Genomics of human adipose tissue macrophages in obesity

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Obesity is one of the top 3 global social burdens

Globally $2 \text{ trillion} \text{ USD in 2012}$
## The global cost of obesity

<table>
<thead>
<tr>
<th>Location</th>
<th>Obesity</th>
<th>Est. cost of obesity Per year</th>
<th>% Health care cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong$^{1,2}$</td>
<td>20.7 %</td>
<td>1.5 million</td>
<td>5 billion HKD</td>
</tr>
<tr>
<td>China$^{3}$</td>
<td>12.9 %</td>
<td>177 million</td>
<td>24 billion Yuan</td>
</tr>
<tr>
<td>United States$^{4}$</td>
<td>39.8 %</td>
<td>93 million</td>
<td>&gt; 300 billion USD</td>
</tr>
</tbody>
</table>

$^{1}$Center for Health Protection, HK  
$^{2}$Ko Obesity Reviews 9 Suppl 1:74  
$^{3}$Qin & Pan Health Economics  
$^{4}$Centers for Disease Control and Prevention, USA  
The Commonwealth Fund
Insulin resistance
Type 2 diabetes
Fatty liver disease
Atherosclerosis
Hypertension
Stroke

Asthma
Sleep apnea
Cancer
Surgical Outcomes
Kidney Disease
Gall bladder disease

Slide courtesy C.N. Lumeng
Interventions to improve health in obesity

- Manage comorbid disease
- Weight loss

Diabetes response to medical/behavioral weight loss

Schauer et al. 2014, NEJM 370:2002
Interventions to improve health in obesity

- Manage comorbid disease
- Weight loss

Diabetes response to **surgical weight loss**

Kothari et al. 2017, SORD 13(6):972
Interventions to improve health in obesity

- Manage comorbid disease
- Weight loss

Diabetes response to **surgical weight loss**

Kothari et al. 2017, SORD 13(6):972
### Obesity in Children: Sustained Risk for Diabetes

<table>
<thead>
<tr>
<th>Duration/age of onset (N = 7,855)†</th>
<th>n (%)‡</th>
<th>BMI at 45 years (kg/m²)</th>
<th>Waist circumference (cm)</th>
<th>HbA₁c ≥7* OR (95% CI)</th>
<th>Unadjusted</th>
<th>Adjusted$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>5,819 (74.1)</td>
<td>25.1 (25.0–25.2)</td>
<td>86.9 (86.6–87.2)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Childhood only</td>
<td>62 (0.8)</td>
<td>26.3 (25.5–27.0)</td>
<td>90.6 (87.9–93.4)</td>
<td>7.01 (1.89–25.89)</td>
<td>4.95 (1.30–18.93)</td>
<td></td>
</tr>
<tr>
<td>Onset in midadulthood</td>
<td>1,171 (14.9)</td>
<td>32.5 (32.3–32.7)</td>
<td>103.5 (102.8–104.1)</td>
<td>2.99 (1.77–5.03)</td>
<td>1.13 (0.61–2.08)</td>
<td></td>
</tr>
<tr>
<td>Onset in young adulthood</td>
<td>652 (8.3)</td>
<td>35.2 (35.0–35.5)</td>
<td>109.2 (108.3–110.0)</td>
<td>16.04 (10.63–24.17)</td>
<td>3.96 (2.10–7.43)</td>
<td></td>
</tr>
<tr>
<td>Onset in childhood</td>
<td>151 (1.9)</td>
<td>37.8 (37.2–38.4)</td>
<td>113.2 (111.4–115.1)</td>
<td>23.86 (13.52–42.14)</td>
<td>4.38 (1.86–10.31)</td>
<td></td>
</tr>
</tbody>
</table>

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*Diabetes Care, volume 34, September 2011*
Overnutrition & Obesity

- Adipose Tissue Expands
- Adipocyte Insulin Resistance
- Increased FFA
- Peripheral Insulin Resistance
- Metabolic Syndrome

Inflammation
Cross Section of Adipose Tissue

- Adipocytes
- Vascular Endothelial Cells
- Macrophages (ATM)
- Caveolin
- F4/80
- Nuclei
## Adipose Tissue in Humans

