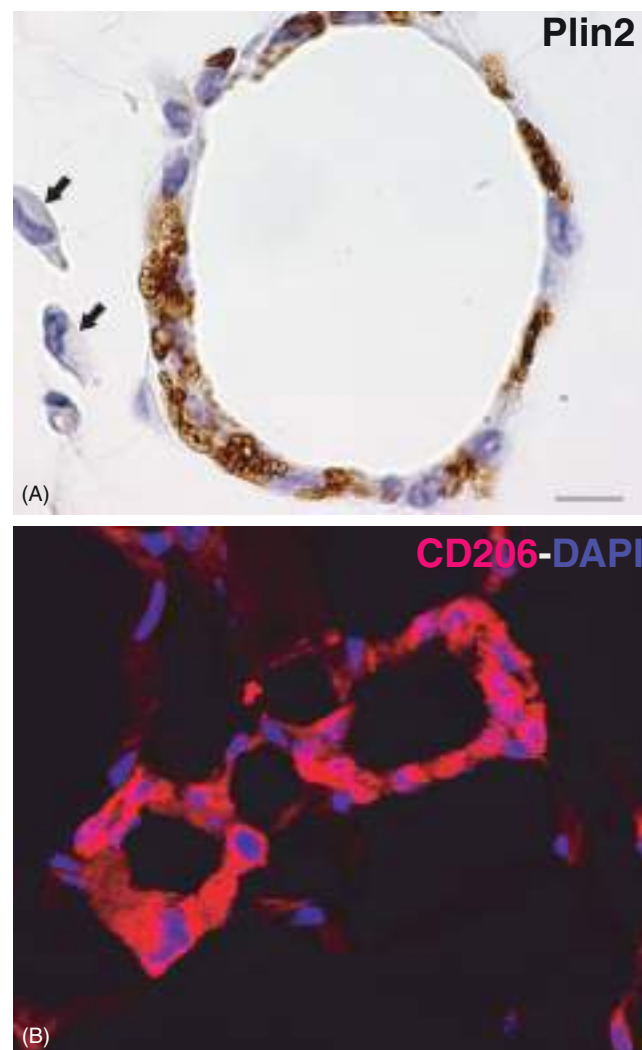


Figure 22 Immunohistochemistry of CLS showing M2 immunoreactivity of macrophages. Bar: in A and B 15 μm . A adapted, with permission, from (179).

MAC2 immunoreactive macrophages are also part of CLS. Immunohistochemistry and electron microscopy analyses revealed that CLS are sites of reabsorption of dead adipocytes. The largest part of adipocytes is formed by their lipid droplet, as a consequence most of the residual debris derived from adipocyte death is the lipid droplet, thus active macrophages reabsorbing the debris of death adipocytes (mainly the lipid droplet) form CLS in obese WAT. CLS are present also in lean WAT but their frequency resulted about thirty times higher in obese WAT (179) with a positive correlation between size of adipocytes and CLS density (612).

Subsequent elegant studies confirmed that adipocytes die both in lean and obese subjects and found that the average life of human adipocytes is about ten years (857).

To confirm that CLS are indeed due to death of adipocytes we used a well-characterized transgenic mouse model in which the death of adipocytes in adult mice is inducible and high synchronized. We used the “fat attac” transgenic model in which apoptosis is induced through forced dimerization of a caspase-8 fusion protein (613). The time course of histomorphological changes in epididymal and mesenteric WAT of these mice upon induction of caspase-8 dimerization showed that several adipocytes lost their immunoreactivity for perilipin1 (marker for alive adipocytes) and showed ultrastructural alterations of their organelles. The first population of inflammatory cells infiltrating the dying fat was made up by neutrophils, lymphocytes, and MAC-2 negative macrophages. In the following stages of the process, MAC-2 positive macrophages substituted for MAC-2 negative macrophages, followed by CLS formation. All perilipin1 negative dead adipocytes formed CLS (613). The reabsorption mechanism of macrophages in CLS has been recently described as exophagy (374).

Visceral adipocytes are more prone to death than subcutaneous adipocytes, as a matter of fact, in spite of a positive correlation between density of CLS and size of adipocytes, obese visceral fat with smaller adipocytes than those of subcutaneous fat show higher density of CLS suggesting that visceral adipocytes have a critical death size (size triggering death) lower than that of subcutaneous adipocytes (163,612). The cause of this different critical death size is unknown but several differences in secreted autocrine or paracrine factors by adipocytes or due to extracellular matrix components could play a role (683). Other explanations have been proposed: at least part of the visceral fat is whitened BAT, thus smaller than subcutaneous WAT and with different cellular properties (333). Very recently, a high propensity for death of whitened brown adipocytes has been shown (473). Interestingly obese db/db mice (lacking leptin receptor) with very large adipocytes (only hypertrophic fat, with visceral adipocytes size of about 11,000 μm^2) show about 3.5 times CLS density in visceral fat than obese ob/ob mice (lacking leptin), with hypertrophy and hyperplasia of fat (with visceral adipocytes size of about 8000 μm^2) (612). In line with these inflammation aspects, db/db mice develop T2 diabetes much earlier than ob/ob mice (513).

Bone marrow derived macrophages in peripheral tissues have been classified as inflammatory (M1) and anti-inflammatory (M2, mainly in phagocytosis state). A wide debate on the M1 or M2 state of macrophages infiltrating the obese fat is still open, but recent work indicates that the obese state recruit macrophages with a complex mixture of M1 and M2 phenotypes (380,819). Moreover, some researchers did not observe an obesity-driven phenotypic switch of macrophages from an M2/anti-inflammatory to an M1/inflammatory state (981).

Among the effects of paracrine influences by the cytokines produced by macrophages, it has been proposed also a positive effect on adipogenesis that should play a role to reconstitute the adipocyte compartment of fat when inflammation produce massive death. In visceral fat of diet-induced obese mice, the number of dead adipocytes can be very high with about 70% of WAT occupied by CLS (873). This massive efferocytosis is followed by a prompt (few weeks) restoration of adipose population that imply a massive impulse to preadipocytes development. It has been shown that M2 macrophages of CLS secrete osteopontin (OPN) that is a potent chemoattractant for the subpopulation of CD44+ (receptor for OPN)/+ adipocyte precursors (505,508), suggesting a role for CLS macrophages in the recruitment of new adipose cells.

Interestingly a recent paper (563) support the idea that adipocyte precursors, under appropriate conditions, can shift toward fibroblast-like cells and be responsible for the characteristic fibrosis of human fat of obese patients.

Pyroptosis Fat inflammation and CLS density are positively correlated with adipocyte size (612,964). To find the cause of death of adipocytes we studied a model of lean mice with hypertrophic adipocytes. Hormone sensitive lipase (HSL) is an important enzyme for lipolysis in adipocytes. Mice lacking this enzyme are lean but their adipocytes are hypertrophic. The density of CLS in fat of these HSL-knockout mice is similar to that found in WAT of genetically obese mice suggesting that size and not obesity per se, is responsible for adipocytes death (160). Interestingly, rosiglitazone administration to obese mice, reduced the size of adipocytes, the CLS density, and improved their glucose metabolism (482).

To try to understand the cause of death in hypertrophic adipocytes we studied the ultrastructure of subcutaneous and visceral fat of two murine models of genetic obesity (336). Several organelle alterations including irregular size and number of mitochondria, dilatation of rough endoplasmic reticulum, hypertrophy of Golgi complex was found in obese adipocytes that also showed glycogen clusters, calcium deposits, and increased amount of collagen fibrils associated with the external membrane. Most of these alterations (with the exception of calcium deposits) were quantitatively more abundant in visceral than in subcutaneous fat in both strains. Furthermore, we also found, occasionally, cholesterol crystals in obese adipocytes. Notably, size and cholesterol content correlate positively in adipocytes (477). In line with



Figure 23 High-resolution scanning electron microscopy of a hypertrophic and degenerating adipocyte from an obese db/db mouse. Bar: 2 μ m. Adapted, with permission, from [336].

these morphologic data suggesting a stressed phenotype of obese adipocytes, we found increased gene expression levels of some cell-stress markers: SOD-1, catalase, and GPX-1 (677).

We also found electron microscopy (both by transmission and scanning microscopy) pictures of degenerating adipocytes extruding lipid droplets with figures suggesting a prompt reaction by macrophages ready to surround and reabsorb the dying adipocyte (Fig. 23). The absence of perilipin1 in about 15% of obese adipocytes at immunohistochemistry corroborated the morphologic suggestion of degenerating adipocytes.

Hypertrophic and stressed adipocytes have hypertrophic Golgi complex and developed rough endoplasmic reticulum that are organelles implied in protein secretion, in line with the well-known hyper production of potent macrophage chemoattractant proteins such as MCP-1, CXCL14, MIP-1 α , MCP-2, MCP-3, and RANTES by obese adipocytes (255,445,878,979). In human obese fat, the hyper production of collagen (365) and amyloid has also been shown (695).

The presence of cholesterol crystals and altered organelles are potential damage-associated-molecular patterns (DAMPs) known as activators of Nucleotide Oligomerization Domain receptors (NOD-like receptors, NLRs) that together with the precursor procaspase-1 and the adaptor ASC (PYCARD: Apoptosis-associated speck-like protein) form one of the protein complex of innate immunity known as

inflammasome (255,715,741). The NLRP3 form of inflammasome has been shown to be activated in obese fat (940), and we observed that the gene expression levels of thioredoxin-interacting protein (TXNIP), which drives NLRP3 inflammasome activation following oxidative stress, were increased in obese fat. NLRP3 activation results in production of activated caspase-1 that in turn processes the cytosolic precursors of the proinflammatory cytokines IL-1 β and IL-18, which are secreted as biologically active cytokines that play important pathogenic roles for the development of insulin resistance (246,425,495,661,871). Caspase-1 is a proinflammatory caspase whose activity can result in a highly inflammatory form of cell death known as pyroptosis (53,566,804).

We detected ASC, NLRP3 and caspase 1 in the cytoplasm of obese adipocytes (in genetically obese db/db and ob/ob as well as in HFD obese mice) by immunohistochemistry strongly supporting the idea that hypertrophic adipocytes die by pyroptosis (336). In line with this hypothesis the caspase-8 induced adipocyte specific death in the FAT ATTAC apoptosis model did not show any inflammasome related protein immunoreactivity in adipocytes (336).

In summary, considering all together, the sequence of events preceding the CLS formation could be: (i) hypertrophy of adipocytes due to positive energy balance, (ii) stress of hypertrophic adipocytes with organelles alteration, (iii) production of MCP1, with macrophages attraction to stressed adipocytes, (iv) degeneration and death of adipocytes by pyroptosis, and (v) CLS formation and reabsorption of debris derived from dead obese adipocytes. Of note, hypertrophic obese adipocytes are very big structures in comparison with the size of macrophages implying the need for multinucleated giant cell formation and a chronic phagocytosis activity. Several cytokines and factors produced by stressed adipocytes and macrophages in their different functional states are likely to be the molecular link between obesity and type2 diabetes (406,546,580,666,997). The lower critical death size of visceral fat offers an explanation to the well-known difference in metabolic-consequences between subcutaneous (pear shaped: protective) and visceral (apple shaped: inducing) obesity (73,693,929).

The concept of Critical Death Size Index As earlier detailed, two cell autonomous aspects are very important drivers of molecular mechanisms inducing the death of obese adipocytes: size and localization in the organ. As a matter of fact, in spite of a positive correlation between size and CLS density for both subcutaneous and visceral fat depots, visceral adipocytes die at lower size both in mice and in humans (103,612). Thus, subcutaneous and visceral adipocytes display a different critical death size (CDS) (163). The CDS seems to be variable not only between subcutaneous and visceral fat but also in different areas of these depots, at different ages, different level of obesity and different genetic backgrounds, thus I propose a new parameter: critical death size index (CDSI) that take into account in the same time of the two variable aspects: size and death proneness indicated

by the CLS density in that specific area of adipose organ. The index results from the ratio between CLS density/area of adipocyte $\times 1000$ and its main purpose is to give evidence to the unhealthy potential of the pathologic enlargement of adipocytes in that specific area of adipose organ. For example, in adult genetically obese db/db mice the inguinal (subcutaneous) adipocytes have an average size of about $14,000 \mu\text{m}^2$ and a CLS density of about 250 CLS/10,000 adipocytes. Thus, the CLS index here is $250/14,000 \times 1000 = 17$. In the same mice, mesenteric adipocytes have an average size of about $11,500 \mu\text{m}^2$ but the CLS density in this depot is about 1500 CLS/10,000 adipocytes, thus here the CLS index is 13, thus 7% higher than that of inguinal fat. In the omentum of the same mice, the CLS index is 40, thus higher than inguinal but lower than mesenteric. Future applications of this index could be useful for a better understanding of the pathologic proneness of different fat depot in the adipose organ.

Adipose Organ Remodeling with Aging

Aging is a physiologic process, and several data link adipose organ remodeling with aging.

BAT quantity reduces with age. In a quantitative study of interscapular BAT of rats of different ages (from 1 week old to 104 weeks old) we showed a progressive decrease of multilocular adipocytes (*bona fide* brown adipocytes) in the interscapular area of adipose organ with a progressive simultaneous increase of unilocular adipocytes (*bona fide* white adipocytes). The percentage of multilocular versus unilocular in young animals was about 90% and progressively reduced to about 50% (793). Interestingly rats 104 weeks old exposed for 4 weeks at 4°C reversed the proportion approaching to that 1 week old, supporting the reversibility of the phenomenon (605).

In line with these old data, several recent papers showed a progressive BAT-WAT conversion also in other parts of the organ (499, 747). Interestingly loss and gain of function data support a role for orexin, a neuropeptide produced by neurons in the lateral hypothalamus (222, 779) in this aging-related remodeling phenomenon (810, 811).

Several data support similar reduction with age and BMI in humans (767, 946). In a case series of about 50 peri-carotid neck biopsies of adult patients we found UCP1 immunoreactive BAT in all patients below 30 years, in 20% to 30% of patients below 50 and in only 1 (60 years old) in 10 patients aged between 60 and 85. Furthermore, we found BAT in 90% of lean and only 10% of overweight patients; none of the obese subjects studied showed BAT (161, 1019).

This BAT reduction with age could be due to several age-related mechanisms including reduction of stem cells reproductive capacities, reduction of T3 production and T4-T3 conversion, reduction in mitochondrial function and of sympathetic nervous system activity (353, 746).

Interestingly PTEN (phosphatase tensin homolog, oncosuppressor gene) transgenic mice have enhanced BAT activity

and live longer than controls (659). This is not surprising considering the antiobesity, antidiabetic, and antiatherosclerotic activity of BAT (26, 40, 625, 809, 865).

The concept of inflammaging imply a key role for chronic inflammatory states as aging factors and low grade of chronic inflammation of obese adipose organ (or meta-inflammation) is a paradigm of this concept (297). As a matter of fact, in meta-inflammation a dramatic increase in the number of cells expressing markers of cellular senescence [such as, p16, p53, and senescence-associated β -galactosidase (SA β -gal)] in visceral adipose tissue (VAT) was found. Senescent cells (probably macrophages and T lymphocytes) secrete several factors, which are collectively known as a senescence-associated secretory phenotype (SASP) (298, 375, 833). These factors damage neighboring cells and contribute to age-related degeneration and inflammation that correspond to inflammaging.

Calorie restriction (CR) is a well-known antiaging mechanism across several species from yeast to primates (14, 65, 235, 295). At least part of the beneficial effects is thought to be due to the loss of adipose tissue that can be mimicked by surgical fat removal (80, 415). The molecular mechanism linking CR to lifespan is largely unknown but several data converge on the idea that adipose organ mediate cell-autonomous and cell-nonautonomous mechanisms linking miRNAs of fat and aging.

Dicer is an endoribonuclease with important effects on the microRNA pathway, which has a significant role in the differentiation and function of WAT and BAT (600, 602). It has been shown that adipose organ dicer reduces with aging and this reduction can be prevented by CR (601).

Dysregulation of miRNAs has been associated with several age-related diseases (245). Furthermore, miRNAs may influence pathways involved in senescence such as p53/p21 and p16/RB and pathways of proteins and transcription factors involved in senescence such as IL-6 and IL-8 and HMAG2 (61, 348).

The proposed cell autonomous mechanism should be related to miRNAs responsible for the renewal mechanisms of adipose tissue. Their alteration would induce insulin resistant hypertrophic adipocytes and inflammaging (601). The nonautonomous mechanisms would imply secretion of miRNAs influencing the rest of the organism (137).

Interestingly, fat specific dicer-KO accelerate aging and mitigates several effects of CR in mice (725).

Adipose Organ Remodeling in Humans

The anatomical organization of human adipose organ is very similar to that described above for murine adipose organ (304): that is, a large and diffuse organ composed by a mixture of WAT and BAT, weighting about 20% of the total body weight (adult lean) and mainly distributed in two compartments of the body: subcutaneous and visceral, but with extensions into several other organs such as muscles, heart, bone marrow, parotid, parathyroid, gastrointestinal apparatus,

lymph nodes, joints, and visual apparatus. The most relevant differences between humans and rodents reside in the relative amount of BAT, its anatomical localization and in the organization of subcutaneous WAT.

Several aspects of human adipose organ have been specifically described in previous paragraphs of this review and only a general view of its anatomy and remodeling is reported here.

BAT

Immunohistochemistry studies and clinical imaging techniques (mainly based on positron emission tomography: PET) have been used quite recently to confirm old data (498) showing the presence of metabolically active BAT in adult humans (211, 608, 626, 776, 938, 948).

Human BAT is composed similarly to murine BAT (Fig. 2): that is, UCP1 immunoreactive multilocular adipocytes organized in polygonal lobules densely innervated by TH immunoreactive (noradrenergic) parenchymal nerve fibers and provided with a dense vascular supply (Fig. 24).

In the adipose organ of adult humans, it is rare to find large areas composed exclusively by BAT. Even the more compact areas are in fact “infiltrated” by unilocular adipocytes, thus resembling the mixed depots of murine adipose organ (1019). Electron microscopy show the typical numerous large and packed with laminar cristae mitochondria. Several adipocyte precursors have been described in pericarotid BAT of human adults. Quantification of these brown adipocyte precursors identified by their specific ultrastructural characteristics (see development paragraph) suggest a frequency of these cell of about 1/5-10 pericapillary areas (1019). Their ultrastructure was very similar to that found in brown adipocyte precursors of hibernomas, where their frequency was about five times higher (561).

In adult female Sv129 mice (obesity-resistant), maintained for ten days at 28°C, quantitative analyses showed that

about 60% of the total number of adipocytes contained in the adipose organ were brown adipocytes. Instead, in the obesity-prone mice B6 (same experimental conditions as Sv129) only about 15% of all adipocytes were brown adipocytes (614, 950), supporting a role for the genetic background for obesity prevention.

Precise anatomical quantification studies of human BAT does not exist, but, in adult lean humans maintained in a standard western-type environment, BAT have been calculated in about 100-150 gr (771, 937), thus representing a very low percentage (about 1%) of the human adipose organ weight, but even a prudent estimate of 50 g of active BAT in adult humans would mean a potential impact on BMR of 2.7% to 5% that outline its potential clinical relevance (210). Furthermore, it must be outlined that BAT activity is highly dependent from age, sex, BMI, and environmental conditions living open the door to the possibility to increase the amount of active BAT in adult humans (917). Like in small mammals, obesity and aging inversely correlate with the presence of BAT and in old rats, we found an about 50% reduction of interscapular BAT that can be entirely reconstituted by cold exposure (605, 793). As a matter of fact, it has been clearly shown also in humans that the amount of active BAT can be increased by acute and chronic cold exposure and by administration of the last generation β 3AR agonist mirabegron and capsinoids (212, 775, 938).

The therapeutic impact of a chronic activation of BAT in adult humans to treat obesity and related metabolic disorders is still unknown, but several data suggest a positive impact on insulin sensitivity of cold exposure (144) in line with previous studies showing a reduced expression of brown adipogenic genes in subcutaneous fat of adult humans with insulin resistance (994).

Furthermore, it must be outlined that under appropriate conditions not only quiescent BAT become active, but also WAT of adult humans can convert to BAT.

Omental fat is a classic WAT of adult humans and the analyses of biopsies from 20 lean adult patients confirmed the presence of only unilocular UCP1 negative adipocytes in this tissue. Omental biopsies from 12 patients suffering from pheochromocytoma (benign tumor of adrenal glands secreting adrenaline and noradrenaline) showed a reduction in size of unilocular adipocytes in all pheochromocytoma patients and browning in half patients (307). Browning was accompanied by a classic remodeling of the tissue with increased density of capillary network and of noradrenergic parenchymal nerve fibers. Interestingly in all patients showing browning we found not only UCP1 immunoreactive multilocular adipocytes but also UCP1+ paucilocular adipocytes (UCP1-PL), probably representing the first step of white-brown conversion (see browning paragraph). The electron microscopy of UCP1-PL revealed the presence of abundant mitochondria with intermediate features between those of white-like and brown-like mitochondria and rare intermediate forms, that is, mitochondria with an area with brown-like characteristic (roundish with laminar cristae) and an area with

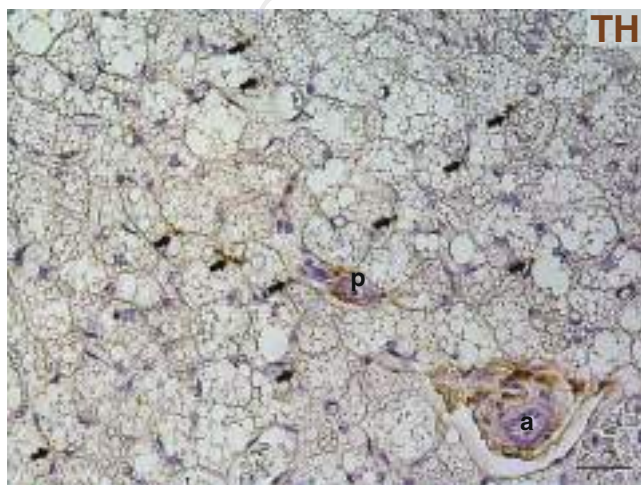


Figure 24 TH immunoreactivity of parenchymal noradrenergic fibers in human brown adipose tissue. Bar: 23 μ m.

white-like characteristic (elongated with randomly oriented cristae). Interestingly, very similar intermediate mitochondria were found in transforming human brown adipocytes maintained in primary culture (152). The master transcription factor of browning PRDM16 was detected by immunohistochemistry only in nuclei of cells with morphologic features of converting adipocytes (i.e., mainly in paucilocular adipocytes and multilocular adipocytes) (307). Some multilocular adipocytes resulted PRDM16 negative suggesting a role for the acquirement of the brown phenotype but not for its maintenance.

The origin of brown adipocytes found in the omental fat of pheochromocytoma patients seems, therefore, to be due to direct conversion of preexisting white adipocytes and data obtained with the proliferation marker Ki67 and quantitative electron microscopy of preadipocytes fully confirmed this idea. Ki67 was negative in multilocular cells (with internal positive cells in a lymph-node). Preadipocytes show specific features that can be visualized by electron microscopy, and a quantitative evaluation showed no difference with omentum samples of normal subjects and allowed us also to exclude the possibility that preexisting preadipocytes developed without proliferation because intermediate forms between preadipocytes and mature multilocular cells were not detected.

Thus, the main phenomenon giving rise to brown adipocytes in omental fat of adult humans in these conditions seems to be a white to brown direct transdifferentiation of mature adipocytes. The fact that visceral human white adipocytes of a depot (omentum), that is usually resistant to browning even in small mammals (169), can convert to brown adipocytes offer a new important therapeutic target (333).

PET studies showed that the activated BAT in humans is distributed in the areas surrounding aorta and its main branches (483), thus in the visceral part of the adipose organ including neck (perisubclavian and pericarotid fat), thorax (periaortic and periintercostal fat), and abdomen (mainly: periaortic and perirenal). It must be outlined that the supraclavicular region is one of the sites of branches of aorta (subclavian arteries) and this site is considered the main or classic anatomical site for BAT in adult humans, but other periaortic regions (such as perirenal fat) have been proven as sites of BAT not only in adults but also in old patients (161, 169).

Thus, the main depot for human BAT is visceral and close to the aorta system. This anatomical location offers an easy finalistic reason: the aorta system is ready to distribute heat produced by BAT to the rest of the body. In small mammals (mice, rats, and ferrets), the main depot for BAT is located in the interscapular and surrounding regions (subscapular and deep cervical). These areas are located in part in the subcutaneous depot (interscapular) and in part in deep intermuscular regions (subscapular and deep cervical) in direct anatomical continuity with the anterior subcutaneous depot and the vascular system is connected with these BAT-rich areas with special vessels that do not exist in humans such as the Sulzer's

vein (624). The reason for this difference could depend on the fact that small mammals need more thermogenesis than humans' due to differences in heat dispersion caused by a different body volume/surface ratio that favors heat dispersion in small mammals (396). This difference requires extra BAT for small mammals that is located outside the trunk, but still in tight connection with the central vascular system through the Sulzer's vein connecting interscapular BAT with azygos vein. This location of BAT mainly in the subcutaneous compartment together with the widely accepted concept of subcutaneous inguinal fat proneness to browning probably contributed to the idea that subcutaneous fat should be the main target for browning. It should be outlined that some parts of this big depot are located deeply among skeletal muscles (subscapular and deep cervical) and therefore they are not strictly located in the subcutaneous compartment (between skin and skeletal muscle fascial plane). Furthermore, most data from murine adipose tissues studies come from epididymal fat used as a paradigm of visceral fat and its resistance to browning contributed to the aforementioned idea of subcutaneous target, but it must be outlined that epididymal fat is not in direct connection with the aorta system and cannot be taken in consideration as representative of the visceral compartment (169).

Thus, if the visceral fat should be the target for browning, the earlier reported data on browning of human omentum are particularly remarkable taking also into consideration the importance of visceral fat for the metabolic consequences deriving from its hypertrophy in obesity (73, 238, 929).

WAT

The vast majority of fat in human adipose organ is WAT (Fig. 25). Subcutaneous WAT is located in the compartment located below skin. Skin is composed by epidermis and dermis. Murine dermis contains WAT (175), human dermis contains WAT only in proximity to adnexa such as bulbs of hairs and sweat glands (169, 251). This WAT is continuous with subcutaneous fat that form a layer below skin of variable thickness and can be subdivided in two parts: superficial and deep (108). The visceral fat is distributed in the trunk similarly to that of murine adipose organ. Mediastinal fat is continuous with that surrounding the aorta and its main branches till the deep areas of the neck and axillae. Interestingly epicardial fat exhibits intermediate features between white and brown adipocytes (773). In the abdomen, it is located in intraperitoneal compartments (mainly: mesenteric and omental fat) or below the parietal peritoneum (mainly: perirenal and pelvic fat) (68, 411).

Distribution and thus, in a broad sense remodeling, because always associated with functional variations of adipocytes (16, 19, 21, 237, 299), of fat in subcutaneous or visceral compartments is mainly regulated by sexual hormones. The gender differences in human adipose organ distribution are more evident than in murine adipose organ (69, 70, 780, 894, 895). Females accumulate fat mainly in the

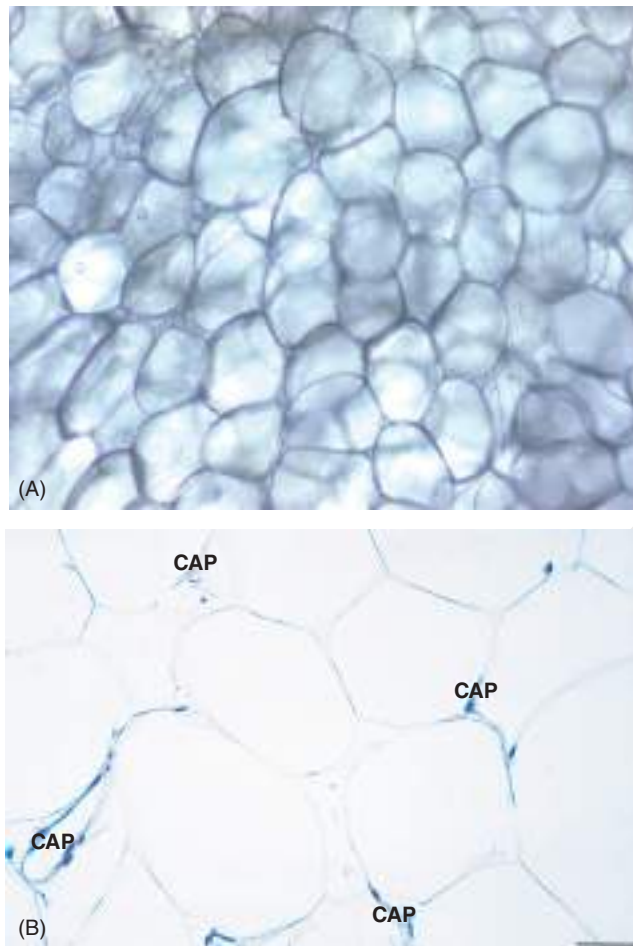


Figure 25 Human subcutaneous white adipose tissue fresh (A) and paraffin embedded (B). Bar: in A, 80 μ m; and in B, 30 μ m. B adapted, with permission, from [153].

subcutaneous compartment with preference of areas corresponding to the mammary glands localization in other mammals, that is, in the breast and in the gluteo-femoral areas. Males have more visceral fat than females: it has been calculated in lean adult subjects adjusted for their total body fat, females have about 60% of the visceral fat found in men (197, 223, 231, 514). The differences disappear after menopause (912, 1004) and studies on subjects under hormonal treatment for female-to-male and male-to-female transsexuals confirmed the importance of sexual hormones in fat distribution (259-261).

These differences are mediated also by different activity of LPL, that is, the enzyme favoring the lipid accumulation in adipocytes by uptake from circulating fatty acids and triglycerides (18, 719). LPL activity is higher in visceral fat of men and in subcutaneous fat of women. Furthermore, testosterone inhibit LPL activity in subcutaneous fat of men. Another mechanism explaining the sexual differences imply the different distribution of adrenergic receptors (AR): estradiol increases the antilipolytic α 2AR in subcutaneous fat (681). The ratio of α 2AR to β ARs in subcutaneous

fat in premenopausal women is high and reversed in visceral fat favoring lipolysis. In men and postmenopausal women, the ratio is reversed offering an explanation for preferred visceral fat accumulation (322, 733, 902). Also, the distribution of the two estrogen receptors ($ER\alpha$ and β) contribute to the different fat localization (615, 670). Mice lacking $ER\alpha$ have an increased body fat with preferential increase in the visceral compartment (217, 390), in line with several data suggesting a lipolytic effect of $ER\alpha$ and opposite effect of $ER\beta$ and with the relative lack of expression of $ER\alpha$ in visceral fat of males (243, 671).

Furthermore, the expression of $ER\alpha$ in central nervous system could play a role in fat distribution. $ER\alpha$ seems to be more expressed in hypothalamic neurons responsible for the adrenergic innervation of visceral fat and a target disruption of $ER\alpha$ in the ventromedial hypothalamus induces visceral obesity in female mice (3, 183, 982).

In line with these data experiments on denervated fat pads and central administration of estrogen demonstrated important peripheral effects due to central nervous system stimuli of $ER\alpha$. Mainly based on these data, it has been proposed a theory explaining the different gender distribution of fat. It is based on three main drivers: sympathetic nervous system (SNS) activity, adrenoceptors, and LPL activity. The increased SNS activity on visceral fat due to the hypothalamic activation by estrogens would result in less visceral fat in women. In addition, the higher leptin production by subcutaneous fat would reinforce the same SNS activity. These stimuli and the aforementioned different distribution of antilipolytic (α 2) and lipolytic (β 1-3) adrenoceptors together with the different LPL activity of adipocytes would explain the different sexual distribution of adipose organ.

Teleological explanations for gender differences in fat distribution have been proposed: the visceral fat would allow a readier disposal for short-term energy requirements typical for men hunting and combatting. The lower lipolytic rates in subcutaneous fat make this depot more appropriate to respond to chronic metabolic challenges such as those due to pregnancy and lactation periods (671).

