New therapies to induce brown adipose tissue

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What is beauty?

Two threats

Limited food supply

Cold temperatures

Venus from Willendorf?
(~ 24,000 B.C.)
Adipose tissue “Critical link Organ”

- Energy storage & release
- Immune Function
- Endocrine Function
- Energy Management
- Reproduction
- Fit for Fight & Flight

Iwen KA, et al, Discovery Medicine 2006:75-81
Brown Fat

• One of two types of fat or adipose tissue (brown and white) found in mammals

• Especially abundant in newborns and hibernating mammals

• Its primary function is to generate body heat in animals or newborns that do not shiver.
Brown adipose tissue (BAT) vs. White adipose tissue (WAT)

<table>
<thead>
<tr>
<th>BAT</th>
<th>WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Multilocular droplets</td>
<td>• Unilocular lipid droplet</td>
</tr>
<tr>
<td>• Burning calories as heat (nonshivering thermogenesis)</td>
<td>• Store nutrients as lipids</td>
</tr>
<tr>
<td>• much higher number of mitochondria (UCP1), capillaries</td>
<td>• The excess WAT accumulation; major risk factor of IR, T2DM and CVD</td>
</tr>
<tr>
<td>• High levels of expression of PRDM16, PGC-1α and type 2 iodothyronine deiodinase (DIO2)</td>
<td></td>
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</tbody>
</table>
BAT (Brown Adipose Tissue)

- Repeated cold exposure
  \[ \rightarrow \text{increase in BAT activity in humans} \]

- BAT activity correlated with the amount of BAT
  - (Van Marken Lichtenbelt, et al. NEJM 2009;360:1518-1525)

- Actual amount of BAT found in adults
  quite low and correlates inversely with BMI and age.
The era of BAT targeting

**Late in 1970s~1980**

- Sympathetic control of BAT/ β₃ ARs in BAT and WAT
- BAT presence after chronic cold or pheo. in human
- Limitation: insensitivity of B3 AR agents, safety lack of evidence of BAT in adult human

**From pharmaceuticals to nutraceuticals**

- ephedrine, caffeine, catechin polyphenols, capsaicin, MCT, PUFA
- control of Sympathetic nerve system

- Limitation: Small effect of daily energy expenditure (50-150kcal/day)

**Late in 2000~**

- Presence of metabolically active BAT by PET/CT by relatively short exposure to mild cold processes that induce the browning of WAT (chechi, metab, 2013)

- Classic adipocytes from myogenic precursor vs brite from that close to WAT lineage (Spiegelman, Diabetes 2013)
PET/CT Scan of Brown Fat

(Zingaretti, FASEB 2009; Cypress, NEJM 2009)
The era of BAT targeting

Late in 1970s~1980
From pharmaceuticals to nutraceuticals

Late in 2000~

presence of metabolically active BAT by PET/CT

Processes that induce the browning of WAT (chechi, metab, 2013)

Classic adipocytes from myogenic precursor vs browning from that close to WAT lineage (Spiegelman, Diabetes 2013)
Induced BAT

Browning of WAT (Brown in white adipocytes) = Beige, Brite Adipocytes
Human UCP1-positive cells more closely resemble mouse beige adipocytes than mouse classic brown adipocytes

