Clinical and Experimental Pharmacology and Physiology (2011) **38**, 872–878

CEPP Frontiers in Research

Frontiers in Research:

Adipokines and Cardiovascular Disease

Regulation of stem cell differentiation in adipose tissue by chronic inflammation

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SUMMARY

1. Recent studies suggest that a local hypoxic response leads to chronic inflammation in the adipose tissue of obese individuals. The adipose tissue hypoxia may reflect a compensatory failure in the local vasculature system in response to obesity.

2. Studies suggest that inflammation stimulates angiogenesis and inhibits adipocyte activities in a feedback manner within the obese adipose tissue. Adipose-derived stem cells (ASC) are able to differentiate into multiple lineages of progenitor cells for adipocytes, endothelial cells, fibroblasts and pericytes. Differentiation of ASC into those progenitors is regulated by the adipose tissue microenvironment.

3. As a major factor in the microenvironment, inflammation may favour ASC differentiation into endothelial cells through the induction of angiogenic factors. At the same time, inflammation inhibits ASC differentiation into adipocytes by suppressing peroxisome proliferator-activated receptor γ activity and the insulin signalling pathway. In this context, inflammation may serve as a signal mediating the competition between adipocytes and endothelial cells for the limited source of ASC.

4. It is a new concept that inflammation mediates signals in the competition between adipocytes and endothelial cells for the limited ASC in obesity. There is a lot of evidence that inflammation promotes endothelial cell differentiation. However, this activity of inflammation remains to be established in adipose tissue. The present article reviews the literature in support of this conclusion.

Key words: adipocytes, adipose tissue, adipose-derived stem cell, cell differentiation, endothelial cells, inflammation, obesity.

ADIPOSE TISSUE INFLAMMATION

Inflammation in obesity

Inflammation occurs in adipose tissue in obesity and exerts a broad impact on energy (glucose and fatty acids) metabolism.[1–5] Obesity-associated inflammation is characterized by increased expression of pro-inflammatory cytokines in adipose tissue and elevation of inflammatory mediators in plasma.[1,6–9] Macrophage infiltration into adipose tissue provides a strong cellular basis for the inflammatory response.[10–12] Inflammation inhibits adipocyte function by suppressing adipokine expression (such as adiponectin) and decreasing triglyceride storage. The molecular mechanisms are related to impairment of insulin signalling and suppression of peroxisome proliferator-activated receptor (PPAR) γ function (discussed below). In adipocytes, inflammation inhibits the insulin signalling pathway by targeting insulin receptor substrates (IRS).[1,13,14] Alternatively, inflammation may impair insulin action by stimulating lipolysis, which leads to the release of free fatty acids (FFA) from the adipocytes. The FFA induce insulin resistance through lipotoxicity.[15] Recent studies from our laboratory suggest that inflammation has important beneficial activities in the body through the control of energy balance and angiogenesis. Systemic inflammation in transgenic mice protects the body against obesity by inducing energy expenditure.[16,17] Local inflammation promotes angiogenesis and improves blood supply to adipose tissues through the induction of angiogenic factors.[18]

"Adipose hypoxia contributes to inflammation"

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doi: 10.1111/j.1440-1681.2011.05596.x

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Origin of inflammation

There are several hypotheses regarding the pathogenesis of obesity-associated inflammation.[19] These hypotheses include activation of toll-like receptor 4 by fatty acids, activation of protein kinase C or c-Jun N-terminal kinase (JNK) by fatty acid derivatives (diaglyceride or ceramide), endoplasmic reticulum (ER) stress, oxidative stress, activation of macrophages by dead adipocytes and activation of the NLR family, pyrin domain containing 3 (NLRP3) inflammasome by lipids.[20–23] Although these possibilities are able to explain some aspects of inflammation and provide mechanisms for metabolic disorders in obesity, their aetiology remains to be identified. It is not clear why FFA, ER stress, oxidative stress and adipocyte death are increased in obesity. In addition, it is not known why adiponectin is reduced and leptin is elevated in adipose tissue. There is not yet a consensus for a single theory to account for the metabolic and endocrine dysfunctions in white adipose tissue associated with obesity. However, the discovery of adipose tissue hypoxia (ATH) in obese mice has provided a potential unifying mechanism.

