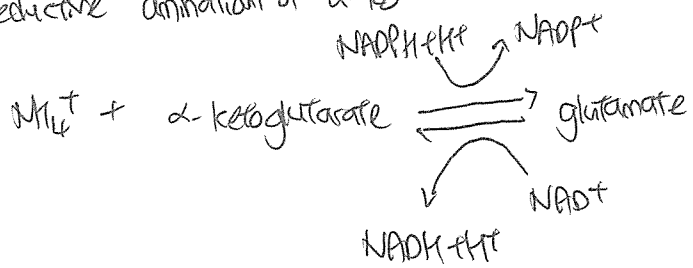
Glutamate Dehydrogenase

- reductive amination of α-KG



- reversible

- glutamate can be used to generate α-ketoglutarate for TCA cycle

Allosteric Regulation

- synthesis of α-ketoglutarate from glutamate
- inhibited by ATP / GTP, stimulated by ADP / GDP

Regulation of Glutamine Synthetase

(irreversible reaction)

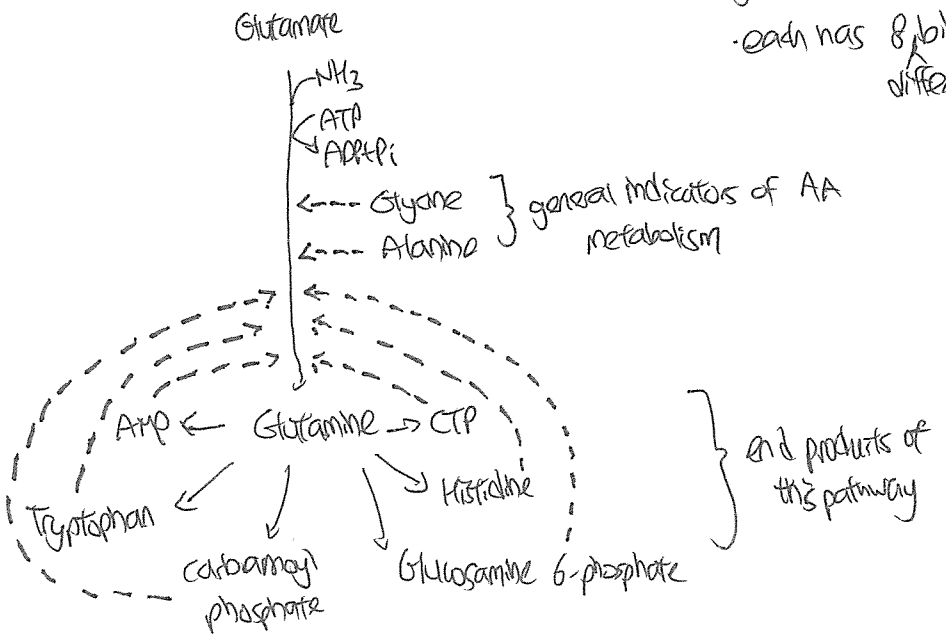
- glutamate + NH_3 + ATP → glutamine + ADP + P_i
- central role in N metabolism
- biosynthesis of amino acids
- purines & pyrimidines
- detoxification of ammonia (particularly important in brain)
- depletion of α-KG

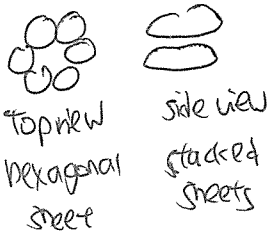
- tightly regulated
- allosteric regulation – cumulative feedback inhibition
- covalent modification

- Glu first reacts with ATP to form γ-glutamyl phosphate + ADP.
- The γ-glutamyl phosphate intermediate then reacts with NH_3 to form Gln + P_i

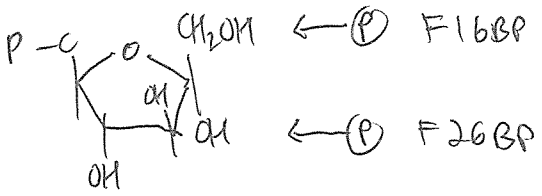
Feedback inhibition by specific inhibitors

- glutamine synthetase has 12 subunits
- each has 8 binding sites, 96 total allosteric effector sites





