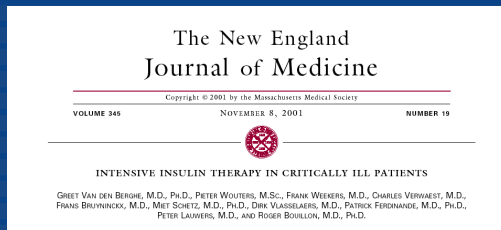


# Metabolic stress, mitochondria and organ failure during critical illness: underlying mechanisms revealing therapeutic potential

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:*

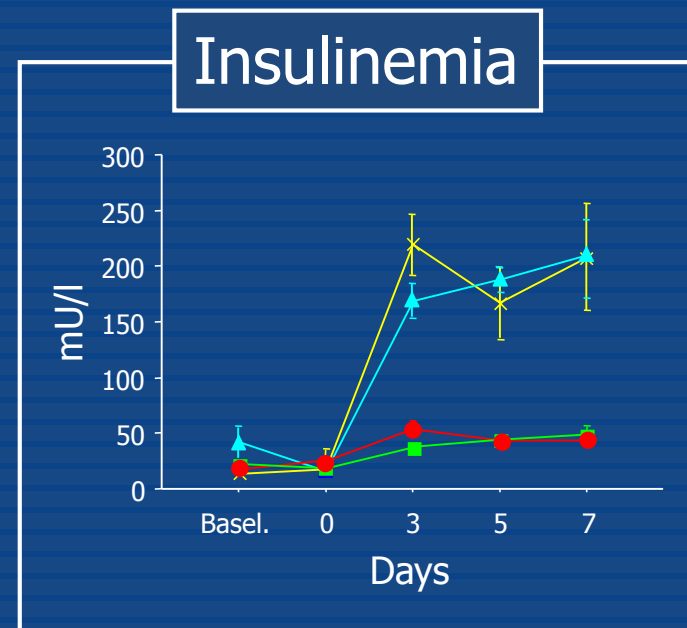
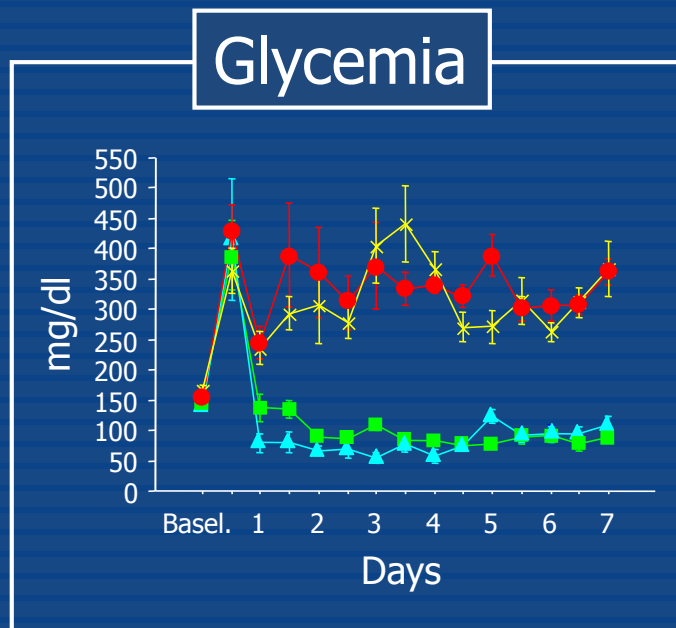
Kidney Int 2009;76(5):512-20

4-arm study:

# blood glucose and insulinemia regulated independently

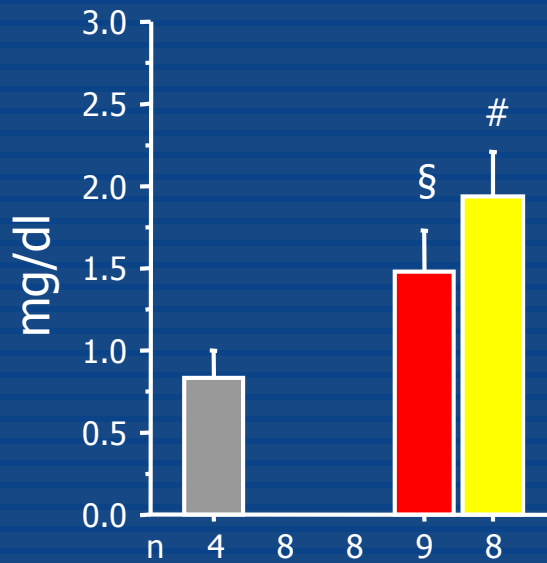
Normoinsulinemia/Normoglycemia  
Hyperinsulinemia/Normoglycemia  
Normoinsulinemia/Hyperglycemia  
Hyperinsulinemia/Hyperglycemia

■ NI/NG  
▲ HI/NG  
● NI/HG  
× HI/HG



# Glucose vs insulin & kidney function

## Plasma creatinine



	Control	Sick			
Glyc.		NG	NG	HG	HG
Ins.		NI	HI	NI	HI

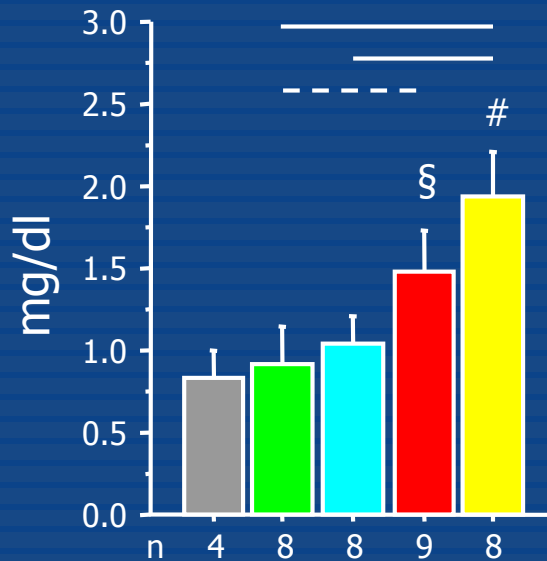
Bars indicate mean + s.e.m.

§, #:  $p \leq 0.1$ ,  $p \leq 0.05$  versus control

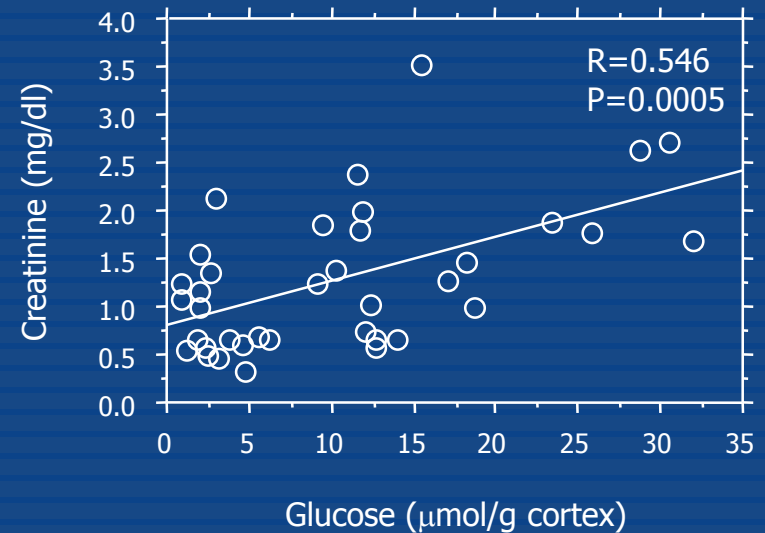
--- — :  $p \leq 0.1$ ,  $p \leq 0.05$  between sick groups

# Glucose vs insulin & kidney function

## Plasma creatinine



	Control	Sick
Glyc.		NG NG HG HG
Ins.		NI HI NI HI



Bars indicate mean + s.e.m.

§, #:  $p \leq 0.1$ ,  $p \leq 0.05$  versus control

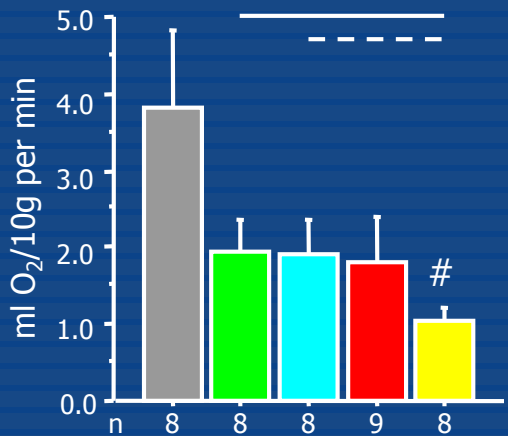
--- — :  $p \leq 0.1$ ,  $p \leq 0.05$  between sick groups

# Mechanisms of organ protection

## Tissue perfusion & DO<sub>2</sub>

## Mitochondrial function

### Cortical O<sub>2</sub> delivery



Control

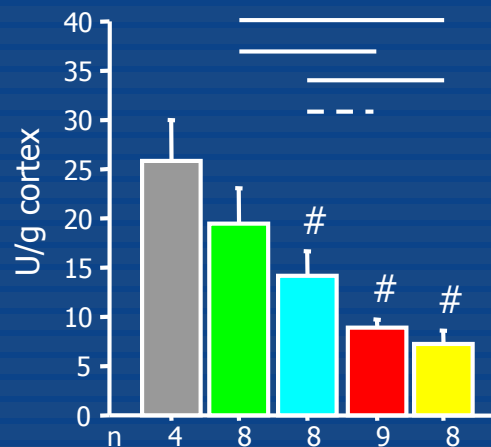
NI/NG

HI/NG

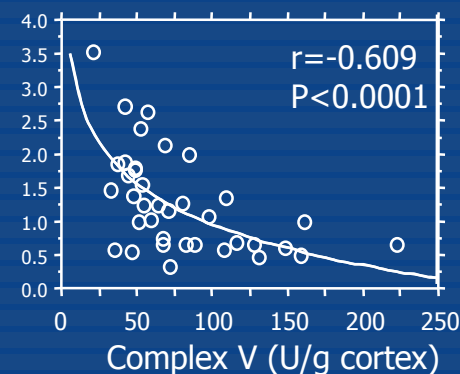
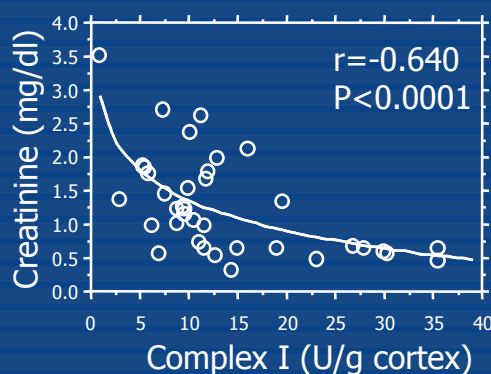
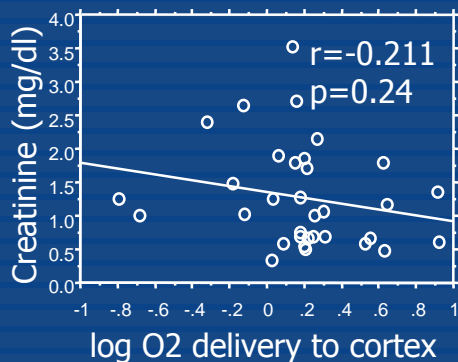
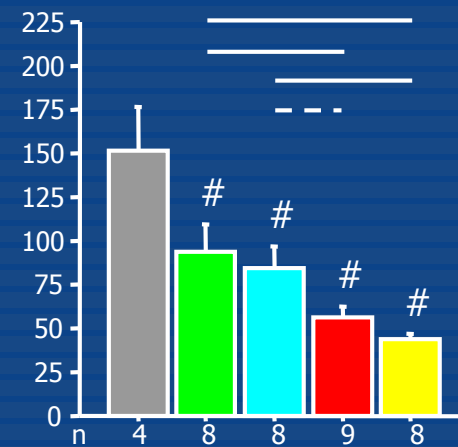
NI/HG

HI/HG

### Complex I



### Complex V



# :  $p \leq 0.05$  versus control

--- — :  $p \leq 0.1$ ,  $p \leq 0.05$  between sick groups

Bars indicate mean + s.e.m.

