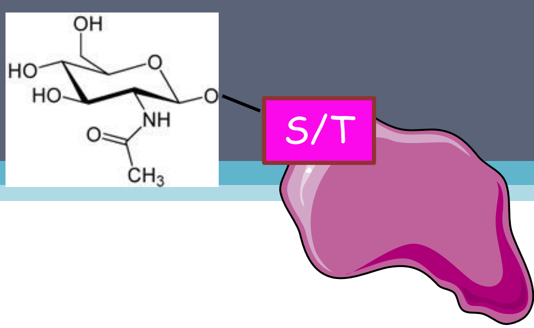
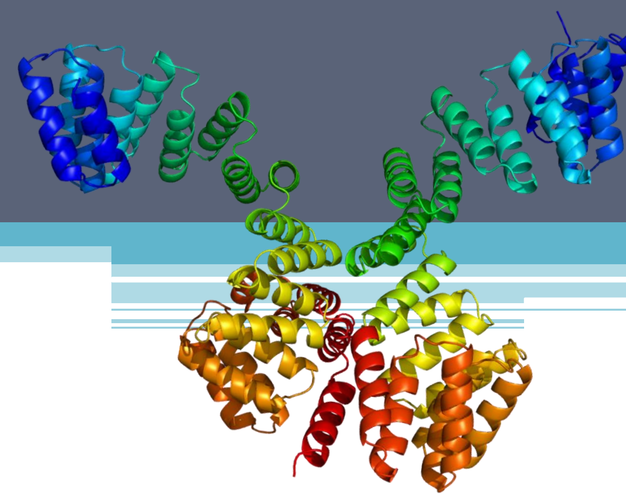
Regulation of hepatic Fatty Acid Synthase properties by O-GlcNAcylation *in vivo* and *ex vivo*

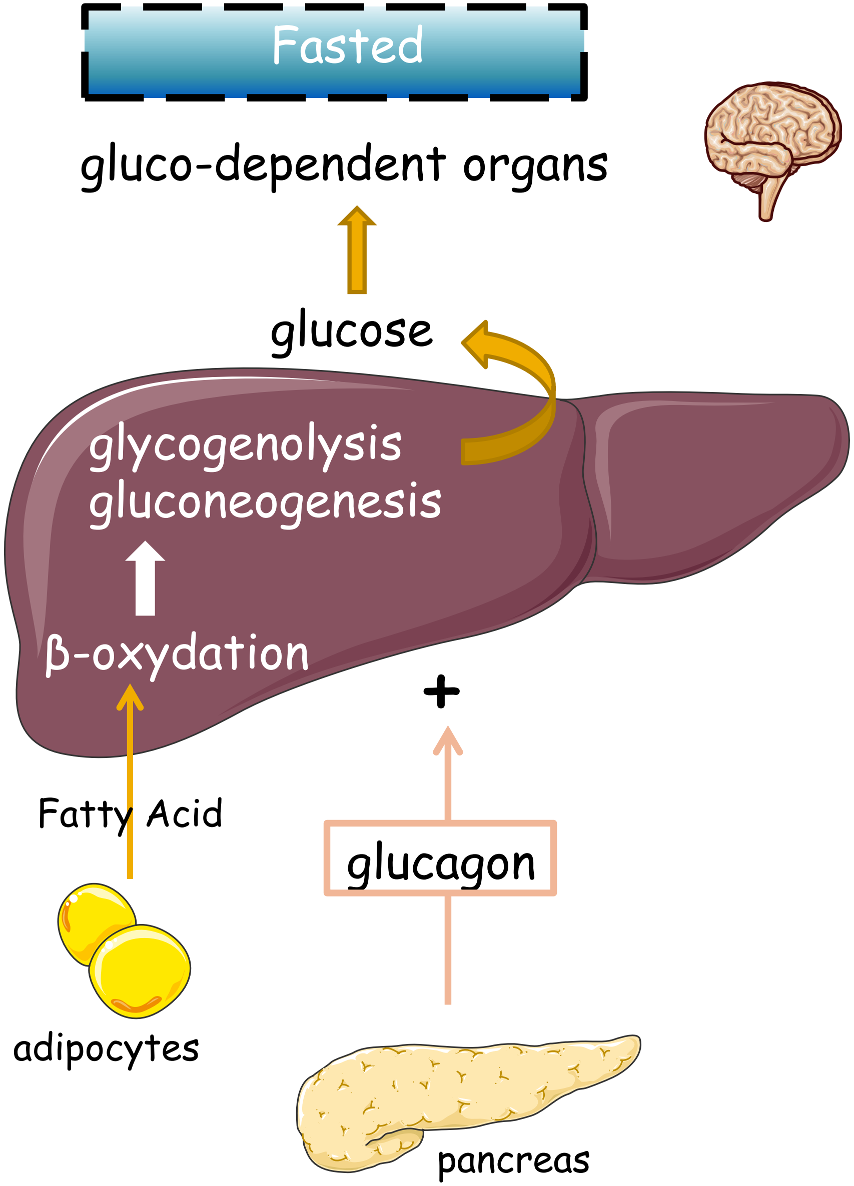




Baldini Steffi, Anne-Marie Mir, Marlène Mortuaire, Céline Guinez and Tony Lefebvre

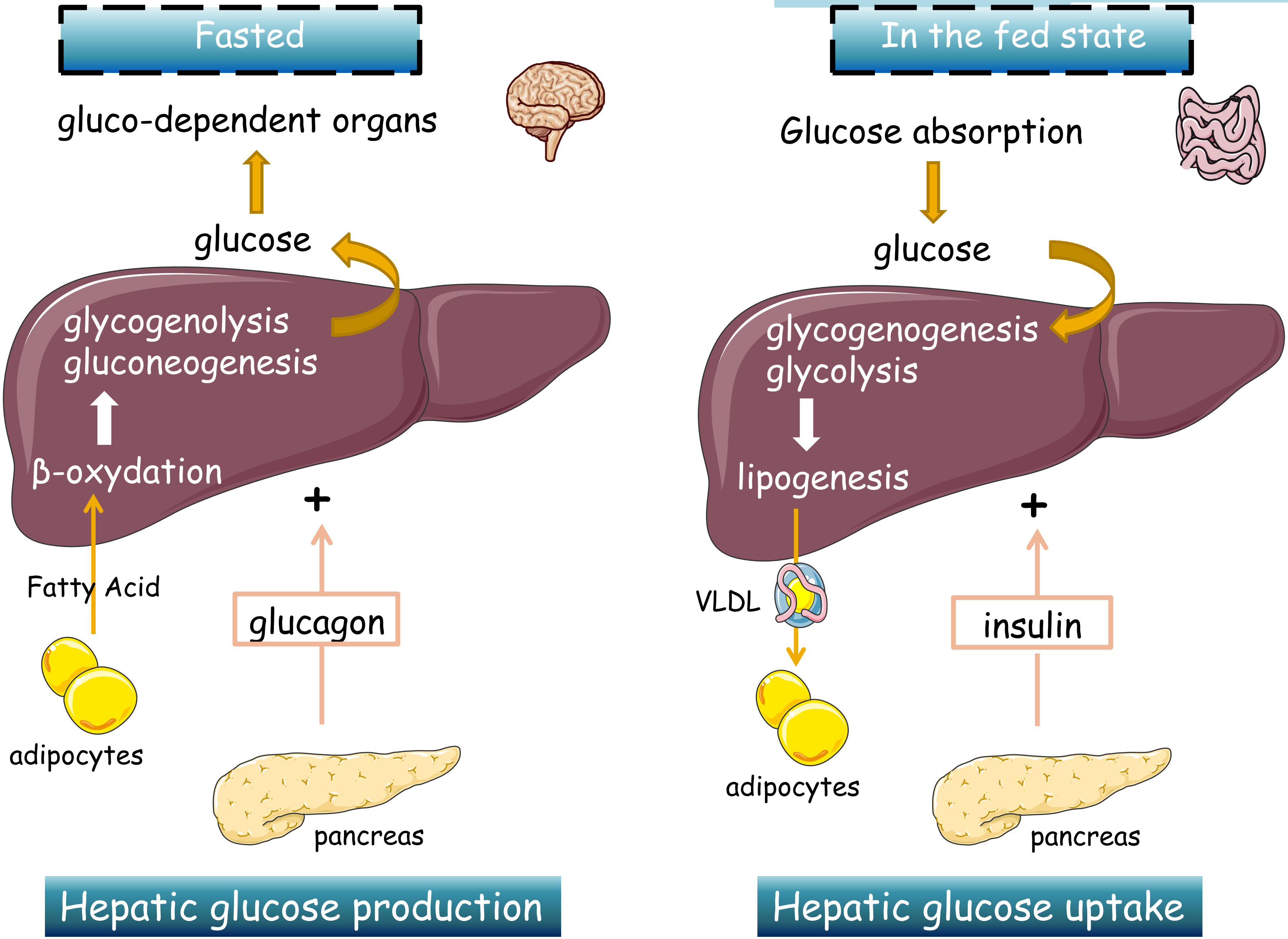
CNRS-UMR 8576, Unit of Structural and Functional Glycobiology, FRABio FR3688, Lille 1 University, 59655 Villeneuve d'Ascq, France

