Integration of Metabolism

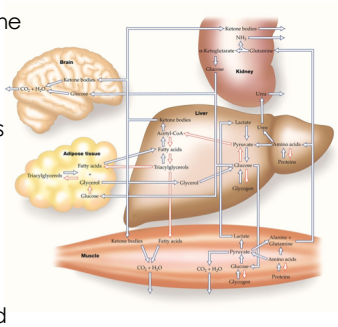
Pathways

We have studied various pathways - all based on the production and usage of Metabolic energy.

-KEY=glucose homeostasis

Pathways include: fatty acids, carbohydrates, amino acids all involved

Need to put together the different pathways and identify the key points and the regulation in different states

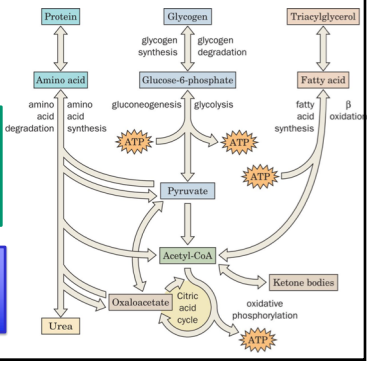


Several common/key metabolic intermediates (AKA -**cross road metabolites**)

- glucose 6 phosphate
- pyruvate
- acetyl CoA

Which are the key Enzymes involved in metabolism of each of these metabolites?

Which pathways take place in which Tissue?



Why do we need to think about the system of metabolism?

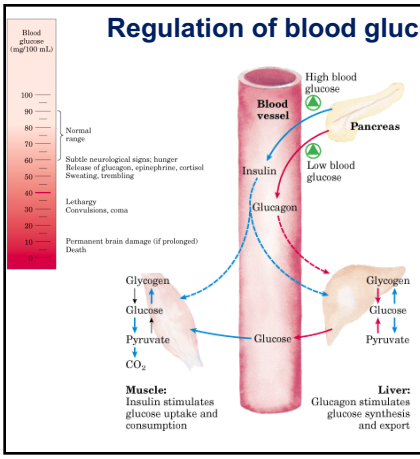
We are more than a "cell"…

TABLE 30.1 Fuel reserves in a typical 70-kg man

Organ	Available energy in kcal (kJ)		
	Glucose or glycogen	Triacylglycerols	Mobilizable proteins
Blood	60 (250)	45 (200)	0 (0)
Liver	4 (1700)	450 (2000)	400 (1700)
Brain	8 (30)	0 (0)	0 (0)
Muscle	1,200 (5000)	450 (2000)	24,000 (100,000)
Adipose tissue	80 (330)	135,000 (560,000)	40 (170)

Source: After G. F. Cahill, Jr. *Clin. Endocrinol. Metab.* 5(1976):398.

Regulation of blood glucose

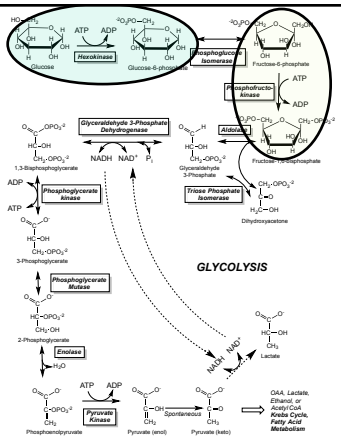


Its about TRANSPORT And Availability

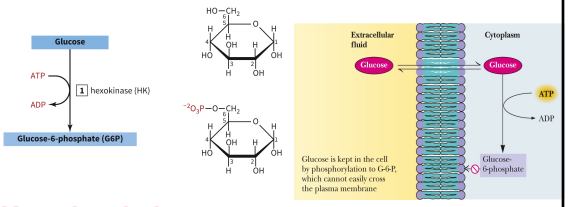
Lets start with Important pathways Controlling glucose and metabolite homeostasis

Glycolysis

HK and PFK are two the most important control points



Reactions of Glycolysis Metabolism
Glucokinase / Hexokinase - (GK/HK)



Key glycolytic enzyme

-1st step in glycolysis; *DG* large, negative

-Hexokinase (and glucokinase) act to phosphorylate glucose and keep "trap" Glucose in the cell

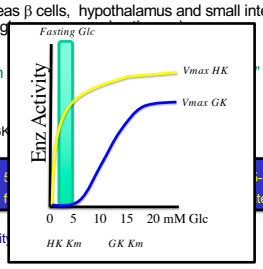
-1 ATP is consumed - is considered an irreversable step. Also a key glycolytic step GK is NOT inhibited by G6P

Reactions of Glycolysis Metabolism Glucokinase / Hexokinase - (GK/HK)

-Glucokinase in liver, pancreas β cells, hypothalamus and small intestine (maintenance of blood glucose levels and g

-Hexokinase in expressed in is non specific for various hexoses

HK K_m for glucose is 0.1 m*M*; GK



Blood Glc: normal 4 - 5 10 mM
Intracellular glucose ranges f te

Therefore - HK has a High affinit

- The function of GK is to remove glucose from the blood following a meal

HK – but not GK is regulated. HK – is allosterically product inhibited (G6P).

