Nutrients, insulin and muscle wasting during critical illness

Sarah Derde
Introduction

- Critical illness: feeding-resistant hypercatabolism
- Imbalance between protein synthesis and breakdown
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  - Imbalance between protein synthesis and breakdown

- Skeletal muscle = main protein source
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Severe muscle weakness
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\[\text{Severe muscle weakness}\]

- rehabilitation: delayed
- mortality risk $\uparrow$
- after hospital discharge: quality of life $\downarrow$
Muscle weakness

- Myofibrillar protein
  - synthesis
  - breakdown

- Ubiquitin proteasome system
- Autophagy

Introduction
Protein degradation pathways:
Ubiquitin-proteasome system

Short-lived proteins
myofibrils

Muscle wasting

MuRF-1
Atrogin-1

Lysosomal system

Introduction
Protein degradation pathways: 
**Autophagy**

Degradation of own cellular components within lysosomes

1. Isolation membrane
2. Autophagosome
3. Autolysosome

- removing damaged proteins
- energy supply

+ accumulation toxic protein aggregates

Introduction
Role of autophagy in muscle wasting?

- Excessive activation → could aggravate muscle wasting
- Impairment/inhibition → could evoke atrophy and myopathy
  - muscle fiber degeneration
  - muscle weakness

- Effect of autophagy during prolonged critical illness?
Inhibitors of catabolism

- Insulin
  - Insulin resistance
- Nutrients
  - Dysfunctional gastro-intestinal tract
  - Intravenous nutrition: ineffective to safely counteract hypercatabolism

Introduction
General hypothesis

**Intravenous nutrition**, while maintaining **normoglycemia**, safely counteracts muscle wasting during prolonged critical illness.
Objectives

1. Effect of strict blood glucose control with intensive insulin therapy on muscle wasting in fed critically ill patients

2. Efficacy in counteracting protein degradation and safety of intravenous nutritional interventions in an animal model

- Effect of fasting versus intravenous glucose load

- Impact of altering nutritional substrate composition
Hypothesis I

Muscle atrophy in fed critically ill patients can be attenuated by intensive insulin therapy

Vanhorebeek et al, J Clin Endocrinol Metab 2011, 96:E633-E645

Study 1: Insulin therapy and muscle wasting
Patient population

2 randomized controlled studies

Surgical ICU
1548 patients

Medical ICU
1200 patients

Non-survivors
75/98

Non-survivors
69/306

ICU stay >14 days
64/252

Blood glucose control

insulin therapy

Conventional
≤ 215 mg/dl

Intensive
80-110 mg/dl

m. Rectus abdominis biopsy

m. Vastus lateralis biopsy

Study 1: Insulin therapy and muscle wasting
- Muscle protein synthesis
- Myofiber size and morphology
- Muscle protein degradation

- Study 1: Insulin therapy and muscle wasting
Synthesis of myofibrillary proteins

MyHC-I  MyHC-IIa  actin

Myosin/actin

* : P ≤ 0.05 versus control

Study 1: Insulin therapy and muscle wasting
Myofiber size: fiber cross-sectional area

Study 1: Insulin therapy and muscle wasting
Morphological analysis of skeletal muscle:

**Rectus abdominis**

<table>
<thead>
<tr>
<th>Inflammation and/or necrosis (n, %)</th>
<th>Present</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 (0)</td>
<td>0.09</td>
</tr>
<tr>
<td>CIT</td>
<td>20 (28)</td>
<td></td>
</tr>
<tr>
<td>IIT</td>
<td>16 (32)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presence of adipocytes (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>CIT</td>
</tr>
<tr>
<td>IIT</td>
</tr>
</tbody>
</table>

Study 1: Insulin therapy and muscle wasting
Myofiber degeneration

Control
Conventional insulin therapy
Intensive insulin therapy

Vacuolization

Myofibers with centralized nuclei

* : p ≤ 0.05 versus control
// (*) : p ≤ 0.1 versus control
// § : p ≤ 0.05  sick versus control

Study 1: Insulin therapy and muscle wasting
Protein degradation: autophagy

Vacuolization ↑

Impaired autophagy

![Graph showing ubiquitin and p62 levels](image)

- *: p ≤ 0.05 versus control
- ($) : p ≤ 0.1 sick versus control

Study 1: Insulin therapy and muscle wasting
Protein degradation: results

**Ubiquitin-proteasome system**

- **MuRF-1**
  - Healthy control
  - Conventional insulin therapy
  - Intensive insulin therapy

- **Atrogin-1**
  - Healthy control
  - Conventional insulin therapy
  - Intensive insulin therapy

**20 S proteasome activity**

- Healthy control
- Conventional insulin therapy
- Intensive insulin therapy

* : p ≤ 0.05 versus control
(§): P ≤ 0.1 sick versus control

Protein degradation: results
Conclusions

### Prolonged critical illness

| gene expression myofibrillary proteins | ↓ |
| myosin/actin ratio | ↓ |
| Myofiber size | ↓ |
| autophagy | ↓ |
| 20 S proteasome | ↑ |

Study 1: Insulin therapy and muscle wasting
2. Efficacy to attenuate protein degradation and safety of intravenous nutrition in an animal model of prolonged critical illness
Hypothesis 1

Increasing the intravenous glucose load, within the physiological range and while maintaining normoglycemia, safely counteracts muscle catabolism

Derde et al, Crit Care Med 2010, 38:602-611
Experimental setup 1

Study 2: increasing the intravenous glucose load

- Baseline glucose levels
- Randomisation
- Third degree burn
- Fluid resuscitation

