The Epicardial Adipose Tissue and the Coronary Arteries: dangerous liaisons

Running title: Epicardial Adipose Tissue and Coronary Artery Disease

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ONLINE SUPPLEMENT
Cellular aspects of human adipose depots (expanded)

Histologically, adipocytes in WAT typically have a round appearance and contain a single large lipid droplet, for which they are usually also referred as “unilocular adipocytes” 1. As a result, the nucleus in these cells is forced to the periphery together with the Golgi apparatus, smooth and rough endoplasmic reticula, free ribosomes, and few but large and elongated mitochondria, whose enzymatic equipment varies in different fat depots 1.

The non-adipocyte component of WAT, known as the “stromal vascular fraction”, contains multipotent stem cells, pre-adipocytes, fibroblasts, pericytes and endothelial cells of blood and lymphatic vessels, in addition to infiltrating inflammatory immune cells 2. In particular, the alternatively activated M2 macrophages, which work in constructive processes such as wound healing and tissue repair, as well as turn-off effectors of harmful immune system activation by producing anti-inflammatory cytokines such as interleukin (IL)-10, predominate in the WAT of lean subjects.

In contrast, the WAT of obese individuals is largely infiltrated by classically activated M1 “killer” macrophages, which secrete high levels of IL-12 and low levels of IL-10 3. A network of structured capillaries ensures the delivery of nutrients and oxygen, and the distribution of a plethora of biologic mediators, including hormones, cytokines and growth factors 4. Of note, the blood vessels in the AT not only provide nutrients and oxygen to feed the adipocytes, but also – as a result of overfeeding – they support an expansion of WAT. This occurs through the formation of new microvessels (angiogenesis) and a remodeling of the entire stromal compartment, so that many authors now consider targeting the vasculature of the adipose tissue as a promising intervention for the treatment of obesity 5.

Functionally, BAT has evolved in mammals to dissipate chemical energy in the form of heat and to help maintain normal body temperature in the newborn 6. Embryologically, brown fat cells derive from progenitors that express myogenic Myf5 and Pax7 transcription factors 7. In line with a common evolutionary relationship between BAT and muscle, brown fat precursors express muscle-like gene
signatures and a comparable mitochondrial proteomic profile. In humans, relatively large depots of BAT are found in infancy, but only small amounts persist in adults. In the latter, BAT depots are essentially distributed in the fat tissues of the neck, thorax, abdomen, in proximity to the heart, lungs, kidneys, adrenals, and the intestine, as well as along the large vessels (the aorta, the carotid arteries, as well as pulmonary and mesenteric vessels). The brown adipocytes differ from the white adipocytes by their polygonal shape, the smaller size, the central location of the nucleus, and numerous small lipid droplets, lending them a multilocular appearance (Fig. 1 Main Text). While the mitochondrial content of white adipocytes is low, brown adipocytes contain well-developed mitochondria that fill most of the cytoplasm. The abundance of mitochondria and the rich vasculature and innervation give BAT a brown color, darker than the WAT. After chronic exposure to low temperatures, the sympathetic nervous system stimulates BAT to activate the shivering thermogenesis response by activating Uncoupling Protein (UCP)-1, which mediates heat production by dissipating the proton electrochemical gradient over the inter-mitochondrial membrane space without generating ATP (Online Fig. 1). Accumulating evidence suggests that BAT is more active in humans than previously appreciated, and plays functional roles in metabolic homeostasis. The amount of BAT is inversely correlated with BMI. The cold-induced activation of human BAT increases the energy expenditure and triggers an improvement in insulin sensitivity, making BAT a potential therapeutic target to be stimulated in order to combat obesity and associated metabolic disease.

**Molecular aspects of human adipose depots**

In the early stages of adipogenesis, the same adipogenic signals trigger a common cascade of activation of the transcription factors, leading to the differentiation of both brown and white adipocyte. Among the first activated transcriptional factors are the CCAAT-enhancer-binding protein (CEBP)-β and CEBP-δ, forming a heterodimer that activates the adipogenic master regulator...
peroxisome proliferator-activated receptor (PPAR)γ\textsuperscript{19,20}. Upon activation, PPARγ promotes transcription of most adiposity-related genes, including those involved in fatty acid synthesis, lipid storage and glucose metabolism\textsuperscript{20}.

The specific brown/beige differentiation is driven by the activity of additional transcription factors and microRNAs (miRNAs), noncoding RNA molecules that regulate the post-transcriptional expression of the target mRNAs. The transcriptional co-regulator PRD1-BF-1-RIZ1 homologous Domain Member (PRMD)16 is recognized as the key driver of brown and beige cell differentiation\textsuperscript{21}. This factor primarily acts by binding to and modulating the activity of several auxiliary transcriptional factors, including C/EBPs, PPARγ, PPARα and PPARγ coactivator (PGC)-1α, up to the final induction of UCP-1 and many other specific thermogenic genes\textsuperscript{22}. Among the main key factors regulating expression and/or activity of PRDM16, are the bone morphogenetic protein (BMP)7, which works by inducing the expression of PRMD16\textsuperscript{23}; and some miRNAs, including miR-133a and miR93b-365\textsuperscript{24-26}, which – conversely – reduce the amount of PRMD16 and the following downstream thermogenic gene expression, and hence the brown and beige adipose tissue development\textsuperscript{25}.

