Non-Lysosomal Glycogen Storage Disorders

Biochemical Diagnosis

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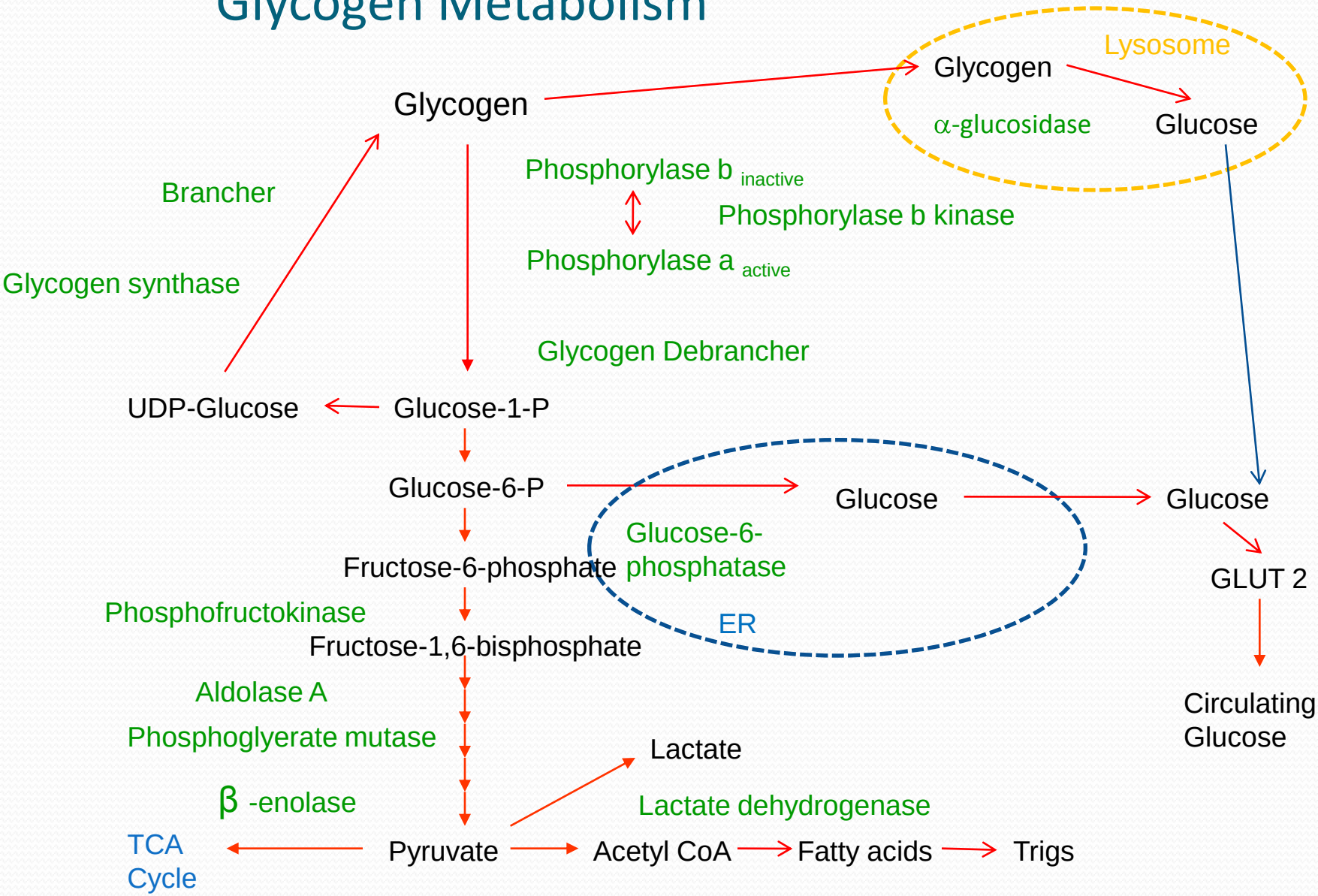
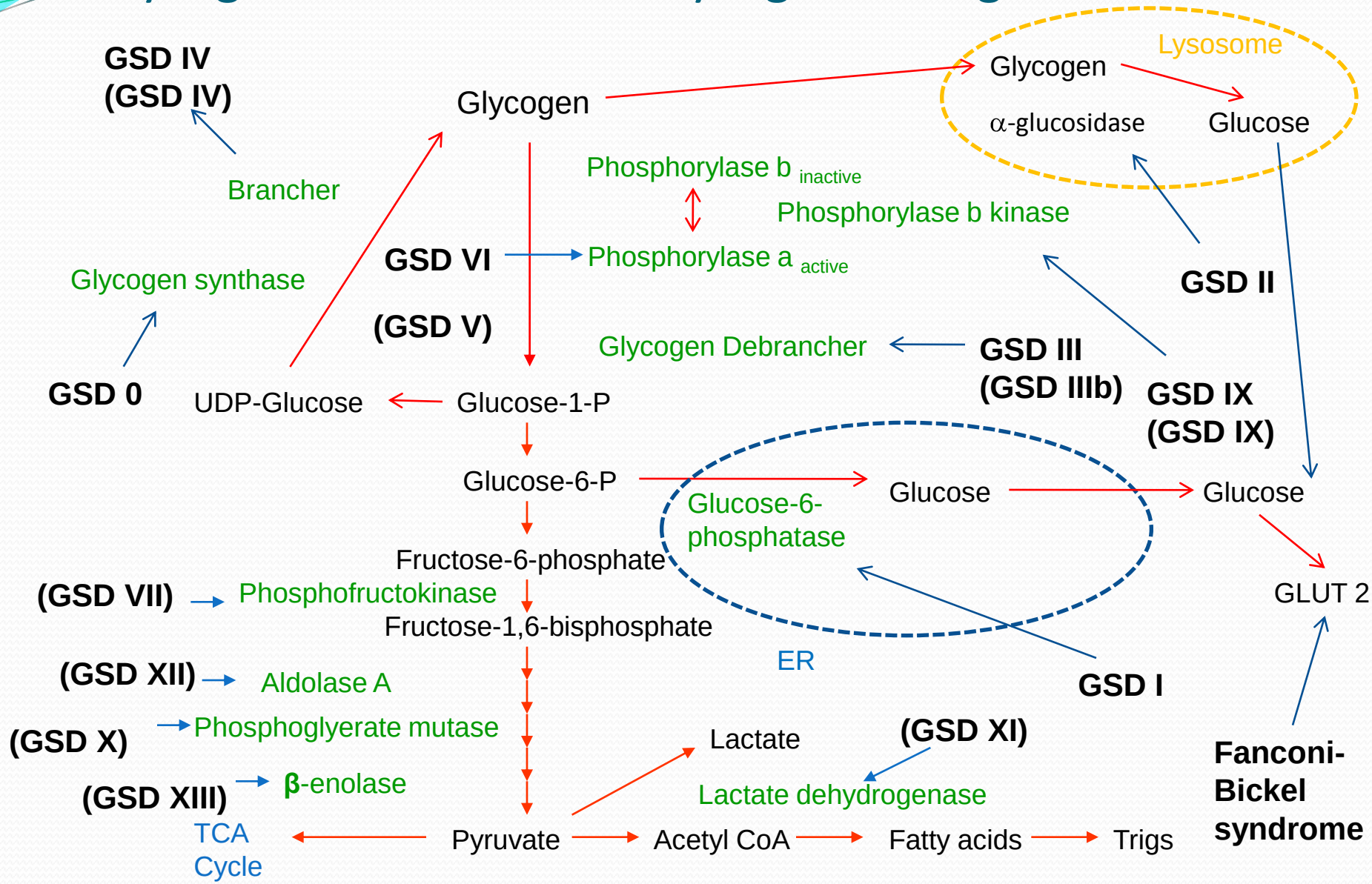
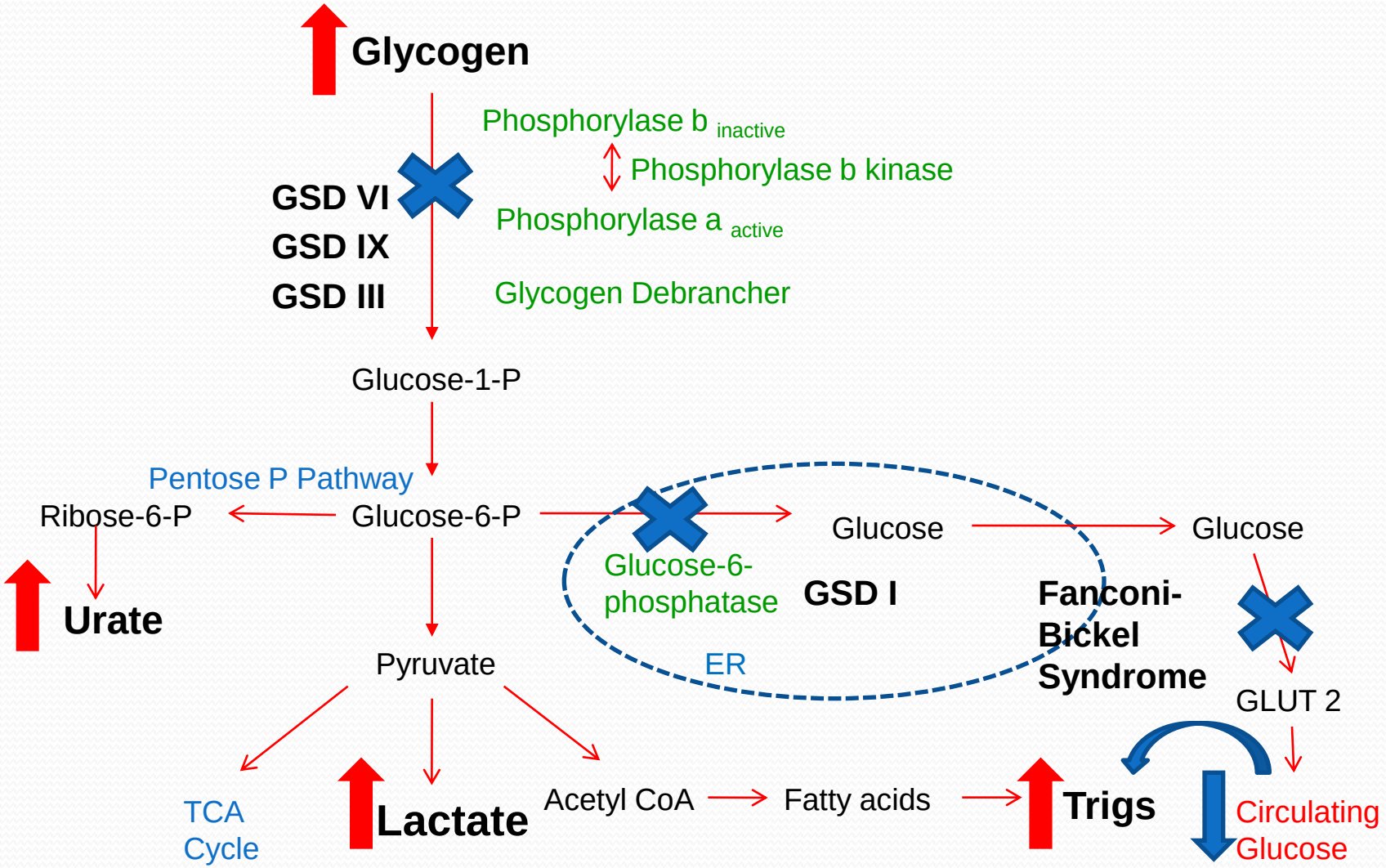
Glycogen Metabolism



