Adipose tissue depots and inflammation: effects on plasticity and resident mesenchymal stem cell function

Lina Badimon and Judit Cubedo

1Cardiovascular Science Institute – ICCC, IIB-Sant Pau, Ciber CV, Hospital de Sant Pau, c/Sant Antoni Mª Claret 167, Barcelona 08025, Spain; and 2Cardiovascular Research Chair UAB, Barcelona, Spain

Received 30 January 2017; revised 4 April 2017; editorial decision 10 April 2017; accepted 10 May 2017; online publish-ahead-of-print 11 May 2017

Abstract

Adipose tissue (AT) is a highly heterogeneous organ. Beside the heterogeneity associated to different tissue types (white, brown, and ‘brite’) and its location-related heterogeneity (subcutaneous, visceral, epicardial, and perivascular, etc.), AT composition, structure, and functionality are highly dependent on individual-associated factors. As such, the pro-inflammatory state associated to the presence of obesity and other cardiovascular risk factors (CVRFs) directly affects AT metabolism. Furthermore, the adipose-derived stem cells (ASCs) that reside in the stromal vascular fraction of AT, besides being responsible for most of the plasticity attributed to AT, is an additional source of heterogeneity. Thus, ASCs directly contribute to AT homeostasis, cell renewal, and spontaneous repair. These ASCs share many properties with the bone-marrow mesenchymal stem cells (i.e. potential to differentiate towards multiple tissue lineages, and angiogenic, antiapoptotic, and immunomodulatory properties). Moreover, ASCs show clear advantages in terms of accessibility and quantity of available sample, their easy in vitro expansion, and the possibility of having an autologous source. All these properties point out towards a potential use of ASCs in regenerative medicine. However, the presence of obesity and other CVRFs induces a pro-inflammatory state that directly impacts ASCs proliferation and differentiation capacities affecting their regenerative abilities. The focus of this review is to summarize how inflammation affects the different AT depots and the mechanisms by which these changes further enhance the obesity-associated metabolic disturbances. Furthermore, we highlight the impact of obesity-induced inflammation on ASCs properties and how those effects impair their plasticity.

Keywords

Adipose tissue depots • Adipose-derived stem cells • Plasticity • Inflammation

This article is part of the Spotlight Issue on Dysfunctional Adipocyte and Cardiovascular Disease.

1. Introduction

The adipose tissue (AT) is a highly heterogeneous organ due not only to the existence of different AT types, i.e. white AT (WAT), brown AT (BAT), and ‘brite’ (brown-in-white), but also because of its multi-depot distribution. Moreover, both WAT and BAT coexist at several locations.1 Indeed, because of their high heterogeneity these AT depots have been even considered as ‘mini-organs’ with autonomous characteristics and functionality.2

Besides its heterogeneity, another key property of AT is its ability to change its structural, cellular and molecular characteristics depending on both physiological and pathological conditions that directly impact its functionality, a term that is known as plasticity.3 This plasticity can be deleterious or beneficial depending on how it affects AT functionality. Moreover, the cellular and structural differences of the different fat depots also influence AT plasticity and remodelling. In fact, important differences exist between the two main WAT depots, visceral AT (VAT), that has been traditionally considered to have a protective role against trauma, and subcutaneous AT (SAT), usually considered an energy storage organ. Both tissues have different structure (i.e. vascular density and innervation), different adipokine expression profiles and thus, different metabolic functions.4,5 Importantly, VAT shows a higher acute inflammatory profile than SAT, being therefore considered a more deleterious WAT depot.6

Therefore, AT represents an extremely dynamic organ that can exhibit important differences in its characteristics depending on...
conditioning/regulatory factors. One of the most evident and common physiological changes observed in AT is the progressive reduction in brown adipocytes with the concomitant increase in white adipocytes observed with age. Interestingly, the inverse effect seems to be observed after cold exposure both in rodents and in humans, representing a clear example of the potential beneficial plasticity of AT. Similarly, physical activity can also promote browning of AT.

