IN VIVO SYSTEMS TO STUDY GLYCOGEN STORAGE DISEASE TYPE 1 (GSD I)

PoLiMeR Teaching Event
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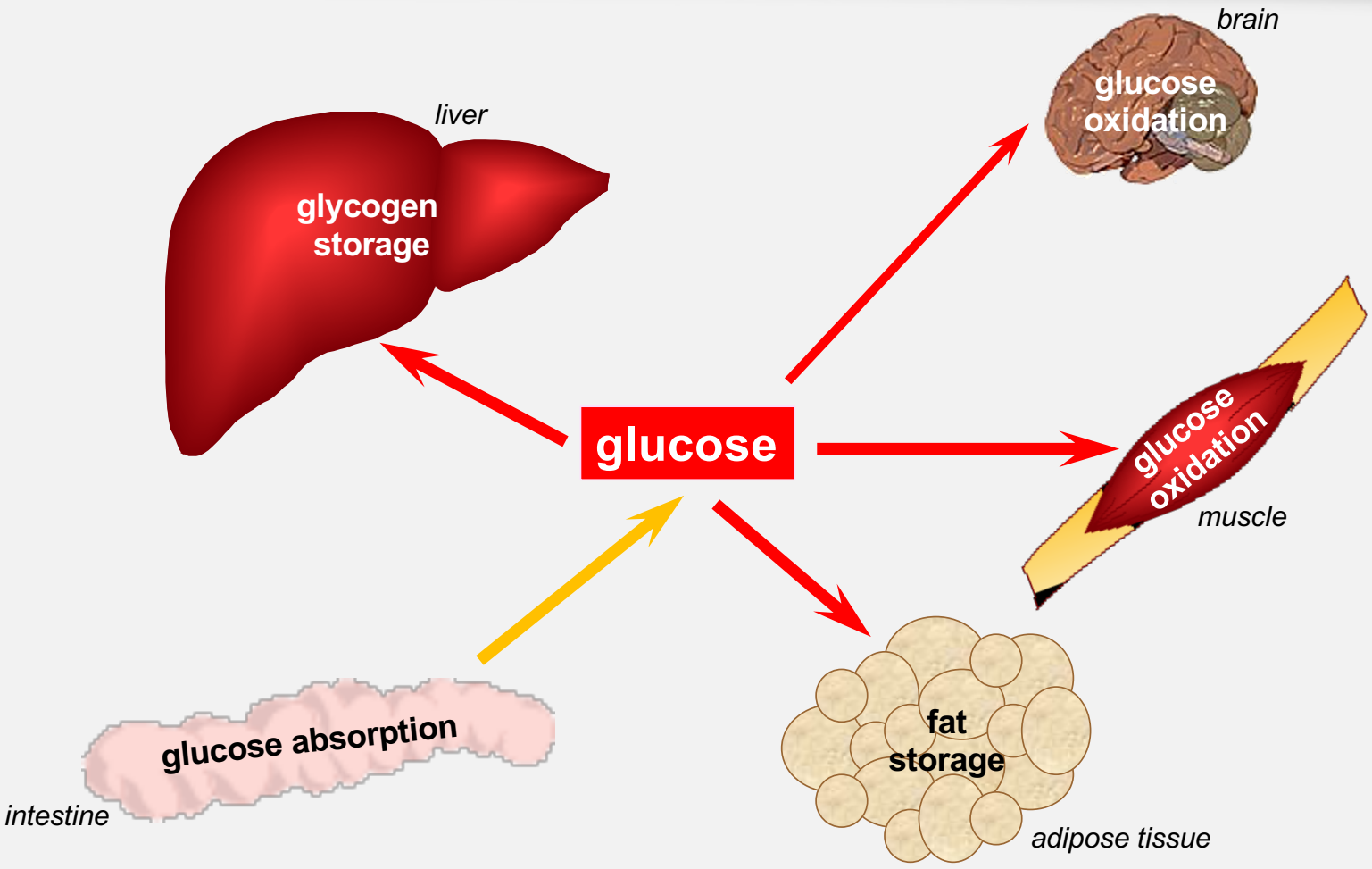