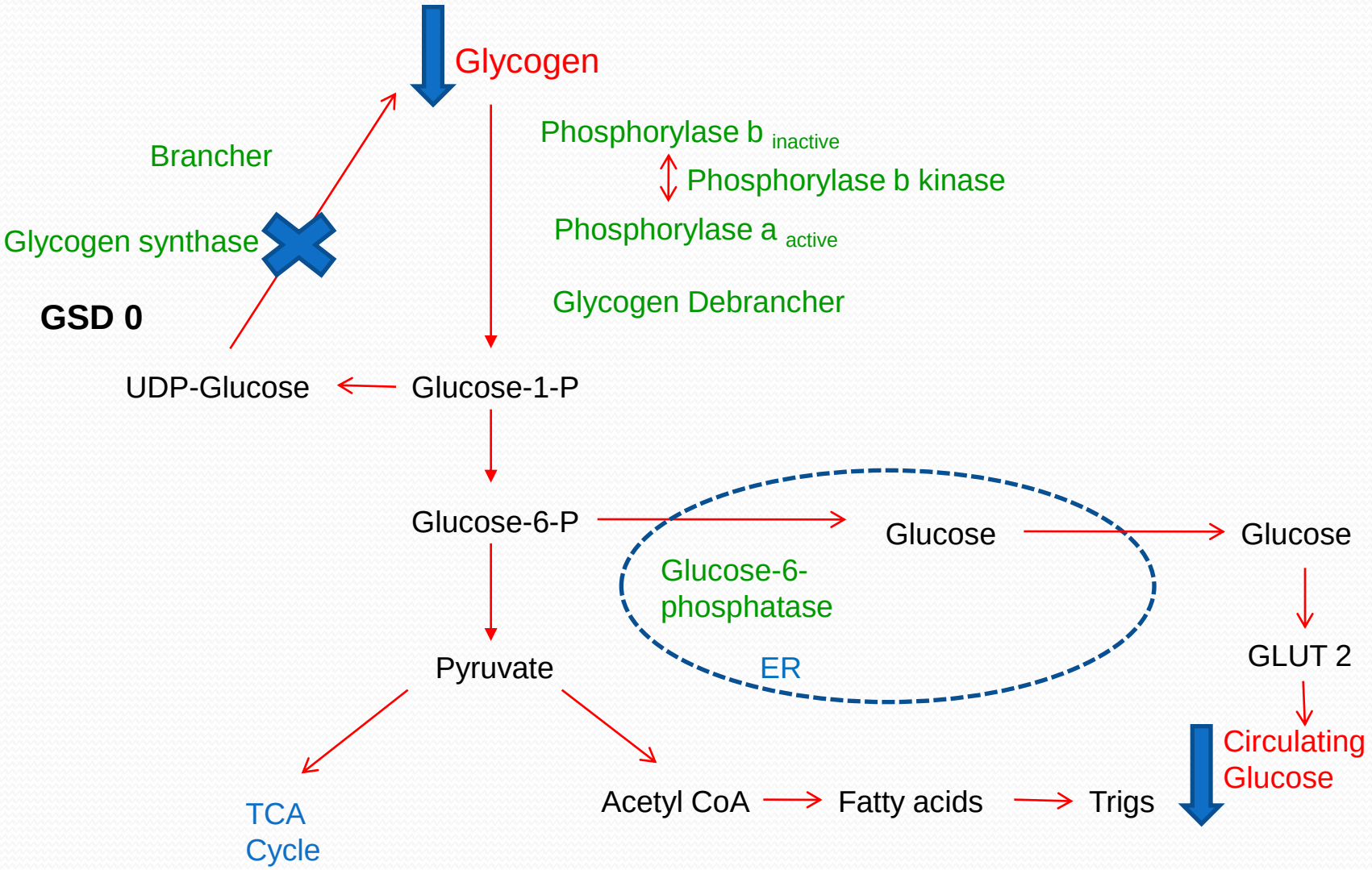
Glycogen Metabolism & Glycogen Storage Disorders



