The origin of intermuscular adipose tissue and its pathophysiological implications

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Vettor R, Milan G, Franzin C, Sanna M, De Coppi P, Rizzuto R, Federspil G. The origin of intermuscular adipose tissue and its pathophysiological implications. Am J Physiol Endocrinol Metab 297: E987–E998, 2009. First published September 8, 2009; doi:10.1152/ajpendo.00229.2009.—The intermuscular adipose tissue (IMAT) is a depot of adipocytes located between muscle bundles. Several investigations have recently been carried out to define the phenotype, the functional characteristics, and the origin of the adipocytes present in this depot. Among the different mechanisms that could be responsible for the accumulation of fat in this site, the dysdifferentiation of muscle-derived stem cells or other mesenchymal progenitors has been postulated, turning them into cells with an adipocyte phenotype. In particular, muscle satellite cells (SCs), a heterogeneous stem cell population characterized by plasticity and self-renewal that allow muscular growth and regeneration, can acquire features of adipocytes, including the abilities to express adipocyte-specific genes and accumulate lipids. Failure to express the transcription factors that direct mesenchymal precursors into fully differentiated functionally specialized cells may be responsible for their phenotypic switch into the adipogenic lineage. We proved that human SCs also possess a clear adipogenic potential that could explain the presence of mature adipocytes within skeletal muscle. This occurs under some pathological conditions (i.e., primary myodystrophies, obesity, hyperglycemia, high plasma free fatty acids, hypoxia, etc.) or as a consequence of thiazolidinedione treatment or simply because of a sedentary lifestyle or during aging. Several pathways and factors (PPARs, WNT growth factors, myokines, GEF-GAP-Rho, p66shc, mitochondrial ROS production, PKCβ) could be implicated in the adipogenic conversion of SCs. The understanding of the molecular pathways that regulate muscle-to-fat conversion and SC behavior could explain the increase in IMAT depots that characterize many metabolic diseases and age-related sarcopenia.

muscle satellite cells; mesenchymal stem cells; myogenesis; adipogenesis

Intermuscular Adipose Tissue and its Pathophysiological Implications

In humans, the anatomic dissection provides a clear distinction into two different important compartments of adipose organ, the subcutaneous adipose tissue (SAT) and the visceral adipose tissue (VAT). Using the new highly sensitive imaging techniques, computed tomography scan, and magnetic resonance imaging (MRI), it is possible to detect and measure other adipose tissue depots that collectively contribute to total body adipose tissue (AT) (11, 125). AT is also present within many organs and tissues and in particular within skeletal muscle, where we can distinguish intermuscular AT (IMAT), paraseal AT, and perimuscular AT, which is not so easily distinguishable from the adjacent AT compartments (73). An absolute or relative VAT expansion has been associated with an increased risk of morbidity and mortality for cardiovascular diseases and metabolic diseases (13, 90). Nevertheless, important differences in the metabolic and functional properties among the different depots within the “VAT” compartment have been recognized. The IMAT compartment includes IMAT that is located between muscle groups and beneath the muscle fascia and intramuscular adipose tissue that is distributed within individual muscles visible on MRI images (42, 62). Recently, attention has focused on the content, localization, and composition of fat within skeletal muscle as determinants of insulin resistance. This fat depot includes most of the intramyocellular triglycerides, adipocytes present between muscle groups (intermuscular fat), and a smaller pool of adipocytes present between muscle fascicles (intramuscular adipocytes).

In morbidly obese patients, weight loss induced by biliary pancreatic diversion provoked a significant amelioration in insulin sensitivity with a parallel change in intramyocellular
but not perivascular or interfibrillar lipid accumulation (48). It is now quite clear that intramyocellular fat is due mostly to triglyceride accumulation within the muscle cell, whereas the perivascular or interfibrillar lipids correspond to adipose cells along the blood vessels and in the intermuscular space and between muscle fascicles (62). With MRI it is possible to define and localize IMAT, which can be found between the muscle bundles, and it is clearly separated from SAT by a well-defined fascia. There is a strong direct linear correlation between total adipose tissue and IMAT in men and women of different ethnic groups (42). A greater amount of IMAT was also seen in subjects with type 2 diabetes mellitus than in healthy controls (41) and in pathological conditions as in familial partial lipodystrophy (44) and in both men and women with the metabolic syndrome (47). More recently, it has been shown that IMAT deposit is greater in acromegaly despite the increased muscle mass, which suggests that increased AT in muscle could be associated with growth hormone-induced insulin resistance (38). Finally, an increment in IMAT has been shown in conditions characterized mostly by a primary decrease of the muscle mass as in primary myopathies (19, 106) during aging (63, 118) but also in sedentary young subjects (74). These changes in the regional body composition with an increase in the AT within muscle affect insulin sensitivity, glucose, and lipid metabolism identifying the phenotype of the normal-weight “metabolically obese” individuals (100).