Control of blood glucose by the liver

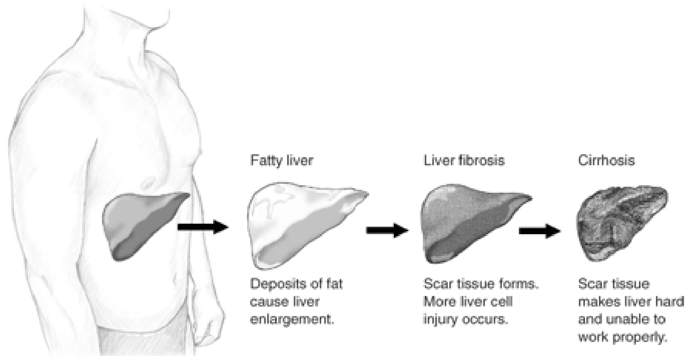


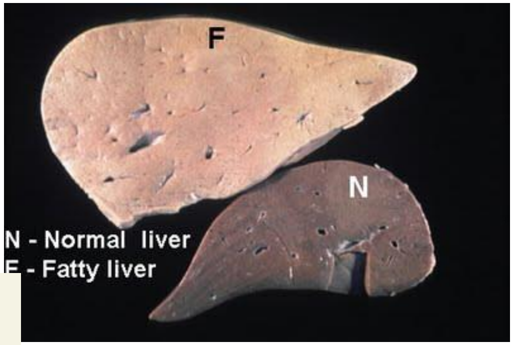
Hepatic glucose production

Control of blood glucose by the liver

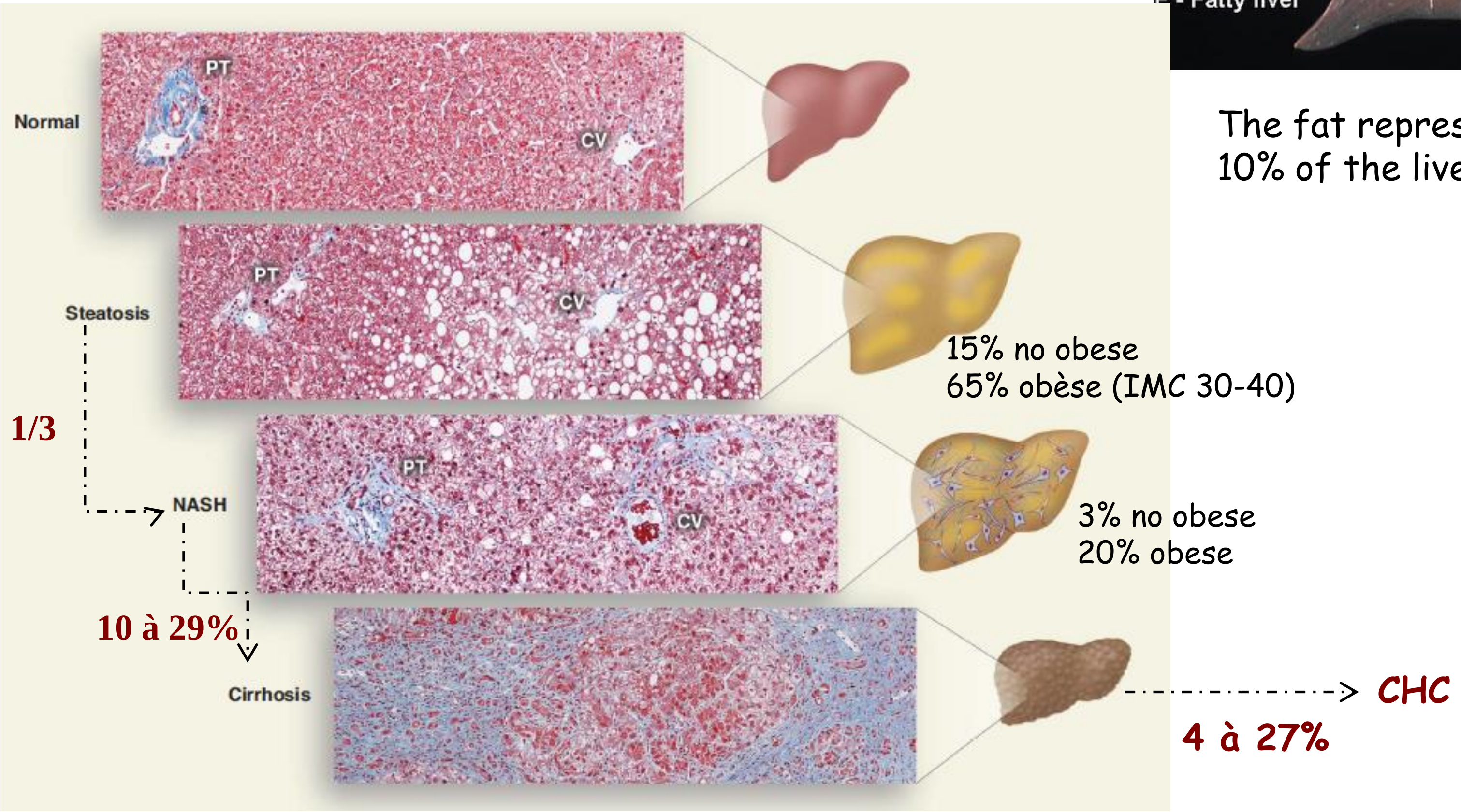


Dysregulation of glucido-lipidic metabolism and hepatic steatosis

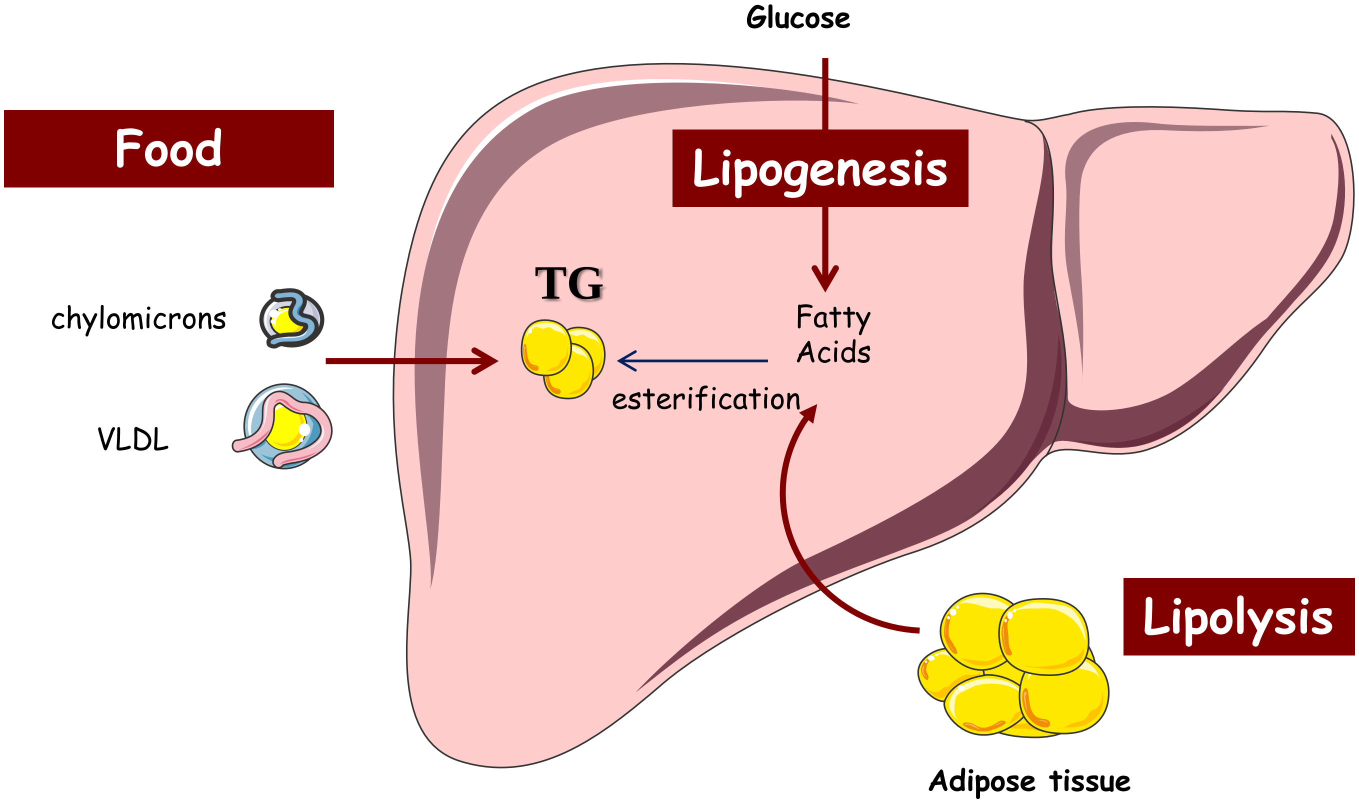




The fat represents at least 5 to 10% of the liver weight

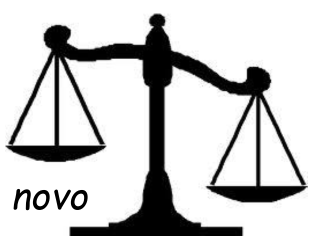


The causes of hepatic steatosis

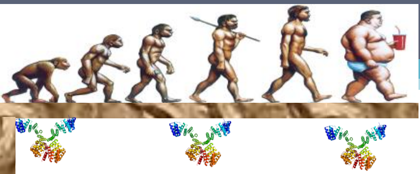


Supply

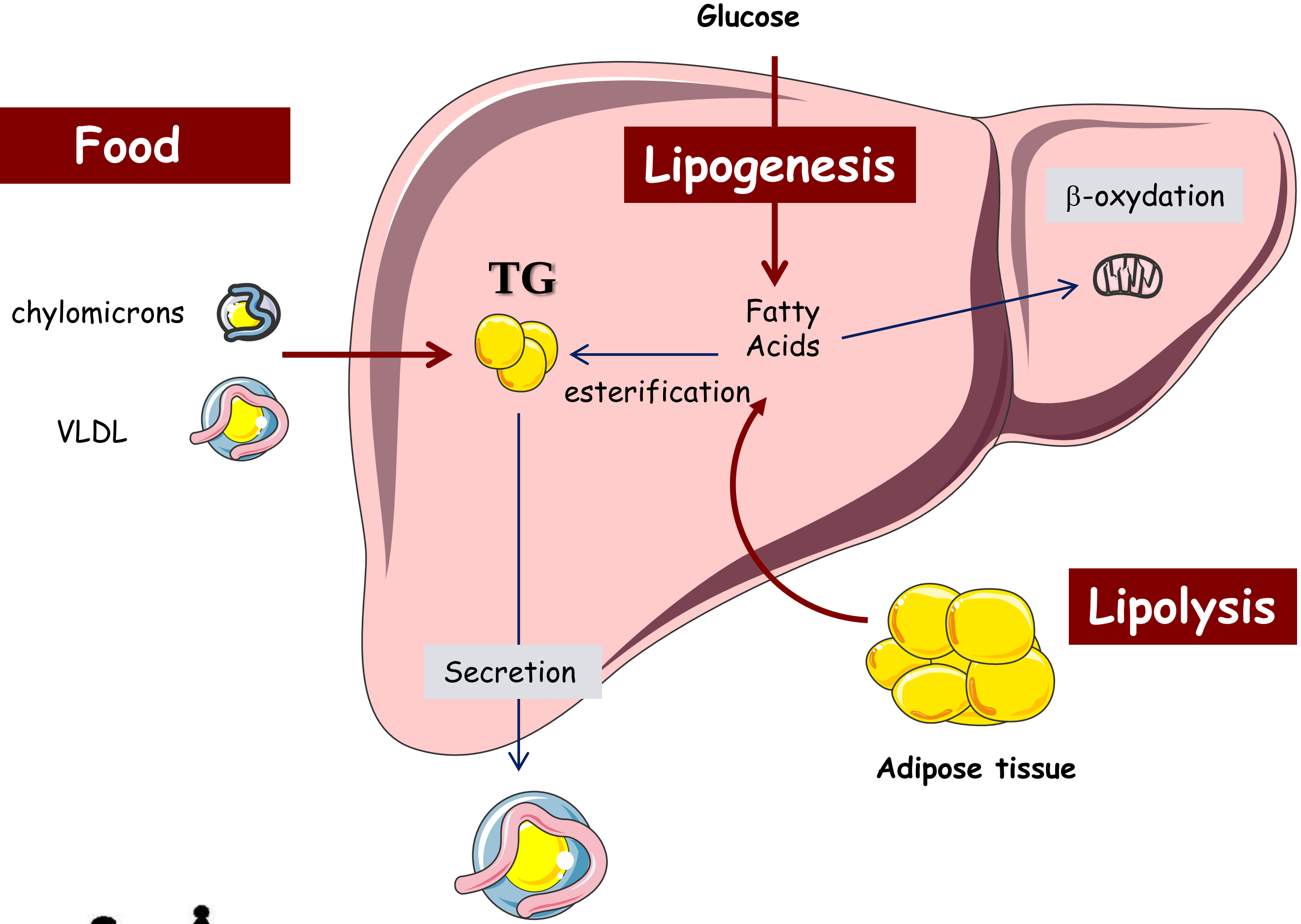
Lipolysis
Lipogenesis *de novo*
Food



STEATOSIS

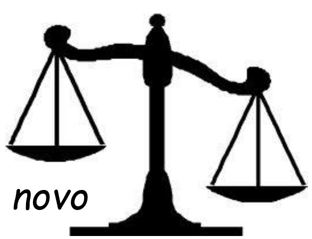


The causes of hepatic steatosis



Supply

Lipolysis
Lipogenesis *de novo*
Food