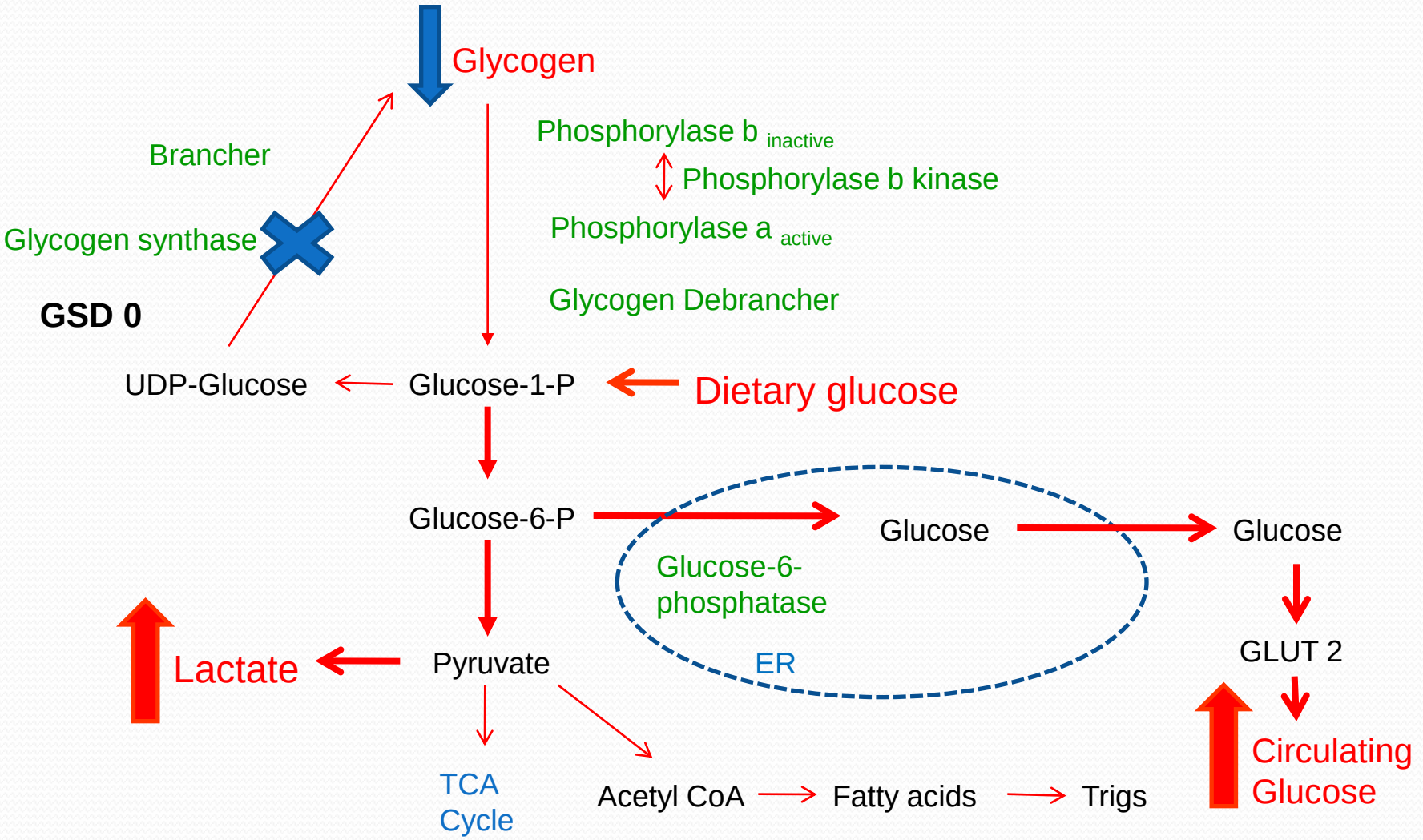
Glycogen Metabolism & Hepatic Glycogen Storage Disorders



