Adipose tissue autophagy in obesity – Contributes to, or protects from, morbidity?

Assaf Rudich
Ben-Gurion University
Be’er-Sheva, Israel

Sunrise, Lahav
Photo: Zvia Rudich
Understanding the intriguing phenotype of the insulin-sensitive obese

BMI = ~45 Kg/m²

The insulin-sensitive obese:

* Lower systemic inflammation: Lower hsCRP, lower systemic IL-6
* Fat distribution: less Visceral fat, more superficial SC fat
* Lower fat deposition in non-adipose tissue/adipocytes (ectopic fat = fatty liver)
* Increased reliance on adipocyte hyperplasia

Hyperplasia

Hypertrophy

*Less adipose tissue inflammation and fibrosis

How does “stressed fat” become dysfunctional?

BMI = ~45 Kg/m²

Stresses:
- Oxidative
- Inflammatory
- Hypoxic
- Metabolic

Dysfunctional:
- Dys-regulated lipolysis
- Insulin resistant
- Abnormal secreted products

Human adipose tissue stress response in obesity

- Inflammatory activation
- Autophagy
- LC3
- Atg5
- ASK1 (MAP3K5)
- JNK / p38MAPK
- LC3 Atg5
- Autophagy

Endocrinology, 148:2955, 2007

“Angry fat”!!
- Stressed
- Dysfunctional
Increased protein levels of autophagy genes in omental (visceral) fat in obesity

Obese

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Questions:

1. Is autophagic flux up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

2. What is the mechanism for autophagy activation in adipose tissue in obesity?

3. What might be the functional/ (pathophysiologial) role of such activation? - Is adipose tissue autophagy “good” or “bad”?
Autophagic flux is activated (1):
more increase in LC3II, p62 and NBR1 with inhibitors

Δ = flux

Autophagic flux is activated (2):

Higher number of LC3-dots (autophagosomes) in human fat explants

**Autophagy in obesity:**
Enhanced in *human* adipose tissue, BUT Inhibited in *mouse* liver!

- Mouse Vs human difference?
- Adipose Vs liver difference?
Autophagy in liver and fat in obesity:
A tissue-difference, not a human-mouse difference

** Liver

- ** Adipose tissue

<table>
<thead>
<tr>
<th></th>
<th>Regular chow</th>
<th>High fat fed</th>
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<tbody>
<tr>
<td>Atg5</td>
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<td>Atg12</td>
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<td>β-tubulin</td>
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<tr>
<td>Atg12</td>
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</table>

**Obesity Facts 5: 710, 2012**
Autophagy in liver and fat in obesity:
A tissue-difference, not a human-mouse difference

Change in autophagy gene expression by 16w high fat feeding in mice

- Atg5
- Atg7
- LC3
- Atg12
- Beclin1

Relative mRNA levels: % change in HFF mice
Questions:

1. Is autophagic \textit{FLUX} up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

\textbf{YES} (even if oppositely-regulated in other tissues!!):

\textbf{NO}:
- Suppressed \textit{in mice}: BBRC 417, 352, 2012
Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

2. What is the mechanism for autophagy activation in adipose tissue in obesity?

3. What might be the functional/ (pathophysiological) role of such activation?
Is Atg’s mRNA increased?

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<th>SC-Obese (n=88)</th>
<th>IA-Obese (n=42)</th>
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<td>57.8 ± 1.5 *</td>
<td>59.1 ± 2.0 ^</td>
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<td>BMI (Kg/m²)</td>
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<td>34.6 ± 0.6 *</td>
<td>33.1 ± 0.8 ^</td>
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<td>Fat area (cm²)</td>
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<tr>
<td>SC</td>
<td>55.3 ± 2.8</td>
<td>851.9 ± 32.3 *</td>
<td>416.1 ± 24.1 #</td>
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<tr>
<td>IA (visceral)</td>
<td>56.6 ± 2.8</td>
<td>165.7 ± 4.4 *</td>
<td>294.5 ± 8.5 #</td>
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<td>diameter (µm)</td>
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<td>SC</td>
<td>94.2 ± 1.1</td>
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<td>OM</td>
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<td>Fasting plasma</td>
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<td>glucose (mmol/l)</td>
<td>5.8 ± 0.1</td>
<td>5.7 ± 0.1</td>
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<td>Fasting plasma</td>
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<tr>
<td>insulin (pmol/l)</td>
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<td>175.7 ± 13.3 *</td>
<td>193.8 ± 16.6 ^</td>
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<td>GIR (µmol/Kg/min)</td>
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<td>59.0 ± 2.7 *</td>
<td>37.1 ± 3.2 #</td>
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<td>HbA1C (%)</td>
<td>5.5 ± 0.1</td>
<td>5.7 ± 0.1 *</td>
<td>5.9 ± 0.1 #</td>
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</tbody>
</table>