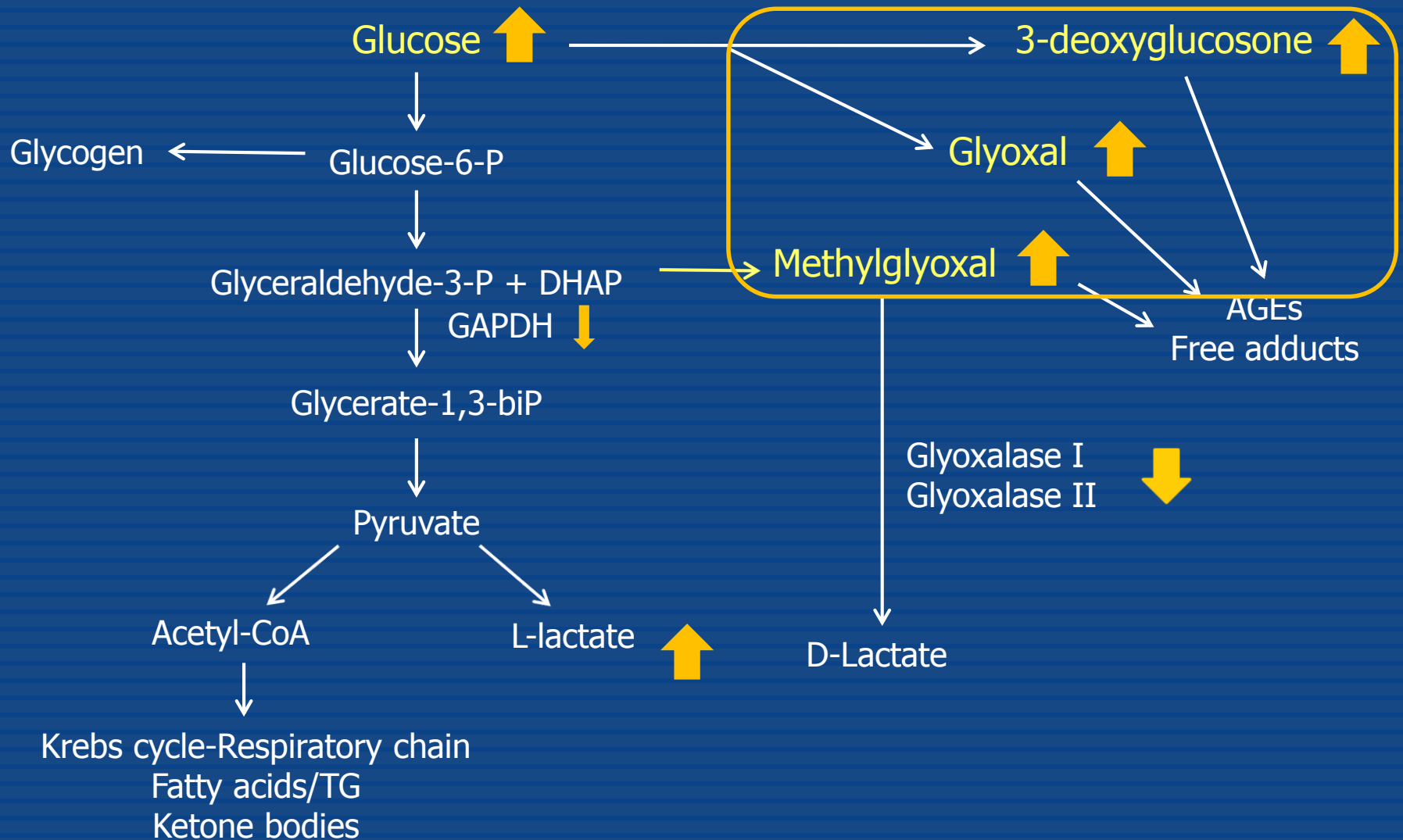


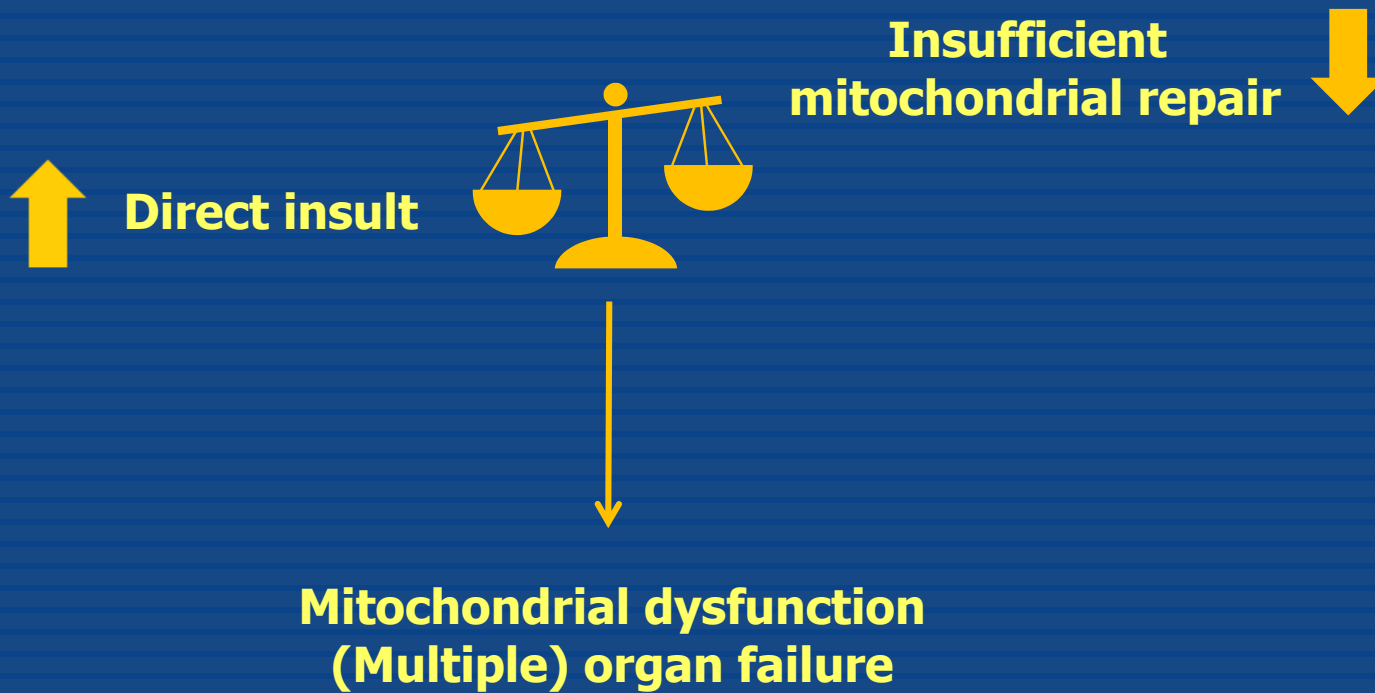
# Conclusion Part 1

## Glycemic control versus Insulin & renal damage

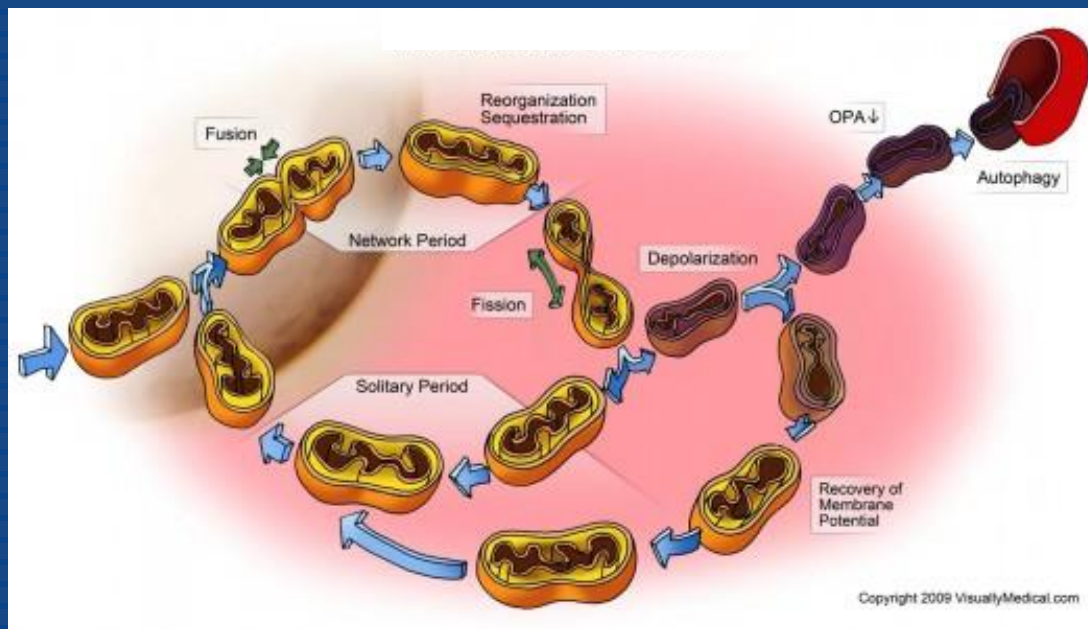


# Mechanisms of glucose toxicity





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



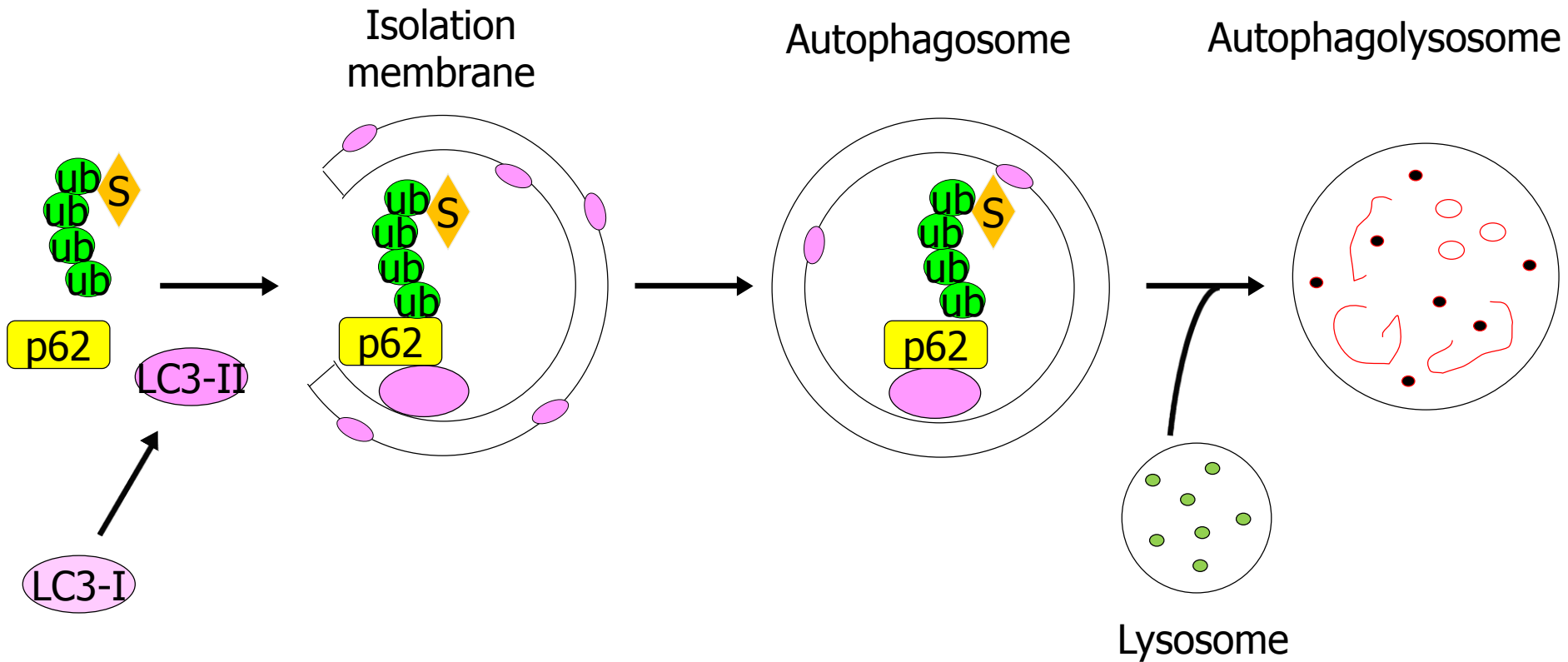
*Data published:*

J Clin Endocrinol Metab 2011;96(4):E633-645

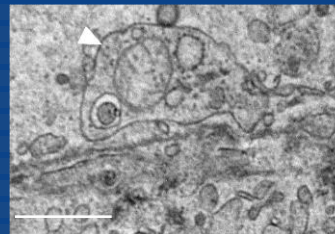
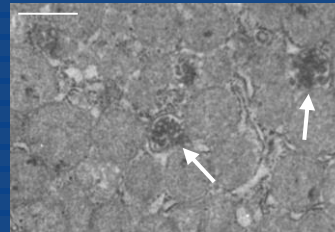
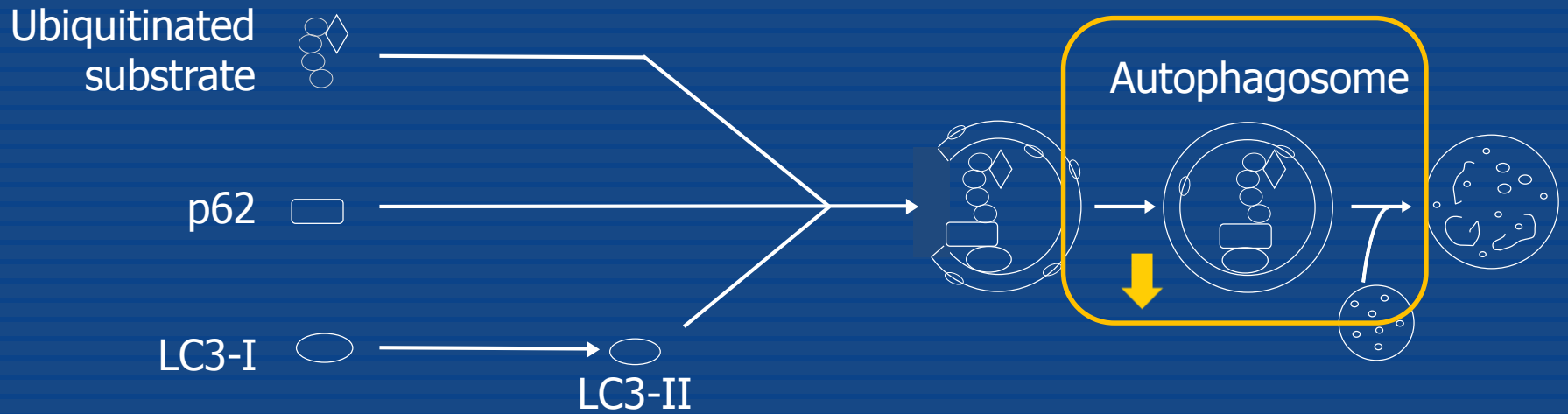