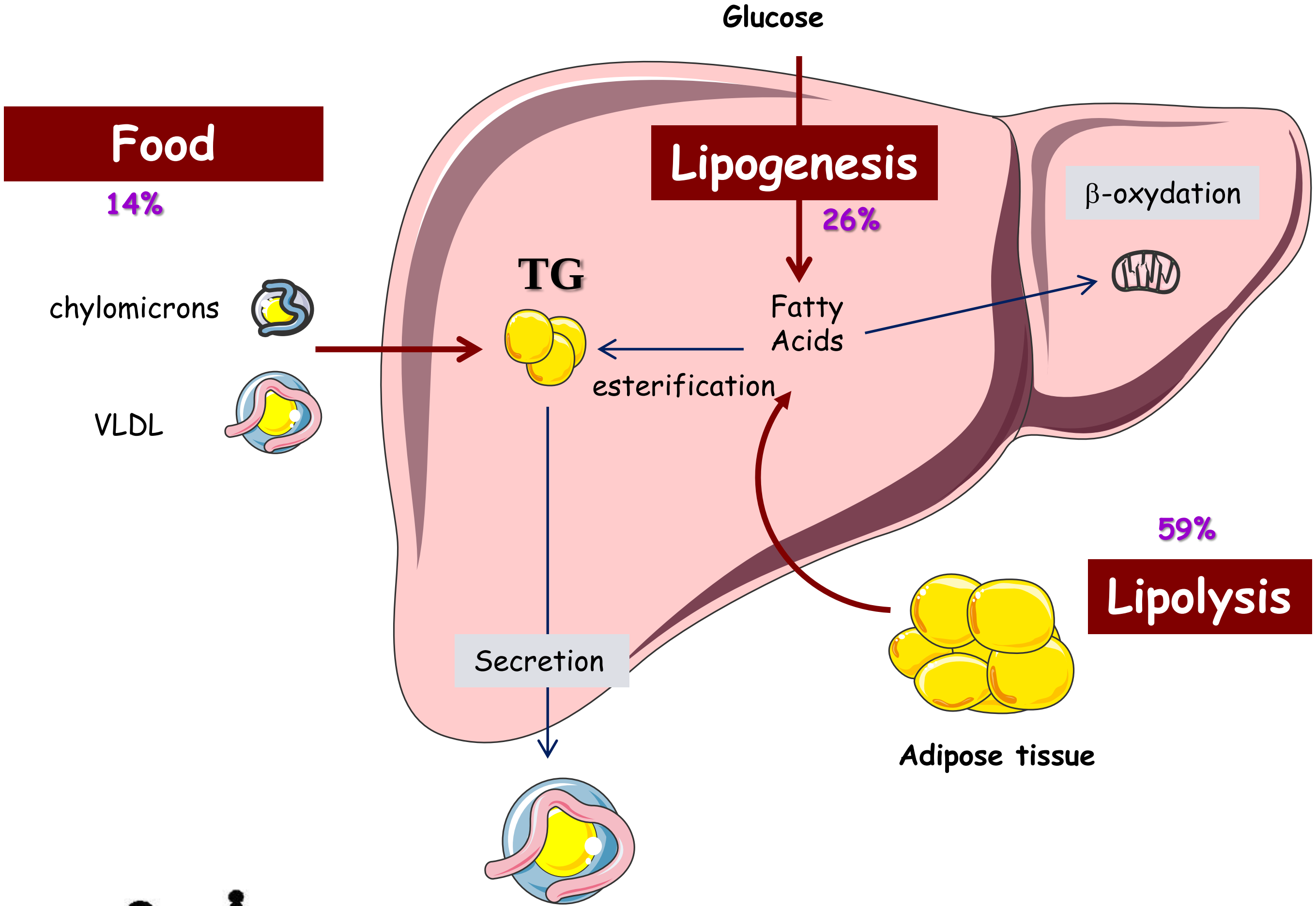


STEATOSIS

Use

β-oxydation
Secretion

The causes of hepatic steatosis



Supply

Lipolysis
Lipogenesis *de novo*
Food

Use

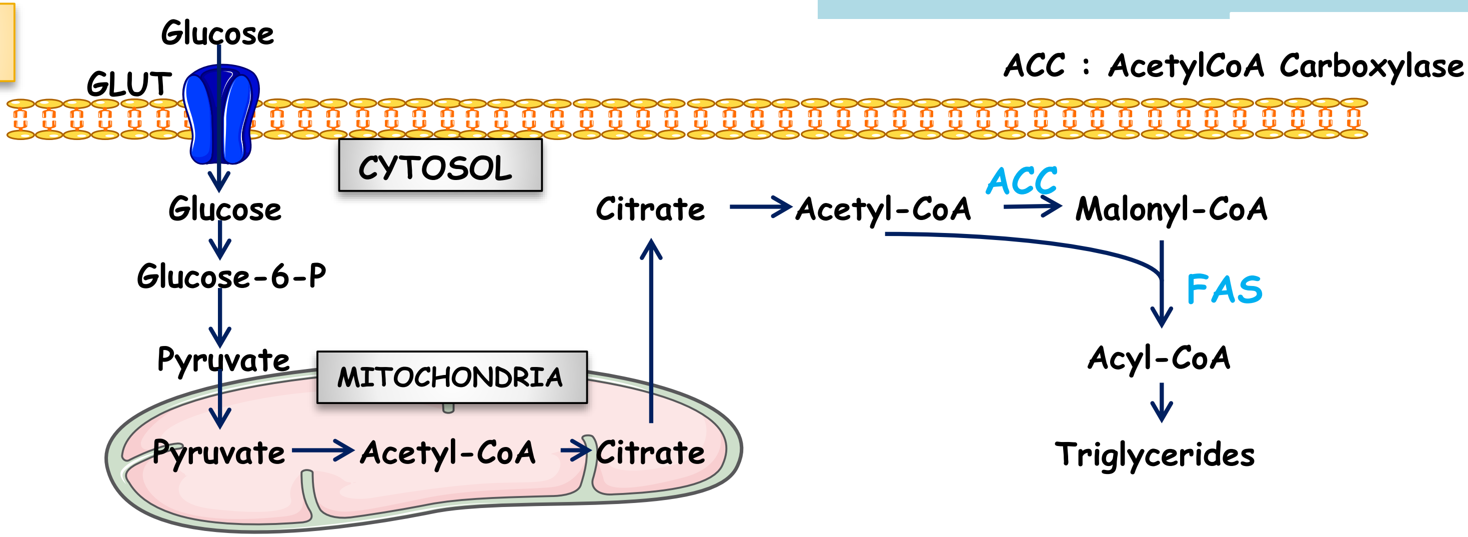
β-oxydation
Secretion

<u>Donnelly *et al.*, 2005</u>

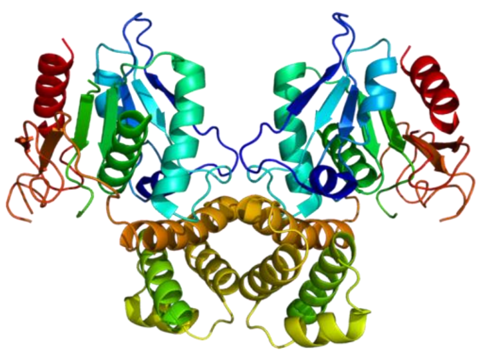
Hepatic steatosis : Fatty acids of triglycerides come from
59% of the lipolyse
26% of the lipogenesis
14 % of the food

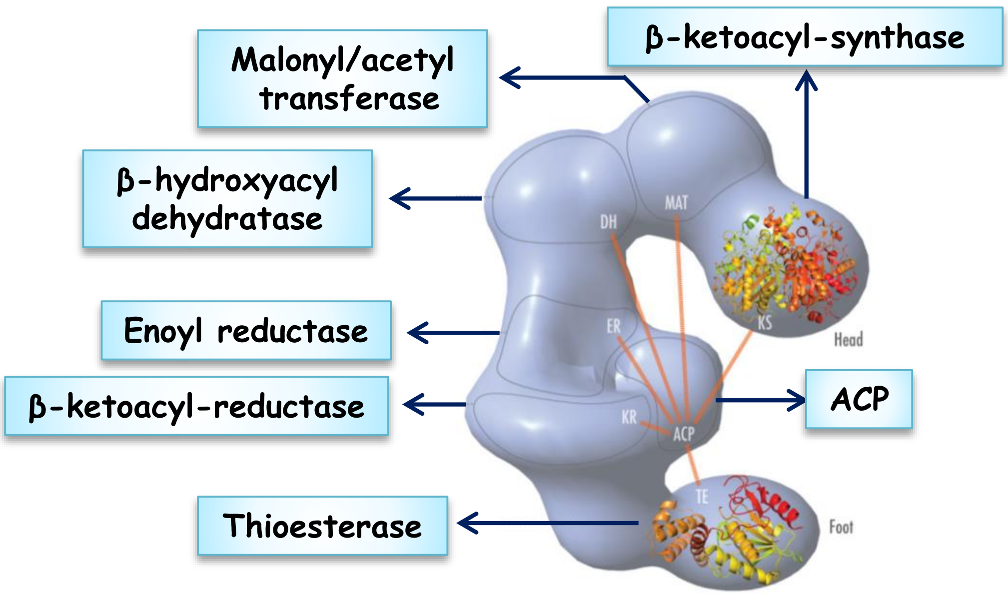
Lipogenesis and Fatty Acid Synthase

Lipogenesis



Fatty Acid Synthase



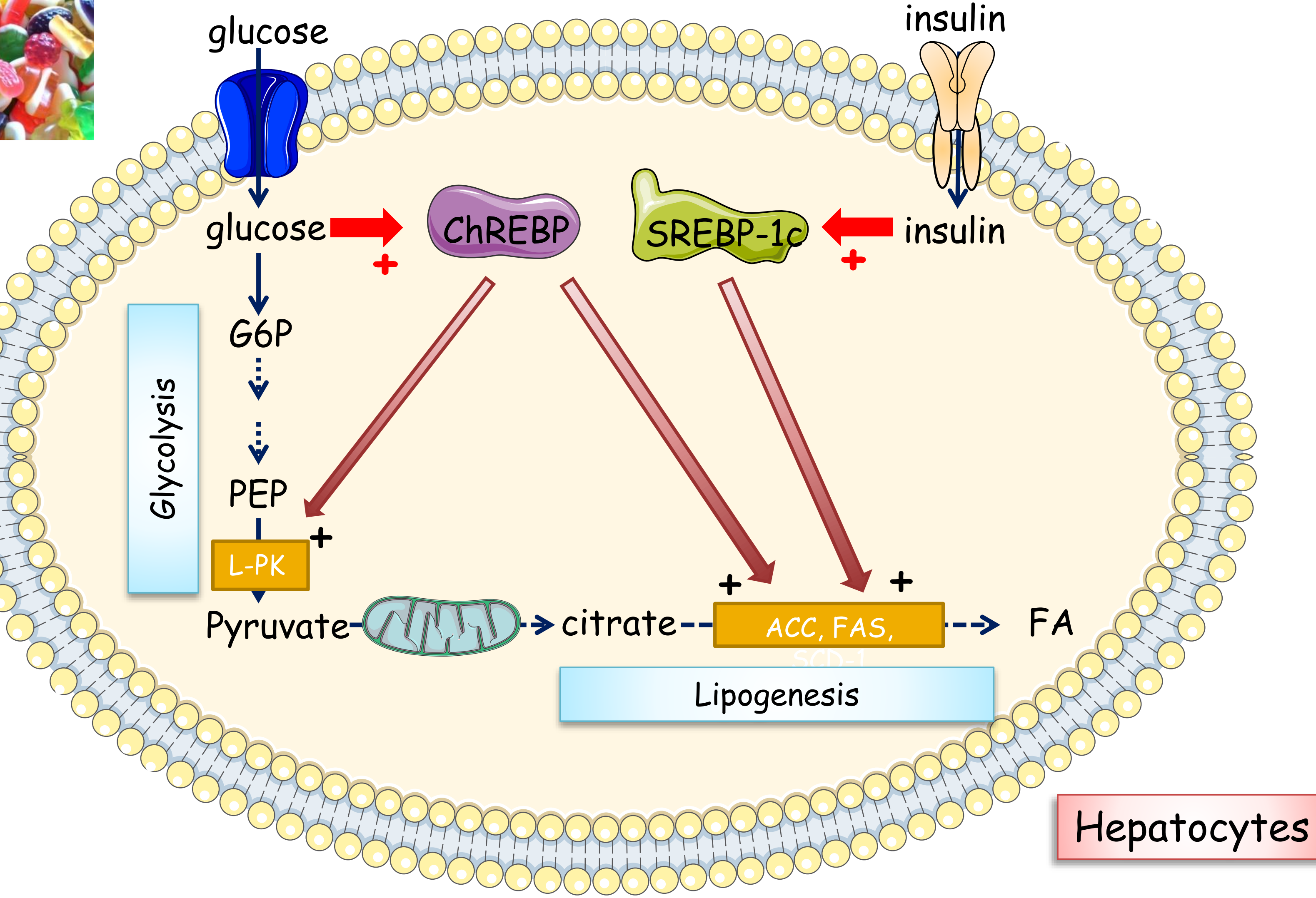


- 2 subunits
- 7 activities
- liver, adipose tissue, mammary gland

<u>Overall reaction</u> : acetylCoA + 7 malonylCoA + 14 NADPH,H^+ → palmitoylCoA (C16:0) + 7CO_2 + 14 $NADP^+$ + 7CoA

