*Cinti S*[1]

# Exercise and the Adipose Organ

## *Training und das adipöse Organ*

REVIEW

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1. UNIVERSITY OF ANCONA, *Dpt Experimental and Clinical Medicine Center of Obesity, Ancona, Italy*

### *Summary*

- **Most of white and brown adipocytes,** in spite of their different functions – storing energy and thermogenesis – are contained together in subcutaneous and visceral depots. The reason for this mixture lies in the fact that adipocytes have plastic properties allowing each to convert to the other: under chronic cold exposure, white convert into brown to support the need for thermogenesis and under obesogenic diet, brown convert into white to satisfy the need for energy storing.
- **The white-brown transdifferentiation** is of medical interest because the browning is associated with obesity resistance and drugs inducing the browning phenomenon curb obesity and related disorders.
- **Type2 diabetes** is the most common disorder associated with visceral obesity. Macrophages infiltrating the obese adipose organ are responsible for the low-grade chronic inflammation dealing to insulin resistance and T2 diabetes. Macrophages form characteristic histopathology figures: crown-like structures (CLS) due to the need for removal of debris deriving from the death of adipocytes. Death of adipocytes is related to their hypertrophy up to the critical death size. Visceral adipocytes have a smaller critical death size, thus offering an explanation for the higher inflammation and morbidity of visceral fat. Physical exercise induces both useful changes of adipose organ: size reduction of adipocytes and browning.
- **Exercise-induced browning** is due to several mechanisms including increased density of noradrenergic fibres in white adipose tissue and to factors produced by skeletal muscles and adipose tissue.

**KEY WORDS:**
Adipose Organ, White Adipose Tissue, Brown Adipose Tissue, Adipocyte, Transdifferentiation, Physical Exercise

### *Zusammenfassung*

- **Die meisten weißen und braunen Fettzellen** sind trotz ihrer verschiedener Funktionen – Energiespeicherung und Thermogenese – gemeinsam in den subkutanen und viszeralen Depots enthalten. Die Ursache liegt darin, dass Fettzellen plastische Eigenschaften haben, die es ihnen ermöglichen, sich ineinander zu verwandeln: unter chronischer Kältebehandlung werden weißen zu braune Fettzellen, um die Thermogenese zu unterstützen. Unter einer adipogenen Diät werden braune zu weißen Fettzellen, um Energie zu speichern.
- **Die weiß-braune Transdifferenzierung** ist von medizinischem Interesse, da das „Browning" mit Adipositasresistenz zusammenhängt. Medikamente, die das „Browning" induzieren, wirken Adipositas und metabolischen Faktoren entgegen.
- **Diabetes Typ II** ist die am häufigsten auftretende Störung, die mit viszeraler Adipositas in Verbindung gebracht wird. Makrophagen infiltrieren das fettleibige adipöse Organ und sind verantwortlich für niedrig-persistierende Entzündungen, die zu Insulinresistenz und Diabetes Typ II führen. Makrophagen weisen charakteristische histopathologische Zeichen auf: Sie bilden „Crown Like Structures" (CLS), um die Ablagerungen abzubauen, die durch das Absterben von Fettzellen zustande kommen. Das Absterben von Fettzellen wird induziert durch eine Hypertrophie bis zu einer „tödlichen Größe", die dann den Zelltod bewirkt. Bei viszeralen Fettzellen ist die „tödliche Organgröße" kleiner als bei anderen Fettzellen. Dies erklärt die höhere Inflammation und Sterberate des viszeralen Fettes offenbart. Körperliche Aktivität ermöglicht zwei nützliche Anpassungen des adipösen Organs: zum einem das Schrumpfen der Größe, zum anderen das „Browning".
- **In weißem Fettgewebe** führt körperliches Training zu einem „Browning" und zusätzlich findet sich dort eine erhöhte Dichte noradrenergener Fasern in weißem Fettgewebe und entsprechende Zytokine, die aus Skelettmuskel und Fettgewebe stammen.

**SCHLÜSSELWÖRTER:**
Adipöses Organ, weißes Fettgewebe, braunes Fettgewebe, Fettzelle, Transdifferenzierung, körperliche Aktivität

## Some Shared but Many Differing Features of White and Brown Adipocytes

In mammals cells with a relevant amount of lipids in their cytoplasm, in physiologic conditions, are defined adipocytes. Thus, the term adipocyte is just descriptive of one of the structural aspects of these cells and it is completely unlinked to their functional role.

Two types of adipocytes are widely recognized: white and brown. These two cell types have a very different morphology and physiology (13).