Phosphofructokinase : isoenzymes and products

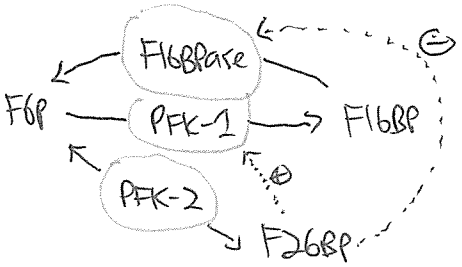


F2,6BP

- coordination of PFK and F1,6BPase activity via allosteric control (glycolysis and gluconeogenesis)
- PFK1 : F6P → F1,6BP
- PFK 2 : F6P → F2,6BP

major : PFK1 (90%)
minor : PFK 2 (10%)

PFK-2, F2,6BP and co-ordinate allosteric control



multiple PFK-2 isoforms

- tissue-specific expression
- activity modulated by phosphorylation
- activity modulated at level of expression
- bifunctional enzymes

* F2,6BP must be present for glycolysis to proceed

PFK-2 : bifunctional enzymes

↳ activated by shape change (2 active sites)

- single polypeptide chains, 2 enzymatic activities (only ① enzymatic activity is active at any given time)

↳ ② inactivated only in higher eukaryote, in lower prokaryote, since 2 enzymatic activities are encoded by 2 genes

glycolysis : activation of PFK-1 (inhibition of F2,6BPase) inhibition of PFK-1

cAMP-dependent protein kinase phosphorylate PFK-2 bifunctional protein, enhancing its F2,6BPase activity. (effect of glucagon)

↳ product of pentose phosphate pathway xylulose 5-phosphate activates phosphoprotein phosphatase 2A, which dephosphorylate the PFK2/F2,6BPase bifunctional protein, leading to ↑ F2,6BP.

gluconeogenesis : inhibition of F1,6BPase ↑ F2,6BP

activation of F1,6BPase ↓ F2,6BP

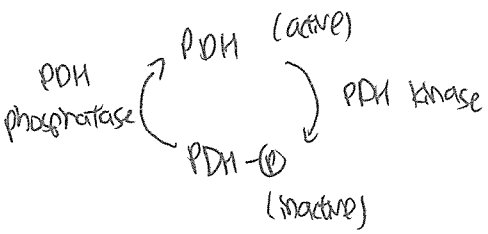
PFK-2 : isoforms (PFKFB)

- single gene, alternative splicing (tissue-specific) generates isoforms that vary in terms of
  - ↳ sensitivity to diff. protein kinases and control
  - ↳ differential ratio of PFK-2 and F2,6BPase activities

PFKFB-1 : liver, heart, skeletal muscle (L-type)
2 : lungs, brain, heart (H-type)
3 : most tissues, low basal but inducible (I-type) → depends on hypoxia
4 : most tissues, minor form

Pyruvate dehydrogenase → reduction → Acetyl CoA, NADH

- multienzyme complex (3 units)
- major control of activity via phosphorylation



PDH kinase regulated allosterically by metabolites

- activated by acetyl CoA, NADH (PDH products)
- inhibited by pyruvate, CoA, NAD (PDH substrates)
- inhibited by ADP (indicator of cellular energy state)

PDH products plentiful → feedback inhibition

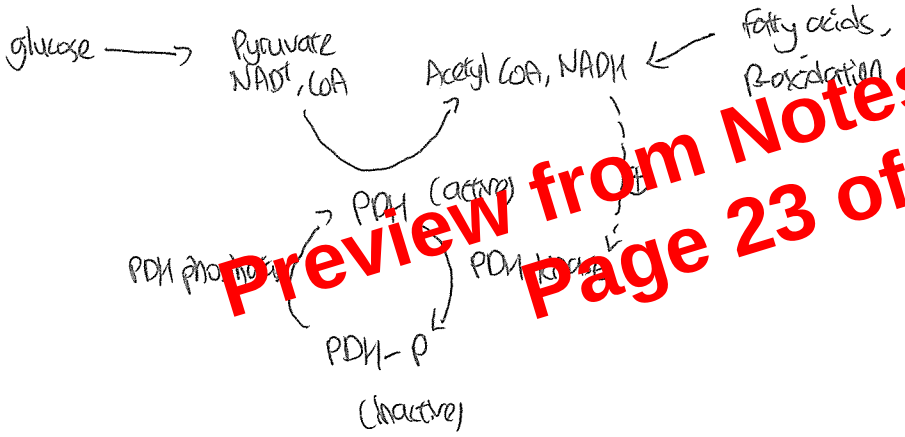
PDH products eg: Acetyl CoA also produced from fatty acid oxidation – allows coordination of glucose and fat oxidation