AT plasticity is also observed in many pathological situations such as in the presence of cardiovascular risk factors (CVRFs), obesity being one of the most determining factors inducing a metabolic shift in adipocytes that leads to dysfunctional AT. Specifically, obese individuals show BAT atrophy in association with increased fat accumulation in VAT and hyperglycaemia. Better understanding of the effects of the individual-related factors, such as gender or age, and the clustering of CVRFs, such as hypercholesterolemia, hyperglycaemia, and obesity-associated subclinical inflammation, on AT heterogeneity and plasticity is critical to design targeted strategies to combat the presence of dysfunctional AT.

2. Obesity-induced inflammation and AT depots

Human and animal model studies have shown that obesity is accompanied by a significant increase in macrophage infiltration, and this recruitment is linked to systemic inflammation, insulin resistance, and oxidative stress. AT and inflammation are closely interrelated, not only because of the chronic low-grade inflammation present in obesity, but also due to the intrinsic ability of adipocytes to secrete both, anti- and pro-inflammatory adipokines, their imbalance being determinant for AT functional profile and the development of metabolic disorders. Adipocytes of obese individuals are larger (hypertrophic adipocytes) than those from lean subjects and change their adipokine secretion profile towards a pro-inflammatory state producing lower levels of adiponectin, but higher levels of tumour necrosis factor-alpha (TNF-alpha), thus contributing to the pro-inflammatory milieu associated to obesity. This obesity-induced inflammation directly impacts AT functionality.

In addition to adipocyte hypertrophy, excessive calorie consumption promotes adipocyte hyperplasia, partly due to the differentiation of stem cells towards adipocytes, process known as adipogenesis. On one hand, newly formed adipocytes have increased sensitive to insulin and an increased uptake of free fatty acids and triglycerides. On the other hand, hypertrophied adipocytes become dysfunctional, loosing their ability to protect against systemic lipotoxicity, and leading to ectopic fat accumulation. Obesity is also characterized by the remodelling of the extracellular matrix in order to allow tissue expansion. Additionally, AT from obese subjects shows a decreased ability to expand the capillary network that surrounds adipocytes leading to adipocyte hypoxia and necrosis. As a result, obese AT is characterized by the presence of crown-like structures (CLS) that are formed by infiltrating macrophages surrounding necrotic adipocytes. However, how obesity and its associated inflammatory status affect AT plasticity is different depending on the background characteristics of each AT depot.

2.1 Differential effects on VAT and SAT

Although overall fat mass has been linked to the development of obesity-associated comorbidities, the accumulation of VAT has shown to be specifically associated to the development of metabolic complications. However, not all the VAT depots exhibit the same behaviour in relation to obesity-associated inflammation and metabolic disturbances. A recent study comparing three different VAT depots, mesenteric, omental, and periaortic, from patients with cardiovascular disease (CVD), has described important differences in their inflammatory profile. Specifically, periaortic VAT showed the highest adipokine secretion ability although its morphological characteristics were not associated to metabolic complications in obesity. On the contrary, authors found that mesenteric AT, although showing a low inflammatory profile depicted morphological features that were consistently associated to metabolic syndrome, with a higher adipocyte size and an increased presence of CLS. Indeed, an important association between the specific mesenteric depot and obesity has been reported. This strong association has been explained by the anatomical location of this fat depot, close to the intestine, in which the adipokines secreted by mesenteric AT could directly influence the secretion of intestinal hormones such as glucagon-like peptide-1 (GLP-1).

Traditionally, a protective role against cardiometabolic disease has been attributed to SAT. SAT depots show an enhanced adipocyte generation capacity compared with VAT that might represent a protective property against metabolic dysfunction. However, obesity does not only affect VAT depots, but it also disrupts SAT homeostasis. Indeed, obesity also induces adipocyte hypertrophy and decreased adipogenesis and angiogenesis in SAT. However, it has been reported that obesity-mediated adipocyte hypertrophy is more frequent in VAT, whereas adipocyte hyperplasia is more commonly found in SAT. This difference is partly explained by the increased propensity to cell death observed in VAT and the higher amount of progenitor cells observed in SAT. Nevertheless, CLS have also been found in subcutaneous fat depots. However, it should be noted that functional and morphological differences among superficial and deep SAT depots have been reported.