White adipocytes are large spherical cells with a single cytoplasmic vacuole that predominate the structural organization of this cell (therefore ›

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**CORRESPONDING ADDRESS:**
Prof. Saverio Cinti, MD
Director Center Obesity
School of Medicine, University of Ancona
(Università Politecnica delle Marche)
Via Tronto 10a, 60020 Ancona, Italy
✉: cinti@univpm.it

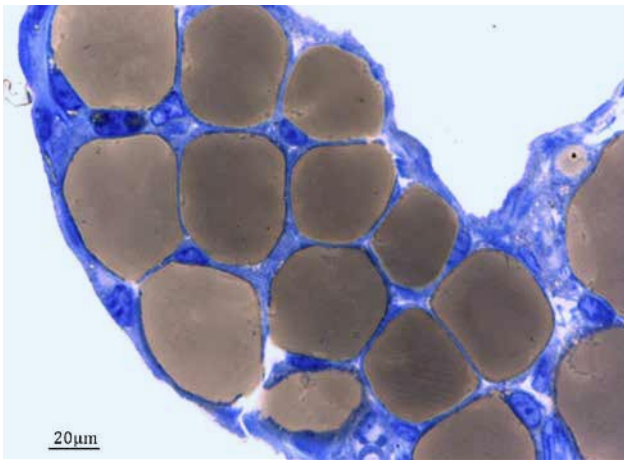


Figure 1

White adipose tissue. Light microscopy of resin embedded tissue. Blue of toluidine staining.

unilocular adipocyte is also used to define white adipocytes). About 90% of its volume is formed by this vacuole composed by triacylglycerides. The rest of the cell is formed by a crescent shaped nucleus squeezed by the lipid droplet and a very thin cytoplasmic rim surrounding the central vacuole (Fig. 1). Further organelles are recognizable in the cytoplasm of white adipocytes, but they are usually small: mitochondria, rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi complex, lysosomes, peroxisomes and pinocytotic vesicles. Each white adipocyte is surrounded by an external lamina mainly composed by collagen IV. The structure of this external lamina (sometimes described also as basal lamina) is formed by an internal part with a fine network of microfibrils and an external part in which sparse collagen fibrils are visible by both transmission and scanning electron microscope (33).

Brown adipocytes are polygonal cells with a size that is about 1/3-1/2 that of white adipocytes. The nucleus is roundish and often located in the central part of the cell (Fig. 2). Cytoplasmic lipids are contained into several small vacuoles (multilocular adipocyte). Mitochondria are numerous, large and packed with cristae. Other organelles are usually poorly developed (Fig. 3). An external lamina similar to that described above is also present on the external surface of brown adipocytes (12).

Thus, white and brown adipocytes display a different anatomy allowing their different physiology. White adipocytes store lipids in order to allow intervals between meals. It should be outlined that this function was of pivotal importance for millions of years when the fasting intervals could last for weeks. This could explain the positive gene selection for white adipocytes.

Brown adipocytes burn lipids to generate heat. Body temperature must remain constant at 37°C in spite of the very variable external temperatures in the environment where humans live: from -50°C to +50°C. Thus, the normal temperature is quite closer to the upper limit than the lower one and therefore thermogenesis is a very important physiologic automatic reaction of the body when exposed to temperatures below thermo neutrality (38). Cold receptors of skin activate afferent nerves able to activate hypothalamic neurons of sympathetic nervous system that directly activate, trough synaptoid-adipocyte noradrenergic junctions, brown adipocytes (4, 9). Gap junctions between brown adipocytes couple electrically these cells and diffuse the adrenergic stimulus to promote heat production via beta-oxidation of fatty acids in these cells. Mitochondria of brown adipocytes are uncoupled due to the presence of the unique uncoupling protein 1 (UCP1), thus the only byproduct derived from fatty acids burning in these cells is heat (8, 55).

Thus, these two cell types, in spite of their evident differences in anatomy and physiology, share the fact that they both contain relevant amount of cytoplasmic lipids and that these lipids are used to satisfy important functional needs for survival of the organism (short term homeostasis).

## Orchestration of Specialized Adipocytes into White and Brown Adipose Tissue

Anatomical studies showed that white and brown adipocytes are contained into dissectible structures located under the skin (subcutaneous depots) or into the trunk (visceral depots, see Fig. 4) (25).

These depots are composed mainly by adipocytes and can be considered all together as a diffuse large organ (adipose organ) in which the parenchymal cell is the adipocyte.