PDH substrates plentiful / energy status poor – favours continued PDH activity

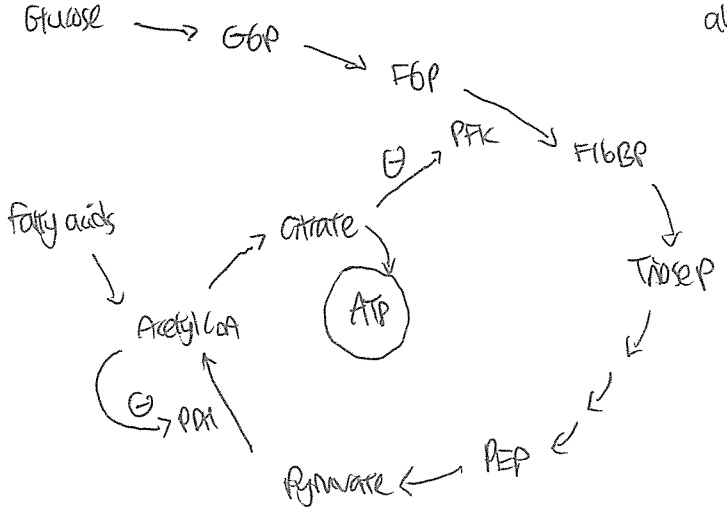
PDH phosphatase activated by insulin signalling

↳ indicator of "knowing" glucose-derived pyruvate

↳ co-ordinates w fatty acid utilisation in starvation



Glucose-fatty acid cycle



In cancer, mutations in regulatory mechanisms allow hijack of body's glucose to grow into large tumours.

In normal cells, glucose utilisation is low

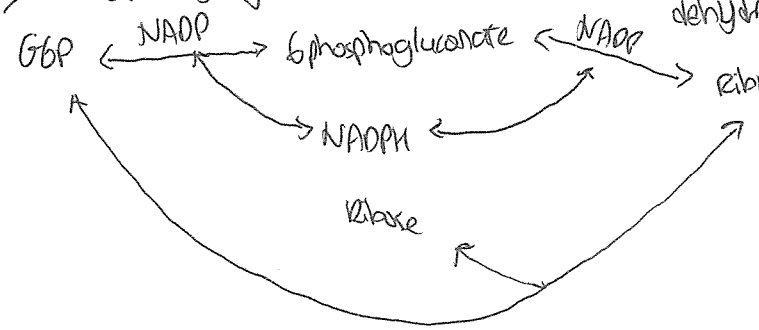
Pentose phosphate pathway (cytosol)

- alternate pathway of glucose catabolism
- 5-50% of all glucose ~~metabolism~~ catabolism (generally < 10%)
  - adipose cells ≈ 50%
  - in tumour cells ≈ 50%
- high contribution in specific cells — active cell division eg: tumour cells
  active fatty acid synthesis eg: adipose

* NADPH used for biosynthetic reaction

oxidative phase

[allosterically stimulated by G6P dehydrogenase NADP+]



* erythrocytes and cells of the lens & cornea are directly exposed to O_2 → damaging free radicals generated by O_2. By maintaining a reducing atmosphere (↑NADPH / NADP+) such cells can prevent oxidative damage.

Pentose phosphate pathway: specific function and regulation

NADPH: for biosynthetic pathways eg: fatty acids, sterols

ribose sugars: for nucleotides, support DNA & RNA synthesis

Regulation: amount G6PDH and 6PGDH protein decreased in starvation

transcription of both genes — activated by insulin
— inhibited by glucagon

Non-oxidative phase: ribulose 5-phosphate is converted back to Glucose 6-phosphate by enzymes: transketolases, transaldolase

Ribulose 5-phosphate is first converted into xylulose 5-phosphate by ribose 5-phosphate epimerase.