Obese individuals with type 2 diabetes mellitus and peripheral neuropathy show an increased IMAT in leg skeletal muscles, and it has been suggested that this fact could explain the low calf muscle strength and power and impaired physical function (54). Femoral-gluteal SAT and femoral-gluteal IMAT distribution varies by sex and race, and these two components have independent and opposing relationships with cardiovascular disease risk factors (129). It is important to underline that lifestyle intervention as the increase in physical activity in overweight men with atherogenic dyslipidemia reduces the thigh IMAT, whereas it increases both HDL and LDL size, the two phenomena being significantly correlated. Reducing IMAT through aerobic exercise was more predictive of the changes in the serum lipid profile than was the reduction in VAT (36).

The presence of adipose tissue within skeletal muscle (IMAT) in addition to the increase in lipid content could play a crucial pathogenetic role and represents a negative prognostic factor for several myopathies, metabolic diseases, and aging. Therefore, investigating the origin and the biology of this adipose tissue depot is important because it could represent a promising pharmacological target.

### Muscle Satellite Stem Cells and the Origin of IMAT

Fat cells surrounding the muscle bundles could derive from different mesenchymal progenitors normally present in the adult skeletal muscle: mesenchymal stem cells, muscle-derived stem cells, or muscle satellite cells (SCs) (Fig. 1). After birth, muscle regeneration is mediated mostly by SCs, a unique population of committed stem cells located adjacent to the plasma membrane of myofibers and first described in 1961 by Mauro (75). These cells represent a heterogeneous population (7, 65, 131) of self-renewable stem cells (39, 66, 85) that are able to differentiate into different cell types (7, 27, 32, 115). In physiological conditions, SCs are quiescent in vivo (53, 108), but they can be activated by increased muscle work such as after-load-induced hypertrophy, prolonged exercise such as the resistance training, and in some pathological conditions such as myotraumas. When activated, SCs proliferate, migrate from the myofibers, and express specific myogenic markers, thus becoming muscle precursor cells (MPCs). MPCs are devoted to muscle repair; they fuse and form new multinucleated cells, allowing the complete repair of the injured muscle (83, 107, 130).

#### Muscle Satellite Stem Cells: Markers, Heterogeneity, and Plasticity

The most widely used marker of quiescent and activated SCs is represented by Pax7 (paired homeobox transcription factor 7) (112), a transcription factor belonging to helix-turn-helix paired-box family involved in muscle development. After ac-
tivation, SCs express a series of myogenic transcription factors (namely Myf5, MyoD, myogenin, and MRF4), defined as MRFs (myogenic regulatory factors), characterized by their helix-loop-helix conformation (Fig. 1). The first MRF in the myogenic cascade is Myf5 (myogenic factor 5), whose expression is detectable in ~90% of SCs (64) and which is involved in activation and proliferation of muscle precursors. MyoD (myogenic differentiation 1), which in vivo promotes muscle formation (122) and blocks proliferation (6), represents the master regulatory gene in the differentiation process of SCs. MyoD expression increases in vitro just before SC activation (91, 111, 112, 128) and in vivo during muscle regeneration (28, 40). Myogenin (also known as MyoG) plays an important role in the late phase of muscle differentiation during both development (95, 116) and regeneration (128). It posses a fundamental role in myotube and myofiber formation (16). Finally, MRF4 (also known as Myf6) plays the major role not only in the terminal differentiation of MPCs (96) but also in the rescue of early myogenesis in Myf5/MyoD double-mutant mice (60).