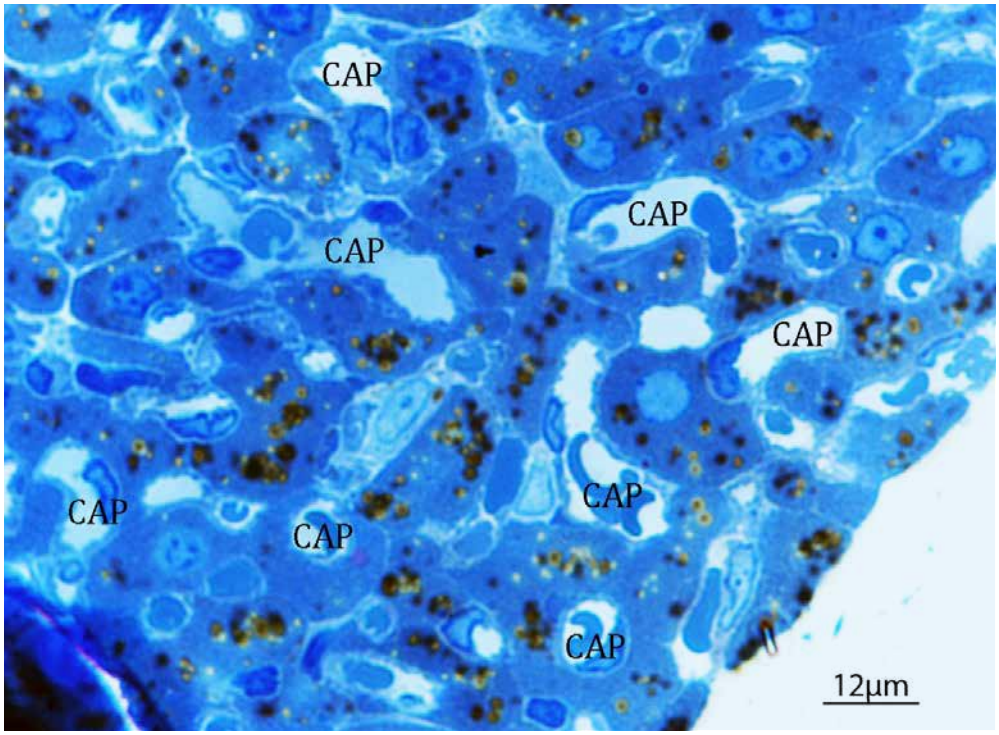


Figure 2

Brown adipose tissue (rat). Light microscopy of resin embedded tissue. Toluidin blue staining. CAP=capillary (some indicated).

The color of this organ is white in the areas mainly containing white adipocytes (white adipose tissue: WAT) and brown in the areas containing mainly brown adipocytes (brown adipose tissue: BAT), thus the name of adipocytes derives from the color they give to the organ.

WAT and BAT have different vascular and nerve supply. The capillary network is about six times denser in BAT than in WAT (51). This high density of vascular network in BAT support the need for both high rate of metabolic activity and for a rapid transport of heat to the rest of the body.

Nerves are mainly represented by noradrenergic parenchymal fibers that are much more represented in BAT than in WAT (30, 31, 32).

Detailed morphometric studies showed that most depots in adult mice of different strains are mixed, i.e.: composed by a mixture of WAT and BAT (47, 49, 62). In brief BAT is prevalent in the areas located near aorta and its main branches and, in mice, in the interscapular area.

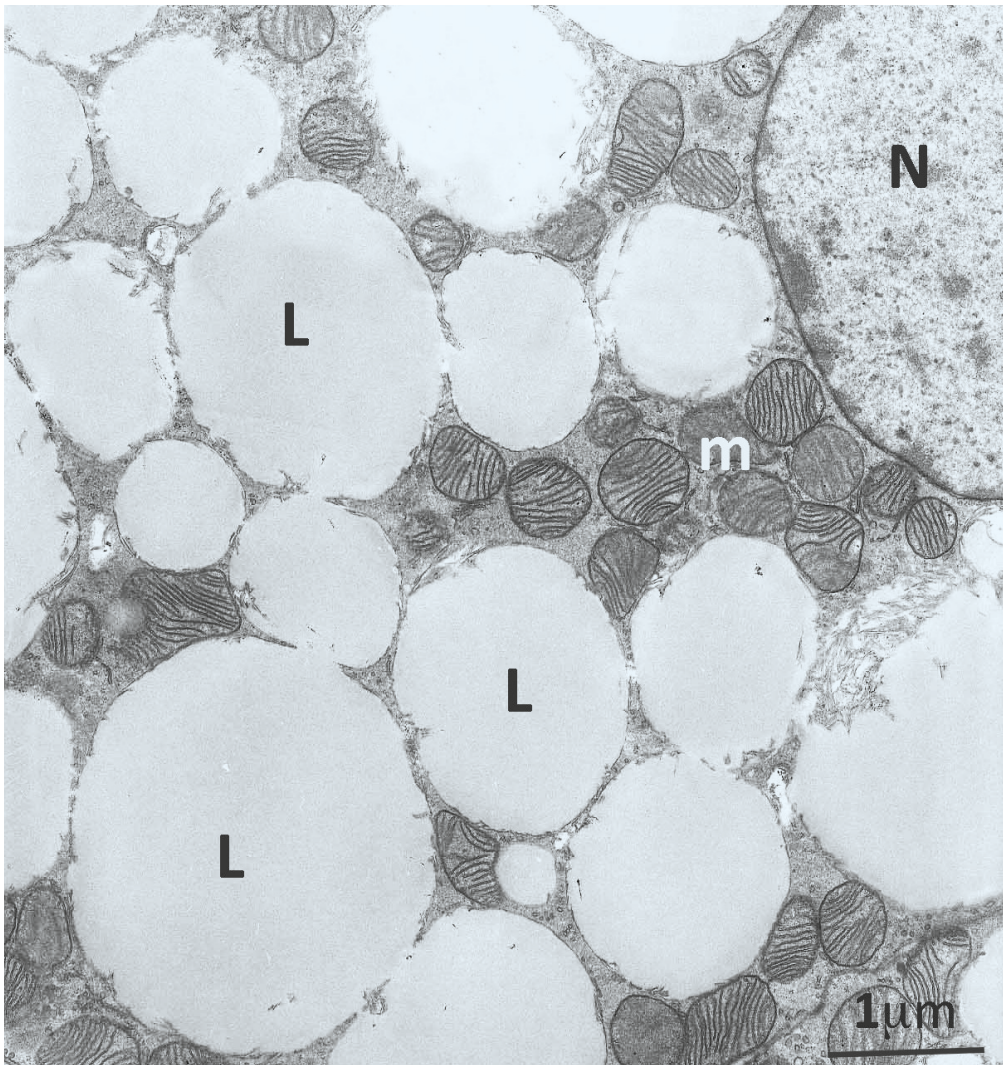


Figure 3

Brown adipose tissue (rat). Electron microscopy showing details of organelles. m mitochondria, L lipid droplets, N nucleus.