J Clin Endocrinol Metab 2012;97(1):E59-64

Crit Care Med 2013; 41(1):182-94

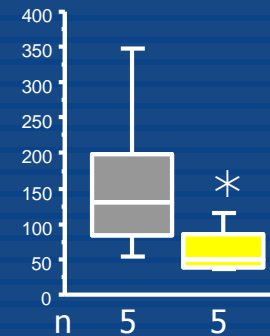
# Mitochondrial repair: autophagy



# Human liver



Number  
autophagic vacuoles



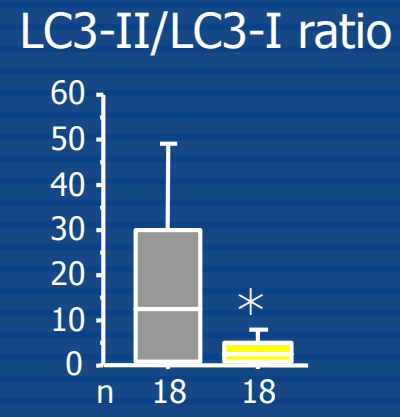
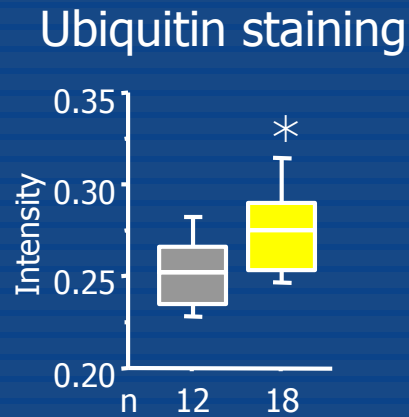
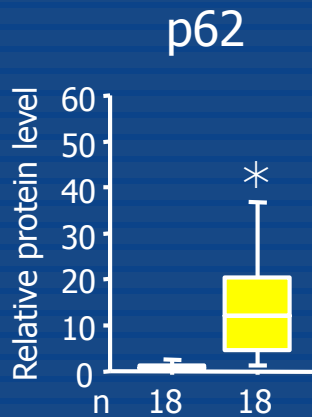
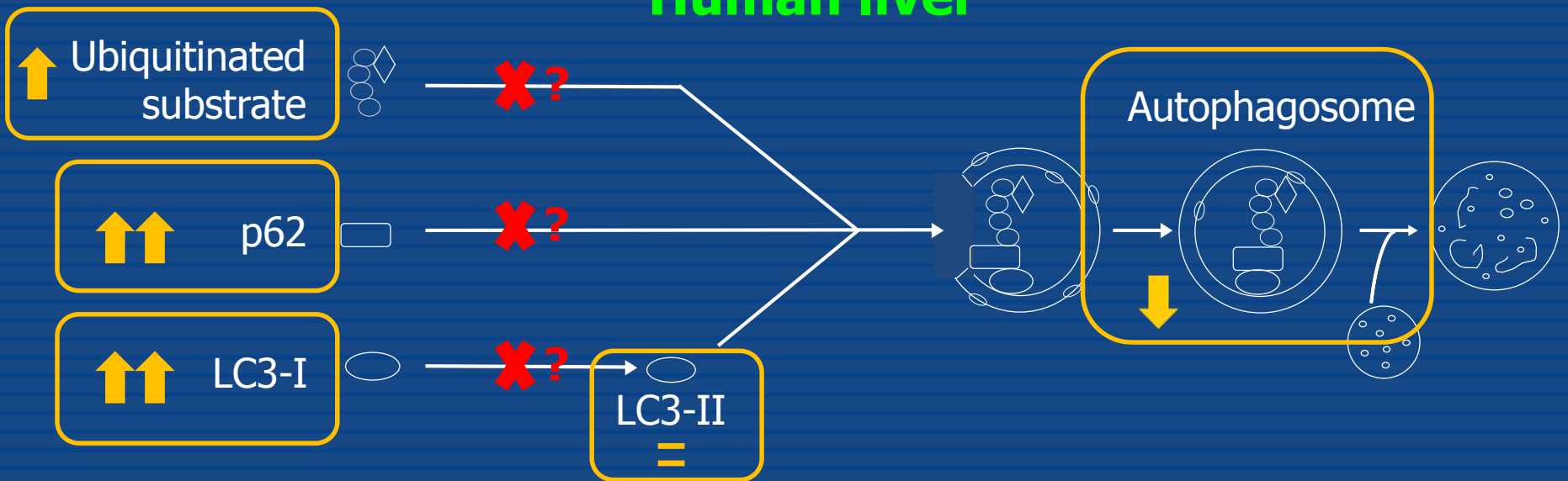
Boxes indicate median/IQR,  
whiskers interdecile range

\* :  $p \leq 0.05$  versus control

■ Control (elective rectal surgery)