Beige adipocytes are diffused among white adipocytes. Similarly to the BAT, beige cells are rich in mitochondria and express the thermogenic UCP-1 protein\textsuperscript{27} (Online Fig. 1). However, although brown and beige cells share the same thermogenic function, they derive from different adipocyte lineages, and show distinct gene signatures and functional and developmental characteristics (Fig. 2 Main Text). For these reasons, they must be considered as different cell types\textsuperscript{28}. Beige cells develop within subcutaneous and intra-abdominal regions from Myf5\textsuperscript{18} precursors or through transdifferentiation of mature white adipocytes\textsuperscript{18} in response to cold or β-adrenergic stimulation, which results in the increased expression of UCP-1 and in the transformation of unilocular stored fat in many small lipid droplets\textsuperscript{29}. However, while brown adipocytes express high levels of UCP-1 and other thermogenic genes under basal (unstimulated) conditions, beige adipocytes express these genes only in response to specific activators, such as β-adrenergic receptor agonists, risin, lactate and others.
(Online Fig. 1)\textsuperscript{27}. Therefore, apart from thermogenesis, it is likely that beige and brown adipocytes have other cell type-specific functions that have not yet been completely recognized.
Online Supplement References


Legend to Table 2:

SAT, subcutaneous adipose tissue; EAT epicardial adipose tissue; MAT, mediastinal adipose tissue. CCL21, C-C motif chemokine ligand 21; CXCR4: C-X-C motif chemokine receptor 4; IL7R: interleukin 7 receptor; CCL2: C-C motif chemokine ligand 2; SELL: selectin L; LTB: lymphotoxin beta; CCL18: C-C motif chemokine ligand 18; CXCL1: C-X-C motif chemokine ligand 1; CXCL5: C-X-C motif chemokine ligand 5; CCL11: C-C motif chemokine ligand 11; CXCR6: C-X-C motif chemokine receptor 6; ICAM3: intercellular adhesion molecule 3; IL1B: interleukin 1 beta; CCL8: C-C motif chemokine ligand 8; CXCL1: C-X-C motif chemokine ligand 1; THBD: thrombomodulin; CCR2: C-C motif chemokine receptor 2; CCL4: C-C motif chemokine ligand 4; ALOX5: arachidonate 5-lipoxygenase; SELPLG: selectin P ligand; CXCL2: C-X-C motif chemokine ligand 2; IL1R1: interleukin 1 receptor type 1; CCL3: C-C motif chemokine ligand 3; PLA2G2A: phospholipase A2 group IIA; SLPI: secretory leukocyte peptidase inhibitor; TIMP1: TIMP metallopeptidase inhibitor 1; HP: haptoglobin; CFB: complement factor B; CXADR Ig-like cell adhesion molecule; FAM20B: FAM20B glycosaminoglycan xylosylkinase; JCHAIN: joining chain of multimeric IgA and IgM; NOS3: nitric oxide synthase 3; CXCL8: C-X-C motif chemokine ligand 8; TCF21: transcription factor 21; CDH19: cadherin 19; SERPINA5: serpin family A member 5; ADORA1: adenosine A1 receptor; TFF3: trefoil factor 3; TRIM55: tripartite motif containing 55; KANK4: KN motif and ankyrin repeat domains 4; G0S2: G0/G1 switch 2; MRAP: melanocortin 2 receptor accessory protein; CGNL1: cingulin like 1; IGKV3D-20: immunoglobulin kappa variable 3D-20; IGLL1: immunoglobulin lambda like polypeptide 1; RARRES1: retinoic acid receptor responder 1; ACTG2: actin gamma 2, smooth muscle; HOXC6: homeobox C6; homeobox A5; CR2: complement C3d receptor 2; CETP: cholesteryl ester transfer protein; VPREB3: V-set pre-B cell surrogate light chain 3; CEMIP: cell migration inducing hyaluronidase 1; CD19: CD19 molecule; UBD: ubiquitin D; BCL11A: BAF chromatin remodeling complex subunit BCL11A; HOXB7: homeobox B7; DEFA1: defensin alpha 1; CTHRC1: collagen triple...
helix repeat containing 1; HOXC8: homeobox C8; HOXA5: homeobox A5; NNAT: neuronatin; N7SK: RNA, 7SK small nuclear; PTGDS: prostaglandin D2 synthase; AIF1: allograft inflammatory factor 1; C5AR1: complement C5a receptor 1; CCL21: C-C motif chemokine ligand 21; CCR1: C-C motif chemokine receptor 1; CD8A: CD8a molecule; CFI: complement factor I; CLEC7A: C-type lectin domain containing 7A; CYFIP2: cytoplasmic FMR1 interacting protein 2; GPR183: G protein-coupled receptor 183; S1PR3: sphingosine-1-phosphate receptor 3; FCGR2A: Fc fragment of IgG receptor IIa; FCGR3A: Fc fragment of IgG receptor IIIa; FCGR3B: Fc fragment of IgG receptor IIIb; FPR1: formyl peptide receptor 1; GPR65: G protein-coupled receptor 65; IGHM: immunoglobulin heavy constant mu; IL6: interleukin 6; IL1RN: interleukin 1 receptor antagonist; LCP2: lymphocyte cytosolic protein 2; LST1: leukocyte specific transcript 1; NCF4: neutrophil cytosolic factor 4; LYZ: lysozyme; NFIL3: nuclear factor, interleukin 3 regulated; NTN3: netrin 3; PROK2: prokineticin 2; PTGS2: prostaglandin-endoperoxide synthase 2; PTX3: pentraxin 3; S100A12: S100 calcium binding protein A12; S100A8: S100 calcium binding protein A8; S100A9: S100 calcium binding protein A9; SEMA4D: semaphorin 4D; SERPINA3: serpin family A member 3; TGM2: transglutaminase 2; TLR1: toll like receptor 1; TLR2: toll like receptor 2; TNFSF13B: TNF superfamily member 13b; SECTM1: secreted and transmembrane 1; OSM: oncostatin M; IGK: immunoglobulin kappa locus; IGKC: immunoglobulin kappa constant; IGKV1D-13: immunoglobulin kappa variable 1D-13; IGL: immunoglobulin lambda locus; COL4A4: collagen type IV alpha 4 chain; COL6A6: collagen type VI alpha 6 chain; THBS3: thrombospondin 3; LAMA2: laminin subunit alpha 2; FN1: fibronectin 1; SERPINE2: serpin family E member 2; PLAT: plasminogen activator, tissue type; ITLN1: intelectin 1; SYT4: synaptotagmin 4; EZR: ezrin; INMT: indolethylamine N-methyltransferase; PRG4: proteoglycan 4; ALOX15: arachidonate 15-lipoxygenase; KCNN3: potassium calcium-activated channel subfamily N member 3; KCNK17: potassium two pore domain channel subfamily K member 17; CXCR5: C-X-C motif chemokine receptor 5; CTSE: cathepsin E; TECRL: trans-2,3-enoyl-CoA reductase like; FLRT3: fibronectin leucine rich transmembrane protein 3; TRDN: triadin; HSPB7: heat shock protein family B (small)
member 7; C7: complement C7; OSR1: odd-skipped related transcription factor 1; ENPP2: ectonucleotide pyrophosphatase/phosphodiesterase 2; NTRK2: neurotrophic receptor tyrosine kinase 2; TBX15: T-box 15; ADRA2A: adrenoceptor alpha 2A; MCOLN3: mucolipin 3; CTSK: cathepsin K; GPC3: glypican 3; RBP7: retinol binding protein 7; CXCL14: C-X-C motif chemokine ligand 14. Orange color: upregulated genes; green color: downregulated genes.