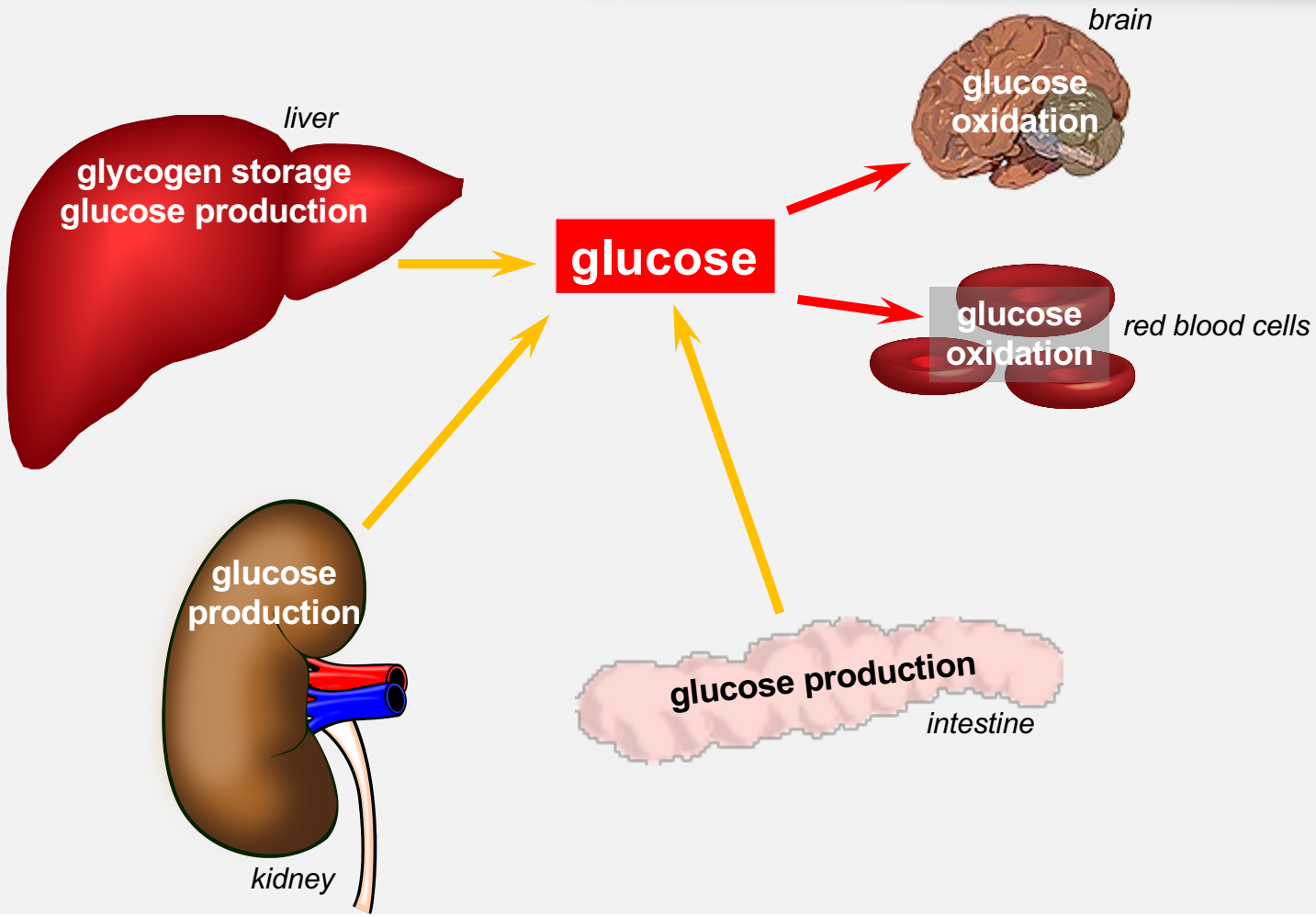
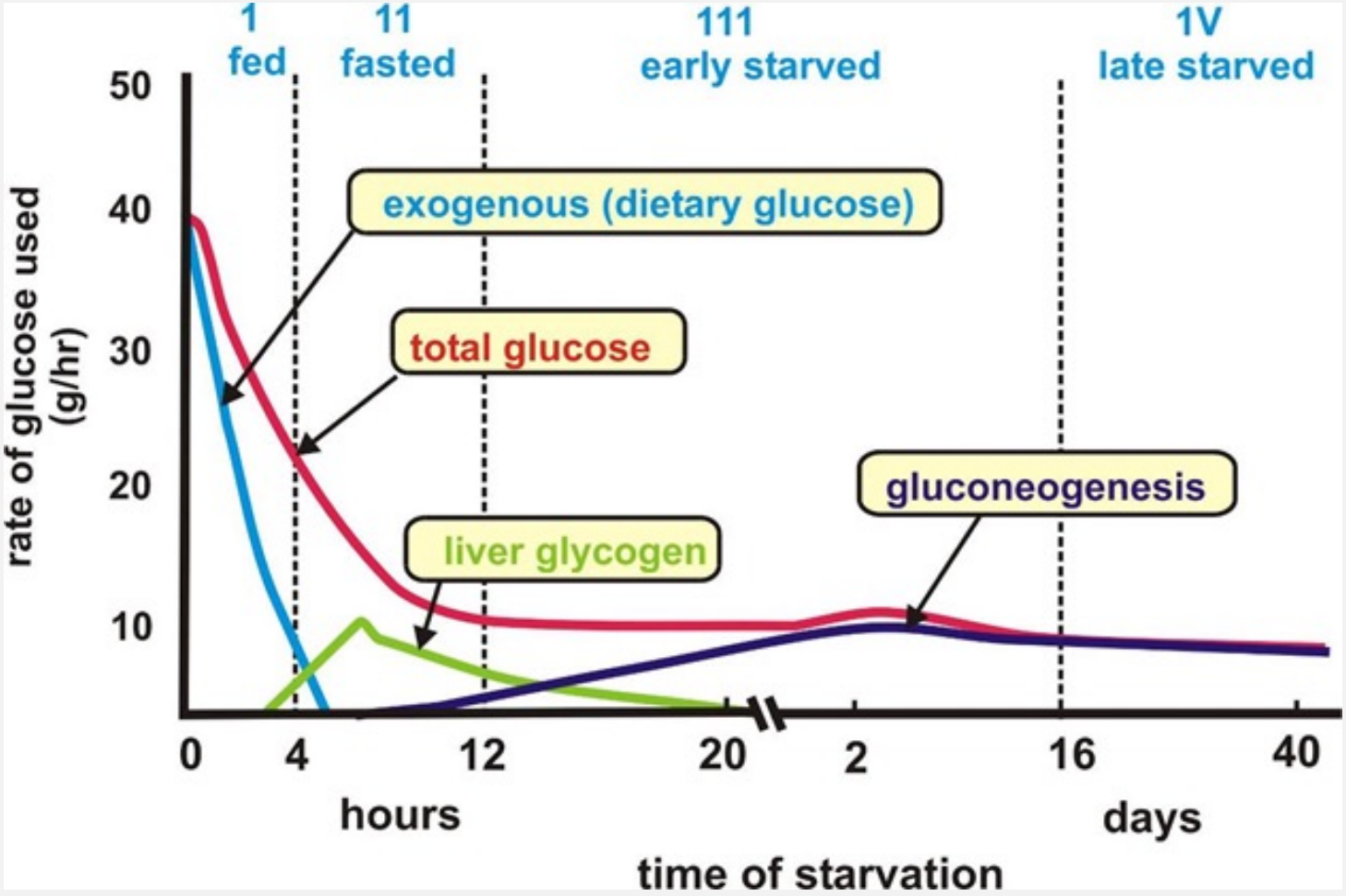
Glucose metabolism after feeding: glucose consumption