The need for a rapid and diffuse dispersion of the heat to whole organism offers a finalistic explanation to this location of BAT. In human newborns the distribution of BAT is similar to that of mice above described, but adult humans lack the interscapular BAT and small amounts of metabolically active BAT is found in the supraclavicular area (in close contact with subclavian arteries) and in the perirenal region (21, 58, 60, 61, 68).

The rest of the organ is mainly composed by WAT. Thus, depots located quite far from aorta and its main branches such as posterior subcutaneous depot, perivescical and epididymal depots are almost pure WAT.

## The Adipose Organ Is Plastic

This mixture of WAT and BAT in this organ is an important feature because it should imply a physiologic rationale given the fact that these two tissues differ so clearly in their functional role.

We found a possible explanation in the plasticity of this organ (18, 34). When chronically exposed to adrenergic stimulus (cold, administration of beta-adrenergic agonists, in cases of noradrenaline secreting tumors) both in mice and humans the amount of BAT in the organ increase (3, 26, 35, 36, 39). Such increase is generally referred as browning (Fig. 4). On the other hand when the energy balance is positive, one of the possible mechanism to store the energy excess is to convert brown adipocytes into white adipocytes. Detailed studies from our group as well as other groups showed that the cytological mechanism of reversible browning is mainly due to direct conversion (transdifferentiation) of white adipocytes into brown adipocytes (3, 35, 39, 62) as we described earlier such conversions are visually characterized as paucilocular adipocytes (Fig. 5) (3). The most reliable technique able to demonstrate the conversion of a cell type into another cell type is the lineage tracing technique. Recent experiments using this technique confirmed our hypothesis (57) even if the proliferative/developmental mechanism has been proven to play a role (63).

Thus a mature cell in adult mammals can reprogram its genoma and reversibly transform into a cell with different functional role under physiologic stimuli (15, 16, 17).

This plasticity could have a therapeutic role in the next future because all data from rodents and humans suggest the beneficial effect of browning to curb obesity and related disorders (1, 11, 27, 28, 37, 40, 42, 56, 59).

## Physical Exercise Serves as a Driving Force of White-To-Brown Conversion

Cold exposure is not the only way to obtain white-to-brown conversion. Physical exercise, particularly a special kind of physical exercise, in enriched environment, induces an increase in ›

hypothalamic BDNF and activates the sympathetic nervous system, is able to induce browning (10, 23). Furthermore, a recently discovered hormone called irisin, which is produced by murine and human skeletal muscles during physical exercise, has been proven to be a potent inducer of white to brown conversion both in vitro and in vivo, with positive metabolic consequences and weight reduction in diet-induced obese mice (7).

Lee et al. found that muscle-related shivering contraction is also an important stimulus for irisin secretion in humans, but some recently published data seems to questioning the real possibility that irisin could be an interesting therapeutical tool (41, 54). Interestingly we recently found a new potential role of iris on bone cortical mass (20).

Very recently another factor produced by exercised skeletal muscle and cold exposed adipocytes have been discovered: meteorin-like. Meteorin-like factor induce browning trough an indirect action on inflammatory cells (53).

Physical exercise seems to be involved in the secretion of heart hormones called atrial and ventricular natriuretic peptides (ANPs and BNPs) (43, 44, 45). The main stimulus for their secretion is stretching of cardiac muscle (24). These hormones act on the kidneys and adrenal glands to maintain homeostasis of body fluids; they are also vasodilators and are "cardioprotective". They signal through specific clearance (NPRC) and activation receptors (NPRA), implying that PKG signaling shares substrates such as p38MAPK with PKA signaling (66). This kinase is an important transcription regulator of UCP1 downstream in the adrenergic signaling cascade, which allows synergic action of NP with adrenergic stimuli. Cold exposure also increases ANP secretion by the heart. Mice lacking NPRC, the negative regulator of NP activity, have inguinal WAT displaying some browning and have increased expression of thermogenic genes in both WAT and BAT (6, 65).

Importantly, activation of the natriuretic system physiologically counteracts the negative effects of catecholamines on the cardiovascular system. Thus, drugs that act on the above mentioned target molecules, such as irisin, meteorin-ilke NPRA or NPRC antagonists, could represent alternative strategies to induce browning of the adipose organ and avoid the activation of the sympathetic nervous system and its negative collateral effects on the cardiovascular system.

## Histopathology of the Obese Adipose Organ

In 2003, two independent laboratories showed that the adipose tissue of obese animals and humans is infiltrated by macrophages, inducing a chronic low-grade inflammation. They also showed that macrophage infiltration is positively correlated to the size of adipocytes and is coincident with the appearance of insulin resistance. Furthermore, they showed that most cytokines involved with insulin resistance are produced by cells in the stroma-vascular fraction (SVF) of adipose tissue, including macrophages, and not in the mature adipocytes (64, 67). We found that over 90% of active MAC2-immunoreactive macrophages surround dead adipocytes both in lean and obese mice, forming characteristic structures called "crown-like structures" (CLSs) (14, 48, 50). Their density is positively correlated to the size of adipocytes, independently of obesity status because hormone sensitive lipase (HSL) knockdown mice are lean but have fat with hypertrophic adipocytes and their fat have the same CLS density than obese fat. Adipose tissue from humans showed similar features (14).