Legend to Online Figure 1

Online Figure 1: Schematic representation of factors that promote the development and the thermogenic activity of beige adipocytes.

The list of factors that activate BAT can be divided into A) physiological stimuli [including 1) factors derived from exposure to cold, in particular norepinephrine (NE) secreted by the nervous system; 2) exercise-induced myokines, including irisin, meteorin-like (METRNL, or Glial Cell Differentiation Regulator), lactate and β-aminoisobutyric acid (BAIBA); and 3) insulin as a post-prandially secreted factor], and B) endocrine stimuli [including the thyroid hormone T3, the natriuretic peptides (NP), the fibroblast growth factor 21 (FGF21), the bone morphogenetic protein 7 (BMP7), the bone morphogenetic protein 8b (BMP8b), orexin (OX), vascular endothelial growth factor (VEGF), and prostaglandins (PGs)]. Protons (H+) are transferred from the matrix via de electron transport chain to the inner membrane space. A proton gradient across the inner membrane arises. Under coupling conditions (interrupted line - - - - -), proton flow via ATPase leads to production of ATP. Under uncoupling conditions (continued line - - - - -), proton flow via uncoupling protein 1 (UCP1) results in extra heat generation.
Online Figure 1

A: Physiological stimuli
- Cold exposure
- Exercise
- Diet, Insulin

B: Endocrine stimuli
- T3, NE, NP, FGF21, Irisin, lactate, METRNL, BAIBA BMP7, BMP8b, OX, VEGF, PGs

White adipocytes → Activated brown/brite adipocytes

Inner-membrane space
- Matrix

- ATP Synthase
- ADP → ATP
- UCP-1
- Heat production

- Respiratory chain
  - I, II, III, IV
  - Coupling
  - Uncoupling
  - H⁺ movement