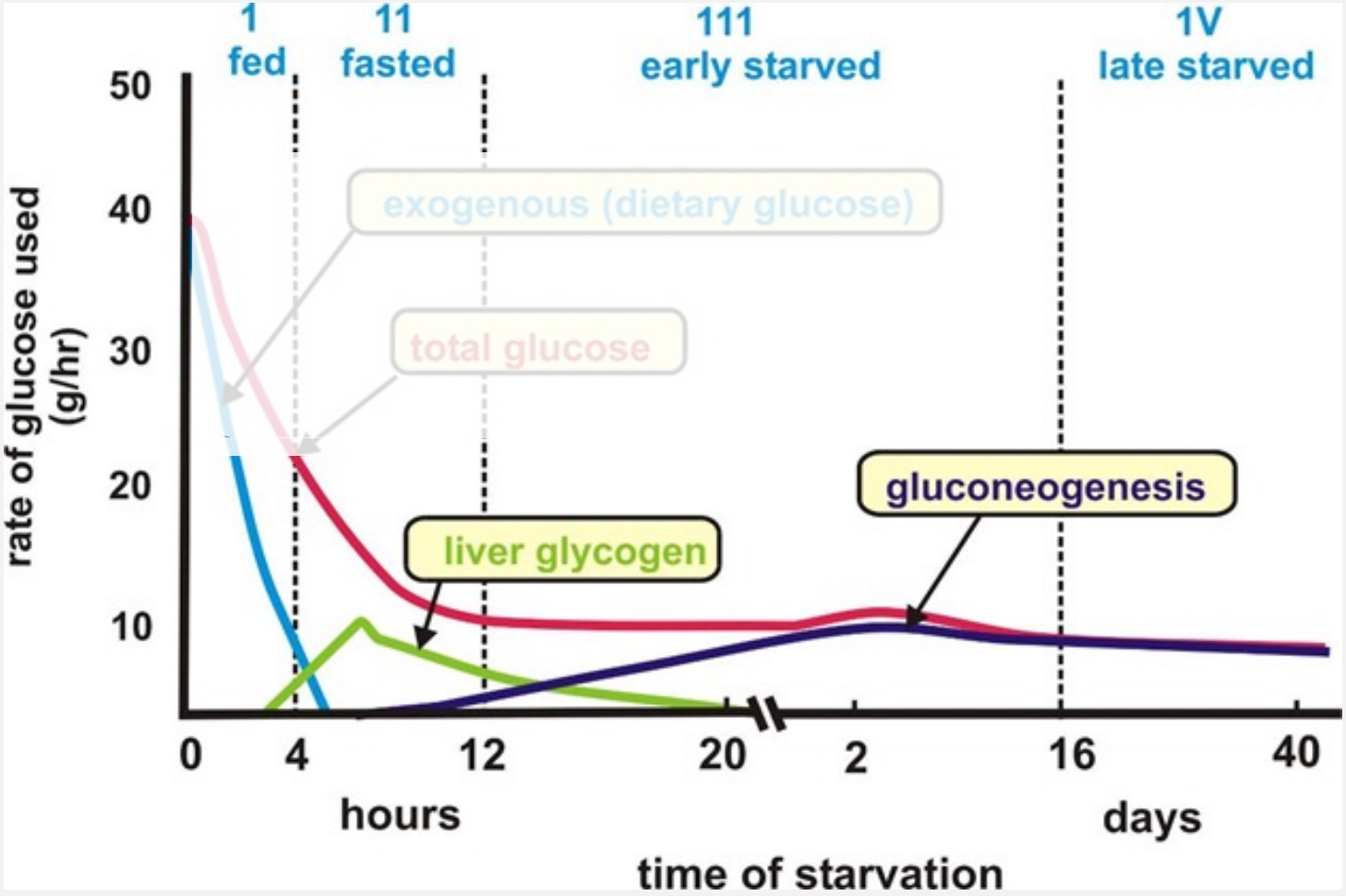
Glucose metabolism upon fasting: glucose production