Glycogen turnover: synthesis of glycogen (occurs at non-reducing end)

UDP provides activation energy for glycogen synthesis from glucose

Glycogenin (1 or 2; 37 or 52kDa)

UDP-glucose ↓ addition to ~~single~~ tyrosine 194 ~~residues~~ on glycogenin (catalysed by glycogenin's intrinsic glucosyltransferase activity)

glycogenin-glucose

UDP-glucose ↓ autocatalysis

glycogenin - [glucose]$_{6-8}$

UDP-glucose ↓ glycogen synthase (GS) — undergo regulation

glycogenin - [glucose]$_{20}$ — after 20 ($C_6H_{12}O_6$ residues attached, branching occurs

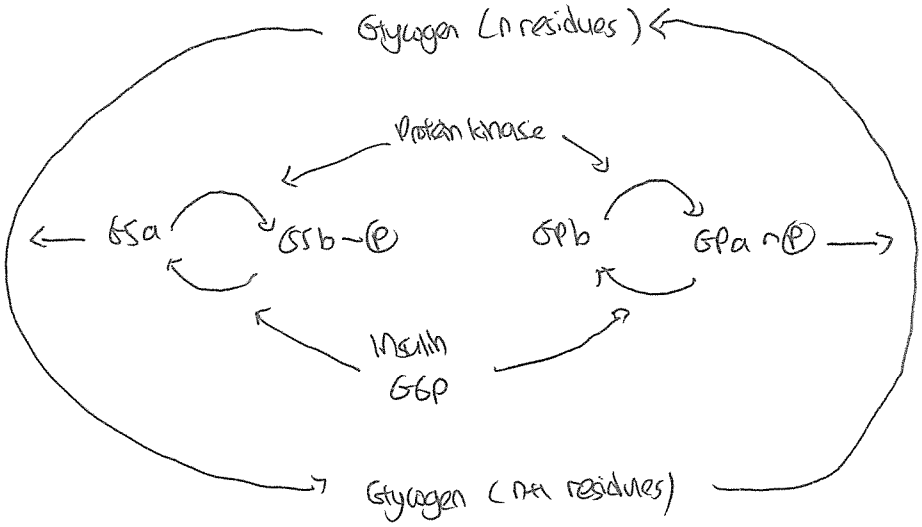
↓ branching enzyme (BE) — little regulation

Glycogenin

UDP-glucose ↓ GS & BE

glycogen → {Glycogenin}

Glycogen turnover : summary of regulation



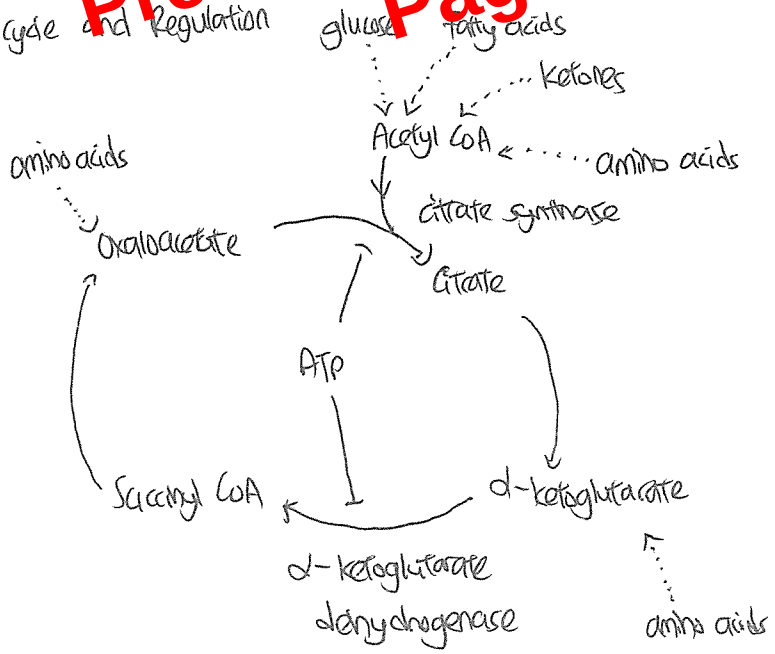
Glycogen turnover : complexity of regulation

* latency coordination

Starved rats, liver extracts, liver cells

* All GP must be inactivated (dephosphorylated) for GS to be activated

Citric Acid cycle and regulation



Advantages of FASI organisation (hydrophobic intermediates, but in cytosol)