Several studies have tried to address the effects of obesity on VAT and SAT and the differential contribution of both fat depots to the development of chronic inflammation. The specific analysis of macrophage infiltration has shown a higher infiltration rate in VAT than in SAT in both, obese and normal-weight individuals. Regarding inflammatory cytokines, it has been recently shown that whereas IL-6 and IL-15 levels were higher in SAT than in VAT depots form obese and normal-weight subjects, obesity was associated with an important shift towards a higher pro-inflammatory profile of VAT with a significant increase in IL-6 and IL-15 in obese subjects compared with non-obese individuals. These results highlight that obesity is associated with a shift towards a pro-inflammatory state specifically in VAT. Furthermore, there was an additive effect of the presence of metabolic syndrome in obese patients that showed significantly higher levels of IL-1β, IL-6, and IL-15 in VAT compared with obese subjects without metabolic syndrome. Indeed, in vitro experiments have shown that VAT-derived adipocytes secrete more pro-inflammatory cytokines than SAT-derived adipocytes. Importantly, a correlation between IL-6 serum levels and the concentration of this cytokine in SAT has been reported, supporting the obesity-mediated increase of fat accumulation in this depot. In addition, by using a novel labelling proteomic approach to analyse the secretome of VAT and SAT depots from obese individuals, a specific increased secretion of extracellular matrix proteins has been detected specifically in SAT. These results underscore a stronger effect of obesity on extracellular matrix
over-production in SAT depots that could lead to fibrosis having a negative influence on AT expansion by impairing adipogenesis.

2.2. Effects on perivascular AT (PVAT)

PVAT represents a key AT depot in the context of CVD due to its intimate contact with arterial beds. Indeed, PVAT is known to exert vasodilatory and anti-inflammatory functions that are blunted in obesity, where the volume of this depot increases proportionally to the increase in VAT.41-43 Thus, PVAT expansion has also been described during obesity.44 Rodent models under high-fat diet have white and brown peri-aortic adipocytes hypertrophy.45 Importantly, local PVAT expansion has been associated to atherosclerotic plaque development and vascular calcifications.46 This association is mediated, at least in part, by the paracrine action of PVAT-derived growth factors that can stimulate vascular smooth muscle cell (VSMC) proliferation.47 Among these factors, visfatin has been shown to induce VSMC growth via ERK 1/2 and p38.48 Indeed, obesity is associated with increased circulating visfatin levels.49 Decreased adiponectin PVAT secretion in obesity could have an additional role as this adipokine exerts an anti-proliferative action on VSMC.50 Other PVAT-derived factors that could contribute to the obesity-associated increase in atherosclerosis development are TNF-α, VEGF, and leptin which have also been implicated in VSMC proliferation and migration.51,52 Inflammation seems associated to adventitial vasa vasorum expansion that by increasing the communication with PVAT propagates the pro-inflammatory signals,53 having a pivotal role in vascular disease development. Thus, because of its location, PVAT is an essential player in the ‘outside-in’ theory supporting that vascular inflammation begins in the adventitia and advances to the media and the intima.54,55 Early obesity induced by high-fat diet in mice has shown to reduce the expression of adipogenesis-related genes in thoracic PVAT, and to induce a decrease in adiponectin and an increase in macrophage inflammatory protein 1-alpha (MIP-1α).56 Importantly, while these changes were seen in PVAT, minimal changes were detected in SAT and VAT depots. Furthermore, high-fat diet also induces an imbalance in anti-oxidative mechanisms in PVAT leading to oxidative stress that amplifies inflammation in this specific fat depot.57,58 Moreover, obesity induces a shift in PVAT from an anti-inflammatory profile towards a pro-inflammatory status that is mainly orchestrated by MCP-1.59 In addition, experimental studies have demonstrated that PVAT from obese mice have an impaired insulin-mediated vasodilation when compared with non-obese animals, and that this effect was reversed by the inhibition of inflammation through the blockade of the c-Jun N-terminal kinase (JNK) signalling pathway.60

Because of the intimate contact between PVAT and the vascular bed, inflammation-mediated changes in PVAT can accelerate and propagate inflammation, oxidative stress and vascular dysfunction contributing to vascular disease progression in the context of obesity.