Matthias Blüher, University of Leipzig, Germany

mRNA levels of key autophagy genes are increased in human OM fat in obesity

Is autophagy regulated transcriptionally??

Human adipose tissue stress response in obesity – inspiration from literature:

Inflammation/Stress

- E2F1
- Oncogene 27: 4860, 2008
- JBC 281: 31309, 2006

Autophagy

- LC3, Atg5
- ASK1 (MAP3K5)
- JNK / p38MAPK

“Angry fat”!!
- Stressed
- Dysfunctional

References:

Endocrinology, 148:2955, 2007
Transcriptional-based regulation of autophagy: Loss-of-function approach

MEF (fibroblasts)  MEF (adipocyte-like cells)  Oil-red O (adipocyte-like cells)

WT  E2F1-/-

MEFs: Dr. Gustavo Leone, Ohio State U, Columbus

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<th>TNFα + IL-1β</th>
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<td>E2F1-/-</td>
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<tr>
<td>E2F1-/-</td>
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E2F1

ASK1

p-JNK

LC3B

Atg12

β-actin

Yulia Haim, unpublished data

E2F1/18S, Hprt1 mRNA level (folds of control)

Normalized to β2-AS, Hprt1 (logarithmic scale)
Transcriptional-based regulation of autophagy: gain-of-function approach

<table>
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<tr>
<th></th>
<th>pCMV empty</th>
<th>pCMV-E2F1</th>
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<td><strong>Atg5</strong></td>
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<td><strong>β-actin</strong></td>
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<td><strong>Cont + Baf</strong></td>
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<tr>
<td><strong>TNFα + Baf</strong></td>
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<tr>
<td><strong>H2O2 + Baf</strong></td>
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Δ = flux

HEK 293 cells

Julia Kovsan, unpublished data
Transcriptional-based regulation of autophagy: gain-of-function approach

Control

-Baf  +Baf

TNFα

-Baf  +Baf

HEK 293 cells

Julia Kovsan, unpublished data
Sensitization to TNFα of several autophagy gene promoters by E2F1 over-expression: Dual luciferase assay

Plasmids: Kind gift from Eun-Kyeong Jo, Chungnam National University School of Medicine, Daejeon, South Korea;; Kenichi Yoshida, Meiji University, Kanagawa, Japan.

Yulia Haim, unpublished results
Higher expression of \textit{E2F1} in omental (visceral) fat associates with \textit{ASK1} and \textit{Atg5} expression (but not \textit{Ki67})

\textit{Matthias Blüher, Iris Shai, Assaf Rudich, unpublished data}

\textbf{Human samples n=500}

\textit{Matthias Blüher, Leipzig}
Protein expression of E2F1 *vis-à-vis* ASK11 and autophagy genes in human adipose tissue

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<tr>
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<td>LC3II</td>
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<tr>
<td>β-actin</td>
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<td><img src="image14.png" alt="Image" /></td>
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</table>

Correlation with E2F1

- $r=0.536$, $p<0.0001$
- $r=0.555$, $p<0.0001$

Tanya Tarnovscki, unpublished data
A chromatin immunoprecipitation (ChIP) protocol for use in whole human adipose tissue

Yulia Haim, Tanya Tarnovscki, Dana Bashari, and Assaf Rudich

**LC3B promoter:**

<table>
<thead>
<tr>
<th>BMI</th>
<th>25</th>
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<th>41</th>
<th>41</th>
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| inputs | | | | | | |
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**cyclin D1 promoter:**

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| inputs | | | | | | |
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**α-RNA pol II**

**α-ICAM**

**Figures:**

1. A graph showing the occupation of LC3 promoter by E2F1 (% of input) with BMI values ranging from 20 to 50. The graph has a linear trend line with an $r^2$ value of 0.9145 and a p-value of 0.0001.

