(Digestion, Glycolysis and general pathways by Amirova M.F.)

CARBOHYDRATES DIGESTION.

Let's have short review on carb representatives. Main monosaccharides are glucose, fructose & galactose.

Main oligosaccharides are disaccharides maltose, sucrose, & lactose. Main polysaccharides are glycogen & starch. Cellulose is also polysaccharide present in diet bun it is indigestible.

Passing through GIT, polysaccharides undergo digestion under the action of salivary amylase resulting in production dextrins. Pancreatic amylase has the same effect oh polysaccharides with one distinction: the time of action of pancreatic amylase is much more longer than time of salivary amylase. Amylase splits polysaccharides on dextrins, maltose and few glucose molecules. Further, in intestine, disaccharides maltose, saccharose & lactose are digested with the subsequent release glucose, fructose& galactose.

There is no digestion of carbs in stomach because of acidic medium ceasing amylase action.

With food cellulose, starch, glycogen and rest oligosaccharides enter the body. Salivary amylase has a little effect on the carbs, mainly dextrins are formed, but isomaltose, maltose are also made.

Cellulose can not be digested because amylase cleaves alpha 1,4-bonds, but nor beta-bond, which is why cellulose passes through GIT largely intact, but protecting GIT from chemical toxins & mechanical damage.

Sucrase –isomaltase complex. sucrase isomaltase function.

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carbohydrates that includes starch, sucrose, and isomaltase. This hydrolysis leads to ATP production after further processing.

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In small intestine, maltase of brush border produces 2 alpha-glucose molecules from maltose, sucrase – beta-glucose & beta-fructose from sucrose, and eventually lactase splits lactose to beta-galactose & glucose.

Isomaltose is glucose connected with next glucose via 1,6-bond. This bond is broken down by isomaltase.

After digestion, monosaccharides are absorbed by intestinal wall. Following ways of absorption are distinguished: passive, facilitated & active. Pentoses are absorbed by passive diffusion; hexoses may enter enterocytes both by facilitated diffusion & active transport. Facilitated diffusion is possible because of glut membrane transporters, mainly insulin-dependent. Insulin-independent GLUT transporters are available in intestine, liver, brain. Active transport of glucose is implemented via sodium-dependent glucose transporter.

The picture shows that channel is closed without signal which is actually insulin, In presence of insulin channel opened & allows glucose penetration into the cell. Both diffusion types: passive & facilitated are implemented by concentration gradient, not against. Conversely, active transport is mainly implemented against concentration gradient. SGLT action is secondary active transport type: ATPase pumps sodium ions outward & potassium – inward, this way creating gradient of these ions. Sodium & potassium strives to return to the cell, and this energy is used to enter 1 glucose in terms of each sodium ion returning intestinal cell. As becomes clear, ATP is used for entry hexoses into the cells by this way.

Soda ions are exchanged for potassium, therefore soda is less in enterocyte, what creates the driving force for glucose uptake.

GLUT 5 transporter is for fructose facilitated diffusion, & it refers to as passive diffusion. SGLT is for glucose & galactose (hexoses), GLUT transporters sort into 1, 2, 3, 4, 5. 4^{th} is insulin-dependent.

Currently, about 75% of world's population is lactose intolerant. Lactose intolerance is a disease when lactase absent in the brush border of the small intestine, which is why lactose, the main carbohydrate in **dairy** products. a sugar of milk can not be digested and undergoes fermentation by the intestinal bacteria. Diarrhea occurs due to undigested lactose, which is osmotic active, ergo attaches water &causes water to move into intestine.**Lactose intolerance**, a digestive disorder caused by the inability to digest **lactose**, can cause various symptoms, including bloating, diarrhea and abdominal cramps. Flatulence &gas are manifestations resulting from fermentation, pain is caused by short chain fatty acids. The severity of symptoms can vary depending of how much lactose patient can tolerate & how much he has eaten.

In norm, lactase is digested to glucose & galactose, the end products, while in lactose intolerance lactose cumulation in intestine results in diarrhea, & products of fermentation – to irritation.

GLYCOLYSIS

What happens after glucose entry into the cell? – Glucose is used as main energy source producing pyruvate; however PPP (with the formation of ribose), glycogenesis, & synthesis of extracellular polysaccharides, namely glycose aminoglycans also occur.

Glycolysis occurs in all cells of the body. It is a sequence of reactions converting glucose to pyruvate or lactate. The term is a merger of 2 words: glykys – sweet & lysis (breakdown).

In glycolysis, 2 pyruvates (or 2 lactates) & 2 ATPs are produced. The pathway occurs in cytoplasm, may occur aerobically or anaerobically (with O2 or without).

Under anaerobic condition, lactate is the end product of glycolysis. In the tissues lacking mitochondria it is the only way to supply the cell with energy. RBC, eye cornea, lens belong to this type of cells.