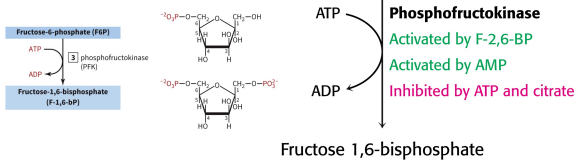
This is how at low blood glc, liver doesn't compete with brain use or with other tissues for available

Major metabolic pathways and control sites

Glycolysis

HK and PFK are two the most important control points

PFK - In the liver, the most important regulator is F-2,6 BiP
- When blood Glc goes down, glucagon triggerred pathway increases phosphatase, decreases kinase (making F-2,6BiP)
- Therefore, F-2,6BiP decreases
- PFK decreases
- Glycolysis slows down



TCA cycle and Oxidative Phosphorylation

Mitochondria – lead to complete oxidation of food stuff and ADP-> ATP

[ATP] controls both pathways

High ATP levels decrease the activities of 2 enzymes:

- Isocitrate dehydrogenase
- Alpha ketoglutarate dehydrogenase

Produces reducing equivalents NADH and $FADH_2$

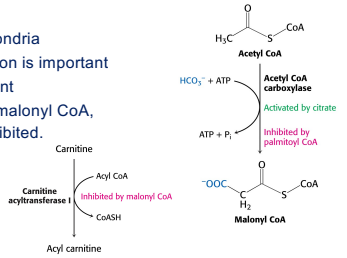
Fa Synthesis and Degradation

Fa's are made in the cytosol

2C donor: MalonylCoA

Acetyl groups are carried from mitochondria to the cytosol as CITRATE
- Citrate: increases the activity of acetylCoA carboxylase which increases fa synthesis

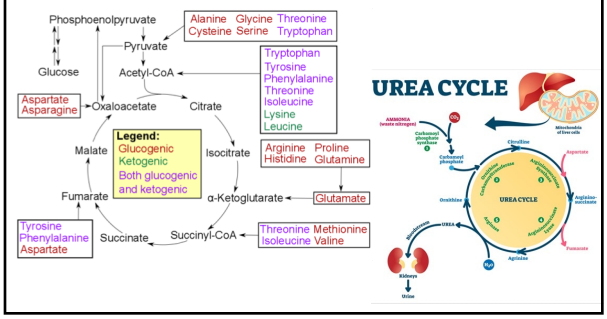
Beta oxidation is in mitochondria
- Acylcarnitine formation is important
- ATP need is important
- If there is too much malonyl CoA, fa degradation is inhibited.



Amino Acid Synthesis & Degradation

Integrated into several pathways including TCA

High protein degradation to amino acids and the further metabolism is clinically detected by high levels of free nitrogen (urea) in urine

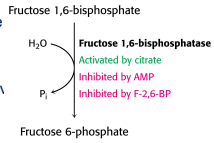


Gluconeogenesis

Glc can be made by the liver from noncarbohydrates

The major entry point of this pathway is pyruvate which is carboxylated to OAA in mitochondria.

Gloconeogenesis and glycolysis are usually reciprocally regulated so one pathway is not activ while the other one is active.

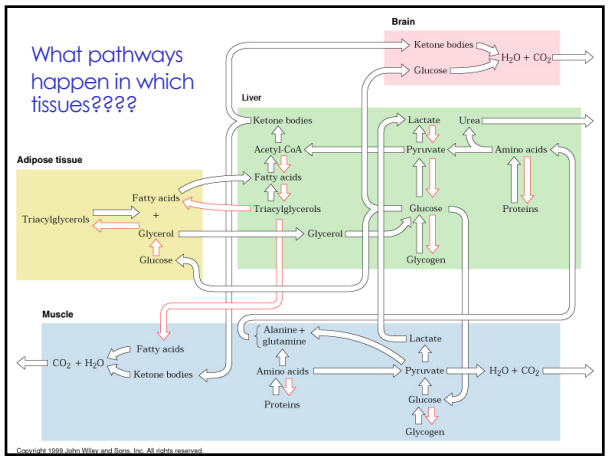


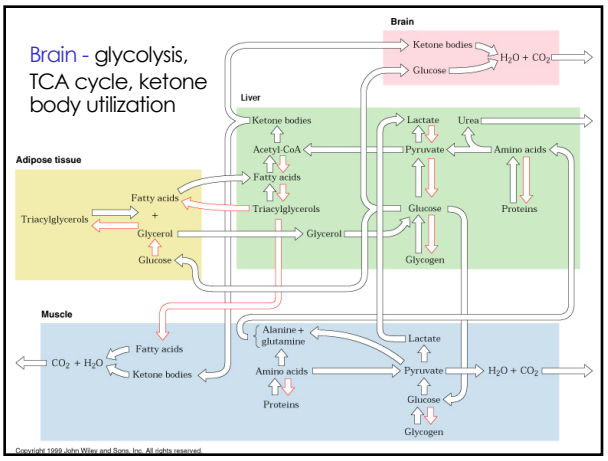
- If F-2,6BiP increases, gluconeogenesis is inhibited and glycolysis is activated.

Glycogen synthesis and degradation

Glycogen synthesis and degradation are coordinately controlled by a hormone-triggered cascade so there is no misunderstanding

Enzymes to remember:
- Phosphorylase
- Glycogen synthase



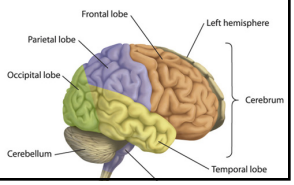


Some organs do not synthesize fuel molecules but are only involved in the use or break down.

The brain's primary fuel source is normally glucose. This energy is used to restore ion gradients for neural function.

The brain uses 10 fold more energy by weight than other tissues!