Human BAT, mainly from classic BAT or beige adipocyte?
It depends where you look

# Classic Brown vs. Beige Adipocyte

<table>
<thead>
<tr>
<th>Immunohistochemistry with anti-Ucp1</th>
<th>Location in humans</th>
<th>Location in mice</th>
<th>Developmental origin in mice</th>
<th>Enriched markers</th>
<th>Key transcription factors</th>
<th>Activators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>Neck Interscapular (newborns) (Perirenal?)</td>
<td>Interscapular Cervical Axillary Perirenal (Endocardial?)</td>
<td>Myf5⁺ cells (dermomyotome)</td>
<td>Zic1, Lhx8, Eva1, Pdk4, Epst1, miR-206, miR-133b</td>
<td>C/ebpβ, Prdm16, Pgc-1α, Ppar-α, Ebf2, TR</td>
<td>Cold, Thiazolidinediones, Natriuretic peptides, Thyroid hormone, Fgf21, Bmp7, Bmp8b, Orexin</td>
</tr>
<tr>
<td>Beige</td>
<td>Supraclavicular (Paraspinal?)</td>
<td>Interspersed within WAT subcutaneous fat &gt; visceral fat</td>
<td>Myf5⁻ cells, Pdgfr-α⁺ (perigonadal)</td>
<td>Cd137, Tbx1, Tmem26, Cited1, Shox2</td>
<td>C/ebpβ, Prdm16, Pgc-1α, (Ppar-α?)</td>
<td>Cold, Thiazolidinediones, Natriuretic peptides, (Thyroid hormone?) Fgf21, Irisin</td>
</tr>
</tbody>
</table>

Regulation of classic brown adipogenesis

Regulation of beige adipocyte

Derived from WAT precursors

*The AMPK–SIRT1–PGC-1α axis*

*The PPAR complex - PGC-1α*
Transcriptional regulation of brown and beige adipocyte development

Beige Adipocyte from a bipotential precursor cell

Two types of brown fat has been conclusively identified and shown to be functional in adult humans: classic brown and beige adipocytes.

Differences in the fat depot’s anatomical location, precursor cells and molecular signatures between brown and beige adipocytes.

Beige adipocytes are generated by both de novo recruitment from progenitor cells and transdifferentiation from white adipocytes.
Using brown adipose tissue as a therapy for obesity

- **in-vivo pharmacological approach using small molecules and growth factors**

- **ex-vivo cell-based approach**
### Mouse models resistant to weight gain through enhanced brown and beige fat activity