CLS density is positively correlated with adipocyte size in both the subcutaneous and visceral fat of obese mice. However, in fat with adipocytes of comparable size, CLS density is higher in visceral fat, suggesting that visceral adipocytes are more fragile and reach a critical size that triggers death, termed the "critical death size" (CDS), earlier than subcutaneous adipocytes (14, 16, 19, 33, 48). These data offer an explanation to the well known clinical notion that visceral obesity is more often associated with other disorders (mainly T2 diabetes) that subcutaneous obesity (5).

Interestingly, physical exercise induce a size reduction of both visceral and subcutaneous adipocytes, thus, considering the above described data, it should have anti-inflammatory consequences on fat.

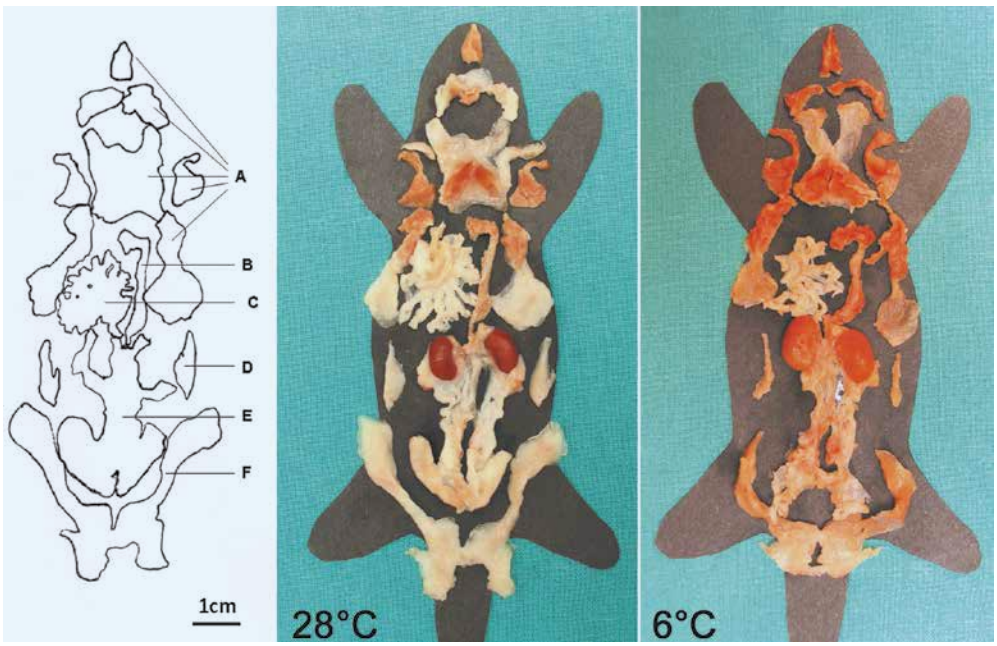


Figure 4

Gross anatomy of the adipose organ of adult female Sv129 mice maintained at 28°C and 6°C for ten days, this last condition is a visually evident example of cold-induced browning of the adipose organ; from (47) with permission.
A anterior subcutaneous depot including: interscapular, subscapular and axillary areas, mainly composed by brown adipose tissue even at 28°C; B mediastinal-periaortic visceral depot, mainly composed by brown adipose tissue even at 28°C; C mesenteric visceral depot, mainly composed by white adipose tissue at 28°C; D retroperitoneal visceral depot, mainly composed by white adipose tissue at 28°C; E abdomino-pelvic visceral depot including perirenal, periovaric, parametrial and perivescical areas, mainly composed by white adipose tissue at 28°C;F posterior subcutaneous depot including: dorso-lumbar, inguinal and gluteal parts, mainly composed by white adipose tissue at 28°C.

## Conclusions

New insights regarding the plasticity of adipocytes and

the adipose organ have been gained in the last few years. White and brown adipocytes can reciprocally convert each other under appropriate stimuli to better satisfy important physiological needs of the organism, including thermogenesis and energy storage. This is not the only example of adipocyte plasticity in the adipose organ because we also showed the ability of white adipocytes of mammary glands to convert reversibly into milk-producing glandular cells during pregnancy and lactation (22, 46, 52). The "browning" effects of the adipose organ can be useful to combat metabolic syndrome, not only because brown adipocytes are more "healthy" than white adipocytes, but also because the simple size reduction of white adipocytes that characterizes the first steps of transdifferentiation can be useful in determining how to avoid triggering of death based on critical size and the consequent chronic low-grade inflammation due to macrophage infiltration. Physical exercise together with cold exposure are both important to trigger healthy plasticity of adipose organ.