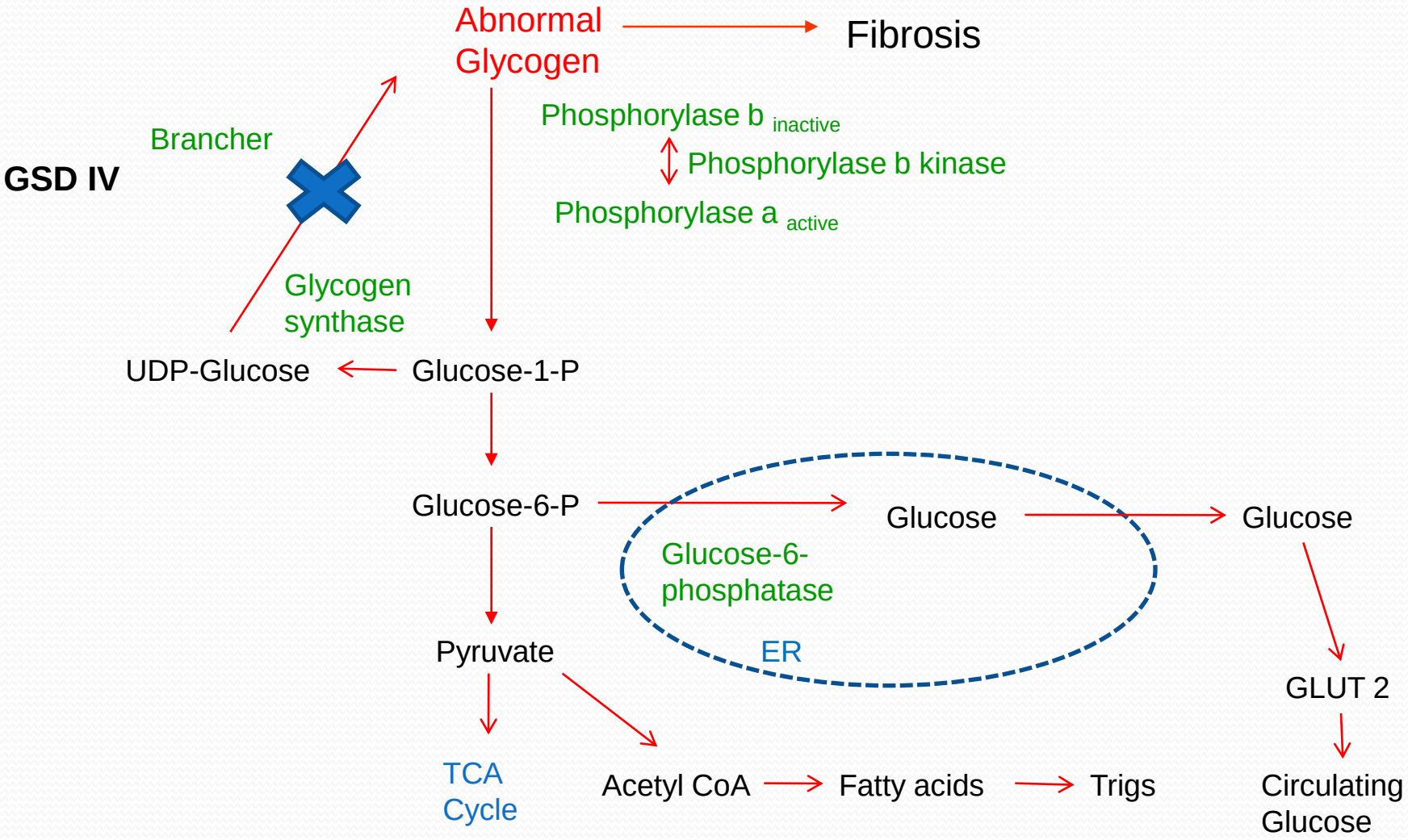
Glycogen Metabolism & Hepatic Glycogen Storage Disorders