<table>
<thead>
<tr>
<th>Gene</th>
<th>Induces beige fat</th>
<th>Increases brown fat</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptg2s2 (also known as Cox2)</td>
<td>Yes</td>
<td>Not determined</td>
<td>Cox2-overexpressing mice have increased beige fat and are resistant to weight gain, demonstrating the role of prostaglandins in the recruitment of beige fat.</td>
</tr>
<tr>
<td>Foxc2</td>
<td>Yes</td>
<td>Yes</td>
<td>Overexpression of Foxc2 in adipose increases the expression of the R1α regulatory subunit of PKA, making the cells more sensitive to catecholamines.</td>
</tr>
<tr>
<td>Prdm16</td>
<td>Yes</td>
<td>No</td>
<td>Mice selectively transgenic for Prdm16 in fat have increased beige fat.</td>
</tr>
<tr>
<td>Pten</td>
<td>Yes</td>
<td>Yes</td>
<td>Increases in Pten levels inhibit PI3K, which drives a thermogenic program.</td>
</tr>
<tr>
<td>Ucp1</td>
<td>Yes</td>
<td>No</td>
<td>Transgenic expression of Ucp1 increases thermogenesis in WAT and prevents weight gain.</td>
</tr>
<tr>
<td>Loss-of-function models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrv2b</td>
<td>No</td>
<td>Yes</td>
<td>Neutralizing antibodies to ActRIIB lead to an increase in BAT mass without affecting WAT. Loss of ActRIIB activates Smad3 signaling to increase expression of thermogenic genes.</td>
</tr>
<tr>
<td>Adrbk1</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased core temperature and thermogenic program in BAT and WAT. Interestingly, the phenotype seems to be age related.</td>
</tr>
<tr>
<td>Acot11 (also known as Them1)</td>
<td>No</td>
<td>Yes</td>
<td>Increased expression of in thermogenic genes in BAT and a decreased expression of markers of inflammation in WAT.</td>
</tr>
<tr>
<td>Aldh1a1</td>
<td>Yes</td>
<td>No</td>
<td>Build up of retinaldehyde leads to activation the retinoic acid receptor, which recruits Pgc-1α to the Ucp1 promoter.</td>
</tr>
<tr>
<td>Arrdc3</td>
<td>Yes</td>
<td>Yes</td>
<td>Arrdc3 interacts directly with β-ARs. Loss of Arrdc3 sensitized adipocytes to catecholamines and thus increased thermogenic programs in BAT and WAT.</td>
</tr>
<tr>
<td>Atg7</td>
<td>Yes</td>
<td>Yes</td>
<td>BAT showed increased amounts of thermogenic proteins, and WAT had increased expression of thermogenic gene signatures. Studies have demonstrated a role for autophagy in adipose development.</td>
</tr>
<tr>
<td>Atf4</td>
<td>Yes</td>
<td>Yes</td>
<td>WAT showed increased expression of Pgc-1α and Ucp2, and BAT was enriched for expression of Ucp1 and Ucp3.</td>
</tr>
<tr>
<td>Bace1</td>
<td>No</td>
<td>Yes</td>
<td>Increased expression of Ucp1 in BAT and of Ucp2 and Ucp3 in skeletal muscle.</td>
</tr>
<tr>
<td>Cidea</td>
<td>No</td>
<td>Yes</td>
<td>Knockout (KO) mice are lean, have increased oxygen consumption and defend core temperature</td>
</tr>
<tr>
<td>Cidec</td>
<td>Yes</td>
<td>No</td>
<td>Increased expression of BAT-specific genes and of mitochondrial genes in WAT. The mechanism is thought to involve loss of pRb and Rip140.</td>
</tr>
<tr>
<td>Chnr1</td>
<td>Yes</td>
<td>Not determined</td>
<td>KO mice are lean. In vitro, cannabinoid receptor type 1m antagonists can induce Ucp1 transcription in white adipocytes.</td>
</tr>
<tr>
<td>Curr (also known as Chrr2)</td>
<td>Not determined</td>
<td>Yes</td>
<td>Increased glucose tolerance and increased Ucp1 expression in BAT.</td>
</tr>
<tr>
<td>Dlk1 (also known as Pref1)</td>
<td>Not determined</td>
<td>Yes</td>
<td>BAT has increased expression of Pgc-1α and Ucp1. C/ebpβ binds and activates the Pref1 promoter.</td>
</tr>
<tr>
<td>Eif4ebp1</td>
<td>Yes</td>
<td>No</td>
<td>Increased metabolic rate, induction of thermogenic genes in WAT depots and increased eIF4F phosphorylation.</td>
</tr>
<tr>
<td>Idb1</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased oxygen consumption and an increased expression of thermogenic genes in BAT. WAT has increased amounts of Ucp1 transcripts and protein.</td>
</tr>
</tbody>
</table>
Pharmacologic approach

1) Activate classic BAT

2) Induce beige adipocyte from progenitor cells
<table>
<thead>
<tr>
<th>Drug</th>
<th>Principal mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephedrine, sympathomimetics</td>
<td>Mixed sympathomimetic</td>
</tr>
<tr>
<td>BRL-26830, L-796568, N-5984</td>
<td>Selective cell-surface b3 adrenergic receptor activators</td>
</tr>
<tr>
<td>BMP family</td>
<td>Cell-surface BMP receptor activator</td>
</tr>
<tr>
<td>FGFs</td>
<td>Cell-surface FGF receptor activator signaling</td>
</tr>
<tr>
<td>Bile acids, INT-777</td>
<td>Cell-surface TGR5 receptor activator</td>
</tr>
<tr>
<td>Glitazars</td>
<td>PPAR alpha/gamma agonist</td>
</tr>
<tr>
<td>GW501516</td>
<td>PPAR delta agonist</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>SIRT1 activator</td>
</tr>
<tr>
<td>FNDC5/Irisin+</td>
<td>Myokine in response to PGC-1α activation</td>
</tr>
<tr>
<td>Orexin</td>
<td>Neurotransmitter</td>
</tr>
</tbody>
</table>

+ stimulates only specific precursor cells within the white but not the BAT
Catecholamine - induction of thermogenesis
Catecholamine-triggered responses to cold by neuronal circuit and endocrine signals

Lipid-derived hormones

• Prostaglandins: fatty acid derivatives generated from cyclooxygenase activity

• Transgenic expression of *Ptgs2* → prevent diet induced obesity
  

• Cold exposure → PG ↑
  → PGC-1α ↑ in mesenchymal progenitors

Cold exposure induces COX1 and COX2 expression in iWAT and iBAT.