The brain stores no glycogen and therefore relies primarily on glucose from the bloodstream.



Brain

Glc is virtually the sole fuel for the human brain, except during prolonged starvation

It consumes 120 g glc per day

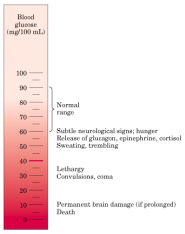
No glycogen stores in the brain

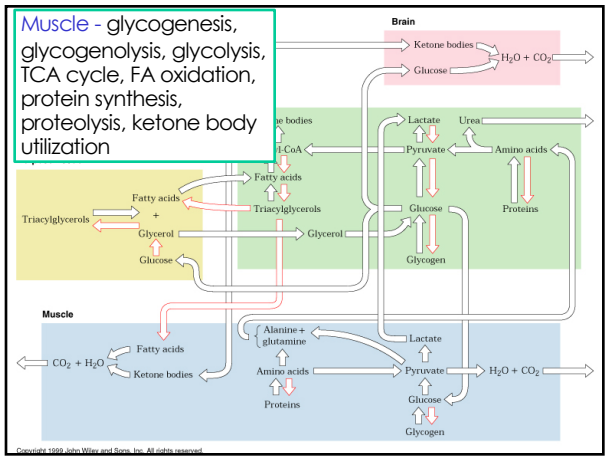
During prolonged starvation acetoacetate is used

Fatty Acids (FA) - do not serve as fuel in the brain because:

- They are bound to albumin in plasma, therefore they cannot pass the blood brain barrier.
- In essence, ketone bodies are transported equivalents of FA's

Because little storage and limited use of sources of fuel (?) brain requires a steady supply of glucose from the blood. Less than 5 mM will make you dizzy and prolong low bld glc or lower glc levels will cause coma and death

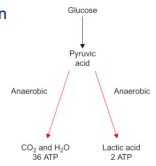




Muscle

Muscle differs from brain in having a large store of glycogen

- 75% of glycogen is in muscle.
- Major sources are glucose, FFA & ketone bodies
- The energy consumption increases with muscle activity
- Contraction of muscle uses ATP (currency of the cell…)
- Initial ATP is actually generated from phosphocreatine



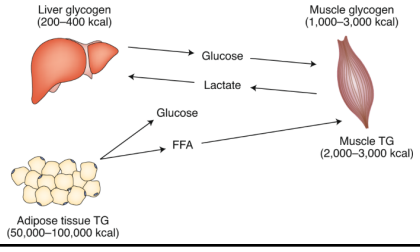


TABLE 30.3 Fuel sources for muscle contraction

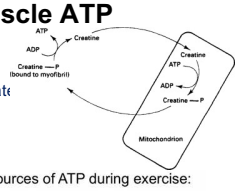
Fuel source	Maximal rate of ATP production (mmol/s)	Total ~P available (mmol)
Muscle ATP		223
Creatine phosphate	73.3	446
Conversion of muscle glycogen into lactate	39.1	6,700
Conversion of muscle glycogen into CO_2	16.7	84,000
Conversion of liver glycogen into CO_2	6.2	19,000
Conversion of adipose-tissue fatty acids into CO_2	6.7	4,000,000

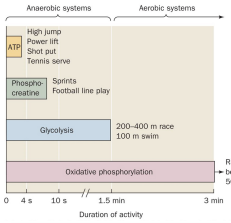
Note: Fuels stored are estimated for a 70-kg person having a muscle mass of 28 kg.
Source: After E. Hultman and R. C. Harris. In *Principles of Exercise Biochemistry*, J. R. Poortmans (Ed.). (Karger, 1988), pp. 78–119.

Sources of Muscle ATP

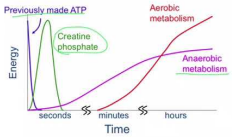
Initial ATP stores are used quickly

rest is generated from phosphocreatine or glycogen and glucose imported from liver, late fatty acids





Sources of ATP during exercise:

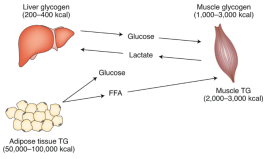


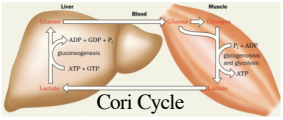
Muscle fatigue is not due to lack of "energy" (glucose or ATP) but instead due to acidic pH from lactate generated during glycolysis

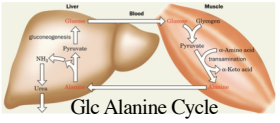
Muscle

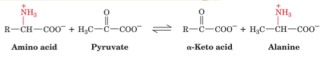
In actively contracting skeletal muscle, the rate of glycolysis far exceeds that of the citric acid cycle, and much of the pyruvate ---> lactate then.