SCs express also nonmyogenic markers such as CD34 (cluster of differentiation 34), a surface glycoprotein that functions as cell-cell adhesion factor. The two isoforms generated from alternatively spliced transcripts, the truncated CD34 (CD34trunc) and the full-length protein (CD34full), are expressed by quiescent and activated SCs, respectively (84, 120). SCs appear to be a heterogeneous cell population. Differences on their morphology, proliferation rate, gene expression profile, and resistance to irradiation suggest that among SCs at least two subpopulations exist, one already committed to enter the myogenic lineage and the other basically devoted to the maintenance of the stem compartment. The two subpopulations of SCs, characterized by a high and a low proliferation rate, have been studied both in vitro (99) and in vivo (107). The “rapidly proliferating” SCs have been recognized to provide myonuclei to growing fibers, whereas SCs with much longer cell cycles probably function as cell reserves (107). Moreover, it has been observed that only high-proliferating SCs are able to fuse with differentiated myotubes and myofibers, whereas slow proliferating cells mainly fuse together, suggesting different roles in muscle growth (99).

It has been shown that SCs are able to enter alternative mesenchymal differentiation pathways and in particular toward adipogenic, osteogenic, and smooth muscle cell lineage (7, 32, 115). When plated under osteogenic conditions, such as the treatment with bone morphogenetic proteins (7) or with β-glycerophosphate, ascorbic acid phosphate, and dexamethasone (32), MPCs change their shape and start to express osteospecific genes, as assessed by cytochemical staining for detection of alkaline phosphatase (AP) and RT-PCR for osteocalcin expression. The coexpression of Pax7 and bone-specific AP has been detected in mononucleated cells located near the plasma membrane of myofibers from muscle biopsies of patients affected by Duchenne dystrophy (52). Moreover, primary human MPCs derived from a healthy woman express both myogenic and osteogenic-specific markers, and muscle stem cells are full of osteogenic differentiation (52).

In summary, SCs express well-known markers of staminality and of skeletal muscle progenitors. Moreover, they express specific markers involved in regulating their activation, proliferation, and differentiation toward fully differentiated adipocytes, osteocytes, and smooth muscle cells.

Adipogenic Differentiation of Muscle Satellite Cells

The ability of myogenic cells to differentiate toward adipogenic lineage was first described using myogenic cell lines as C2C12 myoblasts (49, 55, 56, 110, 124). More recently, it has been observed that this is confirmed also when primary myogenic progenitors are tested. SCs originating from a single myofiber obtained from rat muscles are able to undergo spontaneous adipogenesis (7), and similar findings have been confirmed using human SCs (Fig. 2) (32). Moreover, when cul-

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**Fig. 2.** Human SC (arrows) are quiescent and adherent to muscle fiber (A) and in appropriate culture conditions can proliferate and fuse to form myotubes (B; enlargement in inset). Morphological characterization of adipocytes derived from SC (C) compared with those derived from stromal-vascular fraction of human adipose tissue (D). C and D, insets: Oil Red O staining for lipid droplets. Adapted from De Coppi et al. (32).
tured in adipogenic medium, SCs undergo adipocyte differentiation along with the expression of specific adipogenic markers such as peroxisome proliferator-activated receptor (PPAR)γ2, leptin, and adiponectin. We also observed that the addition of rosiglitazone to the adipogenic cocktail increases the number of SCs that enter the adipogenic lineage, whereas rosiglitazone per se failed to induce the adipogenic differentiation of SCs (Fig. 3).

In a recent paper, Shefer et al. (115) reported the results of the clonal analysis of SCs as assessed by limiting dilution and confirmed once again that adult mouse SCs could spontaneously differentiate into adipocytes. Moreover, the clonal analysis concluded that myocytes and adipocytes originating from a single fiber in culture derive from distinct SCs (7, 115). In fact, in the progeny derived from the same clone, adipogenesis and myogenesis were mutually exclusive. In other words, some clones, called “myogenic,” only express myogenic markers such as MyoD, fuse in multinucleated cells, and do not show any lipid accumulation, whereas the others, called “nonmyogenic,” show an intense adipogenic differentiation without any indication of myogenesis. More recently, we confirmed the existence of two SC subpopulations by clonal analyses, and we showed that a fixed proportion of SCs per fiber is able to enter the mesenchymal alternative pathway. The two SC clone types also have different capacity to regenerate muscle in vivo after injury (unpublished observations).

Although it is clear from all these observations that the SCs have the ability to differentiate into white adipocytes, their susceptibility to become brown adipocytes has not been completely elucidated and is nowadays the object of an intense investigation. A recent study (110) showed that, although some brown fat cells are derived from precursors that originate from Myf5-expressing dermomyotome (which has a key role in regulating muscle differentiation), other brown fat cells have a different origin. Since white adipocytes are Myf5 negative, their origin from muscle stem cells seems to be unlikely, and it has been suggested that they could derive from blood vessel-associated pericyte-like cells originating from the lateral plate mesoderm (121).