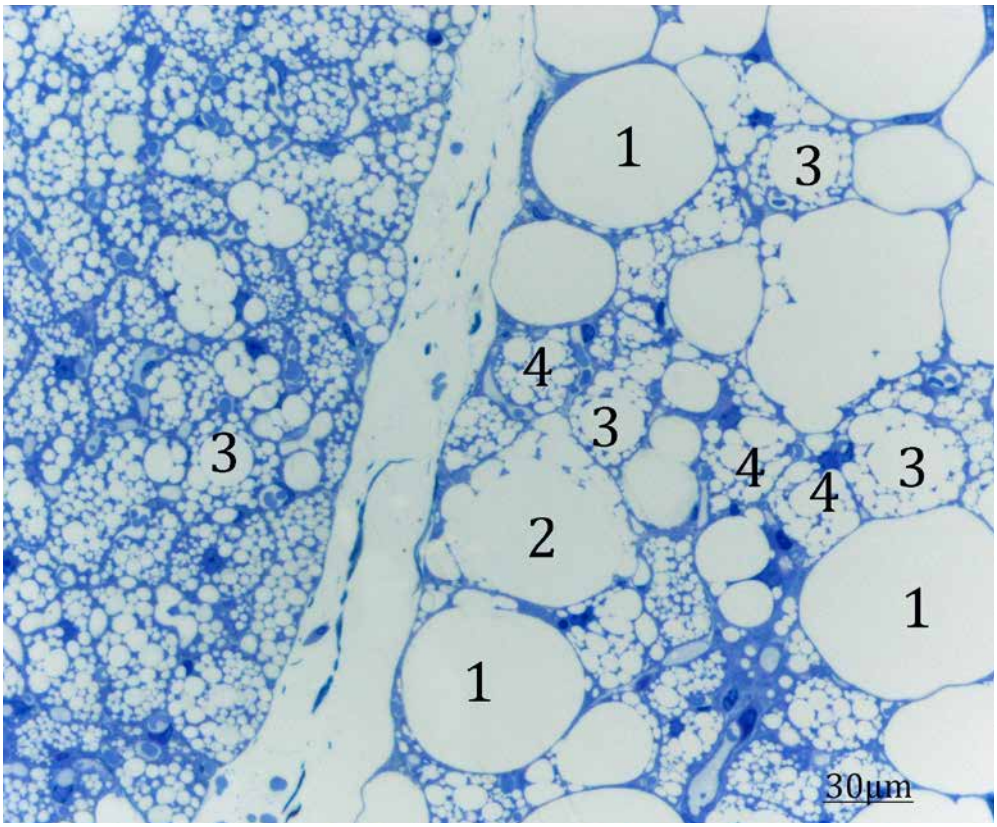


**Figure 5**

Mixed area in anterior subcutaneous depot of adult mouse cold (6°C) exposed for 6 days. Light microscopy of resin embedded tissue. Toludin blue staining. Brown adipocytes are mainly in the left part of the figure. The right part show white (1) and brown adipocytes (4). 2 and 3 are intermediate forms, these intermediate cells increase during browning and support a phenomenon of white into brown adipocyte direct interconversion (browning).

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## References

(1) **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. 2002; 297: 843-845. doi:10.1126/science.1073160

(2) **BARBATELLI G, HEINZELMANN 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. 1994; 26: 667-676. doi:10.1016/0040-8166(94)90051-5

(3) **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. 2010; 298: E1244-E1253. doi:10.1152/ajpendo.00600.2009

(4) **BARTNESS TJ, SONG CK.** Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. J Lipid Res. 2007; 48: 1655-1672. doi:10.1194/jlr.R700006-JLR200

(5) **BJÖRNTORP P.** Metabolic abnormalities in visceral obesity. Ann Med. 1992; 24: 3-5. doi:10.3109/07853899209164137

(6) **BORDICCHIA M, LIU D, AMRI EZ, AILHAUD G, DESSÌ-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. 2012;122: 1022-1036. doi: 10.1172/JCI159701

(7) **BOSTRÖM P, WU J, JEDRYCHOWSKI MP, KORDE A, YE L, LO JC, RASBACH KA, BOSTRÖM EA, CHOI JH, LONG JZ, KAJIMURA S, ZINGARETTI MC, VIND BF, TU H, CINTI S, HØJLUND K, GYGI SP, SPIEGELMAN BM.** A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481: 463-468. doi: 10.1038/nature10777

(8) **CANNON B, HEDIN A, NEDERGAARD J.** Exclusive occurrence of thermogenin antigen in brown adipose tissue. FEBS Lett. 1982; 150: 129-132. doi:10.1016/0014-5793(82)81319-7

(9) **CANNON B, NEDERGAARD J.** Brown adipose tissue: function and physiological significance. Physiol Rev. 2004; 84: 277-359. doi:10.1152/physrev.00015.2003

(10) **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. 2011; 14: 324-338. doi:10.1016/j.cmet.2011.06.020

(11) **CASTEILLA L, PLANAT-BÉNARD V, COUSIN B, SILVESTRE JS, LAHARRAGUE P, CHARRIÈRE G, CARRIÈRE A, PÉNICAUD L.** Plasticity of adipose tissue: a promising therapeutic avenue in the treatment of cardiovascular and blood diseases? Arch Mal Coeur Vaiss. 2005; 98: 922-926.