■ Conventional insulin therapy

# Human liver



Boxes indicate median/IQR, whiskers interdecile range

\* :  $p \leq 0.05$  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

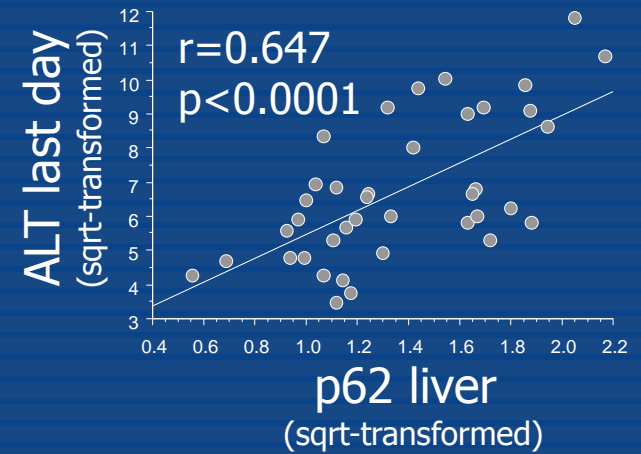
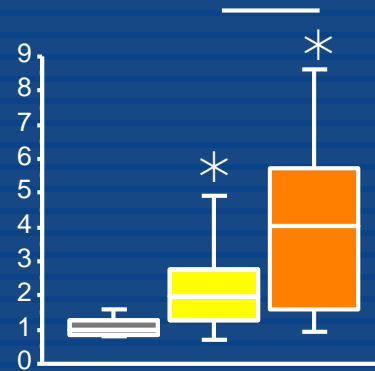
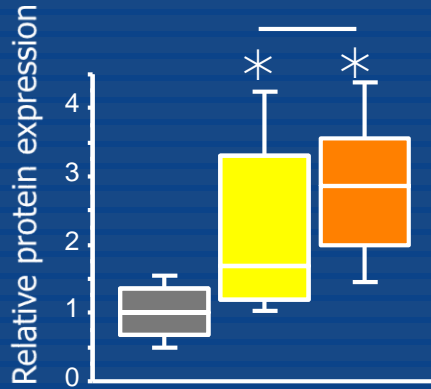


# Autophagy ~ Outcome

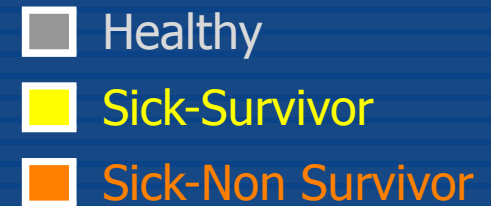
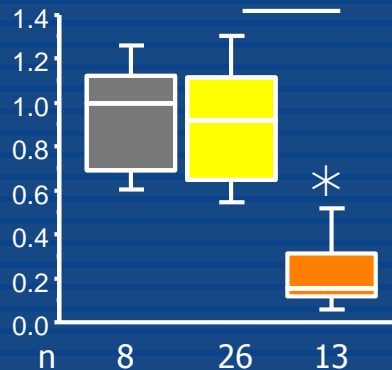
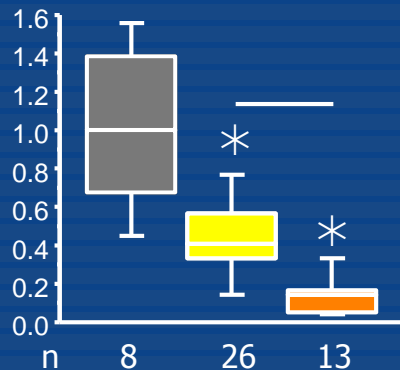
Liver

Kidney

p62



LC3-II/LC3-I ratio



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

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

— :  $p \leq 0.05$ ,  $0.05 < p \leq 0.1$  survivor vs non-survivor

# LIVER

# KIDNEY

3 days

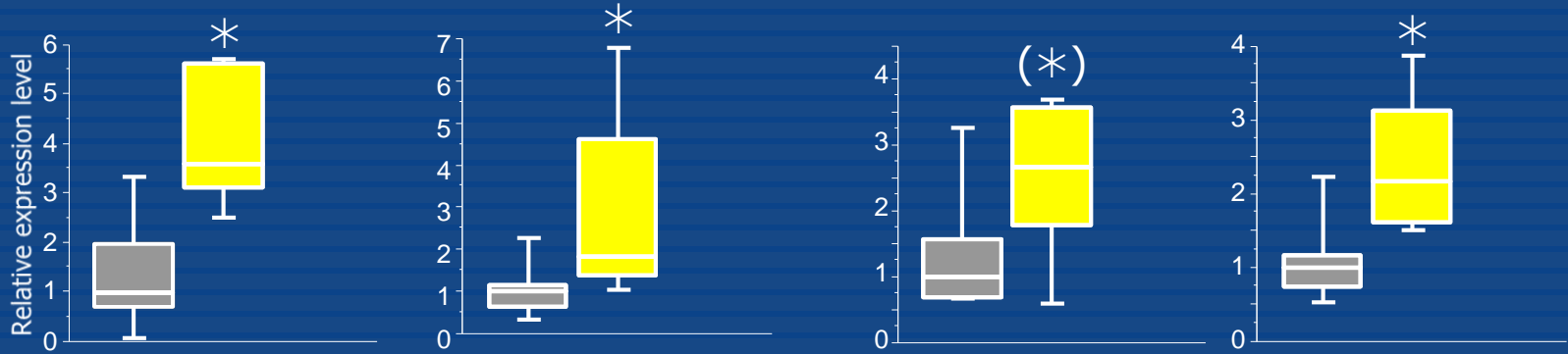
7 days

3 days

7 days

p62 protein

- Control
- Hyper-glycemia
- Normo-glycemia



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

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

————— - - - - - :  $p \leq 0.05$ ,  $0.05 < p \leq 0.1$  between sick groups

# LIVER

# KIDNEY

3 days

7 days

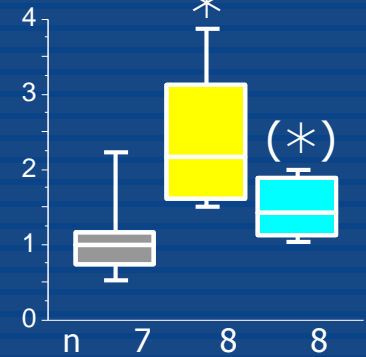
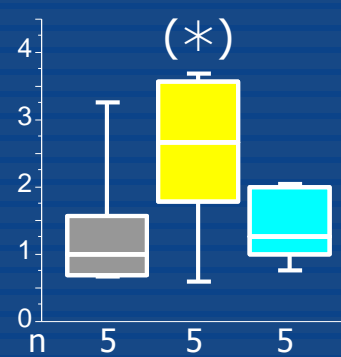
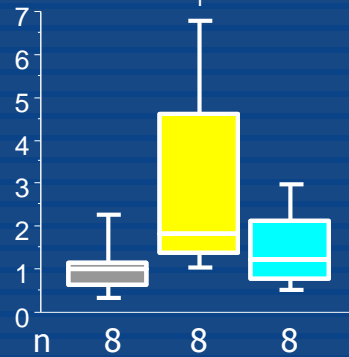
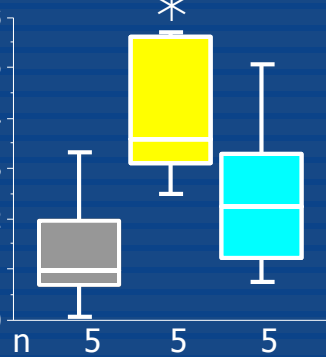
3 days

7 days

p62 protein

- Control
- Hyper-glycemia
- Normo-glycemia

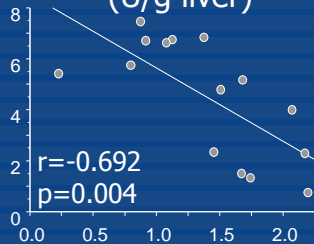
Relative expression level



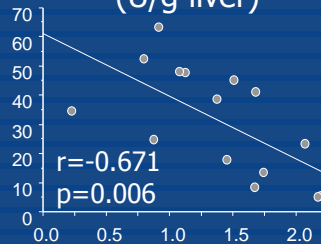
Correlation p62 protein (X-axis) with:

Mitochondrial damage

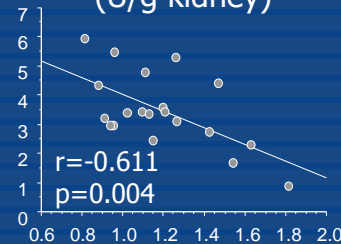
Complex I activ. (U/g liver)



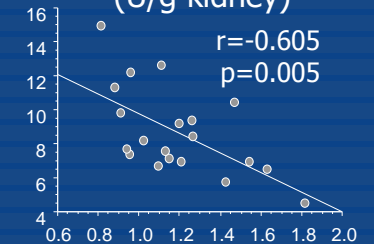
Complex V activ. (U/g liver)



Complex I activ. (U/g kidney)

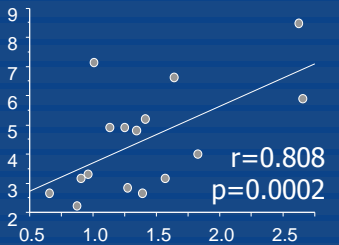


Complex V activ. (U/g kidney)

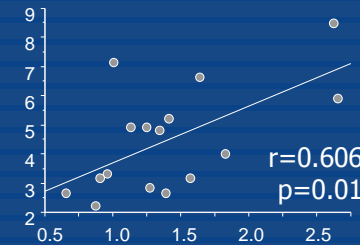


Organ damage

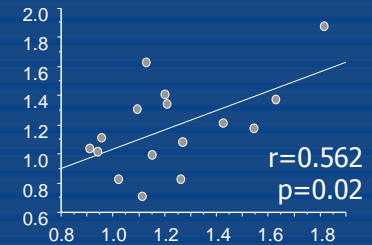
AST last day (U/l)



ALT last day (U/l)



Creatinine last day (mg/dl)

