Effects of dietary niacin supplementation on hepatic expression of FoxO1 and genes involved in glucose production in dairy cows during the transition period

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Background

**Forkhead box protein O1 (FoxO1)**

Transcriptional factor for G6P, PCK1 (Barthel et al., 2005)
Inactivated by phosphorylation as a target of insulin signaling (Barthel et al., 2005)
Background

**glucose output**

- Glucose $\rightarrow$ G6P $\rightarrow$ Glucose-6-P
- PEP $\rightarrow$ PCK1 $\rightarrow$ OAA

**gluconeogenesis**

- Malate $\rightarrow$ PEP $\rightarrow$ OAA
- PEP $\rightarrow$ OAA
- Pyruvate $\rightarrow$ Malate

**phosphorylation**

- Insulin $\rightarrow$ FOXO1 (active)
- Insulin $\rightarrow$ pFOXO1 (inactive)

**Gene expression**

- PCK1, G6P
- Mitochondria
- Cytosol
- Nucleus

- Alanine $\rightarrow$ Propionate
Background

**Forkhead box protein O1 (FoxO1)**
- Transcriptional factor for G6P, PCK1 (Barthel et al., 2005)
- Inactivated by phosphorylation as a target of insulin signaling (Barthel et al., 2005)

**Nicotinic acid (NA)**
- Substrate for NAD, NADH (Niehoff et al., 2009)
- Lipid-lowering effect (Pires et al., 2009, Titgemeyer et al., 2011, Kenez et al., 2014)
- Affects translational and transcriptional regulation (Khan et al., 2013, 2013)
- Reduced phosphorylation of FoxO1 in rats (Choi et al, 2011)
Hypothesis & aims

Hypothesis:

NA supplements and onset of lactation affect FoxO1-mediated regulation of hepatic glucose production and the expression of downstream genes in dairy cows in transition period.

Aims:

To investigate the effects of dietary NA supplements and onset of lactation on expression and extent of phosphorylation of FoxO1 as well as mRNA expression of genes involved in glucose metabolism in dairy cows in transition period fed with diet with high or low concentrate portions.
### Study design

#### 21 pluriparous German Holstein cows

<table>
<thead>
<tr>
<th>Nicotinic acid (NA) supplementation (d-42 – d+21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA (24g/day; N=11)</td>
</tr>
<tr>
<td>Control (0g/day; N = 10)</td>
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</table>
Study design

21 pluriparous German Holstein cows

- Concentrate proportion (%): HC - CON, HC - NA
- LC - CON, LC - NA

- NA supplementation 24g/day

- Days related to calving: -42, -21, 0, 1, 7, 16, 21

- Liver biopsy

- X Liver biopsy
Study design

21 pluriparous German Holstein cows

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<td>NA (24g/day)</td>
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<tr>
<td>Prepartum: LOW concentrate proportion (30% in DM basis)</td>
</tr>
<tr>
<td>LC-NA (n=5)</td>
</tr>
<tr>
<td>Prepartum: HIGH concentrate proportion (60% in DM basis)</td>
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<tr>
<td>HC-NA (n=6)</td>
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Analysis of liver biopsy samples

Protein expression (Western Blot)
- tFoxO1: Total protein of FoxO1
- pFoxO1: Extent of phosphorylation of FoxO1 at serine 256

mRNA expression (real time-qPCR)
- FoxO1
- Insulin Receptors (IRA, IRB)
- GLUT2
- G6P, PCK1, PC, PCCA

Data evaluation
- SAS mixed model for repeated measures for effects of NA, time, and concentrate
FoxO1 Protein and mRNA expression

**Total FoxO1 Protein**

- **LC-CON**
- **HC-CON**
- **LC-NA**
- **HC-NA**

**FoxO1 mRNA**

Time: n.s.
NA: n.s.
Concentrate: n.s.
Extent of phosphorylation of FoxO1 at ser256

- LC-CON
- HC-CON
- LC-NA
- HC-NA

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<th>Days Relative to Calving</th>
<th>Extent of Phosphorylation</th>
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<tr>
<td>d-42</td>
<td>2.0 (LC-CON), 2.2 (HC-CON), 1.9 (LC-NA), 2.1 (HC-NA)</td>
</tr>
<tr>
<td>d1</td>
<td>2.1 (LC-CON), 2.3 (HC-CON), 2.0 (LC-NA), 2.2 (HC-NA)</td>
</tr>
<tr>
<td>d21</td>
<td>2.2 (LC-CON), 2.4 (HC-CON), 2.1 (LC-NA), 2.3 (HC-NA)</td>
</tr>
</tbody>
</table>

- Time: 0.08
- NA: n.s.
- Concentrate: n.s.
- NA x Concentrate: 0.04
- NA x Concentrate x Time: 0.08
mRNA expression of gluconeogenic enzymes

**PCK1**
- Days relative to calving: d-42, d1, d21
- mRNA expression
- Time: <0.01
- NA x time: 0.09

**G6P**
- Days relative to calving: d-42, d1, d21
- mRNA expression
- Time: <0.01
- NA x time: 0.01

**PC**
- Days relative to calving: d-42, d1, d21
- mRNA expression
- Time: <0.01
- NA x NA: n.s.

**PCCA**
- Days relative to calving: d-42, d1, d21
- mRNA expression
- Time: <0.01
- NA x time: 0.04

- CON (n = 10)
- NA (n = 11)
mRNA expression

GLUT2

Time: n.s.
NA x time: <0.01

-CON (n = 10)
-NA (n = 11)

IRA

Time: <0.01
NA x time: n.s.

IRB

Time: 0.04
NA x time: <0.01

mRNA expression

days relative to calving
Summary

**Insulin signaling**

- **FOXO1**
- **PCK1**
- **G6P**
- **Gluconeogenesis**
  - **G6P** → **Glucose-6-P** → **Glucose**
  - **PEP** → **OAA** → **G6P**

**Glucose output**

- **GLUT2**
- **tFOXO1** → **pFOXO1**
- **IRB** → **IRA**
- **Cytosol** → **Nucleus**
- **Mitochondria**

**No effect by time and diet**

- **PCCA** up-regulation by NA at d21

**Down-regulation by NA at d1**

- **Insulin phosphorylation**
  - **Insulin**
  - **No effect by time and diet**
  - **Mitochondria**
  - **Alanine**
  - **Malate**
  - **Pyruvate**

**Up-regulation by NA at d21**

- **Propionate**
- **PCCA**
- **PEP**
- **OAA**

**Insulin signaling**

- **Glucose**
- **Glucose output**
- **Gluconeogenesis**
Conclusion

- NA supplements appeared to induce a reduced insulin sensitivity and increased hepatic gluconeogenesis in dairy cows in transition period.
- Prepartal concentrate portion in the diet had only a marginal effect on the NA action on gene expression.
- Regulation of hepatic gluconeogenesis by FoxO1 appeared to be less important at the levels of transcription, translation and phosphorylation.
Thank you for your attention

This study was supported by Deutsche Forschungsgemeinschaft