In any cases, this sexual dimorphism has important healthy consequences when fat accumulation reaches the overweight or obesity state: only visceral fat accumulation has severe metabolic consequences (see also obese organ paragraph) (236, 480, 701).

The human fat of obese patients is inflamed with all the histology characteristics described earlier for murine obese fat, including the CLS (179).

In patients with unusually large adipocytes, we found structures similar to gigantic CLS that we described as cyst-like-structures (CyLS) (103). In this study, we showed that the size of adipocytes was larger in subcutaneous than in visceral fat in all patients, but the number of CLS was higher in visceral than in subcutaneous fat suggesting that, similarly to murine fat, also in humans the critical death size is smaller in visceral than in subcutaneous fat and offering an explanation to the old notion that visceral fat accumulation is more

dangerous for metabolic consequences than subcutaneous fat accumulation. Diabetic patients showed the highest level of CLS density in line with the idea that inflammation is linked to metabolic consequences (103).

After bariatric surgery and substantial weight loss subcutaneous fat histology showed a reduction in adipocyte size and in CLS density in line with previous work (106), but a diffuse infiltration of macrophages was still present in most diabetic patients in association with a persistence of altered pancreatic β -cell glucose sensitivity.

Adipose Organ Remodeling in Lipodystrophic Mice and Humans

Congenital and acquired lipodystrophies are rare diseases (about 1:1,000,000) affecting mainly the adipose organ (319, 520). It can affect all parts of the adipose organ (generalized), limited to specific areas (partial), or localized (trivial loss of fat). Genetic causes have been identified for *congenital generalized lipodystrophies* in humans including mutations in the following genes:

1. AGPAT2, encoding an acyltransferase resident in endoplasmic reticulum of adipocytes and responsible for de novo phospholipid and triglycerides biosynthesis, (4, 319, 320).
2. BSCL2, encoding seipin, a protein resident in endoplasmic reticulum of adipocytes and involved in the normal formation of lipid droplets, (558, 936).
3. CAV1, encoding caveolin1, a main component of caveolae plasma membrane in several cell types. Adipocyte membranes are rich in caveolae, which increase ten times during differentiation *in vitro* (274) and that are involved in the lipid droplet development (457, 663).

Moreover, familial partial lipodystrophies:

1. LMNA, encoding for two major proteins: prelamin A and lamin C plus lamin A Δ 10 and C2 by alternative splicing. Posttranslational modifications of prelamin A induces the formation of mature lamin A. Lamins are members of intermediate structural nuclear filaments of inner surface of nuclear lamina that are efficiently processed to allow several basic functions of the cells permitted by fine chromatin conformational changes, affecting DNA damage response factors and shuttling transcription factors (104, 116, 362, 607).
2. ZMPSTE24, encoding a prelamin-proteolytic processing zinc metalloproteinase necessary for lamin A formation (842).

3. PPARG, encoding PPAR γ a key transcription factor in adipogenesis (759).

4. AXT2, encoding protein kinase B, a phosphoinositide-dependent serine/threonine kinase involved in postreceptor insulin signaling (325).

Anyway, the most prevalent form of lipodystrophy is an acquired form that occurs in HIV-infected patients chronically treated with protease inhibitor therapy (418).

Several of the generalized forms (both congenital or acquired) have a common trait: fat misdistribution with absence in subcutaneous areas mainly in the lower part of the body and abundance of fat in the visceral compartments of the upper part of the body (268, 659, 799).

This upper-body location corresponds, at least in part, to the usual anatomic distribution of human BAT (see the human adipose organ paragraph) and several data suggest that this fat could indeed be a modified BAT both in murine models and in human patients (see the succeeding text).

Most of the patients suffering for lipodystrophy present symptoms similar to those of obese patients such as insulin resistance, hyperglycemia, hepatic steatosis, hypertension, and dyslipidemia (360, 413, 769, 788, 789). This paradox clearly suggests that either pathologic excess or reduction of adipose organ induce similar metabolic alteration and a balanced energy storage and utilization are necessary for normal homeostatic mechanisms (943) (Fig. 26).

Several murine models of lipodystrophy (787) have been created or described including:

1. Mice with adipocyte-specific expression of diphtheria toxin (766).
2. A-ZIP/F mice, which were rendered lipoatrophic by artificial interference with the adipogenic C/EBP transcription factors (527, 594). About 99% of WAT loss (727).
3. FAT-ATTAC mice, which undergo transient lipoatrophy after temporal induction of adipocyte apoptosis (613, 669).
4. aP2-nSrebp1c mice, which express a truncated, constitutively active Srebp-1c transgene in adipocytes, and whose lipodystrophy phenotype is poorly understood (830). About 70% of WAT loss with abnormal BAT hypertrophy (829).
5. fld ("fatty liver dystrophy") mice, which suffer from severe lipoatrophy because of spontaneous mutations in the Lpin1 gene that is a transcription activator necessary for adipocyte differentiation (upstream of PPAR γ) (685, 688, 730).
6. Mice deficient for Lmna (gene encoding nuclear lamins) (see the preceding text) (208, 258).

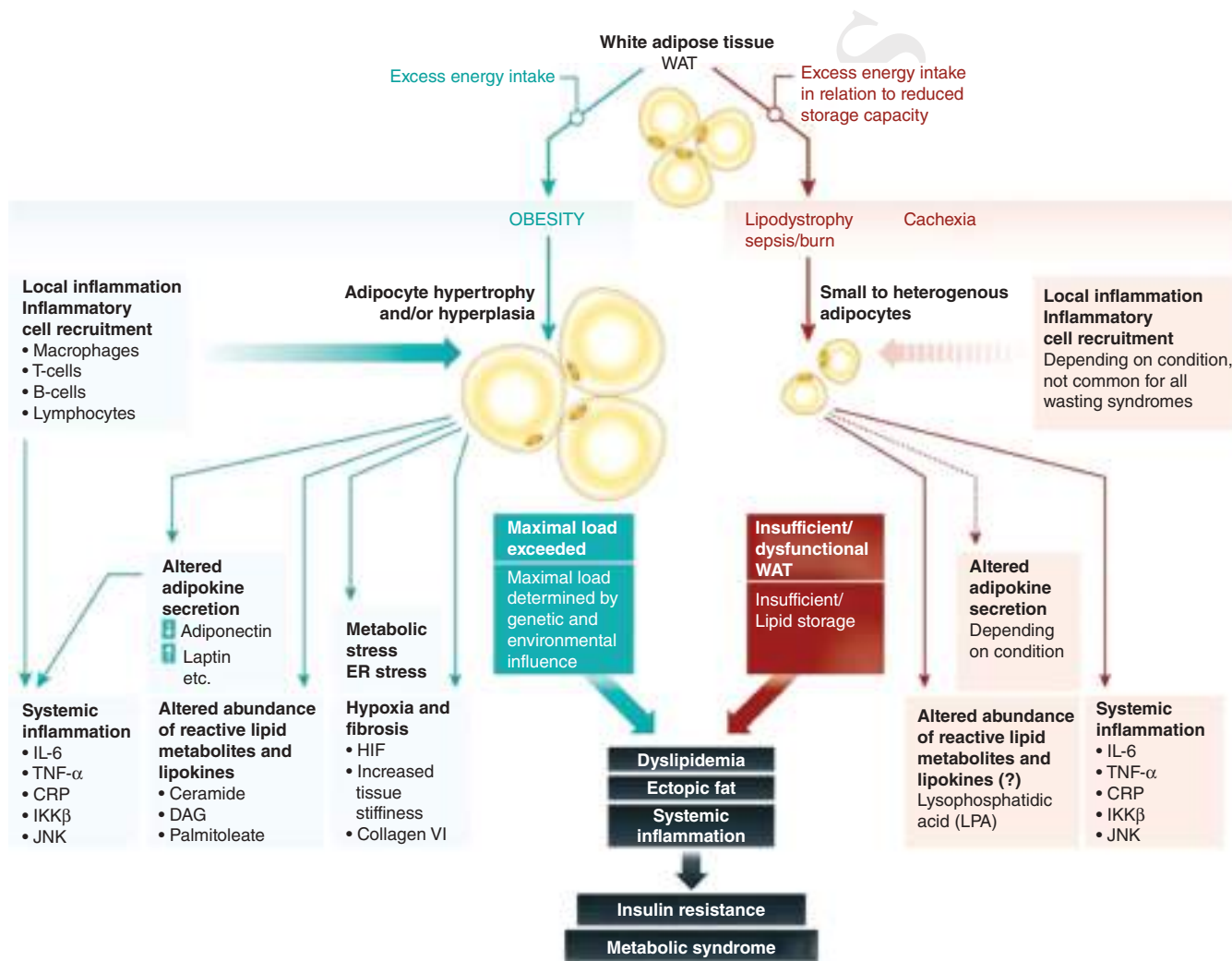


Figure 26 Proposed mechanisms for the paradoxical clinical outcome of obesity and lipodystrophy. Adapted, with permission, from [943].

- Zmpste24 is a gene encoding for a metallopeptidase involved in the processing of Lamin A (684).
- Ribosomal S6 kinase (Rsk2). RSK2 is a member of a family of growth factor-regulated kinases abundantly expressed in WAT and probably involved in a variety of physiologic processes. Its functional role is still elusive. Knockout mice showed about 50% of WAT loss (258).
- Insulin receptor ablation in 80% and 98% of the cells resulted in marked and diffuse lipodystrophy due to lack of differentiation of adipocyte precursors (175, 464). Interestingly, in localized lipodystrophy due to insulin injections in subcutaneous fat in patients with T1 diabetes, we found numerous poorly differentiated adipocytes and slimmed cells (588). We proposed that a localized insulin resistance caused by insulin injections could be responsible for both: lack of development on preadipocytes and prevalent lipolysis in differentiated adipocytes (Fig. 27)
- Irs1/Irs3 double-knockout mice show diffuse lipodystrophy (497).
- Adipocyte-specific Pparg-null mice show diffuse lipodystrophy with hypertrophy of abnormal brown adipocytes (388, 422, 434).
- PTEN-Myf5-Kcnoc¹ PTEN (Phosphatase and Tensin homolog) is a tumor suppressor that counteract the activity of phosphatidylinositol 3-kinase type I (PI3K). Myf5 is expressed by precursors of brown adipocytes in interscapular and perirenal BAT (808). Myf-5 driven ablation of PTEN give rise to a partial lipodystrophy with atrophy of WAT and hypertrophy of abnormal BAT (659, 783).
- Pparg^{ldi}. This partial lipodystrophy was the serendipitous outcome of a genetically engineered allele of peroxisome proliferator-activated receptor (PPAR γ); the phenotype was atrophy of WAT and hypertrophy of abnormal BAT (460).

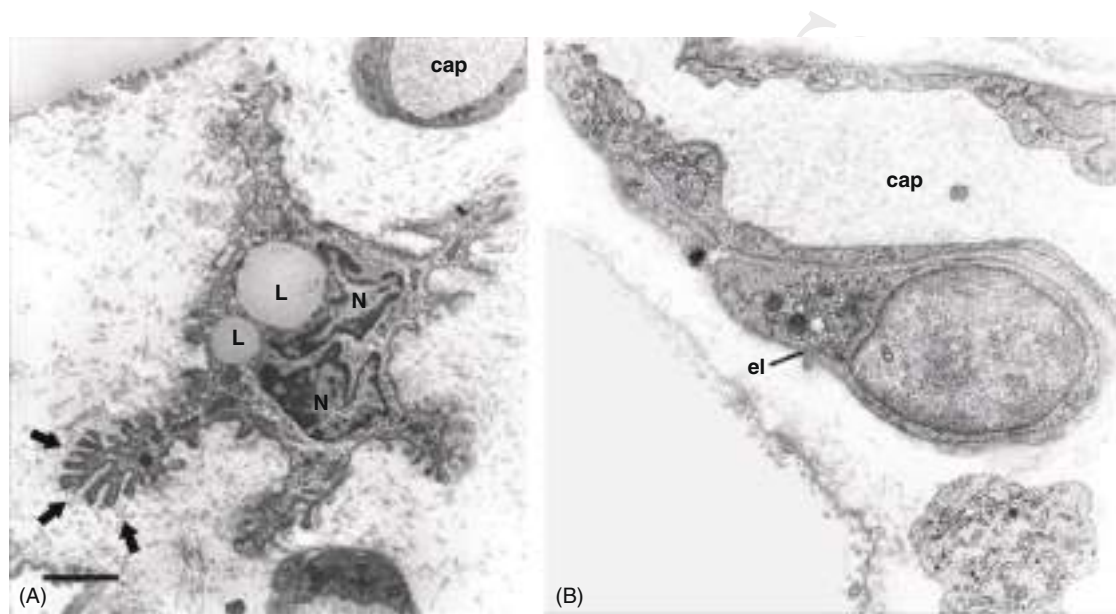


Figure 27 Slimmed adipocyte (A) and adipocyte precursors (B) resulted typical features of localized lipodystrophy secondary to insulin injections. Bar: in A, 1.8 μm ; and in B, 1.2 μm . Adapted, with permission, from (588).

Interestingly, several murine models of lipodystrophy (aforementioned in 4, 6, 7, 11, 12, and 13) showed a misdistribution and remodeling of the adipocytes in the adipose organ with variable atrophy of subcutaneous WAT and hypertrophy of abnormal BAT. The abnormal BAT was mainly characterized by hypertrophic adipocytes with a unilocular-like morphology (i.e., BAT-whitening).

Human lipodystrophy with similar characteristics are:

1. Multiple symmetric lipomatosis (MLS) of type 1 (Madelung disease). Rare acquired disease in adult red wine drinkers with symmetrical accumulation of fat masses in all typical sites for BAT areas and atrophy of WAT in the rest of adipose organ (268). Histology and electron microscopy of biopsies from lipomatosis areas showed unequivocal signs of morphological markers of modified BAT both in mature adipocytes of the tissue and in primary cultures of developing adipocyte precursors from the stroma-vascular fraction extracted from the MLS tissue (176, 639, 1005). Interestingly, the red wine is rich in resveratrol that is a potential stimulator of BAT (489). Recently a mutation in gene coding for hormone sensitive lipase in a case of multiple symmetric lipomatosis has been reported (1020).
2. Familial partial lipodystrophy (FPLD). The most common subtype is FPLD2 (about 500 reported patients) (198, 413). Due to a missense mutation of LMNA, it is an autosomal dominant disorder characterized by subcutaneous fat loss, with pubertal onset, from the upper and lower extremities and often fat accumulation in the face, neck, perineal, and intra-abdominal areas, particularly in women.

3. Highly active antiretroviral therapy–induced lipodystrophy (HAART) in patients infected with human immunodeficiency virus (HIV). This is the most frequent acquired lipodystrophy (128, 289, 645, 923). After 2 to 4 years of HIV-1 protease inhibitors (PIs) or nucleoside reverse transcriptase inhibitors (NRTI) therapy a partial lipodystrophy occurs with loss of subcutaneous fat from the extremities and face and fat accumulation in the BAT areas.

PIs inhibit ZMPSTE24 that is an important enzyme in the process of prelamin A to lamin A, thus inducing accumulation of toxic prelamin A (similar to FPLD2, see the preceding text). NRTI cause mitochondrial dysfunction due to inhibition of mitochondrial polymerase γ . A brown lineage signature has been shown in biopsies from lipodystrophic fat of these patients too (128, 910).

Thus, several murine models of lipodystrophy and the most frequent human congenital and acquired lipodystrophies have the WAT/BAT common trait described earlier.


One very simply explanation come from the general concept of adipose organ remodeling described in this review: mammals have an organ composed by two different tissues: WAT and BAT. Usually different tissues in the same organ cooperate to a single finalistic role useful for the organism homeostasis. Apparently, WAT (energy storing) and BAT (energy dissipation) have quite distinct and even opposite functions, but they cooperate in special occasions by the reversible transdifferentiation property: when chronic cold requires intense thermogenesis WAT browning increase the thermogenic BAT. When

chronic positive energy balance requires more storing tissue, BAT whitening conversion help to reach the goal. Thus, it could be hypothesized that lipoatrophy of WAT induce a transformation of the remaining part of the adipose organ (BAT) that adapt the cells from a thermogenic to a storing cell type. This transformation seems to be incomplete and residual markers of the original BAT tissue can be detected (128, 176, 639, 910, 1005). Of course, other mechanisms could concur to develop the abnormal hypertrophic BAT.

Conclusions

This organ was largely unexplored until about 50 years ago when the obesity became a diffuse clinical condition in the western countries and the first connections with metabolic syndrome were realized. Since then a progressive interest for adipose tissues was demonstrated by an exponential increase in the related scientific literature. Nowadays, the metabolic syndrome represents one of the most important worldwide healthy problem and the key role of adipose organ is widely recognized. Several matters are still largely debated such as the origin of adipocytes and their plastic adaptability to the thermogenic, metabolic and lactating needs of the organism, but several data appear widely accepted such as those related to the concept of meta-inflammation. The lack of specific medical treatment is one of the cause that push the scientific community to tackle the myriad of molecular mechanisms still unexplored in the physiology and physiopathology of this important and complex organ and I sincerely hope that this review could play a role in stimulating new scientists to join the cohort that dedicated their scientific life to study this fascinating organ.

Acknowledgements

The author is grateful to all members of my  quipe for the enthusiastic collaboration in the study of adipose organ anatomy and physiology during the last 40 years. Grant RSA University of Ancona 2016–2017.

References

1. Abdullahi A, Chen P, Stanojcic M, Sadri AR, Coburn N, Jeschke MG. IL-6 signal from the bone marrow is required for the browning of white adipose tissue post burn injury. *Shock* 47: 33-39, 2017.
2. Abdullahi A, Jeschke MG. White adipose tissue browning: A double-edged sword. *Trends Endocrinol Metab* 27: 542-552, 2016.
3. Adler ES, Hollis JH, Clarke IJ, Grattan DR, Oldfield BJ. Neurochemical characterization and sexual dimorphism of projections from the brain to abdominal and subcutaneous white adipose tissue in the rat. *J Neurosci* 32: 15913-15921, 2012.
4. Agarwal AK, Arioglu E, De Almeida S, Akkoc N, Taylor SI, Bowcock AM, Barnes RI, Garg A. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat Genet* 31: 21-23, 2002.
5. Aguilar V, Annicotte JS, Escote X, Vendrell J, Langin D, Fajas L. Cyclin G2 regulates adipogenesis through PPAR gamma coactivation. *Endocrinology* 151: 5247-5254, 2010.
6. Ahn J, Lee H, Jung CH, Jeon TI, Ha TY. MicroRNA-146b promotes adipogenesis by suppressing the SIRT1-FOXO1 cascade. *EMBO Mol Med* 5: 1602-1612, 2013.
7. Akerblad P, Lind U, Liberg D, Bamberg K, Sigvardsson M. Early B-cell factor (O/E-1) is a promoter of adipogenesis and involved in control of genes important for terminal adipocyte differentiation. *Mol Cell Biol* 22: 8015-8025, 2002.
8. Albrecht E, Norheim F, Thiede B, Holen T, Ohashi T, Schering L, Lee S, Brenmoehl J, Thomas S, Drevon CA, Erickson HP, Maak S. Irisin—a myth rather than an exercise-inducible myokine. *Sci Rep* 5: 8889, 2015.
9. Alexander CM, Selvarajan S, Mudgett J, Werb Z. Stromelysin-1 regulates adipogenesis during mammary gland involution. *J Cell Biol* 152: 693-703, 2001.
10. Almind K, Kahn CR. Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. *Diabetes* 53: 3274-3285, 2004.
11. Almind K, Manieri M, Sivitz WI, Cinti S, Kahn CR. Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proc Natl Acad Sci U S A* 104: 2366-2371, 2007.
12. Alva JA, Zovein AC, Monvoisin A, Murphy T, Salazar A, Harvey NL, Carmeliet P, Iruela-Arispe ML. VE-Cadherin-Cre-recombinase transgenic mouse: A tool for lineage analysis and gene deletion in endothelial cells. *Dev Dyn* 235: 759-767, 2006.
13. An Y, Kang Q, Zhao Y, Hu X, Li N. Lats2 modulates adipocyte proliferation and differentiation via hippo signaling. *PloS One* 8: e72042, 2013.
14. Anderson RM, Weindruch R. Metabolic reprogramming, caloric restriction and aging. *Trends Endocrinol Metab* 21: 134-141, 2010.
15. Armani A, Cinti F, Marzolla V, Morgan J, Cranston GA, Antelmi A, Carpinelli G, Canese R, Pagotto U, Quarta C, Malorni W, Matarrese P, Marconi M, Fabbri A, Rosano G, Cinti S, Young MJ, Caprio M. Mineralocorticoid receptor antagonism induces browning of white adipose tissue through impairment of autophagy and prevents adipocyte dysfunction in high-fat-diet-fed mice. *FASEB J* 28: 3745-3757, 2014.
16. Arner E, Westermark PO, Spalding KL, Britton T, Ryden M, Frisen J, Bernard S, Arner P. Adipocyte turnover: Relevance to human adipose tissue morphology. *Diabetes* 59: 105-109, 2010.
17. Arner P, Langin D. Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. *Trends Endocrinol Metab* 25: 255-262, 2014.
18. Arner P, Lithell H, Wahrenberg H, Bronnegard M. Expression of lipoprotein lipase in different human subcutaneous adipose tissue regions. *J Lipid Res* 32: 423-429, 1991.
19. Arner P, Spalding KL. Fat cell turnover in humans. *Biochem Biophys Res Commun* 396: 101-104, 2010.
20. Arribas M, Valverde AM, Benito M. Role of IRS-3 in the insulin signaling of IRS-1-deficient brown adipocytes. *J Biol Chem* 278: 45189-45199, 2003.
21. Arsenault BJ, Beaumont EP, Despres JP, Larose E. Mapping body fat distribution: A key step towards the identification of the vulnerable patient? *Ann Med* 44: 758-772, 2012.
22. Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Meerasahib MF, Gonzalez-Cadavid NF. Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. *Endocrinology* 146: 3547-3557, 2005.
23. Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol* 196: 235-249, 2008.
24. Asano A, Irie Y, Saito M. Isoform-specific regulation of vascular endothelial growth factor (VEGF) family mRNA expression in cultured mouse brown adipocytes. *Mol Cell Endocrinol* 174: 71-76, 2001.
25. Ba K, Yang X, Wu L, Wei X, Fu N, Fu Y, Cai X, Yao Y, Ge Y, Lin Y. Jagged-1-mediated activation of notch signalling induces adipogenesis of adipose-derived stem cells. *Cell Prolif* 45: 538-544, 2012.
26. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB. betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297: 843-845, 2002.
27. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. *Nature* 394: 790-793, 1998.
28. Bagchi M, Kim LA, Boucher J, Walshe TE, Kahn CR, D'Amore PA. Vascular endothelial growth factor is important for brown adipose tissue development and maintenance. *FASEB J* 27: 3257-3271, 2013.
29. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol* 55: 97-179, 1994.

30. Bajzer M, Olivieri M, Haas MK, Pfluger PT, Magrisso IJ, Foster MT, Tschop MH, Krawczewski-Carhuatanta KA, Cota D, Obici S. Cannabinoid receptor 1 (CB1) antagonism enhances glucose utilisation and activates brown adipose tissue in diet-induced obese mice. *Diabetologia* 54: 3121-3131, 2011.
31. Banerjee RR, Lazar MA. Resistin: Molecular history and prognosis. *J Mol Med (Berl)* 81: 218-226, 2003.
32. Banerjee SS, Feinberg MW, Watanabe M, Gray S, Haspel RL, Denking DJ, Kawahara R, Hauner H, Jain MK. The Kruppel-like factor KLF2 inhibits peroxisome proliferator-activated receptor-gamma expression and adipogenesis. *J Biol Chem* 278: 2581-2584, 2003.
33. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. *Trends Cell Biol* 24: 400-406, 2014.
34. Barbatelli G, Heinzlmann M, Ferrara P, Morroni M, Cinti S. Quantitative evaluations of gap junctions in old rat brown adipose tissue after cold acclimation: A freeze-fracture and ultra-structural study. *Tissue Cell* 26: 667-676, 1994.
35. Barbatelli G, Morroni M, Vinesi P, Cinti S, Michetti F. S-100 protein in rat brown adipose tissue under different functional conditions: A morphological, immunocytochemical, and immunochemical study. *Exp Cell Res* 208: 226-231, 1993.
36. Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am J Physiol Endocrinol Metab* 298: E1244-E1253, 2010.
37. Barnard T, Lindberg O. Ultrastructural changes in the chondriome during perinatal development in brown adipose tissue of rats. *J Ultrastruct Res* 29: 293-310, 1969.
38. Barneda D, Frontini A, Cinti S, Christian M. Dynamic changes in lipid droplet-associated proteins in the "browning" of white adipose tissues. *Biochim Biophys Acta* 1831: 924-933, 2013.
39. Barre L, Richardson C, Hirshman MF, Brozinick J, Fiering S, Kemp BE, Goodyear LJ, Witters LA. Genetic model for the chronic activation of skeletal muscle AMP-activated protein kinase leads to glycogen accumulation. *Am J Physiol Endocrinol Metab* 292: E802-E811, 2007.
40. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmüller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J. Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 17: 200-205, 2011.
41. Bartelt A, Heeren J. Adipose tissue browning and metabolic health. *Nat Rev Endocrinol* 10: 24-36, 2014.
42. Bartness TJ. Dual innervation of white adipose tissue: Some evidence for parasympathetic nervous system involvement. *J Clin Invest* 110: 1235-1237, 2002.
43. Bartness TJ, Bamshad M. Innervation of mammalian white adipose tissue: Implications for the regulation of total body fat. *Am J Physiol* 275: R1399-R1411, 1998.
44. Bartness TJ, Ryu V. Neural control of white, beige and brown adipocytes. *Int J Obes Suppl* 5: S35-S39, 2015.
45. Bartness TJ, Shrestha YB, Vaughan CH, Schwartz GJ, Song CK. Sensory and sympathetic nervous system control of white adipose tissue lipolysis. *Mol Cell Endocrinol* 318: 34-43, 2010.
46. Bartness TJ, Song CK. Thematic review series: Adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. *J Lipid Res* 48: 1655-1672, 2007.
47. Bartness TJ, Vaughan CH, Song CK. Sympathetic and sensory innervation of brown adipose tissue. *Int J Obes (Lond)* 34(Suppl 1): S36-S42, 2010.
48. Beiroa D, Imbernon M, Gallego R, Senra A, Herranz D, Villarroya F, Serrano M, Ferno J, Salvador J, Escalada J, Dieguez C, Lopez M, Fruhbeck G, Nogueiras R. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* 63: 3346-3358, 2014.
49. Bengoechea-Alonso MT, Ericsson J. The ubiquitin ligase Fbxw7 controls adipocyte differentiation by targeting C/EBPalpha for degradation. *Proc Natl Acad Sci U S A* 107: 11817-11822, 2010.
50. Bengtsson T, Cannon B, Nedergaard J. Differential adrenergic regulation of the gene expression of the beta-adrenoceptor subtypes beta1, beta2 and beta3 in brown adipocytes. *Biochem J* 347(Pt 3): 643-651, 2000.
51. Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, Harrison SD, MacDougald OA. Regulation of Wnt signaling during adipogenesis. *J Biol Chem* 277: 30998-31004, 2002.
52. Bensaid M, Gary-Bobo M, Esclanon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrie P. The cannabinoid CB1 receptor antagonist SR141716 increases Acip30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 63: 908-914, 2003.
53. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: Host cell death and inflammation. *Nat Rev Microbiol* 7: 99-109, 2009.
54. Bernatchez PN, Soker S, Sirois MG. Vascular endothelial growth factor effect on endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1-dependent. *J Biol Chem* 274: 31047-31054, 1999.
55. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Bluher M. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 54: 2911-2916, 2005.
56. Berry DC, Jiang Y, Graff JM. Mouse strains to study cold-inducible beige progenitors and beige adipocyte formation and function. *Nat Commun* 7: 10184, 2016.
57. Berry DC, Stenlesen D, Zeve D, Graff JM. The developmental origins of adipose tissue. *Development* 140: 3939-3949, 2013.
58. Berry R, Jeffery E, Rodeheffer MS. Weighing in on adipocyte precursors. *Cell Metab* 19: 8-20, 2014.
59. Bertola A, Ciucci T, Rousseau D, Bourlier V, Duffaut C, Bonnafous S, Blin-Wakkach C, Anty R, Iannelli A, Gugenheim J, Tran A, Bouloumie A, Gual P, Wakkach A. Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 61: 2238-2247, 2012.
60. Bezy O, Vernochet C, Gesta S, Farmer SR, Kahn CR. TRB3 blocks adipocyte differentiation through the inhibition of C/EBPbeta transcriptional activity. *Mol Cell Biol* 27: 6818-6831, 2007.
61. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Orjalo AV, Rodier F, Lithgow GJ, Campisi J. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. *Aging (Albany NY)* 1: 402-411, 2009.
62. Bier E, De Robertis EM. EMBRYO DEVELOPMENT. BMP gradients: A paradigm for morphogen-mediated developmental patterning. *Science* 348: aaa5838, 2015.
63. Bing C, Bao Y, Jenkins J, Sanders P, Manieri M, Cinti S, Tisdale MJ, Trayhurn P. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. *Proc Natl Acad Sci U S A* 101: 2500-2505, 2004.
64. Birsoy K, Chen Z, Friedman J. Transcriptional regulation of adipogenesis by KLF4. *Cell Metab* 7: 339-347, 2008.
65. Bishop NA, Guarente L. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. *Nat Rev Genet* 8: 835-844, 2007.
66. Bjorntorp P. Size, number and function of adipose tissue cells in human obesity. *Horm Metab Res* (Suppl 4): 77-83, 1974.
67. Bjorntorp P. Visceral fat accumulation: The missing link between psychosocial factors and cardiovascular disease? *J Intern Med* 230: 195-201, 1991.
68. Bjorntorp P. Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition* 13: 795-803, 1997.
69. Bjorntorp P. Hormonal control of regional fat distribution. *Hum Reprod* 12(Suppl 1): 21-25, 1997.
70. Bjorntorp P. Neuroendocrine factors in obesity. *J Endocrinol* 155: 193-195, 1997.
71. Bjorntorp P. Obesity. *Lancet* 350: 423-426, 1997.
72. Bjorntorp P, Karlsson M, Pertoft H, Pettersson P, Sjöström L, Smith U. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J Lipid Res* 19: 316-324, 1978.
73. Bjorntorp P, Rosmond R. Visceral obesity and diabetes. *Drugs* 58(Suppl 1): 13-18; discussion 75-82, 1999.
74. Blanchette-Mackie EJ, Dwyer NK, Barber T, Coxey RA, Takeda T, Rondinone CM, Theodorakis JL, Greenberg AS, Londos C. Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes. *J Lipid Res* 36: 1211-1226, 1995.
75. Blanchette-Mackie EJ, Scow RO. Continuity of intracellular channels with extracellular space in adipose tissue and liver: Demonstrated with tannic acid and lanthanum. *Anat Rec* 203: 205-219, 1982.
76. Blanchette-Mackie EJ, Scow RO. Lipolysis and lamellar structures in white adipose tissue of young rats: Lipid movement in membranes. *J Ultrastruct Res* 77: 295-318, 1981.
77. Blanchette-Mackie EJ, Scow RO. Membrane continuities within cells and intercellular contacts in white adipose tissue of young rats. *J Ultrastruct Res* 77: 277-294, 1981.
78. Bluher M. Vaspilin in obesity and diabetes: pathophysiological and clinical significance. *Endocrine* 41: 176-182, 2012.
79. Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Balkai S, Pacher P, Schon MR, Jordan J, Stumvoll M. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 55: 3053-3060, 2006.
80. Bluher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299: 572-574, 2003.
81. Bo S, Ciccone G, Baldi I, Gambino R, Mandrile C, Durazzo M, Gentile L, Cassader M, Cavallo-Perin P, Pagano G. Plasma visfatin concentrations after a lifestyle intervention were directly associated with inflammatory markers. *Nutr Metab Cardiovasc Dis* 19: 423-430, 2009.