Glucose is transported between muscle and liver through the blood in two pathways





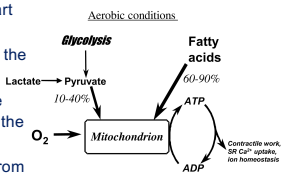


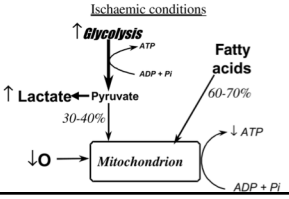


Key – Lactate vs Pyruvate to generate muscle "waste" into glucose in liver

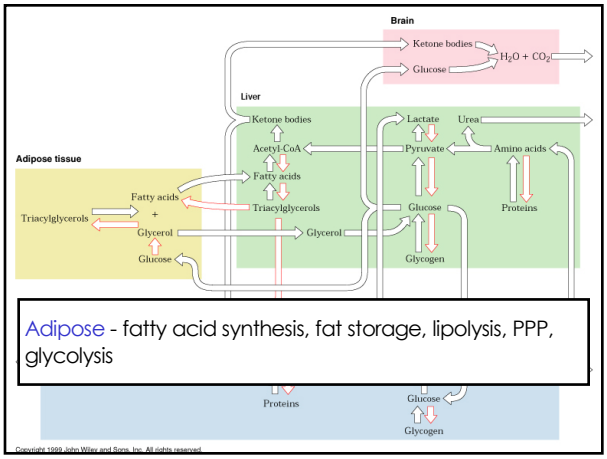
Heart muscle

- For reasons that are not clear, the heart relies mainly on fatty acids.
- Fatty acid supply is more reliable than the fluctuating carbohydrate supply?
- Most organisms have a very extensive supply of FA's; thus the functioning of the heart muscle is protected
- MOST ATP is from OxPhos only 2% from glycolysis in normal conditions





- Moderate loss of blood flow to heart leads to higher % of glycolysis resulting in lower pHi
- Combined with FA oxidation lower oxygen levels.
- Increased NADH inhibits pyruvate leading to higher lactate.
- Combined O_2 and pH acid are damaging



Adipose - fatty acid synthesis, fat storage, lipolysis, PPP, glycolysis

Adipose tissue

The TAGs are stored here.

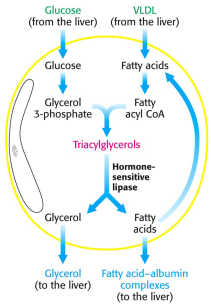
They are enormous reservoir of fuel.

Adipose cells need glucose for the synthesis of TAGs

The glucose level inside adipose cells is a major factor in determining whether fatty acids are released into the blood

Adipose tissue: If too much food → FFA stored.

If Glc and glycogen are NOT enough, TAG→ FFA→ Liver



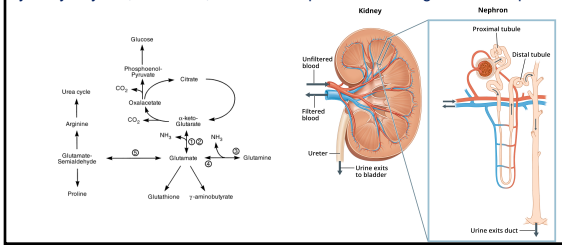
The kidney

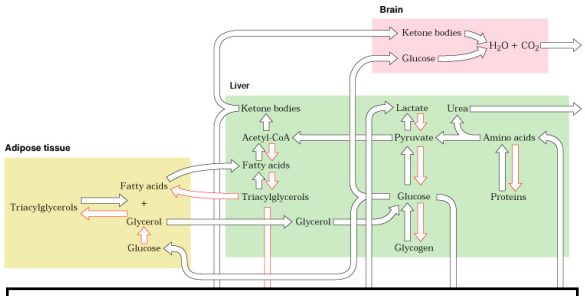
Major role: to make urine

The blood plasma is filtered nearly 60 times each day in the renal tubules.

During starvation the kidney becomes an important site of gluconeogenesis and may contribute as much as half of the blood glucose!

Supports blood pH homeostasis by excreting H^+ with acetoacetate, b-hydroxybutyrate, ammonia, and can re-uptake CO_2 to regulate blood pH





Liver – gluconeogenesis, glycolysis, glycogenesis, glycogenolysis, fatty acid biosynthesis, fatty acid oxidation, ketogenesis, protein synthesis, amino acid degradation, PPP, TCA cycle and urea cycle

Liver

The liver serves as the body's distribution center, detoxification center, and central clearing house.

Metabolic hub

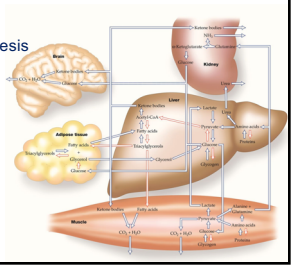
The liver plays an essential role in the integration of metabolism.

Liver removes 2/3 of the glucose from the blood.

Glc------> G-6-P

G-6-P has many fates

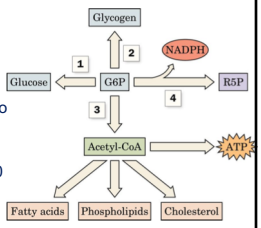
- FA, cholesterol or bile synthesis
- Glycogen synthesis
- PPP



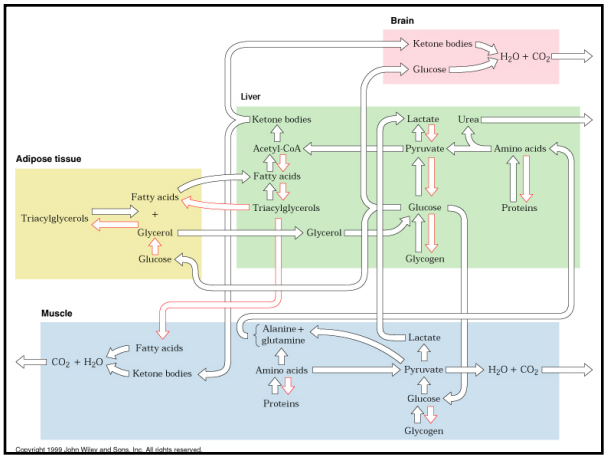
liver

Glucose 6-phosphate serves as crossroad of metabolism

- When fuels are increased, FA's are derived from the diet or synthesized by the liver as TAGs
- They are secreted into the blood in the form of VLDL
- During fasting, the liver converts fa's into ketone bodies
- The liver also plays an essential role in amino acid metabolism - secretes 20-30 g urea/day