Seale et al. (110) also reported a bidirectional cell fate switch between primary myoblasts and brown fat cells regulated by PRDM16 (PRD1-BF1-RIZ1 homologous domain containing 16), a positive transcription factor of brown adipose fat (brown adipose tissue). The knockdown of prdm16 in mice induces myogenesis from primary brown preadipocytes, although this phenomenon is not present in wild-type cells. In addition, they showed an intense brown adipogenesis with uncoupling protein-1 (UCP1) expression when PRDM16 ex-
pression was forced by retroviral system in both C2C12 and primary mouse myoblasts. The role of PPARγ seems to be crucial in view of the fact that PRDM16 overexpression failed to induce adipogenesis in PPARγ-deficient fibroblasts (110). Moreover, it has recently been shown that PRDM16 forms a transcriptional complex with the active form of C/EBPβ, acting as a critical molecular unit that controls the cell fate switch from myoblastic precursors to brown fat cells (58).

In contrast with these recent intriguing observations, our results clearly show that SC-derived adipocytes display a gene expression profile and morphological features characterizing white adipocytes (1, 32). In fact, in our studies we did not observe any morphological or molecular features suggesting the presence of a brown phenotype in adipocytes differentiated from human and rat SCs as well as from neonatal muscle-derived stem cells from newborn mice. This fact has been confirmed in experiments in which we observed the spontaneous shift of SCs toward the adipogenic lineage or when we forced the adipogenic process either by addition of the adipogenic cocktail in the culture medium or by the exposition of the cells to high glucose concentrations.

Further studies will be necessary to solve these discrepancies and to better characterize the origin, phenotype, and metabolic functions of intermuscular adipocytes. Therapeutic strategies aiming to specifically redirect the differentiation capacity of muscle-derived adipocytes from white to brown phenotype would be important to face obesity and its metabolic complications.

Finally, it is important to remember that several studies performed with myoblast cell line and with SCs from rodents and humans clearly prove their potential to enter the canonical adipogenic differentiation process (for details, see Ref. 103). In normal conditions, the adipogenic generation/differentiation of SCs is negligible in healthy muscles; however, the number of SCs for single myofibers (45, 114), their proliferative capacity (57, 109, 114), and the adipogenic potential (123) change with age. Moreover, in well-defined pathological conditions (obesity, type 2 diabetes, primary myopathies, etc.), they could enhance their conversion to mature adipocytes, thus increasing IMAT.

**Molecular Mechanism Involved in Muscle SC Conversion to Fat Cells**

PPARs. With respect to the mechanism involved in SC conversion to fat cells, PPAR family is likely to be a key factor, since it happens for preadipocytes coming from AT. In C2C12 cell line exposed to fatty acids, ectopic expression of PPARδ prevents myotube formation and promotes lipid accumulation, whereas in nonexposed cells myogenesis is not impaired, suggesting that activators of PPARδ are necessary for its activity. Moreover, PPARδ activation leads to an upregulation of PPARγ gene and consequently to a responsiveness to PPARγ activators of myoblasts, as confirmed by the strongly increased adipogenesis in C2C12 cells overexpressing PPARδ treated with pioglitazone and, on the contrary, by the lack of response in C2C12 engineered to express PPARδ dominant-negative mutant (55). The fundamental role of PPARγ is suggested also by the prompt differentiation in adipocytes of G8 myoblastic cell line cotransfected with PPARγ and C/EBPα retroviral vectors and treated with adipogenic inducers (56). Interestingly, in the absence of adipogenic agents, the ectopic expression of PPARγ and C/EBPα inhibits the expression of myogenic factors; in particular, MyoD and MRF4 repression sustained by PPARγ is dependent on the presence of its activators, indicating that the activation of the receptor is necessary for its negative regulation of myogenesis (56). This means that myogenic inhibition by the two transcription factors is temporally and functionally distinct from their ability to stimulate overt adipogenesis.