82. Bonadonna RC, Saccomani MP, Seely L, Zych KS, Ferrannini E, Cobelli C, DeFronzo RA. Glucose transport in human skeletal muscle. The in vivo response to insulin. *Diabetes* 42: 191-198, 1993.
83. Bordinchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang C, Takahashi N, Sarzani R, Collins S. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest* 122: 1022-1036, 2012.
84. Bordinchia M, Pocognoli A, D'Anzeo M, Siquini W, Minardi D, Muzzonigro G, Dessi-Fulgheri P, Sarzani R. Nebivolol induces, via beta3 adrenergic receptor, lipolysis, uncoupling protein 1, and reduction of lipid droplet size in human adipocytes. *J Hypertens* 32: 389-396, 2014.
85. Bost F, Aouadi M, Caron L, Binetruy B. The role of MAPKs in adipocyte differentiation and obesity. *Biochimie* 87: 51-56, 2005.
86. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481: 463-468, 2012.
87. Bottcher Y, Unbehauen H, Kloting N, Ruschke K, Korner A, Schleinitz D, Tonjes A, Enigk B, Wolf S, Dietrich K, Koriath M, Scholz GH, Tseng YH, Dietrich A, Schon MR, Kiess W, Stumvoll M, Bluher M, Kovacs P. Adipose tissue expression and genetic variants of the bone morphogenetic protein receptor 1A gene (BMPRIA) are associated with human obesity. *Diabetes* 58: 2119-2128, 2009.
88. Boucher J, Mori MA, Lee KY, Smyth G, Liew CW, Macotela Y, Rourk M, Bluher M, Russell SJ, Kahn CR. Impaired thermogenesis and adipose tissue development in mice with fat-specific disruption of insulin and IGF-1 signalling. *Nat Commun* 3: 902, 2012.
89. Bouillaud F, Alves-Guerra MC, Ricquier D. UCPs, at the interface between bioenergetics and metabolism. *Biochim Biophys Acta* 1863: 2443-2456, 2016.
90. Bouillaud F, Ricquier D, Mory G, Thibault J. Increased level of mRNA for the uncoupling protein in brown adipose tissue of rats during thermogenesis induced by cold exposure or norepinephrine infusion. *J Biol Chem* 259: 11583-11586, 1984.
91. Bouloumie A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res* 83: 1059-1066, 1998.
92. Bouloumie A, Sengenès C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinases 2 and 9: Involvement in adipose differentiation. *Diabetes* 50: 2080-2086, 2001.
93. Boutens L, Stienstra R. Adipose tissue macrophages: Going off track during obesity. *Diabetologia* 59: 879-894, 2016.
94. Bowers RR, Kim JW, Otto TC, Lane MD. Stable stem cell commitment to the adipocyte lineage by inhibition of DNA methylation: role of the BMP-4 gene. *Proc Natl Acad Sci U S A* 103: 13022-13027, 2006.
95. Braga M, Pervin S, Norris K, Bhasin S, Singh R. Inhibition of in vitro and in vivo brown fat differentiation program by myostatin. *Obesity (Silver Spring)* 21: 1180-1188, 2013.
96. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D. Group 2 innate lymphoid cells promote being of white adipose tissue and limit obesity. *Nature* 519: 242-246, 2015.
97. Brinton RD. Estrogen-induced plasticity from cells to circuits: Predictions for cognitive function. *Trends Pharmacol Sci* 30: 212-222, 2009.
98. Broberger C. Brain regulation of food intake and appetite: Molecules and networks. *J Intern Med* 258: 301-327, 2005.
99. Broeders EP, Nascimento EB, Havekes B, Brans B, Roumans KH, Tailleux A, Schaart G, Kouach M, Charton J, Deprez B, Bouvy ND, Mottaghy F, Staels B, van Marken Lichtenbelt WD, Schrauwen P. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. *Cell Metab* 22: 418-426, 2015.
100. Bronnikov G, Bengtsson T, Kramarova L, Golozoubova V, Cannon B, Nedergaard J. beta1 to beta3 switch in control of cyclic adenosine monophosphate during brown adipocyte development explains distinct beta-adrenoceptor subtype mediation of proliferation and differentiation. *Endocrinology* 140: 4185-4197, 1999.
101. Bronnikov G, Houstek J, Nedergaard J. Beta-adrenergic, cAMP-mediated stimulation of proliferation of brown fat cells in primary culture. Mediation via beta 1 but not via beta 3 adrenoceptors. *J Biol Chem* 267: 2006-2013, 1992.
102. Byun MR, Jeong H, Bae SJ, Kim AR, Hwang ES, Hong JH. TAZ is required for the osteogenic and anti-adipogenic activities of kaempferol. *Bone* 50: 364-372, 2012.
103. Camastra S, Vitali A, Anselmino M, Gastaldelli A, Bellini R, Berta R, Severi I, Baldi S, Astiarraga B, Barbatelli G, Cinti S, Ferrannini E. Muscle and adipose tissue morphology, insulin sensitivity and beta-cell function in diabetic and nondiabetic obese patients: Effects of bariatric surgery. *Sci Rep* 7: 9007, 2017.
104. Camozzi D, Capanni C, Cenni V, Mattioli E, Columbaro M, Squarzone S, Lattanzi G. Diverse lamin-dependent mechanisms interact to control chromatin dynamics. Focus on laminopathies. *Nucleus* 5: 427-440, 2014.
105. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* 17: 819-837, 2013.
106. Canello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, Bouloumie A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clement K. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 54: 2277-2286, 2005.
107. Canello R, Zingaretti MC, Sarzani R, Ricquier D, Cinti S. Leptin and UCP1 genes are reciprocally regulated in brown adipose tissue. *Endocrinology* 139: 4747-4750, 1998.
108. Canello R, Zulian A, Gentilini D, Maestrini S, Della Barba A, Invitti C, Cora D, Caselle M, Liuzzi A, Di Blasio AM. Molecular and morphologic characterization of superficial- and deep-subcutaneous adipose tissue subdivisions in human obesity. *Obesity (Silver Spring)* 21: 2562-2570, 2013.
109. Cannon B, Nedergaard J. Energy dissipation in brown fat. *Experientia Suppl* 32: 107-111, 1978.
110. Cannon B, Nedergaard J. Brown adipose tissue: Function and physiological significance. *Physiol Rev* 84: 277-359, 2004.
111. Cannon B, Nedergaard J, Lundberg JM, Hokfelt T, Terenius L, Goldstein M. 'Neuropeptide tyrosine' (NPY) is co-stored with noradrenaline in vascular but not in parenchymal sympathetic nerves of brown adipose tissue. *Exp Cell Res* 164: 546-550, 1986.
112. Cantile M, Procino A, D'Armiento M, Cindolo L, Cillo C. HOX gene network is involved in the transcriptional regulation of in vivo human adipogenesis. *J Cell Physiol* 194: 225-236, 2003.
113. Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X, During MJ. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab* 14: 324-338, 2011.
114. Cao W, Daniel KW, Robidoux J, Puigserver P, Medvedev AV, Bai X, Floering LM, Spiegelman BM, Collins S. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol* 24: 3057-3067, 2004.
115. Cao W, Medvedev AV, Daniel KW, Collins S. beta-Adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 MAP kinase. *J Biol Chem* 276: 27077-27082, 2001.
116. Capanni C, Mattioli E, Columbaro M, Lucarelli E, Parnaik VK, Novelli G, Wehnert M, Cenni V, Maraldi NM, Squarzone S, Lattanzi G. Altered pre-lamin A processing is a common mechanism leading to lipodystrophy. *Hum Mol Genet* 14: 1489-1502, 2005.
117. Carey DG, Nguyen TV, Campbell LV, Chisholm DJ, Kelly P. Genetic influences on central abdominal fat: A twin study. *Int J Obes Relat Metab Disord* 20: 722-726, 1996.
118. Carmeliet P, Ferreira V, Breier G, Pollefeys T, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoek A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 380: 435-439, 1996.
119. Carnevali LS, Masuda K, Frigerio F, Le Bacquer O, Um SH, Gandin V, Topisirovic I, Sonenberg N, Thomas G, Kozma SC. S6K1 plays a critical role in early adipocyte differentiation. *Dev Cell* 18: 763-774, 2010.
120. Carpentier J, Perrelet A, Orci L. Morphological changes of the adipose cell plasma membrane during lipolysis. *J Cell Biol* 72: 104-117, 1977.
121. Carrer M, Liu N, Grueter CE, Williams AH, Frisard MI, Hulver MW, Bassel-Duby R, Olson EN. Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378*. *Proc Natl Acad Sci U S A* 109: 15330-15335, 2012.
122. Carriere A, Jeanson Y, Berger-Muller S, Andre M, Chenouard V, Arnaud E, Barreau C, Walther R, Galinier A, Wdziekonski B, Villageois P, Louche K, Collas P, Moro C, Dani C, Villarroja F, Casteilla L. Browning of white adipose cells by intermediate metabolites: An adaptive mechanism to alleviate redox pressure. *Diabetes* 63: 3253-3265, 2014.
123. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Curr Hypertens Rep* 10: 93-98, 2008.
124. Castan-Laurell I, Dray C, Attane C, Duparc T, Knauf C, Valet P. Apelin, diabetes, and obesity. *Endocrine* 40: 1-9, 2011.
125. Casteilla L, Planat-Benard V, Cousin B, Silvestre JS, Laharrague P, Carriere G, Carriere A, Penicaud L. Plasticity of adipose tissue: A promising therapeutic avenue in the treatment of cardiovascular and blood diseases? *Arch Mal Coeur Vaiss* 98: 922-926, 2005.
126. Castellucci M, De Matteis R, Meisser A, Canello R, Monsurro V, Islami D, Sarzani R, Marzoni D, Cinti S, Bischof P. Leptin modulates extracellular matrix molecules and metalloproteinases: Possible

- implications for trophoblast invasion. *Mol Hum Reprod* 6: 951-958, 2000.
127. Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, Enerback S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106: 563-573, 2001.
 128. Cereijo R, Gallego-Escuredo JM, Moure R, Villarroya J, Domingo JC, Fontdevila J, Martinez E, Gutierrez Mdel M, Mateo MG, Giral M, Domingo P, Villarroya F. The molecular signature of HIV-1-associated lipomatosis reveals differential involvement of brown and Beige/Brite adipocyte cell lineages. *PLoS One* 10: e0136571, 2015.
 129. Chadt A, Scherneck S, Joost HG, Al-Hasani H. Molecular links between obesity and diabetes: "Diabesity." In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershsman JM, Koch C, Korbonits M, McLachlan J, Purnell J, Rebar R, Singer F, Vinik A, editors. *Endotext*. South Dartmouth (MA), 2000.
 130. Challa TD, Straub LG, Balaz M, Kiehlmann E, Donze O, Rudolfsky G, Ukropec J, Ukropcova B, Wolfrum C. Regulation of de novo adipocyte differentiation through cross talk between adipocytes and preadipocytes. *Diabetes* 64: 4075-4087, 2015.
 131. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111: 1409-1421, 2003.
 132. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: More than just another fat cell hormone? *Diabetes care* 26: 2442-2450, 2003.
 133. Chau YY, Bandiera R, Serrels A, Martinez-Estrada OM, Qing W, Lee M, Slight J, Thornburn A, Berry R, McHaffie S, Stimson RH, Walker BR, Chapuli RM, Schedl A, Hastie N. Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. *Nat Cell Biol* 16: 367-375, 2014.
 134. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol* 142: 295-305, 1998.
 135. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 278: 45021-45026, 2003.
 136. Chen L, Dai YM, Ji CB, Yang L, Shi CM, Xu GF, Pang LX, Huang FY, Zhang CM, Guo XR. MiR-146b is a regulator of human visceral preadipocyte proliferation and differentiation and its expression is altered in human obesity. *Mol Cell Endocrinol* 393: 65-74, 2014.
 137. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: A new form of intercellular communication. *Trends Cell Biol* 22: 125-132, 2012.
 138. Chen Y, Pfeifer A. Brown fat-derived exosomes: Small vesicles with big impact. *Cell Metab* 25: 759-760, 2017.
 139. Chen Y, Siegel F, Kipschull S, Haas B, Frohlich H, Meister G, Pfeifer A. miR-155 regulates differentiation of brown and beige adipocytes via a bistable circuit. *Nat Commun* 4: 1769, 2013.
 140. Chen YH, Heneidi S, Lee JM, Layman LC, Stepp DW, Gamboa GM, Chen BS, Chazenbalk G, Azziz R. miRNA-93 inhibits GLUT4 and is overexpressed in adipose tissue of polycystic ovary syndrome patients and women with insulin resistance. *Diabetes* 62: 2278-2286, 2013.
 141. Chen Z, Torrens JJ, Anand A, Spiegelman BM, Friedman JM. Krox20 stimulates adipogenesis via C/EBP β -dependent and -independent mechanisms. *Cell Metab* 1: 93-106, 2005.
 142. Chevalier C, Stojanovic O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, Rigo D, Fabbiano S, Stevanovic A, Hagemann S, Montet X, Seimbille Y, Zamboni N, Hapfelmeier S, Trajkovski M. Gut microbiota orchestrates energy homeostasis during cold. *Cell* 163: 1360-1374, 2015.
 143. Choi YS, Chakrabarti R, Escamilla-Hernandez R, Sinha S. Elf5 conditional knockout mice reveal its role as a master regulator in mammary alveolar development: Failure of Stat5 activation and functional differentiation in the absence of Elf5. *Dev Biol* 329: 227-241, 2009.
 144. Chondronikola M, Volpi E, Borsheim E, Porter C, Annamalai P, Enerback S, Lidell ME, Saraf MK, Labbe SM, Hurren NM, Yfanti C, Chao T, Andersen CR, Cesani F, Hawkins H, Sidossis LS. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes* 63: 4089-4099, 2014.
 145. Chouchani ET, Kazak L, Jedrychowski MP, Lu GZ, Erickson BK, Szpyt J, Pierce KA, Laznik-Bogoslavski D, Vetrivelan R, Clish CB, Robinson AJ, Gygi SP, Spiegelman BM. Mitochondrial ROS regulate thermogenic energy expenditure and sulfenylation of UCP1. *Nature* 532: 112-116, 2016.
 146. Choy L, Derynck R. Transforming growth factor-beta inhibits adipocyte differentiation by Smad3 interacting with CCAAT/enhancer-binding protein (C/EBP) and repressing C/EBP transactivation function. *J Biol Chem* 278: 9609-9619, 2003.
 147. Choy L, Skillington J, Derynck R. Roles of autocrine TGF-beta receptor and Smad signaling in adipocyte differentiation. *J Cell Biol* 149: 667-682, 2000.
 148. Christian M. Transcriptional fingerprinting of "browning" white fat identifies NRG4 as a novel adipokine. *Adipocyte* 4: 50-54, 2015.
 149. Christian M, White R, Parker MG. Metabolic regulation by the nuclear receptor corepressor RIP140. *Trends Endocrinol Metab* 17: 243-250, 2006.
 150. Chu DT, Malinowska E, Gawronska-Kozak B, Kozak LP. Expression of adipocyte biomarkers in a primary cell culture models reflects preweaning adipobiology. *J Biol Chem* 289: 18478-18488, 2014.
 151. Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta* 1609: 127-143, 2003.
 152. Cigolini M, Cinti S, Brunetti L, Bosello O, Osculati F, Bjorntorp P. Human brown adipose cells in culture. *Exp Cell Res* 159: 261-266, 1985.
 153. Cinti S. *The Adipose Organ*. Milan: Kurtis, 1999.
 154. Cinti S. Adipose tissues and obesity. *Ital J Anat Embryol* 104: 37-51, 1999.
 155. Cinti S. Anatomy of the adipose organ. *Eat Weight Disord* 5: 132-142, 2000.
 156. Cinti S. The adipose organ: Endocrine aspects and insights from transgenic models. *Eat Weight Disord* 6: 4-8, 2001.
 157. Cinti S. The adipose organ: Morphological perspectives of adipose tissues. *Proc Nutr Soc* 60: 319-328, 2001.
 158. Cinti S. Adipocyte differentiation and transdifferentiation: Plasticity of the adipose organ. *J Endocrinol Invest* 25: 823-835, 2002.
 159. Cinti S. The adipose organ. *Prostaglandins Leukot Essent Fatty Acids* 73: 9-15, 2005.
 160. Cinti S. Morphology of the inflammatory state in the adipose organ in obese mice and humans. *Diabetes Obes Metab* 8: 105-103, 2006.
 161. Cinti S. The role of brown adipose tissue in human obesity. *Nutr Metab Cardiovasc Dis* 16: 569-574, 2006.
 162. Cinti S. The adipose organ. In: *Adipose Tissue and Adipokines in Health and Disease*. Humana Pr Inc., 2007, pp. 1-17.
 163. Cinti S. Reversible physiological transdifferentiation in the adipose organ. *Proc Nutr Soc* 68: 340-349, 2009.
 164. Cinti S. Transdifferentiation properties of adipocytes in the adipose organ. *Am J Physiol Endocrinol Metab* 297: E977-E986, 2009.
 165. Cinti S. Plasticity of the adipose organ. In: Granneman La, editor. *Adipose Tissue in Health and Disease*. Wiley-Blackwell, 2010, pp. 49-63.
 166. Cinti S. The adipose organ at a glance. *Dis Model Mech* 5: 588-594, 2012.
 167. Cinti S. The adipose organ. In: Frelut EoM, editor. *Child and Adolescent Obesity*. Springer, 2015.
 168. Cinti S. UCP1 protein: The molecular hub of adipose organ plasticity. *Biochimie* 134: 71-76, 2017.
 169. Cinti S. *Obesity, Type2 Diabetes and the Adipose Organ*. Springer, 2018.
 170. Cinti S, Cancelli R, Zingaretti MC, Ceresi E, De Matteis R, Giordano A, Himms-Hagen J, Ricquier D. CL316,243 and cold stress induce heterogeneous expression of UCP1 mRNA and protein in rodent brown adipocytes. *J Histochem Cytochem* 50: 21-31, 2002.
 171. Cinti S, Cigolini M, Bosello O, Bjorntorp P. A morphological study of the adipocyte precursor. *J Submicrosc Cytol* 16: 243-251, 1984.
 172. Cinti S, Cigolini M, Gazzanelli G, Bosello O. An ultrastructural study of adipocyte precursors from epididymal fat pads of adult rats in culture. *J Submicrosc Cytol* 17: 631-636, 1985.
 173. Cinti S, Cigolini M, Morroni M, Zingaretti MC. S-100 protein in white preadipocytes: An immunoelectronmicroscopic study. *Anat Rec* 224: 466-472, 1989.
 174. Cinti S, de Matteis R, Ceresi E, Pico C, Oliver J, Oliver P, Palou A, Obrador A, Maffei C. Leptin in the human stomach. *Gut* 49: 155, 2001.
 175. Cinti S, Eberbach S, Castellucci M, Accili D. Lack of insulin receptors affects the formation of white adipose tissue in mice. A morphometric and ultrastructural analysis. *Diabetologia* 41: 171-177, 1998.
 176. Cinti S, Enzi G, Cigolini M, Bosello O. Ultrastructural features of cultured mature adipocyte precursors from adipose tissue in multiple symmetric lipomatosis. *Ultrastruct Pathol* 5: 145-152, 1983.
 177. Cinti S, Frederich RC, Zingaretti MC, De Matteis R, Flier JS, Lowell BB. Immunohistochemical localization of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* 138: 797-804, 1997.
 178. Cinti S, Matteis RD, Pico C, Ceresi E, Obrador A, Maffei C, Oliver J, Palou A. Secretory granules of endocrine and chief cells of human stomach mucosa contain leptin. *Int J Obes Relat Metab Disord* 24: 789-793, 2000.
 179. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines

AU: Please provide the publisher name for reference 130.

AU: Please check and verify the journal title for correctness for reference 160.

AU: Please provide the publisher location for reference 162.

AU: Please provide the publisher location for reference 165.

AU: Please provide the publisher location for reference 167.

AU: Please provide the publisher location for reference 169.

- macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* 46: 2347-2355, 2005.
180. Cinti S, Morroni M. Brown adipocyte precursor cells: A morphological study. *Ital J Anat Embryol* 100(Suppl 1): 75-81, 1995.
 181. Cinti S, Zancanaro C, Sbarbati A, Cicolini M, Vogel P, Ricquier D, Fakan S. Immunoelectron microscopical identification of the uncoupling protein in brown adipose tissue mitochondria. *Biol Cell* 67: 359-362, 1989.
 182. Claffey KP, Wilkison WO, Spiegelman BM. Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. *J Biol Chem* 267: 16317-16322, 1992.
 183. Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes* 55: 978-987, 2006.
 184. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gormelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lehoucq Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392: 398-401, 1998.
 185. Cock TA, Auwerx J. Leptin: Cutting the fat off the bone. *Lancet* 362: 1572-1574, 2003.
 186. Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, Lo JC, Zeng X, Ye L, Khandekar MJ, Wu J, Gunawardana SC, Banks AS, Camporez JP, Jurczak MJ, Kajimura S, Piston DW, Mathis D, Cinti S, Shulman GI, Seale P, Spiegelman BM. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* 156: 304-316, 2014.
 187. Cohen P, Spiegelman BM. Cell biology of fat storage. *Mol Biol Cell* 27: 2523-2527, 2016.
 188. Colaianni G, Cinti S, Colucci S, Grano M. Irisin and musculoskeletal health. *Ann N Y Acad Sci* 1402: 5-9, 2017.
 189. Colaianni G, Cuscito C, Mongelli T, Oranger A, Mori G, Brunetti G, Colucci S, Cinti S, Grano M. Irisin enhances osteoblast differentiation in vitro. *Int J Endocrinol* 2014: 902186, 2014.
 190. Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, Lu P, Sartini L, Di Comite M, Mori G, Di Benedetto A, Brunetti G, Yuen T, Sun L, Reseland JE, Colucci S, New MI, Zaidi M, Cinti S, Grano M. The myokine irisin increases cortical bone mass. *Proc Natl Acad Sci U S A* 112: 12157-12162, 2015.
 191. Colaianni G, Mongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Notarnicola A, Severi I, Passeri G, Mori G, Brunetti G, Moretti B, Tarantino U, Colucci SC, Reseland JE, Vettor R, Cinti S, Grano M. Irisin prevents and restores bone loss and muscle atrophy in hind-limb suspended mice. *Sci Rep* 7: 2811, 2017.
 192. Collins S. A heart-adipose tissue connection in the regulation of energy metabolism. *Nat Rev Endocrinol* 10: 157-163, 2014.
 193. Collins S, Sarzani R, Bordicchia M. Coordinate control of adipose 'browning' and energy expenditure by beta-adrenergic and natriuretic peptide signalling. *Int J Obes Suppl* 4: S17-S20, 2014.
 194. Collins S, Surwit RS. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog Horm Res* 56: 309-328, 2001.
 195. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* 108: 1875-1881, 2001.
 196. Contreras GA, Lee YH, Mottillo EP, Granneman JG. Inducible brown adipocytes in subcutaneous inguinal white fat: The role of continuous sympathetic stimulation. *Am J Physiol Endocrinol Metab* 307: E793-E799, 2014.
 197. Corona G, Giagulli VA, Maseroli E, Vignozzi L, Aversa A, Zitzmann M, Saad F, Mannucci E, Maggi M. Testosterone supplementation and body composition: Results from a meta-analysis of observational studies. *J Endocrinol Invest* 39: 967-981, 2016.
 198. Cortes VA, Fernandez-Galilea M. Lipodystrophies: Adipose tissue disorders with severe metabolic implications. *J Physiol Biochem* 71: 471-478, 2015.
 199. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonov A. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* 149: 6018-6027, 2008.
 200. Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112: 423-431, 2003.
 201. Cottrell EC, Mercer JG. Leptin receptors. *Handb Exp Pharmacol* 2012(209): 3-21.
 202. Cousin B, Cinti S, Morroni M, Raimbault S, Ricquier D, Penicaud L, Casteilla L. Occurrence of brown adipocytes in rat white adipose tissue: Molecular and morphological characterization. *J Cell Sci* 103(Pt 4): 931-942, 1992.
 203. Craig BW, Hammons GT, Garthwaite SM, Jarett L, Holloszy JO. Adaptation of fat cells to exercise: Response of glucose uptake and oxidation to insulin. *J Appl Physiol Respir Environ Exerc Physiol* 51: 1500-1506, 1981.
 204. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Bhurung HJ, Giacobino JP, Lazzari L, Huard J, Peault B. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3: 301-313, 2008.
 205. Crossno JT, Jr., Majka SM, Grazia T, Gill RG, Klemm DJ. Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. *J Clin Invest* 116: 3220-3228, 2006.
 206. Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, McKnight GS. Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature* 382: 622-626, 1996.
 207. Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R, Bouloumie A. Macrophages in human visceral adipose tissue: Increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 49: 744-747, 2006.
 208. Cutler DA, Sullivan T, Marcus-Samuels B, Stewart CL, Reitman ML. Characterization of adiposity and metabolism in Lmna-deficient mice. *Biochem Biophys Res Commun* 291: 522-527, 2002.
 209. Cypess AM, Chen YC, Sze C, Wang K, English J, Chan O, Holman AR, Tai I, Palmer MR, Kolodny GM, Kahn CR. Cold but not sympathomimetics activates human brown adipose tissue in vivo. *Proc Natl Acad Sci U S A* 109: 10001-10005, 2012.
 210. Cypess AM, Kahn CR. The role and importance of brown adipose tissue in energy homeostasis. *Curr Opin Pediatr* 22: 478-484, 2010.
 211. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 360: 1509-1517, 2009.
 212. Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A, Kolodny GM. Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. *Cell Metab* 21: 33-38, 2015.
 213. Cypess AM, Zhang H, Schulz TJ, Huang TL, Espinoza DO, Kristiansen K, Unterman TG, Tseng YH. Insulin/IGF-I regulation of necdin and brown adipocyte differentiation via CREB- and FoxO1-associated pathways. *Endocrinology* 152: 3680-3689, 2011.
 214. Daquinag AC, Tseng C, Salameh A, Zhang Y, Amaya-Manzanares F, Dabbini A, Florez F, Xu Y, Tong Q, Kolonin MG. Depletion of white adipocyte progenitors induces beige adipocyte differentiation and suppresses obesity development. *Cell Death Differ* 22: 351-363, 2015.
 215. Darlington GJ, Ross SE, MacDougald OA. The role of C/EBP genes in adipocyte differentiation. *J Biol Chem* 273: 30057-30060, 1998.
 216. Das SS, Hayashi H, Sato T, Yamada R, Hiratsuka M, Hirasawa N. Regulation of dipeptidyl peptidase 4 production in adipocytes by glucose. *Diabetes Metab Syndr Obes* 7: 185-194, 2014.
 217. Davis KE, Neinstadt MD, Sun K, Skiles WM, Bills JD, Zehr JA, Zeve D, Hahner LD, Cox DW, Gent LM, Xu Y, Wang ZV, Khan SA, Clegg DJ. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation, and fibrosis. *Mol Metab* 2: 227-242, 2013.
 218. Davis KE, Moldes M, Farmer SR. The forkhead transcription factor FoxC2 inhibits white adipocyte differentiation. *J Biol Chem* 279: 42453-42461, 2004.
 219. de Jesus LA, Carvalho SD, Ribeiro MO, Schneider M, Kim SW, Harney JW, Larsen PR, Bianco AC. The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. *J Clin Invest* 108: 1379-1385, 2001.
 220. de Jong JM, Larsson O, Cannon B, Nedergaard J. A stringent validation of mouse adipose tissue identity markers. *Am J Physiol Endocrinol Metab* 308: E1085-E1105, 2015.
 221. de Jong JMA, Wouters RTF, Boulet N, Cannon B, Nedergaard J, Petrovic N. The beta3-adrenergic receptor is dispensable for browning of adipose tissues. *Am J Physiol Endocrinol Metab* 312: E508-E518, 2017.
 222. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, II, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 95: 322-327, 1998.
 223. De Maddalena C, Vodo S, Petroni A, Aloisi AM. Impact of testosterone on body fat composition. *J Cell Physiol* 227: 3744-3748, 2012.
 224. De Matteis R, Arch JR, Petroni ML, Ferrari D, Cinti S, Stock MJ. Immunohistochemical identification of the beta(3)-adrenoceptor in intact human adipocytes and ventricular myocardium: Effect of obesity and treatment with ephedrine and caffeine. *Int J Obes Relat Metab Disord* 26: 1442-1450, 2002.
 225. De Matteis R, Cinti S. Ultrastructural immunolocalization of leptin receptor in mouse brain. *Neuroendocrinology* 68: 412-419, 1998.