Recent reports suggest that ATH occurs in obese mice and obese patients.[24–27] Adipose tissue hypoxia represents a novel causative risk factor for the chronic inflammation in obesity.[26] Hypoxia induces inflammation via activation of two major transcription factors, hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-κB, each of which activates the transcription of a variety of angiogenic and/or pro-inflammatory cytokines (Fig. 1). The ATH not only explains the origin of inflammation in adipose tissue, but also provide a mechanism for the pathological responses in adipose tissue, such as

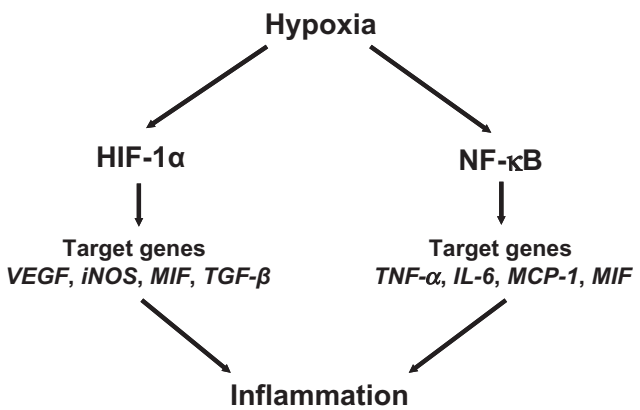

Fig. 1 Induction of an inflammatory response by hypoxia in adipose tissue. Hypoxia activates transcription factors hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-κB. Those transcription factors activate the transcription of inflammatory genes, such as inducible nitric oxide synthase (*iNOS*), macrophage migration inhibitory factor (*MIF*), transforming growth factor β (*TGF-β*), tumour necrosis factor-α (*TNF-α*), interleukin 6 (*IL-6*) and monocyte chemotactic protein 1 (*MCP-1*) among others. Expression of these genes will lead to macrophage infiltration and activation in adipose tissue.

ER stress,[25,28] oxidative stress,[29] adipocyte death,[30] adiponectin reduction[26,31] and leptin induction.[32] Thus, the concept of ATH presents an exciting and testable hypothesis underlying the mechanisms of chronic inflammation, adipose tissue dysfunction and metabolic disorders in obesity.[19]

Cause of ATH

In tissues, oxygen is delivered by the circulation via haemoglobin in red blood cells. A reduction in blood supply is a common mechanism of tissue hypoxia and this is the case for ATH. Adipose tissue blood flow (ATBF; in mL/min per g) is a measure of blood supply in adipose tissue. A reduction in ATBF was first described in 1966 when a decreased rate of radioisotope clearance was detected in the subcutaneous fat of an obese individual.[33] The reduction was confirmed in animal and human studies thereafter.[34–36] In rat models, the reduction in ATBF has been found to be the result of obesity, but not a consequence of insulin resistance. Adipose tissue blood flow was compared in two rat models of Type 2 diabetes with or without obesity.[37] Both obese Zucker rats and non-obese Goto-Kakizaki (GK) rats suffered insulin resistance. The reduction in ATBF was observed in the obese diabetic rats (obese Zucker rats), but not in the non-obese diabetic GK rats, suggesting that the reduction in ATBF is the result of obesity, not insulin resistance. However, in studies of obese humans, the reduction in ATBF has been associated with insulin resistance,[38,39] suggesting a role for reductions in ATBF in the pathogenesis of insulin resistance. In addition to the reduction in ATBF, the increase in adipocyte size associated with obesity may contribute to the hypoxic response in adipose tissue (for further discussion, see Ye *et al.*[26]). *In vivo*, oxygen can defuse approximately 120 μm through tissue.[40] When adipocyte diameters increase to (or above) 120 μm, oxygen diffusion from the capillary will be compromised. Because the diameter of an adipocyte can be 150 μm, the consequences of obesity on tissue oxygenation can be substantial.[41]

"A result of vascular failure in adipose tissue"

Capillary density was reduced in the adipose tissue of obese mice and contributed to ATH.[18] Our initial observation has been confirmed by subsequent independent studies.[27,42,43] Capillary density is determined by angiogenesis, which requires proliferation and tube formation by endothelial cell progenitors. Capillary formation is driven by angiogenic factors, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). A balance between these two angiogenic factors is required for the formation and function of new capillaries.[44] In obese mice, PDGF expression was reduced in adipose tissue, which may contribute to the compensation failure in angiogenesis.[18]