**WNT growth factors.** The development of tissues and organs in multicellular organisms is controlled by the interplay of several signaling pathways that cross talk to provide positional information and induce cell fate specification. Together with other families of secreted factors, WNT (wingless-type mouse mammary tumor virus integration site family) is crucially implicated in these processes. The WNT genes encode a large family of secreted protein growth factors. At present, close to 100 WNT genes have been isolated from various species. In humans, 19 WNT proteins have been identified. They share 27–83% amino acid sequence identity and a conserved pattern of 23 or 24 cysteine residues. During development, WNTs have diverse roles in governing cell fate, proliferation, migration, polarity, and death. In adults, WNTs function in homeostasis, and inappropriate activation of the WNT pathway is implicated in a variety of cancers (61, 67). Some isoforms of the Wnt family have a role in the fate control of SC toward myogenic or adipogenic pathways (82, 91, 98, 126). C2C12 shows spontaneous adipogenic differentiation, accompanied by aP2 expression (as assessed by immunoblot analysis), when expressing a dominant-negative mutation of T cell-specific transcription factor (TCF) that cannot be activated by β-catenin, finally impairing Wnt signaling (98). In addition, myoblasts of Wnt10b−/− mice display a significantly accelerated adipogenesis (confirmed by the expression of adipogenic-specific markers) compared with wild-type mice. In these animals, areas of regeneration after muscle toxic injury accumulate large amounts of lipid, although myogenesis is not completely impaired (126). Taken together, these observations suggest that Wnt10b signaling acts as a myogenesis/adipogenic switch, and when it is expressed, adipogenesis is inhibited. On the contrary, myogenesis is not affected by the lack of Wnt10b probably because of the compensatory overexpression of Wnt7b detected by RT-PCR in Wnt10b−/− mice (126). Interestingly, Wnt expression undergoes a downregulation with aging; 24-mo-old mice-derived myoblasts show a greater adipogenic potential accompanied by a significantly lower expression of Wnt10b gene than 8-mo-old mice-derived myoblasts (126).

**Muscle-derived factors.** Skeletal muscle is generally not considered as a secretory organ, but much evidence is now available regarding the existence of muscular secreted proteins acting both locally as interleukin (IL)-4, vascular endothelial growth factor, and insulin-like growth factor I (IGF-I) and systemically as myostatin and IL-6 (15). Myostatin is the muscular mediator of the “muscle-AT axis”. It acts as strong negative regulator of myogenesis (50, 69, 79), and the lack of it leads to a decreased adipogenesis and to a reduction of fat accumulation, observed also in animal models of obesity (69, 80), suggesting its important role in metabolism. Artaza et al. (4) studied the effects of recombinant myostatin protein and anti-myostatin antibody on myogenic and adipogenic differentiation of mesenchymal multipotent cells. They concluded that
myostatin promotes the differentiation of multipotent mesenchymal cells into the adipogenic lineage and inhibits myogenesis. Myostatin was also seen to inhibit adipogenesis in human bone marrow-derived mesenchymal stem cells and preadipocytes. These effects were mediated in part by activation of Smad3 and cross-communication of the TGFβ/Smad signal to the Wnt/β-catenin/TCF4 pathway, leading to downregulation of PPARγ. The inhibitory effects of myostatin on adipogenesis were blocked by overexpression of PPARγ and RNAi silencing of β-catenin and diminished by overexpression of dominant-negative TCF4 (5). Moreover, myostatin affects cell cycling by affecting the entry of SCs into S phase (3, 76). Interestingly, we observed that in obese patients the mRNA levels of myostatin in muscle biopsies significantly decreased after weight loss due to biliary pancreatic diversion, suggesting that myostatin reduction could represent an attempt to spare muscle mass during caloric restriction or nutrient malabsorption (81).

IL-6 is a pleiotropic cytokine produced by skeletal myocyte in response to contraction (87) and works in a hormone-like fashion to induce lipolysis and fat oxidation. It has been shown that IL-6 treatment of myotubes increases fatty acid oxidation, basal and insulin-stimulated glucose uptake, and translocation of glucose transporter 4 to the plasma membrane. IL-6 mediates anti-inflammatory effects, achieved by regular exercise, by stimulating the production of anti-inflammatory cytokines and by suppressing TNFα production, exerting its beneficial effects against chronic diseases associated with low-grade inflammation such as diabetes and cardiovascular diseases. Several recent studies show that IL-6 signaling may be important in myogenesis. Moreover, it was only recently that IL-6 was shown to play a significant role in SC-mediated muscle hypertrophy (113) and also in the human SC-mediated response to contraction-induced muscle damage (77). Nevertheless, the function of this cytokine in the adipogenic differentiation of SCs and in particular in the origin and expansion of IMAT is far from being elucidated.