2.3. Effects on epicardial AT

Epicardial AT is the specific VAT depot of the heart that is found between the myocardial tissue and the visceral pericardium surrounding both ventricles.61 Because of its anatomical location, and together with PVAT, epicardial AT arises as one of the most important fat depots in relation to CVDs. Indeed, epicardial AT and the myocardium share the same microcirculation having thus a clear functional relationship.62

The presence of established CVD or CVRFs has a direct impact on the epicardial fat depot. Moreover, changes in the phenotype of this specific fat depot can directly impact heart function through the paracrine secretion of pro-inflammatory cytokines, what has been named ‘outside-to-inside cellular cross-talk’.52,63 Obesity induces an increase in epicardial AT thickness that has been associated with atrial enlargement and diastolic function impairment.64 Furthermore, the enlargement of this AT depot has been related to metabolic syndrome through its effects on arterial blood pressure, insulin, and LDL-cholesterol levels, among others.65 These effects have been related to the deleterious effects of toxic lipid intermediates through the activation of the inflammasome and the induction of mitochondrial dysfunction and apoptosis.66

Changes in epicardial AT have also been reported in patients with established coronary artery disease (CAD). As such, PET studies have shown that epicardial AT volume is a better independent predictor of myocardial ischaemia than coronary artery calcium score.67 This is consistent with the fact that patients with unstable angina show higher epicardial AT thickness than patients with stable angina. Furthermore, CAD patients have a higher ratio of pro-inflammatory to anti-inflammatory macrophages in epicardial AT than subjects without CAD,68 contributing to this pro-inflammatory scenario in those patients. Indeed, a transcriptomic study on human epicardial AT has demonstrated a co-ordinated change in the expression of several genes involved in key cellular functions in CAD patients compared with subjects without CAD.69

All these evidence support the notion that epicardial AT changes directly affect cardiac function and thus CVD development.

3. Adipose-derived stem cells (ASCs)

AT is composed mainly of two different cell categories: adipocytes, which are the main parenchymal cell type; and the stromal vascular fraction (SVF) containing the remaining cellular components. This SVF includes preadipocytes, fibroblasts, endothelial cells, immune cells and multipotent stem cells.70 Adipocytes are the main cellular component of AT in terms of volume as they occupy more than 90% of the tissue due to their unique morphology containing a single large lipid droplet. However, the SVF is the main cellular fraction in terms of quantity in the AT of both lean and obese subjects. In 2013, the International Fat Applied Technology Society reached a consensus on the minimal phenotypic criteria to characterize the adherent stromal/stem cell population from AT. Thus, it is now accepted that ASCs are characterized by CD39+/CD44+/CD73+/CD90+/CD105+/CD45+/CD31- cells.71 However, the expression of ASCs surface markers, such as CD34, may change with division, and thus different ASC subpopulations may exist in vivo, further contributing to AT heterogeneity.

ASCs, contributing to tissue homeostasis, cell renewal and spontaneous repair, exert a physiological role in AT (Figure 1). These ASCs share many properties with bone-marrow mesenchymal stem cells such as their potential to differentiate towards multiple tissue lineages, their ability to secrete angiogenic and anti-apoptotic cytokines, and their immunomodulatory properties. Specifically, ASCs are able to differentiate into cardiomyocytes, muscle myoblasts, osteoblasts, chondrocytes, hepatocytes, pancreatic cells, endothelial cells, and haematopoietic-supporting cells, among others.72-78 making them an excellent target in the research field of regenerative medicine. Importantly, in vitro and in vivo animal studies have revealed that ASC-conditioned media confers functional improvement and attenuation of injury in a similar way to that afforded by ASCs,79,80 suggesting that ASCs-effects could be mediated via complex paracrine actions. Indeed, ASCs have the capacity to secrete several growth factors and cytokines (i.e. transforming growth factor β1 (TGF-
beta1), vascular endothelial growth factor, insulin-like growth factor, or hepatocyte growth factor, among others), explaining some of the observed effects of ASCs stimulating the recovery of damaged tissues. In addition, ASCs are also targets of different growth factors as they have been shown to express different growth factor receptors that are partly responsible for their plasticity. One of the most relevant examples of the plasticity of ASCs is their ability to secrete angiogenic factors under hypoxic conditions, enabling them to survive in an ischaemic environment where they provide a reservoir of the necessary growth factors to promote angiogenesis.