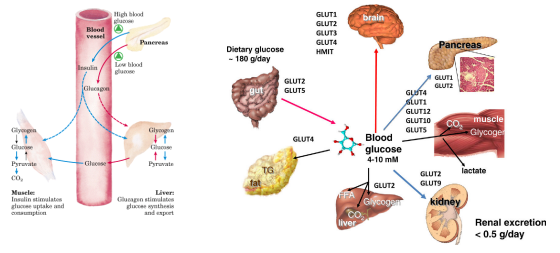
Liver meets its own energy by using α-ketoacids



Insulin and Glucose Transport

Glucose can NOT easily or freely diffuse across membranes. Thermodynamics of shedding water and crossing a hydrophobic barrier

- Glucose Transporters (GLUT) many isoforms each with unique expression, glucose affinity and specificity.



Insulin and Glucose Transport

Glucose can NOT easily or freely diffuse across membranes.
Thermodynamics of shedding water and crossing a hydrophobic barrier

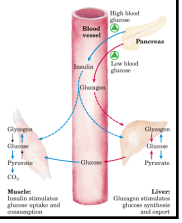
- GLUT 4 is expressed in skeletal muscle, cardiac muscle and adipose tissue. ONLY GLUT4 is insulin responsive, 5 mM Km.
- GLUT 2 (pancreas and Liver) is not insulin depended and low affinity (15-20 mM Km).

This allows glucose transport in pancreas and liver only when glc levels are high – after meal. Controlling pancreas function and liver sparing glc for brain.

- Liver and pancreas have glucokinase (low affinity)

which also support sparing glucose for rest of body and not full metabolism of glucose until high conc.

Metabolism in pancreas is primarily Glc -> pyruvate
Glc must be metabolized by β–Islets of pancreas to stimulate insulin release via ATP levels controlling Calcium and insulin secreation



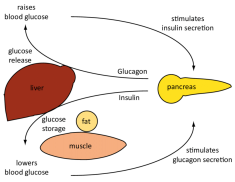
Insulin and Glucose Transport

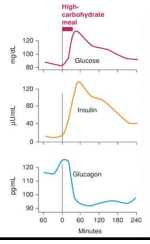
Insulin binds to its receptor (many tissues) and in Muscle cells will lead to GLUT4 surface translocation.

Thus glucose metabolism at high concentrations will lead to insulin stimulation of glucose storage from blood to muscles

Post-meal glucose leads to insulin release, GLUT4 activation resulting in low blood sugar – Insulin blocks liver use of glucose (glycogen production and glycolysis)

Glucagon released reverses effect of insulin





Hormonal Control of Fuel Metabolism

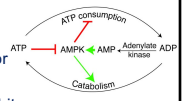
Nervous stimulation of adrenal glands release Epinephrine and norepinephrine

Tissue	Insulin	Glucagon	Epinephrine
Muscle	↑ Glucose uptake	No effect	↑ Glycogenolysis
	↑ Glycogen synthesis		
Adipose tissue	↑ Glucose uptake	↑ Lipolysis	↑ Lipolysis
	↑ Lipogenesis		
	↓ Lipolysis		
Liver	↑ Glycogen synthesis	↓ Glycogen synthesis	↓ Glycogen synthesis
	↑ Lipogenesis	↑ Glycogenolysis	↑ Glycogenolysis
	↓ Gluconeogenesis		↑ Gluconeogenesis

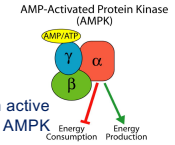
AMP Dependent Protein Kinase (AMPK)

AMPK is a highly conserved master regulator of metabolism.
Binds and phosphorylates (it is a kinase) other proteins

- Activated in response to energy stress by sensing increases in ADP:ATP ratio (normally about 1 ADP for every 1000 ATP).
- AMPK leads to pathways that generate ATP and inhibit ATP using biosynthetic pathways



γ - ATP(inhibitory) AMP (Activation)
α – Ser/Thr kinase domain



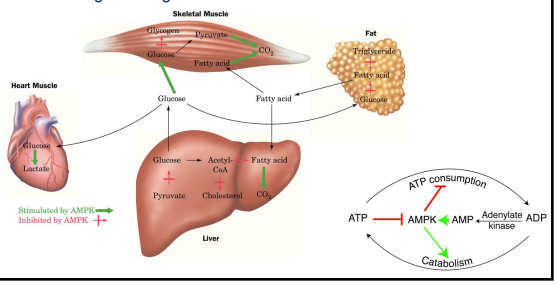
Inactive until phosphorylated by γ subunit. AMP open active site so other kinases can phosphorylate and activate AMPK

Adenylate Kinase (2ADP –> AMP + ATP) helps signal low energy state and creates AMP to activate AMPK

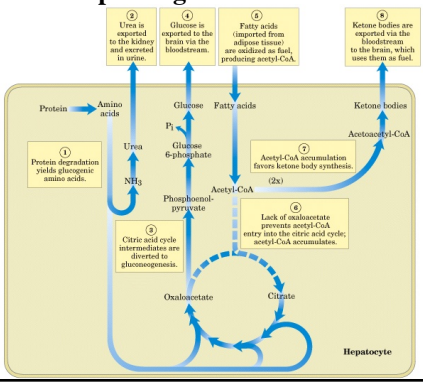
AMP Dependent Protein Kinase (AMPK)

AMPK targets bifunctional enzyme in heart increasing [F2,6P] activating PFK1 and glycolysis.