Regulation of FAS

- **At the transcriptional level** by the transcription factors,ChREBP and SREBP-1c

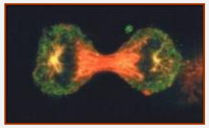


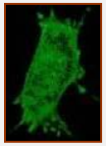
- **At the post translational level** by phosphorylation

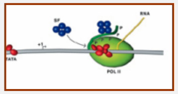
… Hundreds proteins bearing O-GlcNAc have been identified

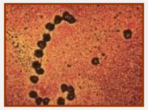
Enzymes of metabolism
Proteins of stress
Proteins of cytoskeleton
Transcription Factors
OGT/OGA
Kinases/phosphatases
Proteasome
Proteins of pore nuclear
Ribosomals proteins

… Involved in many cellular processes


Cell cycle


Apoptosis

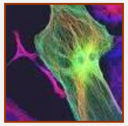

Translation

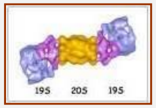

Transcription


Development


Signaling


Cell trafficking


Cellular architecture



Proteins degradation

… dynamism disturbed in certain diseases


Type 2 diabetes

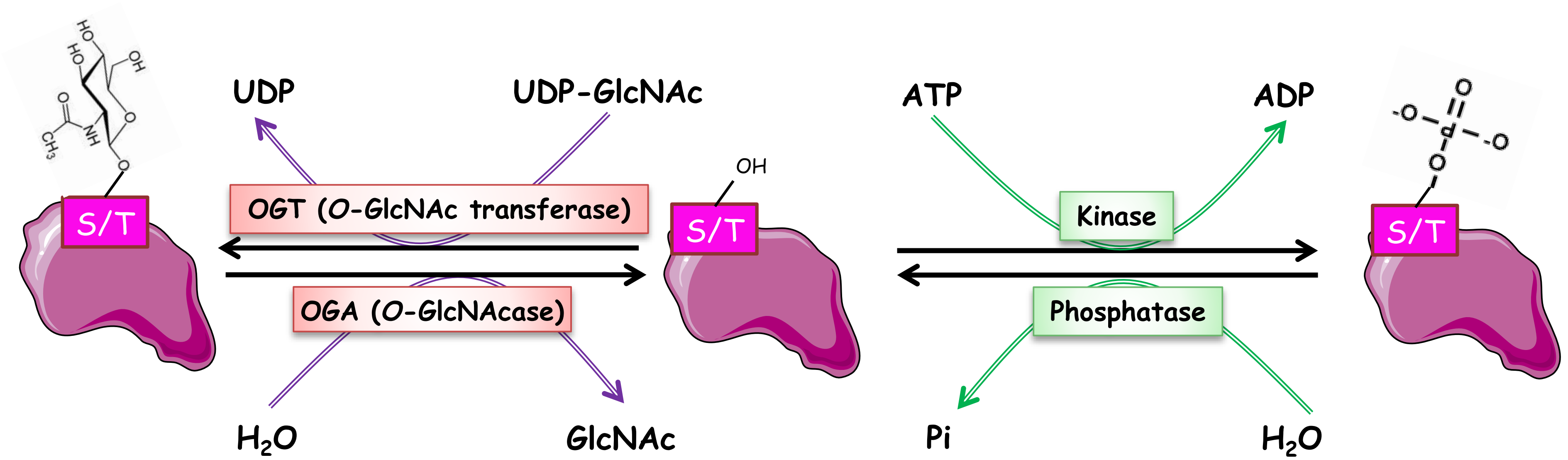

Neurological diseases


Cardiovascular diseases

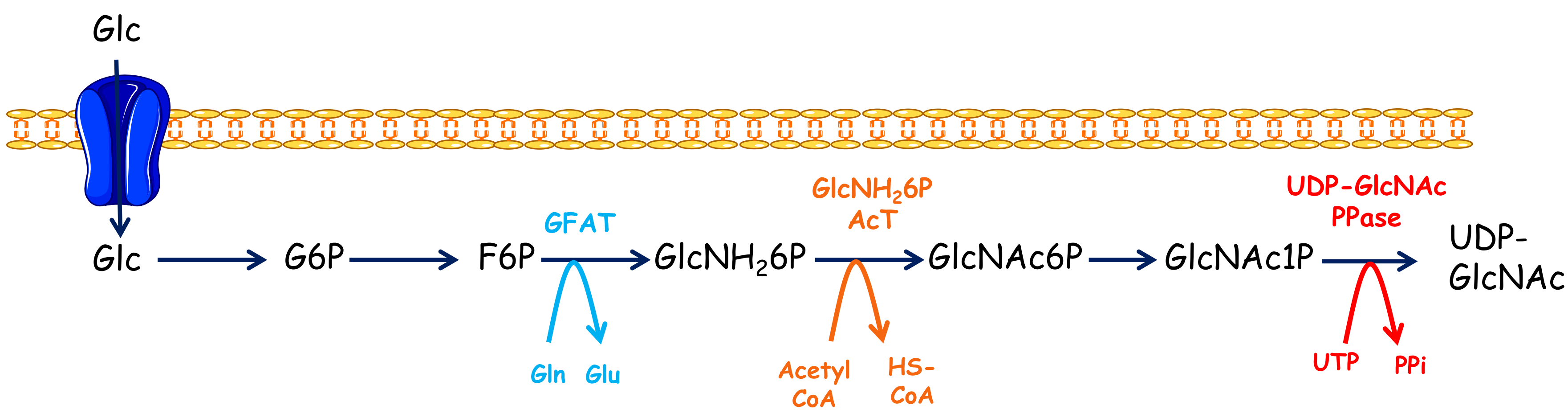

Cancers

O-N-acetylglucosaminylation (O-GlcNAcylation)

O-GlcNAcylation

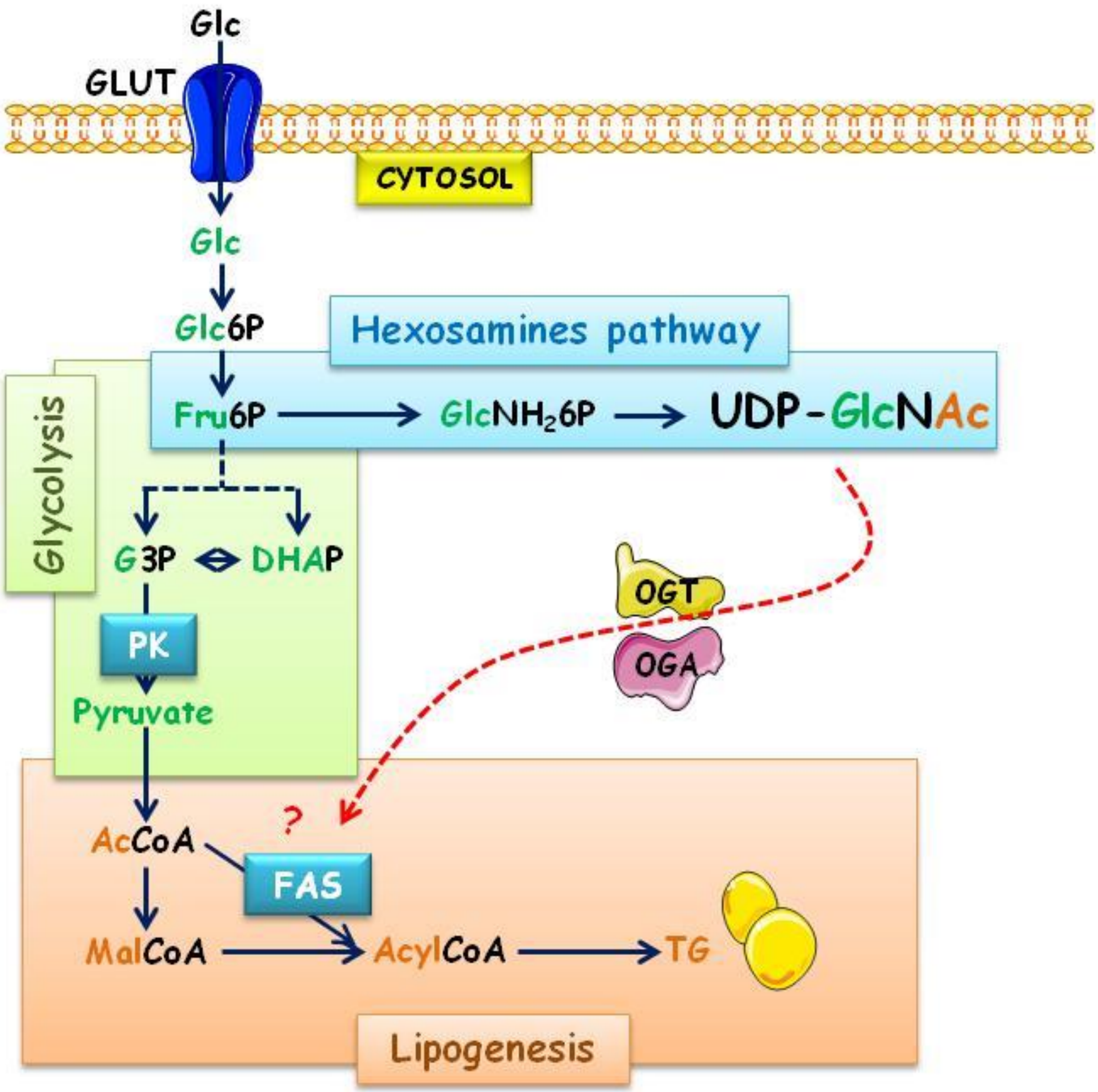


The Hexosamine Biosynthesis Pathway (HBP)



Hypothesis

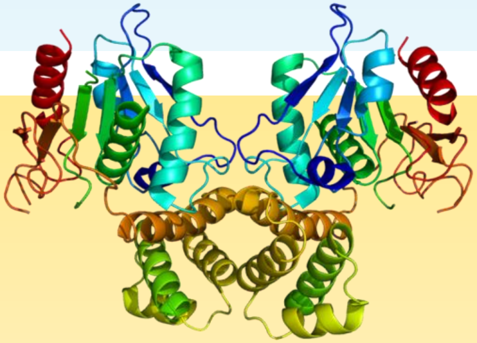
- FAS expression and *O*-GlcNAcylation level depend on glucose concentrations



Relation between *O*-GlcNAcylation, expression and activity of FAS

- Is FAS *O*-GlcNAcylated ?