226. De Matteis R, Dashtipour K, Ognibene A, Cinti S. Localization of leptin receptor splice variants in mouse peripheral tissues by immunohistochemistry. *Proc Nutr Soc* 57: 441-448, 1998.
227. De Matteis R, Lucertini F, Guescini M, Polidori E, Zeppa S, Stocchi V, Cinti S, Cuppini R. Exercise as a new physiological stimulus for brown adipose tissue activity. *Nutr Metab Cardiovasc Dis* 23: 582-590, 2013.
228. De Matteis R, Puxeddu R, Riva A, Cinti S. Intralobular ducts of human major salivary glands contain leptin and its receptor. *J Anat* 201: 363-370, 2002.
229. De Matteis R, Ricquier D, Cinti S. TH-, NPY-, SP-, and CGRP-immunoreactive nerves in interscapular brown adipose tissue of adult rats acclimated at different temperatures: an immunohistochemical study. *J Neurocytol* 27: 877-886, 1998.
230. De Matteis R, Zingaretti MC, Murano I, Vitali A, Frontini A, Giannulis I, Barbatelli G, Marcucci F, Bordinchia M, Sarzani R, Raviola E, Cinti S. In vivo physiological transdifferentiation of adult adipose cells. *Stem Cells* 27: 2761-2768, 2009.
231. De Pergola G. The adipose tissue metabolism: Role of testosterone and dehydroepiandrosterone. *Int J Obes Relat Metab Disord* 24(Suppl 2): S59-S63, 2000.
232. Denis GV. Bromodomain coactivators in cancer, obesity, type 2 diabetes, and inflammation. *Discov Med* 10: 489-499, 2010.
233. Denis GV, Nikolajczyk BS, Schnitzler GR. An emerging role for bromodomain-containing proteins in chromatin regulation and transcriptional control of adipogenesis. *FEBS Lett* 584: 3260-3268, 2010.
234. DeOme KB, Faulkin LJ, Jr., Bern HA, Blair PB. Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Res* 19: 515-520, 1959.
235. Derous D, Mitchell SE, Wang L, Green CL, Wang Y, Chen L, Han JJ, Promislow DEL, Lusseau D, Douglas A, Speakman JR. The effects of graded levels of calorie restriction: XI. Evaluation of the main hypotheses underpinning the life extension effects of CR using the hepatic transcriptome. *Aging (Albany NY)* 9: 1770-1824, 2017.
236. Despres JP. Abdominal obesity as important component of insulin-resistance syndrome. *Nutrition* 9: 452-459, 1993.
237. Despres JP. Body fat distribution and risk of cardiovascular disease: An update. *Circulation* 126: 1301-1313, 2012.
238. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 444: 881-887, 2006.
239. Dessi-Fulgheri P, Sarzani R, Tamburrini P, Moraca A, Espinosa E, Cola G, Giantomassi L, Rappelli A. Plasma atrial natriuretic peptide and natriuretic peptide receptor gene expression in adipose tissue of normotensive and hypertensive obese patients. *J Hypertens* 15: 1695-1699, 1997.
240. Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. *Nat Rev Mol Cell Biol* 8: 870-879, 2007.
241. Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia* 51: 1356-1367, 2008.
242. Di Marzo V, Cote M, Matias I, Lemieux I, Arsenault BJ, Cartier A, Piscitelli F, Petrosino S, Almeras N, Despres JP. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: Associations with changes in metabolic risk factors. *Diabetologia* 52: 213-217, 2009.
243. Dieudonne MN, Leneuve MC, Giudicelli Y, Pecquery R. Evidence for functional estrogen receptors alpha and beta in human adipose cells: Regional specificities and regulation by estrogens. *Am J Physiol Cell Physiol* 286: C655-C661, 2004.
244. Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 148: 293-300, 2003.
245. Dimmeler S, Nicotera P. MicroRNAs in age-related diseases. *EMBO Mol Med* 5: 180-190, 2013.
246. Dinarello CA, Donath MY, Mandrup-Poulsen T. Role of IL-1beta in type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes* 17: 314-321, 2010.
247. Ding F, Yao J, Zhao L, Mao Z, Chen S, Brinton RD. Ovariectomy induces a shift in fuel availability and metabolism in the hippocampus of the female transgenic model of familial Alzheimer's. *PLoS One* 8: e59825, 2013.
248. Dodson MV, Fernyhough ME. Mature adipocytes: Are there still novel things that we can learn from them? *Tissue Cell* 40: 307-308, 2008.
249. Domingos AI, Vaynshteyn J, Voss HU, Ren X, Gradinaru V, Zang F, Deisseroth K, de Araujo IE, Friedman J. Leptin regulates the reward value of nutrient. *Nat Neurosci* 14: 1562-1568, 2011.
250. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, Geczy CL. Functions of S100 proteins. *Curr Mol Med* 13: 24-57, 2013.
251. Driskell RR, Jahoda CA, Chuong CM, Watt FM, Horsley V. Defining dermal adipose tissue. *Exp Dermatol* 23: 629-631, 2014.
252. Drori S, Girmun GD, Tou L, Szwaya JD, Mueller E, Xia K, Shivdasani RA, Spiegelman BM. Hic-5 regulates an epithelial program mediated by PPARgamma. *Genes Dev* 19: 362-375, 2005.
253. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell* 100: 197-207, 2000.
254. Duerschmid C, He Y, Wang C, Li C, Bournat JC, Romere C, Saha PK, Lee ME, Phillips KJ, Jain M, Jia P, Zhao Z, Farias M, Wu Q, Milewicz DM, Sutton VR, Moore DD, Butte NF, Krashes MJ, Xu Y, Chopra AR. Asprosin is a centrally acting orexigenic hormone. *Nat Med* 23: 1444-1453, 2017.
255. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464: 1357-1361, 2010.
256. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 365: 1415-1428, 2005.
257. Eguchi J, Yan QW, Schones DE, Kamal M, Hsu CH, Zhang MQ, Crawford GE, Rosen ED. Interferon regulatory factors are transcriptional regulators of adipogenesis. *Cell Metab* 7: 86-94, 2008.
258. El-Hashimi K, Dufresne SD, Hirshman MF, Flier JS, Goodyear LJ, Bjorbaek C. Insulin resistance and lipodystrophy in mice lacking ribosomal S6 kinase 2. *Diabetes* 52: 1340-1346, 2003.
259. Elbers JM, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am J Physiol* 276: E317-E325, 1999.
260. Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ. Long-term testosterone administration increases visceral fat in female to male transsexuals. *J Clin Endocrinol Metab* 82: 2044-2047, 1997.
261. Elbers JM, Giltay EJ, Teerlink T, Scheffer PG, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol (Oxf)* 58: 562-571, 2003.
262. Elsen M, Raschke S, Eckel J. Browning of white fat: Does irisin play a role in humans? *J Endocrinol* 222: R25-R38, 2014.
263. Elsen M, Raschke S, Sommerfeld M, Gassenhuber H, Eckel J. Comment on Wu and Spiegelman. Irisin ERKs the fat. *Diabetes* 63: 381-383, 2014. *Diabetes* 63: e16, 2014.
264. Elsen M, Raschke S, Tennagels N, Schwahn U, Jelenik T, Roden M, Romacho T, Eckel J. BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. *Am J Physiol Cell Physiol* 306: C431-C440, 2014.
265. Emmett MJ, Lim HW, Jager J, Richter HJ, Adlanmerini M, Peed LC, Briggs ER, Steger DJ, Ma T, Sims CA, Baur JA, Pei L, Won KJ, Seale P, Gerhart-Hines Z, Lazar MA. Histone deacetylase 3 prepares brown adipose tissue for acute thermogenic challenge. *Nature* 546: 544-548, 2017.
266. Engeli S, Janke J, Gorzelniak K, Bohnke J, Ghose N, Lindschau C, Luft FC, Sharma AM. Regulation of the nitric oxide system in human adipose tissue. *J Lipid Res* 45: 1640-1648, 2004.
267. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension* 35: 1270-1277, 2000.
268. Enzi G, Busetto L, Sergi G, Coin A, Inelmen EM, Vindigni V, Bassetto F, Cinti S. Multiple symmetric lipomatosis: A rare disease and its possible links to brown adipose tissue. *Nutr Metab Cardiovasc Dis* 25: 347-353, 2015.
269. Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravichandran LV, Sun Y, Koo S, Perera RJ, Jain R, Dean NM, Freier SM, Bennett CF, Lollo B, Griffey R. MicroRNA-143 regulates adipocyte differentiation. *J Biol Chem* 279: 52361-52365, 2004.
270. Estep M, Armistead D, Hossain N, Elarainy H, Goodman Z, Baranova A, Chandhoke V, Younossi ZM. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 32: 487-497, 2010.
271. Esteves CL, Kelly V, Breton A, Taylor AI, West CC, Donadeu FX, Peault B, Seckl JR, Chapman KE. Proinflammatory cytokine induction of 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) in human adipocytes is mediated by MEK, C/EBPbeta, and NF-kappaB/RelA. *J Clin Endocrinol Metab* 99: E160-E168, 2014.
272. Fajas L, Egler V, Reiter R, Hansen J, Kristiansen K, Debril MB, Miard S, Auwerx J. The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation. *Dev Cell* 3: 903-910, 2002.
273. Fajas L, Landsberg RL, Huss-Garcia Y, Sardet C, Lees JA, Auwerx J. E2Fs regulate adipocyte differentiation. *Dev Cell* 3: 39-49, 2002.
274. Fan JY, Carpentier JL, van Obberghen E, Grunfeld C, Gorden P, Orci L. Morphological changes of the 3T3-L1 fibroblast plasma membrane upon differentiation to the adipocyte form. *J Cell Sci* 61: 219-230, 1983.
275. Fang L, Guo F, Zhou L, Stahl R, Grams J. The cell size and distribution of adipocytes from subcutaneous and visceral fat is associated with type 2 diabetes mellitus in humans. *Adipocyte* 4: 273-279, 2015.

276. Farmer SR. Transcriptional control of adipocyte formation. *Cell Metab* 4: 263-273, 2006.
277. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 341: 879-884, 1999.
278. Fasshauer M, Bluher M. Adipokines in health and disease. *Trends Pharmacol Sci* 36: 461-470, 2015.
279. Faust IM, Johnson PR, Stern JS, Hirsch J. Diet-induced adipocyte number increase in adult rats: A new model of obesity. *Am J Physiol* 235: E279-E286, 1978.
280. Faust IM, Miller WH, Jr. Effects of diet and environment on adipocyte development. *Int J Obes* 5: 593-596, 1981.
281. Fawcett D. *The Cytochemical Foundations of Histology*. London: Butterworths, 1981.
282. Fawcett DW. *A Comparative Atlas of the Histological Organization and Cytochemical Reactions in Brown and White Adipose Tissue*, 1952.
283. Fawcett DW. Differences in physiological activity in brown and white fat as revealed by histochemical reactions. *Science* 105: 123, 1947.
284. Febbraio MA. Role of interleukins in obesity: Implications for metabolic disease. *Trends Endocrinol Metab* 25: 312-319, 2014.
285. Feldman BJ, Streeter RS, Farese RV, Jr., Yamamoto KR. Myostatin modulates adipogenesis to generate adipocytes with favorable metabolic effects. *Proc Natl Acad Sci U S A* 103: 15675-15680, 2006.
286. Fernyhough ME, Hausman GJ, Guan LL, Okine E, Moore SS, Dodson MV. Mature adipocytes may be a source of stem cells for tissue engineering. *Biochem Biophys Res Commun* 368: 455-457, 2008.
287. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 380: 439-442, 1996.
288. Fiore M, Chaldakov GN, Aloe L. Nerve growth factor as a signaling molecule for nerve cells and also for the neuroendocrine-immune systems. *Rev Neurosci* 20: 133-145, 2009.
289. Fiorenza CG, Chou SH, Mantzoros CS. Lipodystrophy: Pathophysiology and advances in treatment. *Nat Rev Endocrinol* 7: 137-150, 2011.
290. Fischer K, Ruiz HH, Jhun K, Finan B, Oberlin DJ, van der Heide V, Kalinovich AV, Petrovic N, Wolf Y, Clemmensen C, Shin AC, Divanovic S, Brombacher F, Glasmacher E, Keipert S, Jastroch M, Nagler J, Schramm KW, Medrikova D, Collden G, Woods SC, Herzig S, Homann D, Jung S, Nedergaard J, Cannon B, Tschop MH, Muller TD, Buettner C. Alternatively activated macrophages do not synthesize catecholamines or contribute to adipose tissue adaptive thermogenesis. *Nat Med* 23: 623-630, 2017.
291. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonov A, Flier JS, Maratos-Flier E, Spiegelman BM. FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev* 26: 271-281, 2012.
292. Flanagan JN, Linder K, Mejhert N, Dungner E, Wahlen K, Decaunes P, Ryden M, Bjorklund P, Arver S, Bhasin S, Bouloumie A, Arner P, Dahlman I. Role of follistatin in promoting adipogenesis in women. *J Clin Endocrinol Metab* 94: 3003-3009, 2009.
293. Floyd ZE, Stephens JM. STAT5A promotes adipogenesis in non-precursor cells and associates with the glucocorticoid receptor during adipocyte differentiation. *Diabetes* 52: 308-314, 2003.
294. Fontaine C, Cousin W, Plaisant M, Dani C, Peraldi P. Hedgehog signaling alters adipocyte maturation of human mesenchymal stem cells. *Stem Cells* 26: 1037-1046, 2008.
295. Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. *Science* 328: 321-326, 2010.
296. Foster MT, Bartness TJ. Sympathetic but not sensory denervation stimulates white adipocyte proliferation. *Am J Physiol Regul Integr Comp Physiol* 291: R1630-R1637, 2006.
297. Franceschi C. Healthy ageing in 2016: Obesity in geroscience—is cellular senescence the culprit? *Nat Rev Endocrinol* 13: 76-78, 2017.
298. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging'. *Trends Endocrinol Metab* 28: 199-212, 2017.
299. Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 27: 875-888, 2003.
300. Fried SK, Ricci MR, Russell CD, Laferrere B. Regulation of leptin production in humans. *J Nutr* 130: 3127S-3131S, 2000.
301. Friedman JM. Leptin at 14 y of age: An ongoing story. *Am J Clin Nutr* 89: 973S-979S, 2009.
302. Friedman JM. Obesity in the new millennium. *Nature* 404: 632-634, 2000.
303. Frisch RE, McArthur JW. Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* 185: 949-951, 1974.
304. Frontini A, Cinti S. Distribution and development of brown adipocytes in the murine and human adipose organ. *Cell Metab* 11: 253-256, 2010.
305. Frontini A, Giordano A, Cinti S. Endothelial cells of adipose tissues: A niche of adipogenesis. *Cell Cycle* 11: 2765-2766, 2012.
306. Frontini A, Rousset S, Cassard-Doulcier AM, Zingaretti C, Ricquier D, Cinti S. Thymus uncoupling protein 1 is exclusive to typical brown adipocytes and is not found in thymocytes. *J Histochem Cytochem* 55: 183-189, 2007.
307. Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S. White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. *Biochim Biophys Acta* 1831: 950-959, 2013.
308. Frydelund-Larsen L, Akerstrom T, Nielsen S, Keller P, Keller C, Pedersen BK. Visfatin mRNA expression in human subcutaneous adipose tissue is regulated by exercise. *Am J Physiol Endocrinol Metab* 292: E24-E31, 2007.
309. Fu M, Rao M, Bouras T, Wang C, Wu K, Zhang X, Li Z, Yao TP, Pestell RG. Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. *J Biol Chem* 280: 16934-16941, 2005.
310. Fuh G, Li B, Crowley C, Cunningham B, Wells JA. Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. *J Biol Chem* 273: 11197-11204, 1998.
311. Fukasawa KM, Fukasawa K, Sahara N, Harada M, Kondo Y, Nagatsu I. Immunohistochemical localization of dipeptidyl aminopeptidase IV in rat kidney, liver, and salivary glands. *J Histochem Cytochem* 29: 337-343, 1981.
312. Fukuoka A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science* 307: 426-430, 2005.
313. Fukunaka A, Fukada T, Bhin J, Suzuki L, Tsuzuki T, Takamine Y, Bin BH, Yoshihara T, Ichinoseki-Sekine N, Naito H, Miyatsuka T, Takamiya S, Sasaki T, Inagaki T, Kitamura T, Kajimura S, Watada H, Fujitani Y. Zinc transporter ZIP13 suppresses beige adipocyte biogenesis and energy expenditure by regulating C/EBP- β expression. *PLoS Genet* 13: e1006950, 2017.
314. G AAAS. Secretion of cholesterol ester transfer protein by adipose tissue. In: Angel A AH, Bouchard C, Lau D, Leiter L, Mendelson R, editors. *Obesity Research: Proceedings of the Seventh International Congress on Obesity*. London: John Libbey & Company, 1996, pp. 13-19.
315. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 316: 129-139, 2010.
316. Gan L, Liu Z, Feng F, Wu T, Luo D, Hu C, Sun C. Foxc2 coordinates inflammation and browning of white adipose by leptin-STAT3-PRDM16 signal in mice. *Int J Obes (Lond)* 42: 252-259, 2017.
317. Garces C, Ruiz-Hidalgo MJ, Font de Mora J, Park C, Miele L, Goldstein J, Bonvini E, Porras A, Laborda J. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. *J Biol Chem* 272: 29729-29734, 1997.
318. Garcia-Serrano S, Gutierrez-Repiso C, Gonzalo M, Garcia-Arnes J, Valdes S, Soriguer F, Perez-Valero V, Alaminos-Castillo MA, Francisco Cobos-Bravo J, Moreno-Ruiz FJ, Rodriguez-Canete A, Rodriguez-Pacheco F, Garcia-Escobar E, Garcia-Fuentes E. C-peptide modifies leptin and visfatin secretion in human adipose tissue. *Obesity (Silver Spring)* 23: 1607-1615, 2015.
319. Garg A. Acquired and inherited lipodystrophies. *N Engl J Med* 350: 1220-1234, 2004.
320. Garg A, Wilson R, Barnes R, Arioglu E, Zaidi Z, Gurakan F, Kocak N, O'Rahilly S, Taylor SI, Patel SB, Bowcock AM. A gene for congenital generalized lipodystrophy maps to human chromosome 9q34. *J Clin Endocrinol Metab* 84: 3390-3394, 1999.
321. Garten A, Petzold S, Barnikol-Oettler A, Korner A, Thasler WE, Kratzsch J, Kiess W, Gebhardt R. Nicotinamide phosphoribosyltransferase (NAMPT/PBEF/visfatin) is constitutively released from human hepatocytes. *Biochem Biophys Res Commun* 391: 376-381, 2010.
322. Gavin KM, Cooper EE, Hickner RC. Estrogen receptor protein content is different in abdominal than gluteal subcutaneous adipose tissue of overweight-to-obese premenopausal women. *Metabolism* 62: 1180-1188, 2013.
323. Gavin KM, Gutman JA, Kohrt WM, Wei Q, Shea KL, Miller HL, Sullivan TM, Erickson PF, Helm KM, Acosta AS, Childs CR, Muscelwhite E, Varela-Garcia M, Kelly K, Majka SM, Klemm DJ. De novo generation of adipocytes from circulating progenitor cells in mouse and human adipose tissue. *FASEB J* 30: 1096-1108, 2016.
324. Ge K, Guermah M, Yuan CX, Ito M, Wallberg AE, Spiegelman BM, Roeder RG. Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis. *Nature* 417: 563-567, 2002.
325. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I. A family with severe insulin resistance and diabetes due to a mutation in AKT2. *Science* 304: 1325-1328, 2004.

326. Gerin I, Dolinsky VW, Shackman JG, Kennedy RT, Chiang SH, Burant CF, Steffensen KR, Gustafsson JA, MacDougald OA. LXR-beta is required for adipocyte growth, glucose homeostasis, and beta cell function. *J Biol Chem* 280: 23024-23031, 2005.
327. Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: Tracking obesity to its source. *Cell* 131: 242-256, 2007.
328. Gettys TW, Rohlfes EM, Prpic V, Daniel KW, Taylor IL, Collins S. Age-dependent changes in beta-adrenergic receptor subtypes and adenylyl cyclase activation in adipocytes from Fischer 344 rats. *Endocrinology* 136: 2022-2032, 1995.
329. Giordano A, Cesari P, Capparuccia L, Castellucci M, Cinti S. Sema3A and neuropilin-1 expression and distribution in rat white adipose tissue. *J Neurocytol* 32: 345-352, 2003.
330. Giordano A, Coppari R, Castellucci M, Cinti S. Sema3a is produced by brown adipocytes and its secretion is reduced following cold acclimation. *J Neurocytol* 30: 5-10, 2001.
331. Giordano A, Frontini A, Castellucci M, Cinti S. Presence and distribution of cholinergic nerves in rat mediastinal brown adipose tissue. *J Histochem Cytochem* 52: 923-930, 2004.
332. Giordano A, Frontini A, Cinti S. Adipose organ nerves revealed by immunohistochemistry. *Methods Mol Biol* 456: 83-95, 2008.
333. Giordano A, Frontini A, Cinti S. Convertible visceral fat as a therapeutic target to curb obesity. *Nat Rev Drug Discov* 15: 405-424, 2016.
334. Giordano A, Frontini A, Murano I, Tonello C, Marino MA, Carruba MO, Nisoli E, Cinti S. Regional-dependent increase of sympathetic innervation in rat white adipose tissue during prolonged fasting. *J Histochem Cytochem* 53: 679-687, 2005.
335. Giordano A, Morroni M, Carle F, Gesuita R, Marchesi GF, Cinti S. Sensory nerves affect the recruitment and differentiation of rat periovarian brown adipocytes during cold acclimation. *J Cell Sci* 111(Pt 17): 2587-2594, 1998.
336. Giordano A, Murano I, Mondini E, Perugini J, Smorlesi A, Severi I, Barazzoni R, Scherer PE, Cinti S. Obese adipocytes show ultrastructural features of stressed cells and die of pyroptosis. *J Lipid Res* 54: 2423-2436, 2013.
337. Giordano A, Nisoli E, Tonello C, Cancelli R, Carruba MO, Cinti S. Expression and distribution of heme oxygenase-1 and -2 in rat brown adipose tissue: The modulatory role of the noradrenergic system. *FEBS Lett* 487: 171-175, 2000.
338. Giordano A, Perugini J, Kristensen DM, Sartini L, Frontini A, Kajimura S, Kristiansen K, Cinti S. Mammary alveolar epithelial cells convert to brown adipocytes in post-lactating mice. *J Cell Physiol* 232: 2923-2928, 2017.
339. Giordano A, Smorlesi A, Frontini A, Barbatelli G, Cinti S. White, brown and pink adipocytes: The extraordinary plasticity of the adipose organ. *Eur J Endocrinol*, 2014.
340. Giordano A, Song CK, Bowers RR, Ehlen JC, Frontini A, Cinti S, Bartness TJ. White adipose tissue lacks significant vagal innervation and immunohistochemical evidence of parasympathetic innervation. *Am J Physiol Regul Integr Comp Physiol*, 2009.
341. Giordano A, Tonello C, Bulbarelli A, Cozzani V, Cinti S, Carruba MO, Nisoli E. Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. *FEBS Lett* 514: 135-140, 2002.
342. Giralt M, Cereijo R, Villarroya F. Adipokines and the endocrine role of adipose tissues. *Handb Exp Pharmacol* 233: 265-282, 2016.
343. Giralt M, Cereijo R, Villarroya F. Adipokines and the endocrine role of adipose tissues. In: Switzerland SH, editor, *Metabolic Control, Handbook of Experimental Pharmacology*. Springer, 2015, pp. 265-282.
344. Giralt M, Gavalda-Navarro A, Villarroya F. Fibroblast growth factor-21, energy balance and obesity. *Mol Cell Endocrinol* 418(Pt 1): 66-73, 2015.
345. Gnad T, Scheibler S, von Kugelgen I, Scheele C, Kilic A, Glode A, Hoffmann LS, Reverte-Salisa L, Horn P, Mutlu S, El-Tayeb A, Kranz M, Deuther-Conrad W, Brust P, Lidell ME, Betz MJ, Enerback S, Schrader J, Yegutkin GG, Muller CE, Pfeifer A. Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. *Nature* 516: 395-399, 2014.
346. Goldfine AB, Crunkhorn S, Costello M, Gami H, Landaker EJ, Niinobe M, Yoshikawa K, Lo D, Warren A, Jimenez-Chillaron J, Patti ME. Necdin and E2F4 are modulated by rosiglitazone therapy in diabetic human adipose and muscle tissue. *Diabetes* 55: 640-650, 2006.
347. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem* 85: 825-836, 2002.
348. Gorospe M, Abdelmohsen K. Microregulators come of age in senescence. *Trends Genet* 27: 233-241, 2011.
349. Gosset M, Berenbaum F, Salvat C, Sautet A, Pigenet A, Tahiri K, Jacques C. Crucial role of visfatin/pre-B cell colony-enhancing factor in matrix degradation and prostaglandin E2 synthesis in chondrocytes: Possible influence on osteoarthritis. *Arthritis Rheum* 58: 1399-1409, 2008.
350. Goto M, Osada S, Imagawa M, Nishizuka M. FAD104, a regulator of adipogenesis, is a novel suppressor of TGF-beta-mediated EMT in cervical cancer cells. *Sci Rep* 7: 16365, 2017.
351. Graham TE, Kahn BB. Tissue-specific alterations of glucose transport and molecular mechanisms of intertissue communication in obesity and type 2 diabetes. *Horm Metab Res* 39: 717-721, 2007.
352. Grahn TH, Zhang Y, Lee MJ, Sommer AG, Mostoslavsky G, Fried SK, Greenberg AS, Puri V. FSP27 and PLIN1 interaction promotes the formation of large lipid droplets in human adipocytes. *Biochem Biophys Res Commun* 432: 296-301, 2013.
353. Graja A, Schulz TJ. Mechanisms of aging-related impairment of brown adipocyte development and function. *Gerontology* 61: 211-217, 2015.
354. Granneman JG, Li P, Zhu Z, Lu Y. Metabolic and cellular plasticity in white adipose tissue I: Effects of beta3-adrenergic receptor activation. *Am J Physiol Endocrinol Metab* 289: E608-E616, 2005.
355. Gray SL, Dalla Nora E, Backlund EC, Manieri M, Virtue S, Noland RC, O'Rahilly S, Cortright RN, Cinti S, Cannon B, Vidal-Puig A. Decreased brown adipocyte recruitment and thermogenic capacity in mice with impaired peroxisome proliferator-activated receptor (P465L PPARgamma) function. *Endocrinology* 147: 5708-5714, 2006.
356. Greenberg AS, Egan JJ, Wek SA, Garty NB, Blanchette-Mackie EJ, Londos C. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J Biol Chem* 266: 11341-11346, 1991.
357. Gregoire FM. Adipocyte differentiation: From fibroblast to endocrine cell. *Exp Biol Med (Maywood)* 226: 997-1002, 2001.
358. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev* 78: 783-809, 1998.
359. Gregor MF, Misch ES, Yang L, Hummasti S, Inouye KE, Lee AH, Biebert B, Hotamisligil GS. The role of adipocyte XBP1 in metabolic regulation during lactation. *Cell Rep* 3: 1430-1439, 2013.
360. Grundy SM. Adipose tissue and metabolic syndrome: Too much, too little or neither. *Eur J Clin Invest* 45: 1209-1217, 2015.
361. Gu Z, Eleswarapu S, Jiang H. Identification and characterization of microRNAs from the bovine adipose tissue and mammary gland. *FEBS Lett* 581: 981-988, 2007.
362. Guenantin AC, Briand N, Bidault G, Afonso P, Berezat V, Vatiere C, Lascals O, Caron-Debarle M, Capeau J, Vigouroux C. Nuclear envelope-related lipodystrophies. *Semin Cell Dev Biol* 29: 148-157, 2014.
363. Guermah M, Ge K, Chiang CM, Roeder RG. The TBN protein, which is essential for early embryonic mouse development, is an inducible TAFII implicated in adipogenesis. *Mol Cell* 12: 991-1001, 2003.
364. Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP. Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest* 102: 412-420, 1998.
365. Guglielmi V, Cardellini M, Cinti F, Corcosinho F, Cardolini I, D'Adamo M, Zingaretti MC, Bellia A, Lauro D, Gentileschi P, Federici M, Cinti S, Sbraccia P. Omental adipose tissue fibrosis and insulin resistance in severe obesity. *Nutr Diabetes* 5: e175, 2015.
366. Guo W, Flanagan J, Jasuja R, Kirkland J, Jiang L, Bhasin S. The effects of myostatin on adipogenic differentiation of human bone marrow-derived mesenchymal stem cells are mediated through cross-communication between Smad3 and Wnt/beta-catenin signaling pathways. *J Biol Chem* 283: 9136-9145, 2008.
367. Gupta RK, Arany Z, Seale P, Mepani RJ, Ye L, Conroe HM, Roby YA, Kulaga H, Reed RR, Spiegelman BM. Transcriptional control of preadipocyte determination by Zfp423. *Nature* 464: 619-623, 2010.
368. Gupta RK, Mepani RJ, Kleiner S, Lo JC, Khandekar MJ, Cohen P, Frontini A, Bhowmick DC, Ye L, Cinti S, Spiegelman BM. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. *Cell Metab* 15: 230-239, 2012.
369. Gupta RK MR, Kleiner S, LoJC, Khandekar MJ, Cohen P, Frontini A, Bhowmick DC, Ye L, Cinti S, Spiegelman BM. Isolation and localization of committed primary preadipocytes through Zfp423. *Cell Metab* 15: 230-239, 2012.
370. Gustafson B, Hammarstedt A, Hedjazifar S, Hoffmann JM, Svensson PA, Grimsby J, Rondonne C, Smith U. BMP4 and BMP antagonists regulate human white and Beige adipogenesis. *Diabetes* 64: 1670-1681, 2015.
371. Gustafson B, Hammarstedt A, Hedjazifar S, Smith U. Restricted adipogenesis in hypertrophic obesity: The role of WISP2, WNT, and BMP4. *Diabetes* 62: 2997-3004, 2013.
372. Gustafson B, Smith U. The WNT inhibitor Dickkopf 1 and bone morphogenetic protein 4 rescue adipogenesis in hypertrophic obesity in humans. *Diabetes* 61: 1217-1224, 2012.
373. Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* 19: 31-44, 2018.

AU: Please provide volume and page numbers for reference 339.

AU: Please provide volume and page numbers for reference 340.

AU: Please provide the publisher location for reference 343.

AU: Please check authors for reference 369.