In addition to angiogenesis, a decrease in vasodilation is another possible mechanism contributing to reductions in ATBF. This

possibility is supported by literature on angiotensin (Ang) II, a serum peptide capable of inducing vasoconstriction. Angiotensin II is a component of the renin–angiotensin system (RAS) and a product of AngI after digestion by angiotensin-converting enzyme (ACE). Angiotensin II acts on both AT_1 and AT_2 receptors. In obesity, AngII activity is increased in the adipose tissue and in the circulation, thereby leading to increased vasoconstriction and reduced vasodilation. Consistent with this model, pharmacological AngII inhibitors enhance blood perfusion in adipose tissue.[45] In addition, inflammatory cytokines (such as tumour necrosis factor (TNF)-α) inhibit vasodilation via the induction of vasoconstriction.[46,47]

ADIPOSE TISSUE STEM CELLS

Cell types and adipocyte turnover

Adipose tissue contains a heterogeneous population of cells, including mature adipocytes, endothelial cells, fibroblast cells, lymphoid cells, macrophages, pericytes and pre-adipocytes or adipose-derived stromal/stem cells (ASC; see below).[48–51] The growth and expansion of adipose tissue in obesity is determined primarily by adipocyte hypertrophy and hyperplasia. Triglyceride accumulation is responsible for adipocyte hypertrophy, whereas increased adipocyte differentiation contributes to hyperplasia. Studies based on ^{14}C labelling suggest that the average adipocyte lifespan is approximately 10 years.[52] Adipocytes undergo apoptosis and necrosis at the end of their lifespan.[53,54] This type of cell death is accelerated in adipose tissue by obesity.[54] Regarding the cause of adipocyte death, we have reported that ATH contributes to both the necrosis and apoptosis of adipocytes in obesity.[30] To sustain tissue growth, new adipocytes are generated from pre-adipocytes and/or ASC to replace the dead adipocytes and to increase total adipocyte numbers in the fat pads. When the generation of new adipocytes cannot meet the demand for triglyceride storage, adipocyte hypertrophy will take place to store triglyceride through an increase in cell size. The increased adipocyte death and enlarged cell size in obesity are associated with chronic inflammation and insulin resistance. Both inflammation and hypoxia inhibit new adipocyte generation from pre-adipocyte differentiation.[30,55] Impaired compensatory vascularization contributes to hypoxia and the inflammatory response in the adipose tissue of obese individuals.[56]

''Stem cell is required for adipocyte turnover''

Adipose-derived stem cells

After digestion by collagenase, adipose tissue can be divided into two fractions, the adipocyte fraction and a stromal vascular fraction (SVF).[57,58] In the SVF, there is a plastic adherent subpopulation known as the ASC.[58] The ASC are multipotent cells that can differentiate along the adipocyte, chondrocyte, epithelial cell, hepatocyte, myocyte, neuronal-like and osteoblast lineage pathways.[57–61] *In vitro*, ASC have the capacity of self-renewal and maintain their capacity for multilineage differentiation at the clonal level, consistent with the definition of a 'stem cell'.[61–63] In part, the differentiation and proliferation of ASC are determined by the microenvironment where the stem cells are maintained (i.e. the stem cell niche). The microenvironment of the ASC is changed during chronic inflammation and ATH associated with obesity. This implies that the metabolic conditions associated with obesity have consequences potentially extending to the stem cell level.

Differentiation of ASC in adipose tissue

Inhibition of ASC differentiation into pre-adipocytes represents one mechanism by which adipocyte hyperplasia can be suppressed in adipose tissue. It has been reported that pre-adipocyte numbers are reduced in adipose tissue in obesity and that this reduction is associated with impaired glucose metabolism.[64] Pre-adipocytes derive from ASC in adipose tissue and their generation may be inhibited by obesity-associated responses. Endothelial cells are the source of paracrine factors that influence pre-adipocyte generation. Endothelial cells are required for the formation of new blood vessels that control blood supply to the adipose tissue. Endothelial progenitor cells are derived from ASC[65] or circulating bone marrow-derived cells.[66] Differentiation of endothelial progenitor cells into endothelial cells is required for the formation of new blood vessels in adipose tissue.[67–71] Inhibition of endothelial progenitor cell recruitment may lead to the suppression of adipose tissue growth and remodelling in obesity (for a review, see Daquinag *et al.*[72]). When obese murine models, such as leptin-deficient *ob/ob* mice, were treated with the anti-angiogenic compounds TNP-470 (a compound with β-cyclodextrin) or angiostatin, they exhibited a time- and dose-dependent weight loss similar to that observed with leptin administration alone.[73] Similarly, weight loss was achieved in mice treated with a peptide targeting the vascular-associated protein prohibitin in adipose tissue.[74] When this peptide was coupled to a pro-apoptotic molecule, it prevented and reversed weight gain in wild-type mice on a high-fat diet.[74]