Indomethacin prevents cold-induced UCP1 expression in iWAT.

Cold-induced UCP1 expression is attenuated in iWAT in COX2 KO mice.

Lipid-derived hormones

- Prostaglandins: fatty acid derivatives generated from cyclooxygenase activity

- Transgenic expression of *Ptgs2* → prevent diet induced obesity
  
  (Vegiopoulos, A. *et al.* *Science* 2010:1158–1161)

- Cold exposure → PG ↑ → PGC-1α ↑ in mesenchymal progenitors
  
  (Madsen, L. *et al.* *PLoS ONE* 2010:e11391)
New brown- and beige-fat recruiters and activators

- Irisin
- FGF-21
- Natriuretic peptides
- BAT recruiters/activators
IRISIN (the Greek Goddess Iris)
Fndc5/Irisin

• Drive brown-fat-like development of white fat and thermogenesis

• Increase in mice and humans by exercise training

• Stimulate the browning of WAT & enhance glucose tolerance

Suppresses weight gain

Irisin induces browning of white adipose tissues in vivo and protects against diet-induced obesity and diabetes.

Fndc5/Irisin

- Beneficial effect on improvement of the metabolic parameters in mice

- Still unknown
  - Irisin receptor?
  - Transcriptional process?
  - Regulated process on the cleavage of Fndc5 into irisin?
  - What effect on other tissues?
FGF-21

- Increase in glucose uptake & energy expenditure without change in food intake (Kharitonenkov et al. 2005; Coskun et al. 2008)

- The hepatic effects of FGF21 → increased fatty acid oxidation (Coskun et al. 2008)

Adapted from Gregory G, et al. Cell Metabolism 2013:333
FGF21 induces adipose thermogenic gene expression and browning of WAT.

FGF21 regulates PGC-1α protein content of adipocytes in vitro and in vivo.

FGF21-KO display an impaired response to a cold challenge.

Natriuretic peptides (ANP/BNP)

- reduce blood volume, blood pressure and CO

- In mice, promote beige adipocyte development in WAT & increase thermogenic gene expression in BAT

- In humans, ass. with weight loss

Adapted from Richard EK. Cardiovas physiol. 2007
Model for parallel β-AR and NPRA activation of p38 MAPK to trigger expression of the brown fat thermogenic gene program.

- Trigger lipolysis and browning through activation of PKG and P38

- A/BNP infusion increased $O_2$ consumption, EE, and expression of brown adipocyte markers

Candidates for BAT recruiters/activators

- **BMP7/Bmp8b**
  - Stimulate BAT growth and reduce weight gain in mice
  - Amplify thermogenic response
  - Injection into brain $\rightarrow$ sympathetic outflow and weight loss

- **Thyroid hormone**
  - induces the expression of thermogenic genes

- **Orexin**
  - Augment BAT function
    - by sympathetic outflow and BAT precursor differentiation
Secreted factors that recruit brown adipocytes, beige adipocytes or both
Hormonal control of browning

Adapted from Nature review endocrinology 2013
Ex-vivo: cell base approach

1. Biopsy progenitor cells
2. In-vivo differentiation
3. Transplant back to donor

Mesenchymal stem cell → Brown preadipocyte → Brown adipocyte → Activated brown adipocyte
Challenges

• only identified recently as having effects on the biology of brown and beige fat

→ very few studies in human cells and tissues

• still be efficiently activated even in thermoneutrality?

• rebound weight gain? or compensation?
Summary (2)

• There are now an extensive variety of factors and pathways that could potentially be targeted for therapeutic effects

• There is growing evidence from animal models showing enhancement of the function through inducing/activating brown adipocytes

• Brown adipose tissue is a critical regulator of metabolic health in mice; yet, whether induction of browning will be a promising avenue to treat metabolic disorders in humans remains unclear
Thank you