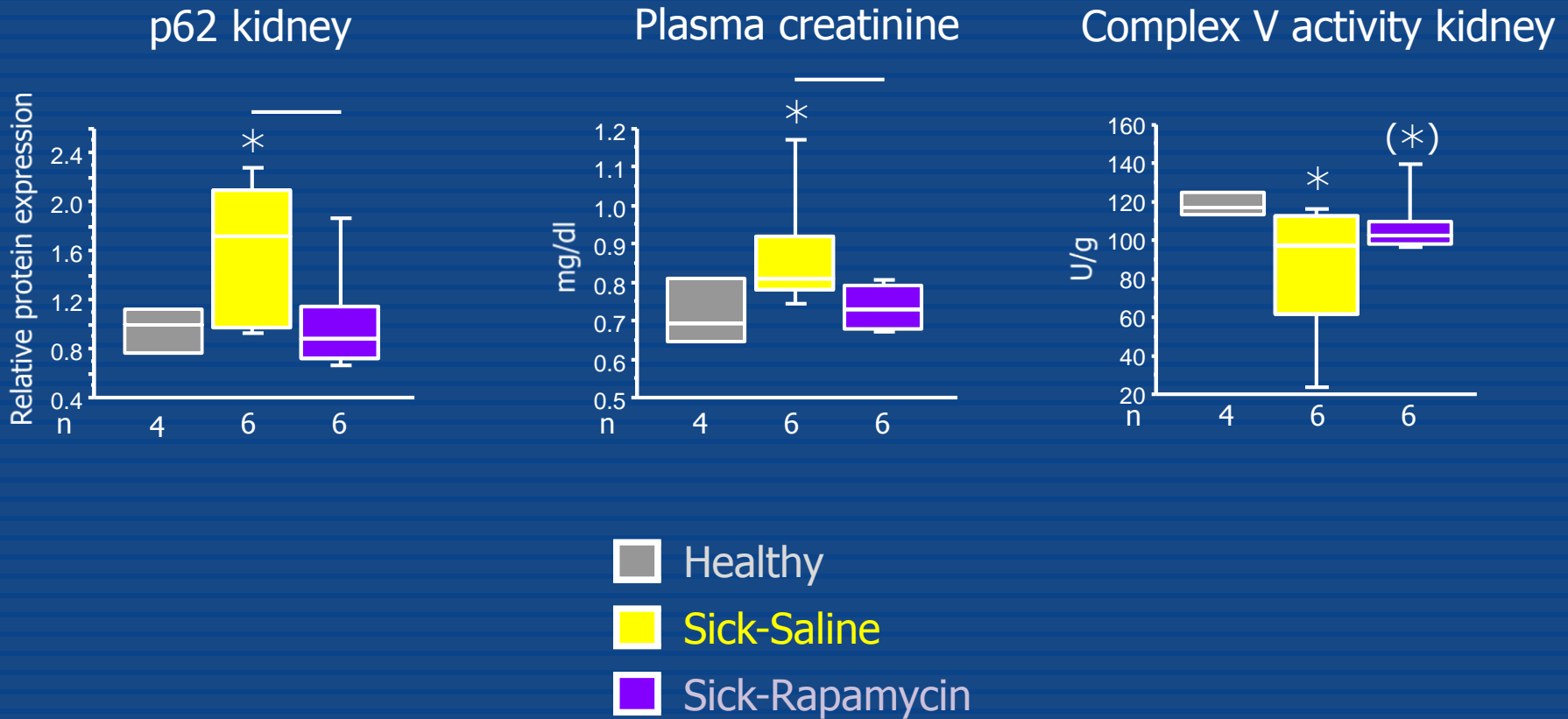


Boxes indicate median/IQR, whiskers interdecile range. Pearson correlation calculated after square root transformation of p62 and markers of organ damage

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

————— - - - - - :  $p \leq 0.05$ ,  $0.05 < p \leq 0.1$  between sick groups

# Activation of autophagy

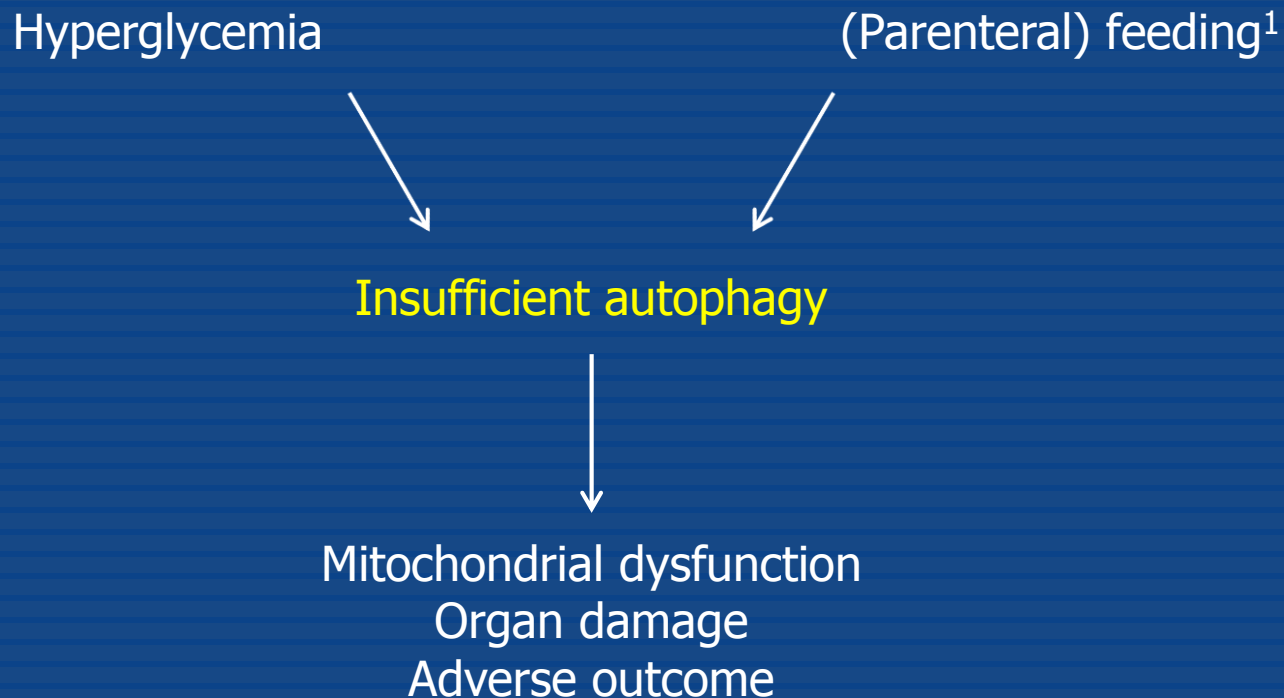


Boxes indicate median/IQR, whiskers interdecile range

\*, (\*):  $p \leq 0.05$ ,  $0.05 < p \leq 0.1$  vs healthy

—:  $p \leq 0.05$ ,  $0.05 < p \leq 0.1$  between sick groups

# Conclusion Part 2: Role of intact mitochondrial repair in critical illness



## Early versus Late Parenteral Nutrition in Critically Ill Adults

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

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

*Data published:*

J Am Soc Nephrol 2013; 24(6):995-1005

# Incidence of AKI

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

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



# Recovery from AKI

	Early PN	Late PN	p
<b>AKI stage 1</b>			
Days with AKI in ICU <sup>1</sup>	1 (1-2)	2 (1-3)	0.4
<b>AKI stage 2</b>			
<b>Days with AKI in ICU<sup>1</sup></b>	<b>5 (3-9)</b>	<b>4 (2-6)</b>	<b>0.04</b>
<b>AKI stage 3</b>			
Days with AKI in ICU <sup>1</sup>	12 (7-21)	11 (6-21)	0.2

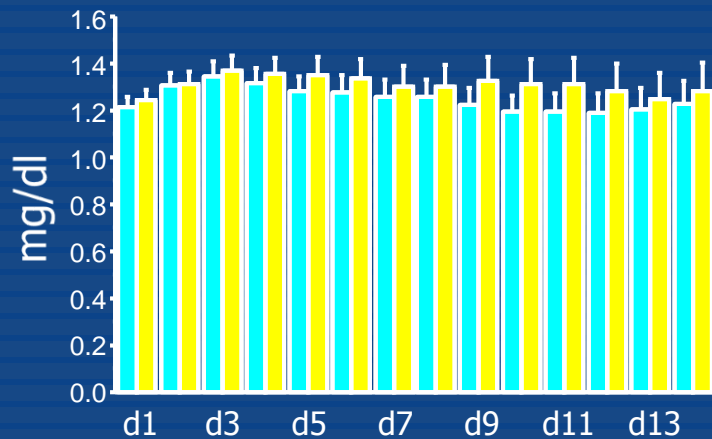
<sup>1</sup> Data show median (interquartile range) for ICU survivors only

# Recovery from AKI

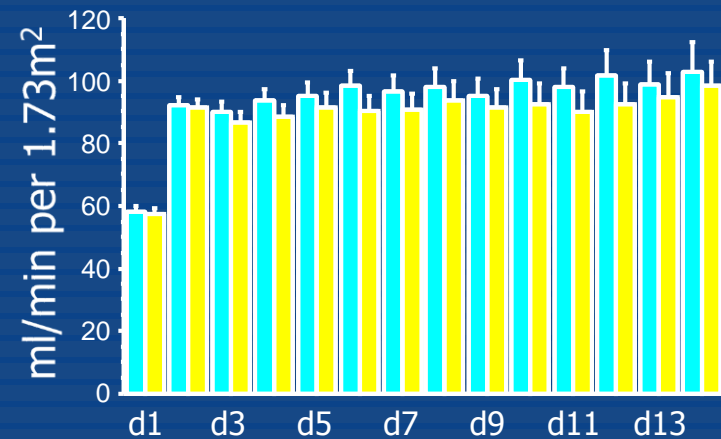
	Early PN	Late PN	p
<b>AKI stage 1</b>			
Days with AKI in ICU <sup>1</sup>	1 (1-2)	2 (1-3)	0.4
<b>Alive and AKI-free at hospital discharge, n (%)</b>	<b>168 (76.7)</b>	<b>148 (75.1)</b>	<b>0.7</b>
<b>AKI stage 2</b>			
Days with AKI in ICU <sup>1</sup>	5 (3-9)	4 (2-6)	0.04
<b>Alive and AKI-free at hospital discharge, n (%)</b>	<b>63 (63.6)</b>	<b>68 (63.6)</b>	<b>0.9</b>
<b>AKI stage 3</b>			
Days with AKI in ICU <sup>1</sup>	12 (7-21)	11 (6-21)	0.2
<b>Alive and AKI-free at hospital discharge, n (%)</b>	<b>86 (34.4)</b>	<b>98 (37.5)</b>	<b>0.5</b>

<sup>1</sup> Data show median (interquartile range) for ICU survivors only

## Plasma creatinine



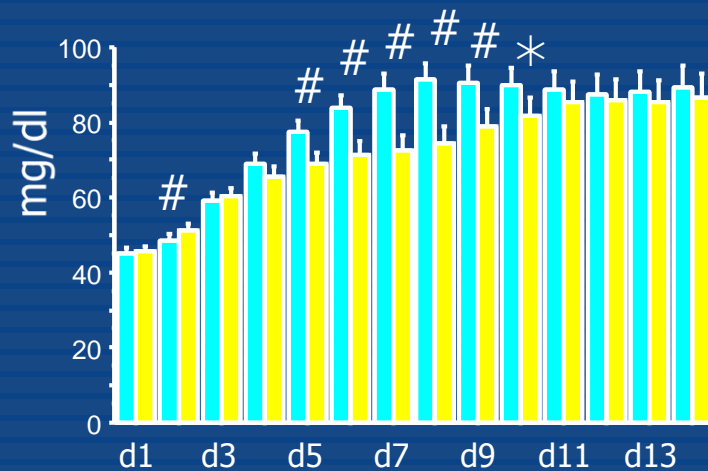
## Creatinine clearance<sup>1</sup>



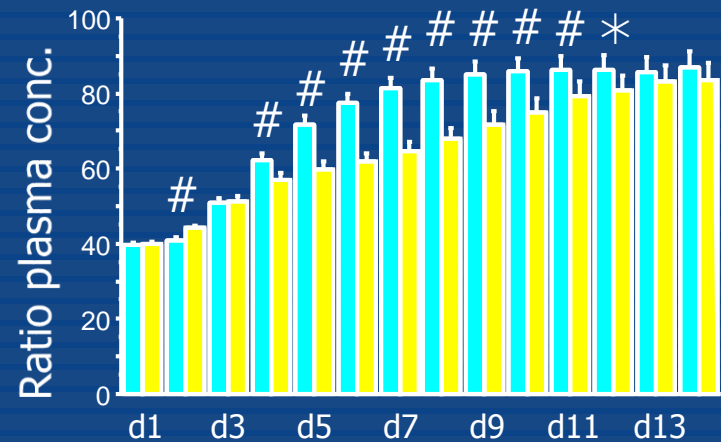
■ Early PN

■ Late PN

## Plasma urea



## Urea/creatinine ratio



Early PN	2312	1438	975	736	601	517	432	No.
Late PN	2328	1399	913	655	524	436	365	in ICU