374. Haka AS, Barbosa-Lorenzi VC, Lee HJ, Falcone DJ, Hudis CA, Dannenberg AJ, Maxfield FR. Exocytosis of macrophage lysosomes leads to digestion of apoptotic adipocytes and foam cell formation. *J Lipid Res* 57: 980-992, 2016.
375. Hall BM, Balan V, Gleiberman AS, Strom E, Krasnov P, Virtuoso LP, Rydkina E, Vujcic S, Balan K, Gitlin I, Leonova K, Polinsky A, Chernova OB, Gudkov AV. Aging of mice is associated with p16(Ink4a)- and beta-galactosidase-positive macrophage accumulation that can be induced in young mice by senescent cells. *Aging (Albany NY)* 8: 1294-1315, 2016.
376. Hallenborg P, Feddersen S, Francoz S, Murano I, Sundekilde U, Petersen RK, Akimov V, Olson MV, Lozano G, Cinti S, Gjersten BT, Madsen L, Marine JC, Blagoev B, Kristiansen K. Mdm2 controls CREB-dependent transactivation and initiation of adipocyte differentiation. *Cell Death Differ* 19: 1381-1389, 2012.
377. Hamam D, Ali D, Vishnubalaji R, Hamam R, Al-Nbaheen M, Chen L, Kassem M, Aldahmash A, Alajez NM. microRNA-320/RUNX2 axis regulates adipocytic differentiation of human mesenchymal (skeletal) stem cells. *Cell Death Dis* 5: e1499, 2014.
378. Hammarstedt A, Hedjazifar S, Jenndahl L, Gogg S, Grunberg J, Gustafson B, Klimcakova E, Stich V, Langin D, Laakso M, Smith U. WSP2 regulates preadipocyte commitment and PPARgamma activation by BMP4. *Proc Natl Acad Sci U S A* 110: 2563-2568, 2013.
379. Hams E, Locksley RM, McKenzie AN, Fallon PG. Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice. *J Immunol* 191: 5349-5353, 2013.
380. Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, Davis RJ. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science* 339: 218-222, 2013.
381. Hansen JB, Jorgensen C, Petersen RK, Hallenborg P, De Matteis R, Boye HA, Petrovic N, Enerback S, Nedergaard J, Cinti S, te Riele H, Kristiansen K. Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc Natl Acad Sci U S A* 101: 4112-4117, 2004.
382. Hansen JB, Kristiansen K. Regulatory circuits controlling white versus brown adipocyte differentiation. *Biochem J* 398: 153-168, 2006.
383. Hansen JB, Petersen RK, Larsen BM, Bartkova J, Alsner J, Kristiansen K. Activation of peroxisome proliferator-activated receptor gamma bypasses the function of the retinoblastoma protein in adipocyte differentiation. *J Biol Chem* 274: 2386-2393, 1999.
384. Hao Q, Hansen JB, Petersen RK, Hallenborg P, Jorgensen C, Cinti S, Larsen PJ, Steffensen KR, Wang H, Collins S, Wang J, Gustafsson JA, Madsen L, Kristiansen K. ADD1/SREBP1c activates the PGC1-alpha promoter in brown adipocytes. *Biochim Biophys Acta* 1801: 421-429, 2010.
385. Harbour JW, Dean DC. Rb function in cell-cycle regulation and apoptosis. *Nat Cell Biol* 2: E65-E67, 2000.
386. Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ. The biology of white adipocyte proliferation. *Obes Rev* 2: 239-254, 2001.
387. He Q, Huang HY, Zhang YY, Li X, Qian SW, Tang QQ. TAZ is downregulated by dexamethasone during the differentiation of 3T3-L1 preadipocytes. *Biochem Biophys Res Commun* 419: 573-577, 2012.
388. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 100: 15712-15717, 2003.
389. Heiker JT, Klotting N, Kovacs P, Kuettner EB, Strater N, Schultz S, Kern M, Stumvoll M, Blüher M, Beck-Sickinger AG. Vaspin inhibits kallikrein 7 by serpin mechanism. *Cell Mol Life Sci* 70: 2569-2583, 2013.
390. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci U S A* 97: 12729-12734, 2000.
391. Hiasa M, Togawa N, Moriyama Y. Vesicular nucleotide transport: A brief history and the vesicular nucleotide transporter as a target for drug development. *Curr Pharm Des* 20: 2745-2749, 2014.
392. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H, Kanwar YS. Visceral adipose tissue-derived serine protease inhibitor: A unique insulin-sensitizing adipocytokine in obesity. *Proc Natl Acad Sci U S A* 102: 10610-10615, 2005.
393. Higuchi K, Masaki T, Gotoh K, Chiba S, Katsuragi I, Tanaka K, Kakuma T, Yoshimatsu H. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. *Endocrinology* 148: 2690-2697, 2007.
394. Hileman SM, Pierroz DD, Flier JS. Leptin, nutrition, and reproduction: Timing is everything. *J Clin Endocrinol Metab* 85: 804-807, 2000.
395. Hilton C, Neville MJ, Karpe F. MicroRNAs in adipose tissue: Their role in adipogenesis and obesity. *Int J Obes (Lond)* 37: 325-332, 2013.
396. Himms-Hagen J. Brown Adipose Tissue and Cold-Acclimation, 1986.
397. Himms-Hagen J. Brown adipose tissue and cold-acclimation. In: Trayhurn P, Nicholls AD, editors. *Brown Adipose Tissue*. London: Edward Arnold, 1986, p. 214.
398. Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G, Cinti S. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am J Physiol Cell Physiol* 279: C670-C681, 2000.
399. Hinoi E, Nakamura Y, Takada S, Fujita H, Iezaki T, Hashizume S, Takahashi S, Odaka Y, Watanabe T, Yoneda Y. Growth differentiation factor-5 promotes brown adipogenesis in systemic energy expenditure. *Diabetes* 63: 162-175, 2014.
400. Hiraike Y, Waki H, Yu J, Nakamura M, Miyake K, Nagano G, Nakaki R, Suzuki K, Kobayashi H, Yamamoto S, Sun W, Aoyama T, Hirota Y, Ohno H, Oki K, Yoneda M, White AP, Tseng YH, Cypess AM, Larsen TJ, Jespersen NZ, Scheele C, Tsutsumi S, Aburatani H, Yamauchi T, Kadowaki T. NFIA co-localizes with PPARgamma and transcriptionally controls the brown fat gene program. *Nat Cell Biol* 20: 1007-1017, 2018.
401. Holubec H, Payne CM, Bernstein H, Dvorakova K, Bernstein C, Waltmire CN, Warneke JA, Garewal H. Assessment of apoptosis by immunohistochemical markers compared to cellular morphology in ex vivo-stressed colonic mucosa. *J Histochem Cytochem* 53: 229-235, 2005.
402. Hondares E, Gallego-Escuredo JM, Flachs P, Frontini A, Cereijo R, Goday A, Perugini J, Kopecky P, Giralt M, Cinti S, Kopecky J, Villarroya F. Fibroblast growth factor-21 is expressed in neonatal and pheochromocytoma-induced adult human brown adipose tissue. *Metabolism* 63: 312-317, 2014.
403. Hondares E, Iglesias R, Giralt A, Gonzalez FJ, Giralt M, Mampel T, Villarroya F. Thermogenic activation induces FGF21 expression and release in brown adipose tissue. *J Biol Chem* 286: 12983-12990, 2011.
404. Hong KY, Bae H, Park I, Park DY, Kim KH, Kubota Y, Cho ES, Kim H, Adams RH, Yoo OJ, Koh GY. Perilipin+ embryonic preadipocytes actively proliferate along growing vasculatures for adipose expansion. *Development* 142: 2623-2632, 2015.
405. Hotamisligil GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. *J Intern Med* 245: 621-625, 1999.
406. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140: 900-917, 2010.
407. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* 259: 87-91, 1993.
408. Hovey RC, Trott JF. Morphogenesis of mammary gland development. *Adv Exp Med Biol* 554: 219-228, 2004.
409. Howlett AC. Pharmacology of cannabinoid receptors. *Annu Rev Pharmacol Toxicol* 35: 607-634, 1995.
410. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271: 10697-10703, 1996.
411. Hu HH, Nayak KS, Goran MI. Assessment of abdominal adipose tissue and organ fat content by magnetic resonance imaging. *Obes Rev* 12: e504-e515, 2011.
412. Huang Z, Zhong L, Lee JTH, Zhang J, Wu D, Geng L, Wang Y, Wong CM, Xu A. The FGF21-CCL11 axis mediates being of white adipose tissues by coupling sympathetic nervous system to type 2 immunity. *Cell Metab* 26: 493-508 e494, 2017.
413. Huang-Doran I, Sleight A, Rochford JJ, O'Rahilly S, Savage DB. Lipodystrophy: Metabolic insights from a rare disorder. *J Endocrinol* 207: 245-255, 2010.
414. Hudak CS, Sul HS. Pref-1, a gatekeeper of adipogenesis. *Front Endocrinol (Lausanne)* 4: 79, 2013.
415. Huffman DM, Barzilai N. Role of visceral adipose tissue in aging. *Biochim Biophys Acta* 1790: 1117-1123, 2009.
416. Hummasti S, Hong C, Bensinger SJ, Tontonoz P. HRSLS3 is a PPARgamma-selective target gene that promotes adipocyte differentiation. *J Lipid Res* 49: 2535-2544, 2008.
417. Hummasti S, Laffitte BA, Watson MA, Galardi C, Chao LC, Ramamurthy L, Moore JT, Tontonoz P. Liver X receptors are regulators of adipocyte gene expression but not differentiation: Identification of apoD as a direct target. *J Lipid Res* 45: 616-625, 2004.
418. Hussain I, Garg A. Lipodystrophy syndromes. *Endocrinol Metab Clin North Am* 45: 783-797, 2016.
419. Hutley L, Shurety W, Newell F, McGeary R, Pelton N, Grant J, Herington A, Cameron D, Whitehead J, Prins J. Fibroblast growth factor 1: A key regulator of human adipogenesis. *Diabetes* 53: 3097-3106, 2004.
420. Iacobellis G, Di Gioia C, Petramala L, Chiappetta C, Serra V, Zinamosca L, Marinelli C, Ciardi A, De Toma G, Letizia C. Brown fat expresses adiponectin in humans. *Int J Endocrinol* 2013: 126751, 2013.
421. Imai T, Jiang M, Chambon P, Metzger D. Impaired adipogenesis and lipolysis in the mouse upon selective ablation of the retinoid X receptor alpha mediated by a tamoxifen-inducible chimeric Cre recombinase (Cre-ERT2) in adipocytes. *Proc Natl Acad Sci U S A* 98: 224-228, 2001.

AU: Please provide volume and page numbers for reference 400.

AU: Please check ref. 396.

422. Imai T, Takakuwa R, Marchand S, Dentz E, Bornert JM, Messaddeq N, Wendling O, Mark M, Desvergne B, Wahli W, Chambon P, Metzger D. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci U S A* 101: 4543-4547, 2004.
423. Inagaki T, Sakai J, Kajimura S. Transcriptional and epigenetic control of brown and beige adipose cell fate and function. *Nat Rev Mol Cell Biol* 17: 480-495, 2016.
424. Iyama K, Ohzono K, Usuku G. Electron microscopical studies on the genesis of white adipocytes: Differentiation of immature pericytes into adipocytes in transplanted preadipose tissue. *Virchows Arch B Cell Pathol Incl Mol Pathol* 31: 143-155, 1979.
425. Jager J, Gremeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 148: 241-251, 2007.
426. Jakkaraju S, Zhe X, Pan D, Choudhury R, Schuger L. TTPs are tension-responsive proteins involved in myogenic versus adipogenic differentiation. *Dev Cell* 9: 39-49, 2005.
427. Jeanson Y, Ribas F, Galinier A, Arnaud E, Ducos M, Andre M, Chenouard V, Villarroya F, Casteilla L, Carriere A. Lactate induces FGF21 expression in adipocytes through a p38-MAPK pathway. *Biochem J* 473: 685-692, 2016.
428. Jimenez M, Barbatelli G, Allevi R, Cinti S, Seydoux J, Giacobino JP, Muzzin P, Preitner F. Beta 3-adrenoceptor knockout in C57BL/6J mice depresses the occurrence of brown adipocytes in white fat. *Eur J Biochem* 270: 699-705, 2003.
429. Jimenez MA, Akerblad P, Sigvardsson M, Rosen ED. Critical role for Ebf1 and Ebf2 in the adipogenic transcriptional cascade. *Mol Cell Biol* 27: 743-757, 2007.
430. Jimenez-Pretner M, Berney X, Uldry M, Vitali A, Cinti S, Ledford JG, Thorens B. Plac8 is an inducer of C/EBPbeta required for brown fat differentiation, thermoregulation, and control of body weight. *Cell Metab* 14: 658-670, 2011.
431. Jin W, Takagi T, Kanesashi SN, Kurahashi T, Nomura T, Harada J, Ishii S. Schnurri-2 controls BMP-dependent adipogenesis via interaction with Smad proteins. *Dev Cell* 10: 461-471, 2006.
432. Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA, Rossi FM. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol* 12: 153-163, 2010.
433. Johnson PR, Hirsch J. Cellularity of adipose depots in six strains of genetically obese mice. *J Lipid Res* 13: 2-11, 1972.
434. Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, Shiota M, Kesterson RA, Kahn BB, Magnuson MA. Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci U S A* 102: 6207-6212, 2005.
435. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U S A* 97: 12735-12740, 2000.
436. Jordan SD, Kruger M, Willmes DM, Redemann N, Wunderlich FT, Bronneke HS, Merkwirth C, Kashkar H, Olkkonen VM, Bottger T, Braun T, Seibler J, Bruning JC. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nat Cell Biol* 13: 434-446, 2011.
437. Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity. *Ann Med* 32 (Suppl 1): 78-84, 2000.
438. Jumabay M, Abdmoulen R, Ly A, Cubberly MR, Shahmirian LJ, Heydarkhan-Hagvall S, Dumesic DA, Yao Y, Bostrom KI. Pluripotent stem cells derived from mouse and human white mature adipocytes. *Stem Cells Transl Med* 3: 161-171, 2014.
439. Jung KM, Clapper JR, Fu J, D'Agostino G, Guijarro A, Thongkham D, Avanesian A, Astarita G, DiPatrizio NV, Frontini A, Cinti S, Diano S, Piomelli D. 2-arachidonoylglycerol signaling in forebrain regulates systemic energy metabolism. *Cell Metab* 15: 299-310, 2012.
440. Kajimura S. Adipose tissue in 2016: Advances in the understanding of adipose tissue biology. *Nat Rev Endocrinol* 13: 69-70, 2017.
441. Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gyi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature* 460: 1154-1158, 2009.
442. Kalupahana NS, Massiera F, Quignard-Boulange A, Ailhaud G, Voy BH, Wasserman DH, Moustaid-Moussa N. Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose intolerance, and insulin resistance. *Obesity (Silver Spring)* 20: 48-56, 2012.
443. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach. *Regul Pept* 119: 77-81, 2004.
444. Kammoun HL, Kraakman MJ, Febbraio MA. Adipose tissue inflammation in glucose metabolism. *Rev Endocr Metab Disord* 15: 31-44, 2014.
445. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494-1505, 2006.
446. Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, Lee CH. Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. *Cell Metab* 7: 485-495, 2008.
447. Karbiener M, Pisani DF, Frontini A, Oberreiter LM, Lang E, Vegiopoulos A, Mossenbock K, Bernhardt GA, Mayr T, Hildner F, Grillari J, Ailhaud G, Herzig S, Cinti S, Amri EZ, Scheiderer M. MicroRNA-26 family is required for human adipogenesis and drives characteristics of brown adipocytes. *Stem Cells* 32: 1578-1590, 2014.
448. Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A, Anzala O, Jaoko W, Mutua G, Ruzagira E, Mulenga J, Sanders EJ, Mwangome M, Allen S, Bwanika A, Bahemuka U, Awuondo K, Omosa G, Farah B, Amornkul P, Birungi J, Yates S, Stoll-Johnson L, Gilmour J, Stevens G, Shutes E, Manigart O, Hughes P, Dally L, Scott J, Stevens W, Fast P, Kamali A. CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLoS One* 4: e4401, 2009.
449. Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* 7: 2839-2849, 2015.
450. Kawwass JF, Summer R, Kallen CB. Direct effects of leptin and adiponectin on peripheral reproductive tissues: A critical review. *Mol Hum Reprod* 21: 617-632, 2015.
451. Kazama T, Fujie M, Endo T, Kano K. Mature adipocyte-derived differentiated fat cells can transdifferentiate into skeletal myocytes in vitro. *Biochem Biophys Res Commun* 377: 780-785, 2008.
452. Kennaway DJ, Varcos TJ, Voultsios A, Boden MJ. Global loss of bmal1 expression alters adipose tissue hormones, gene expression and glucose metabolism. *PLoS One* 8: e65255, 2013.
453. Kennell JA, Gerin I, MacDougall OA, Cadigan KM. The microRNA miR-8 is a conserved negative regulator of Wnt signaling. *Proc Natl Acad Sci U S A* 105: 15417-15422, 2008.
454. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: The history, pathophysiology and complications. *West J Med* 137: 379-399, 1982.
455. Kerr J. *Cell Biology: A Laboratory Handbook*. Academic Press, London, 1994.
456. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89: 2548-2556, 2004.
457. Kim CA, Delepine M, Boutet E, El Mourabit H, Le Lay S, Meier M, Nemani M, Bridel E, Leite CC, Bertola DR, Semple RK, O'Rahilly S, Dugail I, Capeau J, Lathrop M, Magre J. Association of a homozygous nonsense caveolin-1 mutation with Berardinelli-Seip congenital lipodystrophy. *J Clin Endocrinol Metab* 93: 1129-1134, 2008.
458. Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 10: 1096-1107, 1996.
459. Kim JH, Cho HT, Kim YJ. The role of estrogen in adipose tissue metabolism: Insights into glucose homeostasis regulation. *Endocr J* 61: 1055-1067, 2014.
460. Kim S, Huang LW, Snow KJ, Ablamunits V, Hasham MG, Young TH, Paulk AC, Richardson JE, Affourtit JP, Shalom-Barak T, Bult CJ, Barak Y. A mouse model of conditional lipodystrophy. *Proc Natl Acad Sci U S A* 104: 16627-16632, 2007.
461. Kim YH, Barclay JL, He J, Luo X, O'Neill HM, Keshvari S, Webster JA, Ng C, Hutley LJ, Prins JB, Whitehead JP. Identification of carboxypeptidase X (CPX)-1 as a positive regulator of adipogenesis. *FASEB J* 30: 2528-2540, 2016.
462. Kimmel AR, Sztalryd C. Perilipin 5, a lipid droplet protein adapted to mitochondrial energy utilization. *Curr Opin Lipidol* 25: 110-117, 2014.
463. Kiskinis E, Chatzeli L, Curry E, Kafrou M, Frontini A, Cinti S, Montana G, Parker MG, Christian M. RIP140 represses the "brown-in-white" adipocyte program including a futile cycle of triacylglycerol breakdown and synthesis. *Mol Endocrinol* 28: 344-356, 2014.
464. Kitamura T, Kitamura Y, Nakae J, Giordano A, Cinti S, Kahn CR, Efstratiadis A, Accili D. Mosaic analysis of insulin receptor function. *J Clin Invest* 113: 209-219, 2004.
465. Klemm DJ, Leitner JW, Watson P, Nesterova A, Reusch JE, Goalstone ML, Draznin B. Insulin-induced adipocyte differentiation. Activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation. *J Biol Chem* 276: 28430-28435, 2001.
466. Klötting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Bluher M. Vasp gene expression in human adipose

- tissue: Association with obesity and type 2 diabetes. *Biochem Biophys Res Commun* 339: 430-436, 2006.
467. Klötting N, Graham TE, Berndt J, Kralisch S, Kovacs P, Wason CJ, Fasshauer M, Schon MR, Stumvoll M, Bluher M, Kahn BB. Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. *Cell Metab* 6: 79-87, 2007.
 468. Klyde BJ, Hirsch J. Increased cellular proliferation in adipose tissue of adult rats fed a high-fat diet. *J Lipid Res* 20: 705-715, 1979.
 469. Klyde BJ, Hirsch J. Isotopic labeling of DNA in rat adipose tissue: Evidence for proliferating cells associated with mature adipocytes. *J Lipid Res* 20: 691-704, 1979.
 470. Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell* 75: 1389-1399, 1993.
 471. Kopinke D, Roberson EC, Reiter JF. Ciliary hedgehog signaling restricts injury-induced adipogenesis. *Cell* 170: 340-351 e312, 2017.
 472. Kortum RL, Costanzo DL, Haferbier J, Schreiner SJ, Razidlo GL, Wu MH, Volle DJ, Mori T, Sakaue H, Chaika NV, Chaika OV, Lewis RE. The molecular scaffold kinase suppressor of Ras 1 (KSR1) regulates adipogenesis. *Mol Cell Biol* 25: 7592-7604, 2005.
 473. Kotzbeck P, Giordano A, Mondini E, Murano I, Severi I, Venema W, Cecchini MP, Kershaw EE, Barbatelli G, Haemmerle G, Zechner R, Cinti S. Brown adipose tissue whitening leads to brown adipocyte death and adipose tissue inflammation. *J Lipid Res* 55: 208-218, 2014.
 474. Koutnikova H, Cock TA, Watanabe M, Houten SM, Champy MF, Dierich A, Auwerx J. Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPAR gamma hypomorphic mice. *Proc Natl Acad Sci U S A* 100: 14457-14462, 2003.
 475. Kovacs P, Geyer M, Berndt J, Klötting N, Graham TE, Botcher Y, Enigk B, Tonjes A, Schleinitz D, Schon MR, Kahn BB, Bluher M, Stumvoll M. Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. *Diabetes* 56: 3095-3100, 2007.
 476. Kozak LP. Genetic variation in brown fat activity and body weight regulation in mice: Lessons for human studies. *Biochim Biophys Acta* 1842: 370-376, 2014.
 477. Krause BR, Hartman AD. Adipose tissue and cholesterol metabolism. *J Lipid Res* 25: 97-110, 1984.
 478. Krist J, Wieder K, Klötting N, Oberbach A, Kralisch S, Wiesner T, Schon MR, Gartner D, Dietrich A, Shang E, Lohmann T, Dressler M, Fasshauer M, Stumvoll M, Bluher M. Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. *Obes Facts* 6: 57-69, 2013.
 479. Krol E, Martin SA, Huhtaniemi IT, Douglas A, Speakman JR. Negative correlation between milk production and brown adipose tissue gene expression in lactating mice. *J Exp Biol* 214: 4160-4170, 2011.
 480. Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 72: 1150-1162, 1983.
 481. Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T, Suzuki R, Satoh H, Tsuchida A, Moroi M, Sugi K, Noda T, Ebinuma H, Ueta Y, Kondo T, Araki E, Ezaki O, Nagai R, Tobe K, Terauchi Y, Ueki K, Minokoshi Y, Kadowaki T. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab* 6: 55-68, 2007.
 482. Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J. n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. *Diabetologia* 52: 941-951, 2009.
 483. Kuji I, Imabayashi E, Minagawa A, Matsuda H, Miyauchi T. Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. *Ann Nucl Med* 22: 231-235, 2008.
 484. Kukla M, Ciupinska-Kajor M, Kajor M, Wylezol M, Zwirska-Korczala K, Hartleb M, Berdowska A, Mazur W. Liver visfatin expression in morbidly obese patients with nonalcoholic fatty liver disease undergoing bariatric surgery. *Pol J Pathol* 61: 147-153, 2010.
 485. Kuo MM, Kim S, Tseng CY, Jeon YH, Choe S, Lee DK. BMP-9 as a potent brown adipogenic inducer with anti-obesity capacity. *Biomaterials* 35: 3172-3179, 2014.
 486. Labbe SM, Caron A, Lanfray D, Monge-Rofarello B, Bartness TJ, Richard D. Hypothalamic control of brown adipose tissue thermogenesis. *Front Syst Neurosci* 9: 150, 2015.
 487. Labbe SM, Mouchiroud M, Caron A, Secco B, Freinkman E, Lamoureaux G, Gelinat Y, Lecomte R, Bosse Y, Chimin P, Festuccia WT, Richard D, Laplante M. mTORC1 is required for brown adipose tissue recruitment and metabolic adaptation to cold. *Sci Rep* 6: 37223, 2016.
 488. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res* 48: 275-297, 2009.
 489. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127: 1109-1122, 2006.
 490. Lamers D, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwers DM, Eckardt K, Kaufman JM, Ryden M, Muller S, Hanisch FG, Ruige J, Arner P, Sell H, Eckel J. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes* 60: 1917-1925, 2011.
 491. Langin D. In and out: adipose tissue lipid turnover in obesity and dyslipidemia. *Cell Metab* 14: 569-570, 2011.
 492. Langin D, Lucas S, Lafontan M. Millennium fat-cell lipolysis reveals unsuspected novel tracks. *Horm Metab Res* 32: 443-452, 2000.
 493. Lapinskas EJ, Palmer J, Ricardo S, Hertzog PJ, Hammacher A, Pritchard MA. A major site of expression of the ets transcription factor Elf5 is epithelia of exocrine glands. *Histochem Cell Biol* 122: 521-526, 2004.
 494. Laque A, Yu S, Qualls-Creekmore E, Gettys S, Schwartzburg C, Bui K, Rhodes C, Berthoud HR, Morrison CD, Richards BK, Munzberg H. Leptin modulates nutrient reward via inhibitory galanin action on orexin neurons. *Mol Metab* 4: 706-717, 2015.
 495. Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356: 1517-1526, 2007.
 496. Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH, Astrup A. Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* 76: 780-788, 2002.
 497. Laustsen PG, Michael MD, Crute BE, Cohen SE, Ueki K, Kulkarni RN, Keller SR, Lienhard GE, Kahn CR. Lipotrophic diabetes in Irs1(-/-)/Irs3(-/-) double knockout mice. *Genes Dev* 16: 3213-3222, 2002.
 498. Lean J, James P. *Brown Adipose Tissue in Man in Brown Adipose Tissue*. Edward Arnold, 1986, pp 339-365.
 499. Lecoultrre V, Ravussin E. Brown adipose tissue and aging. *Curr Opin Clin Nutr Metab Care* 14: 1-6, 2011.
 500. Lee JA, Park HS, Song YS, Jang YJ, Kim JH, Lee YJ, Heo YS. Relationship between vaspin gene expression and abdominal fat distribution of Korean women. *Endocr J* 58: 639-646, 2011.
 501. Lee JO, Kim N, Lee HJ, Lee YW, Kim SJ, Park SH, Kim HS. Resistin, a fat-derived secretory factor, promotes metastasis of MDA-MB-231 human breast cancer cells through ERM activation. *Sci Rep* 6: 18923, 2016.
 502. Lee MJ, Jeon ES, Lee JS, Cho M, Suh DS, Chang CL, Kim JH. Lysophosphatidic acid in malignant ascites stimulates migration of human mesenchymal stem cells. *J Cell Biochem* 104: 499-510, 2008.
 503. Lee MW, Odegaard JJ, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, Yun K, Locksley RM, Chawla A. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* 160: 74-87, 2015.
 504. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. *Cell Metab* 19: 302-309, 2014.
 505. Lee YH, Petkova AP, Granneman JG. Identification of an adipogenic niche for adipose tissue remodeling and restoration. *Cell Metab* 18: 355-367, 2013.
 506. Lee YH, Petkova AP, Konkar AA, Granneman JG. Cellular origins of cold-induced brown adipocytes in adult mice. *FASEB J* 29: 286-299, 2015.
 507. Lee YH, Petkova AP, Mottillo EP, Granneman JG. In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. *Cell Metab* 15: 480-491, 2012.
 508. Lee YH, Thacker RI, Hall BE, Kong R, Granneman JG. Exploring the activated adipogenic niche: Interactions of macrophages and adipocyte progenitors. *Cell Cycle* 13: 184-190, 2014.
 509. Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. PPARgamma and the global map of adipogenesis and beyond. *Trends Endocrinol Metab* 25: 293-302, 2014.
 510. Lefterova MI, Zhang Y, Steger DJ, Schupp M, Schug J, Cristancho A, Feng D, Zhuo D, Stoeckert CJ, Jr., Liu XS, Lazar MA. PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes Dev* 22: 2941-2952, 2008.
 511. Lehr S, Hartwig S, Lamers D, Famulla S, Muller S, Hanisch FG, Cuvelier C, Ruige J, Eckardt K, Ouwers DM, Sell H, Eckel J. Identification and validation of novel adipokines released from primary human adipocytes. *Mol Cell Proteomics* 11: M111 010504, 2012.
 512. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. *Proteomics Clin Appl* 6: 91-101, 2012.
 513. Leibel RL. Molecular physiology of weight regulation in mice and humans. *Int J Obes (Lond)* 32(Suppl 7): S98-S108, 2008.

AU: Please provide the publisher location for reference 498.

514. Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. *Am J Clin Nutr* 58: 463-467, 1993.
515. Levi-Montalcini R. Tissue and nerve growth promoting factors. Biological aspects of specific growth promoting factors. *Proc R Soc Med* 58: 357-360, 1965.
516. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. *Nature* 444: 1022-1023, 2006.
517. Li L, B Li, Li M, Niu C, Wang G, Li T, Król E, Jin W, Speakman JR. Speakman Brown adipocytes can display a mammary basal myoepithelia cell phenotype in vivo. *Molecular Metabolism* in print: 1-14, 2017.
518. Li N, Yang Q, Walker RG, Thompson TB, Du M, Rodgers BD. Myostatin attenuation in vivo reduces adiposity, but activates adipogenesis. *Endocrinology* 157: 282-291, 2016.
519. Li WC, Yu WY, Quinlan JM, Burke ZD, Tosh D. The molecular basis of transdifferentiation. *J Cell Mol Med* 9: 569-582, 2005.
520. Lightbourne M, Brown RJ. Genetics of lipodystrophy. *Endocrinol Metab Clin North Am* 46: 539-554, 2017.
521. Lijnen HR, Maquoi E, Hansen LB, Van Hoef B, Frederix L, Collen D. Matrix metalloproteinase inhibition impairs adipose tissue development in mice. *Arterioscler Thromb Vasc Biol* 22: 374-379, 2002.
522. Lilla J, Stickens D, Werb Z. Metalloproteases and adipogenesis: A weighty subject. *Am J Pathol* 160: 1551-1554, 2002.
523. Lin J, Patel SR, Cheng X, Cho EA, Levitan I, Ullenbruch M, Phan SH, Park JM, Dressler GR. Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat Med* 11: 387-393, 2005.
524. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. *FEBS J* 276: 2348-2358, 2009.
525. Lindgren EM, Nielsen R, Petrovic N, Jacobsson A, Mandrup S, Cannon B, Nedergaard J. Noradrenaline represses PPAR (peroxisome-proliferator-activated receptor) gamma2 gene expression in brown adipocytes: Intracellular signalling and effects on PPARgamma2 and PPARgamma1 protein levels. *Biochem J* 382: 597-606, 2004.
526. Lindquist JM, Rehnmark S. Ambient temperature regulation of apoptosis in brown adipose tissue. Erk1/2 promotes norepinephrine-dependent cell survival. *J Biol Chem* 273: 30147-30156, 1998.
527. Linhart HG, Ishimura-Oka K, DeMayo F, Kibe T, Repka D, Poindexter B, Bick RJ, Darlington GJ. C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. *Proc Natl Acad Sci U S A* 98: 12532-12537, 2001.
528. Lipinski MM, Jacks T. The retinoblastoma gene family in differentiation and development. *Oncogene* 18: 7873-7882, 1999.
529. Liu D, Bordicchia M, Zhang C, Fang H, Wei W, Li JL, Guilherme A, Guntur K, Czech MP, Collins S. Activation of mTORC1 is essential for beta-adrenergic stimulation of adipose browning. *J Clin Invest* 126: 1704-1716, 2016.
530. Liu W, Bi P, Shan T, Yang X, Yin H, Wang YX, Liu N, Rudnicki MA, Kuang S. miR-133a regulates adipocyte browning in vivo. *PLoS Genet* 9: e1003626, 2013.
531. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott M, Meigs J, Mozaffarian D, Nichol G, O'Donnell C, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Steinberger J, Thom T, Wasserthiel-Smoller S, Wong N, Wyllie-Rosett J, Hong Y, American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics—2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 119: 480-486, 2009.
532. Lo JC, Ljubicic S, Leibiger B, Kern M, Leibiger IB, Moede T, Kelly ME, Chatterjee Bhowmick D, Murano I, Cohen P, Banks AS, Khandekar MJ, Dietrich A, Flier JS, Cinti S, Bluher M, Danial NN, Berggren PO, Spiegelman BM. Adipsin is an adipokine that improves beta cell function in diabetes. *Cell* 158: 41-53, 2014.
533. Loncar D. Convertible adipose tissue in mice. *Cell Tissue Res* 266: 149-161, 1991.
534. Loncar D. Development of thermogenic adipose tissue. *Int J Dev Biol* 35: 321-333, 1991.
535. Loncar D, Afzelius BA, Cannon B. Epididymal white adipose tissue after cold stress in rats. I. Nonmitochondrial changes. *J Ultrastruct Mol Struct Res* 101: 109-122, 1988.
536. Loncar D, Afzelius BA, Cannon B. Epididymal white adipose tissue after cold stress in rats. II. Mitochondrial changes. *J Ultrastruct Mol Struct Res* 101: 199-209, 1988.
537. Loncar D, Bedrica L, Mayer J, Cannon B, Nedergaard J, Afzelius BA, Svajger A. The effect of intermittent cold treatment on the adipose tissue of the cat. Apparent transformation from white to brown adipose tissue. *J Ultrastruct Mol Struct Res* 97: 119-129, 1986.
538. Long JZ, Svensson KJ, Bateman LA, Lin H, Kamenecka T, Lokurkar IA, Lou J, Rao RR, Chang MR, Jedrychowski MP, Paulo JA, Gygi SP, Griffin PR, Nomura DK, Spiegelman BM. The secreted enzyme PM20D1 regulates lipidated amino acid uncouplers of mitochondria. *Cell* 166: 424-435, 2016.
539. Long JZ, Svensson KJ, Tsai L, Zeng X, Roh HC, Kong X, Rao RR, Lou J, Lokurkar I, Baur W, Castellot JJ, Jr., Rosen ED, Spiegelman BM. A smooth muscle-like origin for beige adipocytes. *Cell Metab* 19: 810-820, 2014.
540. Lonnqvist F, Nordfors L, Jansson M, Thorne A, Schalling M, Arner P. Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. *J Clin Invest* 99: 2398-2404, 1997.
541. Lopez-Mejia IC, Castillo-Armengol J, Lagarrigue S, Fajas L. Role of cell cycle regulators in adipose tissue and whole body energy homeostasis. *Cell Mol Life Sci*, 2017.
542. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 394: 897-901, 1998.
543. Louet JF, O'Malley BW. Coregulators in adipogenesis: what could we learn from the SRC (p160) coactivator family? *Cell Cycle* 6: 2448-2452, 2007.
544. Lowe CE, O'Rahilly S, Rochford JJ. Adipogenesis at a glance. *J Cell Sci* 124: 2681-2686, 2011.
545. Lowell BB, V SS, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366: 740-742, 1993.
546. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest* 121: 2111-2117, 2011.
547. Luo X, Hutley LJ, Webster JA, Kim YH, Liu DF, Newell FS, Widberg CH, Bachmann A, Turner N, Schmitz-Peiffer C, Prins JB, Yang GS, Whitehead JP. Identification of BMP and activin membrane-bound inhibitor (BAMBI) as a potent negative regulator of adipogenesis and modulator of autocrine/paracrine adipogenic factors. *Diabetes* 61: 124-136, 2012.
548. Lynes MD, Leiria LO, Lundh M, Bartelt A, Shamsi F, Huang TL, Takahashi H, Hirshman MF, Schlein C, Lee A, Baer LA, May FJ, Gao F, Narain NR, Chen EY, Kiebish MA, Cypess AM, Bluher M, Goodyear LJ, Hotamisligil GS, Stanford KI, Tseng YH. The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. *Nat Med* 23: 631-637, 2017.
549. Ma Y, Gao M, Sun H, Liu D. Interleukin-6 gene transfer reverses body weight gain and fatty liver in obese mice. *Biochim Biophys Acta* 1852: 1001-1011, 2015.
550. Macchi V, Porzionato A, Sarasin G, Petrelli L, Guidolin D, Rossato M, Fontanella CG, Natali A, De Caro R. The infrapatellar adipose body: A histotopographic study. *Cells Tissues Organs* 201: 220-231, 2016.
551. MacDonald PC, Madden JD, Brenner PF, Wilson JD, Siiteri PK. Origin of estrogen in normal men and in women with testicular feminization. *J Clin Endocrinol Metab* 49: 905-916, 1979.
552. MacDougald OA, Lane MD. Adipocyte differentiation. When precursors are also regulators. *Curr Biol* 5: 618-621, 1995.
553. Machinal-Quelin F, Dieudonne MN, Leneveu MC, Pecquery R, Giudicelli Y. Proadipogenic effect of leptin on rat preadipocytes in vitro: Activation of MAPK and STAT3 signaling pathways. *Am J Physiol Cell Physiol* 282: C853-C863, 2002.
554. Maddaluno L, Rudini N, Cuttano R, Bravi L, Giampietro C, Corada M, Ferrarini L, Orsenigo F, Papa E, Boulday G, Tournier-Lasserre E, Chapon F, Richichi C, Retta SF, Lampugnani MG, Dejana E. EndMT contributes to the onset and progression of cerebral cavernous malformations. *Nature* 498: 492-496, 2013.
555. Madsen L, Pedersen LM, Lillefosse HH, Fjaere E, Bronstad I, Hao Q, Petersen RK, Hallenborg P, Ma T, De Matteis R, Araujo P, Mercader J, Bonet ML, Hansen JB, Cannon B, Nedergaard J, Wang J, Cinti S, Voshol P, Doskeland SO, Kristiansen K. UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. *PLoS One* 5: e11391, 2010.
556. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50: 2094-2099, 2001.
557. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, et al. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155-1161, 1995.
558. Magre J, Delpech M, Khallouf E, Gedde-Dahl T, Jr., Van Maldergem L, Sobel E, Papp J, Meier M, Megarbane A, Bachy A, Verloes A, d'Abronzio FH, Seemanova E, Assan R, Baudin N, Bourut C, Czernichow P, Huet F, Grigorescu F, de Kerdanet M, Lacombe D, Labrune P, Lanza M, Loret H, Matsuda F, Navarro J, Nivelon-Chevalier A, Polak M, Robert JJ, Tric P, Tubiana-Rufi N, Vigouroux C, Weissenbach J, Savasta S, Maassen JA, Trygstad O, Bogalho P, Freitas P, Medina JL, Bonnicci F, Joffe BI, Loyson G, Panz VR, Raal FJ, O'Rahilly S, Stephenson T, Kahn CR, Lathrop M, Capeau J, Group BW.