- intermediates do not leave the enzyme complex – FA synthesis occurs in cytosol
- ↑ efficiency – intermediates concentrated, reactions coordinated
- coordinated regulation of expression – regulate levels of just 1 polypeptide

Precursors for FA synthesis

Acetyl CoA – from glycolysis (pyruvate) and amino acids
- mainly generated in mitochondria

NADPH – from pentose phosphate pathway
- from transport of acetyl-CoA into cytosol (via citrate shuttle)
- from malic enzymes (malate → pyruvate)

For every Acetyl CoA transported across mitochondrial membrane, 1 NADPH generated

– Acetyl CoA + oxaloacetate → citrate (citrate synthase)
– citrate transported from mitochondrial matrix into cytosol by a citrate transporter on the inner mitochondrial membrane.
Citrate Lyase then break down citrate into acetyl CoA + oxaloacetate
Oxaloacetate then converted to malate, (malate dehydro
then pyruvate, then transported back into mitochondria by pyruvate transporter
(major pathway)

malate only transported back to mitochondria by malate-α-KG transporter (minor pathway)

Regulation of FA synthesis

- maximal when carbohydrate & ATP abundant
- excess carbohydrate converted to FA and stored as fat

Control mechanisms

- enzyme activity (fine control – covalent modification, allostery (ACC)
- enzyme amount (course control – transcription, translation, degradation (FAS & ACC)

Multi-level control of ACC

global control: insulin, glucagon, adrenaline regulate enzyme activity according to status of whole organism

local control: allosteric effectors (citrate, FA, AMP) regulate enzyme activity according to conditions in individual cells

long-term control: regulation of ACC synthesis and degradation by changes in diet/nutritional status

Hormonal signals

insulin → signals for ↑ glucose, stimulates synthesis of fat, glycogen and protein
→ inhibits breakdown of fat, glycogen and protein
→ stimulate glucose transport into cells

glucagon – signal for ↓ glucose
– inhibit synthesis of fat, glycogen and protein
– stimulates breakdown of fat, glycogen and protein

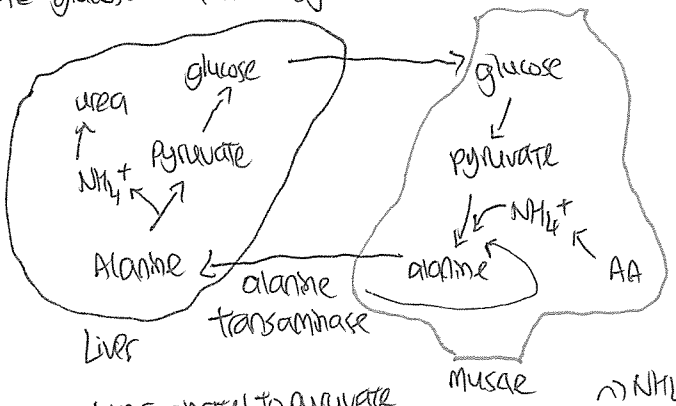
adrenaline – signal energy needed immediately
– inhibit synthesis of fat, glycogen & protein
– stimulates breakdown .....

Transporting Nitrogen to Liver

. urea is produced in liver

- The alanine cycle is used to transport nitrogen to the liver

The glucose – alanine cycle



1) transfer of amino groups to liver for disposal

2) transfer carbon from muscle protein to the liver for glucose synthesis

↳ catabolism of branched chain AA in muscle, provide carbon for gluconeogenesis in the liver

1) alanine transaminated to pyruvate
amino group converted to urea
pyruvate converted to glucose

1) NH_4^+ from AA transferred onto pyruvate
alanine transported to liver

* Serum levels of alanine transaminase and creatine kinase and Asp transaminase can be measure to determine severity of heart & liver damage after a heart attack. This is because these enzymes leak out of heart after heart attack.