2. Another graph showing the occupation of LC3 promoter by E2F1 (% of input) with BMI values ranging from 20 to 50. The graph has a linear trend line with an $r^2$ value of 0.03143 and a p-value of 0.6020.
2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?

**Our suggestion:**

- Transcriptionally-based;
- Direct, and/or *by sensitizing* the tissue to stress
Questions:

1. Is autophagic FLUX up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?

3. What might by the functional/ (pathophysiological) role of such activation? - Is adipose tissue autophagy “good” or “bad”?
Adipose tissue autophagy early in the course of obesity-associated cardio-metabolic morbidity

BMI = ~45 Kg/m²

BMI and age-matched, n=30 pairs

<table>
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<tr>
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<th>Insulin sensitive obese (n=30)</th>
<th>Insulin resistant obese (n=30)</th>
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<tr>
<td>Age</td>
<td>44.6 ± 0.4</td>
<td>44.9 ± 0.4</td>
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<tr>
<td>Sex (% male)</td>
<td>33</td>
<td>37</td>
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<tr>
<td>BMI (Kg/m²)</td>
<td>45.1 ± 0.2</td>
<td>45.2 ± 0.2</td>
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<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2 ± 0.0</td>
<td>5.7 ± 0.1*</td>
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<tr>
<td>Fasting plasma insulin</td>
<td>29.8 ± 2.6</td>
<td>104.7 ± 5.6*</td>
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<tr>
<td>GIR (µmol/Kg/min)</td>
<td><strong>89.4 ± 1.7</strong></td>
<td><strong>33.0 ± 2.5</strong></td>
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<td>HbA1C (%)</td>
<td>5.3 ± 0.0</td>
<td>5.7 ± 0.1*</td>
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GIR – glucose infusion rate;

- Normoglycemic
- No CV disease

Obese -/+ Insulin resistant

Omental fat autophagy activation co-appears with insulin resistance, but precedes obesity-associated morbidity!

But when it is activated – does it act to limit disease progression, or does it contribute to pathogenesis?
Activated autophagy is potentially protective:

3MA-mediated autophagy inhibition in human fat explants enhances pro-inflammatory cytokines secretion/expression.
Activated autophagy is potentially detrimental: Inhibiting adipose tissue autophagy prevents hepatocyte insulin resistance in a fat-hepatocyte co-culture system

Noa Slutsky, unpublished data
Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?

3. What might by the functional/ (pathophysiological) role of such activation? - Is adipose tissue autophagy “good” or “bad”? The “jury is still out”:

   - Autophagy may limit further adipose inflammation. But,
   - May be involved in adipocyte insulin resistance and dysfunctional fat-liver crosstalk!
Thanks to:

**BGU**
- Nava Bashan
- Iris Shai
- Yulia Haim
- Tanya Tarnovscki
- Noa Slutsky
- Ori Nov
- Ilana Harman-Boehm
- Boris Kirshtein
- Julia Kovsan

**BIU**
- Doron Ginsberg

**Leipzig, Germany**
- Matthias Blüher
- Nora Kloting

**Columbus, OH**
- Gustavo Leone

**Zurich, Switzerland**
- Daniel Konrad
- Stephan Wuest

**Daejeon, Korea**
- Eun-Kyeong Jo

**WIS**
- Zevi Elazar
- Michael Walker

**GIF, BSF, ISF, Israeli ministry of Health**

*Sde-Boker, Negev*  
*(Photo: Zvia Rudich)*

... and thanks for your attention!
Inhibition of autophago-lysosome function protects against adipocyte TNF-induced insulin resistance.
E2F1 mRNA in subcutaneous (SC) and omental (Om) human adipose tissue

Matthias Blüher, University of Leipzig, Germany
Loss of function approach to prove causality

Inflammation/Stress

E2F1

ASK1 (MAP3K5)

LC3 Atg5

Autophagy

JNK / p38MAPK
**E2F1** mRNA in subcutaneous *(SC)* and omental *(Om)* human adipose tissue

Matthias Blüher, University of Leipzig, Germany
Higher expression of E2F1 in omental (visceral) fat associates with a more morbid obese phenotype.