The exact localization of ASCs within the AT has not been totally identified, but there are evidence suggesting a perivascular location. Importantly, ASCs also contribute to AT heterogeneity. Indeed, ASCs from different depots show a different ability to grow and differentiate (i.e. VAT vs. BAT) and even ASCs from different anatomical areas also exhibit different characteristics. As such, ASCs derived from SAT and VAT when differentiated in vivo, show important differences inherent to the source tissue. The adipogenic differentiation capacity of SAT-derived ASCs is higher than the one of ASCs from the VAT. This limited capacity of VAT ASCs to differentiate into new adipocytes could partly explain the hypertrophy of existing adipocytes as a response to fat accumulation in obesity. As opposed to this, the greater differentiation capacity of SAT ASCs induces the formation of new adipocytes with smaller lipid vacuoles. These existing differences between ASCs from different fat depots appear to be the result of both, epigenetic regulation in the early developmental phases and the specific environmental influence of each AT depot, representing a key factor contributing to the differences in AT plasticity of different depots. Furthermore, specific individual physiological and pathological conditions could induce additional changes on ASCs even leading to the loss of their properties limiting their stemness and their regenerative potential. These individual conditioning factors (age, CVRFs, etc.) affect ASCs properties and represent a potential limitation for their autologous use in the clinical setting for reparative purposes.

4. Inflammation-induced changes on ASCs

Importantly, it has been described that several individual-related factors such as age or gender can modify the number and the proliferation, differentiation, and angiogenic capacity of ASCs. Specifically, ageing has been associated with a loss of the ASC growth and differentiation potential. Thus, younger ages (25–30 years) show a high differentiation rate in all AT depots, whereas in older individuals the differentiation rate is only high in the arm and thigh SAT depots. This loss of ASC differentiation potential can be partly driven by the pro-inflammatory profile that characterizes ageing. In fact, inflammation is believed to play a key role in governing ASCs differentiation and plasticity. A gene expression profiling study has demonstrated a differential time-dependent expression signature of pro- and anti-inflammatory cytokines between ASCs undergoing osteogenic differentiation and ASCs undergoing adipogenic differentiation. Some of the pro-inflammatory cytokines that showed an early up-regulation in ASCs that are differentiating to osteoblasts, such as IL-1 and TNF-alpha, had been shown to suppress PPAR-gamma expression, thus inhibiting adipogenic differentiation. Obesity induces an important dysregulation not only of adipocytes but also of ASCs plasticity that contributes to the induction of adipocyte hypertrophy and hyperplasia through the increase in pro-inflammatory cytokines. Therefore, the pro-inflammatory milieu that characterizes obesity and other

Figure 1 ASCs functionality in AT. ASCs influence AT homeostasis through their ability to: differentiate towards different lineages, express different growth factors receptors and secrete cytokines and different mediators. In addition, ASCs are responsible, at least in part, of AT depot-related heterogeneity.
cardiovascular associated metabolic disturbances directly influences ASCs plasticity by affecting their proliferation and differentiation potential, finally determining their regenerative potential (Figure 2). The understanding of the mechanisms by which inflammation affects ASCs plasticity is crucial in order to be able to develop therapeutic approaches based on autologous delivery of ASCs. Indeed, previous work of our group has demonstrated a role for sphingosine kinase-1 (SK1) in the regulation of the pro-inflammatory response in ASCs and a potential therapeutic role of the targeted inhibition of this enzyme in the attenuation of the chronic inflammatory state in obesity-related metabolic disturbances.100

4.1 Effects on ASC differentiation and proliferation

The potential of ASCs to differentiate, grow and proliferate is determined by the surrounding environment. Thus, the presence of metabolic disturbances, such as that induced by obesity, can clearly affect ASCs differentiation and proliferation capacity.