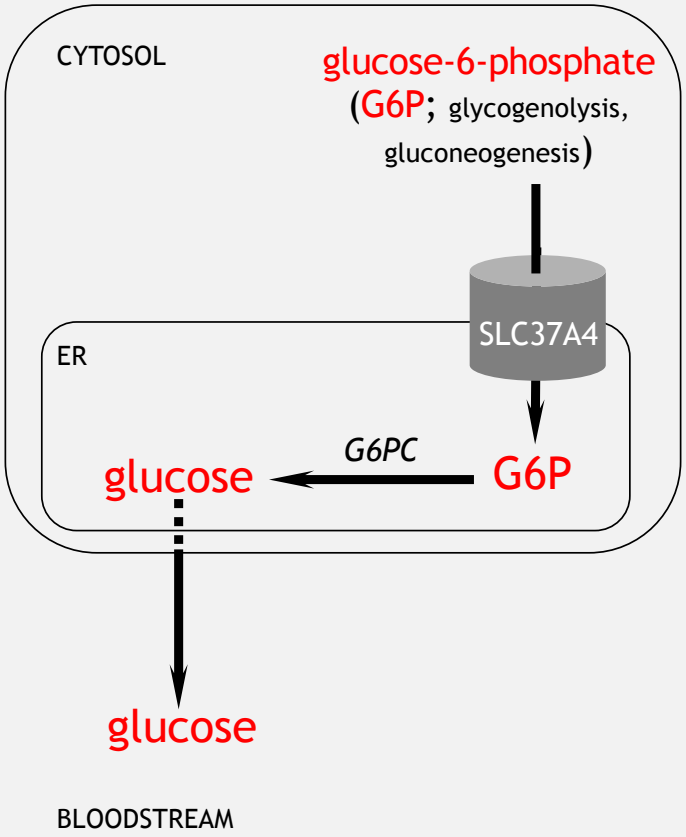


Sources of endogenous glucose production upon fasting

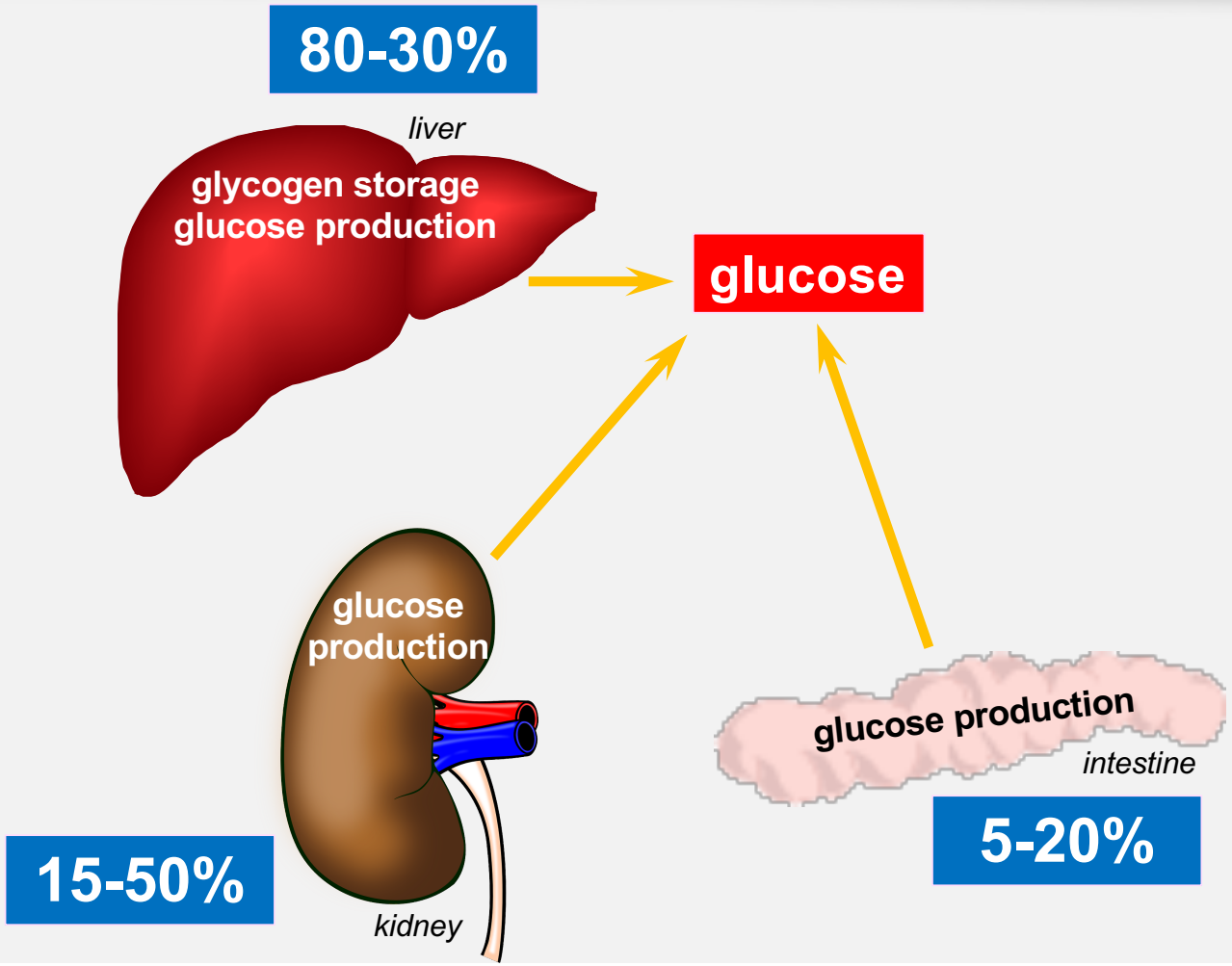


Glucose-6-phosphatase (G6PC) mediates the final step of glycogen breakdown and gluconeogenesis





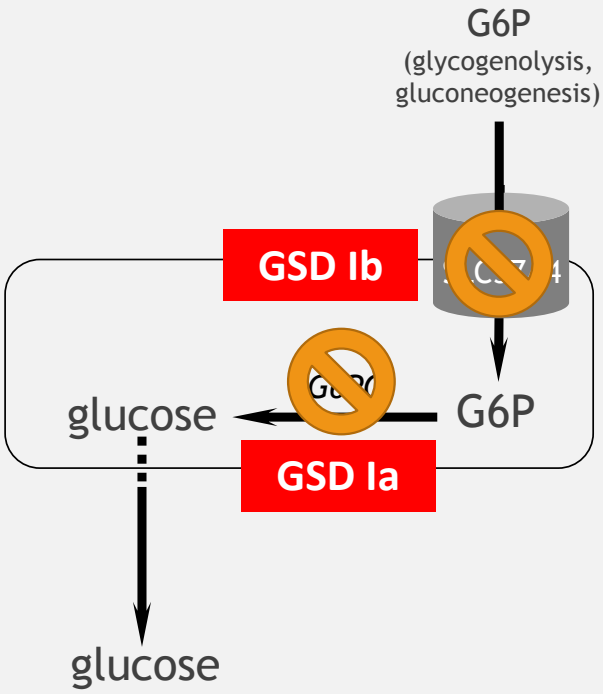
Endogenous glucose production



Glycogen Storage Disease type 1 (GSD I):
endogenous glucose production is impaired

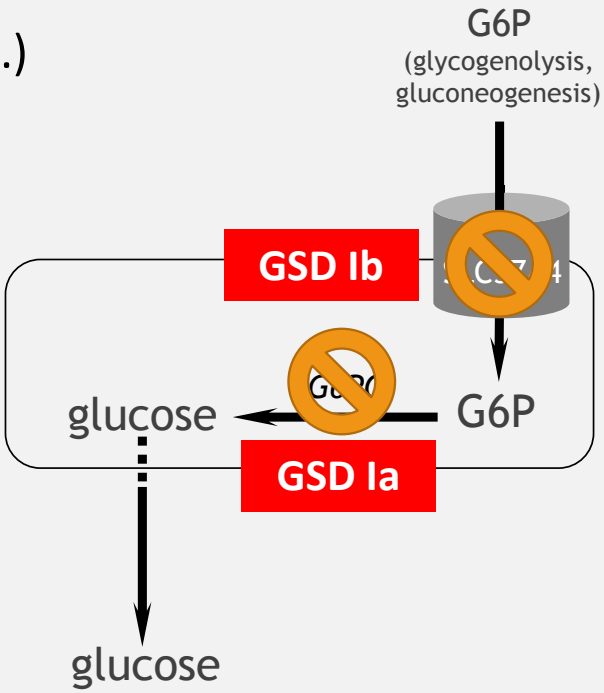
- Inborn Error of Metabolism, overall 1:100,000
  - Type Ia: *G6PC1* mutations
  - Typa Ib: *SLC37A4* mutations

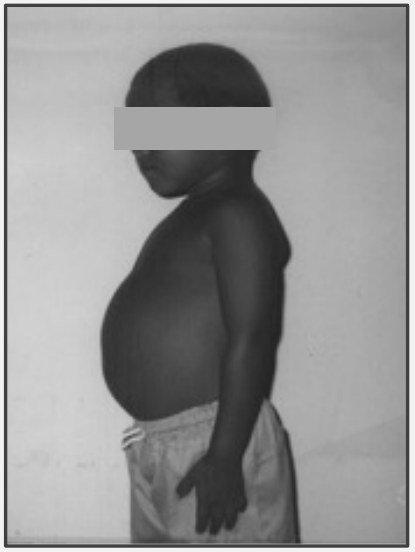
- G6PC1 is expressed in liver, kidney, intestine
- SLC37A4 is ubiquitously expressed



Glycogen Storage Disease type 1 (GSD I)

- **Biochemical symptoms**
  Low blood glucose levels (fasting, fever, ..)
  Enlarged liver, enlarged kidney
  Fatty liver disease
  High blood lipid levels
  High blood lactate levels
  High blood uric acid levels





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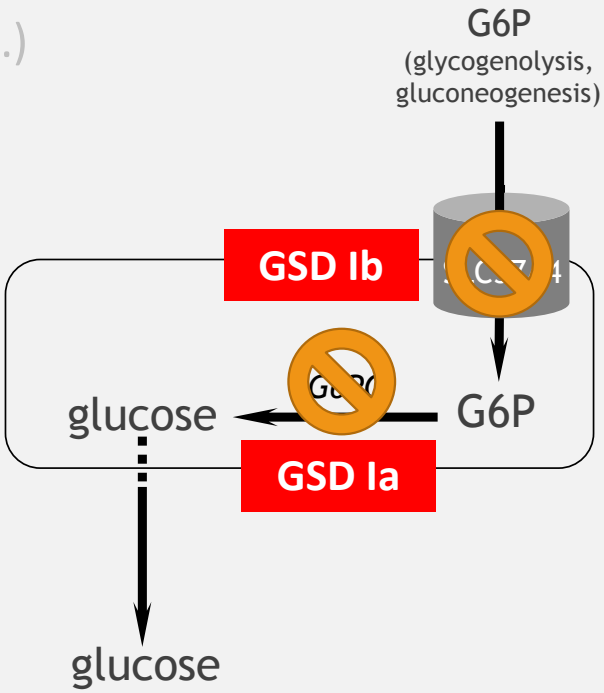
- **Dietary management**
  Gastric drip feeding
  Uncooked cornstarch (every 2-4 hour)