REGULATION OF ADIPOSE TISSUE STEM CELLS BY INFLAMMATION

Inhibition of adipogenesis

Inflammation may inhibit ASC differentiation into pre-adipocytes by suppressing the transcription factor PPARγ and the insulin signalling pathway (Fig. 2). The nuclear receptor PPARγ is a lipid sensor that promotes lipid accumulation through gene transcription. Inhibition

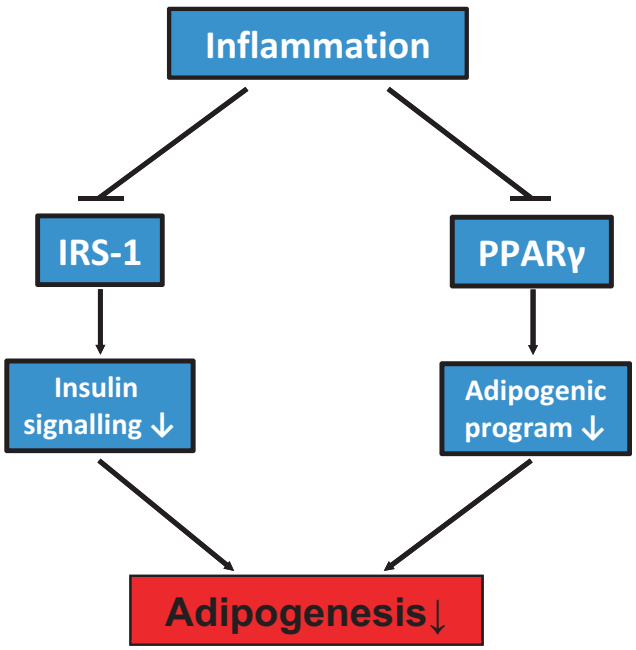

Fig. 2 Regulation of adipocyte differentiation by inflammation. Insulin receptor substrate (IRS)-1 mediates the insulin receptor signal in adipocytes for glucose and fatty acid uptake. Inhibition of IRS-1 function by inflammation leads to suppression of triglyceride synthesis in adipocytes. Inflammation inhibits peroxisome proliferator-activated receptor (PPAR) γ activity to block the transcriptional programme for adipocyte differentiation. Insulin receptor substrate-1 and PPARγ are two representative targets of inflammation in the inhibition of adipocyte differentiation.

of PPARγ activity by TNF-α leads to the suppression of adipocyte differentiation and is involved in the pathogenesis of several conditions, including insulin resistance. The activity of PPARγ is regulated by TNF-α at both the pre- and post-translational levels.[75] Activation of serine kinases, including IκB kinases (IKK), extracellular signal-regulated kinase (ERK), JNK and p38, underlies the TNF-α inhibition of PPARγ activity. Of the four kinases, IKK is a dominant signalling molecule in the regulation of PPARγ by TNF-α; IKK activates the transcription factor NF-κB, which, in turn, suppresses PPARγ activity.[75]

Insulin is required for adipocyte differentiation. Insulin activates its receptor to induce glucose and FFA uptake by adipocytes. Glucose and FFA are building materials in the biosynthesis of triglyceride, which is stored in the cytoplasm of adipocytes and is often used as a marker of adipocyte differentiation. In addition, insulin inhibits the hydrolysis of triglyceride in adipocytes by suppressing lipases.[76] When the insulin signalling pathway is impaired due to insulin resistance, triglyceride synthesis will be reduced and the hydrolysis of triglyceride will be enhanced. Under these conditions, adipocyte differentiation will be inhibited due to a lack of triglyceride. Inflammation inhibits insulin signalling by targeting IRS-1 (Fig. 2).

Molecular mechanism

There are three models for TNF-α inhibition of PPARγ,[75] as outlined below.

1. The expression of PPARγ is reduced at the mRNA level by TNF-α through the inhibition of the CCAAT box enhancer binding protein (C/EBP) family. This is observed in 3T3-L1 adipocytes treated with TNF-α for 24 h or longer.[75] The mechanism is related to inhibition of C/EBPδ expression by TNF-α. It has been shown that C/EBPδ activates the PPARγ gene promoter through direct protein–DNA interaction.[75] When C/EBPδ expression is reduced by TNF-α, PPARγ gene transcription will be suppressed.
2. There is no change in PPARγ mRNA expression, but its activity remains decreased. This mechanism was demonstrated in cells transfected with a PPARγ expression vector.[77] In this model, the ligand-dependent transcriptional activity of PPARγ is reduced as a result of loss of PPARγ DNA-binding activity. It was shown that the inhibition of DNA-binding activity was dependent on a direct association between NF-kB and PPARγ.[77]
3. The transcriptional activity of PPARγ is inhibited by TNF-α via the activation of a nuclear corepressor.[78] In this mechanism, the DNA-binding activity of PPARγ is not reduced by TNF-α itself. Instead, the DNA-bound PPARγ is inactivated by histone deacetylase 3.