### Obese Patients: Diabetic vs. Non-Diabetic
- Adult 21-60y
- BMI > 30 kg/m²
- Bariatric surgery (University of Michigan & Veterans Administration Hospital)

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=10)</th>
<th>NDM (n=18)</th>
<th>DM (n=18)</th>
<th>p-value (NDM v. DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female)</td>
<td>10%</td>
<td>44%</td>
<td>33%</td>
<td>0.733</td>
</tr>
<tr>
<td>Age (mean, years)</td>
<td>62</td>
<td>45</td>
<td>48</td>
<td>0.465</td>
</tr>
<tr>
<td>BMI (mean, kg/m²)</td>
<td>27</td>
<td>48</td>
<td>44</td>
<td>0.049</td>
</tr>
<tr>
<td>HbA1c (mean)</td>
<td>5.3%</td>
<td>5.7%</td>
<td>7.2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mean, mg/dL)</td>
<td>not done</td>
<td>95</td>
<td>144</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Comorbid conditions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep apnea</td>
<td>10%</td>
<td>61%</td>
<td>83%</td>
<td>0.264</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40%</td>
<td>56%</td>
<td>89%</td>
<td>0.060</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>40%</td>
<td>28%</td>
<td>78%</td>
<td>0.007</td>
</tr>
<tr>
<td>Average conditions/pt</td>
<td>0.9</td>
<td>1.4</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

Robert O’Rourke, MD
Diabetic Obese Patients Have Inflammatory Signatures

Adipose Tissue Dominant Gene Expression Signatures

Visceral Adipose Tissue Biological Pathway Analysis

- Diabetic vs. Non-Diabetic
- Hematopoietic cell lineage: 2.05652E-06
- Phagosome: 3.64292E-06
- Osteoclast differentiation: 0.000018546
- Chemokine signaling pathway: 4.01278E-05
- Salmonella infection: 5.87082E-05
- HTLV-I infection: 5.87082E-05
- Cytokine-cytokine receptor interaction: 5.87082E-05
- Rheumatoid arthritis: 6.39245E-05
- Tuberculosis: 6.39245E-05
- Complement and coagulation cascades: 8.08137E-05

N=10/group

iPathways (Advaita)

Slide data courtesy C.N. Lumeng
Obesity Activates Adipose Tissue Macrophages

Adipose tissue macrophage (ATM)

Adipose tissue signals in obesity

ATMs in obesity
- Increased quantity
- Altered phenotypes
- Activation of T cells

Tissue dysfunction
- Inflammation
- Diabetes
Isolating and Defining Human ATMs

**Methods**
1. Collect human adipose tissue
2. Digest tissue to single cell level
3. Isolate all non-adipocytes (called SVCs)
4. Flow cytometry analysis with macrophage markers
Patients With Metabolic Dysfunction Have Higher CD206⁺ ATMs

**Visceral Adipose Tissue**
RNA-sequencing of Human ATMs Subtypes

ATM subsets

CD206^+  
CD11c^+

CD206 APC-Cy7  
CD11c PE-Cy7

Flow  
Sort  
RNA-seq
CD206\(^+\) ATMs Diverge From Other Subsets

A. Differentially expressed genes

<table>
<thead>
<tr>
<th>Comparison</th>
<th>DE genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD206(^+) vs. CD11c(^+)</td>
<td>831</td>
</tr>
<tr>
<td>CD206(^+) vs. DP</td>
<td>656</td>
</tr>
<tr>
<td>CD11c(^+) vs. DP</td>
<td>62</td>
</tr>
</tbody>
</table>

B. Principal component analysis

C. Dendrogram

Bioinformatics Core, University of Michigan
• CD206+ ATMs are a distinct type associated with diabetes

• Can we use data to understand their function?
Biological Pathways That Distinguish CD206+ ATMs

**Significant Pathways**
- Hematopoietic cell lineage
- Regulation of actin cytoskeleton
- Tuberculosis
- Cell adhesion molecules (CAMs)
  - Cytokine-cytokine receptor interaction
- Malaria
- Jak-STAT signaling pathway
- Natural killer cell mediated cytotoxicity
- Glycolysis / Gluconeogenesis
- Endocytosis
- PPAR signaling pathway
- Phagosome
- MAPK signaling pathway
- Focal adhesion