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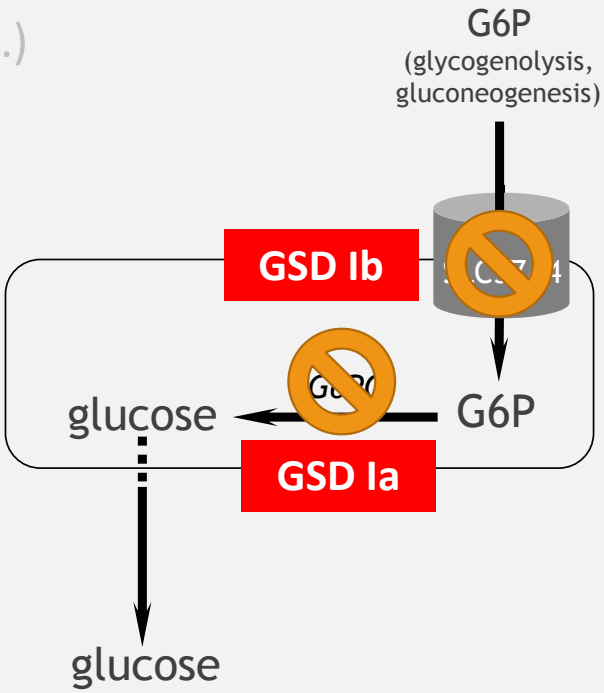
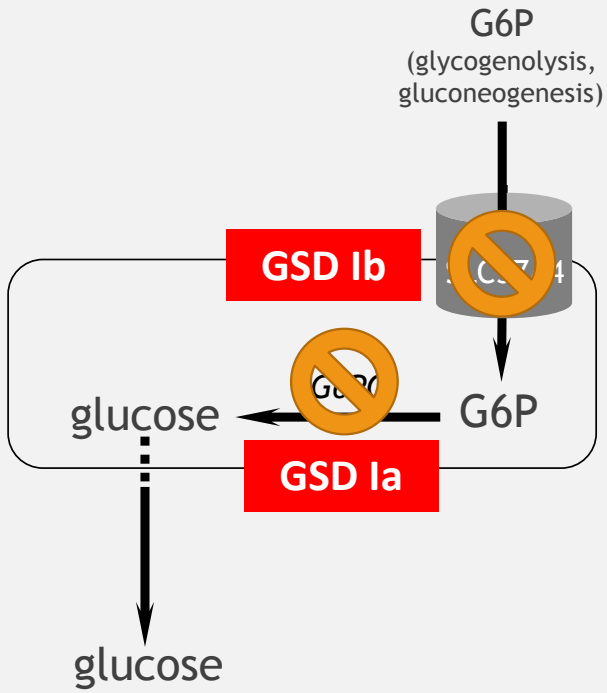


- **Dietary management**
  Gastric drip feeding
  Uncooked cornstarch (every 2-4 hour)

-> effective to reduce mortality,
but does not prevent GSD I complications!

Glycogen Storage Disease type 1 (GSD I)

- **Long-term complications**
  Growth retardation
  Epilepsy
  Osteoporosis
  Gout
  Abdominal obesity
  Renal failure
  Pulmonary hypertension
  PCOS
  Anemia
  Platelet dysfunction
  Neutropenia (GSD Ib)
  Inflammatory Bowel Disease (GSD Ib)
  Periodontitis (GSD Ib)



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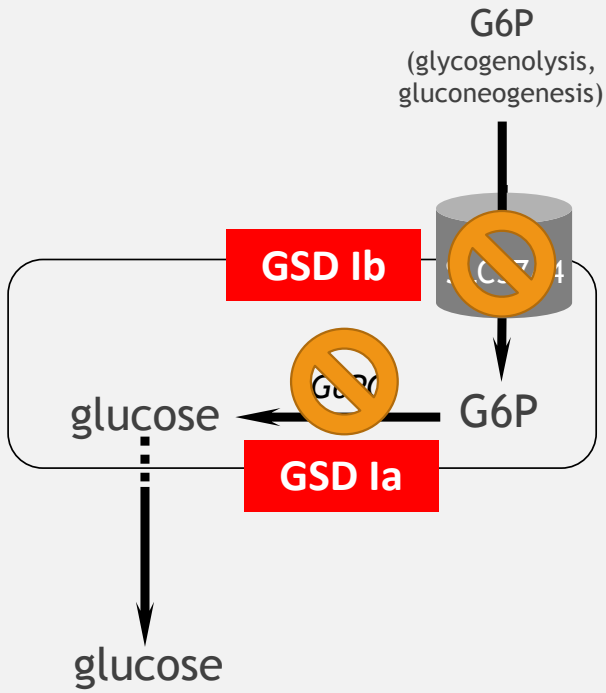
  Neutropenia (GSD Ib)
  Inflammatory Bowel Disease (GSD Ib)
  Periodontitis (GSD Ib)
  Liver tumors (>2/3 patients)



GSD I research agenda

improving care for and quality of life of GSD I patients

- Developing methods to improve (home-side) monitoring of GSD I symptoms and signs
- Understanding differences in severity of/risk for symptoms/complications between individual patients
- Elucidating the mechanisms of biochemical symptoms and long-term complications
- Establishing the relationship between biochemical symptoms and long-term complications
- Developing preventive/curative treatments for GSD I

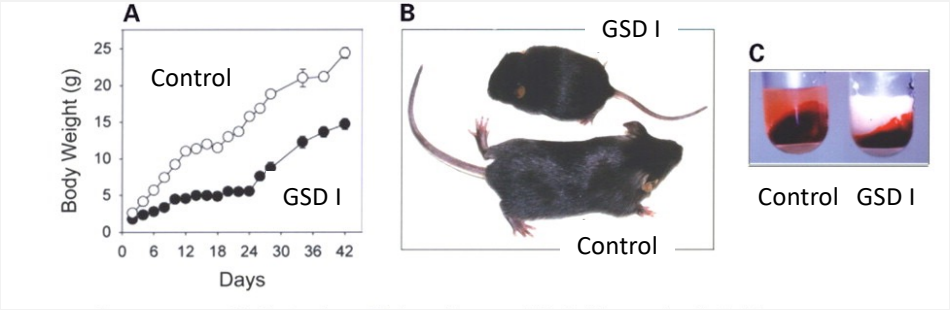
In vivo models for GSD I research

- Clinical research: restricted by age and vurnerability of patients, patient numbers, access to relevant organ tissues, ethical considerations
- To ensure long term efficacy and **safety**: preclinical research essential!