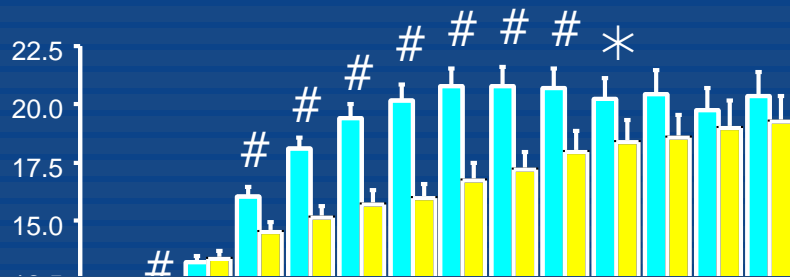
\* 0.001 < p ≤ 0.01; # p ≤ 0.001 between sick groups

Bar graphs indicate mean and 95% CI

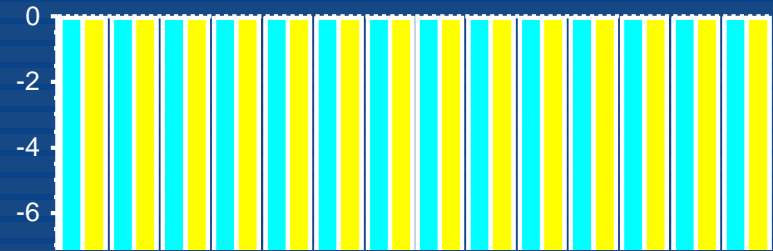
<sup>1</sup>Excluded: dialyzed patients (n=428) and patients with missing samples on more than 2 consecutive days (n=584)

# Nitrogen loss and balance over time in ICU

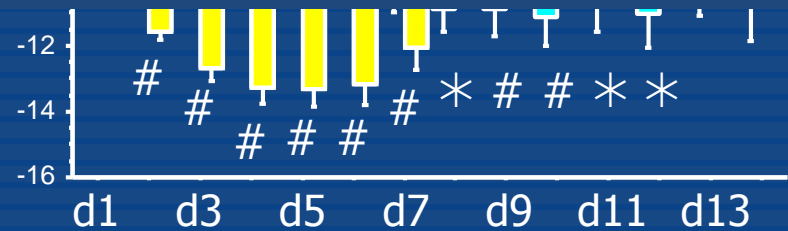
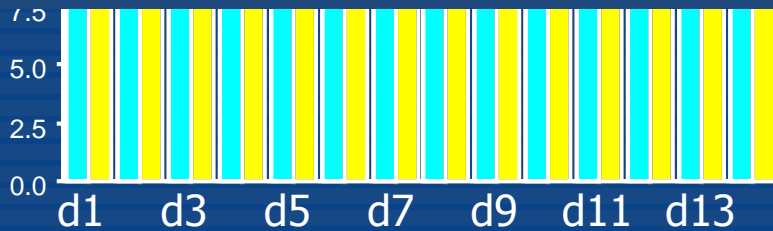
## Nitrogen loss (g)



## Nitrogen balance (g)



63% of extra nitrogen administration net wasted!



	d1	d3	d5	d7	d9	d11	d13
Early	1801	1003	622	436	336	276	220
Late PN	1827	989	598	402	298	234	191

No. in ICU	d1	d3	d5	d7	d9	d11	d13	Early	Late PN
1801	1827	1003	989	598	402	298	234	276	220

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

Bar graphs indicate mean and 95% CI

■ Early PN

■ Late PN

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

# Conclusion Part 3

## Early versus late PN & AKI

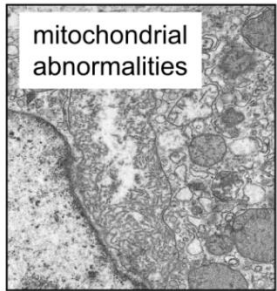
### Early PN:

- 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)



