Metabolic stress, mitochondria and organ failure during critical illness: underlying mechanisms revealing therapeutic potential

Jan Gunst, MD, PhD
Metabolic interventions can affect outcome

Tight glycemic control with intensive insulin therapy

Early parenteral nutrition

Van den Berghe et al. NEJM 2001
Van den Berghe et al. NEJM 2006
Vlasselaers et al. Lancet 2009
Casaer et al. NEJM 2011
Study aims

- Mechanisms of organ protection by preventing hyperglycemia with insulin
- Detailed impact of early vs late PN on the kidney
Part 1: Glycemic control vs insulin & renal damage

Data published:
Kidney Int 2009;76(5):512-20
4-arm study: blood glucose and insulinenia regulated independently
Glucose vs insulin & kidney function

Plasma creatinine

Bars indicate mean + s.e.m.

§, #: p≤0.1, p≤0.05 versus control

--- --- --- --- --- : p≤0.1, p≤0.05 between sick groups
Glucose vs insulin & kidney function

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<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyc.</td>
<td>NG NG HG HG</td>
<td></td>
</tr>
<tr>
<td>Ins.</td>
<td>NI HI NI HI</td>
<td></td>
</tr>
</tbody>
</table>

R=0.546
P=0.0005
Mechanisms of organ protection

Tissue perfusion & DO$_2$

Cortical O$_2$ delivery

Complex I

Complex V

Mitochondrial function

Bars indicate mean ± s.e.m.

# : p≤0.05 versus control

--- --- : p≤0.1, p≤0.05 between sick groups

- : r=-0.211
- : p=0.24

- : r=-0.609
- : P<0.0001

- : r=-0.640
- : P<0.0001
Conclusion Part 1
Glycemic control versus Insulin & renal damage

Normoglycemia → Protection against mitochondrial damage → Renal protection

Hyperinsulinemia
Mechanisms of glucose toxicity

Glucose $\rightarrow$ Glucose-6-P $\rightarrow$ Glyceraldehyde-3-P + DHAP $\rightarrow$ Glycerate-1,3-biP $\rightarrow$ Pyruvate $\rightarrow$ Acetyl-CoA

Krebs cycle-Respiratory chain
Fatty acids/TG
Ketone bodies

GAPDH

L-lactate $\rightarrow$ D-Lactate

Glyoxalase I
Glyoxalase II

Methylglyoxal $\rightarrow$ AGES
Free adducts

Glyoxal $\rightarrow$ 3-deoxyglucosone

Mechanisms of glucose toxicity
Mitochondrial dysfunction
(Multiple) organ failure

Direct insult

Insufficient mitochondrial repair
Part 2: Role of intact mitochondrial repair in critical illness

Data published:
J Clin Endocrinol Metab 2011;96(4):E633-645
J Clin Endocrinol Metab 2012;97(1):E59-64
Mitochondrial repair: autophagy
LC3-I

Ubiquitinated substrate

p62

LC3-II

Autophagosome

Human liver

Boxes indicate median/IQR, whiskers interdecile range

*: p ≤ 0.05 versus control

Control (elective rectal surgery)

Conventional insulin therapy
Human liver

- Ubiquitinated substrate
- p62
- LC3-I

Autophagosome

p62
Ubiquitin staining
LC3-II/LC3-I ratio

Boxes indicate median/IQR, whiskers interdecile range
*: p ≤ 0.05 versus control

Control
Conventional insulin therapy
Mitochondrial repair in vivo?

Human post mortem biopsies: relation with outcome?

Rabbit model of prolonged critical illness: survivors vs. non-survivors
Autophagy ~ Outcome

Liver

p62

Relative protein expression

LC3-II/LC3-I ratio

Kidney

ALT last day (sqrt-transformed)

p62 liver (sqrt-transformed)

r=0.647
p<0.0001

Boxes indicate median/IQR, whiskers interdecile range. Organs could not be sampled in 5/18 non-surviving animals.

*, (※) : p≤0.05, 0.05<p≤0.1 vs control

Healthy

Sick-Survivor

Sick-Non Survivor

: p≤0.05, 0.05<p≤0.1 survivor vs non-survivor
Mitochondrial protection by preventing hyperglycemia (in part) explained by maintaining autophagy more efficient?

Boxes indicate median/IQR, whiskers interdecile range. Pearson correlation calculated after square root transformation of p62 and markers of organ damage

* , (•) : p≤0.05, 0.05<p≤0.1 vs control

---

**Liver**
- 3 days: Control vs Hyperglycemia: p≤0.05, 0.05<p≤0.1
- 7 days: Control vs Hyperglycemia: p≤0.05

**Kidney**
- 3 days: Control vs Hyperglycemia: p≤0.05, 0.05<p≤0.1
- 7 days: Control vs Hyperglycemia: p≤0.05
Correlation p62 protein \((x\text{-axis})\) with:

**Mitochondrial damage**
- Complex I activ. \((\text{U/g liver})\)
  - \(r=-0.692\), \(p=0.004\)
- Complex V activ. \((\text{U/g liver})\)
  - \(r=-0.671\), \(p=0.006\)
- Complex I activ. \((\text{U/g kidney})\)
  - \(r=-0.611\), \(p=0.004\)
- Complex V activ. \((\text{U/g kidney})\)
  - \(r=-0.605\), \(p=0.005\)

**Organ damage**
- AST last day \((\text{U/l})\)
  - \(r=0.808\), \(p=0.0002\)
- ALT last day \((\text{U/l})\)
  - \(r=0.606\), \(p=0.01\)
- Creatinine last day \((\text{mg/dl})\)
  - \(r=0.562\), \(p=0.02\)

Boxes indicate median/IQR, whiskers interdecile range. Pearson correlation calculated after square root transformation of p62 and markers of organ damage.