- Animal models
  - allow to investigate genetic defect in relation to GSD I symptoms and complications
  - enable systematic experimentation, collection of relevant organ tissues
- Available animal models
  - transgenic mouse (>1996 (Ia); >2003 (Ib)) and (natural point mutation) dogs (>2001)
  - acute pharmacological model for GSD Ib: S4048 (>2001)

In vivo models for GSD I research

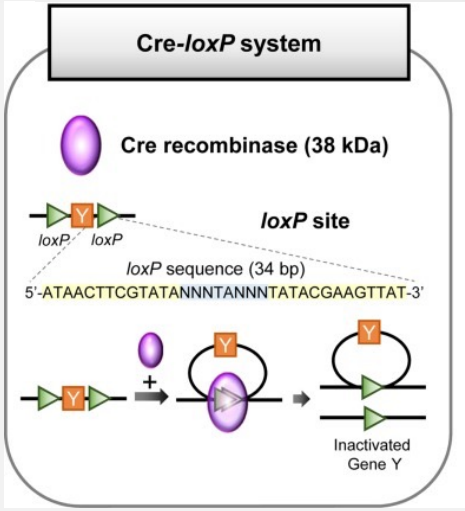
- Genetic animal models: deficiency/inactivation of G6PC/SLC37A4 in all cells/organs recapitulate severe biochemical symptoms



- GSD Ia mice/dogs and GSD Ib mice require daily glucose injections for survival
- GSD I mice/dogs have a limited lifespan -> limits research on long-term complications

In vivo models for GSD I research

- Solution: conditional *G6pc/Slc37a4* knockout mice (>2011)
  targeted gene deletion: (inducible) Cre-LoxP system
  Cre recombinase is expressed by a cell-type specific promoter
  deletion is induced after birth



- GSD Ia: hepatocytes (albumin-CRE), kidney cells (kap-CRE), enterocytes (villin-CRE)

  These mouse models have provided insight into
  contribution of liver, kidney and intestine to GSD I symptoms and long-term complications
  inter-organ communcation / glucose production compensation

- GSD Ib: hepatocytes (albumin-CRE)

Mutel et al., 2011
Resaz et al., 2014
Penhoat et al., 2014
Clar et al., 2014
Rajas et al., 2015
Raggi et al., 2018

In vivo models for GSD I research

- *G6pc/Slc37a4* knockout mice: either 50 or 100% deletion of the gene/function

  Limitation: does not allow to investigate heterogeneity in symptoms and complications observed in GSD I patients

  GSD Ia patients: 0-23% residual G6PC activity

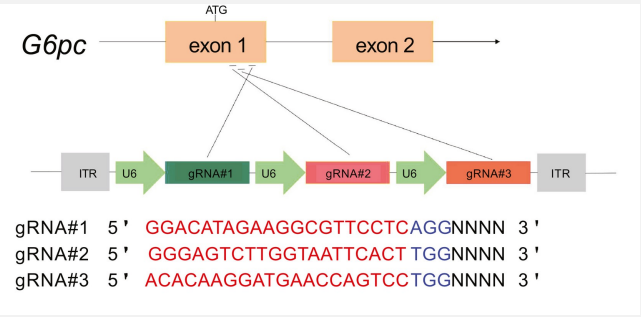
- Solution: CRISPR-cas9 mediated somatic gene editing *in vivo*

  CRISPR-cas9 is used to generate a (conditional) knockout mouse model

  targeted cell-type deletion: expression of cas9 is expressed by a cell-type specific promoter

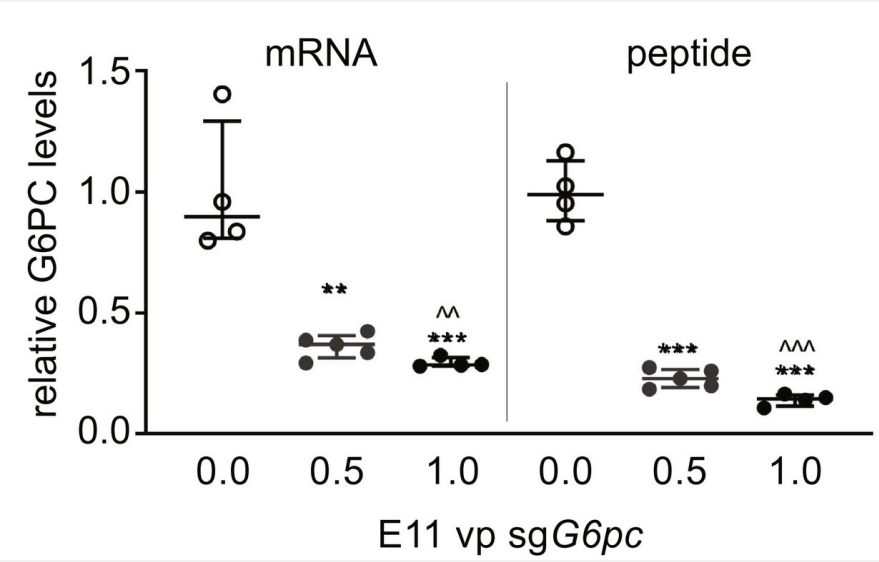
  single guide RNAs targeting *G6pc* or *Slc37a4 are* administered by viral delivery

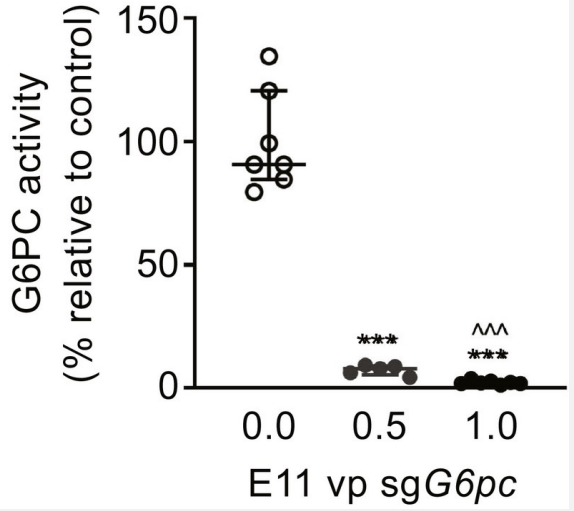
  mutation is induced after birth

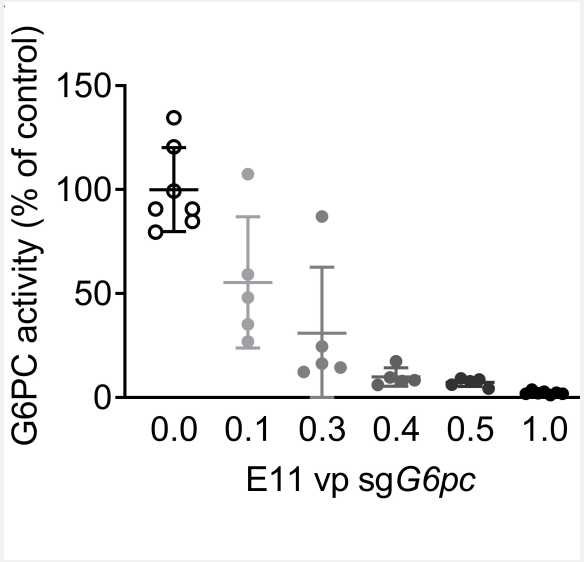


Fedoseienko et al., 2018
Rutten et al., 2021

CRISPR-cas9 mediated somatic gene editing allows to model heterogeneity in GSD Ia biochemical symptoms in mice