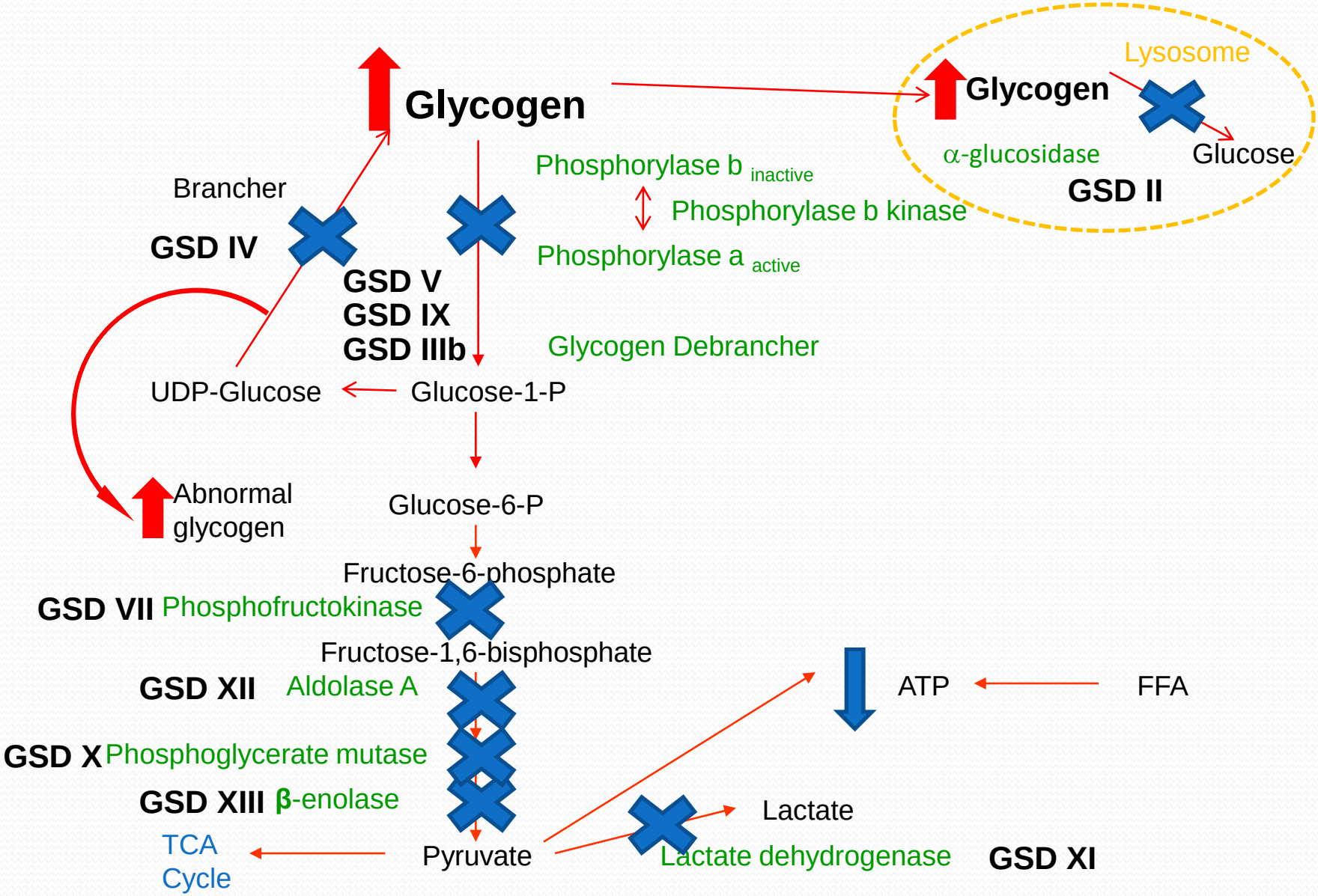
Glycogen Metabolism & Hepatic Glycogen Storage Disorders



Glycogen Metabolism & Hepatic Glycogen Storage Disorders



Glycogen Metabolism & Muscle Glycogen Storage Disorders



Glycogen Storage Diseases

Predominately Hepatic GSDs:

GSD I – glucose-6-phosphatase or transport systems in ER

GSD III – debranching enzyme

GSD IX – liver phosphorylase b kinase

GSD VI – liver phosphorylase

GSD IV – branching enzyme

GSD 0 – glycogen synthase

Predominately Muscle GSDs:

GSD II – acid a-glucosidase

GSD V – muscle phosphorylase

GSD VII - muscle phosphofructokinase

Rare Muscular forms:

GSD X - phosphoglycerate mutase

GSD XI - LDH

GSD XII – Aldolase A

GSD XIII – β-enolase

Glycogen Storage Diseases

Muscle variant forms of (hepatic) GSDs:

GSD IXd – muscle phosphorylase b kinase

GSD IIIb - debranching enzyme

GSD IV – branching enzyme, neuromuscular form

Liver and muscle affected:

GSD III –debranching enzyme

GSD IV – branching enzyme

GSD IX – phosphorylase b kinase

GSD	Hepato-megaly	Muscle symptoms	Glucose homeostasis	Other Biochemistry
GSD 0	No	None	Fasting ketotic hypoglycaemia	Post-prandial hyperglycaemia, and raised lactate
GSD I	Yes	None	Severe (ketotic) hypoglycaemia	Raised lipids, urate, lactate, AST/ALT, proteinuria, anaemia, +/- neutopenia
GSD II	No	Truncal & proximal muscle weakness. More severe infantile form.	No overt effect	Raised CK,vacuolated lymphocytes
GSD III	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia	Raised lipids, AST/ALT, CK may be raised
GSD IV Hepatic	Yes	Myopathy can occur	Normal until end stage liver disease	Raised AST/ALT, CK can be raised
GSD V	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD VI	Yes	None	Fasting ketotic hypoglycaemia	Raised AST/ALT, lipids
GSD VII	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD IX liver form	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia can occur	Raised AST/ALT, lipids, CK can be raised
Fanconi-Bickel Syn. (GSD XI)	Yes	None	Ketotic hypoglycaemia	Raise AST/ALT, Abnormal renal biochemistry including tubular markers.

Laboratory Tests for the Investigation of Suspected GSD

- Blood glucose
  - Pre and post feed
  - If hypoglycaemia include insulin, FFA, ketones etc
- Blood lactate
  - Pre and post feed
- Urate
- LFTs
- Lipids
- CK
- FBC (including WBCs)
- U&E, tubular proteins, protein/albumin, phosphate
- LDH

- Initial differential diagnostic tests:
  - Bloodspot carnitine
  - RBC Gal-1-PUT
  - PAA
  - UOAs
- Further Tests
  - Vacuolated lymphocytes
  - Tissue Histology
  - Blood GSD screen
  - Bloodspot α–glucosidase
  - Glucagon stimulation test
  - Tissue Enzymology
  - Genetics

Glycogen storage disease screen:

- Minimum 5ml blood in lithium heparin
- Red cells – glycogen and phosphorylase b kinase
- White cells – debrancher and phosphorylase
  - (brancher)
- Batch consists of 8 samples (manageable no. of assay tubes)
- Screen takes operator one a week to complete