GEF-GAP-Rho. Several stimuli acting upon guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that control the activation state of the small GTPase Rho could affect mesenchymal stem cell fate. Rho GTPTase and its regulator, p190-B RhoGAP, are important components of the master switch regulating the mesenchymal stem cells entering either adipogenesis or myogenesis. Cells derived from embryos lacking p190-B RhoGAP exhibit excessive Rho activity and are defective for adipogenesis but undergo myogenesis in response to IGF-I exposure. In vitro, activation of Rho kinase by Rho inhibits adipogenesis and is required for myogenesis. The activation state of Rho following IGF-I signaling is determined by the tyrosine phosphorylation status of p190-B RhoGAP and its resulting subcellular relocalization. Moreover, adjusting Rho activity is sufficient to alter the differentiation program of adipocyte and myocyte precursors. Together, these results identify the Rho GTPTase as an essential modulator of IGF-I signals that direct the adipogenesis/myogenesis cell fate decision (119). Rho family GEFs are molecular regulators of the mesenchymal cell fate decisions that occur during development and repair of tissue damage. The Rho-specific GEF “GEFT” is modulated during skeletal muscle regeneration and exerts a powerful promotion of skeletal muscle regeneration in vivo. Studies in muscle cell lines confirm that endogenous GEFT is transcriptionally upregulated during myogenic differentiation and downregulated during adipogenic differentiation. GEFT inhibits insulin-induced adipogenesis in 3T3-L1 preadipocytes, and its forced expression promotes myogenesis of C2C12 cells, whereas a dominant-negative mutant of GEFT inhibits this process (17).

All pathways and factors reported above (PPARs, WNT growth factors, myokinnes, GEF-GAP-Rho) could be implicated in the adipogenic conversion of SCs, and therefore, their alterations/changes in pathological conditions could be an interesting research field (for details, see Ref. 103).

Preservation of Stem Cell Niche, Regenerative Capacity, and Tissue Repairing

We reported above that the alterations in metabolic conditions characterized by increasing insulin resistance, the decreased oxygen supply, and changes in the local metabolic milieu could altogether stimulate SCs to enter the alternative mesenchymal lineage differentiation pathway and ultimately justify the increase in IMAT. Thus it is becoming more and more evident that the preservation of stem cell niche components is critical for maintaining the regenerative capacity and the muscular lineage orientation of SCs.

Myoblasts harvested from adult skeletal muscle quickly change their fate and their regenerative capacity and lose their self-renewal capacity during in vitro expansion (12, 97, 128). On the contrary, direct implantation of freshly isolated SCs or single fibers with resident SCs is extremely effective to regenerate damaged muscle tissue (14, 27). There is now increasing evidence that cells sense the mechanical properties of their matrix and respond by phenotypic change (37) possibly by differentiating away from their precursor state (e.g., in the case of rigid culture plastic). Support for the importance of the matrix in the stem cell niche also comes from observations that bone marrow integrity during injury accelerates natural healing (102). Not only loss of the matrix context but also loss of the cellular context may cause SC fate change. After muscle injury, macrophages are important for removal of dead cells and the dead parts of muscle fibers. Macrophage infiltration of adipose tissue to remove the dead adipocytes explains the appearance of the “low-grade chronic inflammation” present in obesity and type 2 diabetes (26). Moreover, macrophages also enter the atherosclerotic plaque as foam cells, and also in this case the inflammatory process plays a pathogenetic role in the progression of the arterial wall lesion (21, 71). Macrophages have also been shown to be able to directly stimulate SC proliferation and delay their differentiation. In this context it is important to remind that, although acute inflammation is a trigger for stem cell proliferation, chronic inflammation appears to be detrimental on stem cell recruitment and tissue repair. The vascular microenvironment also plays a crucial role on SC fate control. More than 60% of SCs are located very close to and receive signals from endothelial cells, although they are not in direct contact with them. The number of capillaries per muscle fiber has been shown to correlate to the number of SCs, and loss of capillaries leads to loss of SCs, pointing to some sort of interaction (22). In addition, transwell experiments showed that endothelial cells have a positive effect on myoblast proliferation, which is mediated by growth factors (22). Capillary density, the distance of muscle cells
from capillary, and the fiber type play a role in determining the in vivo insulin action in obese subjects (68).