(12) **CINTI S.** The Adipose Organ. Milan: Kurtis; 1999.

(13) **CINTI S.** The adipose organ. Prostaglandins Leukot Essent Fatty Acids. 2005; 73: 9-15. doi:10.1016/j.plefa.2005.04.010

(14) **CINTI S, MITCHELL G, BARBATELLI G, MURANO I, CERESI E, FALOIA E, WANG S, FORTIER M, GREENBERG AS, OBIN MS.** Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005; 46: 2347-2355. doi:10.1194/jlr.M500294-JLR200

(15) **CINTI S.** Transdifferentiation properties of adipocytes in the Adipose Organ. Am J Physiol Endocrinol Metab. 2009; 297: E977-986. doi:10.1152/ajpendo.00183.2009

(16) **CINTI S.** Reversible physiological transdifferentiation in the adipose organ. Proc Nutr Soc. 2009; 68: 340-349. doi:10.1017/S0029665109990140

(17) **CINTI S.** Between brown and white: novel aspects of adipocyte differentiation. Ann Med. 2011; 43: 104-115. doi:10.3109/07853890.2010.535557

(18) **CINTI S.** The adipose organ at a glance. Dis Model Mech. 2012; 5: 588-594. doi:10.1242/dmm.009662

(19) **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. 2014; 156: 304-316. doi:10.1016/j.cell.2013.12.021

(20) **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 M6, CINTI S, GRANO M.** The myokine irisin increases cortical bone mass. Proc Natl Acad Sci USA. 2015; 112: 12157-12162. doi:10.1073/pnas.1516622112

(21) **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. 2009; 360: 1509-1517. doi:10.1056/NEJMoa0810780

(22) **DE MATTEIS R, ZINGARETTI MC, MURANO I, VITALI A, FRONTINI A, GIANNULIS I, BARBATELLI G, MARCUCCI F, BORDICCHIA M, SARZANI R, RAVIOLA E, CINTI S.** In vivo physiological transdifferentiation of adult adipose cells. Stem Cells. 2009; 27: 2761-2768. doi:10.1002/stem.197

(23) **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. Nutrition, metabolism, and cardiovascular diseases. Nutr Metab Cardiovasc Dis. 2013; 23: 582-590. doi:10.1016/j.numecd.2012.01.013

(24) **DIETZ JR.** Mechanisms of atrial natriuretic peptide secretion from the atrium. Cardiovasc Res. 2005; 68: 8-17. doi:10.1016/j.cardiores.2005.06.008

(25) **FRONTINI A, CINTI S.** Distribution and development of brown adipocytes in the murine and human adipose organ. Cell Metab. 2010; 11: 253-256. doi:10.1016/j.cmet.2010.03.004

(26) **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. 2013; 1831: 950-959. doi:10.1016/j.bbalip.2013.02.005

(27) **GHORBANI M, HIMMS-HAGEN J.** Appearance of brown adipocytes in white adipose tissue during CL 316,243-induced reversal of obesity and diabetes in Zucker fa/fa rats. Int J Obes Relat Metab Disord. 1997; 21: 465-475.

(28) **GHORBANI M, HIMMS-HAGEN J.** Treatment with CL 316,243, a beta 3-adrenoceptor agonist, reduces serum leptin in rats with diet- or aging-associated obesity, but not in Zucker rats with genetic (fa/fa) obesity. Int J Obes Relat Metab Disord. 1998; 22: 63-65.

(29) **GIORDANO A, NISOLI E, TONELLO C, CANCELLO 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. 2000; 487: 171-175. doi:10.1016/S0014-5793(00)02217-1

(30) **GIORDANO A, FRONTINI A, CASTELLUCCI M, CINTI S.** Presence and distribution of cholinergic nerves in rat mediastinal brown adipose tissue. J Histochem Cytochem. 2004; 52: 923-930.

(31) **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. 2005; 53: 679-687.

(32) **GIORDANO A, FRONTINI A, CINTI S.** Adipose organ nerves revealed by immunohistochemistry. Methods Mol Biol. 2008; 456: 83-95. doi:10.1007/978-1-59745-245-8_6

(33) **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. 2013; 54: 2423-2436. doi:10.1194/jlr.M038638

(34) **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; 170: R159-171. doi: 10.1530/EJE-13-0945

(35) **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. 2005; 289: E608-E616. doi:10.1152/ajpendo.00009.2005

(36) **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. 1998; 102: 412-420. doi:10.1172/JCI3155

(37) **HIMMS-HAGEN J.** Defective brown adipose tissue thermogenesis in obese mice. Int J Obes. 1985; 9: 17-24.

(38) **HIMMS-HAGEN J.** Brown adipose tissue and cold-acclimation. In: Trayhurn P, Nicholls aD, eds. Brown adipose tissue. London: Edward Arnold; 1986: 214.

(39) **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. 2000; 279: C670-C681.

(40) **KOPECKY J, HODNY Z, ROSSMEISL M, SYROVY I, KOZAK LP.** Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. Am J Physiol. 1996; 270: E768-E775.

(41) **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. 2014; 19: 302-309. doi:10.1016/j.cmet.2013.12.017

(42) **LOWELL BB, S-SUSULIC V, 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. 1993; 366: 740-742.