\(\ast\), \((\ast)\) : \(p \leq 0.05\), \(0.05 < p \leq 0.1\) vs control

\(---\), \(-----\) : \(p \leq 0.05\), \(0.05 < p \leq 0.1\) between sick groups
Activation of autophagy

- **p62 kidney**

- **Plasma creatinine**

- **Complex V activity kidney**

Boxes indicate median/IQR, whiskers interdecile range

*, (**: p≤0.05, 0.05<p≤0.1 vs healthy

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* : p≤0.05, 0.05<p≤0.1 between sick groups
Conclusion Part 2:
Role of intact mitochondrial repair in critical illness

Hyperglycemia \(\rightarrow\) (Parenteral) feeding\(^1\) \(\rightarrow\) Insufficient autophagy \(\rightarrow\) Mitochondrial dysfunction
\hspace{1em} \begin{align*}
\text{Organ damage} \\
\text{Adverse outcome}
\end{align*}

\(^1\) Derde et al. Endocrinology 2012
Early versus Late Parenteral Nutrition in Critically Ill Adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Late-Initiation Group (N = 2328)</th>
<th>Early-Initiation Group (N = 2312)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified RIFLE category — no. (%) ✱</td>
<td>104 (4.6)</td>
<td>131 (5.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Renal-replacement therapy — no. (%)</td>
<td>201 (8.6)</td>
<td>205 (8.9)</td>
<td>0.77</td>
</tr>
<tr>
<td>Median duration of renal-replacement therapy (interquartile range) — days</td>
<td>7 (3–16)</td>
<td>10 (5–23)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Part 3: Detailed impact of early vs late PN on AKI

Data published:
## Incidence of AKI

<table>
<thead>
<tr>
<th></th>
<th>Early PN n (%)</th>
<th>Late PN n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI (any)</td>
<td>568 (24.9)</td>
<td>565 (24.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>AKI Stage 1</td>
<td>219 (9.6)</td>
<td>197 (8.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>AKI Stage 2</td>
<td>99 (4.3)</td>
<td>107 (4.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>AKI Stage 3</td>
<td>250 (11.0)</td>
<td>261 (11.4)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

AKI stage 1 and 2 were defined as peak creatinine $\geq 1.5-2x$, respectively $1.5-2x$ baseline value. AKI stage 3 was defined as peak creatinine $\geq 2x$ baseline value OR Creatinine $>4$ mg/dl (and $\geq 0.5$ mg/dl rise) OR new renal replacement therapy
## Recovery from AKI

<table>
<thead>
<tr>
<th>AKI stage</th>
<th>Early PN</th>
<th>Late PN</th>
<th>p</th>
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<tbody>
<tr>
<td>AKI stage 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with AKI in ICU</td>
<td>1 (1-2)</td>
<td>2 (1-3)</td>
<td>0.4</td>
</tr>
<tr>
<td>AKI stage 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with AKI in ICU</td>
<td>5 (3-9)</td>
<td>4 (2-6)</td>
<td>0.04</td>
</tr>
<tr>
<td>AKI stage 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with AKI in ICU</td>
<td>12 (7-21)</td>
<td>11 (6-21)</td>
<td>0.2</td>
</tr>
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1 Data show median (interquartile range) for ICU survivors only
## Recovery from AKI

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<th>Late PN</th>
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<tr>
<td><strong>AKI stage 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with AKI in ICU(^1)</td>
<td>1 (1-2)</td>
<td>2 (1-3)</td>
<td>0.4</td>
</tr>
<tr>
<td>Alive and AKI-free at hospital discharge, n (%)</td>
<td>168 (76.7)</td>
<td>148 (75.1)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>AKI stage 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with AKI in ICU(^1)</td>
<td>5 (3-9)</td>
<td>4 (2-6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Alive and AKI-free at hospital discharge, n (%)</td>
<td>63 (63.6)</td>
<td>68 (63.6)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>AKI stage 3</strong></td>
<td></td>
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<td>0.2</td>
</tr>
<tr>
<td>Alive and AKI-free at hospital discharge, n (%)</td>
<td>86 (34.4)</td>
<td>98 (37.5)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^1\) Data show median (interquartile range) for ICU survivors only
Plasma creatinine

Creatinine clearance

Plasma urea

Urea/creatinine ratio

Early PN

Late PN

* 0.001 < p ≤ 0.01; # p ≤ 0.001 between sick groups

Bar graphs indicate mean and 95% CI

Excluded: dialyzed patients (n=428) and patients with missing samples on more than 2 consecutive days (n=584)
Nitrogen loss and balance over time in ICU

Nitrogen loss (g)

Nitrogen balance (g)

63% of extra nitrogen administration net wasted!

Early PN

Late PN

Excluded: dialyzed patients (n=428) and patients with missing samples on more than 2 consecutive days (n=584)

* 0.001<p≤0.01; # p≤0.001 between sick groups

Bar graphs indicate mean and 95% CI
Early PN:

- No major impact on incidence and recovery of AKI prolonged stage 2 AKI?

- Inefficient to reverse the negative nitrogen balance
  Increased ureagenesis
  prolonged duration of renal replacement therapy?  
  (as supported by multiple regression-data not shown)
Critical illness

- Mitochondrial abnormalities

- Insufficient autophagy

  → Multiple organ failure

  → Hypercatabolism

  → Mortality risk
Critical illness

Hyperglycemia

oxidative stress
dicarbonyls

mitochondrial abnormalities

Insufficient autophagy

Multiple organ failure

Hypercatabolism

Mortality risk
Future perspectives

Open perspectives for therapies that **activate autophagy** in critical illness,
to stimulate damage removal,
especially in combination with therapies that are able to
effectively **suppress excessive catabolism** of healthy, lean tissue.
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