AU: Please provide volume and page numbers for reference 541.

- Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* 28: 365-370, 2001.
559. Maines MD. The heme oxygenase system: A regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517-554, 1997.
 560. Maines MD. Heme oxygenase: Function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 2: 2557-2568, 1988.
 561. Manieri M, Murano I, Fianchini A, Brunelli A, Cinti S. Morphological and immunohistochemical features of brown adipocytes and preadipocytes in a case of human hibernoma. *Nutr Metab Cardiovasc Dis* 20: 567-574, 2009.
 562. Mannella P, Brinton RD. Estrogen receptor protein interaction with phosphatidylinositol 3-kinase leads to activation of phosphorylated Akt and extracellular signal-regulated kinase 1/2 in the same population of cortical neurons: A unified mechanism of estrogen action. *J Neurosci* 26: 9439-9447, 2006.
 563. Marcelin G, Ferreira A, Liu Y, Atlan M, Aron-Wisnewsky J, Peloux V, Botbol Y, Ambrosini M, Fradet M, Rouault C, Henegar C, Hulot JS, Poutou C, Torcivia A, Nail-Barthelemy R, Bichet JC, Gautier EL, Clement K. A PDGFRalpha-mediated switch toward CD9high adipocyte progenitors controls obesity-induced adipose tissue fibrosis. *Cell Metab* 25: 673-685, 2017.
 564. Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: A review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 26: 1407-1433, 2002.
 565. Martinelli R, Nardelli C, Pilone V, Buonomo T, Liguori R, Castano I, Buono P, Masone S, Persico G, Forestieri P, Pastore L, Sacchetti L. miR-519d overexpression is associated with human obesity. *Obesity (Silver Spring)* 18: 2170-2176, 2010.
 566. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: Master switches of inflammation. *Cell Death Differ* 14: 10-22, 2007.
 567. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 15: 2727-2729, 2001.
 568. Massiera F, Seydoux J, Geloan A, Quignard-Boulange A, Turban S, Saint-Marc P, Fukamizu A, Negrel R, Ailhaud G, Teboul M. Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. *Endocrinology* 142: 5220-5225, 2001.
 569. Massmann GA, Zhang J, Seong WJ, Kim M, Figueroa JP. Sex-dependent effects of antenatal glucocorticoids on insulin sensitivity in adult sheep: Role of the adipose tissue renin angiotensin system. *Am J Physiol Regul Integr Comp Physiol* 312: R1029-R1038, 2017.
 570. Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, Petrosino S, Hoareau L, Festy F, Pasquali R, Roche R, Maj M, Pagotto U, Monteleone P, Di Marzo V. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 91: 3171-3180, 2006.
 571. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 277: 37487-37491, 2002.
 572. Matsumoto T, Kano K, Kondo D, Fukuda N, Iribe Y, Tanaka N, Matsubara Y, Sakuma T, Satomi A, Otaki M, Ryu J, Mugishima H. Mature adipocyte-derived differentiated fat cells exhibit multilineage potential. *J Cell Physiol* 215: 210-222, 2008.
 573. Maumus M, Peyrafitte JA, D'Angelo R, Fournier-Wirth C, Bouloumie A, Casteilla L, Sengenès C, Bourin P. Native human adipose stromal cells: Localization, morphology and phenotype. *Int J Obes (Lond)* 35: 1141-1153, 2011.
 574. Maumus M, Sengenès C, Decaunes P, Zakaroff-Girard A, Bourlier V, Lafontan M, Galitzky J, Bouloumie A. Evidence of in situ proliferation of adult adipose tissue-derived progenitor cells: Influence of fat mass microenvironment and growth. *J Clin Endocrinol Metab* 93: 4098-4106, 2008.
 575. Maurizi G, Poloni A, Mattiucci D, Santi S, Maurizi A, Izzi V, Giuliani A, Mancini S, Zingaretti MC, Perugini J, Severi I, Falconi M, Vivarelli M, Rippo MR, Corvera S, Giordano A, Leoni P, Cinti S. Cover Image, Volume 232, Number 10, October 2017. *J Cell Physiol* 232: i, 2017.
 576. Maurizi G, Poloni A, Mattiucci D, Santi S, Maurizi A, Izzi V, Giuliani A, Mancini S, Zingaretti MC, Perugini J, Severi I, Falconi M, Vivarelli M, Rippo MR, Corvera S, Giordano A, Leoni P, Cinti S. Human white adipocytes convert into "Rainbow" adipocytes in vitro. *J Cell Physiol* 232: 2887-2899, 2017.
 577. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 6: 483-495, 2004.
 578. McDonald ME, Li C, Bian H, Smith BD, Layne MD, Farmer SR. Myocardin-related transcription factor A regulates conversion of progenitors to beige adipocytes. *Cell* 160: 105-118, 2015.
 579. McGregor RA, Choi MS. microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med* 11: 304-316, 2011.
 580. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunity* 41: 36-48, 2014.
 581. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BR. Conversion of vascular endothelial cells into multipotent stem-like cells. *Nat Med* 16: 1400-1406, 2010.
 582. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, Perrin C, Jimenez-Linan M, Blount M, Dixon J, Zahn D, Thresher RR, Aparicio S, Carlton M, Colledge WH, Kettunen MI, Seppanen-Laakso T, Sethi JK, O'Rahilly S, Brindle K, Cinti S, Oresic M, Burcelin R, Vidal-Puig A. The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. *Diabetes* 54: 1706-1716, 2005.
 583. Meissburger B, Stachorski L, Roder E, Rudofsky G, Wolfrum C. Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) controls adipogenesis in obesity in mice and in humans. *Diabetologia* 54: 1468-1479, 2011.
 584. Meissburger B, Ukropec J, Roeder E, Beaton N, Geiger M, Teupser D, Civan B, Langhans W, Nawroth PP, Gasperiakova D, Rudofsky G, Wolfrum C. Adipogenesis and insulin sensitivity are regulated by retinoid-related orphan receptor gamma. *EMBO Mol Med* 3: 637-651, 2011.
 585. Mercader J, Ribot J, Murano I, Feddersen S, Cinti S, Madsen L, Kristiansen K, Bonet ML, Palou A. Haploinsufficiency of the retinoblastoma protein gene reduces diet-induced obesity, insulin resistance, and hepatosteatosis in mice. *Am J Physiol Endocrinol Metab* 297: E184-E193, 2009.
 586. Mercader J, Ribot J, Murano I, Felipe F, Cinti S, Bonet ML, Palou A. Remodeling of white adipose tissue after retinoic acid administration in mice. *Endocrinology* 150: 300-309, 2007.
 587. Mikkelsen TS, Xu Z, Zhang X, Wang L, Gimble JM, Lander ES, Rosen ED. Comparative epigenomic analysis of murine and human adipogenesis. *Cell* 143: 156-169, 2010.
 588. Milan G, Murano I, Costa S, Pianta A, Tiengo C, Zulato E, Centobene C, Bruttomesso D, Cinti S, Vettor R. Lipodystrophy induced by subcutaneous insulin infusion: Ultrastructural analysis and gene expression profiling. *J Clin Endocrinol Metab* 95: 3126-3132, 2010.
 589. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, Xu D, Sattar N, McInnes IB, Liew FY. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res* 107: 650-658, 2010.
 590. Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrier S, Alves SE. Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J Comp Neurol* 491: 81-95, 2005.
 591. Min SY, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH, Noh HL, Kim JK, Cooper MP, Fitzgibbons T, Brehm MA, Corvera S. Human 'brite/beige' adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. *Nat Med* 22: 312-318, 2016.
 592. Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Yagui K, Saito Y. Roles of degree of fat deposition and its localization on VEGF expression in adipocytes. *Am J Physiol Endocrinol Metab* 288: E1128-E1136, 2005.
 593. Modica S, Wolfrum C. Bone morphogenic proteins signaling in adipogenesis and energy homeostasis. *Biochim Biophys Acta* 1831: 915-923, 2013.
 594. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C. Life without white fat: A transgenic mouse. *Genes Dev* 12: 3168-3181, 1998.
 595. Molofsky AB, Nussbaum JC, Liang HE, Van Dyken SJ, Cheng LE, Mohapatra A, Chawla A, Locksley RM. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 210: 535-549, 2013.
 596. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387: 903-908, 1997.
 597. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. *Cell Metab* 19: 512-526, 2014.
 598. Morgan SA, Hassan-Smith ZK, Lavery GG. Mechanisms in endocrinology: Tissue-specific activation of cortisol in Cushing's syndrome. *Eur J Endocrinol* 175: R83-R89, 2016.
 599. Morgan SA, McCabe EL, Gathercole LL, Hassan-Smith ZK, Lamer DP, Bujalska IJ, Stewart PM, Tomlinson JW, Lavery GG. 11beta-HSD1 is the major regulator of the tissue-specific effects of circulating

AU: Please provide volume and page numbers for reference 586.

- glucocorticoid excess. *Proc Natl Acad Sci U S A* 111: E2482-E2491, 2014.
600. Mori M, Nakagami H, Rodriguez-Araujo G, Nimura K, Kaneda Y. Essential role for miR-196a in brown adipogenesis of white fat progenitor cells. *PLoS Biol* 10: e1001314, 2012.
 601. Mori MA, Raghavan P, Thomou T, Boucher J, Robida-Stubbs S, Macotela Y, Russell SJ, Kirkland JL, Blackwell TK, Kahn CR. Role of microRNA processing in adipose tissue in stress defense and longevity. *Cell Metab* 16: 336-347, 2012.
 602. Mori MA, Thomou T, Boucher J, Lee KY, Lallukka S, Kim JK, Torriani M, Yki-Jarvinen H, Grinspoon SK, Cypess AM, Kahn CR. Altered miRNA processing disrupts brown/white adipocyte determination and associates with lipodystrophy. *J Clin Invest* 124: 3339-3351, 2014.
 603. Mori T, Sakaue H, Iguchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsuki Y, Ogawa W, Hiramatsu R, Kasuga M. Role of Kruppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. *J Biol Chem* 280: 12867-12875, 2005.
 604. Moro C, Lafontan M. Natriuretic peptides and cGMP signaling control of energy homeostasis. *Am J Physiol Heart Circ Physiol* 304: H358-H368, 2013.
 605. Morroni M, Barbatelli G, Zingaretti MC, Cinti S. Immunohistochemical, ultrastructural and morphometric evidence for brown adipose tissue recruitment due to cold acclimation in old rats. *Int J Obes Relat Metab Disord* 19: 126-131, 1995.
 606. Morroni M, Giordano A, Zingaretti MC, Boiani R, De Matteis R, Kahn BB, Nisoli E, Tonello C, Pisoschi C, Luchetti MM, Marelli M, Cinti S. Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. *Proc Natl Acad Sci U S A* 101: 16801-16806, 2004.
 607. Muchir A, Worman HJ. The nuclear envelope and human disease. *Physiology (Bethesda)* 19: 309-314, 2004.
 608. Muller S, Balaz M, Stefanicka P, Varga L, Amri EZ, Ukropec J, Wollscheid B, Wolfrum C. Proteomic analysis of human brown adipose tissue reveals utilization of coupled and uncoupled energy expenditure pathways. *Sci Rep* 6: 30030, 2016.
 609. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev* 35: 992-1019, 2014.
 610. Munzberg H, Morrison CD. Structure, production and signaling of leptin. *Metabolism* 64: 13-23, 2015.
 611. Murano I, Barbatelli G, Giordano A, Cinti S. Noradrenergic parenchymal nerve fiber branching after cold acclimatization correlates with brown adipocyte density in mouse adipose organ. *J Anat* 214: 171-178, 2009.
 612. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, Cinti S. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J Lipid Res* 49: 1562-1568, 2008.
 613. Murano I, Rutkowski JM, Wang QA, Cho YR, Scherer PE, Cinti S. Time course of histomorphological changes in adipose tissue upon acute lipotrophy. *Nutr Metab Cardiovasc Dis* 23: 723-731, 2013.
 614. Murano I, Zingaretti CM, Cinti S. The Adipose Organ of Sv129 mice contains a prevalence of brown adipocytes and shows plasticity after cold exposure. *Adipocytes* 1: 121-130, 2005.
 615. Naaz A, Zakroczymski M, Heine P, Taylor J, Saunders P, Lubahn D, Cooke PS. Effect of ovariectomy on adipose tissue of mice in the absence of estrogen receptor alpha (ERalpha): A potential role for estrogen receptor beta (ERbeta). *Horm Metab Res* 34: 758-763, 2002.
 616. Nagashima T, Ohinata H, Kuroshima A. Involvement of nitric oxide in noradrenaline-induced increase in blood flow through brown adipose tissue. *Life Sci* 54: 17-25, 1994.
 617. Nakae J, Kitamura T, Kitamura Y, Biggs WH, 3rd, Arden KC, Accili D. The forkhead transcription factor Foxo1 regulates adipocyte differentiation. *Dev Cell* 4: 119-129, 2003.
 618. Nakajima I, Muroya S, Tanabe R, Chikuni K. Extracellular matrix development during differentiation into adipocytes with a unique increase in type V and VI collagen. *Biol Cell* 94: 197-203, 2002.
 619. Nakajima I, Yamaguchi T, Ozutsumi K, Aso H. Adipose tissue extracellular matrix: Newly organized by adipocytes during differentiation. *Differentiation* 63: 193-200, 1998.
 620. Nakata M, Manaka K, Yamamoto S, Mori M, Yada T. Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca(2+) influx through L-type channels in mouse islet beta-cells. *Endocr J* 58: 305-313, 2011.
 621. Nanbu-Wakao R, Morikawa Y, Matsumura I, Masuho Y, Muramatsu MA, Senba E, Wakao H. Stimulation of 3T3-L1 adipogenesis by signal transducer and activator of transcription 5. *Mol Endocrinol* 16: 1565-1576, 2002.
 622. Napolitano L. The differentiation of white adipose cells. An electron microscope study. *J Cell Biol* 18: 663-679, 1963.
 623. Napolitano L, Fawcett D. The fine structure of brown adipose tissue in the newborn mouse and rat. *J Biophys Biochem Cytol* 4: 685-692, 1958.
 624. Nechad M. Structure and development of brown adipose tissue. In: Trayhurn P, Nicholls D, editors. *Brown Adipose Tissue*. London: Edward Arnold, 1986.
 625. Nedergaard J, Bengtsson T, Cannon B. New powers of brown fat: Fighting the metabolic syndrome. *Cell Metab* 13: 238-240, 2011.
 626. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 293: E444-E452, 2007.
 627. Nedergaard J, Cannon B. The changed metabolic world with human brown adipose tissue: Therapeutic visions. *Cell Metab* 11: 268-272, 2010.
 628. Nedergaard J, Lindberg O. The brown fat cell. *Int Rev Cytol* 74: 187-286, 1982.
 629. Negrel R, Dani C. Cultures of adipose precursor cells and cells of clonal lines from animal white adipose tissue. *Methods Mol Biol* 155: 225-237, 2001.
 630. Nemir M, Bhattacharyya D, Li X, Singh K, Mukherjee AB, Mukherjee BB. Targeted inhibition of osteopontin expression in the mammary gland causes abnormal morphogenesis and lactation deficiency. *J Biol Chem* 275: 969-976, 2000.
 631. Neville MC, Medina D, Monks J, Hovey RC. The mammary fat pad. *J Mammary Gland Biol Neoplasia* 3: 109-116, 1998.
 632. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480: 104-108, 2011.
 633. Ni B, Farrar JS, Vaitkus JA, Celi FS. Metabolic effects of FGF-21: Thermoregulation and beyond. *Front Endocrinol (Lausanne)* 6: 148, 2015.
 634. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 336: 1262-1267, 2012.
 635. Nieves BJ, D'Amore PA, Bryan BA. The function of vascular endothelial growth factor. *Biofactors* 35: 332-337, 2009.
 636. Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta* 1492: 203-206, 2000.
 637. Nisoli E, Briscini L, Giordano A, Tonello C, Wiesbrock SM, Uysal KT, Cinti S, Carruba MO, Hotamisligil GS. Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity. *Proc Natl Acad Sci U S A* 97: 8033-8038, 2000.
 638. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO. Mitochondrial biogenesis in mammals: The role of endogenous nitric oxide. *Science* 299: 896-899, 2003.
 639. Nisoli E, Regianini L, Briscini L, Bulbarelli A, Busetto L, Coin A, Enzi G, Carruba MO. Multiple symmetric lipomatosis may be the consequence of defective noradrenergic modulation of proliferation and differentiation of brown fat cells. *J Pathol* 198: 378-387, 2002.
 640. Nisoli E, Regianini L, Bulbarelli A, Briscini L, Breacale R, Carruba MO. Protective effects of noradrenaline against tumor necrosis factor-alpha-induced apoptosis in cultured rat brown adipocytes: Role of nitric oxide-induced heat shock protein 70 expression. *Int J Obes Relat Metab Disord* 25: 1421-1430, 2001.
 641. Nisoli E, Tonello C, Benarese M, Liberini P, Carruba MO. Expression of nerve growth factor in brown adipose tissue: Implications for thermogenesis and obesity. *Endocrinology* 137: 495-503, 1996.
 642. Nisoli E, Tonello C, Briscini L, Carruba MO. Inducible nitric oxide synthase in rat brown adipocytes: Implications for blood flow to brown adipose tissue. *Endocrinology* 138: 676-682, 1997.
 643. Nisoli E, Tonello C, Carruba MO. Nerve growth factor, beta3-adrenoceptor and uncoupling protein 1 expression in rat brown fat during postnatal development. *Neurosci Lett* 246: 5-8, 1998.
 644. Nnodim JO. Development of adipose tissues. *Anat Rec* 219: 331-337, 1987.
 645. Non LR, Escota GV, Powderly WG. HIV and its relationship to insulin resistance and lipid abnormalities. *Transl Res* 183: 41-56, 2017.
 646. Norheim F, Langley TM, Hjorth M, Holen T, Kielland A, Stadheim HK, Gulseth HL, Birkeland KI, Jensen J, Drevon CA. The effects of acute and chronic exercise on PGC-1alpha, irisin and browning of subcutaneous adipose tissue in humans. *FEBS J* 281: 739-749, 2014.
 647. Norman D, Mukherjee S, Symons D, Jung RT, Lever JD. Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: A plurality of innervation. *J Neurocytol* 17: 305-311, 1988.
 648. Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, Thornton EE, Krummel MF, Chawla A, Liang HE, Locksley RM. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 502: 245-248, 2013.
 649. O'Rahilly S. Life without leptin. *Nature* 392: 330-331, 1998.

650. Oakes SR, Naylor MJ, Asselin-Labat ML, Blazek KD, Gardiner-Garden M, Hilton HN, Kazlauskas M, Pritchard MA, Chodosh LA, Pfeffer PL, Lindeman GJ, Visvader JE, Ormandy CJ. The Ets transcription factor Elf5 specifies mammary alveolar cell fate. *Genes Dev* 22: 581-586, 2008.
651. Ognjanovic S, Ku TL, Bryant-Greenwood GD. Pre-B-cell colony-enhancing factor is a secreted cytokine-like protein from the human amniotic epithelium. *Am J Obstet Gynecol* 193: 273-282, 2005.
652. Oh IS, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature* 443: 709-712, 2006.
653. Ohno H, Shinoda K, Ohyama K, Sharp LZ, Kajimura S. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature* 504: 163-167, 2013.
654. Ohno-Shosaku T, Tanimura A, Hashimoto Y, Kano M. Endocannabinoids and retrograde modulation of synaptic transmission. *Neuroscientist* 18: 119-132, 2012.
655. Oishi Y, Manabe I, Tobe K, Tsushima K, Shindo T, Fujii K, Nishimura G, Maemura K, Yamauchi T, Kubota N, Suzuki R, Kitamura T, Akira S, Kadowaki T, Nagai R. Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. *Cell Metab* 1: 27-39, 2005.
656. Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, Yamaguchi M, Tanabe H, Kimura-Someya T, Shirouzu M, Ogata H, Tokuyama K, Ueki K, Nagano T, Tanaka A, Yokoyama S, Kadowaki T. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature* 503: 493-499, 2013.
657. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 72: 219-246, 2010.
658. Ono H, Oki Y, Bono H, Kano K. Gene expression profiling in multipotent DFAT cells derived from mature adipocytes. *Biochem Biophys Res Commun* 407: 562-567, 2011.
659. Ortega-Molina A, Efevan A, Lopez-Guadamillas E, Munoz-Martin M, Gomez-Lopez G, Canamero M, Mulero F, Pastor J, Martinez S, Romanos E, Mar Gonzalez-Barroso M, Rial E, Valverde AM, Bischoff JR, Serrano M. Pten positively regulates brown adipose function, energy expenditure, and longevity. *Cell Metab* 15: 382-394, 2012.
660. Ortega-Molina A, Serrano M. PTEN in cancer, metabolism, and aging. *Trends Endocrinol Metab* 24: 184-189, 2013.
661. Osborn O, Brownell SE, Sanchez-Alavez M, Salomon D, Gram H, Bartfai T. Treatment with an Interleukin 1 beta antibody improves glycemic control in diet-induced obesity. *Cytokine* 44: 141-148, 2008.
662. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 18: 363-374, 2012.
663. Ostermeyer AG, Paci JM, Zeng Y, Lublin DM, Munro S, Brown DA. Accumulation of caveolin in the endoplasmic reticulum redirects the protein to lipid storage droplets. *J Cell Biol* 152: 1071-1078, 2001.
664. Otrrock ZK, Makarem JA, Shamseddine AI. Vascular endothelial growth factor family of ligands and receptors: Review. *Blood Cells Mol Dis* 38: 258-268, 2007.
665. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 103: 1057-1063, 2001.
666. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306: 457-461, 2004.
667. Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R, Milan G, Rossato M, Federspil G, Vettor R. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab* 91: 3165-3170, 2006.
668. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 27: 73-100, 2006.
669. Pajvani UB, Trujillo ME, Combs TP, Iyengar P, Jelicks L, Roth KA, Kistis RN, Scherer PE. Fat apoptosis through targeted activation of caspase 8: A new mouse model of inducible and reversible lipodystrophy. *Nat Med* 11: 797-803, 2005.
670. Pallottini V, Bulzomi P, Galluzzo P, Martini C, Marino M. Estrogen regulation of adipose tissue functions: Involvement of estrogen receptor isoforms. *Infect Disord Drug Targets* 8: 52-60, 2008.
671. Palmer BF, Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol* 402: 113-119, 2015.
672. Pan D, Huang L, Zhu LJ, Zou T, Ou J, Zhou W, Wang YX. Jmjd3-mediated H3K27me3 dynamics orchestrate brown fat development and regulate white fat plasticity. *Dev Cell* 35: 568-583, 2015.
673. Pan D, Mao C, Quattrocchi B, Friedline RH, Zhu LJ, Jung DY, Kim JK, Lewis B, Wang YX. MicroRNA-378 controls classical brown fat expansion to counteract obesity. *Nat Commun* 5: 4725, 2014.
674. Panettiere P, Accorsi D, Marchetti L, Minicozzi AM, Orsini G, Bernardi P, Benati D, Conti G, Sbarbati A. The trochanteric fat pad. *Eur J Histochem* 55: e16, 2011.
675. Park HK, Ahima RS. Physiology of leptin: Energy homeostasis, neuroendocrine function and metabolism. *Metabolism* 64: 24-34, 2015.
676. Pasarica M, Gowronska-Kozak B, Burk D, Remedios I, Hymel D, Gimble J, Ravussin E, Bray GA, Smith SR. Adipose tissue collagen VI in obesity. *J Clin Endocrinol Metab* 94: 5155-5162, 2009.
677. Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocr Rev* 31: 364-395, 2010.
678. Pedersen BK. IL-6 signalling in exercise and disease. *Biochem Soc Trans* 35: 1295-1297, 2007.
679. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88: 1379-1406, 2008.
680. Pedersen BK, Fischer CP. Beneficial health effects of exercise—the role of IL-6 as a myokine. *Trends Pharmacol Sci* 28: 152-156, 2007.
681. Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating alpha2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alpha. Implications for the female fat distribution. *J Clin Endocrinol Metab* 89: 1869-1878, 2004.
682. Peeraully MR, Jenkins JR, Trayhurn P. NGF gene expression and secretion in white adipose tissue: Regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. *Am J Physiol Endocrinol Metab* 287: E331-E339, 2004.
683. Pellegrinelli V, Carobbio S, Vidal-Puig A. Adipose tissue plasticity: How fat depots respond differently to pathophysiological cues. *Diabetologia* 59: 1075-1088, 2016.
684. Pendas AM, Zhou Z, Cadinanos J, Freije JM, Wang J, Hultenby K, Astudillo A, Wernerson A, Rodriguez F, Tryggvason K, Lopez-Otin C. Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice. *Nat Genet* 31: 94-99, 2002.
685. Peterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet* 27: 121-124, 2001.
686. Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARGamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* 285: 7153-7164, 2010.
687. Pfeifer A. NRG4: An endocrine link between brown adipose tissue and liver. *Cell Metab* 21: 13-14, 2015.
688. Phan J, Reue K. Lipin, a lipodystrophy and obesity gene. *Cell Metab* 1: 73-83, 2005.
689. Philbrick KA, Wong CP, Branscum AJ, Turner RT, Iwaniec UT. Leptin stimulates bone formation in ob/ob mice at doses having minimal impact on energy metabolism. *J Endocrinol* 232: 461-474, 2017.
690. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* 429: 771-776, 2004.
691. Pierleoni C, Verdenelli F, Castellucci M, Cinti S. Fibronectins and basal lamina molecules expression in human subcutaneous white adipose tissue. *Eur J Histochem* 42: 183-188, 1998.
692. Pisani DF, Beranger GE, Corinus A, Giroud M, Ghandour RA, Altirriba J, Chambard JC, Mazure NM, Bendahhou S, Duranton C, Michiels JF, Frontini A, Rohner-Jeanrenaud F, Cinti S, Christian M, Barhanin J, Amri EZ. The K+ channel TASK1 modulates beta-adrenergic response in brown adipose tissue through the mineralocorticoid receptor pathway. *FASEB J* 30: 909-922, 2016.
693. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjonneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulos A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duynhoven FJ, Hallmans G, Weinhall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quiros JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* 359: 2105-2120, 2008.
694. Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Penicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. *Circulation* 109: 656-663, 2004.
695. Poitou C, Viguerie N, Cancellio R, De Matteis R, Cinti S, Stich V, Coussieu C, Gauthier E, Courtine M, Zucker JD, Barsh GS, Saris W, Bruneau P, Basdevant A, Langin D, Clement K. Serum amyloid A: Production by human white adipocyte and regulation by obesity and nutrition. *Diabetologia* 48: 519-528, 2005.