AMPK via –phosphorylation of key metabolic enzymes, blocks lipid production and gluconeogenesis in liver



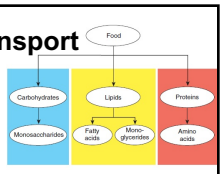
Fuel metabolism in the liver during prolonged starvation



Food absorption and transport

Macromolecues (food) must get “into” the body.

Broken down into smaller functional units and transported into liver and other organs via lymph or blood



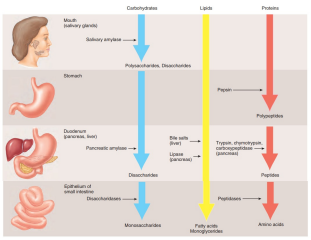
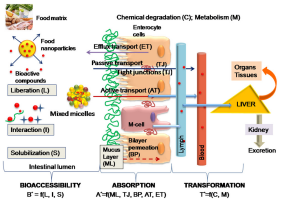


Figure Digestion of Carbohydrates, Lipids, and Proteins



The well-fed state

After the consumption

Glc, aa’s and lipids are transported to the blood

The fed state The secretion of insulin increases.

Insulin increases the uptake of Glc into the liver by GLUT2

Insulin also increases the uptake of Glc by muscle and adipose tissue

Early fasting state

The blood Glc decreases several hors after a meal

Insulin decreases and glucagon increases

So, glucagon signals the starved state

It mobilizes the glycogen by cAMP pathway

Target liver

Net result: Increase glucose in blood

The refed state

Fat process same as fed state

The liver does not initially absorb glc from the blood, but rather leaves it for the peripheral tissues

Liver stays in gluconeogenic mode

Newly made Glc is used to make glycogen

As blood Glc increases the liver completes the replenishment of its glycogen stores

Metabolic adaptation in prolonged starvation minimize protein degradation

What are the adaptations if fasting is prolonged to the point of starvation?

- 70 kg person has fuel reserve ~ 161,000 kcal
- The energy need for a 24 hr cycle 1600-6000 kcal
- So, fuels are ok for 1-3 months!

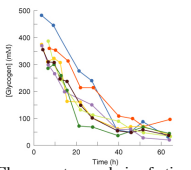
The very first priority of metabolism in starvation

- Providing Glc to the brain and other tissues

The second priority of metabolism in starvation is to preserve protein, which is accomplished by shifting from glc to FA’s

TABLE 30.3 Fuel sources for muscle contraction

Fuel source	Maximal rate of ATP production (mmol/s)	Total ~P available (mmol)
Muscle ATP		223
Creatine phosphate	73.3	446
Conversion of muscle glycogen into lactate	39.1	6,700
Conversion of muscle glycogen into CO_2	16.7	84,000
Conversion of liver glycogen into CO_2	6.2	19,000
Conversion of adipose-tissue fatty acids into CO_2	6.7	4,000,000



Glycogen storage during fasting

Metabolic adaptation in prolonged starvation minimize protein degradation

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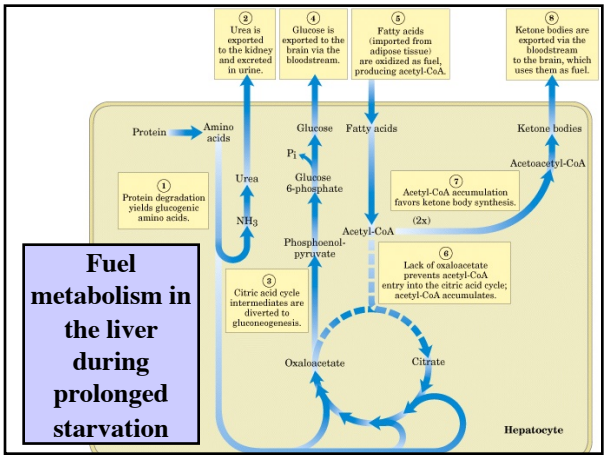


TABLE 30.2 Fuel metabolism in starvation

Fuel exchanges and consumption	Amount formed or consumed in 24 hours (grams) 3d day	40th day
Fuel use by the brain		
Glucose	100	40
Ketone bodies	50	100
All other use of glucose	50	40
Fuel mobilization		
Adipose-tissue lipolysis	180	180
Muscle-protein degradation	75	20
Fuel output of the liver		
Glucose	150	80
Ketone bodies	150	150

Food intake and starvation induce metabolic changes

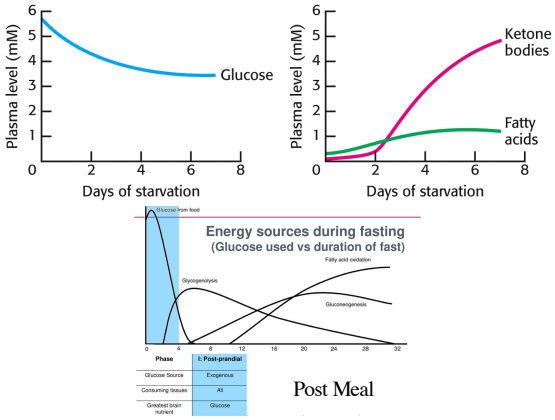
Starved-fed cycle has 3 stages:

- Postabsorbtive state
- Early fasting during the night
- The refed state after breakfast

Use of Energy Stores.