Under aerobic condition pyruvate is not converted to lactate, but undergoes further degradation resulting in water & CO2 formation. Brain has very little stocks of glucose, ergo it nearly completely depends of glucose levels in the blood.

During glycolysis energy is released, ergo it is exergonic pathway. Energy released is used for synthesis of ATP. To enter the process, first glucose must be activated, & for this purpose ATP is spent. The whole pathway may be divided on 3 stages: 1) priming phase, which is energy investment stage, 2) splitting phase, when 2 3carbon compounds are made from 1 6carbon glucose, & 3) energy generation phase, as ATPs are produced in this phase.

Expenditure incurred: 2ATPs splitted to 2 ADPs. Energy gain are 4 ATPs. But as we spent 2 ATPs to start process, total gain is 4-2=2 ATPs. During glycolysis, $2NADH_2$ are also formed from 2 NAD.

In this picture all reactions of any stage with reactant, products & enzymes are represented. We see reactions of investment stage, where ATPs are spent. The goal is to raise glucose to the level, active enough to initiate the required responses. For this purpose, eventually fr-1,6-bisph. Is formed.

We have only 2 reactions of splitting stage, with interconversion of trioses from ketose ph. to aldose phosphate.

& last stage, energy release & formation of ATPs.

Since energy investment & splitting are doth preparatory steps, we may also sort reactions of glycolysis to 2 stages, not 3 ones: preparatory & payoff phases.

Ergo, preparatory phase covers 5 first reactions, resulting in cleavage of hexose chain. Fr-1,6-bisphosphate produced as result of investment, is very symmetrical & can be

divided to 2 relatively equivalent 3carbon containing molecules: Glycerald.-3-ph & DHAP.

Preparatory phase, first 5 reactions are represented in this picture. Phase ends with formation of 2 identical trioses, which are isomers of each other & can be interconverted. Aid is formation of 2 glyceraldehydes-3-phosphates.

With 6^{th} reaction begins payoff phase of glycolysis., which constitutes last reactions of pathway. Payoff phase produces 4 ATPs, but 2 ATPs are subtracted because 2ATPs were spent for activation of glucose, hence, a total gain of ATPs in glycolysis is 2molecules.

In payoff phase, 2ATPs in 7^{th} & 2ATPs in 10^{th} reaction are produced. 6th reactions ends with formation NADH2. Since 2 glycer-aldehydes made from 1 mol of glucose enter this reaction, the number of NADH2 is also 2: 2NAD2 are formed.

1) Glycolysis starts with irreversible conversion gl. to gl-6-ph by assistance hexokinase with consumption of ATP. Hexokinase present in any cell. Hexokinase can convert any hexose, e.g. galactose, mannose to their 6-phosphate derivatives. There is also glucokinase, available only in the liver, & converting only Gl. to Gl-6-ph. By the way, any enzyme transferring phosphoric group with participation of ATP is called "kinase". Kinase can transmit phosphoric group from or to ATP.

Carbonyl group is re-arranged from C1 to C2 thus forming ketose(Fructose) from aldose (Glucose). To be correct, in this reaction aldose Gl-6-ph is reversibly converted to ketose Fr-6-ph. Fr is a keto-isomer to Glucose, that is why enzyme is called isomerase, to be correct, Phosphogluco-isomerase.

3) This step is also irreversible. Fr-6-Ph is converted to fr-1,6-bisph. By enzyme ***Phosphofructo-kinase.*** Enzyme name suggests participation of ATP in reaction, which plays a role of phosphoric group donor. Pi is added to position 1 of fructose. This is the second ATP consumed in glycolysis..

4) Fr-1,6-bisphosphate is symmetric enough, hence it is splitted by aldolase to 2 trioses: glyceraldehyde-3-ph & DHAP. The enzyme is fully called Fr-6-ph aldolase. There is also liver aldolase present only in the liver & capable to split Fr-1-Ph.

5) Since 95% of trioses formed in reaction, are in DHAP form, but in next step only Gl.aldehyde-3-ph used, isomerase converts DHAP to Glyceraldehyde-3-ph (Glyceraldehyde is doubled). By this reason, after this step, all products are doubled.

5th step detailed. To be correct, enzyme catalyzing interconversion of triose phosphates, is called triose-phosphate isomerase Reaction is reversible. It means that if needed, pathway may run in opposite direction, upwards.

6) 2 molecules of glyceraldehydes-3-ph obtained in previous step, undergo oxidation. This reaction comprises 2 steps. The enzyme that catalyzes the 6th reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates glyceraldehyde 3-phosphate, and adds an inorganic phosphate to its product, producing 1,3-bisphosphoglycerate.