Mitochondrial Biogenesis and Function in Muscle SC Differentiation

Cell differentiation involves a phenotypic shift toward a more specialized functional state. This process is fundamental to embryonic development, during which primitive stem cells adopt lineage specification and terminal commitment (31). Critical to these functional adaptations is gene expression regulation, which correlates dynamically with the cell’s phenotype. In particular, myogenesis does not occur without an increase in mitochondrial biogenesis and expression of related genes (34). During the differentiation process from their mesenchymal precursors, muscle cells and adipocytes change their gene and protein expression and the cellular structures according to their final functional activity. In particular, myofibers burn substrates to produce energy (ATP) for mechanical work, whereas adipocytes store energy as triglycerides, and to this end myofibers are rich in mitochondria, and adipocytes drastically reduce their oxidative organelles. An impairment in mitochondrial biogenesis could give rise to muscle cells with a reduced oxidative capacity or to allow muscle stem cell to differentiate toward the adipogenic lineage.

Mitochondrial dysfunction contributes to several human diseases, such as obesity, hyperlipidemia, and type 2 diabetes (33, 72, 86). It is interesting to remember that studies using the proton magnetic resonance spectroscopy technique have shown decreased mitochondrial activity and increased intramyocellular fat content in insulin-resistant children of parents with type 2 diabetes, a group with a strong tendency to develop diabetes later in life (88). Therefore, activated mitochondria pace myogenesis, and a reduced mitochondria biogenesis leads to increased IMAT formation.

Little is known about muscle satellite cell-to-adipocyte conversion with regard to the cellular metabolism and the relative change in mitochondria. It should be kept in mind that skeletal muscle mitochondrial number and function might contribute considerably to oxidative stress by either being activated in excess or having decreased oxidative capacity (51), and this could have important consequences on muscle stem cell differentiation.

Fig. 4. High glucose induces adipocyte differentiation of muscle-derived stem cells (MDSC). BODIPY, a fluorescent dye specific for lipid droplets and staining of MDSC cultured in low-glucose DMEM (LG; A) or high-glucose DMEM (HG; B). Scanning electron microscopy of MDSC cultured on Hyaff 11 sponges in LG (C) and HG (D). Oil Red O staining of MDSCs grown in LG (E) or HG (F). Lipid droplets are in red, and biomaterial fibers are in blue (magnification: ×20) Adapted from Aguiari et al. (1).
Mitochondrial biogenesis requires coordinated changes in the metabolic enzymes of oxidative phosphorylation, TCA cycle, and fatty acid oxidation. Expression of the hundreds of nuclear-encoded mitochondrial genes is coordinated by a few transcription factors that coregulate gene networks. Nuclear respiratory factor (NRF)-1 and NRF-2 regulate many of the genes encoding oxidative phosphorylation proteins (104, 105). PPARs regulate genes encoding enzymes and transporters of fatty acid oxidation (10). Most studies of the control of mitochondrial gene expression implicate PPARγ coactivator-1α (PGC-1α) as a “master controller” of mitochondrial biogenesis coactivating nuclear hormone receptors (PPARs, retinoic acid receptors, and thyroid hormone receptors) as well as NRF-1 (94). PGC-1α may provide an important link between myogenesis and mitochondrial biogenesis. The myogenic transcription factor myocyte enhancer factor 2 induces PGC-1α expression (30), and PGC-1α overexpression can stimulate the formation of slow oxidative muscle (70). The fusion of mitochondria has recently been shown to play a crucial role in embryonic development, antiapoptotic events, the maintenance of an unchanged mitochondrial DNA (mtDNA) pool, and protection against free radical-induced lesions (8, 20, 59). In some types of cells, especially those with high energy requirements, mitochondria are occasionally organized into networks with a concomitant increase in the expression of mitofusins (Mfn)1/2 (18). This network is a highly dynamic structure of individual organelles that regularly fuse and divide (127).

Mfn2 appears to play an important role in cell viability. The inhibition of mitochondrial fission has been reported to block apoptosis (59). To explain this phenomenon, high levels of fused mitochondria have been suggested to constitute a method of defense against the accumulation of oxidative lesions. Such lesions occur during cellular aging when the extensive oxidative metabolism of mitochondria increases the production of reactive oxygen species (ROS) and consequently mtDNA damage and cell death leading to organ dysfunction, as recently shown in muscles of obese Zucker rats, where a decreased level of Mfn2 was observed (9).