RBC glycogen

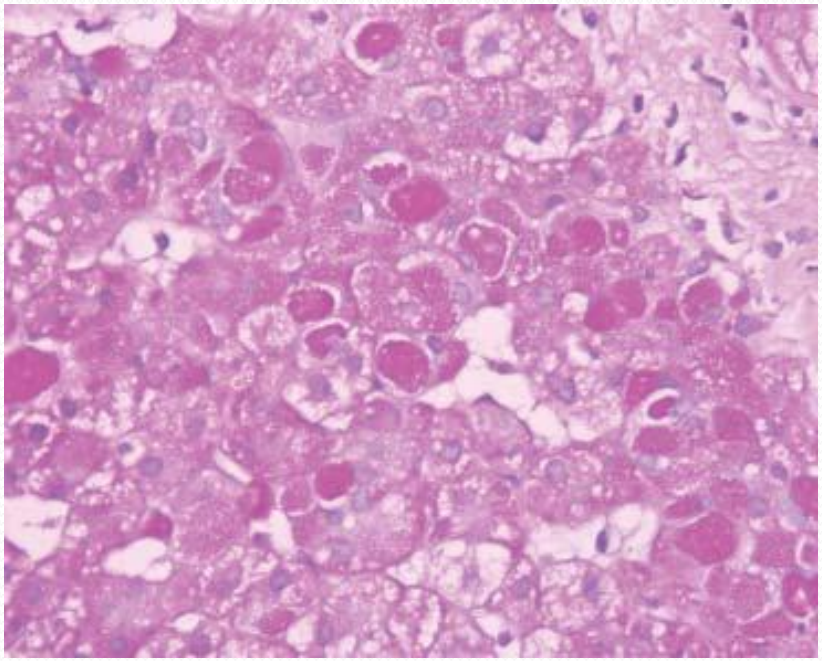
- Relatively non invasive assessment of glycogen storage
- Not elevated in GSD I, II or IV
- Most useful for confirmation of GSD III
- GSD IX – may be elevated to a lesser degree.
- Assay takes 3 days to complete
- Relatively stable
- Available in RBCs, liver and muscle

Glycogen levels in GSDs

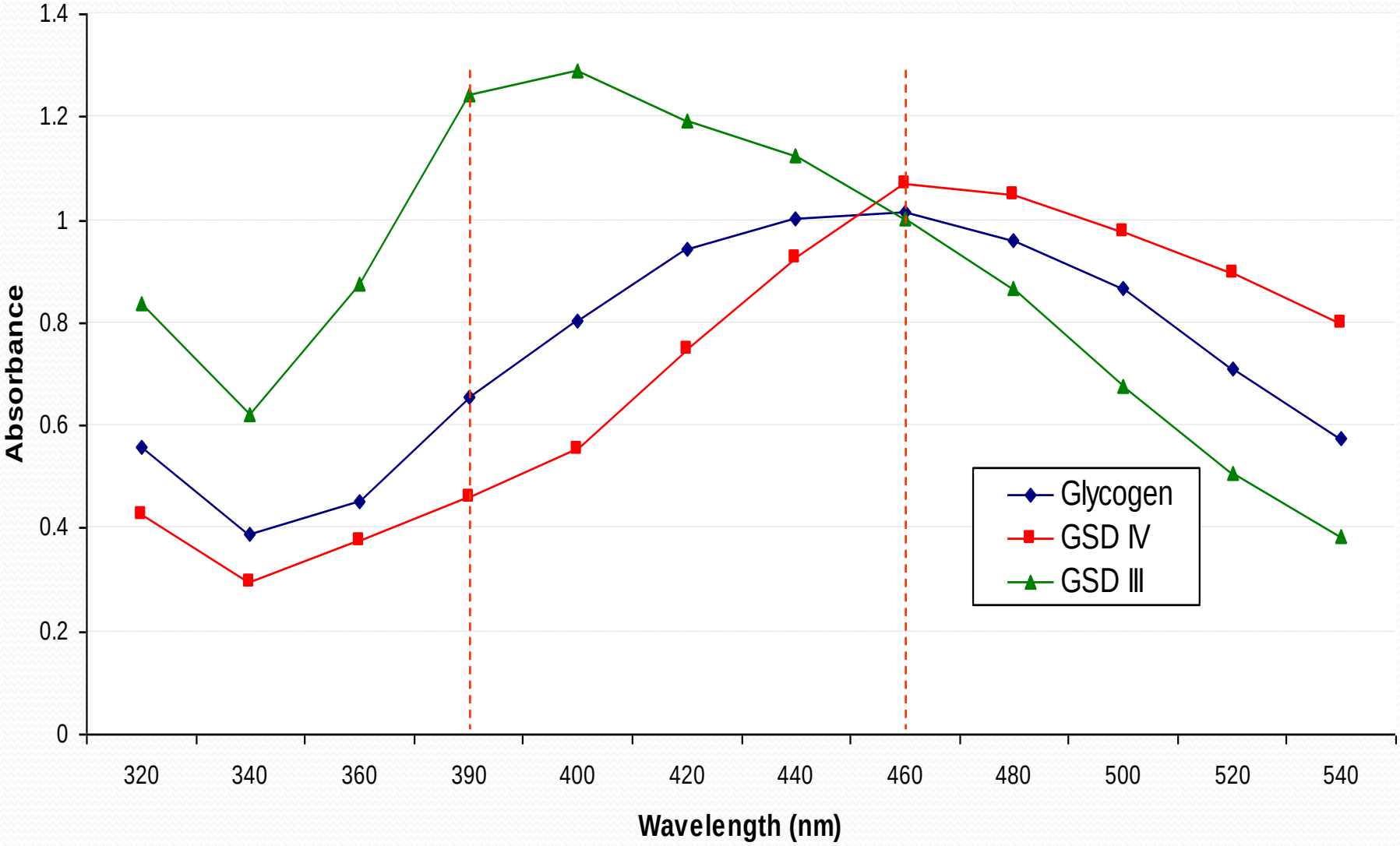
GSD	RBC Glycogen	Tissue glycogen	Histology
GSD 1	Normal	Raised liver glycogen	PAS pos cyoplasmic glycogen, significant lipid accumulation
GSD II	Normal	Raised muscle glycogen	PAS pos lysosomal glycogen
GSD III	Significantly raised	Significantly raised liver glycogen	PAS pos cyoplasmic abnormal glycogen, some lipid accumulation
GSD IV	Normal	**Muscle glycogen conc may be normal**	PAS positive amylopectin like cytoplasmic glycogen (polyglucosan)
GSD V	Normal	Muscle glycogen may be normal	PAS pos cyoplasmic glycogen
GSD VI	Normal	Raised liver glycogen	PAS pos cyoplasmic glycogen,
GSD VII	Normal	**Muscle glycogen may be normal**	PAS pos cyoplasmic glycogen,
GSD IX	Often mild/mod raised	Usually raised liver glycogen	PAS pos cyoplasmic glycogen,

GSD IV: Liver Histology

- Liver biopsy showing diffuse deposition of PAS positive amylopectin like material in hepatocytes (PAS stain).