# Summary

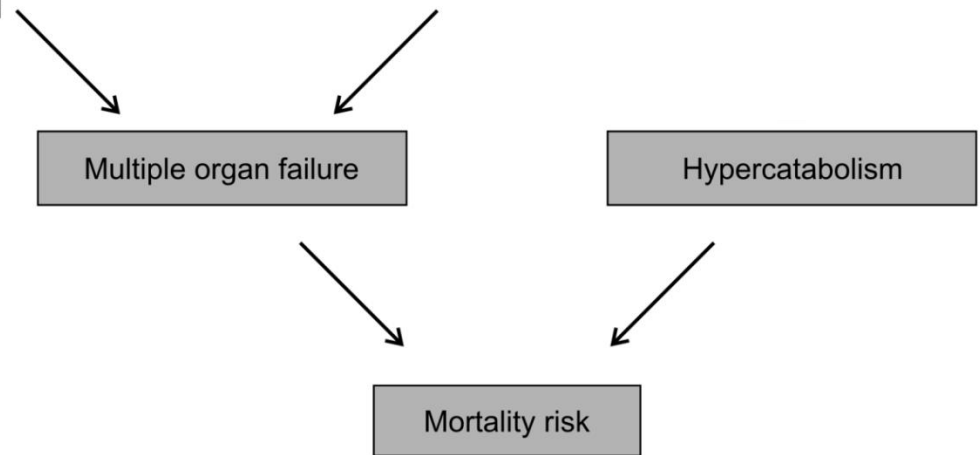
Critical illness



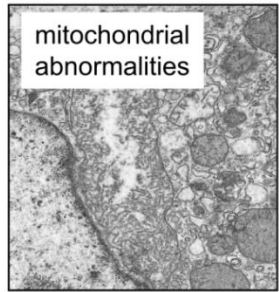
Multiple organ failure

Hypercatabolism

Mortality risk



Critical illness

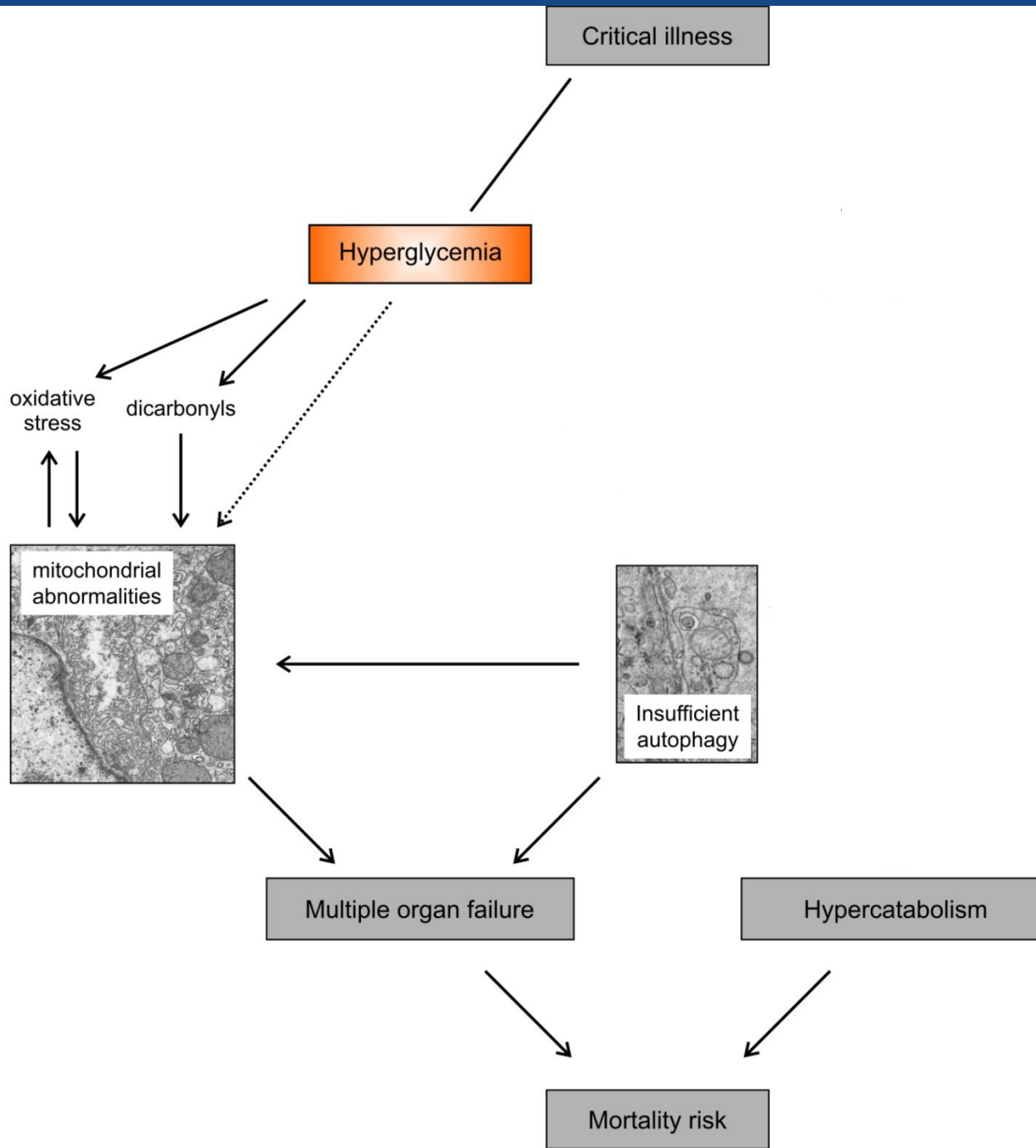


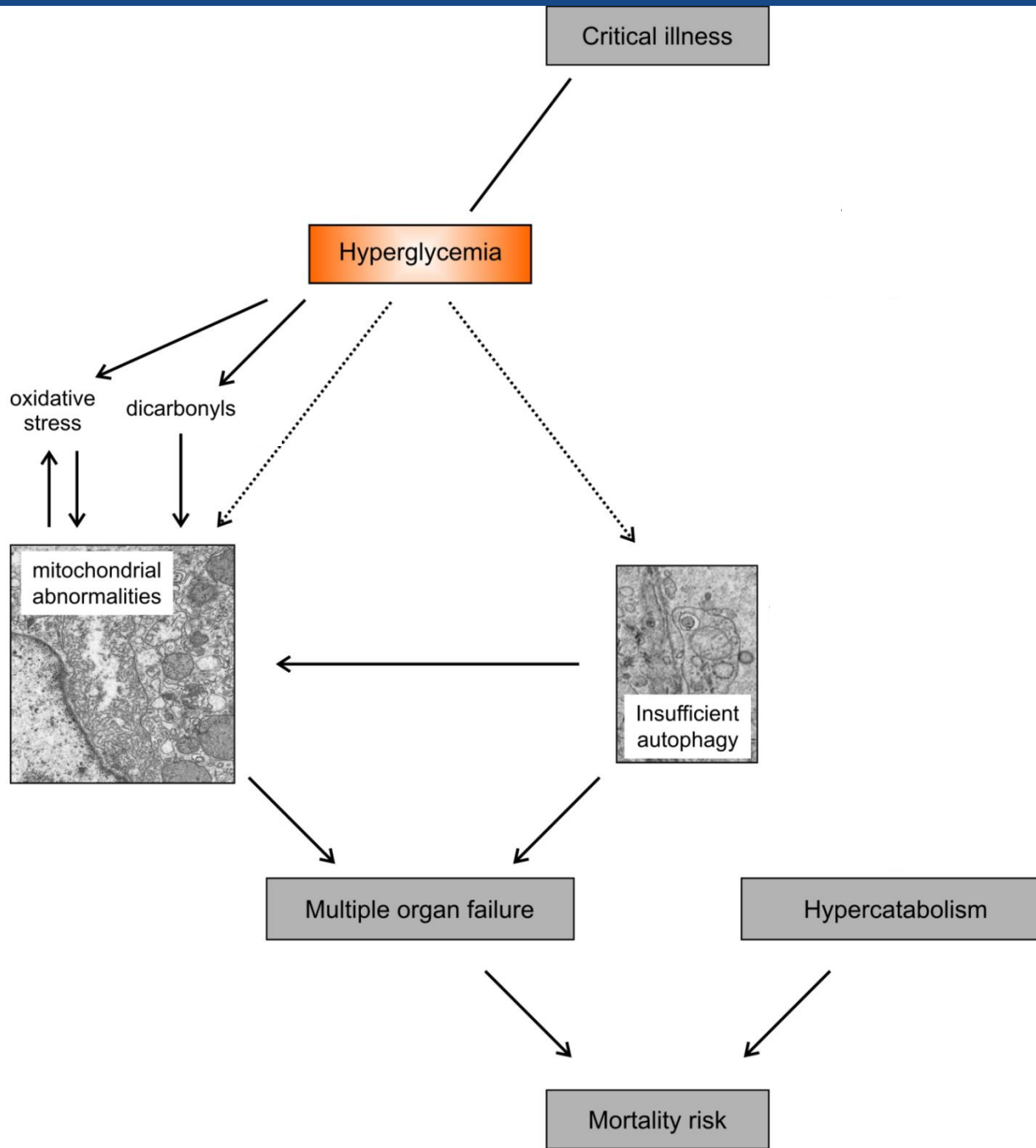
Multiple organ failure

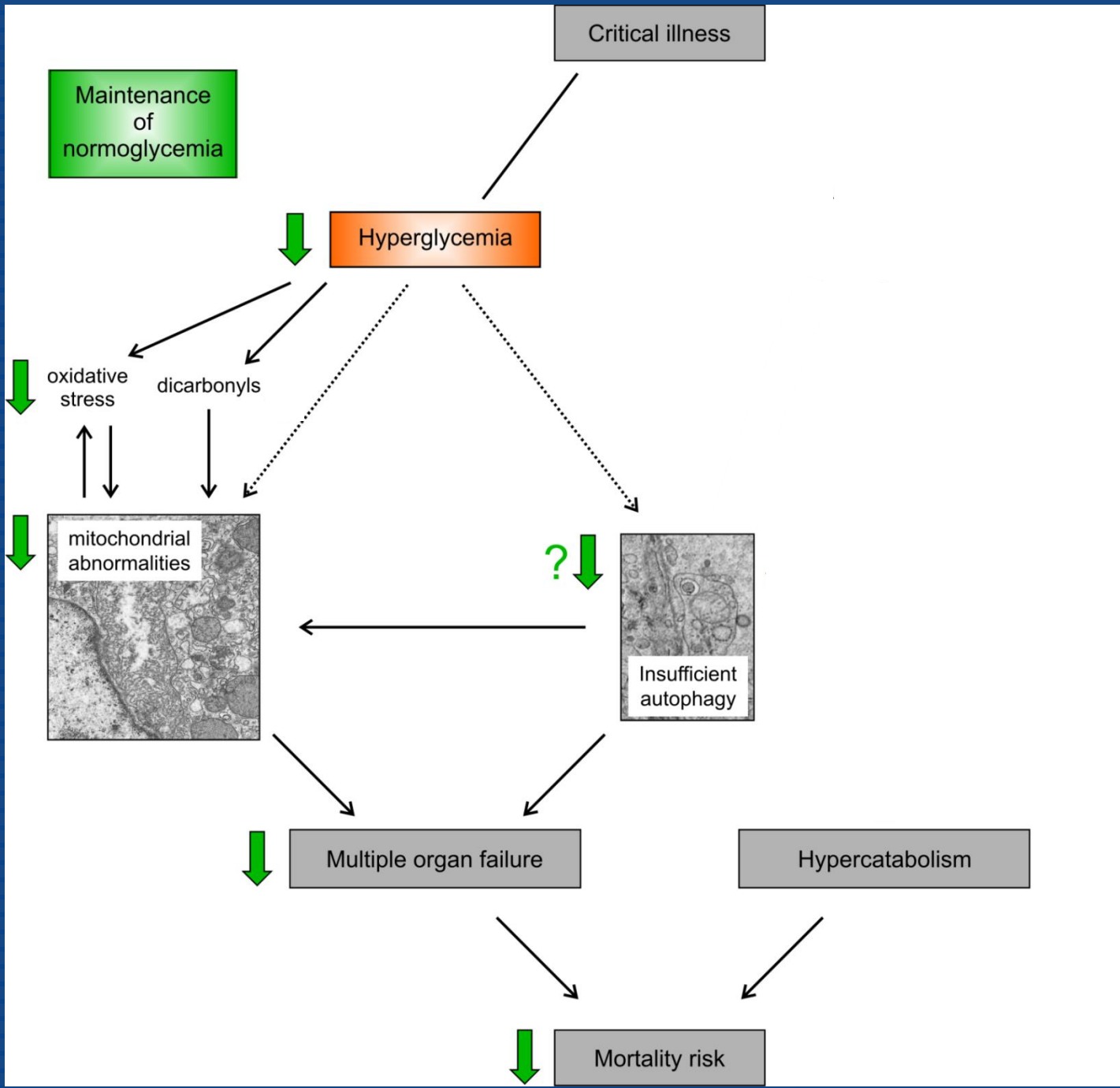