Aminotransferases are also crucial in monitoring people whom are exposed to chemicals like chloroform, CCl_4

Regulation of AA degradation

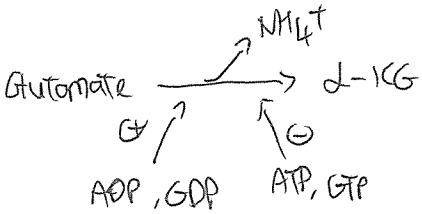
- Enzymes have high K_m → AA degraded & oxidised in proportion to concentration
- Supply of AA regulates activity

Fed : supply determined by delivery of dietary AA

Fasted : supply determined by breakdown of store of body protein

- regulatory point : deamination – glutamate dehydrogenase

Glutamate dehydrogenase



- allosterically regulated by nucleotides
- AA degradation is regulated by energy charge

* ~~mutation in GDH gene →~~

* mutation in GTP binding site of GDH leads to permanent activation of GDH → hyperinsulinism–hyperammonemia syndrome

[elevated levels of ammonia in blood stream & hypoglycaemia]

AA biosynthesis

- Assimilation of ammonia into glutamate / glutamine
- majority of AA obtain their nitrogen from Gln / Glu
- pathways for AA biosynthesis are diverse
- carbon skeletons come from intermediates of glycolysis, pentose phosphate pathway and CAC

Physiological Metabolic Integration: Starvation / feeding

Fed state	zero
Post-absorptive	6 hours
Starved	15 hours
	days

post-absorptive

* Species differentiation
* experimental model: rat, mouse
  ↳ pig = best model for metabolism

balance
- regulation
- integration
- anabolism
- catabolism
- nutrient
- endocrine control

Calories and Nutrition

Daily minimum intake: 1000 - 2000 ~~total~~ calories

	Composition (%)	
	Diet	Body stores
carbohydrates	40-45	0.5
lipid	40	80
protein	15-20	19.5

Body stores "reserves"

carbohydrate
- blood glucose (20g)
- liver glycogen (70g)
- muscle glycogen (200g)

1000 calories (<1 day, 2670kJ)

protein
- tissues (muscles) (8-12kg)

40,000 calories (about 10 days, 100,000kJ)

lipid
- adipose tissue triglyceride (10-20kg)

180,000 calories (about 60 days, 420,000kJ)

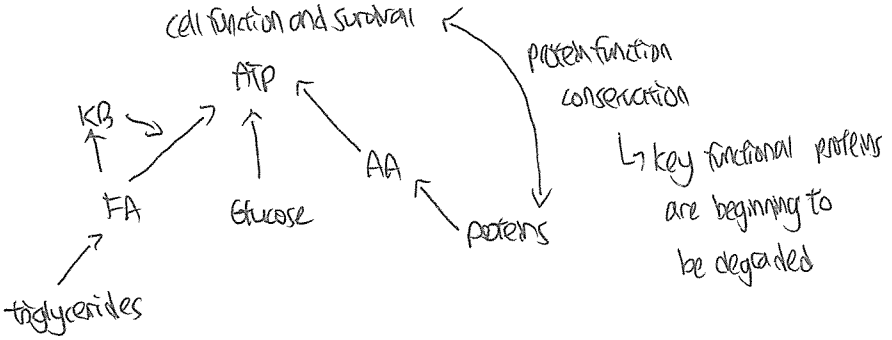
Importance of glucose metabolism during starvation:

ATP production in terms of
- Anaerobic conditions (ETC, O_2)
- cells without mitochondria (no citric acid cycle, ETC)
- cells without alternative nutrient supply

	Glucose used /day (g) during starvation	End product
brain (neurones)	125	lactate (90%)
Blood-brain barrier		CO_2 (10%)
RBC, renal medulla (no mitochondria)	50	lactate, pyruvate
muscle (anaerobic exercise)	50	alanine