Iodine spectrum of glycogen



Glycogen Debrancher

- Glycogen debrancher: amyloglucosidase activity & oligoglucanotransferase activity
  - GSD IIIa: Glycogen debrancher deficiency. Liver & muscle involvement
  - GSD IIIb: : Glycogen debrancher deficiency. muscle involvement
  - GSD IIIc: Amyloglucosidase only
  - Type IIId Oligoglucanotransferase only
- All due to mutations in AGL (1p21)

Glycogen Debrancher

- Available in WBCs, fibroblasts and liver
- Relatively stable
- Reliable for diagnosis of GSD III in WBCs
- Assay required phosphorylase limit dextrin substrate (not commercially available, takes 7 days to synthesise in house)

Glycogen Phosphorylase

- Liver and muscle isoenzymes
  - Liver glycogen phosphorylase deficiency: GSD VI, PYGL (14q21-q22) mutations
  - Myophosphorylase deficiency: GSD V, PYGM (11q13) mutations
  - Total phosphorylase and phosphorylase a in WBCs, liver, muscle and fibroblasts available

Glycogen Phosphorylase

- GSD V will have normal phosphorylase activity in WBCs, fibroblasts and liver.
- Confirmed GSD VI cases described with very high residual enzyme activity in leucocytes
- Very labile enzyme
- WBCs must be prepared within 24 hours
- Storage at -80°C improves stability
- Heterozygotes for GSD V increased risk of statin induced myopathy

Phosphorylase b Kinase

- Four Subunit
  - α subunit: regulatory, X allele , muscle & liver forms
  - β subunit: regulatory
  - γ subunit: catalytic
  - δ subunit: Calcium binding

Phosphorylase b Kinase Deficiency (GSD IX)

- GSD IXa/ VIII/ XLG
  - Def liver α subunit
  - PHKA 2 Mutations (Xp22)
  - X-linked
  - Low activity in liver & RBCs
  - Varient form (XLG2) normal activity in liver & RBCs
- GSD IXb
  - Def β subunit,
  - PHKB (16q12-q13)
  - low activity in liver , muscle & RBCs
  - +/- myopathy
- GSD IXc
  - Def γ subunit
  - PHKG2 (16p12.1-p11.2)
  - Low activity in liver
- GSD IXd
  - Def muscle α subunit
  - PHKA1 (Xq13) mutations
  - Muscle specific form
  - Low activity in muscle

Phosphorylase b Kinase

- Available in RBCs, fibroblasts, liver and muscle
- Even in confirmed cases total enzyme deficiency may not be seen in vitro.
  - Some cases have phosphorylase b kinase deficiency in liver but normal activity in red cells
  - Muscle forms will not be detected in RBCs
  - Mutations have been found that cause a deficiency in vivo but not in vitro
- Phosphorylase in leucocytes:
  - Ratio of the active form to total – low in cases of phosphorylase b kinase deficiency. In some cases of phosphorylase b kinase deficiency the red cell glycogen may be raised BUT not always.
- Problem with stability, improved with storage at -80˚C

Results which may suggest a defect in the phosphorylase activating system

	1	2	3	Control ranges
Red cells:				
glycogen:	17	29	681*	(10 – 120 mg/gHb)
Phos b kinase	15.7	9*	ND*	(10 – 90 mg/g Hb)
White cell enzymes:				
Phosphorylase a (-AMP)	0.70	0.12*	0.48	(0.3 – 3.7 ug/hr/mg ptn)
Total phosphorylase (+AMP)	4.2	2.4	4.6	(2.4 – 10.4 ug/hr/mg ptn)
Phos a/total ratio	0.17*	0.05*	0.10*	(0.42 – 0.78)

Glycogen Brancher Deficiency

- Mutations in GBE1 (3p12)
  - **Classic GSD IV:** Early infancy hepatomegaly with progressive cirrhosis, liver failure, splenomegaly, failure to thrive. Very low or undetectable glycogen brancher activity (1-10% activity)
  - **Perinatal severe GSD IV:** hydrops fetalis, polyhydramnios.
  - **Late onset GSD IV:** nonprogressive mild form
  - **Neuromuscular GSD IV:** hypotonia, cardiomyopathy ,muscle atrophy. Late onset progressive myopathy.
  - **Adult polyglucosan body disease:** Associated with mild mutations. Upper and lower motor neurone involvement and neurogenic bladder.

Glycogen Brancher

- Glycogen branching enzyme available in WBCs, liver, muscle , fibroblasts, amniocytes or choriocytes
- Not automatically part of the blood GSD screen unless specifically requested or clinical details include ‘liver cirrhosis/failure’.
- Reported cases of high residual activity in WBCs in some neuromuscular forms.
- May be normal in adult polyglucosan body disease.

GSD I: Enzymatic Diagnosis

GSD Ia: Deficiency of glucose-6-phosphatase (mutations in G6PC (17q21))

GSD Ib: Deficiency glucose-6-phosphate ER transport protein (T1 transport protein) (mutations in G6PT1 (11q23))

GSD Ic: Deficiency of phosphate translocator (T2b transport protein) (mutations in G6PT1 (11q23))

GSD Id: Deficiency of glucose translocator (GLUT 7 transport protein) (mutations in SLC2A7 (1p36.2))

- Glucose-6-phosphatase available in frozen and fresh liver
- Frozen liver: glucose-6-phosphatase only for GSD Ia
- Fresh liver: Intact microsomal system including transport proteins and the hydrolase system (GSD 1 non-1a)
- Genetics testing recommended as first line testing

Other GSDs

- **Phosphofructokinase:**
  - Tetradimer, M,L & P subunits
  - Available in muscle at GOSH
  - Deficient GSD VII:
    - Severe infantile form: Respiratory failure
    - Mild adult form: Exercise intolerance
    - Mutations in PFKM (12q13.3)
- **Glycogen synthase**
  - GSD 0
  - Reduced hepatic glycogen
  - Enzymology available in liver (Rotterdam)
  - Genetics confirmation
- **Phosphoglycerate mutase:**
  - M and B subunits, MM homodimer most common in muscle
  - Deficiency recently designated GSD X
  - Exercise intolerance, cramps
  - ~12 patients described
  - Mutations described in M subunit (7p13-p12)
  - Enzymology in muscle available (Rotterdam)