- Relations between FAS *O*-GlcNAcylation and nutritional conditions

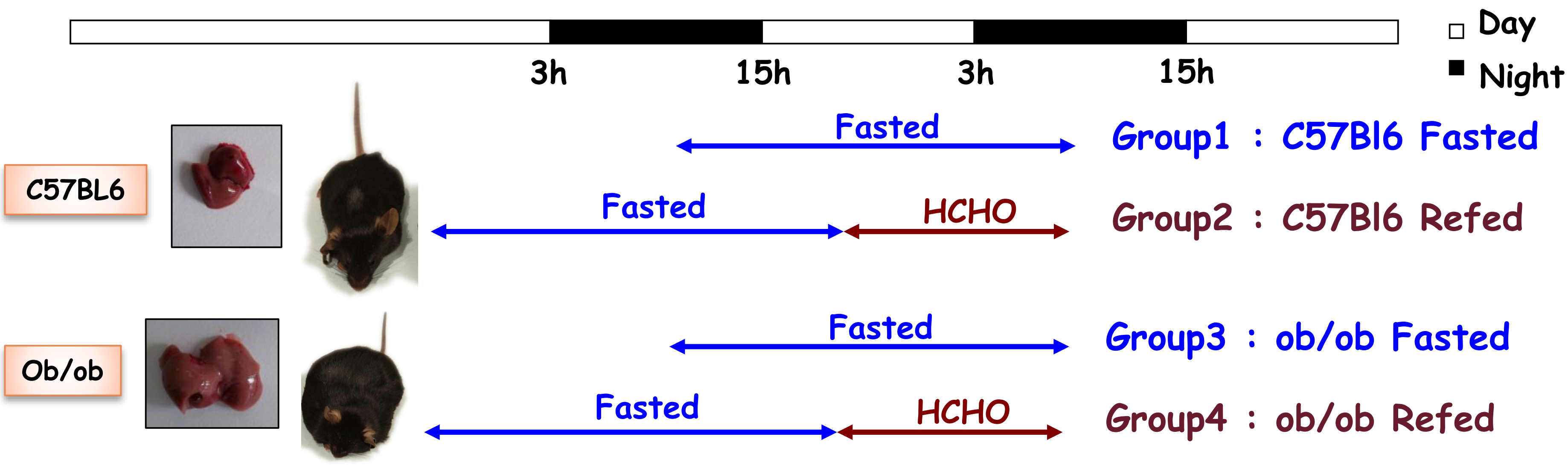


- Do *O*-GlcNAcylation levels interfere with:

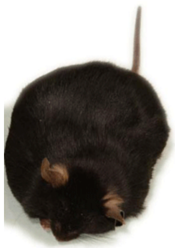
- FAS expression
- FAS stabilization
- FAS activity

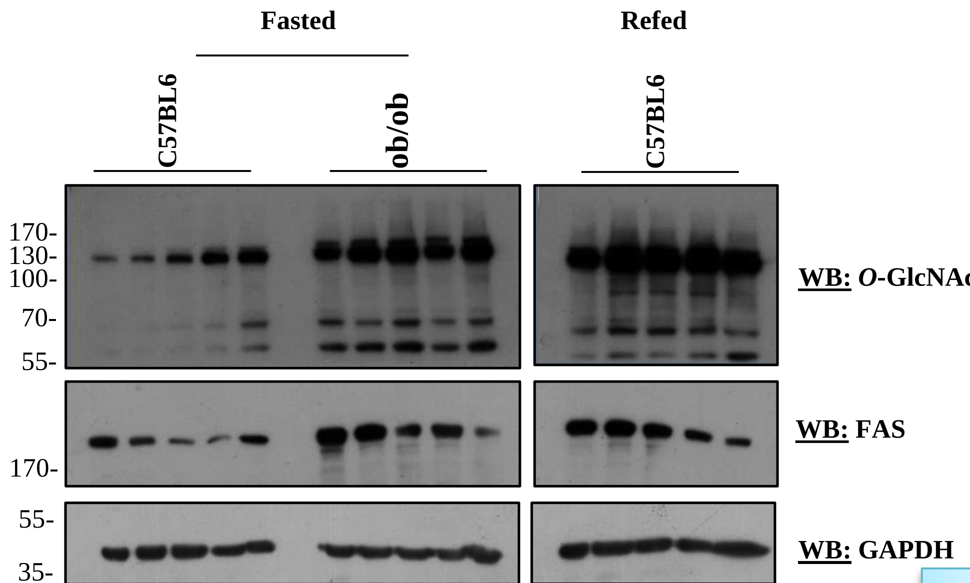
O-GlcNAcylation levels and FAS expression in physiopathological models

Model 1 : Use of mice C57BL6 wild type or ob/ob

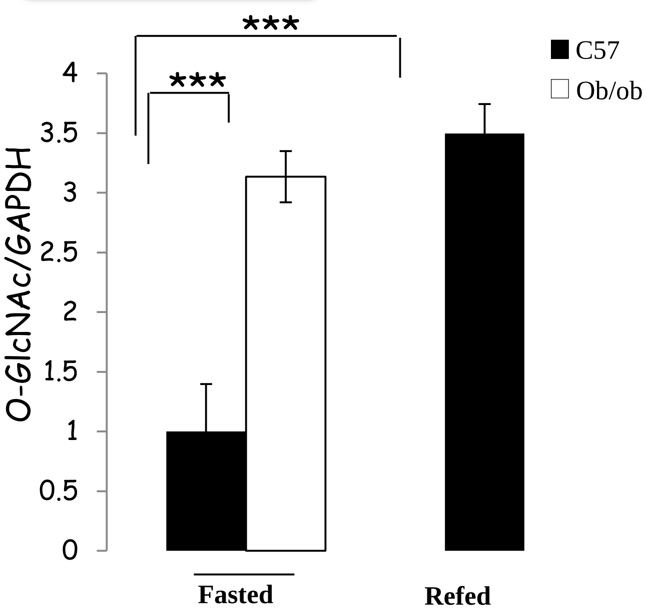


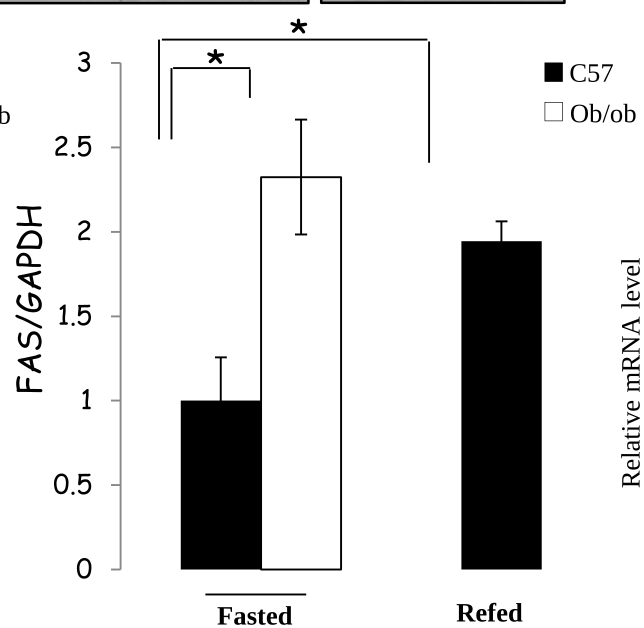
O-GlcNAcylated protein levels and FAS expression



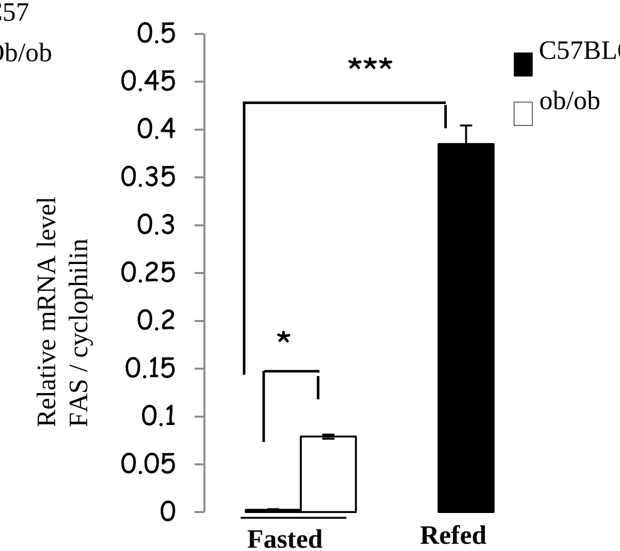


Quantification





FAS ARNm



O-GlcNAcylation levels and FAS expression in physiopathological models

Model 2 : Use of mice C57BL6 fed a chow diet or fed a High Carbohydrate Diet.

Chow Diet (CD)

65% carbohydrate, 24% protein, 11% fat

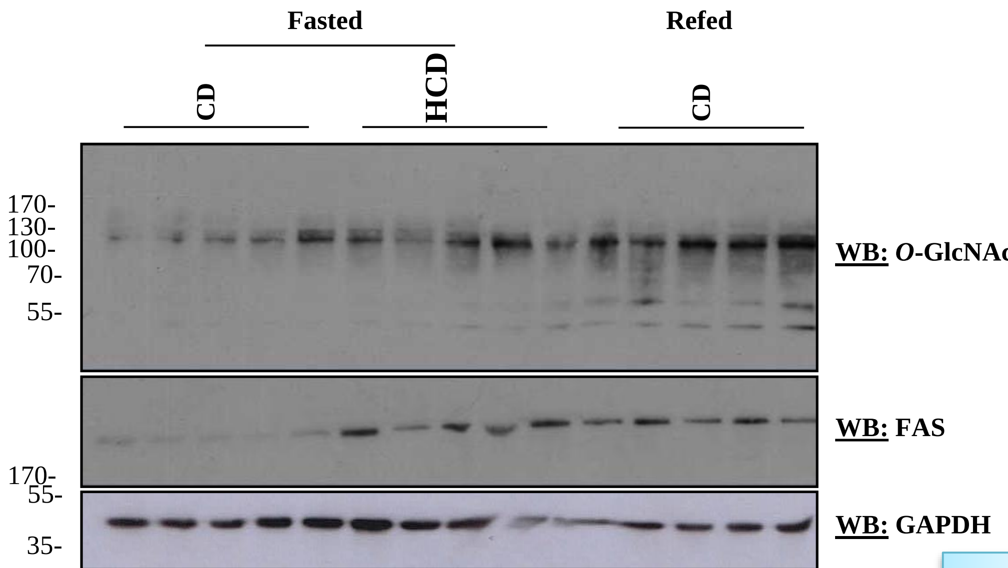
- Fasted → Group1 : CD Fasted
- Fasted → HCHO → Group2 : CD Refed

High Carbohydrate Diet (HCD)

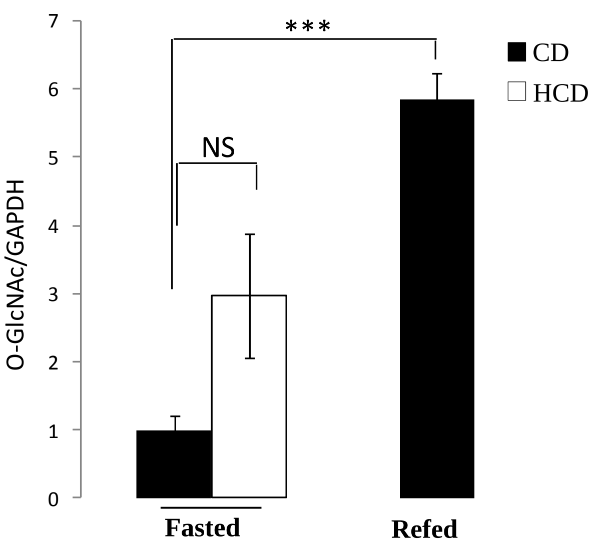
75% carbohydrate, 22% protein, 3% fat

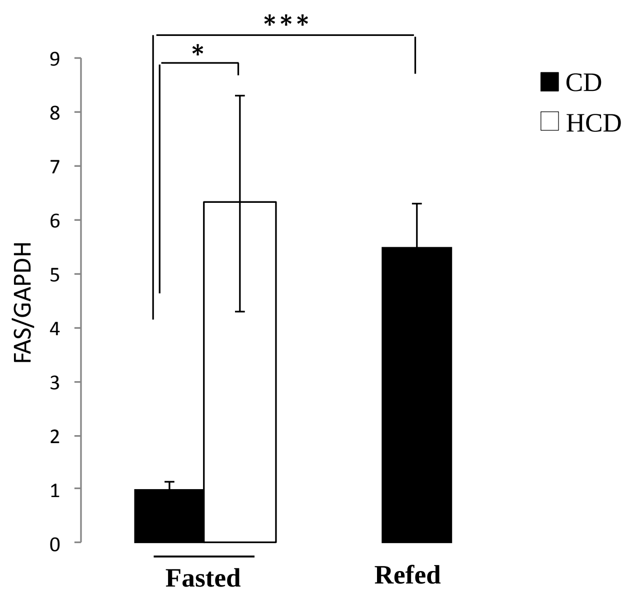
- Fasted → Group3 : HCD Fasted
- Fasted → HCHO → Group4 : HCD Refed