- Glycogen -> glucose
- TAG -> FA
- Longer term – Proteins ->aa-> TCA, Ketone, glucose (depending on tissue)

Main goal is to maintain blood glc homeostasis and energy for brain, kidney and other key orgns!



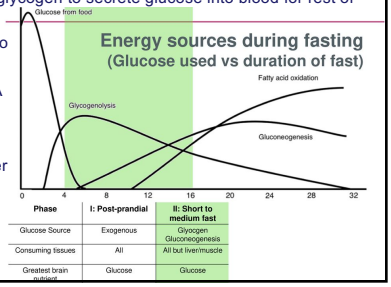
Early fasting state

The blood Glc decreases several hours after a meal

Insulin decreases and glucagon increases

Glucagon signals the starved state

- It mobilizes the glycogen by cAMP pathway
- Target liver is using its glycogen to secrete glucose into blood for rest of body
- Glucose is not taken into muscles spared for brain
- Glucagon stimulates FA release from adipose and muscle uses FA in βOX
- Gluconeogenesis in liver begins



After 3 days of starvation

Liver forms ketone bodies

Ketone body synthesis from Acetyl~CoA is increased because TCA is not running (gluconeogenesis depletes the supply of oxaloacetate) and results in a build-up of Acetyl~CoA

So, liver makes lots of KBs

The brain begins to use acetoacetate by reversing KB synthesis.

After 3 days, 1/3 of the energy comes from KBs for the brain

The heart also uses KBs

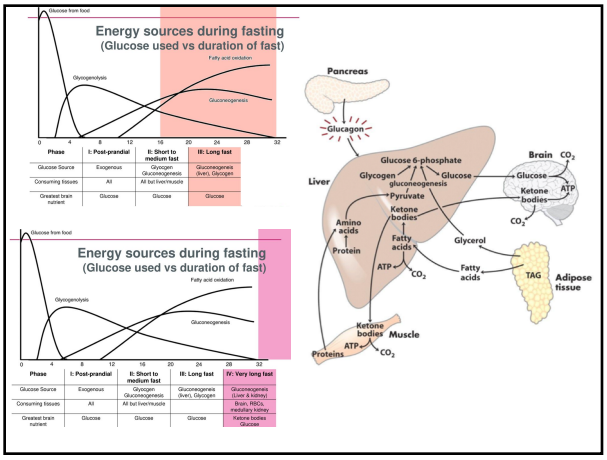
After long term fasting the body expends glycogen reserves within about 24 hours. After 48 hours of fasting.

The liver then moves from glycogenesis to gluconeogenesis and ketogenesis.

Peripheral tissues including the brain moves from glucose as the main fuel to ketone bodies.

Skeletal and heart muscles rely on fatty acids when resting and use glucose from glycogen or the bloodstream during exercise.

Heart tissue is rich in mitochondria and is mostly an aerobic tissue and also utilizes ketone bodies when blood glucose becomes low.



The refed state

- Fat process same as fed state
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- Liver stays in gluconeogenic mode
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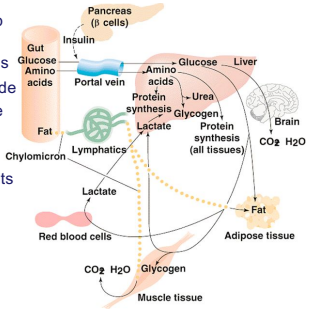

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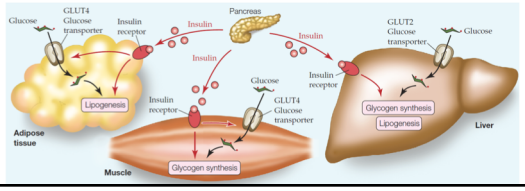


Metabolic changes during exercise

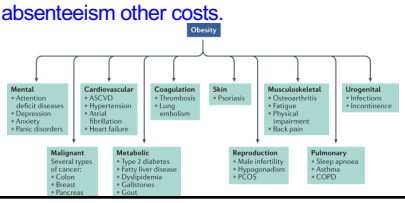
Sprinting and marathon running are powered by different fuels to maximize power output

- A 100 meter sprinter uses:
  - Stored ATP
  - Creatine phosphate
  - Anaerobic glycolysis of muscle glycogen
- A 1000 meter runner
  - Oxidative phosphorylation starts.
- Marathon requires a different selection of fuels
  - A nice cooperation between muscle, liver and adipose tissue
  - Total glycogen stores (103 mol of ATP) are insufficient to provide 150 mol of ATP.
  - Fat breakdown is needed.

The US 2020 treatment market is $8.4 billion and predicted to be $27.1 billion in 2028. 2020 Cancer therapeutic market is $136 billion for comparison

This market potential has caused pharmaceutical companies to prioritize the identification of novel anti-obesity products and consequently the number of drugs in development has risen 5-fold over the past 7 years largely due to an increase in preclinical research activities.

Obesity-related medical care costs >$190 billion with over 4 billion in absenteeism other costs.