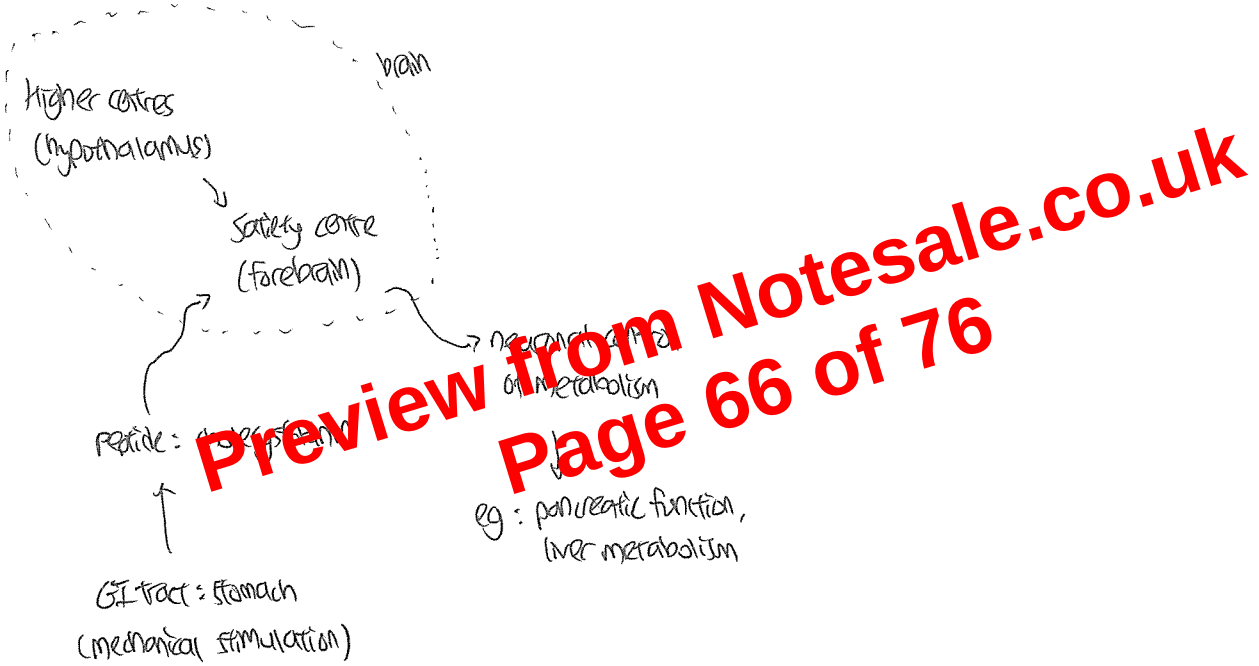
* these cells can only use glucose as energy source

Prolonged starvation

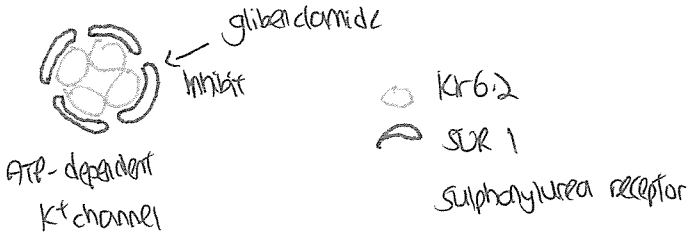


Critical roles of insulin & glucagon in starvation

	insulin	glucagon
change in plasma in starvation	↓	↑
effects of injection on plasma: glucose conc.	↓	↑
FA concentration	↓	↑
AA concentration	↓	↑

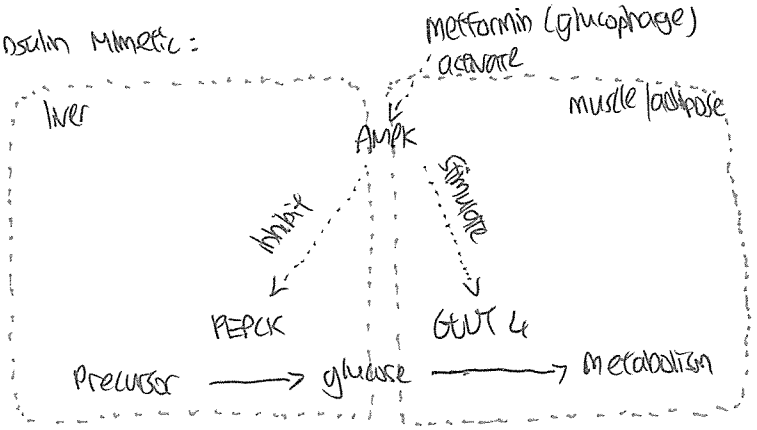


Insulin secretagogues



SUR 1 - binds sulphonylurea compounds (oral delivery)
↳ glibenclamide, tolbutamide
- closure of K^+ channels, insulin release

