Adipose tissue hypoxia is related to increased mtDNA copies and decreased VEGF-A in fat dairy cows

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Adaption to Lactation in Dairy cows

Adapted from Yilmaz & Hotamisligil, 2013
Angiogenesis

Formation of new capillaries and blood vessels from pre-existing ones

Vascular Endothelial Growth Factor A (VEGF-A):

- key regulator of angiogenesis
- stimulates proliferation of endothelial cells
- upregulated through hypoxia in adipose tissue (AT) (Zhang et al., 1997)
- decreased VEGF-A protein amounts in fat dairy cows (Laubenthal et al., 2014)
Hypoxia in AT

Insufficient oxygen supply of a body region

Increase of intercapillary distance between adipocytes

**Hypoxia Inducible Factor-1α (HIF-1α)**

- major hypoxia marker (Lemoine et al., 2013)
- upregulated during hypoxia in obese humans and mice (Mason et al., 2007)
- induces VEGF-A in response to hypoxia (Cao et al., 2007)
Mitochondrial DNA (mtDNA) copy number

= abundance of mitochondria per cell

• modulated by physiological and environmental changes

• lipogenesis impairs mtDNA in human AT (Kaaman et al., 2007)

• increased mtDNA copy numbers in AT of overconditioned cows (Laubenthal et al., 2014)
Hypotheses

Decreasing angiogenesis in AT of overconditioned cows might lead to local hypoxia

Compensation of hypoxic condition by increasing numbers of mitochondria
Experimental design

non-lactating; non-pregnant; 4 - 6 years; n = 8

Body weight gain: 243 ± 33.3 kg

Increase of BCS: 2.31 ± 0.12 to 4.53 ± 0.14
### Material and Methods

#### HIF-1α

<table>
<thead>
<tr>
<th>Immunohistochemistry</th>
<th>mtDNA copy number/cell</th>
<th>VEGF-A</th>
<th>Adipocyte area</th>
</tr>
</thead>
<tbody>
<tr>
<td>polyclonal rabbit anti HIF-1α</td>
<td>12S rRNA gene/β-globin gene</td>
<td>monoclonal mouse anti VEGF-A /mouse anti β-actin</td>
<td>Area (µm²) of 100 randomly selected adipocytes</td>
</tr>
</tbody>
</table>

### Statistics

Non-parametric Wilcoxon-test, Spearman correlation coefficient (SPSS; mean ± SEM)
HIF-1α in AT of overconditioned cows

Local hypoxia in AT

Adipose tissue, 200x-fold

$P = 0.065$
Relationships between HIF-1α and VEGFA, mtDNA and adipocyte area

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value 1</th>
<th>Value 2</th>
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</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>0.336</td>
<td>0.240</td>
</tr>
<tr>
<td>BCS (5-point scale)</td>
<td>0.448</td>
<td>0.094</td>
</tr>
<tr>
<td>Adipocyte area (µm²)</td>
<td>0.661</td>
<td>0.007</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>0.654</td>
<td>0.008</td>
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<tr>
<td>mtDNA copy number</td>
<td>0.550</td>
<td>0.034</td>
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</tbody>
</table>

Determined as significant if $P < 0.05$

$P = 0.036$
Summary & Conclusions

**Hypoxia**

- mtDNA copies $\uparrow$
- Compensation of hypoxic state
- Improving AT mitochondrial function?

**HIF-1$\alpha$ $\uparrow$**

- VEGF-A $\downarrow$
- Impaired angiogenesis

- Dependent on time of hypoxia?
- Inhibition of AT-overexpansion?
Thanks for your attention

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<table>
<thead>
<tr>
<th>Week</th>
<th>Proportion of the daily ration (g/kg DM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Straw</td>
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<tr>
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<tr>
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<tr>
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<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7 - 16</td>
<td>0</td>
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