Health Consequences:

Heart disease and stroke - The leading causes of death and disability for both men and women in the United States

Diabetes - Type 2, or non-insulin-dependent diabetes mellitus

Cancer - Increased risk of cancer of the uterus, gallbladder, cervix, ovary, breast, and colon in women; increased risk of cancer of the colon, rectum, and prostate in men

Sleep apnea - Interrupted breathing during sleep

Osteoarthritis - Wearing away of the joints, which often affects the knees, hips, and lower back

Gallbladder disease - Risk of gallbladder disease and gallstones increases as weight increases

Obesity

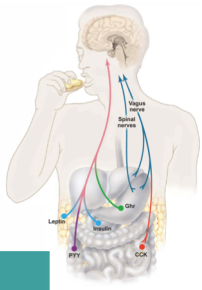
It is an epidemic

Two signal hormones classes

- Orexigenic (stimulate appetite)
- Anorexigenic (decrease appetite)

Important signal molecules

- Insulin, Leptin, Ghrelin and Incretins



ΔE=Q-E Is it about thermodynamics?

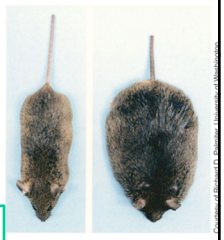
First major clue to energy control was found in ob/ob mouse

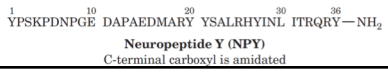
- Missing/defective gene for leptin - decreases satiation

Hypothalamus is a major locus for food intake regulation

- Orexins: promoters of food intake: neuropeptide Y
- Anorexin- inhibition of food intake: proopiomelanocortin and cocaine-related transcript

Leptin reduces concentration of neuropeptide Y – which stimulates appetite and increase fat storage

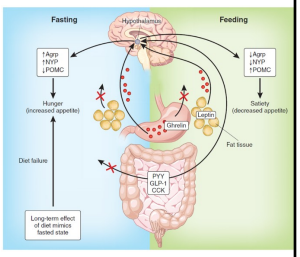




Neuropeptide Y (NPY)
C-terminal carboxyl is amidated

Food Hormones

Leptin, discovered in 1995, is a hormone which suppresses the appetite. Produced primarily in fat tissue, leptin circulates generally in proportion to fat stores. It encourages people to stop eating when their fat cells are full. A newly discovered hormone called ghrelin (pronounced GRELL-in) seems to have the opposite effect.

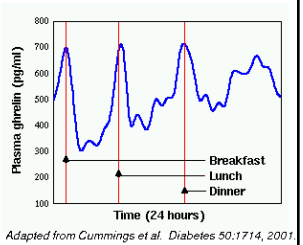


Ghrelin boosts levels of NPY

- The gut produces PYY an appetite suppressing hormone

Food Hormones

Experiments on rats and mice found ghrelin to be a powerful appetite stimulant; rodents given the hormone immediately began feeding. And British researchers last year found that human

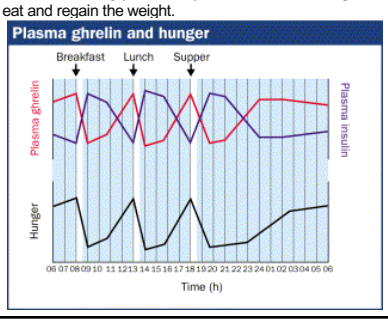


Adapted from Cummings et al. Diabetes 50:1714, 2001.

volunteers injected with ghrelin became ravenous and immediately increased their food intake.

Food Hormones

When we've stored enough fat, leptin tells us to stop eating. Ghrelin, on the other hand, tells us to eat and store fat. In ancient times, ghrelin helped us prepare for the next famine. In modern times, however, it acts as the evil twin of leptin. When we go on a diet and lose weight, ghrelin makes us hungry. The body thinks we are starving and encourages us to eat and regain the weight.



The Hunt for Ghrelin Begins

1970's - Tulane - Peptide library was made to find growth hormone stimulating activity

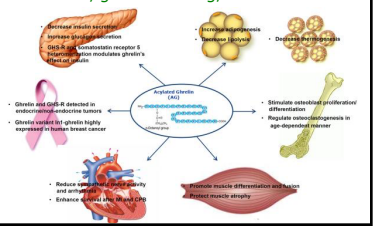
Eli Lily found little growth and instead adiposity and food intake

Merk found the receptor but not the ligand

Most receptors in hypothalamus - not consistent with GH release

Chronic ghrelin administration durably stimulates food intake and suppresses energy expenditure, increasing body weight, whereas acute ghrelin blockade does the opposite. Circulating ghrelin levels increase with weight loss resulting from low-calorie diets, chronic exercise, cancer anorexia, cardiac or hepatic cachexia, gastric banding, and anorexia nervosa.

Independent of its orexigenic effect, ghrelin promotes adiposity and elevates blood glucose through inhibition of insulin secretion



Ghrelin Animal Studies

Injection of ghrelin didn't increase GH

Low doses increased rat feeding by 35% and higher doses by over 300%

Distention of abdomen by water did not increase circulating ghrelin but when carbohydrate was added ghrelin levels dropped

Changing Ghrelin Levels

Fasting ghrelin is significantly higher in lean than obese 857 pM vs. 325 pM

However:

30 min after eating the lean group's ghrelin droped 40% and then reach background (10-20 pM), while the obese subjects levels remained high

Circulating Leptin also dropped in the lean group but not the obese group