O-GlcNAcylated protein levels and FAS expression

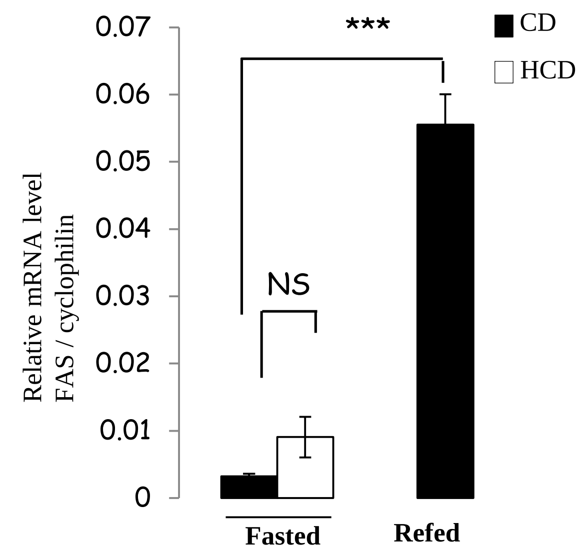


Quantification

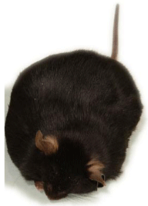


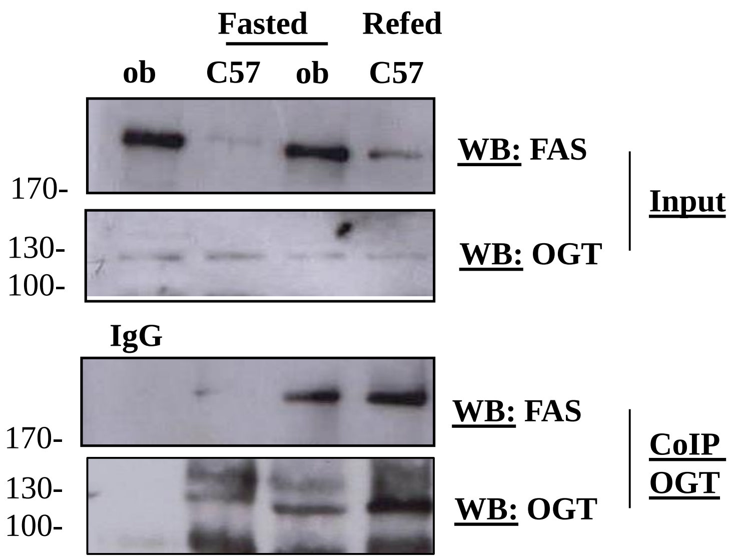


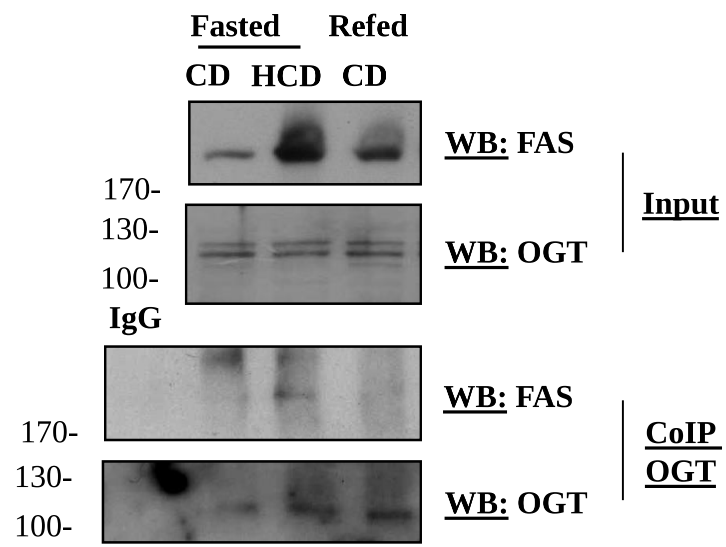
FAS ARNm



Interaction FAS/OGT



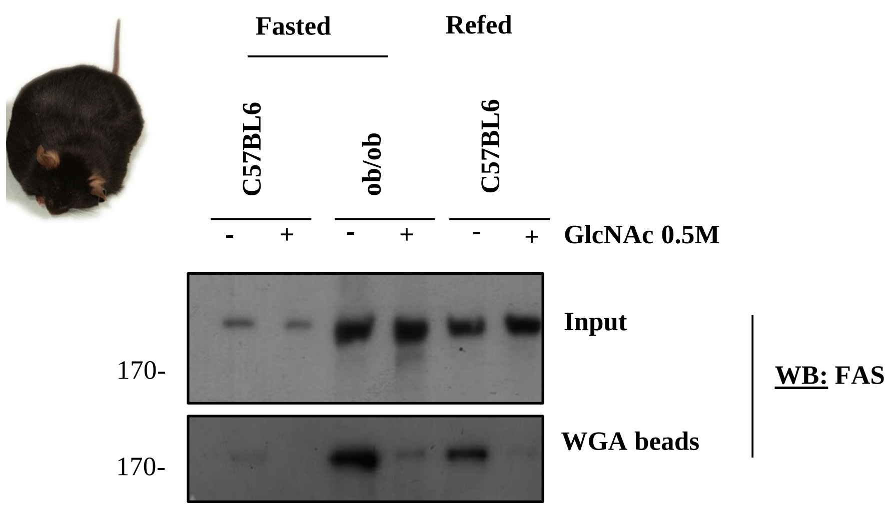


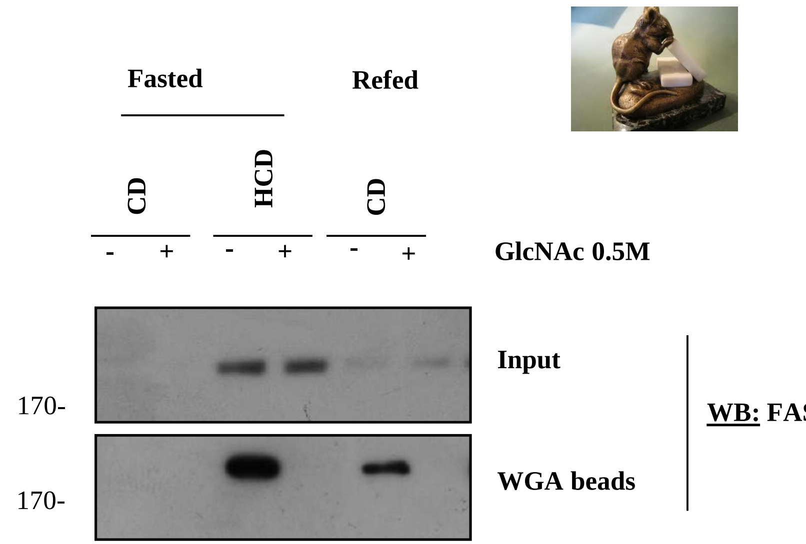


➡ FAS and OGT are partners of interaction

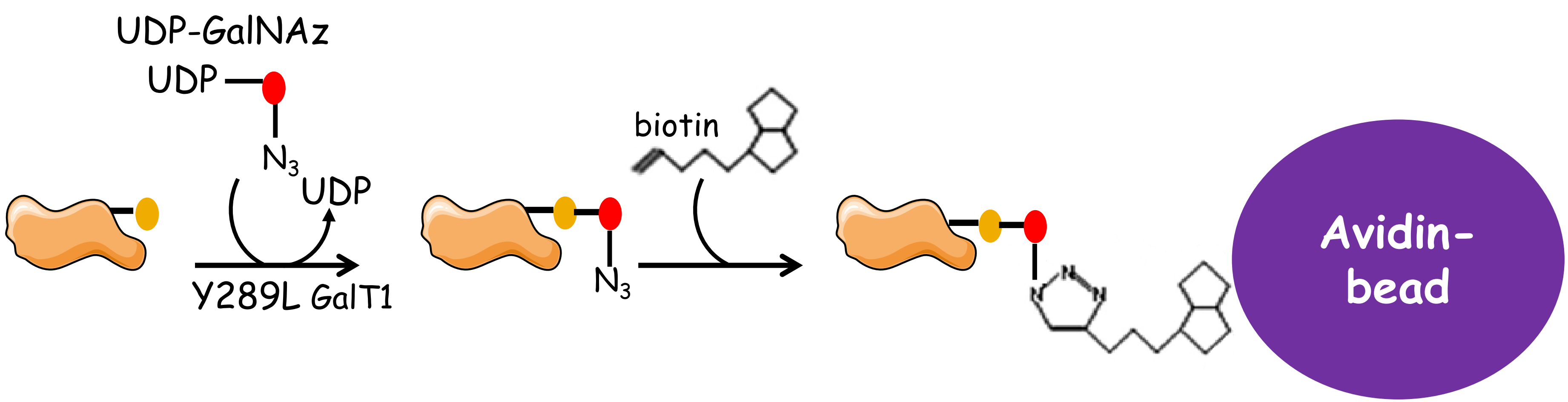
FAS O-GlcNAcylation

❑ Use of lectins : Wheat Germ Agglutinin



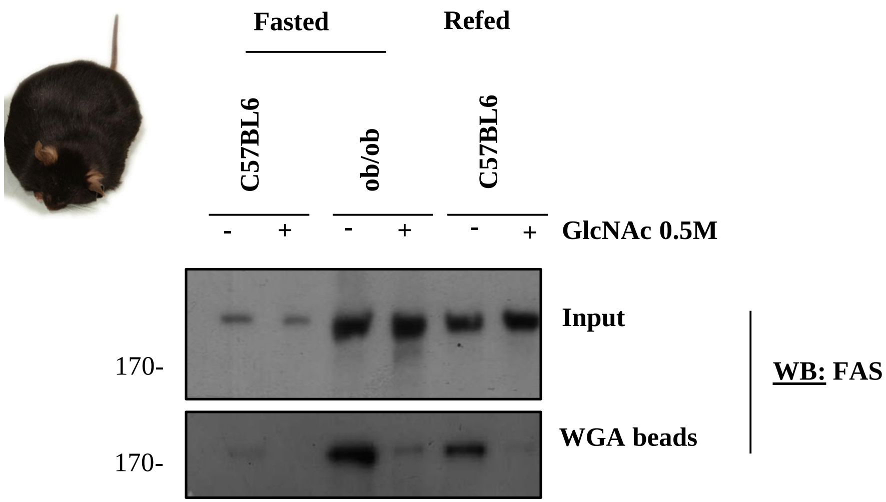


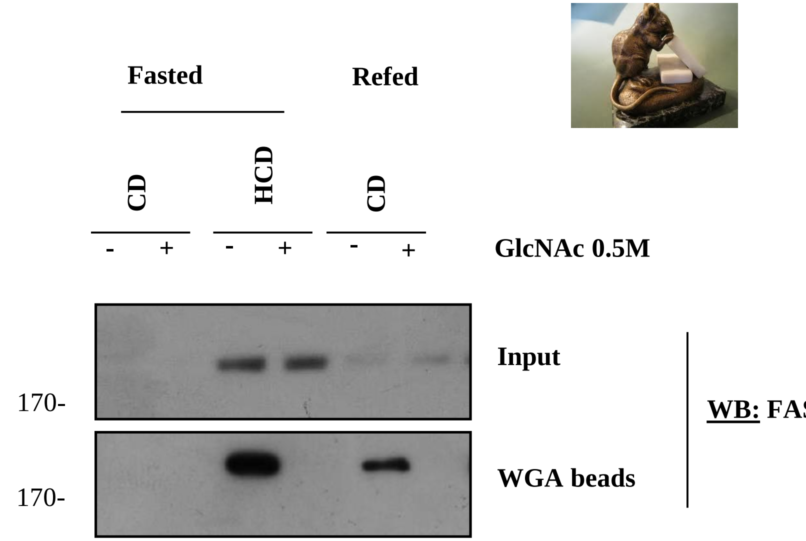
❑ click chemistry



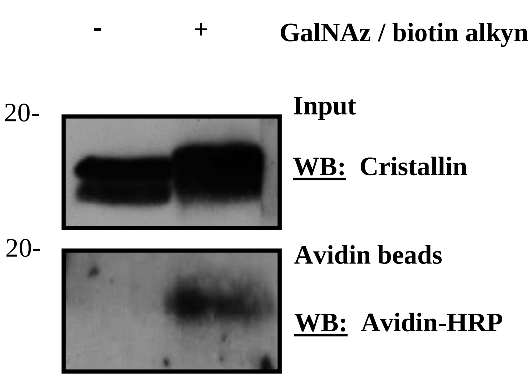
FAS O-GlcNAcylation

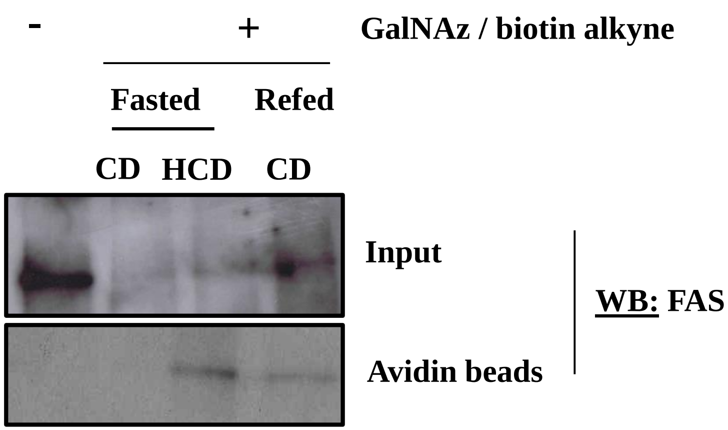
❑ Use of lectins : Wheat Germ Agglutinin