Rutten et al., 2021

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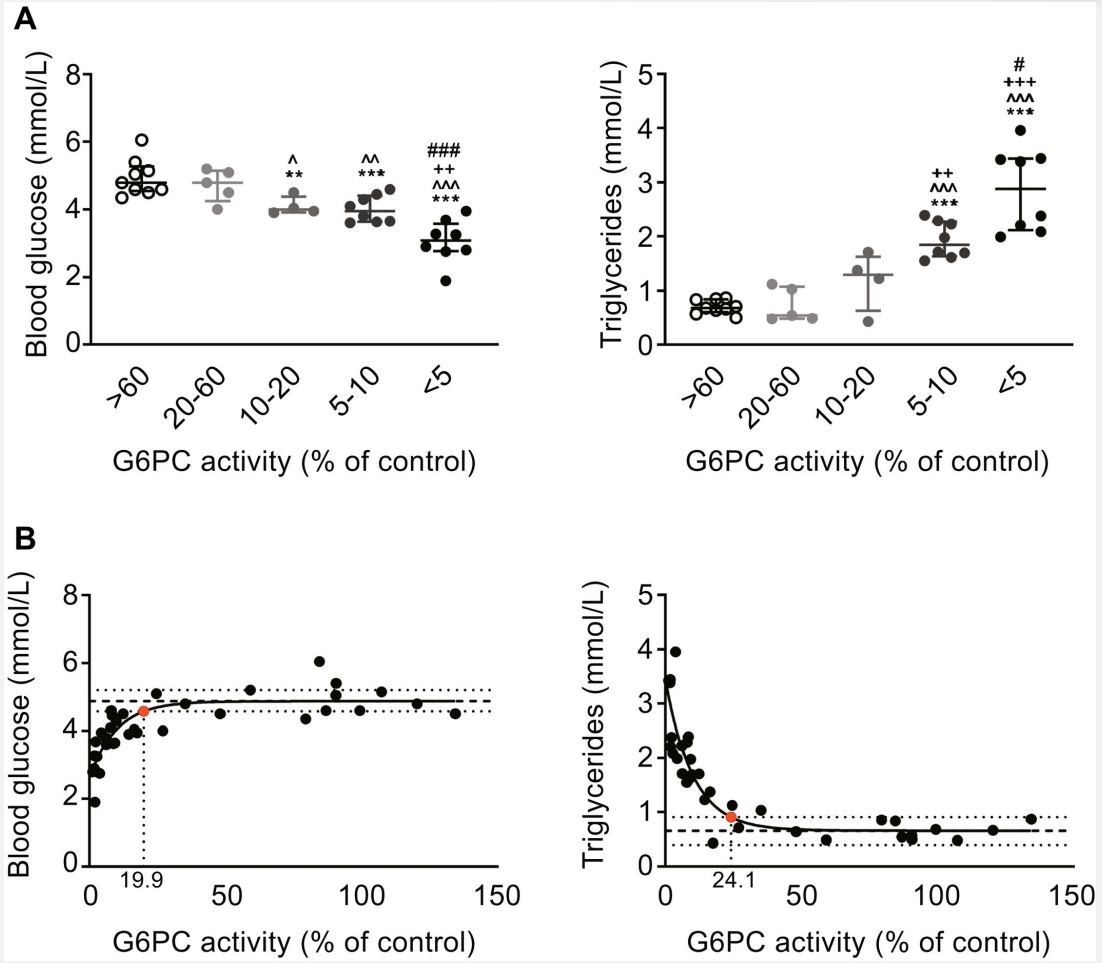