An interesting advancement in the field has been the identification of the gene encoding the 66-kDa isoform of the growth factor adaptor shc as a pure “aging” gene (i.e., its ablation causes lifespan extension with no associated phenotypic alterations). p66shc is potentiated by oxidative stress through the phosphorylation of a critical serine residue (Ser36) and translocates to mitochondria. Within mitochondria, p66shc binds cytochrome c and acts as an oxidoreductase, shuttling electrons to molecular oxygen, thus generating ROS (46). Mitochondrial ROS production initiates a feed-forward cycle that, by causing permeability transition pore opening and mitochondrial dysfunction or by activating other ROS-dependent pathways, eventually leads to apoptotic cell death (89).

We recently demonstrated that hyperglycemia causes adipocytic differentiation of mesenchymal stem cells originating from both adipose and muscle tissue (Fig. 4) (1). In this process, ROS play a primary signaling role because the differentiation can be triggered also by the simple application of an oxidative stress and occurs via a signaling pathway impinging on PKCβ and the 66-kDa isoform of the growth factor adapter shc (p66shc) (Fig. 5). These data add complexity to the notion of ROS as damaging species in age-related organ dysfunction, because they suggest that transdifferentiation (in the case of skeletal muscle) or forced differentiation (in the case of the adipose tissue) of mesenchymal precursors into adipocytes may play a primary pathogenic role. Clarifying the specificity (and possibly the molecular basis) of the ROS-mediated signals in apoptosis and cell differentiation is a goal of utmost interest for understanding the pathological process and designing novel therapeutic approaches.

Conclusive Remarks

In recent years, a new paradigm in anatomy and physiology has been put forward: white and brown adipose tissues are contained together into a dissectable “adipose organ” (23–25). It is composed by two main subcutaneous depots and several visceral depots. All depots are anatomically defined by a cleavage plane that allows a precise dissection of depots from.
surrounding structures. However, there are other adipose tissue depots in which fat cells are interspersed within or surrounding other cells, tissues, or organs (perivascular, epicardial, bone marrow, intermuscular). The view of AT as a passive mesenchymal scaffold has been ruled out by focusing our attention on the functional aspects and the physiological or pathological consequences of its presence or abnormal expansion in these sites.

Progressive loss of contractile mass is commonly accompanied by an increase of IMAT (35, 101, 117). A series of pathological conditions are characterized by relative or absolute muscle wasting (sarcopenia) because of the impairment in the repairing process after injuries, because of inherited muscle genetic defect (mitochondrial or metabolic), or because of degenerative process during aging or in the presence of cancer (93). In all of these conditions, a replacement of the functional muscle tissue with white adipose tissue is a well-established phenomenon.

In recent years, both tissue and hormonal factors that determine the balance between anabolism and catabolism in healthy, diseased, and aging human skeletal muscle have been studied extensively. Most of the molecular mechanisms associated with muscle atrophy have now been elucidated (78). Moreover, it is clear that SCs possess a central role particularly when systemic disorders causing the loss in muscle mass are taken into account. The increase in IMAT is tightly correlated with the presence of the cluster of metabolic and cardiovascular abnormalities identifying the metabolic syndrome. It is possible to speculate that a primary insult to the muscle could induce its differentiation toward adipose tissue. In fact, the high-glucose-induced muscle stem cell to enter the adipogenic lineage (1) supports this view. On the other hand, the expansion of IMAT could induce some changes in muscle metabolism and insulin sensitivity because of the release of adipokines and metabolites from fat cells surrounding muscle fibers.

Because of their particular position close to muscle fibers, it is possible to hypothesize that the biology of intermuscular adipocytes may differ from that of adipocytes from other sites, but only a few data have been reported in animals (43, 92), and no data are available in humans. The close anatomic contact between fat and muscle cells implies a reciprocal influence; in fact, several myokines (IL-6, myostatin, follistatin) and metabolites coming from the muscle could in turn affect the surrounding adipose tissue function. Physiological situations, for instance, physical exercise, and pharmacological treatment (32) could influence the rate of SCs entering the adipogenic lineage and modifying the adipokine expression profile and the secretory pattern of fat cells within the muscle bundles.

Finally, the ectopic brown adipose tissue in intermuscular depots with regulatable expression of UCP1 has recently been shown in a particular strain of mice (2), and the potential for conversion of muscle stem cells into brown adipocytes expressing UCP1 has also been proven in humans (29).

Therefore, the further and better understanding of the molecular pathways that regulates gain or loss of muscle mass and muscle-to-fat conversion is crucial for treating muscle wasting-associated disorders with their physical and metabolic complication.

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