Resultant reaction: In this step, two main events take place: 1) glyceraldehyde (aldehyde) is oxidized to glycerate (an acid) by the coenzyme nicotinamide adenine dinucleotide (NAD); 2) the product molecule is phosphorylated by the addition of a free phosphate group to position 1 forming !.3-bis derivative from 3-phosphate derivative.

7) Substrate level phosphorylation. ***Phosphoglycerate kinase*** (name “kinase” suggests participation of ATP) transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and **3-phosphoglycerate.** In this step, 1,3 bisphoglycerate is rich in energy enough to synthesize ATP directly without other participants. The phosphoric group is transferred to ADP and appears in structure of ATP. Such kind of ATP formation is called Substrate level phosphorylation. This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP. Since there were two 3-carbon products from stage 1 of glycolysis, we actually have two molecules of 1,3 bisphoglycerate , &

hence, synthesize two molecules of ATP at this step. With this synthesis of 2ATPs, we have cancelled the first two molecules of ATP that we used.

8) The enzyme ***Phosphoglycero mutase*** relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form **2-phosphoglycerate.** The enzyme *mutase* is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another.

9) Reaction of dehydration 2-ph. glycerate is shown in the picture.The enzyme ***Enolase*** removes a molecule of water from 2-phosphoglycerate (or *dehydrating* the 2 phosphoglycerate) to form phosphoenolpyruvic acid (PEP).

PEP posess high energy bond of phosphoric group, which is further transmitted to ATP.

10) Last step of glycolysis. The final step of glycolysis is substrate level phosphorylation: the enzyme ***Pyruvate kinase*** transfers a Pi from phosphoenolpyruvate (PEP) to ADP to form **ATP. End product of glycolysis is pyruvic acid**

As the enzyme's name **pyruvate kinase** suggests, this reaction involves the transfer of a phosphate group. The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP. Again, since there are two molecules of PEP, here we actually generate **2 ATP** molecules.

Balance sheet.

Glucose + + 2ADP + 2Pi + $2NAD^+ + 2H^+$ ⟶ 2 pyruvate + 2ATP + 2 $NADH_2$

In glycolysis, for oxidation of glucose 2ATPs are spent, 4 gained: tottaly 2 remain. & 2NADH2 are produced. NADPH2 comprises reducing equivalents in the form of 2 active H+, which under aerobic condition sent to ETC.

In the general scheme of glycolysis. bifurcation & formation of 2 pyruvates along with 2NADH2, as well as consumption of 2ATP with formation 4ATPs is visible.

Immediately upon finishing glycolysis, the cell must continue respiration in either an aerobic or anaerobic direction; this choice is made based on the circumstances of the particular cell. A cell that can perform aerobic respiration, and which finds itself in the presence of oxygen will continue on to the aerobic way. If a cell able to perform aerobic respiration is in a situation where there is no oxygen (such as muscles under extreme exertion).

In anaerobic condition pyruvate forms lactate, ergo **2 $NADH_2$** produced in previous step, are re-oxidized & 2 NAD+ again participate in glycolysis. 2H+ are transmitted to pyruvate with formation lactate. Enzyme catalyzing reaction is NAD requiring LDH.

END PRODUCT OF ANAEROBIC GLYCOSE breakdown: LACTATE. Net equation: Totally 2 ATPs ARE GAINED

The table shows reactions with consumption & yielding of ATP.

Steps 1 and 3 = – 2ATP
Steps 7 and 10 = + 4 ATP
Eventually: Net "visible" ATP produced = 2.

Mature RBCs lack mitochondria & hence use anaerobic breakdown of glucose only.Cardiac muscle in ishemia, sceletal muscle during hard work also use this way foe energy production resuling in cumulation of lactate.
In B1 deficiency, Hypoxia (O2 deficiency), liver issufficiency & some other circumstances intermediate metabolism (metabolism inside of cell) is failed &

anaerobic breakdown of glusose is prevails instead of aerobic resulting in lactate raise in the cell & blood with decrease of pH. Since the energy is meager during anaerobic decomposition of glucose, overrun, overconsumption of sugar occur in the body.
Lactate in blood in norm is less than 15 mg/dL.
In strenuous exercise, respiratory disease, cancer mild lactacidemia occurs.
Severe forms occur in severe circulatory impaiment.
3 irreversible reactions regulate rate of glycolysis.
*Hexokinase is inhibited by Gl-6-ph (produxt).
*Ph. Fr.kinase is rate limiting enzyme. Raise in ATP decline this enzyme activity, as well as citrate, which raised in fat synthesis indicationg high energy availability.
Low energy indicators, such as ADP, AMP increase Ph.Fr.kinase activity.
Fr-2,6 bisphosphate facilitates flux glucose through glucolysis versus Gl.neogenesis.
* Pyruvate kinase is inhibited by ATP, & acetyl-CjA, fatty acids as well, because these compounds serve as alternative fuels for TCA cycle.
Fr-1,6-bisphosphate, also known as Harden-Young ester, is activator of this enzyme
The most common pathway for glucose breakdown is its aerobic decomposition, when pyruvate produced from glucose enters immediately I & II general pathways. O2 availability is required.
Relatively recently the effect of glucose less consumption & inhibition of glycolysis in presence of O2 was found. Ph. Fr.kinase inhibition & flux of glucose to aerobic process was discovered by Pasteur. Ths is because cell may produce much more energy currency in aerobic pathway than in anaerobic.
Pyruvate formed in cytoplasm, enters mitochondrion for further degradation. In this step, NADH2 is produced. The product of reaction is acetyl-CoA.

