The reduction of feed intake and gluconeogenesis during hyperketonemia in dairy cows indicates a signal of abundant energy availability.
Typical metabolic changes during negative energy balance in early lactation

Gross et al., JDS 2011
Citric acid cycle

fatty acids, glucose (adipose, feed)

acetyl-CoA

oxaloacetate

citric acid cycle

succinate

propionate

rumen: starch, sugars

ATP, NADH
Ketone bodies are increasingly synthesized because of depletion of the citric acid cycle through the use of oxaloacetate for gluconeogenesis.
During high ketone body concentration:
- reduced feed intake
- disturbed immune function

Questions:
- are the effects caused directly by ketone bodies?
- why this regulation?

Experimental approach:
Administration of BHBA to study effects
- on feed intake (Dummerstorf)
- on metabolism and immune response (Bern)
Studies in Dummerstorf

Effects of BHBA on feed intake
BHBA in Plasma and CSF in Early Lactation

The increase of plasma BHBA is partially also transferred to CSF.

Laeger et al., JDS 2013
BHBA injection into lateral brain ventricle (in vivo)

BHBA at a high dosage caused an inhibition of feed intake.

Kuhla et al., JDS 2011
BHBA application to hypothalamic neurons (in vitro)

Hypothalamic GT1-7 cells

Incubation with BHBA (6 mM)

BHBA reduced the expression of AGRP likely via inhibition of AMPK.
Effects of elevated plasma BHBA concentration through BHBA infusion (during mid-lactation, at a non-negative energy status) on metabolism and immune response during LPS-induced mastitis
• **Beta- hydroxybutyrate** (HyperB, n=5 animals)

**Clamped infusion for 56 h:**
BHBA concentration measured every 15 min, and infusion rate adjusted accordingly

Goal → **plasma BHBA concentration: 1.5 to 2.0 mmol/L**

• **NaCl** (control, n=8 animals)
  0.9 % saline solution
48-56 h of infusion:

200 \( \mu g \) of *Escherichia coli* LPS

LPS  0.9\% NaCl
BHBA inf-rate: 8.5 ± 0.6 µmol/kg/min

HyperB group: 1.74 ± 0.02 mmol/L

NaCl group: 0.59 ± 0.02 mmol/L

P < 0.001
Glucose (48 h)

**HyperB: 3.47 ± 0.11 mmol/L**  
**NaCl: 4.11 ± 0.08 mmol/L**  
*P < 0.01*

Glucose (mmol/L)

Hours relative to the start of the infusion

Time of day

Day 1  Day 2  Day 3
HyperB: 12.7± 1.4 µU/ml

NaCl: 13.9± 1.1 µU/ml

Insulin (µU/ml)

Hours relative to the start of the infusion

Time of day

Day 1

Day 2

Day 3
Plasma glucagon (pg/ml)

**NaCl**: $107.7 \pm 2.6$ pg/ml

**HyperB**: $87.6 \pm 3.42$ pg/ml

$P < 0.01$

Hours relative to the start of the infusion

Time of day

- **Day 1**: 9h, 21h
- **Day 2**: 9h, 21h
- **Day 3**: 9h
# Results

## Hormones and metabolites (means ± SEM of AUC)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>day 2, (means ± SEM)</th>
<th>ANOVA, (P-Value, group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>HyperB</td>
<td>3.47 ± 0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>4.11 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>INS, mU/L</td>
<td>HyperB</td>
<td>12.7 ± 1.4</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>13.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>BHBA, mmol/L</td>
<td>HyperB</td>
<td>1.74 ± 0.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>0.59 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>NEFA, mmol/L</td>
<td>HyperB</td>
<td>0.06 ± 0.03</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>0.09 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>HyperB</td>
<td>3.77 ± 0.31</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>3.97 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>HyperB</td>
<td>97.4 ± 3.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>107.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>IGF-1, ng/mL</td>
<td>HyperB</td>
<td>90 ± 4.5</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>80 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>HyperB</td>
<td>0.15 ± 0.01</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>0.14 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>HyperB</td>
<td>2.41 ± 0.55</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>2.62 ± 0.43</td>
<td></td>
</tr>
</tbody>
</table>
Results (plasma)

Beta-hydroxybutyrate (BHBA): infusion rate and plasma concentration

- BHBA_inf
- HyperB
- NaCl

* different between two groups
⊕ ●, different from d2 (within group)

Plasma BHBA (mmol/L)

BHBA infusion rate (µmol/kg/min)

Time after LPS challenge (min)

inf.-rate conc. 24-48h average

P < 0.0001

BHBA concentration

BHBA infusion rate

Veterinary Physiology
Glucose (LPS challenge)

Plasma glucose (mmol/L)

- HyperB
- NaCl

AUC/min (30-510):
- HyperB = 3.8 ± 0.1
- NaCl = 4.3 ± 0.1

$P < 0.05$

* different between two groups
\* different from d2 (within group)

$P = 0.03$
**Glucagon (LPS challenge)**

- **Time after LPS challenge (min)**: av. -24 to -48 h
- **Plasma glucagon (pg/ml)**
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300
  - 350
  - 400

- **AUC/min (30-510):**
  - HyperB = 161.2 ± 19.3
  - NaCl = 221.7 ± 15.3

- **P < 0.05**

- HyperB
- NaCl
- HyperB
- NaCl

- *different between two groups
- ○ different from d2 (within group)
Insulin (LPS challenge)

AUC/min (30-510):
HyperB = 34.8 ± 6.9
NaCl = 35.0 ± 5.4

* different between two groups
○, different from d2 (within group)

P = 0.97

P = 0.92

avg. -24 to -48 h
30 90 150 210 270 330 390 450 510
Plasma insulin (µU/ml)

HyperB
NaCl

AUC/min (30-510):
SCC (LPS challenge)

AUC/min (0-450):
HyperB = 6.1 \pm 0.08
NaCl = 5.7 \pm 0.06

\[ P < 0.01 \]

* \( P < 0.05 \)
(time points, between groups)
mRNA abundance (LPS)

Change of mRNA abundance

(IL-8)  

(IL-10)

Target genes

* P< 0.05
Conclusions

Reduced feed intake and gluconeogenesis during hyperketonemia: a paradox reaction to a signal of abundant energy availability?

Metabolization of ketone bodies instead of using other energy sources and to save oxaloacetat for the citric acid cycle?

If so, this adaptation does not consider the specific needs of glucose for lactose synthesis in lactating dairy cows.
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Thank you for your attention!