696. Polak P, Cybulski N, Feige JN, Auwerx J, Ruegg MA, Hall MN. Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration. *Cell Metab* 8: 399-410, 2008.
697. Poloni A, Maurizi G, Anastasi S, Mondini E, Mattiucci D, Discepoli G, Tiberi F, Mancini S, Partelli S, Maurizi A, Cinti S, Olivieri A, Leoni P. Plasticity of human dedifferentiated adipocytes toward endothelial cells. *Exp Hematol* 43: 137-146, 2015.
698. Poloni A, Maurizi G, Foia F, Mondini E, Mattiucci D, Ambrogini P, Lattanzi D, Mancini S, Falconi M, Cinti S, Olivieri A, Leoni P. Glial-like differentiation potential of human mature adipocytes. *J Mol Neurosci* 55: 91-98, 2015.
699. Poloni A, Maurizi G, Leoni P, Serrani F, Mancini S, Frontini A, Zingaretti MC, Siquini W, Sarzani R, Cinti S. Human dedifferentiated adipocytes show similar properties to bone marrow-derived mesenchymal stem cells. *Stem Cells* 30: 965-974, 2012.
700. Pond C. The evolution of mammalian adipocytes. In: Symonds M, editor. *Adipose Tissue Biology*. Springer, 2012, pp. 1-59.
701. Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'Homme D, Lupien PJ, Tremblay A, Bouchard C. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes* 41: 826-834, 1992.
702. Poulos SP, Dodson MV, Culver MF, Hausman GJ. The increasingly complex regulation of adipocyte differentiation. *Exp Biol Med (Maywood)* 241: 449-456, 2016.
703. Principe A, Melgar-Lesmes P, Fernandez-Varo G, del Arbol LR, Ros J, Morales-Ruiz M, Bernardi M, Arroyo V, Jimenez W. The hepatic apelin system: A new therapeutic target for liver disease. *Hepatology* 48: 1193-1201, 2008.
704. Prins JB, O'Rahilly S. Regulation of adipose cell number in man. *Clin Sci (Lond)* 92: 3-11, 1997.
705. Prokesh A SA, Perugini J, Manieri M, Ciarmela P, Mondini E, Trajanoski Z, Kristiansen K, Giordano A, Bogner-Strauss JG, Cinti S. Molecular aspects of adipocyte epithelial transdifferentiation in mouse mammary gland. *Stem Cells* 32: 2756-2766, 2014.
706. Prokesh A, Smorlesi A, Perugini J, Manieri M, Ciarmela P, Mondini E, Trajanoski Z, Kristiansen K, Giordano A, Bogner-Strauss JG, Cinti S. Molecular aspects of adipocyte epithelial transdifferentiation in mouse mammary gland. *Stem Cells* 32: 2756-2766, 2014.
707. Puerta M, Abelenda M, Rocha M, Trayhurn P. Effect of acute cold exposure on the expression of the adiponectin, resistin and leptin genes in rat white and brown adipose tissues. *Horm Metab Res* 34: 629-634, 2002.
708. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): Transcriptional coactivator and metabolic regulator. *Endocr Rev* 24: 78-90, 2003.
709. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS. Adiponectin acts in the brain to decrease body weight. *Nat Med* 10: 524-529, 2004.
710. Qian SW, Tang Y, Li X, Liu Y, Zhang YY, Huang HY, Xue RD, Yu HY, Guo L, Gao HD, Liu Y, Sun X, Li YM, Jia WP, Tang QQ. BMP4-mediated brown fat-like changes in white adipose tissue alter glucose and energy homeostasis. *Proc Natl Acad Sci U S A* 110: E798-E807, 2013.
711. Qin S, LaRosa G, Campbell JJ, Smith-Heath H, Kassam N, Shi X, Zeng L, Buthcher EC, Mackay CR. Expression of monocyte chemoattractant protein-1 and interleukin-8 receptors on subsets of T cells: correlation with transendothelial chemotactic potential. *Eur J Immunol* 26: 640-647, 1996.
712. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* 157: 1292-1308, 2014.
713. Quarta C, Bellochio L, Mancini G, Mazza R, Cervino C, Braulte LJ, Fekete C, Latorre R, Nanni C, Bucci M, Clemens LE, Heldmaier G, Watanabe M, Leste-Lassere T, Maitre M, Tedesco L, Fanelli F, Reuss S, Klaus S, Srivastava RK, Monory K, Valerio A, Grandis A, De Giorgio R, Pasquali R, Nisoli E, Cota D, Lutz B, Marsicano G, Pagotto U. CB(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metab* 11: 273-285, 2010.
714. Rahman S, Lu Y, Czernik PJ, Rosen CJ, Enerback S, Lecka-Czernik B. Inducible brown adipose tissue, or beige fat, is anabolic for the skeleton. *Endocrinology* 154: 2687-2701, 2013.
715. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, Eklund KK. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: A novel link between cholesterol metabolism and inflammation. *PLoS One* 5: e11765, 2010.
716. Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S, Kern W, Hillhouse EW, Lehnert H, Tan BK, Randeve HS. Identification of nesfatin-1 in human and murine adipose tissue: A novel depot-specific adipokine with increased levels in obesity. *Endocrinology* 151: 3169-3180, 2010.
717. Ramel MC, Hill CS. Spatial regulation of BMP activity. *FEBS Lett* 586: 1929-1941, 2012.
718. Ramesh N, Mortazavi S, Unniappan S. Nesfatin-1 stimulates glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide secretion from STC-1 cells in vitro. *Biochem Biophys Res Commun* 462: 124-130, 2015.
719. Ramirez ME, McMurry MP, Wiebke GA, Felten KJ, Ren K, Meikle AW, Iyerius PH. Evidence for sex steroid inhibition of lipoprotein lipase in men: Comparison of abdominal and femoral adipose tissue. *Metabolism* 46: 179-185, 1997.
720. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell* 157: 1279-1291, 2014.
721. Raschke S, Eckel J. Adipo-myokines: Two sides of the same coin—mediators of inflammation and mediators of exercise. *Mediators Inflamm* 2013: 320724, 2013.
722. Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, Jung R, Wisloff U, Tjonna AE, Raastad T, Hallen J, Norheim F, Drevon CA, Romacho T, Eckardt K, Eckel J. Evidence against a beneficial effect of irisin in humans. *PLoS One* 8: e73680, 2013.
723. Razzoli M, Frontini A, Gurney A, Mondini E, Cubuk C, Katz LS, Cero C, Bolan PJ, Dopazo J, Vidal-Puig A, Cinti S, Bartolomucci A. Stress-induced activation of brown adipose tissue prevents obesity in conditions of low adaptive thermogenesis. *Mol Metab* 5: 19-33, 2016.
724. Rebbapragada A, Benchabane H, Wrana JL, Celeste AJ, Attisano L. Myostatin signals through a transforming growth factor beta-like signaling pathway to block adipogenesis. *Mol Cell Biol* 23: 7230-7242, 2003.
725. Reis FC, Branquinho JL, Brandao BB, Guerra BA, Silva ID, Frontini A, Thomou T, Sartini L, Cinti S, Kahn CR, Festuccia WT, Kowaltowski AJ, Mori MA. Fat-specific Dicer deficiency accelerates aging and mitigates several effects of dietary restriction in mice. *Aging (Albany NY)* 8: 1201-1222, 2016.
726. Reitman ML. How does fat transition from white to Beige? *Cell Metab* 26: 14-16, 2017.
727. Reitman ML, Mason MM, Moitra J, Gavrilova O, Marcus-Samuels B, Eckhaus M, Vinson C. Transgenic mice lacking white fat: models for understanding human lipodystrophic diabetes. *Ann N Y Acad Sci* 892: 289-296, 1999.
728. Remillard P, Shen G, Milne R, Maheux P. Induction of cholesteryl ester transfer protein in adipose tissue and plasma of the fructose-fed hamster. *Life Sci* 69: 677-687, 2001.
729. Rettberg JR, Yao J, Brinton RD. Estrogen: A master regulator of bioenergetic systems in the brain and body. *Front Neuroendocrinol* 35: 8-30, 2014.
730. Reue K, Zhang P. The lipin protein family: Dual roles in lipid biosynthesis and gene expression. *FEBS Lett* 582: 90-96, 2008.
731. Reusch JE, Colton LA, Klemm DJ. CREB activation induces adipogenesis in 3T3-L1 cells. *Mol Cell Biol* 20: 1008-1020, 2000.
732. Richard AJ, Hang H, Stephens JM. Pyruvate dehydrogenase complex (PDC) subunits moonlight as interaction partners of phosphorylated STAT5 in adipocytes and adipose tissue. *J Biol Chem* 292: 19733-19742, 2017.
733. Richelsen B, Pedersen SB, Moller-Pedersen T, Bak JF. Regional differences in triglyceride breakdown in human adipose tissue: Effects of catecholamines, insulin, and prostaglandin E2. *Metabolism* 40: 990-996, 1991.
734. Richert MM, Schwertfeger KL, Ryder JW, Anderson SM. An atlas of mouse mammary gland development. *J Mammary Gland Biol Neoplasia* 5: 227-241, 2000.
735. Ricquier D. UCP1, the mitochondrial uncoupling protein of brown adipocyte: A personal contribution and a historical perspective. *Biochimie* 134: 3-8, 2017.
736. Ricquier D, Mory G, Hemon P. Effects of chronic treatments upon the brown adipose tissue of young rats. I. Cold exposure and hyperthyroidism. *Pflügers Arch* 362: 241-246, 1976.
737. Ricquier D, Mory G, Nechad M, Hemon P. Effects of cold adaptation and re-adaptation upon the mitochondrial phospholipids of brown adipose tissue. *J Physiol (Paris)* 74: 695-702, 1978.
738. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 47: 241-259, 2006.
739. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 30: 332-338, 2014.
740. Rim JS, Xue B, Gawronska-Kozak B, Kozak LP. Sequestration of thermogenic transcription factors in the cytoplasm during development of brown adipose tissue. *J Biol Chem* 279: 25916-25926, 2004.
741. Robbins GR, Wen H, Ting JP. Inflammasomes and metabolic disorders: Old genes in modern diseases. *Mol Cell* 54: 297-308, 2014.
742. Roberts LD, Bostrom P, O'Sullivan JF, Schinzel RT, Lewis GD, Dejam A, Lee YK, Palma MJ, Calhoun S, Georgiadi A, Chen MH,

AU: Please provide the publisher location for reference 700.

AU: Please provide volume and page numbers for reference 705.

- Ramachandran VS, Larson MG, Bouchard C, Rankinen T, Souza AL, Clish CB, Wang TJ, Estall JL, Soukas AA, Cowan CA, Spiegelman BM, Gerszten RE. beta-Aminoisobutyric acid induces browning of white fat and hepatic beta-oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab* 19: 96-108, 2014.
743. Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Belen Crujeiras A, Seoane LM, Casanueva FF, Pardo M. FND5C/irisin is not only a myokine but also an adipokine. *PLoS One* 8: e60563, 2013.
744. Roche R, Hoareau L, Bes-Houtmann S, Gonthier MP, Laborde C, Baron JF, Haffaf Y, Cesari M, Festy F. Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes. *Histochem Cell Biol* 126: 177-187, 2006.
745. Rochford JJ, Semple RK, Laudes M, Boyle KB, Christodoulides C, Mulligan C, Lelliott CJ, Schinner S, Hadaschik D, Mahadevan M, Sethi JK, Vidal-Puig A, O'Rahilly S. ETO/MTG8 is an inhibitor of C/EBPbeta activity and a regulator of early adipogenesis. *Mol Cell Biol* 24: 9863-9872, 2004.
746. Rogers NH. Brown adipose tissue during puberty and with aging. *Ann Med* 47: 142-149, 2015.
747. Rogers NH, Smith RG. Brown-to-white transition in subcutaneous fat: Linking aging and disease. *Aging (Albany NY)* 4: 728-729, 2012.
748. Rohrborn D, Eckel J, Sell H. Shedding of dipeptidyl peptidase 4 is mediated by metalloproteases and up-regulated by hypoxia in human adipocytes and smooth muscle cells. *FEBS Lett* 588: 3870-3877, 2014.
749. Romacho T, Sanchez-Ferrer CF, Peiro C. Visfatin/Nampt: An adipokine with cardiovascular impact. *Mediators Inflamm* 2013: 946427, 2013.
750. Romacho T, Villalobos LA, Cercas E, Carraro R, Sanchez-Ferrer CF, Peiro C. Visfatin as a novel mediator released by inflamed human endothelial cells. *PLoS One* 8: e78283, 2013.
751. Romere C, Duerrschmid C, Bournat J, Constable P, Jain M, Xia F, Saha PK, Del Solar M, Zhu B, York B, Sarkar P, Rendon DA, Gaber MW, LeMaire SA, Coselli JS, Milewicz DM, Sutton VR, Butte NF, Moore DD, Chopra AR. Asprosin, a fasting-induced glucogenic protein hormone. *Cell* 165: 566-579, 2016.
752. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O, Andris F. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur J Immunol* 32: 3225-3234, 2002.
753. Ronkainen J, Huusko TJ, Soinen R, Mondini E, Cinti F, Makela KA, Kovalainen M, Herzig KH, Jarvelin MR, Sebert S, Savolainen MJ, Saloniemi T. Fat mass- and obesity-associated gene Fto affects the dietary response in mouse white adipose tissue. *Sci Rep* 5: 9233, 2015.
754. Ronkainen J, Mondini E, Cinti F, Cinti S, Sebert S, Savolainen MJ, Saloniemi T. Fto-deficiency affects the gene and MicroRNA expression involved in brown adipogenesis and browning of white adipose tissue in mice. *Int J Mol Sci* 17: 255, 2016.
755. Rosell M, Hondares E, Iwamoto S, Gonzalez FJ, Wabitsch M, Staels B, Olmos Y, Monsalve M, Giralt M, Iglesias R, Villarroya F. Peroxisome proliferator-activated receptors-alpha and -gamma, and cAMP-mediated pathways, control retinol-binding protein-4 gene expression in brown adipose tissue. *Endocrinology* 153: 1162-1173, 2012.
756. Rosell M, Kaforou M, Frontini A, Okolo A, Chan YW, Nikolopoulou E, Millership S, Fenech ME, MacIntyre D, Turner JO, Moore JD, Blackburn E, Gullick WJ, Cinti S, Montana G, Parker MG, Christian M. Brown and white adipose tissues: Intrinsic differences in gene expression and response to cold exposure in mice. *Am J Physiol Endocrinol Metab* 306: E945-E964, 2014.
757. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Spiegelman BM. C/EBPalpha induces adipogenesis through PPARgamma: A unified pathway. *Genes Dev* 16: 22-26, 2002.
758. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol* 7: 885-896, 2006.
759. Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell* 4: 611-617, 1999.
760. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. *Cell* 156: 20-44, 2014.
761. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 14: 1293-1307, 2000.
762. Rosenwald M, Efthymiou V, Opitz L, Wolftrum C. SRF and MKL1 independently inhibit brown adipogenesis. *PLoS One* 12: e0170643, 2017.
763. Rosenwald M, Perdikari A, Rulicke T, Wolftrum C. Bi-directional interconversion of brite and white adipocytes. *Nat Cell Biol* 15: 659-667, 2013.
764. Ross DA, Rao PK, Kadesch T. Dual roles for the Notch target gene Hes-1 in the differentiation of 3T3-L1 preadipocytes. *Mol Cell Biol* 24: 3505-3513, 2004.
765. Ross SE, Erickson RL, Gerin I, DeRose PM, Bajnok L, Longo KA, Misk DE, Kuick R, Hanash SM, Atkins KB, Andresen SM, Nebb HI, Madsen L, Kristiansen K, MacDougald OA. Microarray analyses during adipogenesis: Understanding the effects of Wnt signaling on adipogenesis and the roles of liver X receptor alpha in adipocyte metabolism. *Mol Cell Biol* 22: 5989-5999, 2002.
766. Ross SR, Graves RA, Spiegelman BM. Targeted expression of a toxin gene to adipose tissue: Transgenic mice resistant to obesity. *Genes Dev* 7: 1318-1324, 1993.
767. Rossato M. Aging and brown adipose tissue activity decline in human: Does the brain extinguish the fire? *Aging Clin Exp Res* 28: 579-581, 2016.
768. Rossmeis M, Barbatelli G, Flachs P, Brauner P, Zingaretti MC, Marelli M, Janovska P, Horakova M, Syrový I, Cinti S, Kopecky J. Expression of the uncoupling protein 1 from the aP2 gene promoter stimulates mitochondrial biogenesis in unilocular adipocytes in vivo. *Eur J Biochem* 269: 19-28, 2002.
769. Rubio-Cabezas O, Puri V, Murano I, Saudek V, Semple RK, Dash S, Hyden CS, Bottomley W, Vigouroux C, Magre J, Raymond-Barker P, Murgatroyd PR, Chawla A, Skepper JN, Chatterjee VK, Suliman S, Patch AM, Agarwal AK, Garg A, Barroso I, Cinti S, Czech MP, Argente J, O'Rahilly S, Savage DB, Consortium LDS. Partial lipodystrophy and insulin resistant diabetes in a patient with a homozygous nonsense mutation in CIDEC. *EMBO Mol Med* 1: 280-287, 2009.
770. Ruiz de Azua I MG, Srivastava RK, Rey AA, Cardinal P, Tedesco L, Zingaretti MC, Sassman A, Quarta C, Schwitter C, Conrad A, Wetschureck N, Vemuri VK, Makriyannis A, Hartwig J, Medez-Lago M, Bindila L, Monory K, Giordano A, Cinti S, Marsicano G, Offermans S, Nisoli E, Pagotto U, Cota D, Lutz B. Adipocyte CB1 receptor regulates energy homeostasis and alternatively activated macrophages. *J Clin Invest* 127: 4148-4162, 2017.
771. Ruth MR, Wellman T, Mercier G, Szabo T, Apovian CM. An automated algorithm to identify and quantify brown adipose tissue in human 18F-FDG-PET/CT scans. *Obesity (Silver Spring)* 21: 1554-1560, 2013.
772. Ryden M, Uzunel M, Hard JL, Borgstrom E, Mold JE, Arner E, Mejhert N, Andersson DP, Widlund Y, Hassan M, Jones CV, Spalding KL, Svahn BM, Ahmadian A, Frisen J, Bernard S, Mattsson J, Arner P. Transplanted bone marrow-derived cells contribute to human adipogenesis. *Cell Metab* 22: 408-417, 2015.
773. Sacks HS, Fain JN, Bahouth SW, Ojha S, Frontini A, Budge H, Cinti S, Symonds ME. Adult epicardial fat exhibits beige features. *J Clin Endocrinol Metab* 98: E1448-E1455, 2013.
774. Sahuri-Arisoylu M, Brody LP, Parkinson JR, Parkes H, Navaratnam N, Miller AD, Thomas EL, Frost G, Bell JD. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int J Obes (Lond)* 40: 955-963, 2016.
775. Saito M. Capsaicin and related food ingredients reducing body fat through the activation of TRP and brown fat thermogenesis. *Adv Food Nutr Res* 76: 1-28, 2015.
776. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes* 58: 1526-1531, 2009.
777. Sakamoto N, Segawa K, Kanzaki M, Ohashi T, Sato M. Role of p120-catenin in the morphological changes of endothelial cells exposed to fluid shear stress. *Biochem Biophys Res Commun* 398: 426-432, 2010.
778. Sakaue H, Ogawa W, Nakamura T, Mori T, Nakamura K, Kasuga M. Role of MAPK phosphatase-1 (MKP-1) in adipocyte differentiation. *J Biol Chem* 279: 39951-39957, 2004.
779. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 1 page following 696, 1998.
780. Salans LB, Cushman SW, Weismann RE. Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients. *J Clin Invest* 52: 929-941, 1973.
781. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 14: 1431-1437, 1994.
782. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Gil A, Ruiz JR. Role of exercise in the activation of brown adipose tissue. *Ann Nutr Metab* 67: 21-32, 2015.
783. Sanchez-Gurmaches J, Hung CM, Sparks CA, Tang Y, Li H, Guertin DA. PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. *Cell Metab* 16: 348-362, 2012.

AU: Please provide volume and page numbers for reference 754.

784. Sarruf DA, Iankova I, Abella A, Assou S, Miard S, Fajas L. Cyclin D3 promotes adipogenesis through activation of peroxisome proliferator-activated receptor gamma. *Mol Cell Biol* 25: 9985-9995, 2005.
785. Sarzani R, Paci VM, Zingaretti CM, Pierleoni C, Cinti S, Cola G, Rappelli A, Dessi-Fulgheri P. Fasting inhibits natriuretic peptides clearance receptor expression in rat adipose tissue. *J Hypertens* 13: 1241-1246, 1995.
786. Sarzani R, Salvi F, Dessi-Fulgheri P, Rappelli A. Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: An integrated view in humans. *J Hypertens* 26: 831-843, 2008.
787. Savage DB. Mouse models of inherited lipodystrophy. *Dis Model Mech* 2: 554-562, 2009.
788. Savage DB, Semple RK, Clatworthy MR, Lyons PA, Morgan BP, Cochran EK, Gorden P, Raymond-Barker P, Murgatroyd PR, Adams C, Scobie I, Mufti GJ, Alexander GJ, Thiru S, Murano I, Cinti S, Chaudhry AN, Smith KG, O'Rahilly S. Complement abnormalities in acquired lipodystrophy revisited. *J Clin Endocrinol Metab* 94: 10-16, 2009.
789. Savage DB, Tan GD, Acerini CL, Jebb SA, Agostini M, Gurnell M, Williams RL, Umpleby AM, Thomas EL, Bell JD, Dixon AK, Dunne F, Boiani R, Cinti S, Vidal-Puig A, Karpe F, Chatterjee VK, O'Rahilly S. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. *Diabetes* 52: 910-917, 2003.
790. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 168: 960-976, 2017.
791. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 169: 361-371, 2017.
792. Sbarbati A, Accorsi D, Benati D, Marchetti L, Orsini G, Rigotti G, Panetti P. Subcutaneous adipose tissue classification. *Eur J Histochem* 54: e48, 2010.
793. Sbarbati A, Morroni M, Zancanaro C, Cinti S. Rat interscapular brown adipose tissue at different ages: A morphometric study. *Int J Obes* 15: 581-587, 1991.
794. Sbarbati A, Zancanaro C, Cigolini M, Cinti S. Brown adipose tissue: A scanning electron microscopic study of tissue and cultured adipocytes. *Acta Anat (Basel)* 128: 84-88, 1987.
795. Schaffler A, Scholmerich J. Innate immunity and adipose tissue biology. *Trends Immunol* 31: 228-235, 2010.
796. Schaffler A, Scholmerich J, Salzberger B. Adipose tissue as an immunological organ: Toll-like receptors, C1q/TNFs and CTRPs. *Trends Immunol* 28: 393-399, 2007.
797. Scherer PE, Bickel PE, Kotler M, Lodish HF. Cloning of cell-specific secreted and surface proteins by subtractive antibody screening. *Nat Biotechnol* 16: 581-586, 1998.
798. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270: 26746-26749, 1995.
799. Schleinitz D, Bottcher Y, Blüher M, Kovacs P. The genetics of fat distribution. *Diabetologia* 57: 1276-1286, 2014.
800. Schleinitz D, Kloting N, Bottcher Y, Wolf S, Dietrich K, Tonjes A, Breitfeld J, Enigk B, Halbritter J, Korner A, Schon MR, Jenkner J, Tseng YH, Lohmann T, Dressler M, Stumvoll M, Blüher M, Kovacs P. Genetic and evolutionary analyses of the human bone morphogenetic protein receptor 2 (BMP2) in the pathophysiology of obesity. *PLoS One* 6: e16155, 2011.
801. Schmieder RE, Hilgers KF, Schlaich MP, Schmidt BM. Renin-angiotensin system and cardiovascular risk. *Lancet* 369: 1208-1219, 2007.
802. Schneider-Picard G, Carpentier JL, Orci L. Quantitative evaluation of gap junctions during development of the brown adipose tissue. *J Lipid Res* 21: 600-607, 1980.
803. Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin complexes have distinct biochemical characteristics. *Endocrinology* 149: 2270-2282, 2008.
804. Schroder K, Tschopp J. The inflammasomes. *Cell* 140: 821-832, 2010.
805. Schulz TJ, Huang P, Huang TL, Xue R, McDougall LE, Townsend KL, Cypess AM, Mishina Y, Gussoni E, Tseng YH. Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. *Nature* 495: 379-383, 2013.
806. Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, Cerletti M, McDougall LE, Giorgadze N, Tchekoniya T, Schrier D, Falb D, Kirkland JL, Wagers AJ, Tseng YH. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc Natl Acad Sci U S A* 108: 143-148, 2011.
807. Schwartz DR, Lazar MA. Human resistin: Found in translation from mouse to man. *Trends Endocrinol Metab* 22: 259-265, 2011.
808. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454: 961-967, 2008.
809. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest* 121: 96-105, 2011.
810. Sellayah D, Bharaj P, Sikder D. Orexin is required for brown adipose tissue development, differentiation, and function. *Cell Metab* 14: 478-490, 2011.
811. Sellayah D, Sikder D. Orexin restores aging-related brown adipose tissue dysfunction in male mice. *Endocrinology* 155: 485-501, 2014.
812. Seo JB, Moon HM, Kim WS, Lee YS, Jeong HW, Yoo EJ, Ham J, Kang H, Park MG, Steffensen KR, Stulnig TM, Gustafsson JA, Park SD, Kim JB. Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor gamma expression. *Mol Cell Biol* 24: 3430-3444, 2004.
813. Shan T, Liu J, Wu W, Xu Z, Wang Y. Roles of notch signaling in adipocyte progenitor cells and mature adipocytes. *J Cell Physiol* 232: 1258-1261, 2017.
814. Shan T, Liu W, Kuang S. Fatty acid binding protein 4 expression marks a population of adipocyte progenitors in white and brown adipose tissues. *FASEB J* 27: 277-287, 2013.
815. Shan T, Xiong Y, Zhang P, Li Z, Jiang Q, Bi P, Yue F, Yang G, Wang Y, Liu X, Kuang S. Lkb1 controls brown adipose tissue growth and thermogenesis by regulating the intracellular localization of CRTC3. *Nat Commun* 7: 12205, 2016.
816. Shan T, Zhang P, Jiang Q, Xiong Y, Wang Y, Kuang S. Adipocyte-specific deletion of mTOR inhibits adipose tissue development and causes insulin resistance in mice. *Diabetologia* 59: 1995-2004, 2016.
817. Shao M, Hepler C, Vishvanath L, MacPherson KA, Busbuso NC, Gupta RK. Fetal development of subcutaneous white adipose tissue is dependent on Zfp423. *Mol Metab* 6: 111-124, 2017.
818. Shao M, Ishibashi J, Kusminski CM, Wang QA, Hepler C, Vishvanath L, MacPherson KA, Spurgin SB, Sun K, Holland WL, Seale P, Gupta RK. Zfp423 maintains white adipocyte identity through suppression of the Beige cell thermogenic gene program. *Cell Metab* 23: 1167-1184, 2016.
819. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. *Diabetes* 59: 1171-1181, 2010.
820. Shen JF, Sugawara A, Yamashita J, Ogura H, Sato S. Dedifferentiated fat cells: An alternative source of adult multipotent cells from the adipose tissues. *Int J Oral Sci* 3: 117-124, 2011.
821. Shen WJ, Patel S, Miyoshi H, Greenberg AS, Kraemer FB. Functional interaction of hormone-sensitive lipase and perilipin in lipolysis. *J Lipid Res* 50: 2306-2313, 2009.
822. Shi C, Huang F, Gu X, Zhang M, Wen J, Wang X, You L, Cui X, Ji C, Guo X. Adipogenic miRNA and meta-signature miRNAs involved in human adipocyte differentiation and obesity. *Oncotarget* 7: 40830-40845, 2016.
823. Shi C, Zhang M, Tong M, Yang L, Pang L, Chen L, Xu G, Chi X, Hong Q, Ni Y, Ji C, Guo X. miR-148a is associated with obesity and modulates adipocyte differentiation of mesenchymal stem cells through Wnt signaling. *Sci Rep* 5: 9930, 2015.
824. Shi H, Clegg DJ. Sex differences in the regulation of body weight. *Physiol Behav* 97: 199-204, 2009.
825. Shi H, Seeley RJ, Clegg DJ. Sexual differences in the control of energy homeostasis. *Front Neuroendocrinol* 30: 396-404, 2009.
826. Shi H, Song CK, Giordano A, Cinti S, Bartness TJ. Sensory or sympathetic white adipose tissue denervation differentially affects depot growth and cellularity. *Am J Physiol Regul Integr Comp Physiol* 288: R1028-R1037, 2005.
827. Shimba S, Ishii N, Ohta Y, Ohno T, Watabe Y, Hayashi M, Wada T, Aoyagi T, Tezuka M. Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proc Natl Acad Sci U S A* 102: 12071-12076, 2005.
828. Shimba S, Wada T, Hara S, Tezuka M. EPAS1 promotes adipose differentiation in 3T3-L1 cells. *J Biol Chem* 279: 40946-40953, 2004.
829. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401: 73-76, 1999.
830. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: Model for congenital generalized lipodystrophy. *Genes Dev* 12: 3182-3194, 1998.
831. Shinkai H. Cholesteryl ester transfer-protein modulator and inhibitors and their potential for the treatment of cardiovascular diseases. *Vasc Health Risk Manag* 8: 323-331, 2012.
832. Shinoda K, Ohyama K, Hasegawa Y, Chang HY, Ogura M, Sato A, Hong H, Hosono T, Sharp LZ, Scheel DW, Graham M, Ishihama Y, Kajimura S. Phosphoproteomics identifies CK2 as a negative regulator of Beige adipocyte thermogenesis and energy expenditure. *Cell Metab* 22: 997-1008, 2015.

833. Shirakawa K, Yan X, Shinmura K, Endo J, Kataoka M, Katsumata Y, Yamamoto T, Anzai A, Isobe S, Yoshida N, Itoh H, Manabe I, Sekai M, Hamazaki Y, Fukuda K, Minato N, Sano M. Obesity accelerates T cell senescence in murine visceral adipose tissue. *J Clin Invest* 126: 4626-4639, 2016.
834. Sidman RL. Histogenesis of brown adipose tissue in vivo and in organ culture. *Anat Rec* 124: 581-601, 1956.
835. Sidossis L, Kajimura S. Brown and beige fat in humans: Thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest* 125: 478-486, 2015.
836. Siersbaek R, Nielsen R, Mandrup S. Transcriptional networks and chromatin remodeling controlling adipogenesis. *Trends Endocrinol Metab* 23: 56-64, 2012.
837. Silva JE, Larsen PR. Adrenergic activation of triiodothyronine production in brown adipose tissue. *Nature* 305: 712-713, 1983.
838. Silvestri C, Di Marzo V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metab* 17: 475-490, 2013.
839. Silvestri C, Ligresti A, Di Marzo V. Peripheral effects of the endocannabinoid system in energy homeostasis: Adipose tissue, liver and skeletal muscle. *Rev Endocr Metab Disord* 12: 153-162, 2011.
840. Simpson ER. Sources of estrogen and their importance. *J Steroid Biochem Mol Biol* 86: 225-230, 2003.
841. Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, Speed C, Jones M. Aromatase—a brief overview. *Annu Rev Physiol* 64: 93-127, 2002.
842. Sinensky M, Fantle K, Trujillo M, McLain T, Kupfer A, Dalton M. The processing pathway of prelamins A. *J Cell Sci* 107(Pt 1): 61-67, 1994.
843. Singh R, Braga M, Pervin S. Regulation of brown adipocyte metabolism by myostatin/follistatin signaling. *Front Cell Dev Biol* 2: 60, 2014.
844. Sjöström L, Narbro K, Sjöström CD, Karason K, Larsson B, Wedel H, Lystig T, Sullivan M, Bouchard C, Carlsson B, Bengtsson C, Dahlgren S, Gummesson A, Jacobson P, Karlsson J, Lindroos AK, Lönroth H, Naslund I, Olbers T, Stenlof K, Torgerson J, Agren G, Carlsson LM, Swedish Obese Subjects S. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* 357: 741-752, 2007.
845. Skarn M, Namlos HM, Noordhuis P, Wang MY, Meza-Zepeda LA, Myklebost O. Adipocyte differentiation of human bone marrow-derived stromal cells is modulated by microRNA-155, microRNA-221, and microRNA-222. *Stem Cells Dev* 21: 873-883, 2012.
846. Skurk T, Hauner H. Obesity and impaired fibrinolysis: Role of adipose production of plasminogen activator inhibitor-1. *Int J Obes Relat Metab Disord* 28: 1357-1364, 2004.
847. Skurk T, Hauner H. Primary culture of human adipocyte precursor cells: Expansion and differentiation. *Methods Mol Biol* 806: 215-226, 2012.
848. Slavin BG. Fine structural studies on white adipocyte differentiation. *Anat Rec* 195: 63-72, 1979.
849. Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J Biol Chem* 263: 9402-9408, 1988.
850. Smith U. Abdominal obesity: A marker of ectopic fat accumulation. *J Clin Invest* 125: 1790-1792, 2015.
851. Smorlesi A, Frontini A, Giordano A, Cinti S. The adipose organ: white-brown adipocyte plasticity and metabolic inflammation. *Obes Rev* 13(Suppl 2): 83-96, 2012.
852. Son YH, Ka S, Kim AY, Kim JB. Regulation of adipocyte differentiation via microRNAs. *Endocrinol Metab (Seoul)* 29: 122-135, 2014.
853. Song G, Xu G, Ji C, Shi C, Shen Y, Chen L, Zhu L, Yang L, Zhao Y, Guo X. The role of microRNA-26b in human adipocyte differentiation and proliferation. *Gene* 533: 481-487, 2014.
854. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21: 70-71, 1999.
855. Sornelli F, Fiore M, Chaldakov GN, Aloe L. Adipose tissue-derived nerve growth factor and brain-derived neurotrophic factor: Results from experimental stress and diabetes. *Gen Physiol Biophys* 28 Spec No: 179-183, 2009.
856. Soukas A, Cohen P, Socci ND, Friedman JM. Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 14: 963-980, 2000.
857. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P. Dynamics of fat cell turnover in humans. *Nature* 453: 783-787, 2008.
858. Spiegelman BM, Choy L, Hotamisligil GS, Graves RA, Tontonoz P. Regulation of adipocyte gene expression in differentiation and syndromes of obesity/diabetes. *J Biol Chem* 268: 6823-6826, 1993.
859. Spiegelman BM, Flier JS. Adipogenesis and obesity: Rounding out the big picture. *Cell* 87: 377-389, 1996.
860. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 104: 531-543, 2001.
861. Spiegelman BM, Hotamisligil GS. Through thick and thin: Wasting, obesity, and TNF alpha. *Cell* 73: 625-627, 1993.
862. Srivastava S, Kashiwaya Y, King MT, Baxa U, Tam J, Niu G, Chen X, Clarke K, Veech RL. Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. *FASEB J* 26: 2351-2362, 2012.
863. Stallknecht B, Vinten J, Ploug T, Galbo H. Increased activities of mitochondrial enzymes in white adipose tissue in trained rats. *Am J Physiol* 261: E410-E414, 1991.
864. Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise effects on white adipose tissue: Being and metabolic adaptations. *Diabetes* 64: 2361-2368, 2015.
865. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest* 123: 215-223, 2013.
866. Starowiec KM, Cristino L, Matias I, Capasso R, Racioppi A, Izzo AA, Di Marzo V. Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. *Obesity (Silver Spring)* 16: 553-565, 2008.
867. Stenlund SM, Ruud J, Karakasilioti I, Backes H, Engstrom Ruud L, Timper K, Hess ME, Tsaozidou E, Mauer J, Vogt MC, Paeger L, Bremser S, Klein AC, Morgan DA, Frommolt P, Brinkkötter PT, Hamerschmidt P, Benzing T, Rahmouni K, Wunderlich FT, Kloppenburg P, Bruning JC. AgRP neurons control systemic insulin sensitivity via myostatin expression in brown adipose tissue. *Cell* 165: 125-138, 2016.
868. Stengel A. Nesfatin-1—more than a food intake regulatory peptide. *Peptides* 72: 175-183, 2015.
869. Stepan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 409: 307-312, 2001.
870. Stern JS, Batchelor BR, Hollander N, Cohn CK, Hirsch J. Adipose-cell size and immunoreactive insulin levels in obese and normal-weight adults. *Lancet* 2: 948-951, 1972.
871. Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, van den Berg S, Romijn J, Rensen PC, Joosten LA, Netea MG, Kanneganti TD. Inflammation is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci U S A* 108: 15324-15329, 2011.
872. Stock MaC. S. Adipose tissue: Structure and function of brown adipose tissue. In: *Cyclopedia of Food Sciences and Nutrition* (2nd ed.). Elsevier, pp. 29-34.
873. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, II, Defuria J, Jick Z, Greenberg AS, Obin MS. Adipocyte death, adipose tissue remodeling and obesity complications. *Diabetes* 55: 1473-1478, 2006.
874. Suarez-Zamorano N, Fabbiano S, Chevalier C, Stojanovic O, Colin DJ, Stevanovic A, Veyrat-Durebex C, Tarallo V, Rigo D, Germain S, Ilievskia M, Montet X, Seimille Y, Hapfelmeier S, Trajkovski M. Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nat Med* 21: 1497-1501, 2015.
875. Sugihara H, Yonemitsu N, Miyabara S, Toda S. Proliferation of unilocular fat cells in the primary culture. *J Lipid Res* 28: 1038-1045, 1987.
876. Sugihara H, Yonemitsu N, Miyabara S, Yun K. Primary cultures of unilocular fat cells: Characteristics of growth in vitro and changes in differentiation properties. *Differentiation* 31: 42-49, 1986.
877. Suh JM, Gao X, McKay J, McKay R, Salo Z, Graff JM. Hedgehog signaling plays a conserved role in inhibiting fat formation. *Cell Metab* 3: 25-34, 2006.
878. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest* 121: 2094-2101, 2011.
879. Sun L, Trajkovski M. MiR-27 orchestrates the transcriptional regulation of brown adipogenesis. *Metabolism* 63: 272-282, 2014.
880. Sun L, Xie H, Mori MA, Alexander R, Yuan B, Hattangadi SM, Liu Q, Kahn CR, Lodish HF. Mir193b-365 is essential for brown fat differentiation. *Nat Cell Biol* 13: 958-965, 2011.
881. Sun T, Fu M, Bookout AL, Kliewer SA, Mangelsdorf DJ. MicroRNA let-7 regulates 3T3-L1 adipogenesis. *Mol Endocrinol* 23: 925-931, 2009.
882. Susulic VS, Frederick RC, Lawitts J, Tozzo E, Kahn BB, Harper ME, Himms-Hagen J, Flier JS, Lowell BB. Targeted disruption of the beta 3-adrenergic receptor gene. *J Biol Chem* 270: 29483-29492, 1995.
883. Svensson KJ, Long JZ, Jedrychowski MP, Cohen P, Lo JC, Serag S, Kir S, Shinoda K, Tartaglia JA, Rao RR, Chedotal A, Kajimura S, Gygi SP, Spiegelman BM. A secreted Slit2 fragment regulates adipose tissue thermogenesis and metabolic function. *Cell Metab* 23: 454-466, 2016.
884. Takahashi N, Kawada T, Yamamoto T, Goto T, Taimatsu A, Aoki N, Kawasaki H, Taira K, Yokoyama KK, Kamei Y, Fushiki T. Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of

AU: Please provide the publisher location for reference 872.