"Inflammation targets PPARγ to inhibit adipogenesis"

Nevertheless, all three mechanisms are dependent on activation of the IKK/NF-κB pathway because TNF-α activity was abolished by the super repressor IκBα.[78,79] Furthermore, the inhibitory activities of TNF-α impact both PPARγ1 and PPARγ2.[77,78,80]

Tumour necrosis factor-α activates intracellular signalling pathways through cell membrane receptors. It activates many signalling pathways, such as the IKK/NF-κB, mitogen-activated protein kinase (JNK, ERK and p38) and apoptosis pathways, through its receptors.[81,82] In the absence of its activators, NF-κB stays in the cytoplasm. This inhibition is mediated by IκBα, which prevents NF-κB shuttling between the cytoplasm and nucleus.[83] Degradation of IκBα is controlled by a phosphorylation-mediated and proteasome-dependent mechanism that is initiated by activation of IKK2 (IKKβ).[84] In the TNF-α signalling pathways, activation of IKK, ERK and JNK has been reported to inhibit the transcriptional activity of PPARγ,[75] but p38 was reported to enhance the function of PPARγ.[85–88]

CONCLUSIONS

The current literature suggests that multiple cell types within adipose tissue share a common progenitor, namely the ASC. Differentiation of each lineage of progenitor cells from the ASC is modulated by the physical microenvironment within adipose tissue. In obesity, there may be a competition between these differentiation pathways for the limited number of ASC (Fig. 3). This competition has metabolic consequences when the tissue is undergoing a rapid expansion leading to obesity. Inflammation and angiogenesis are two important microenvironmental factors that determine the outcome in the competition for lineage commitment of progenitor cells. Inflammation may suppress the generation of pre-adipocytes from ASC by inhibition of adipocyte differentiation. In contrast, inflammation may promote the differentiation of ASC into endothelial precursors. As a result, the dynamics of adipocyte turnover in adipose tissue will be interrupted. This imbalance can be restored by drugs such as thiazolidinedione, which induces pre-adipocyte differentiation into adipocytes. Lack of endothelial cells impairs angiogenesis and leads to ATH, which triggers the compensatory inflammatory response. This engages a feedback response, whereby inflammation promotes ASC differentiation into endothelial cells to improve the blood supply in adipose tissue through angiogenesis. Although these possibilities are supported by considerable evidence, direct proof of the possibilities remains to be obtained in adipose tissue in obesity.

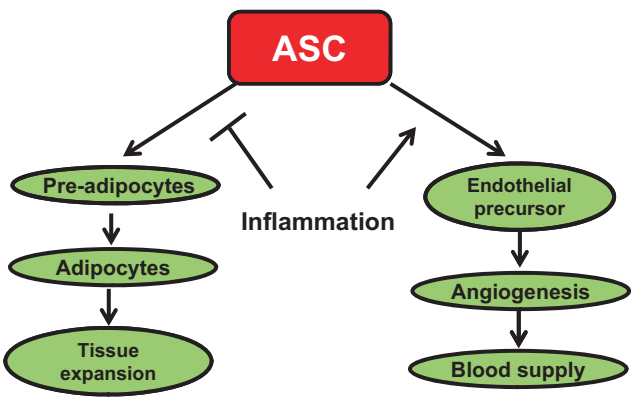

Fig. 3 Regulation of adipose-derived stromal/stem cells (ASC) differentiation by inflammation. The ASC are the common progenitors of adipocytes and endothelial cells. Inflammation inhibits ASC differentiation into adipocytes, but promotes ASC differentiation into endothelial cells. In this way, inflammation mediates the competition between adipocytes and endothelial cells for the limited source of stem cells in adipose tissue in obesity. This possibility represents a new activity of chronic inflammation in adipose tissue.

ACKNOWLEDGEMENTS

The authors' work reported herein was supported by National Institutes of Health grants (DK068036 and DK085495 to JY) and the Pennington Biomedical Research Foundation (to JG).

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