Regulation of adaptive behaviour during fasting by hypothalamic Foxa2

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Foxa2

- The forkhead transcription factor Foxa2 belongs to the FOXO family. (FOX = Forkhead Box)
- The forkhead box is the DNA-binding domain
- FOXO transcription factors are involved in different cellular processes (development, metabolism, differentiation,...)
- Foxa2 is a downstream target of the insulin signalling
Regulation of FoxO by Nuclear Shuttling in Response to AKT Mediated Phosphorylation

Winged-helix family of trx factors are involved in development, metabolism, cell differentiation

Phosphorylation stimulates nuclear export (NES) and prevents nuclear import (NLS)
Regulation of FoxO by Nuclear Shuttling in Response to AKT Mediated Phosphorylation

Survival Signals

AKT

pT308
pS473

FOXO3a

PP2A

pT32
pS253
pS315

14-3-3

FoxO3a

PP2A

(Nuclear Translocation/Activation)

FOXO3a

PP2A

p

p

14-3-3

14-3-3

14-3-3

Growth factor/ Cytokine withdrawal

(Cytoplasm)
Nuclear/cytosolic shuttling in response to insulin

Lean/Insulin sensitive

Fasted
Low Insulin

Fed
High Insulin

PI3-kinase

PKB/AKT

Foxa2 nuclear active

PPA2

Foxa2-P cytosol inactive

Liver

Fatty acid oxidation genes
Ketogenesis genes
Aim of the study

Investigate whether Foxa2 regulates the expression of MCH and orexin in the hypothalamus.

- MCH and orexin regulation is feeding-dependent
- MCH regulates food intake
- Orexin is a positive stimulus for physical activity
Expression pattern of Foxa2 in the central nervous system

- Foxa2 is expressed in MCH and orexin neurons
Subcellular distribution of Foxa1 and Foxa2

- Insulin-treated mice: Foxa2 is detected in the cytosol
- PBS-treated mice: nuclear localization of Foxa2
**Fasted state:** nuclear Foxa2 localization

**Fed state:** cytosolic Foxa2 localization
In fasted mice MCH and orexin expression is increased.
Fampa2 influences promoter activities

Constitutive active Foxa2 increased MCH and orexin reporter activity
Insulin inhibits MCH and orexin expression

Glucose had no effect on MCH- and orexin- mRNA levels, whereas insulin showed an inhibitory effect.
Effect of high-fat diet on Foxa2 localization

- In fasted obese mice Foxa2 was detected in the cytosol
In fasted HFD C57Bl/6 mice Foxa2 is expressed only in the cytoplasm
Expression of MCH and orexin in hypothalamus of obese mice

Expression of MCH and orexin in mice on a HFD were similar in the fed and fasted state
Generation of a conditional mutant mouse model with a constitutive active Foxa2

Constitutively active Foxa2 allele

\[ \text{Foxa2T156A}^{\text{flox/flox}} \]

Cre recombinase expressed under a neuron specific promoter

\[ \text{Nes-Cre/+} \]

\[ \text{Fed mice} \]

\[ \text{Nes-Cre/+; Foxa2T156A}^{\text{flox/flox}} \]
Insulin

Nes-Cre/+  Foxa2T156A^{flox/flox}

Nes-Cre/+; Foxa2T156A^{flox/flox}

a

Hypothalamus

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>Nuclear</th>
<th>T156^{fl/fl} NesCre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxa2</td>
<td></td>
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<tr>
<td>Lsd1</td>
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<tr>
<td>Gapdh</td>
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<td>y-Tub</td>
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</table>

T156^{fl/fl}  

Nes-Cre/+; T156^{fl/fl}
F Paxa2T156A expression & energy homeostasis (1)
Foxa2T156A expression & energy homeostasis (2)
**Foxa2 activation in the hypothalamus**

*Foxa2T156A^flox/flox*  
*Foxa2T156A^flox/flox* 

**Ad-Cre virus**  
**Ad-GFP virus**

**b**  
Rel. expression levels  
***  

**c**  
Food [g/24 h]  
***  

**d**  
O₂ production [L/24 h]  
**  

**e**  
CO₂ consumption [L/24 h]  
**  

**f**  
Movement [counts/24 h]  
***  

**Nuclear**  
**Cytoplasmic**

- **Ad-GFP**  
- **Ad-Cre**

- Foxa2
- γ-Tubulin
Foxa2 activation in the hypothalamus

**a**

Relative expression levels

- **Ad-GFP**
- **Ad-T156A**

**b**

Food intake (g/24 h)

- **Ad-GFP**
- **Ad-T156A**

- **f**

O₂ consumption (L/24 h)

- **Ad-GFP**
- **Ad-T156A**

**c**

O₂ consumption (L/24 h)

- **Ad-GFP**
- **Ad-T156A**

**d**

X-movement (counts x 10^3/24 h)

- **Ad-GFP**
- **Ad-T156A**

**Ad-Foxa2T156A**

**Ad-GFP**

*C57BL/6*

*C57BL/6*
Foxa2 activation & High Fat Diet

Nes-Cre/+  Foxa2T156A$^{flox/flox}$  Nes-Cre/+; Foxa2T156A$^{flox/flox}$

High Fat Diet

**Graphs:**

- **b:** Glucose (mg dl$^{-1}$)
- **c:** Insulin (ng ml$^{-1}$)
- **d:** FFA (µmol l$^{-1}$)
Foxa2 activation & High Fat Diet

Nes-Cre/+  Foxa2T156A\textsuperscript{flox/flox}  Nes-Cre/+; Foxa2T156A\textsuperscript{flox/flox}

High Fat Diet

(e) Food consumption (g per 24 h)

(g) Body fat (percentage of body mass)

(h) Lean body mass (percentage of body mass)
Findings extend FoxA2 functions to the CNS
FoxA2 is a metabolic sensor in neurons

→ In hyperinsulinemic mice with diet-induced obesity, Foxa2 is permanently inactive in the hypotalamus.

→ Constitutive activation of brain Foxa2 in obese mice leads to improved glucose homeostasis, decreased fat and increased lean body mass linked to an increased physical activity.

This study offers a molecular explanation for the inverse correlation between insulin resistance/hyperinsulinemia and physical activity/energy consumption.