Blood glucose levels:
- Baseline: 60-110 mg/dl
- Hyperglycemia: 250-350 mg/dl

Kcal/day

- Low
- Moderate
- High
- High

- (-)
- Low
- Moderate
- High

Study 2: increasing the intravenous glucose load
Safety evaluation: survival & organ function

- Muscle protein degradation

Study 2: increasing the intravenous glucose load
Safety: Survival and organ function

Study 2: increasing the intravenous glucose load

- Survival:
  - glucose load: (-), Low, Moderate, High
  - glycemia: Normal, High

- Liver (ALT)

- Kidney (Creatinine)

- Myocardium (HFABP)

§: p < 0.05 versus all other groups
* : p < 0.05 versus high/hg rabbits
#: p < 0.05 versus healthy control rabbits

Healthy reference range

Study 2: increasing the intravenous glucose load
Muscle proteolysis

**Weight loss**

<table>
<thead>
<tr>
<th>Glucose Load</th>
<th>Glycemia</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>(-)</td>
<td>Normal</td>
<td>13</td>
</tr>
<tr>
<td>Low</td>
<td>Normal</td>
<td>13</td>
</tr>
<tr>
<td>Moderate</td>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>Normal</td>
<td>12</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>8</td>
</tr>
</tbody>
</table>

% change from baseline

-80
-40
0
40
80
120

*
$: p < 0.05 versus high/hg rabbits // @: p < 0.05 versus moderate/ng rabbits // &: p < 0.05 versus no/ng rabbits // #: p < 0.05 versus healthy control rabbits // +: p < 0.05 versus low/ng rabbits // §: healthy reference range // §: p < 0.05 versus all other groups

**Urea**

% change from baseline

-120
-80
-40
0
40
80
120

*:
@:
$: p < 0.05 versus high/hg rabbits

Study 2: increasing the intravenous glucose load
Muscle proteolysis: Ub-proteasome

Study 2: increasing the intravenous glucose load

* p < 0.05 versus high/hg rabbits
@ p < 0.05 versus moderate/ng rabbits
$ p < 0.05 versus no/ng rabbits
# p < 0.05 versus healthy control rabbits
+: p > 0.05 versus low/ng rabbits
/ : healthy reference range
Conclusion

- Increasing intravenous glucose load within physiological range while normoglycemia is maintained
  - Safe with regard to organ function and survival
  - Reduces biochemical markers of catabolism as compared with fasting
  - Optimum may be reached with moderate glucose intake

- High glucose load / hyperglycemia: protective effect nutrition

- Study 2: increasing the intravenous glucose load
Hypothesis 3

Impact of feeding on the catabolic pathways may be nutrient-specific

Derde et al, Endocrinology. 2012 May;153(5):2267-76

Study 3: altering substrate composition
Experimental setup 2

- randomisation
  - Anaesthesia
  - Placing catheters
  - Third degree burn wound
  - Fluid resuscitation

3 ml blood sample

<table>
<thead>
<tr>
<th>Day -1</th>
<th>Day 0, Instrumentation</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
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Continuous parenteral nutrition according to randomization

Titrating glucose levels to normoglycemia

Study 3: altering substrate composition

- F
- G
- AA
- L
- Control

mean bloodglucose (mg/dl)

(mean bloodglucose (mg/dl))

- Glucose
- Amino acids
- Lipids

Study 3: altering substrate composition
- Safety evaluation: survival & weight loss
- Muscle fiber size: cross-sectional area
- Muscle protein degradation
  - Ubiquitin proteasome pathway
  - Autophagy
Safety: Survival and weight loss

- Survival: no mortality difference among groups

- Feeding attenuated weight loss observed in fasted critically ill rabbits

- Study 3: altering substrate composition
Muscle fiber size: cross-sectional area

cross sectional area (pixels²)

- fasted critically ill
- fed critically ill, extra glucose
- fed critically ill, extra lipids
- fed critically ill, extra amino acids
- healthy reference range

Study 3: altering substrate composition
Muscle proteolysis: Ub-proteasome

Study 3: altering substrate composition

- *: p ≤ 0.05 versus healthy control rabbits
- †: p ≤ 0.05 versus fasted critically ill rabbits
- ‡: p ≤ 0.05 versus critically ill rabbits from the glucose group

- Fasted critically ill
- Fed critically ill, extra glucose
- Fed critically ill, extra lipids
- Fed critically ill, extra amino acids
- Healthy reference range
Muscle proteolysis: autophagy

Study 3: altering substrate composition

Rabbits with severe vacuolization

* : p ≤ 0.05 versus healthy control rabbits; † p ≤ 0.05 versus fasted critically ill rabbits; ‡ p ≤ 0.05 versus critically ill rabbits from the glucose group
Conclusions

- Moderate amount of intravenous nutrition
  - Suppression atrophy at level of gene expression and activity
    - minor effect of nutrient composition: AA most effective?
    - reduced fiber size most preserved with extra AA
  - Suppression of autophagy
    - accumulation toxic protein aggregates /damaged organelles in skeletal muscle most pronounced with AA
  - Most anti-catabolic intervention (AA) may have been most toxic

- Study 3: altering substrate composition
General conclusion and perspectives

- Prevention of hyperglycemia in the fed condition is crucial to prevent mortality

- Intravenous nutrition while maintaining normoglycemia catabolism

  BUT possible toxic side effects by inhibition autophagy!! clinical setting?

- Pharmacological intervention to stimulate autophagy
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