High-fat diet induction of obesity in an experimental pig model has shown to induce increased circulating TNF-α levels, consistent with the obesity-induced pro-inflammatory phenotype.101 Furthermore, ASCs from obese animals showed increased senescence and an increased adipogenic and osteogenic potential compared with ASCs from lean animals. Importantly, this animal model corresponded to a model of early obesity without the clustering of other CVRFs that can influence ASCs plasticity. Interestingly, we have described that ASCs from morbidly obese patients obtained from SAT at the moment of bypass gastric surgery revealed a lower proliferation and adipogenic differentiation potential compared with ASCs from non-obese subjects that underwent liposuction.88 It is important to highlight, that morbidly obese patients represent a late stage in the development of obesity where metabolic syndrome is already established and where the pro-inflammatory signals have been maintained for a long period. In fact, by using transcriptomic approaches we have demonstrated that ASCs from morbidly obese patients lose their stemness showing a pre-adipocyte-like differentiated phenotype, and that the impairment in the transcriptomic profile is higher in ASCs from obese patients with metabolic syndrome and clustering of several CVRFs than in ASCs from obese metabolically healthy patients.87 Taken together these evidence underscore the additive effect of each CVRF and metabolic disturbance on ASCs plasticity.

Besides affecting ASCs proliferative potential and differentiation profile, metabolic disturbances affect other key properties of stem cells. As such, ASCs from obese and type-2-diabetic patients have shown higher migration, invasion and phagocytosis capacity than that from lean subjects.102 These changes were accompanied by an increase in the

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/113/9/1064/3819204)
expression of pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α and monocyte chemoattractant protein (MCP-1), and the activation of the NLRP3 (NOD- and pyrin domain-containing like receptor 3) inflammasome signalling pathway, which is associated to innate immunity. Importantly, the increase in inflammatory markers was observed both in VAT and SAT of obese and type-2-diabetic patients. The increase in migration, invasion and phagocytosis in ASCs from obese and type-2-diabetic subjects was accompanied by a higher metabolic activity (increased glycolytic phenotype, decreased succinate dehydrogenase activity and increased succinate release) probably due to a high metabolic ATP demand, comparable to that observed in cancerous cells. In fact, the same authors found increased mRNA levels and activity of MMP-2 and MMP-9 in ASCs from obese and type-2-diabetic subjects.

Diabetes has also been shown to affect ASCs plasticity. By using a rat model of diabetes, we have found a co-ordinated inhibition in the gene expression profile of Notch, Wnt, and fibroblast growth factor (FGF) in ASCs from SAT of diabetic rats compared with a non-diabetic control group, together with an increased ASC-commitment to the adipogenic differentiation, invasion and phagocytosis in ASCs from obese and type-2-diabetic subjects.

Among the described properties of ASCs, their pro-angiogenic function is one of the most important abilities in the context of cardiovascular regenerative medicine. This angiogenic potential of ASCs is their high immunomodulatory activity that is even more potent in the regulation of ASC angiogenic potential. Another key property of ASCs is their participation in autologous cell-based therapy.

Diabetes has also been shown to affect ASCs plasticity. By using a rat model of diabetes, we have found a co-ordinated inhibition in the gene expression profile of Notch, Wnt, and fibroblast growth factor (FGF) in ASCs from SAT of diabetic rats compared with a non-diabetic control group, together with an increased ASC-commitment to the adipogenic differentiation, invasion and phagocytosis in ASCs from obese and type-2-diabetic subjects.