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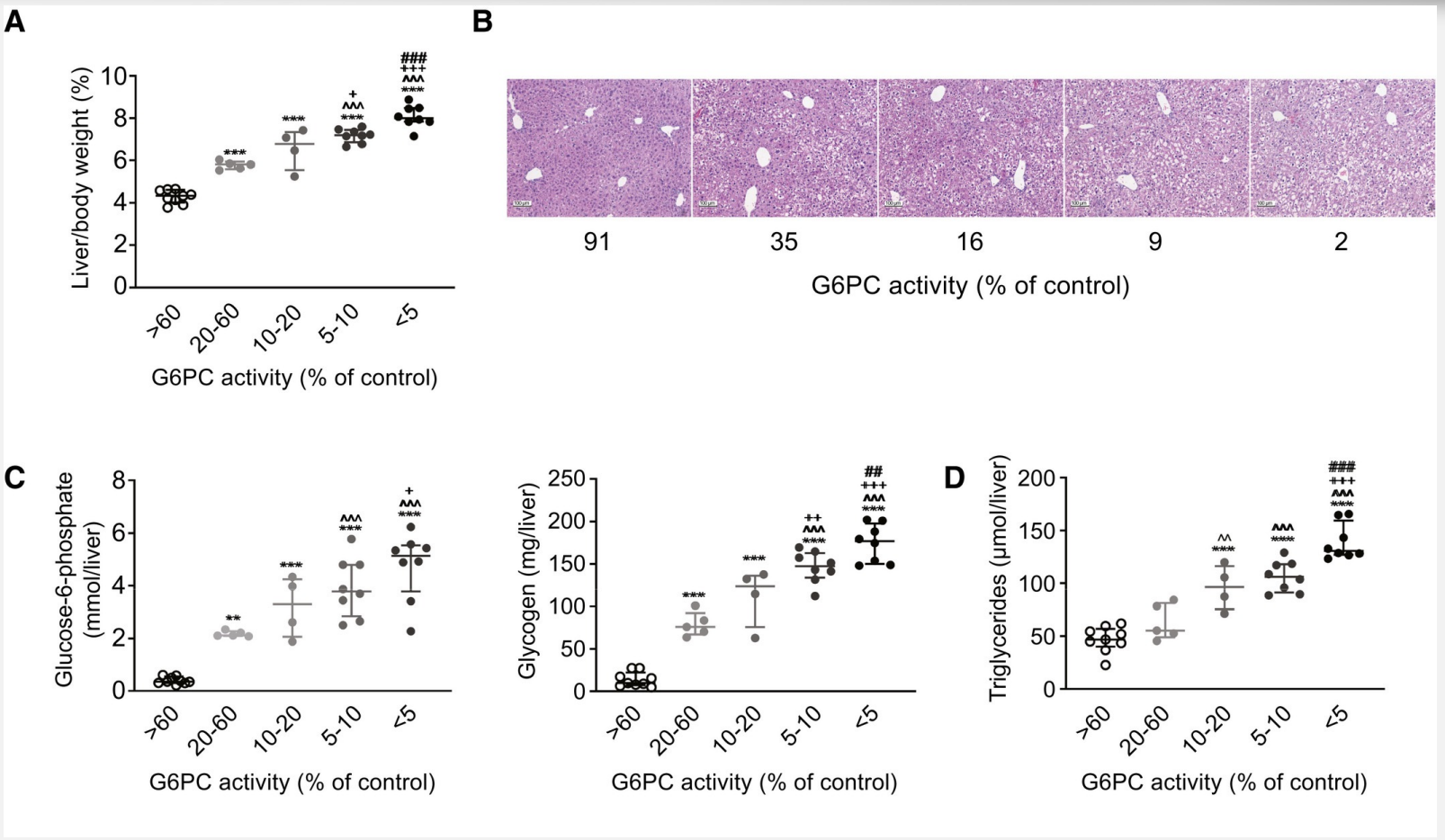
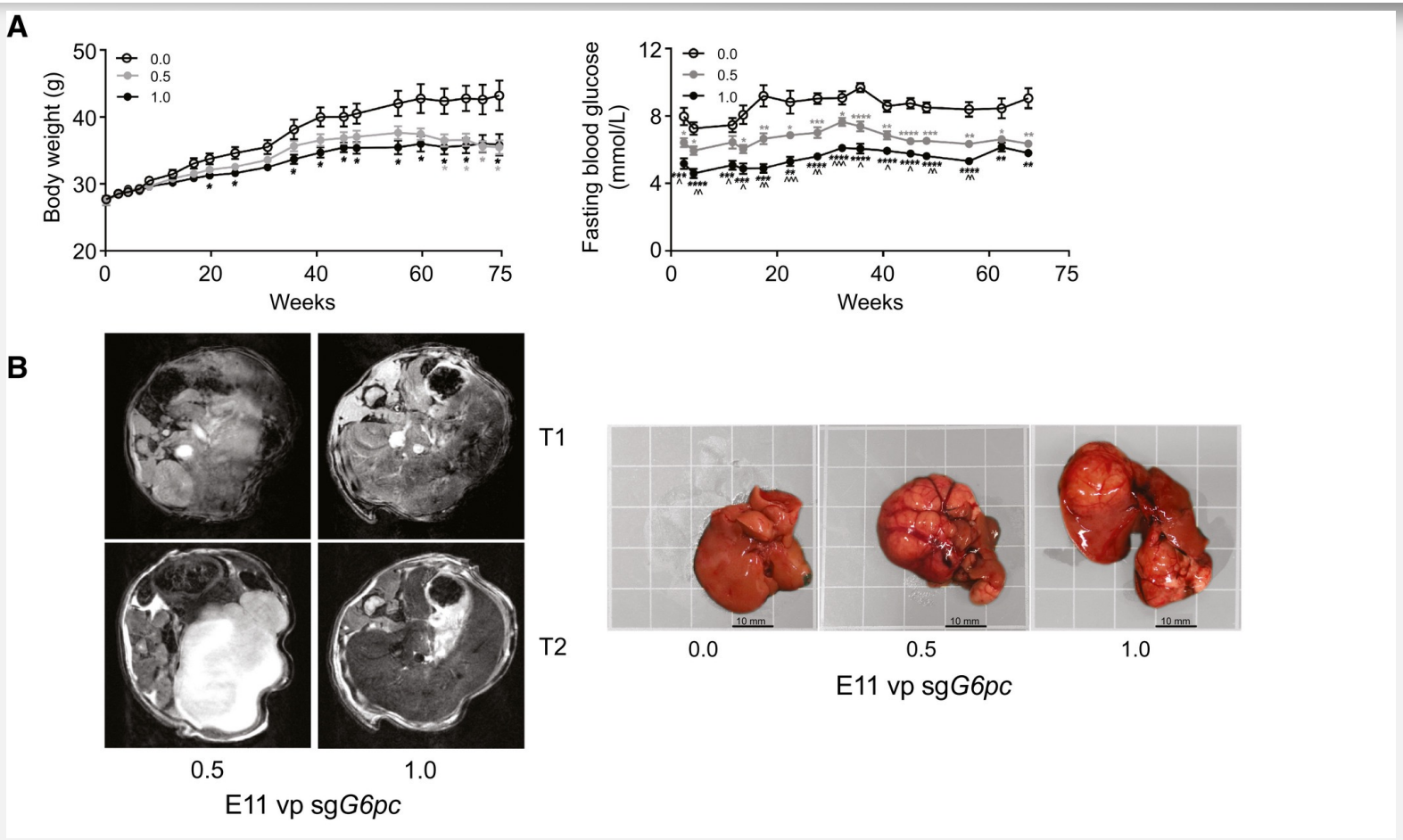


Rutten et al., 2021

CRISPR-cas9 mediated *G6pc* editing in hepatocytes induces persistent fasting hypoglycemia and liver tumor formation



Rutten et al., 2021

GSD I *in vivo* models: benefits

- Exhibit physiologically meaningful G6PC expression/function (<-> *ex vivo/in vitro* models)
- Cas9-mediated somatic gene editing allows to model heterogeneity in biochemical symptoms in mice -> personalized medicine for GSD I patients
- Allow to systematically investigate the relationship between biochemical symptoms and long-term complications along the course of GSD I
- Allow to systematically investigate the mechanisms of biochemical symptoms and long-term complications along the course of GSD I
- Allow to evaluate efficacy and safety of potential new treatments for GSD I

GSD I *in vivo* models: (current) limitations

- Patient-specific mutations are not yet systematically compared
- Dietary management inedequately covered in animal models
- Organ interaction does not allow to dissect the contribution of specific organ tissues to GSD I biochemical symptoms, e.g. contribution of liver to endogenous glucose production

GSD I *in vivo* models: take home messages

- In vivo models are essential for GSD I preclinical research and for development of effective and safe new therapies for GSD I patients
- As *ex vivo/in vitro* (patient specific) models are evolving rapidly, these should be considered as an complementary or alternative approach to address specific reserach questions