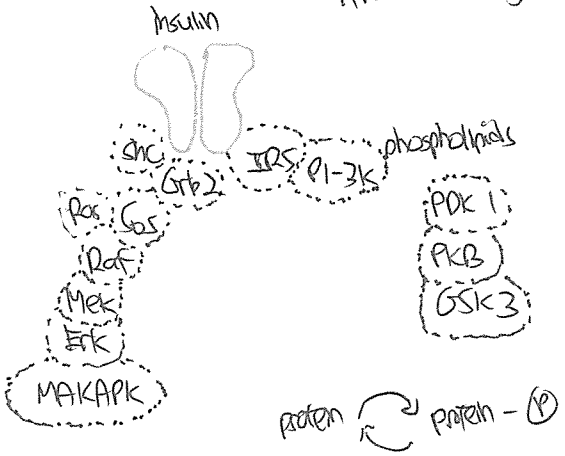
Insulin Mimetic:



1st line of drug of choice for T2DM - especially overweight/obese
- intracellular insulin-mimetic signalling - stimulate [illegible] signalling
- ↑ glucose uptake muscle/adipose
- ↓ glucose [illegible]
- metformin ↑ AMP concentration

Insulin sensitisers

Avandia (rosiglitazone)



protein ⇌ protein - (P)

- metabolic changes
- protein turnover
- gene expression
- cell division
- apoptosis

Site of insulin resistance - human ↳ animal diabetics

Experimental "knock out" animals (IRS 2)

Therapies for obesity:

1) lifestyle and genetic background

2) decrease food intake – Sibutramine (Meridia, Reductil) } decrease food absorption
↳ inhibit neurotransmitter reuptake (5HT)
↳ gastric bands (decrease stomach size)

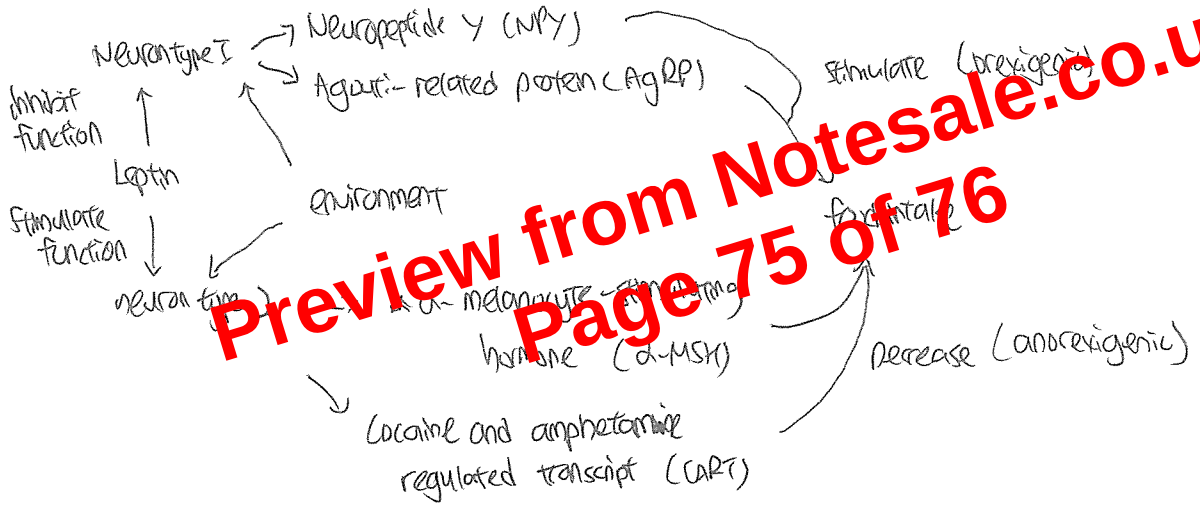
3) block nutrients absorption from gut – orlistat (Xenical)
↳ inhibit pancreatic lipase - lipids not absorbed, flow through the GI tract
(nitrophenol)

4) ~~inhibit~~ ↑ thermogenesis – Ephedrine or caffeine, β3 adrenergic receptor agonist
↳ ↑ mitochondrial oxidation, UCP-1 expression

5) Modulate fat storage / metabolism (surgery)
↳ maintain brown adipocyte phenotype (stem cell differentiation)

6) Modulate central controller system for body weight setting
↳ Leptin? lack of knowledge of key factors

Primary target neurones: Arcuate nucleus of the hypothalamus (therapeutic target potential)



2° target neurons: lateral hypothalamus
↳ default function of neurons to inhibit food intake by unknown "downstream" mechanisms