❑ click chemistry





O-GlcNAcylation levels and FAS expression in physiopathological models

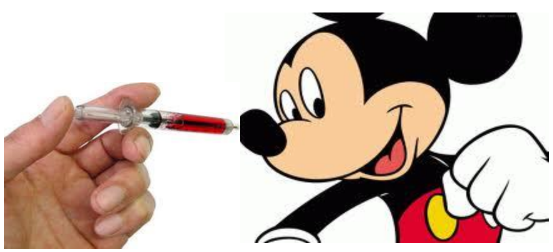
Model 3 : Use of mice C57BL6 presenting an inhibition of OGA

Day 0 — Day 14

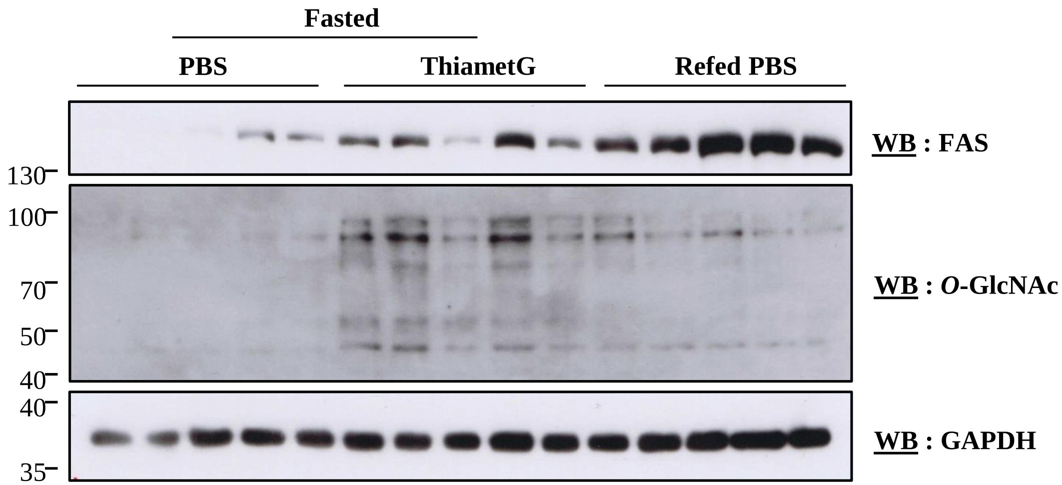
Daily injection of PBS/ThiametG
20 mg/ kg/ day

Fasted → Group1 : Fasted

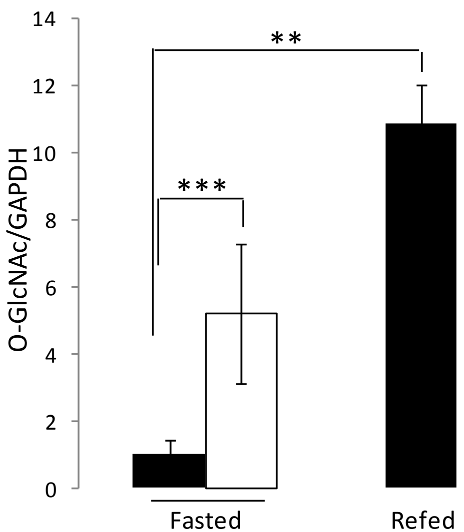
Fasted → HCHO → Group2 : Refed

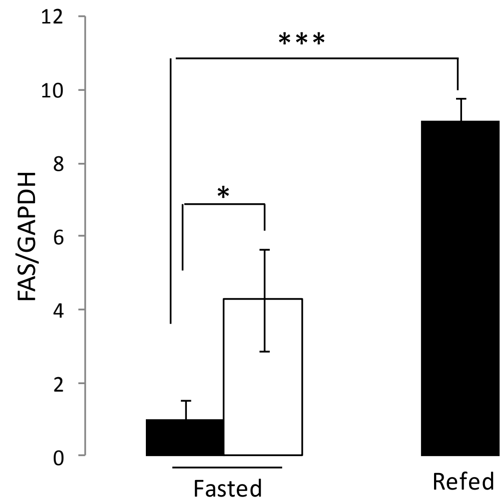


O-GlcNAcylated protein levels and FAS expression

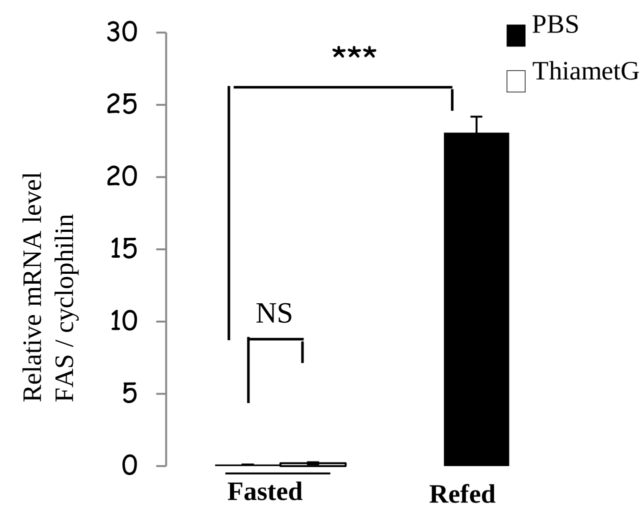


Quantification



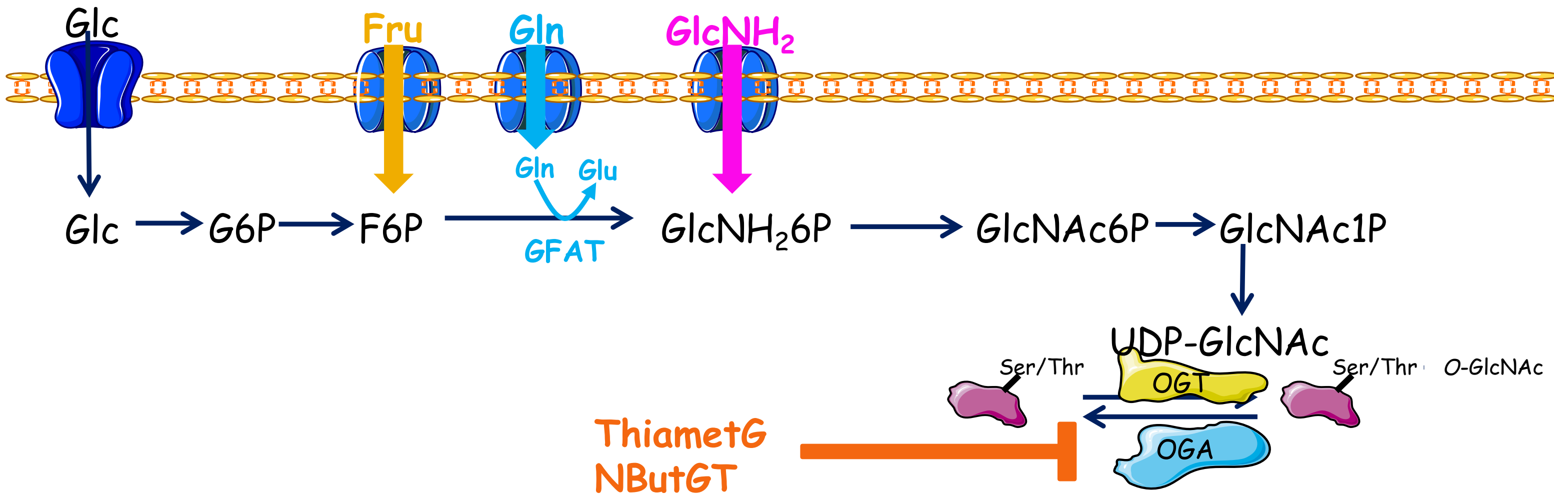


FAS ARNm



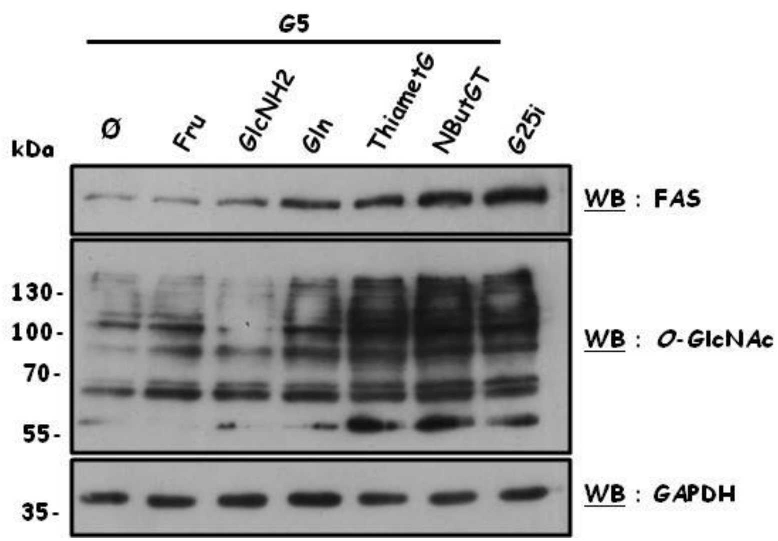
➢ *Ex vivo*

- ❑ Cancer human hepatocytes cell line : HepG2
- ❑ Mouse primary hepatocytes cultures