4.2 Effects on ASC functionality

Changes in the differentiation and proliferation potential of ASCs can have a clear impact on their functionality and thus in their potential use in regenerative medicine. However, inflammation and metabolic disturbances also impair the main ASCs abilities for which they have been considered an important tool for regenerative therapies. Among the described properties of ASCs, their pro-angiogenic function is one of the most important abilities in the context of cardiovascular regenerative medicine. This angiogenic potential of ASCs is mediated through the release of paracrine factors. Indeed, we have seen in an experimental pig model that co-administration of allogenic ASCs and their conditioned media synergistically contribute to the neovascularization of the infarcted myocardium as compared with the delivery of conditioned media or ASC alone through a co-ordinated up-regulation of the pro-angiogenic protein interactome. However, this ability may be impaired in ASCs homed in obese AT, as we have demonstrated that ASCs from SAT of morbidly obese patients secrete higher levels of the anti-angiogenic factor TSP-1 than ASCs from lean individuals, and that ASC-conditioned media from morbidly obese patients show a lower pro-angiogenic capacity than that from lean subjects. In line with this observation, ASCs obtained from SAT of diabetic rats show an impaired ability to form microvessel networks in an in vivo plug angiogenesis assay compared with a non-diabetic control group. Similarly, the presence of a clustering of CVRFs in a rat model induces the loss of the pro-angiogenic paracrine effects on endothelial cells of ASCs from epicardial AT compared with that of rats without CVRFs. Interestingly, an over-activation of the Notch signalling pathway was found to be responsible for this cardiovascular risk-driven loss of ASC angiogenic potential. These results underscore, an AT depot-specific role of Notch signalling in the regulation of ASC angiogenic potential. Another key property of ASCs is their high immunomodulatory activity that is even more potent than that of other mesenchymal stem cells. Importantly, obesity also blunts ASCs immunomodulatory capacities.

5. Reversibility of changes on AT depots and resident ASCs

5.1 AT structural changes

WL induces different changes in the gene expression profile of both depots evidencing once more the differences in the plasticity between VAT and SAT. Interestingly, a recent meta-analysis evaluating all available studies analysing the effect of different WL strategies (diet, exercise, pharmacologic treatment, and bariatric surgery) has revealed that although SAT loss is greater than VAT loss, the percent of change is higher in VAT than in SAT. There is also evidence of a time-dependent effect in the differential fat loss between both depots, being SAT changes associated to short term WL after bariatric surgery, whereas VAT decline is more evident in longer follow-up studies. In both depots, fat loss seems to be proportional to BMI decrease, in normal weight, obese and severely obese subjects, and is associated with a reduction in insulin plasma levels and an improved homeostatic model assessment (HOMA) index. Thus, the decrease in visceral and subcutaneous fat depots induced by WL is able to restore, at least in part, the AT metabolic homeostasis. WL in severely obese individuals has also shown to revert some of the obesity-associated changes in epicardial AT by reducing tissue thickness. A recent study using advanced MRI technology has revealed that bariatric surgery is associated with a reduction of subcutaneous, visceral and epidermal fat at 6-month follow-up. A crucial event behind the beneficial effects of WL is the reduction in adipocyte hypertrophy. Patients undergoing bariatric surgery have shown a significant decrease in adipocyte size reaching a diameter similar to that of lean controls but without changes in adipocyte numbers. Importantly, the reduction in adipocyte size is correlated with the improvements in insulin sensitivity even at the long term follow-up after the surgery. However, the exact intracellular mechanism leading to the decrease in adipocyte size after surgery is yet unknown.

5.2 Reversibility of the pro-inflammatory status

A key feature in the reversible nature of obesity-induced changes in AT is the attenuation of inflammatory and immune pathways both in SAT and VAT. Specifically, WL has been associated with an improvement of the pro-inflammatory state in SAT with a decrease in inflammatory cytokines and immune cells infiltration. Systemic levels of two important pro-inflammatory cytokines, IL-6 and MCP-1, have been consistently shown to decrease in obese patients after surgery, together with a significant decrease in the genes expression of these cytokines in AT depots. Similarly, circulating levels of CRP are also decreased after surgery being this decrease still evident after 10-years follow-up. This decrease in CRP levels has been associated to an improvement in
cardiovascular risk. Importantly, these changes in pro-inflammatory cytokines after WL seem to have an impact on AT infiltrated macrophages with a shift in the balance pro-/anti-inflammatory macrophages after bariatric surgery.122

All these observations undoubtedly support that WL attenuates the pro-inflammatory profile induced by obesity.