AU: Please provide volume and page numbers for reference 873.

- peroxisome proliferator-activated receptor gamma. *J Biol Chem* 277: 16906-16912, 2002.
885. Takeda S, Eleftheriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducey P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 111: 305-317, 2002.
 886. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res* 34: 1255-1274, 1993.
 887. Tan BK, Adya R, Randeve HS. Omentin: A novel link between inflammation, diabetes, and cardiovascular disease. *Trends Cardiovasc Med* 20: 143-148, 2010.
 888. Tan BK, Chen J, Digby JE, Keay SD, Kennedy CR, Randeve HS. Increased visfatin messenger ribonucleic acid and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome: Parallel increase in plasma visfatin. *J Clin Endocrinol Metab* 91: 5022-5028, 2006.
 889. Tanaka T, Yoshida N, Kishimoto T, Akira S. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. *EMBO J* 16: 7432-7443, 1997.
 890. Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM. White fat progenitor cells reside in the adipose vasculature. *Science* 322: 583-586, 2008.
 891. Tang YF, Zhang Y, Li XY, Li C, Tian W, Liu L. Expression of miR-31, miR-125b-5p, and miR-326 in the adipogenic differentiation process of adipose-derived stem cells. *OMICS* 13: 331-336, 2009.
 892. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263-1271, 1995.
 893. Tavassoli M. In vivo development of adipose tissue following implantation of lipid-depleted cultured adipocyte. *Exp Cell Res* 137: 55-62, 1982.
 894. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: An update. *Physiol Rev* 93: 359-404, 2013.
 895. Tchoukalova YD, Koutsari C, Karpyak MV, Votruba SB, Wendland E, Jensen MD. Subcutaneous adipocyte size and body fat distribution. *Am J Clin Nutr* 87: 56-63, 2008.
 896. Tews D, Fischer-Posovszky P, Fromme T, Klingenspor M, Fischer J, Ruther U, Marienfeld R, Barth TF, Moller P, Debatin KM, Wabitsch M. FTO deficiency induces UCP-1 expression and mitochondrial uncoupling in adipocytes. *Endocrinology* 154: 3141-3151, 2013.
 897. Than A, He HL, Chua SH, Xu D, Sun L, Leow MK, Chen P. Apelin enhances brown adipogenesis and browning of white adipocytes. *J Biol Chem* 290: 14679-14691, 2015.
 898. Thomas C, Gioiello A, Noriga L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Matak C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* 10: 167-177, 2009.
 899. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140: 1630-1638, 1999.
 900. Thompson AC, Bruss MD, Nag N, Kharitonov A, Adams AC, Hellerstein MK. Fibroblast growth factor 21 is not required for the reductions in circulating insulin-like growth factor-1 or global cell proliferation rates in response to moderate calorie restriction in adult mice. *PLoS One* 9: e111418, 2014.
 901. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 61: 364-371, 2012.
 902. Tomicek NJ, Lancaster TS, Korzick DH. Increased estrogen receptor beta in adipose tissue is associated with increased intracellular and reduced circulating adiponectin protein levels in aged female rats. *Genet Med* 8: 325-333, 2011.
 903. Tominaga K, Kondo C, Johmura Y, Nishizuka M, Imagawa M. The novel gene fad104, containing a fibronectin type III domain, has a significant role in adipogenesis. *FEBS Lett* 577: 49-54, 2004.
 904. Tonello C, Giordano A, Cozzi V, Cinti S, Stock MJ, Carruba MO, Nisoli E. Role of sympathetic activity in controlling the expression of vascular endothelial growth factor in brown fat cells of lean and genetically obese rats. *FEBS Lett* 442: 167-172, 1999.
 905. Tong Q, Dalgin G, Xu H, Ting CN, Leiden JM, Hotamisligil GS. Function of GATA transcription factors in preadipocyte-adipocyte transition. *Science* 290: 134-138, 2000.
 906. Tong Q, Tsai J, Tan G, Dalgin G, Hotamisligil GS. Interaction between GATA and the C/EBP family of transcription factors is critical in GATA-mediated suppression of adipocyte differentiation. *Mol Cell Biol* 25: 706-715, 2005.
 907. Tontonoz P, Kim JB, Graves RA, Spiegelman BM. ADD1: A novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol Cell Biol* 13: 4753-4759, 1993.
 908. Tontonoz P, Spiegelman BM. Fat and beyond: The diverse biology of PPARgamma. *Annu Rev Biochem* 77: 289-312, 2008.
 909. Topol LZ, Bardot B, Zhang Q, Resau J, Huillard E, Marx M, Calothy G, Blair DG. Biosynthesis, post-translation modification, and functional characterization of Drm/Gremlin. *J Biol Chem* 275: 8785-8793, 2000.
 910. Torriani M, Srinivasa S, Fitch KV, Thomou T, Wong K, Petrow E, Kahn CR, Cypess AM, Grinspoon SK. Dysfunctional subcutaneous fat with reduced dicer and brown adipose tissue gene expression in HIV-infected patients. *J Clin Endocrinol Metab* 101: 1225-1234, 2016.
 911. Tosh D, Slack JM. How cells change their phenotype. *Nat Rev Mol Cell Biol* 3: 187-194, 2002.
 912. Toth MJ, Tchernof A, Sites CK, Poehlman ET. Effect of menopausal status on body composition and abdominal fat distribution. *Int J Obes Relat Metab Disord* 24: 226-231, 2000.
 913. Townsend KL, Suzuki R, Huang TL, Jing E, Schulz TJ, Lee K, Taniguchi CM, Espinoza DO, McDougall LE, Zhang H, He TC, Kokkotou E, Tseng YH. Bone morphogenetic protein 7 (BMP7) reverses obesity and regulates appetite through a central mTOR pathway. *FASEB J* 26: 2187-2196, 2012.
 914. Trajkovski M, Ahmed K, Esau CC, Stoffel M. MyomiR-133 regulates brown fat differentiation through Prdm16. *Nat Cell Biol* 14: 1330-1335, 2012.
 915. Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, Zavan M, Heim MH, Stoffel M. MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* 474: 649-653, 2011.
 916. Tran KV, Gealekman O, Frontini A, Zingaretti MC, Morroni M, Giordano A, Smorlesi A, Perugini J, De Matteis R, Sbarbati A, Corvera S, Cinti S. The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. *Cell Metab* 15: 222-229, 2012.
 917. Trayhurn P. Recruiting brown adipose tissue in human obesity. *Diabetes* 65: 1158-1160, 2016.
 918. Trayhurn P, Bing C, Wood IS. Adipose tissue and adipokines—energy regulation from the human perspective. *J Nutr* 136: 1935S-1939S, 2006.
 919. Trayhurn P, Douglas JB, McGuckin MM. Brown adipose tissue thermogenesis is 'suppressed' during lactation in mice. *Nature* 298: 59-60, 1982.
 920. Trayhurn P, Nicholls D. *Brown Adipose Tissue*. London: Edward Arnold, 1986.
 921. Tseng YH, Butte AJ, Kokkotou E, Yehoor VK, Taniguchi CM, Kriacianus KM, Cypess AM, Niinobe M, Yoshikawa K, Patti ME, Kahn CR. Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necdin. *Nat Cell Biol* 7: 601-611, 2005.
 922. Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN, Kahn CR. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454: 1000-1004, 2008.
 923. Tsoukas MA, Farr OM, Mantzoros CS. Leptin in congenital and HIV-associated lipodystrophy. *Metabolism* 64: 47-59, 2015.
 924. Tsukiyama-Kohara K, Poulin F, Kohara M, DeMaria CT, Cheng A, Wu Z, Gingras AC, Katsume A, Elchebly M, Spiegelman BM, Harper ME, Tremblay ML, Sonenberg N. Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. *Nat Med* 7: 1128-1132, 2001.
 925. Turing AM. The chemical basis of morphogenesis. *Bull Math Biol* 52: 153-197, 1953; discussion 119-152, 1990.
 926. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JL. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444: 1027-1031, 2006.
 927. Turner RT, Philbrick KA, Kuah AF, Branscum AJ, Iwaniec UT. Role of estrogen receptor signaling in skeletal response to leptin in female ob/ob mice. *J Endocrinol* 233: 357-367, 2017.
 928. Uezumi A, Fukada S, Yamamoto N, Takeda S, Tsuchida K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat Cell Biol* 12: 143-152, 2010.
 929. Vague J. The degree of masculine differentiation of obesities: A factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 4: 20-34, 1956.
 930. Vaidyanathan V, Bastarrachea RA, Higgins PB, Voruganti VS, Kamath S, DiPatrizio NV, Piomelli D, Comuzzie AG, Parks EJ. Selective cannabinoid-1 receptor blockade benefits fatty acid and triglyceride metabolism significantly in weight-stable nonhuman primates. *Am J Physiol Endocrinol Metab* 303: E624-E634, 2012.
 931. Valerio A, Nisoli E. Nitric oxide, interorganelle communication, and energy flow: A novel route to slow aging. *Front Cell Dev Biol* 3: 6, 2015.
 932. Valet P, Grujic D, Wade J, Ito M, Zingaretti MC, Soloveva V, Ross SR, Graves RA, Cinti S, Lafontan M, Lowell BB. Expression of human alpha 2-adrenergic receptors in adipose tissue of beta 3-adrenergic receptor-deficient mice promotes diet-induced obesity. *J Biol Chem* 275: 34797-34802, 2000.

933. Valverde AM, Arribas M, Mur C, Navarro P, Pons S, Cassard-Doulcier AM, Kahn CR, Benito M. Insulin-induced up-regulated uncoupling protein-1 expression is mediated by insulin receptor substrate 1 through the phosphatidylinositol 3-kinase/Akt signaling pathway in fetal brown adipocytes. *J Biol Chem* 278: 10221-10231, 2003.
934. Valverde AM, Lorenzo M, Navarro P, Benito M. Phosphatidylinositol 3-kinase is a requirement for insulin-like growth factor I-induced differentiation, but not for mitogenesis, in fetal brown adipocytes. *Mol Endocrinol* 11: 595-607, 1997.
935. van Baak MA, Hul GB, Toubro S, Astrup A, Gottesdiener KM, DeSmet M, Saris WH. Acute effect of L-796568, a novel beta 3-adrenergic receptor agonist, on energy expenditure in obese men. *Clin Pharmacol Ther* 71: 272-279, 2002.
936. Van Maldergem L, Berardinelli-Seip Congenital Lipodystrophy. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford RJ, Smith RJH, Stephens K, editors. *GeneReviews(R)*. Seattle (WA): University of Washington, 2003.
937. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 301: R285-R296, 2011.
938. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 360: 1500-1508, 2009.
939. Van RL, Bayliss CE, Roncari DA. Cytological and enzymological characterization of adult human adipocyte precursors in culture. *J Clin Invest* 58: 699-704, 1976.
940. Vandannagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 17: 179-188, 2011.
941. Vapola MH, Rokka A, Sormunen RT, Alhonen L, Schmitz W, Conzelmann E, Warri A, Grunau S, Anttonenkov VD, Hiltunen JK. Peroxisomal membrane channel Pmp2 in the mammary fat pad is essential for stromal lipid homeostasis and for development of mammary gland epithelium in mice. *Dev Biol* 391: 66-80, 2014.
942. Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Berriel Diaz M, Rozman J, Hrahe de Angelis M, Nusing RM, Meyer CW, Wahli W, Klingenspor M, Herzig S. Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. *Science* 328: 1158-1161, 2010.
943. Vegiopoulos A, Rohm M, Herzig S. Adipose tissue: Between the extremes. *EMBO J* 36: 1999-2017, 2017.
944. Vettor R, Pagano C. The role of the endocannabinoid system in lipogenesis and fatty acid metabolism. *Best Pract Res Clin Endocrinol Metab* 23: 51-63, 2009.
945. Viengchareun S, Zennaro MC, Pascual-Le Tallec L, Lombers M. Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. *FEBS Lett* 532: 345-350, 2002.
946. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One* 6: e17247, 2011.
947. Villarroya F, Cereijo R, Villarroya J, Giral M. Brown adipose tissue as a secretory organ. *Nat Rev Endocrinol* 13: 26-35, 2017.
948. Virtanen KA, Lidell ME, Orava J, Heglin M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 360: 1518-1525, 2009.
949. Vishvanath L, MacPherson KA, Hepler C, Wang QA, Shao M, Spurgin SB, Wang MY, Kusminski CM, Morley TS, Gupta RK. Pdgfrbeta+ Mural preadipocytes contribute to adipocyte hyperplasia induced by high-fat-diet feeding and prolonged cold exposure in adult mice. *Cell Metab* 23: 350-359, 2016.
950. Vitali A, Murano I, Zingaretti MC, Frontini A, Ricquier D, Cinti S. The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. *J Lipid Res* 52: 100-109, 2011.
951. Wada N, Hirako S, Takenoya F, Kageyama H, Okabe M, Shioda S. Leptin and its receptors. *J Chem Neuroanat* 61-62: 191-199, 2014.
952. Wagoner B, Hausman DB, Harris RB. Direct and indirect effects of leptin on preadipocyte proliferation and differentiation. *Am J Physiol Regul Integr Comp Physiol* 290: R1557-R1564, 2006.
953. Wajchenberg BL. Subcutaneous and visceral adipose tissue: Their relation to the metabolic syndrome. *Endocr Rev* 21: 697-738, 2000.
954. Wang C, Liu W, Nie Y, Qaher M, Horton HE, Yue F, Asakura A, Kuang S. Loss of MyoD promotes fate transdifferentiation of myoblasts into brown adipocytes. *EBioMedicine* 16: 212-223, 2017.
955. Wang F, Mullican SE, DiSpirito JR, Peed LC, Lazar MA. Lipodystrophy and severe metabolic disturbance in mice with fat-specific deletion of PPARgamma. *Proc Natl Acad Sci U S A* 110: 18656-18661, 2013.
956. Wang GX, Zhao XY, Lin JD. The brown fat secretome: Metabolic functions beyond thermogenesis. *Trends Endocrinol Metab* 26: 231-237, 2015.
957. Wang GX, Zhao XY, Meng ZX, Kern M, Dietrich A, Chen Z, Cozocov Z, Zhou D, Okunade AL, Su X, Li S, Bluhm M, Lin JD. The brown fat-enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. *Nat Med* 20: 1436-1443, 2014.
958. Wang Q, Li YC, Wang J, Kong J, Qi Y, Quigg RJ, Li X. miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. *Proc Natl Acad Sci U S A* 105: 2889-2894, 2008.
959. Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat Med* 19: 1338-1344, 2013.
960. Wang W, Seale P. Control of brown and beige fat development. *Nat Rev Mol Cell Biol* 17: 691-702, 2016.
961. Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. *Hypertension* 51: 8-14, 2008.
962. Wang ZV, Schraw TD, Kim JY, Khan T, Rajala MW, Follenzi A, Scherer PE. Secretion of the adipocyte-specific secretory protein adiponectin critically depends on thiol-mediated protein retention. *Mol Cell Biol* 27: 3716-3731, 2007.
963. Wei J, Li H, Wang S, Li T, Fan J, Liang X, Li J, Han Q, Zhu L, Fan L, Zhao RC. let-7 enhances osteogenesis and bone formation while repressing adipogenesis of human stromal/mesenchymal stem cells by regulating HMGA2. *Stem Cells Dev* 23: 1452-1463, 2014.
964. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796-1808, 2003.
965. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 351: 987-997, 2004.
966. Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vazquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, Lopez M, Vidal-Puig A. BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. *Cell* 149: 871-885, 2012.
967. Wikstrom JD, Mahdavi K, Liesa M, Sereda SB, Si Y, Las G, Twigg G, Petrovic N, Zingaretti C, Graham A, Cinti S, Corkey BE, Cannon B, Nedergaard J, Shirihai OS. Hormone-induced mitochondrial fission is utilized by brown adipocytes as an amplification pathway for energy expenditure. *EMBO J* 33: 418-436, 2014.
968. Wilfred BR, Wang WX, Nelson PT. Energizing miRNA research: A review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol Genet Metab* 91: 209-217, 2007.
969. Wolf Y, Boura-Halfon S, Cortese N, Haimon Z, Sar Shalom H, Kuperman Y, Kalchenko V, Brandis A, David E, Segal-Hayoun Y, Chappell-Maor L, Yaron A, Jung S. Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. *Nat Immunol* 18: 665-674, 2017.
970. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. *Biochem Biophys Res Commun* 384: 105-109, 2009.
971. Writing Group M, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenland K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J. American Heart Association Statistics C. Stroke Statistics S. Heart disease and stroke statistics—2010 update: A report from the American Heart Association. *Circulation* 121: e46-e215, 2010.
972. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 332: 243-247, 2011.
973. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150: 366-376, 2012.
974. Wu X, Ge H, Lemon B, Vonderfecht S, Weiszmam J, Hecht R, Gupte J, Hager T, Wang Z, Lindberg R, Li Y. FGF19-induced hepatocyte proliferation is mediated through FGFR4 activation. *J Biol Chem* 285: 5165-5170, 2010.
975. Wu Y, Zuo J, Zhang Y, Xie Y, Hu F, Chen L, Liu B, Liu F. Identification of miR-106b-93 as a negative regulator of brown adipocyte differentiation. *Biochem Biophys Res Commun* 438: 575-580, 2013.
976. Xia Z, Sniderman AD, Cianflone K. Acylation-stimulating protein (ASP) deficiency induces obesity resistance and increased energy expenditure in ob/ob mice. *J Biol Chem* 277: 45874-45879, 2002.
977. Xia Z, Stanhope KL, Digitale E, Simion OM, Chen L, Havel P, Cianflone K. Acylation-stimulating protein (ASP)/complement C3adesArg

- deficiency results in increased energy expenditure in mice. *J Biol Chem* 279: 4051-4057, 2004.
978. Xu G, Ji C, Song G, Zhao C, Shi C, Song L, Chen L, Yang L, Huang F, Pang L, Zhang N, Zhao Y, Guo X. MiR-26b modulates insulin sensitivity in adipocytes by interrupting the PTEN/PI3K/AKT pathway. *Int J Obes (Lond)* 39: 1523-1530, 2015.
 979. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821-1830, 2003.
 980. Xu H, Sethi JK, Hotamisligil GS. Transmembrane tumor necrosis factor (TNF)-alpha inhibits adipocyte differentiation by selectively activating TNF receptor 1. *J Biol Chem* 274: 26287-26295, 1999.
 981. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW, Jr. Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. *Cell Metab* 18: 816-830, 2013.
 982. Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, Zou F, Gent LM, Hahner LD, Khan SA, Elias CF, Elmquist JK, Clegg DJ. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. *Cell Metab* 14: 453-465, 2011.
 983. Xu Z, Yu S, Hsu CH, Eguchi J, Rosen ED. The orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II is a critical regulator of adipogenesis. *Proc Natl Acad Sci U S A* 105: 2421-2426, 2008.
 984. Xue Y, Petrovic N, Cao R, Larsson O, Lim S, Chen S, Feldmann HM, Liang Z, Zhu Z, Nedergaard J, Cannon B, Cao Y. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. *Cell Metab* 9: 99-109, 2009.
 985. Yadav H, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, Zerfas P, Zhigang D, Wright EC, Stuelten C, Sun P, Lonning S, Skarulis M, Sumner AE, Finkel T, Rane SG. Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. *Cell Metab* 14: 67-79, 2011.
 986. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423: 762-769, 2003.
 987. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7: 941-946, 2001.
 988. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 13: 332-339, 2007.
 989. Yamauchi T, Oike Y, Kamon J, Waki H, Komeda K, Tsuchida A, Date Y, Li MX, Miki H, Akanuma Y, Nagai R, Kimura S, Saheki T, Nakazato M, Naitoh T, Yamamura K, Kadowaki T. Increased insulin sensitivity despite lipodystrophy in Crebbp heterozygous mice. *Nat Genet* 30: 221-226, 2002.
 990. Yang L, Shi CM, Chen L, Pang LX, Xu GF, Gu N, Zhu LJ, Guo XR, Ni YH, Ji CB. The biological effects of hsa-miR-1908 in human adipocytes. *Mol Biol Rep* 42: 927-935, 2015.
 991. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356-362, 2005.
 992. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC, Gong DW. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab* 290: E1253-E1261, 2006.
 993. Yang SH, Liu R, Perez EJ, Wen Y, Stevens SM, Jr., Valencia T, Brun-Zinkernagel AM, Prokai L, Will Y, Dykens J, Koulen P, Simpkins JW. Mitochondrial localization of estrogen receptor beta. *Proc Natl Acad Sci U S A* 101: 4130-4135, 2004.
 994. Yang X, Enerback S, Smith U. Reduced expression of FOXC2 and brown adipogenic genes in human subjects with insulin resistance. *Obes Res* 11: 1182-1191, 2003.
 995. Ye L, Wu J, Cohen P, Kazak L, Khandekar MJ, Jedrychowski MP, Zeng X, Gygi SP, Spiegelman BM. Fat cells directly sense temperature to activate thermogenesis. *Proc Natl Acad Sci U S A* 110: 12480-12485, 2013.
 996. Yin H, Pasut A, Soleimani VD, Bentzinger CF, Antoun G, Thorn S, Seale P, Fernando P, van Ijcken W, Grosveld F, Dekemp RA, Boushel R, Harper ME, Rudnicki MA. MicroRNA-133 controls brown adipose determination in skeletal muscle satellite cells by targeting Prdm16. *Cell Metab* 17: 210-224, 2013.
 997. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, Ofrecio JM, Wollam J, Hernandez-Carretero A, Fu W, Li P, Olefsky JM. Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity. *Cell* 171: 372-384 e312, 2017.
 998. Yoo EJ, Chung JJ, Choe SS, Kim KH, Kim JB. Down-regulation of histone deacetylases stimulates adipocyte differentiation. *J Biol Chem* 281: 6608-6615, 2006.
 999. Yoshida H, Kanamori Y, Asano H, Hashimoto O, Murakami M, Kawada T, Matsui T, Funaba M. Regulation of brown adipogenesis by the Tgf-beta family: Involvement of Srebp1c in Tgf-beta- and Activin-induced inhibition of adipogenesis. *Biochim Biophys Acta* 1830: 5027-5035, 2013.
 1000. Young P, Arch JR, Ashwell M. Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett* 167: 10-14, 1984.
 1001. Yu C, Markan K, Temple KA, Deplewski D, Brady MJ, Cohen RN. The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor gamma transcriptional activity and repress 3T3-L1 adipogenesis. *J Biol Chem* 280: 13600-13605, 2005.
 1002. Yu H, He K, Wang L, Hu J, Gu J, Zhou C, Lu R, Jin Y. Stk40 represses adipogenesis through translational control of CCAAT/enhancer-binding proteins. *J Cell Sci* 128: 2881-2890, 2015.
 1003. Zamani N, Brown CW. Emerging roles for the transforming growth factor- β superfamily in regulating adiposity and energy expenditure. *Endocr Rev* 32: 387-403, 2011.
 1004. Zamboni M, Armellini F, Milani MP, De Marchi M, Todesco T, Robbi R, Bergamo-Andreis IA, Bosello O. Body fat distribution in pre- and post-menopausal women: metabolic and anthropometric variables and their inter-relationships. *Int J Obes Relat Metab Disord* 16: 495-504, 1992.
 1005. Zancanaro C, Sbarbati A, Morroni M, Carraro R, Cigolini M, Enzi G, Cinti S. Multiple symmetric lipomatosis. Ultrastructural investigation of the tissue and preadipocytes in primary culture. *Lab Invest* 63: 253-258, 1990.
 1006. Zaragosi LE, Wdziekonski B, Villageois P, Keophiphath M, Maumus M, Tchoknia T, Bourlier V, Mohsen-Kanson T, Ladoux A, Elabd C, Scheideler M, Trajanoski Z, Takashima Y, Amri EZ, Lacasa D, Sengenès C, Ailhaud G, Clement K, Bouloumie A, Kirkland JL, Dani C. Activin plays a critical role in proliferation and differentiation of human adipose progenitors. *Diabetes* 59: 2513-2521, 2010.
 1007. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 13: 952-961, 2007.
 1008. Zhang H, Guan M, Townsend KL, Huang TL, An D, Yan X, Xue R, Schulz TJ, Winnay J, Mori M, Hirshman MF, Kristiansen K, Tsang JS, White AP, Cypess AM, Goodyear LJ, Tseng YH. MicroRNA-455 regulates brown adipogenesis via a novel HIF1an-AMPK-PGC1alpha signaling network. *EMBO Rep* 16: 1378-1393, 2015.
 1009. Zhang J, Fu M, Cui T, Xiong C, Xu K, Zhong W, Xiao Y, Floyd D, Liang J, Li E, Song Q, Chen YE. Selective disruption of PPARgamma 2 impairs the development of adipose tissue and insulin sensitivity. *Proc Natl Acad Sci U S A* 101: 10703-10708, 2004.
 1010. Zhang R, Wang D, Xia Z, Chen C, Cheng P, Xie H, Luo X. The role of microRNAs in adipocyte differentiation. *Front Med* 7: 223-230, 2013.
 1011. Zhang W, Shu C, Li Q, Li M, Li X. Adiponectin affects vascular smooth muscle cell proliferation and apoptosis through modulation of the mitofusin-2-mediated Ras-Raf-Erk1/2 signaling pathway. *Mol Med Rep* 12: 4703-4707, 2015.
 1012. Zhang Y, Matheny M, Zolotukhin S, Tumer N, Scarpace PJ. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: Influence of beta3-adrenergic agonists, retinoic acid, leptin and fasting. *Biochim Biophys Acta* 1584: 115-122, 2002.
 1013. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432, 1994.
 1014. Zhao X, Yang Y, Sun BF, Shi Y, Yang X, Xiao W, Hao YJ, Ping XL, Chen YS, Wang WJ, Jin KX, Wang X, Huang CM, Fu Y, Ge XM, Song SH, Jeong HS, Yanagisawa H, Niu Y, Jia GF, Wu W, Tong WM, Okamoto A, He C, Rendtlew Danielsen JM, Wang XJ, Yang YG. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. *Cell Res* 24: 1403-1419, 2014.
 1015. Zhao Y, Zhang H. Update on the mechanisms of homing of adipose tissue-derived stem cells. *Cytotherapy* 18: 816-827, 2016.
 1016. Zhou L, Park SY, Xu L, Xia X, Ye J, Su L, Jeong KH, Hur JH, Oh H, Tamori Y, Zingaretti CM, Cinti S, Argente J, Yu M, Wu L,

- Ju S, Guan F, Yang H, Choi CS, Savage DB, Li P. Insulin resistance and white adipose tissue inflammation are uncoupled in energetically challenged Fsp27-deficient mice. *Nat Commun* 6: 5949, 2015.
1017. Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. *Biochem Biophys Res Commun* 432: 612-617, 2013.
1018. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 414: 782-787, 2001.
1019. Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* 23: 3113-3120, 2009.
1020. Zolotov S, Xing C, Mahamid R, Shalata A, Sheikh-Ahmad M, Garg A. Homozygous LIPE mutation in siblings with multiple symmetric lipomatosis, partial lipodystrophy, and myopathy. *Am J Med Genet A* 173: 190-194, 2017.
1021. Zovein AC, Hofmann JJ, Lynch M, French WJ, Turlo KA, Yang Y, Becker MS, Zanetta L, Dejana E, Gasson JC, Tallquist MD, Iruela-Arispe ML. Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell* 3: 625-636, 2008.