Hypercatabolism

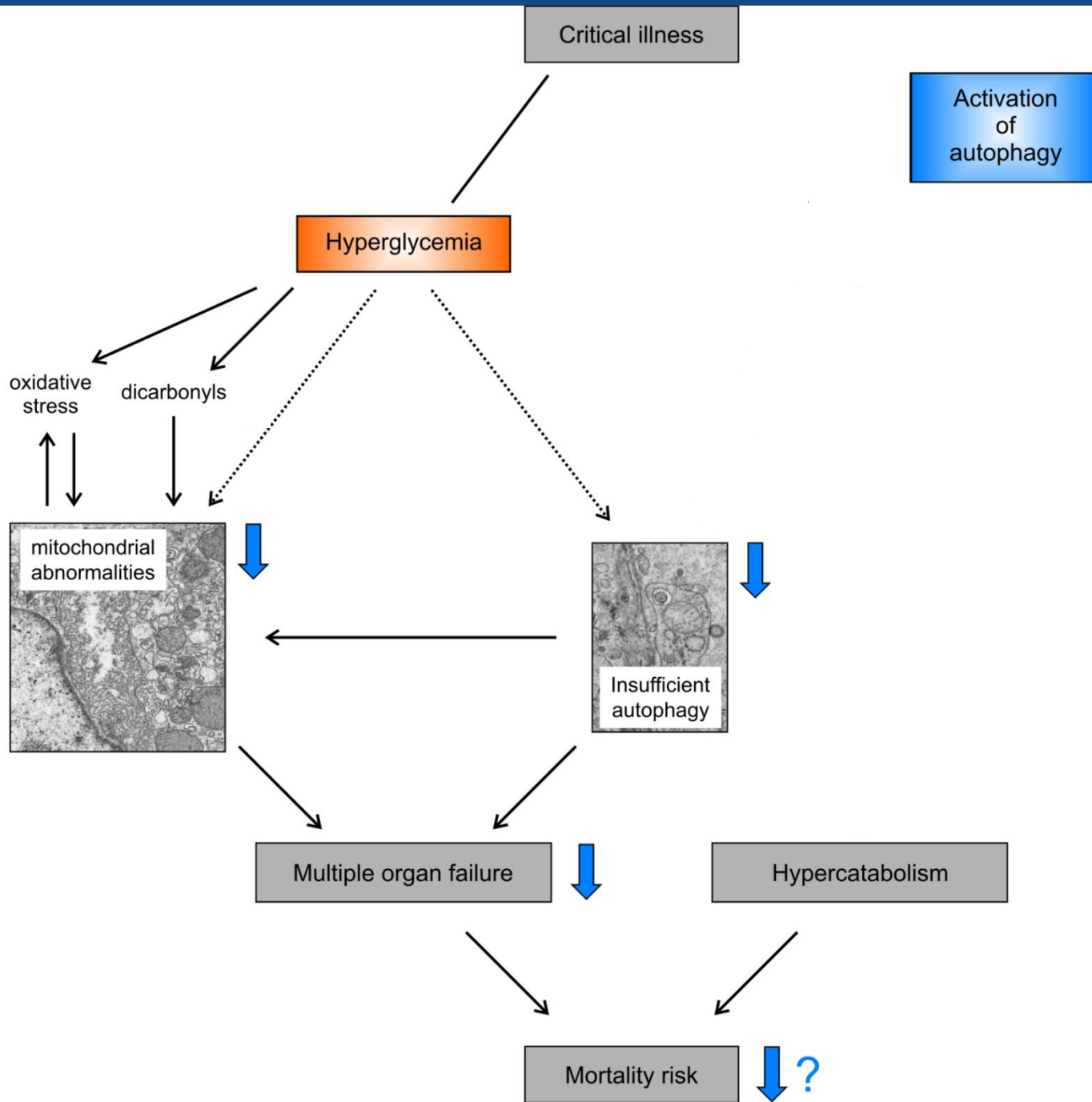
Mortality risk

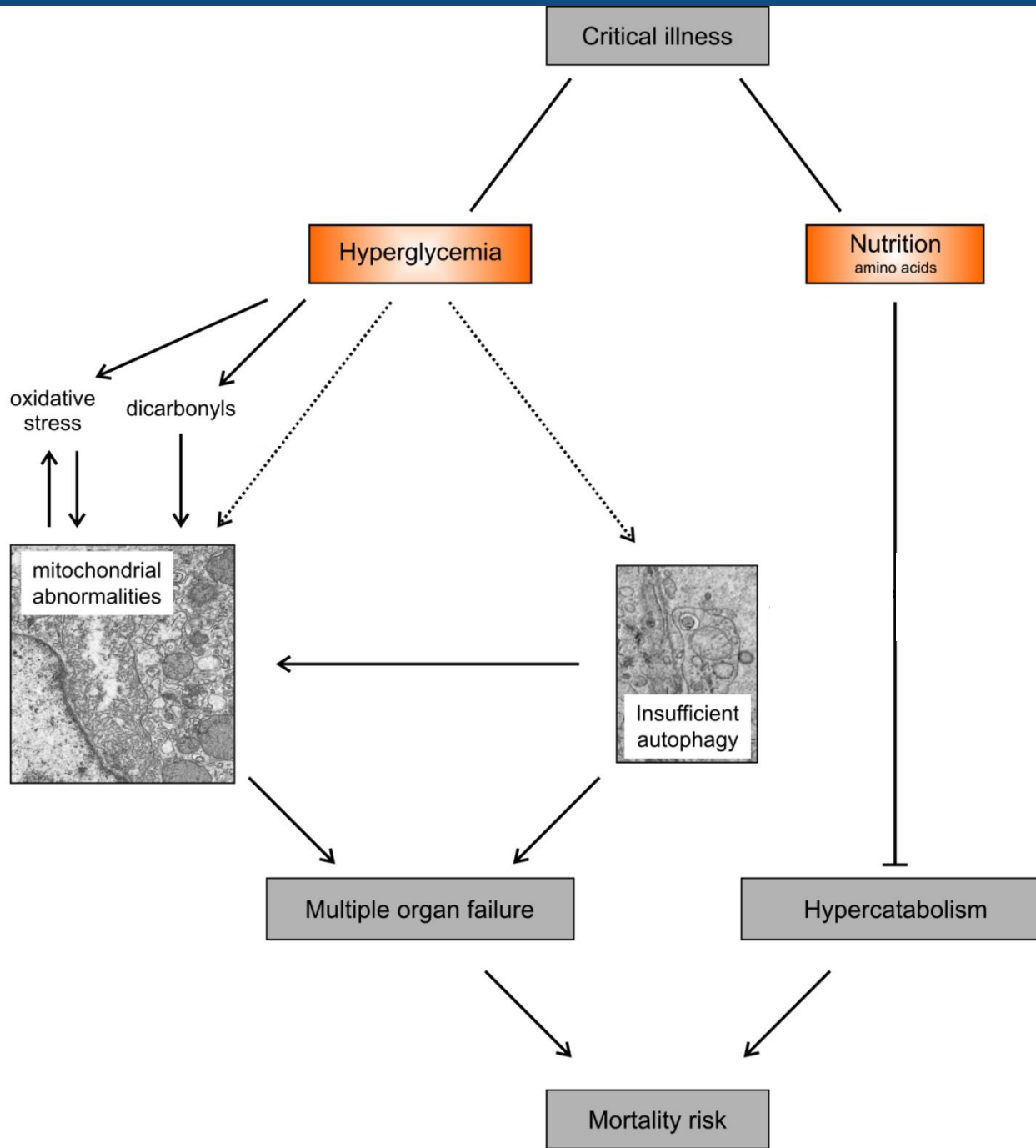


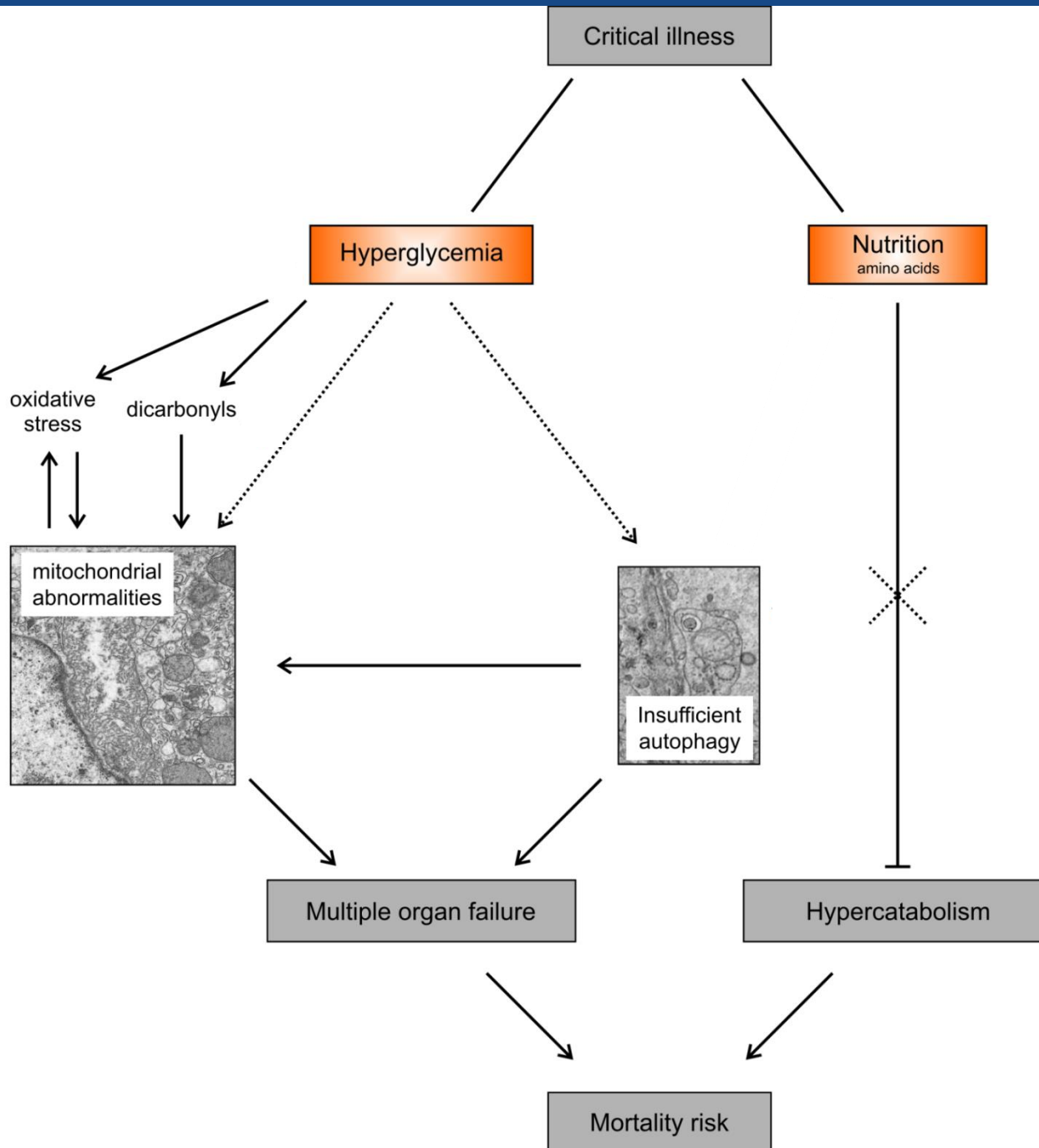


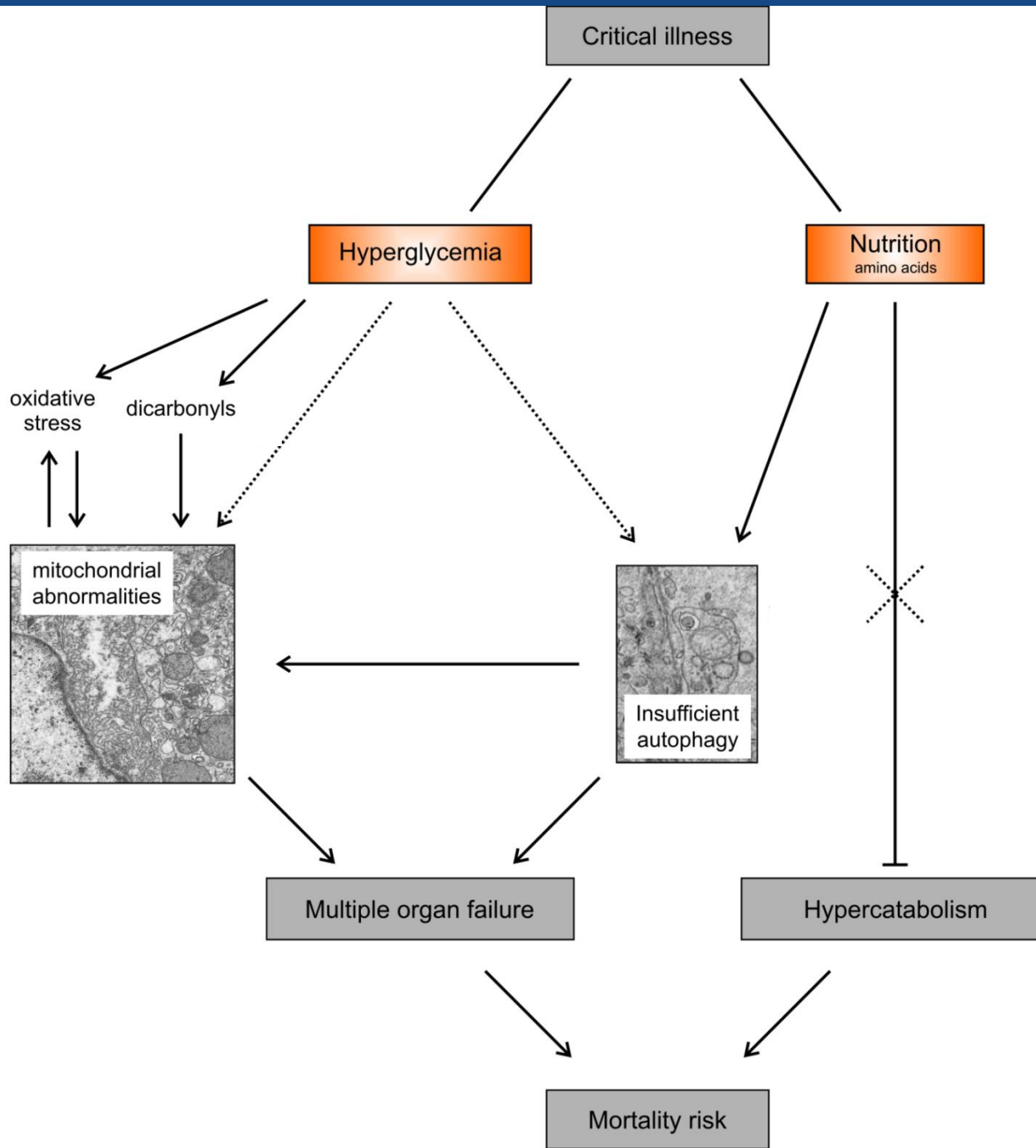












# Future perspectives



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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