**Cytokines**
- CD206+ DP CD11c+
  - Log 2 FPKM

**Cell metabolism**
- Glycolysis / Gluconeogenesis

**Internalization**
- HLA-DRA
- HLA-DRB
- CD84
- CD1 discriminatory
- CLEC9A
- MARCO
- FCGR2A
- HLA-DQA1
- TUBB3
- STX7
- CD74
- CD97
- LIR1

*iPathways (Advaita)*
CD206+ ATMs: Greater Expression of Genes Related to Internalization

Scavenger receptors
- CD209
- CD163L1
- MRC1
- COLEC12
- STAB1
- CD163
- MARCO
- SCARB1
- CD14
- CD36
- MSR1
- CD68
- CXCL16
- SCARB2
- CLEC7A
- LY75
- OLR1

Endocytosis
- IL2RA
- DAB2
- FOLR2
- WWP1
- AP2A2
- SNX6
- MVB12B
- PLD1
- CAV1
- CSF1R
- EPS15
- BIN1
- SH3GL1
- SNX2
- CLTC
- RABEP1
- PSD4
- WAS
- HLA-F
- FLT1
- CXCR4
- CCR5
- IL2RG

Phagosome
- CD209
- MRC1
- COLEC12
- MARCO
- NCF4
- CD14
- CD36
- FCGR2B
- FCGR2A
- STX7
- ITGB2
- TCIRG1
- HLA-F
- CORO1A
- TUBA4A
- FCAR
- OLR1

Log fold change
CD206\(^+\) ATMs:

High Expression of Antigen Presentation Gene HLA-DR

**HLA-DR\(^{hi}\) are CD206\(^+\)**

Gated on all ATMs

![Flow cytometry plots showing CD206+ ATMs have higher HLA-DR across samples.](image)

- **CD206 APC-Cy7**
- **CD11c PE-Cy7**

- **HLA-DR PE-Cy5**

Flow cytometry plots indicate that CD206+ ATMs have higher HLA-DR expression compared to CD206- ATMs, highlighting the role of CD206 in antigen presentation.
Why are CD206+ ATMs increased in human diabetes?

Cues in obesity
- Fatty acids
- Lipoproteins
- GC

↑ CD206+ ATMs
- Detect environmental cues
- Antigen presentation

➢ Tissue dysfunction
➢ Inflammation
➢ Diabetes

MODEL
Human Monocytes

- Find sites of tissue damage
- Differentiate into macrophages, dendritic cells
- Modulate inflammation
Three Subtypes of Human Circulating Blood Monocytes

Gated on Live CD45+ PBMCs

CD14 FITC

CD16 PE

Mo^{NC}

Mo^{INT}

Mo^{C}
Reprogramming Human Blood Monocytes

• Polymer nanoparticles interact with mouse monocytes through scavenger receptors*
  – PLA: Poly(lactic acid)
  – PLG: Poly(lactide-co-glycolide)

• Goal: Test interaction with human monocytes from obese patients

*Getts et al. 2014 Science Translational Medicine 6(219)
Classical Monocytes Interact Most with NPs
Human Diabetic Obese Monocytes Have Unique Interaction with Nanoparticles

Gating: all monocytes

5 μg/ml PLG $^{-20\,\text{mV}}$

Obese
$\text{OB}^{\text{DM}}$ Diabetic Obese
• Genomics characterization of human monocytes in disease
  – RNA-seq, Hi-C
  – Identify distinct functional phenotypes identified by transcription factor (TF) networks
  – Modulate phenotypes via NPs, TFs
Prevent chronic obesity!

1. Higher risk of disease: Cardiovascular, diabetes
2. Sustained disease risk in 50% of bariatric surgery patients
3. Can **permanently** damage adipose tissue
   – inflammation may not resolve with weight loss
4. Obesity can reprogram immune cells into a potentially harmful phenotype (“metabolic phenotype”)

Weight loss may not resolve damage from chronic obesity – but not known
Conclusions

Using data to understand function and programming of human immune cells

ATMs:
1. CD206+ ATMs are a distinct type that is associated with metabolic disease
2. CD206+ ATMs primary function in environment detection, internalization, and antigen presentation

Monocytes:
1. Have distinct response to obesity
2. Interact differently with nanoparticles in metabolic disease
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