Matthias Blüher, Iris Shai, Assaf Rudich, unpublished data
Summary & possible conclusions:

1. “Angry fat” exhibits increased E2F1 expression, at both the mRNA and protein levels.

2. In human adipose tissue, E2F1 correlates with ASK1 and autophagy genes, and exhibits BMI-correlated increased binding to their promoters.

3. In cells, increased E2F1 expression:
   i. induces ASK1 and Atg’s expression;
   ii. plays a permissive/sensitizing role for their induction by inflammatory and oxidative stress signals.

4. In the absence of E2F1, adipocyte-like MEFs exhibit decreased basal and inflammation-induced ASK1 and Autophagy.

→ E2F1 may be a co-regulator of two arms of the adipose stress signaling cascade in obesity, activating and sensitizing them to inflammatory and oxidative stress.
What about other E2Fs?

**mRNA levels (x100, AU)**

**E2F1 (n=196)**

- Lean
- SC - Obese
- Vis - Obese

**E2F3 (n=130)**

- Lean
- SC - Obese
- Vis - Obese

**E2F4 (n=130)**

- Lean
- SC - Obese
- Vis - Obese

- Lean SC - Obese Vis - Obese

- Lean SC - Obese Vis - Obese

- Lean SC - Obese Vis - Obese

- Lean SC - Obese Vis - Obese

- Lean SC - Obese Vis - Obese

- Lean SC - Obese Vis - Obese
E2F1 over-expression:

i. activates the human ASK1 promoter,

ii. is *permissive* for inflammation-induced promoter activation.

ASK1 promoter activity in HEK293 cells

Yulia Haim, unpublished data
E2F1 binding site in the ASK1 promoter includes a -384 bp site

Mutations of a putative E2F1 binding sequence in the hASK1 promoter

Human ASK1 promoter

ASK1 promoter activity assay

Unpublished data
JNK-phosphorylation based input on ASK1 promoter activity

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<th>pCMV-E2F1</th>
<th>TNFα</th>
<th>SP 600125</th>
<th>E2F1</th>
<th>pJNK</th>
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</tbody>
</table>

Avi Shtevi, unpublished data
E2F1 and JNK phosphorylation-based activation of ASK1 promoter

TNFα

Ask1 mRNA

nucleus

C-Jun

JNK

MKK4

ASK1

SP

E2F1

AP1?
ASK1 promoter activation by over-expression of E2F1, E2F2, E2F3, E2F4 and their combinations
Stress signaling in Intra-abdominal fat in human obesity: *Ask1 is an upstream component*

MAP3K

- ASK1 (MAP3K5)
- MLK3
- Tak1

MAP2K

- MKK7
- MKK4
- MKK3
- MKK6

MAPK

- JNK
- p38MAPK
- MAPKAP2
- C-Jun

Endocrinology, 148:2955, 2007
Stress signaling in Intra-abdominal fat in human obesity: *Ask1* is an upstream component

MAP3K

- **ASK1**
- **MLK3**
- **Tak1**

MAP2K

- **MKK7**
- **MKK4**
- **MKK3**
- **MKK6**

MAPK

- **JNK**
- **p38MAPK**

**C-Jun**

**MAPKAP2**


Phosphorylation and transcription -based regulation of ASK1 (MAP3K5)

Inflammation

Oxidative stress

\( \text{Ca}^{2+} \)

\( \text{CaMKII} \)

\( \text{ER stress} \)

Cell death, differentiation, survival, cytokine production, etc.