Other GSDs

- **Glucose transporter-2**
  - Fanconi-Bickel syndrome (GSD XI)
  - GLUT2 (3q26) mutations
  - Impaired glucose and galactose transport in liver and renal tubules
  - Hepatic and tubular glycogen storage
  - Proximal renal tubular dysfunction, failure to thrive, hepatosplenomegaly
  - Raised blood glucose & galactose post feed
  - Fasting Hypoglycaemia
  - Diagnosis by molecular genetics

- **Lactate Dehydrogenase**
  - GSD XI
  - Paradoxically low serum LDH
  - Mutations in M subunit (11p15.4) described in Japanese population
  - Exercise intolerance & cramps +/- skin lesions
  - Confirmation by genetics available

Other GSDs

- **Aldolase A**
  - Fructose-1,6-bisphosphate → glyceraldehyde-3-phosphate & dihydroxyacetone phosphate
  - 16q22-q24 mutations
  - GSD XII
  - Exercise intolerance and weakness
  - 2 patients described
  - Enzymology (RBCs or muscle, available in Rotterdam)

- **β-Enolase deficiency**
  - 17p12 mutations
  - GSD XIII
  - Single patient with exercise intolerance

Biomarkers in GSDs

- Serum Biotinidase:
  - Consistently mild/moderately elevated in GSD Ia & Ib
  - Also variably elevated in some cases of GSD III, VI and IX
  - Mechanism unknown

Paesold-Burda et al 2007

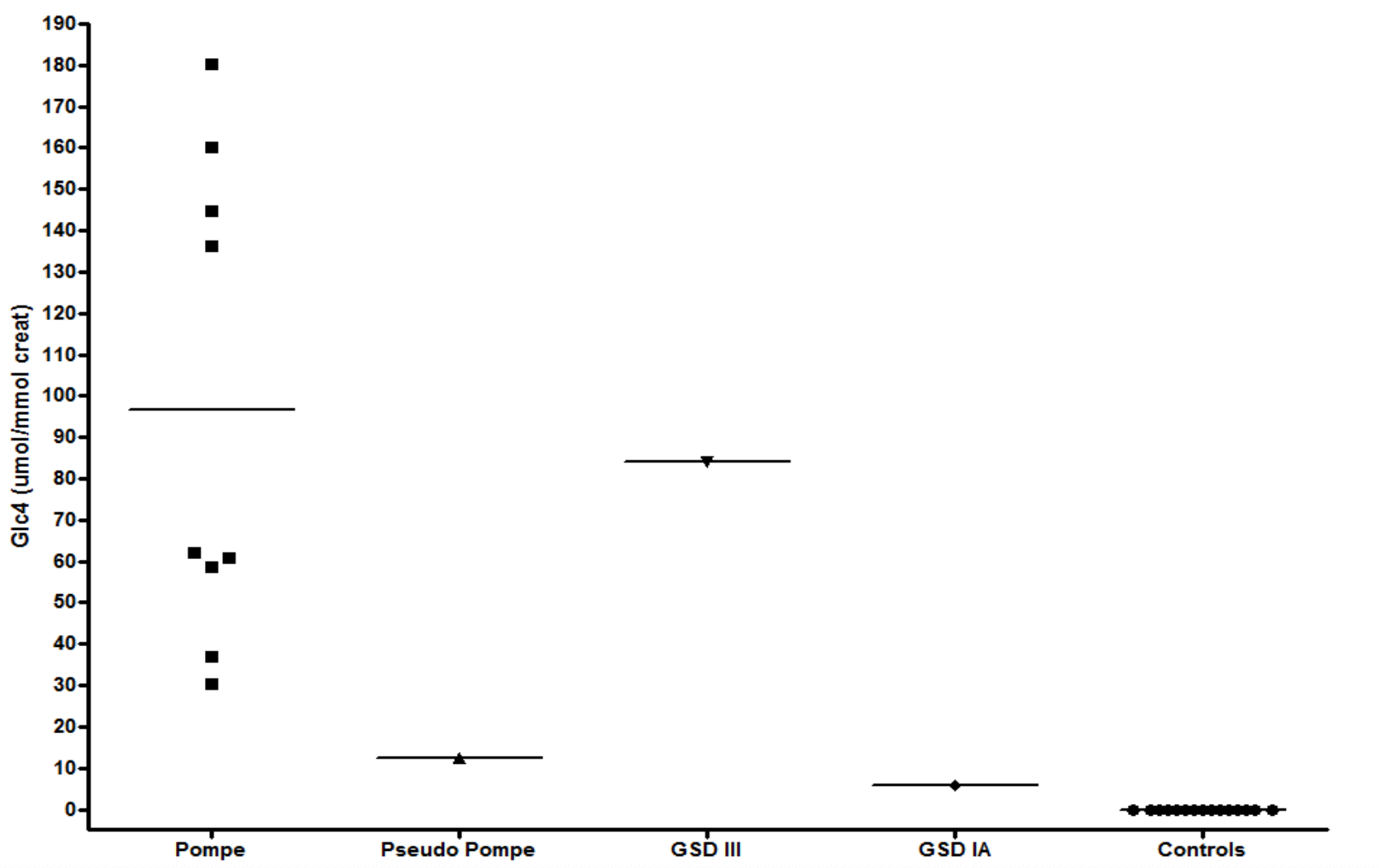
- Urine Tetrasaccharides:
  - Level of Glc_4, is elevated in urine and plasma of GSD II patients by HPLC & electrospray ionisation TMS

An et al. 2000 Analyt Biochem 287, 136, Young et al 2003

  - Glc4 also increased in GSD III & VI

Oberholzer et al. (1990) Clin Chem 36, 1381.

Urine Glc4 in GSDs by HPLC



Summary

- Variable presentation of glycogen storage disorders
- Initial biochemical investigation can provide diagnostic clues
- Enzymatic diagnosis is not always definitive particularly in blood
  - High residual activity
  - Tissue specific isoenzymes
  - Specimen deterioration
- Sometimes biopsy and/or genetic testing is required to confirm diagnosis

Acknowledgements

- Simon Heales
- Amanda Lam
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- Vicki Manwaring
- Enzyme Laboratory at GOSH

Glucagon Stimulation Test

Glucose & lactate measured at 0, 2, 30, 60, 90 and 120 mins post glucagon

GSD	Glucose	Lactate	Post-prandial
I	Reduced	Increased	Greater fall in Gl
III	Normal	Normal	Glycaemic response
VI	Increased	Normal	Greater response
III fasted	Reduced	Normal	