5.3 Changes in adipokine secretion profile

A hallmark of healthy AT function is its adipokine secretion profile which is significantly altered in obesity. WL due to bariatric surgery has shown to have a healthy adipokine secretion by increasing adiponectin and decreasing leptin levels123,124 pointing out to a regain in AT endocrine function. Even normalization in the secretion of other less studied adipokines, such as visfatin and chemerin, has also been reported.125,126 Importantly, it has been shown that changes in adiponectin occur before a significant WL can be observed suggesting that the shift in adipokine secretion profile is not dependent on fat loss.127

All these evidence point out towards an important role of the WL-mediated restoration of adipokine secretion profile and the metabolic improvements observed in AT.

5.4 Influence of ASCs plasticity

WL-mediated effects on ASCs could be one of the mechanisms behind the reversibility of obesity-related AT changes. However, contradictory findings have been reported regarding the relation between WL and the recovery of ASCs properties. It has been described that obese subjects under long-term calorie restriction show ASCs with reduced DNA damage and improved survival compared with ASCs from obese subjects, without affecting their adipogenic capacity.128 Contrarily, a superior in vitro adipogenic potential has been reported for ASCs isolated from subjects who formerly had obesity when compared with non-obese individuals.129 However, complete WL has to be analysed cautiously when evaluating and comparing the results obtained from different studies. A recent experimental study using ASCs from SAT of formerly obese, obese and non-obese mice has shown that while proliferative and migration abilities are recovered after WL, angiogenic and adipogenic differentiation abilities are not restored. Importantly, the same authors showed that although formerly obese mice had the same number of mitochondria they showed lower ROS levels and decreased oxygen consumption.130 Many studies have focused on the analysis of the potential mechanisms behind the reversibility of the ASC dysfunction induced by metabolic disturbances. The use of microarray gene profiling approaches on SAT-derived ASCs from normal weight subjects, obese individuals and long-term weight-losing formerly obese donors, has revealed that long-term WL mainly induces the expression of the insulin-like growth factor 1 (IGF-1) and the GTP-binding RAS-like 3 (DIRAS3) genes.131,132 Specifically, the same authors demonstrated that DIRAS3 downstream regulates Akt-mTOR signalling in ASCs, inhibits adipogenesis and activates the recycling process of autophagy in these cells.131

These evidences seem to suggest that WL is able to restore some of the ASCs capacities. However, a deleterious signal could still persist. Indeed, it has been reported that ASCs from SAT of post-bariatric surgery ex-obese women do not fully recover their adipogenic potential and their cytokine secretion profile.133 Additional research is needed in order to delineate the necessary WL (partial vs. complete) to reverse the obesity-associated ASC pathological phenotype and to identify the signalling pathways associated to the restoration of ASC function that could be different in each AT depot.

6. Concluding remarks

AT is a highly heterogeneous organ and its physiological functionality depends on the interactions between adipocytes and the cells of the stromal fraction.135 The dysregulation of these interactions by the presence of individual conditioning factors (age, CVRFs, etc.) leads to AT dysfunction and metabolic disturbances. AT heterogeneity gets even more complex when ASCs are considered. Indeed, experimental studies using ASCs from both animals and humans have proved that ASCs plasticity appears as a key driver of the inherent differences in the expansion capacities of different AT depots.83

The identification of the key mechanism that triggers the restoration of AT functional and metabolic transition of the obesity-associated pro-inflammatory state by the application of WL approaches would help to develop novel therapeutic strategies for obesity and metabolic diseases. Among these potential mechanisms the restoration of ASCs functionality could play an essential role in regaining the healthy AT phenotype. In this context, further research is needed in order to delineate the exact mechanism governing ASCs plasticity.

Acknowledgements

This work has been supported by grants to L.B. from the Spanish Ministry of Economy and Competitiveness of Science [SAF2016-76819-R]; Institute of Health Carlos III, ISCIII [TERCEL RD16/0011/0018 and Ciber CB16/11/0041]; FEDER ‘Una Manera de Hacer Europa’, the Secretary of University and Research, Department of Economy and Knowledge of the Government of Catalonia [2014SGR1303]; and ‘CERCA Programme/Generalitat de Catalunya’ Spain.

Conflict of interest: none declared.

References

Inflammation, adipose tissue depots, and stem cells