JNK, p38MAPK

\( \text{IRB1} \)

IRE1

\( \text{TRAF2} \)

\( \text{Trx} \)

\( \text{Tr} \)

\( \text{Ask1 mRNA} \)

nucleus

D. Ginsberg: JBC 281: 31309, 2006
OM-Ask1 mRNA level is an independent predictor of whole-body insulin resistance

<table>
<thead>
<tr>
<th>(Dependent variable: Glucose infusion rate (GIR) during clamp)</th>
<th>Standard coefficients (Beta)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong>: Age-adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM-Ask1</td>
<td>-0.547</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SC-Ask1</td>
<td>-0.021</td>
<td>0.797</td>
</tr>
<tr>
<td><strong>Model 2</strong>: Adjusted for Age, Sex, BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM-Ask1</td>
<td>-0.526</td>
<td>&lt;0.001</td>
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<tr>
<td>SC-Ask1</td>
<td>0.002</td>
<td>0.983</td>
</tr>
<tr>
<td><strong>Model 3</strong>: Adjusted for Age, Sex, BMI, HDL-c, LDL-c, TG, FFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM-Ask1</td>
<td>-0.336</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SC-Ask1</td>
<td>-0.100</td>
<td>0.894</td>
</tr>
<tr>
<td><strong>Model 4</strong>: Adjusted for Age, Sex, BMI, HDL-c, LDL-c, TG, FFA, leptin, adiponectin, IL-6</td>
<td>-0.308</td>
<td>0.001</td>
</tr>
<tr>
<td>SC-Ask1</td>
<td>-0.009</td>
<td>0.913</td>
</tr>
</tbody>
</table>

Glucose: Dependent variable (infusion rate (GIR) during clamp)

Transcriptional activation of ASK1 in intra-abdominal adipocytes cell line: potential role of inflammation and oxidative stress

Unpublished data
Transcriptional activation of *ASK1* in intra-abdominal adipocytes: potential role of inflammation and oxidative stress

SGBS human pre-adipocytes

Inflammation

ER stress

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>4h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>5.0</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Intra-abdominal (mouse) adipocytes

AS*K1* / HPRT-β-actin mRNA (folds of control)

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>TNFα</th>
<th>TNFα + ActD. (1 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Unpublished data

E2F1

Ask1 mRNA

nucleus
AP is activated (3):

mRNA levels of key autophagy genes are increased in human OM fat in obesity, particularly if fat is accumulated intra-abdominally!

E2F1 is required for basal and inflammation-induced stress signaling and autophagy

WT MEFs - Fibroblasts

E2F1<sup>−/−</sup> MEFs - adipocyte like

Control

TNFα+IL-1β

Baf A. - + - + - + - +

E2F1

WT E2F1<sup>−/−</sup> WT E2F1<sup>−/−</sup>

Unpublished data
Superficial subcutaneous fat – a metabolically-safe place to store excess calories!

WC – 110 cm
- 33.6%
- 26.2%
- 38%

WC – 112 cm
- 14.5%
- 27.2%
- 53.7%

N=73, type 2 diabetic patients

Beta coefficients

- Glucose
- HbA1c
- TG

- BP
- Heart rate variability

Rachel Golan, et al., Diabetes Care 2012
Take home messages before we go on:

1. Visceral fat accumulation is associated with a more detrimental cardio-metabolic obesity sub-phenotype than accumulation of subcutaneous fat.

2. Adipose tissue expansion by adipocyte hypertrophy is more detrimental than expansion by hyperplasia.

3. Accumulation of superficial subcutaneous fat, a depot likely expanding predominantly by hyperplasia, may be cardio-metabolically protective.

4. “Angry fat” is more hypertrophic (larger cells), inflamed and fibrotic, and may become dysfunction by mounting a “tissue stress response”.

What is the molecular makeup of human adipose tissue stress response?
Autophagy in liver and fat in obesity: A tissue-difference, not a human-mouse difference

Julia Kovsan, unpublished data
AP is activated (1):
more increase in LC3II, p62 and NBR1 with inhibitors

\[ \text{Sc} \quad \text{Om} \]
- Baf+Leu - Baf+Leu

LC3 I  
LC3 II  
β actin  
p62  
NBR1  
β actin  

Fold increase in OM-p62 with autophagy inhibitors

\[ r = 0.440, \quad p = 0.030 \]

BMI (Kg/m²)

non obese  
obese  

AP is activated (2):

Higher number of LC3-dots (autophagosomes) in human fat explants

IF: LC3

Adipocyte
Non-adipocyte

Autophagy in liver and fat in obesity:
A tissue-difference, not a human-mouse difference

Unpublished data
Gain of function approach to prove causality

Inflammation/Stress

E2F1

ASK1 (MAP3K5) LC3 Atg5

Autophagy

JNK / p38MAPK