I GENERAL PATHWAY (Pyruvate DH complex).

In Pyruvate DH complex, 3 enzymes are merged together. Pyruvate DH is also termed pyruvate decarboxylase. Pyruvate, once entering this complex, is immediately

converted to acetyl CoA. No intermediate, but the last product is released into medium of cell.

5 coenzymes of Pyruvate DH complex are:TPP, Lipoate & FAD are merged with appropriate enzymes, while CoA & NAD stayfreely in the enviroment of matrix.

As all catabolic pathway enzymes, PDH complex is inhibited by its product, acetyl CoA, asa well as by phosphorylation: Phosphorylation leads to a strong decrease in PDH activity.

II general pathway utilizes nearly 2/3 of total O2 consume by organism. In CAC acetyl-CoA is completely combusted to CO2 & water.

II GENERAL PATHWAY (KREBS CYCLE, TCA – TRİCARBOXYLİC ACİD CYCLE)

As I genersl pathway, II genersl pathway also occurs in mitochondria, in close proximity to ETC generating NADH2 & FADH2 to ETC.By this way, nearly 70% of cell ATP is produced.At the outset of cycle, tricarboxylic acids participate, which is why cycle is called TCA.

First energy extraction reactions are performed with copounds which are mainly reduced. These substrates are: Citrate – alpha-KG. Latter reactants are mainly oxidized: Succinyl Co A – OA..

Acetyl CoA entry to cycle launches the cascade of reactions catalysed by enzymes: citrate synthase, then series of other enzymes, and finally -malate DH.

Liberation of energy ends with transfer of reducing equivalents to 3NADs with formation 3NADH2 & 1FAD (FADH2 is formed). 1GTP is formed from GDP in substrate level phosphorylation when high energy of thioether bond in succinyl CoA is used for convertion GDP to GTP.

In 1 cycle of TCA totally 10 ATPs are produced:

Thanks to 3NADH2, 7,5 ATPs are produced in ETC.

FADH2 produces 1.5 ATPs in ETC. Plus 1GTP equal to 1ATP, produced in substrate level phosphorylation. Totally 10 ATPs.

1 NADH2 is produced in I general pathway (2,5 ATPs). Summarizing all ATPs produced in I & II general pathways, we obtain 12.5 ATPs produced in both general pathways. Pyruvate loosing all 3 carbons is burned in I & II pathways yielding 3 CO2. ATP & NADH2 inhibit action of main regulatory enzymes of I & II general pathways: PDH, Isocitrate DH, alpa –KG DH.Additionally, PDH is inhibited by its product, actyl-CoA, & alpa –KG DH – by product succinyl CoA. PDH is activated also by pyruvate, a reactant of reaction. ADP & NAD+, the signals of low energy state, activate all key enzymes of general pathways.

Citrate synthase, an irreversible reaction of TCA, is additionally inhibited by citrate (end product of reaction) & succinyl – CoA.

Fumarase deficiency results in mitochondrial encefalopathy, in which brain along with sceletal muscles are affected.

During aerobic glucose breakdown first, glucose is degraded to 2 pyruvates yielding 2 ATPs. 2NADH2 are formed in this step. Then 2 pyruvates enter subsequently I & II general pathways, the reducing equivalents are passed from NADH2& FADH2 to ETC proximately closed with these enzymes. Mentioned process ends by yielding 12.5 ATPs by each pyruvate. Ergo, 2pyruvates give 25 ATPs. CO2 & Water, the waste products of pyruvate combuston are released from mitochondria to the cytoplsm, & further with lungs and kidneys.

Additional to 25 ATPs formed in I & II general pathways mentioned above, 2 ATPs produced in anaerobic step of glucose breakdown, are summarized with 5ATPs produsedby $2NADH_2$.

Totally 25+2+5= 32 ATPs. Sometimes 30ATPs are produced, when cytosolic NADH2 passes H+ not to NAD dependent malate-aspartate suttle system, but to FAD-dependent glycerol phosphate system. As each FAD produsedby 1,5 ATPs, hence 2FAD give 3 ATPs instead of 5, that’s why the end result’ll be 30ATPs. This system is active, e.g. in the brain.