(43) **MORO C, CRAMPES F, SENGENES C, DE GLISEZINSKI I, GALITZKY J, THALAMAS C, LAFONTAN M, BERLAN M.** Atrial natriuretic peptide contributes to physiological control of lipid mobilization in humans. FASEB J. 2004; 18: 908-910.

(44) **MORO C, POLAK J, HEJNOVA J, KLIMCAKOVA E, CRAMPES F, STICH V, LAFONTAN M, BERLAN M.** Atrial natriuretic peptide stimulates lipid mobilization during repeated bouts of endurance exercise. Am J Physiol Endocrinol Metab. 2006; 290: E864-E869. doi:10.1152/ajpendo.00348.2005

(45) **MORO C, PILLARD F, DE GLISEZINSKI I, KLIMCAKOVA E, CRAMPES F, THALAMAS C, HARANT I, MARQUES MA, LAFONTAN M, BERLAN M.** Exercise-induced lipid mobilization in subcutaneous adipose tissue is mainly related to natriuretic peptides in overweight men. Am J Physiol Endocrinol Metab. 2008; 295: E505-E513. doi:10.1152/ajpendo.90227.2008

(46) **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 USA. 2004; 101: 16801-16806. doi:10.1073/pnas.0407647101

(47) **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. 2005; 1: 121-130.

(48) **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. 2008; 49: 1562-1568. doi:10.1194/jlr.M800019-JLR200

(49) **MURANO I, BARBATELLI G, GIORDANO A, CINTI S.** Noradrenergic parenchymal nerve fiber branching after cold acclimatisation correlates with brown adipocyte density in mouse adipose organ. J Anat. 2009; 214: 171-178. doi:10.1111/j.1469-7580.2008.01001.x

(50) **MURANO I, RUTKOWSKI JM, WANG QA, CHO YR, SCHERER PE, CINTI S.** Time course of histomorphological changes in adipose tissue upon acute lipoatrophy. Nutrition, metabolism, and cardiovascular diseases. Nutr Metab Cardiovasc Dis. 2013; 23: 723-731. doi:10.1016/j.numecd.2012.03.005

(51) **NECHAD M.** Structure and development of brown adipose tissue in: Brown Adipose Tissue. Ed.: Paul Trayhurn and David Nicholls, Edward Arnold, London; 1986.

(52) **PROKESCH 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 adipoepithelial transdifferentiation in mouse mammary gland. Stem Cells. 2014; 32: 2756-2766. doi:10.1002/stem.1756

(53) **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 JAG, SPIEGELMAN BM.** Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell. 2014; 157: 1279-1291. doi:10.1016/j.cell.2014.03.065

(54) **RASCHKE S, ELSEN M, GASSENHUBER H, SOMMERFELD M, SCHWAHN U, BROCKMANN B, JUNG R, WISLØFF U, TJØNNA AE, RAASTAD T, HALLÉN J, NORHEIM F, DREVON CA, ROMACHO T, ECKARDT K, ECKEL J.** Evidence against a beneficial effect of irisin in humans. PLoS ONE. 2013; 8: e73680. doi:10.1371/journal.pone.0073680

(55) **RICQUIER D.** Molecular biology of brown adipose tissue. Proc Nutr Soc. 1989; 48: 183-187. doi:10.1079/PNS19890028

(56) **ROSEN ED, SPIEGELMAN BM.** What we talk about when we talk about fat. Cell. 2014; 156: 20-44. doi:10.1016/j.cell.2013.12.012

(57) **ROSENWALD M, PERDIKARI A, RULICKE T, WOLFRUM C.** Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol. 2013; 15: 659-667. doi:10.1038/ncb2740

(58) **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. 2009; 58: 1526-1531. doi:10.2337/db09-0530

(59) **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. 2011; 121: 96-105. doi:10.1172/JCI44271

(60) **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. 2009; 360: 1500-1508. doi:10.1056/NEJMoa0808718

(61) **VIRTANEN KA, LIDELL ME, ORAVA J, HEGLIND M, WESTERGREN R, NIEMI T, TAITTONEN M, LAINE J, SAVISTO NJ, ENERBÄCK S, NUUTILA P.** Functional brown adipose tissue in healthy adults. N Engl J Med. 2009; 360: 1518-1525. doi:10.1056/NEJMoa0808949

(62) **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. 2012; 53: 619-629. doi:10.1194/jlr.M018846

(63) **WANG QA, TAO C, GUPTA RK, SCHERER PE.** Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med. 2013; 19: 1338-1344. doi:10.1038/nm.3324

(64) **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. 2003; 112: 1796-1808. doi:10.1172/JCI200319246

(65) **WHITTLE AJ, VIDAL-PUIG A.** NPs - heart hormones that regulate brown fat? J Clin Invest. 2012; 122: 804-807. doi:10.1172/JCI62595

(66) **WOODARD GE, ROSADO JA.** Natriuretic peptides in vascular physiology and pathology. Int Rev Cell Mol Biol. 2008; 268: 59-93. doi:10.1016/S1937-6448(08)00803-4

(67) **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. 2003; 112: 1821-1830. doi:10.1172/JCI200319451

(68) **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. 2009; 23: 3113-3120. doi:10.1096/fj.09-133546