Mouse primary hepatocytes culture

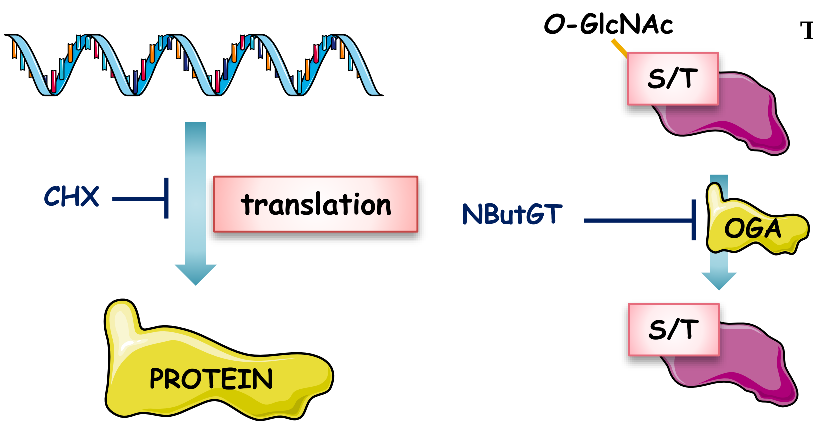
Expression of FAS

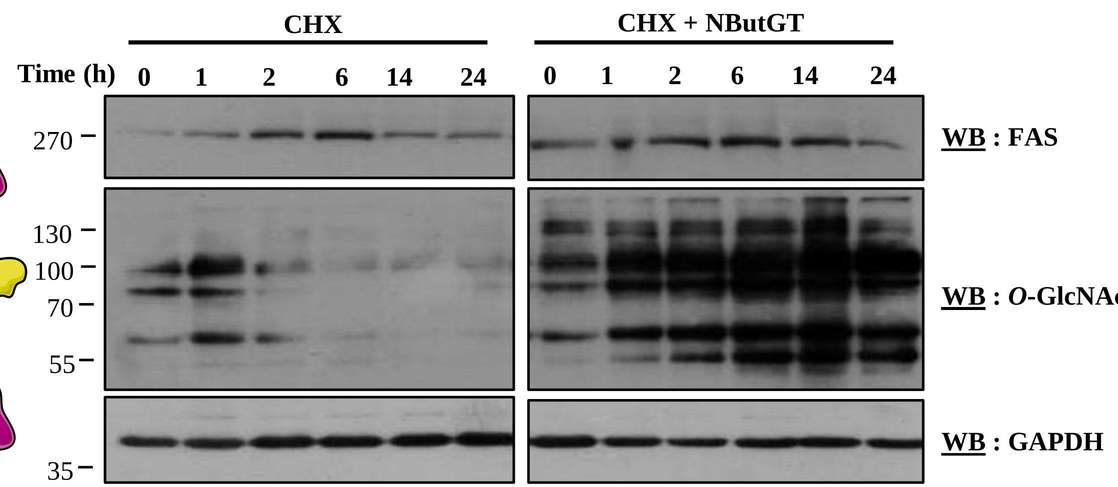


Role of O-GlcNAcylation on FAS stabilisation

HepG2

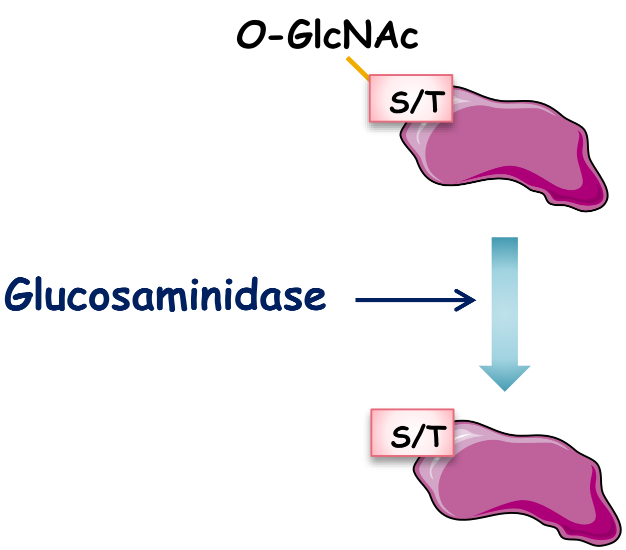
Kinetic with cycloheximide

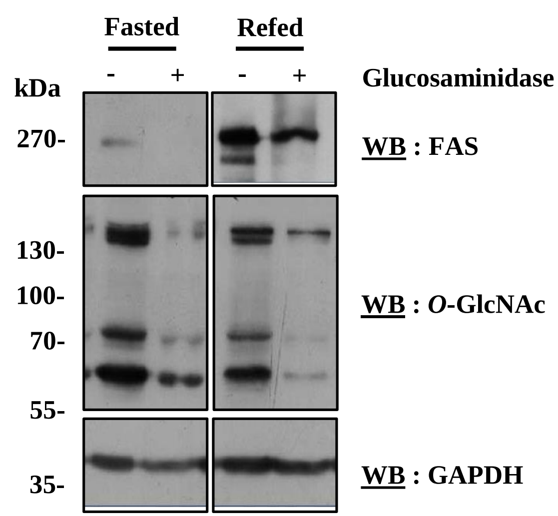




Liver mice

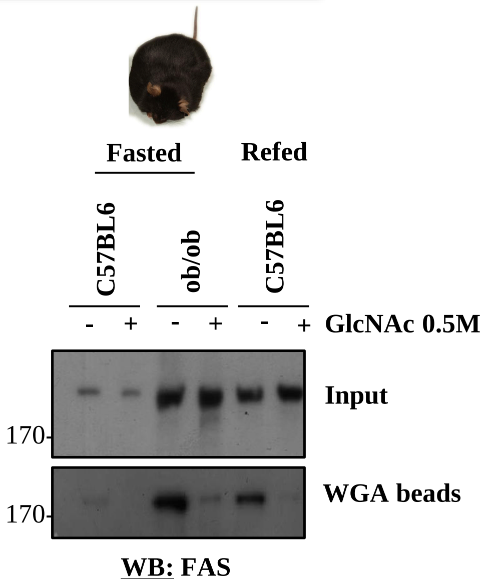
Treatment with β-N-acetylglucosaminidase

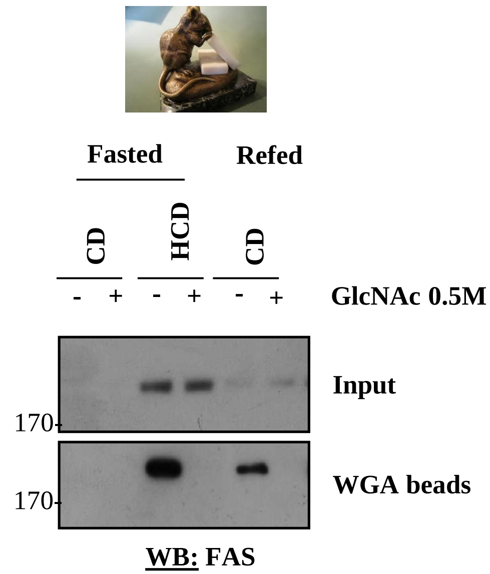




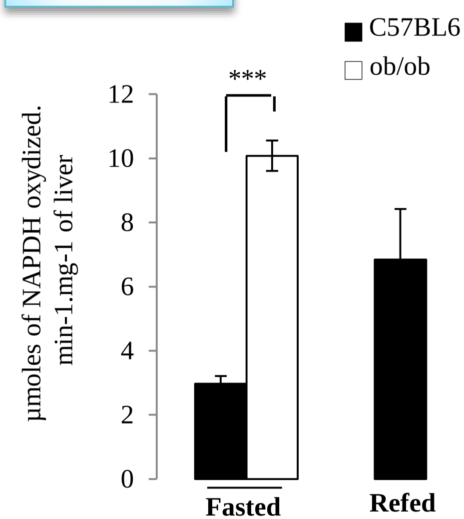
Role of *O*-GlcNAcylation on FAS activity

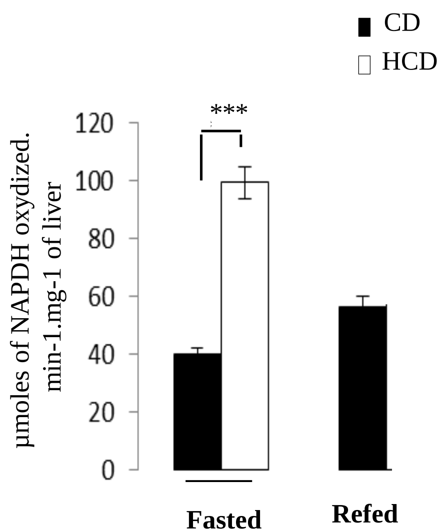
FAS O-GlcNAcylation

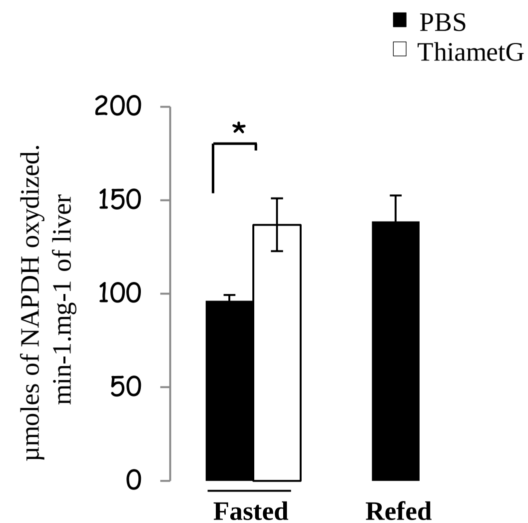




FAS activity



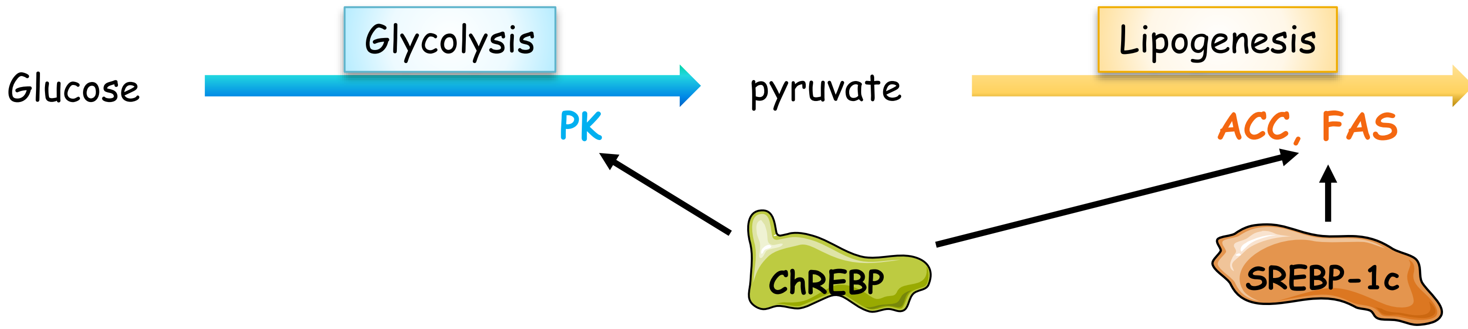




Conclusions

FAS regulation :

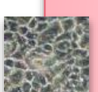
❑ by transcription factors



❑ by *O*-GlcNAcylation

➢ FAS interacts with OGT and it's *O*-GlcNAcylated

➢Roles of the *O*-GlcNAcylation :

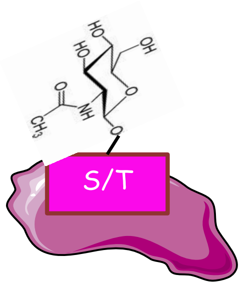


❑ Increase of global *O*-GlcNAcylation levels is correlated with an increase of FAS expression through a reduction of its degradation.

❑ FAS activity is in correlation with its *O*-GlcNAcylation

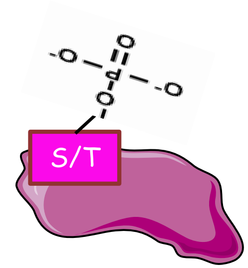
Relation *O*-GlcNAcylation/ubiquitinylation of FAS

Hypothesis

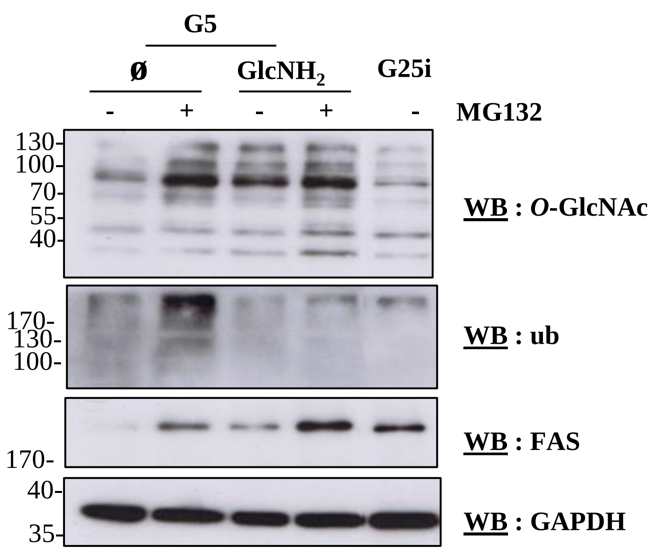


O-GlcNAcylation
Stabilisation

Phosphorylation
Ubiquitinylation
Degradation



Preliminary results



Methodology

- Perform siRNA to silent OGT
- Evaluate FAS ubiquitinylation in function of *O*-GlcNAcylation levels

The MG132 can restore FAS expression at G5 similar to the condition G25. **FAS seems to be degraded by the proteasome in the liver**

ugsf
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RÉGION NORD PAS DE CALAIS