Biochemistry 673 Your Name: Key

Regulation of Metabolism Assoc. Prof. Jason Kahn

Final Exam (150 points total) December 19, 2007

You have 120 minutes for this exam.

Explanations should be concise and clear.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the of the exam time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. Energy Metabolism and Diabetes (40 pts):

(a; 24 pts) Give the primary function that we discussed for each of the following pathways. Indicate whether it consumes or produces NADH and ATP, and the primary carbon inputs and outputs.

Example: Pentose phosphate pathway: Oxidizes glucose (via glucose-6-phosphate) to provide ribose and reducing equivalents for biosynthesis, especially of fat. Produces NADPH, no direct effect on ATP. Input is G6P, output is CO_2 and ribose.

Glycolysis under anaerobic conditions:

- Converts glucose (G6P) to lactate, first step in glucose catabolism — under aerobic conditions would → Acetyl CoA. (+1 for pyruvate or acetyl CoA) +2 pathway +2 +2 eukaryotes
- Produces net 2 ATP, rapidly and without requiring O_2. +1
- No net effect on NADH if pyruvate is reduced to lactate. — it's a fermentation +1

8

TCA cycle:

- Complete oxidation of carbon from glucose or fat, metabolic hub. +2
- Input is Acetyl-CoA [other correct answers OK], output CO_2 +1
- Passes electrons to ox-phos in the form of NADH, $FADH_2$ — +1
- Produces ATP and GTP, NADH +1 +1 needed for aerobic metabolism - efficient

8

Gluconeogenesis:

- Converts pyruvate (or oxaloacetate OK) to glucose to replenish blood glucose +1 +2 during hunger or from muscle lactate. +1
- Consumes ATP +2 — it's the reverse of a downhill pathway
- Consumes NADH +1 starting from pyruvate or OAA, neutral from lactate

8

Score for page: ____

(b; 6 pts) List three ways in which insulin causes blood glucose to decrease.

- Stimulates glycogen synthesis to store glucose
- Stimulates anabolic / proliferative processes like protein synthesis, cell growth + division – consumes energy.
- Stimulates transport of GLUT4 to ~~muscle~~ muscle cell membrane to take up glucose into muscle cells.
- inhibit glycogenolysis, gluconeogenesis

+2 each

(c; 6 pts) In terms of pathways (not purposes) briefly explain why protein can be converted to sugar but fat cannot. Why do starving mammals avoid digesting too much protein to make sugar, even though it would seem metabolically simpler than the alternatives?

- Carbons from fat enter the TCA cycle at acetyl-CoA (2-carbon unit). Both carbons are ejected as CO_2 so there is no net synthesis of OAA, the precursor to gluconeogenesis. +2
- Carbons from most amino acids are converted to pyruvate or α-KG etc. so they enter TCA after decarboxylation or just go to OAA → we get net synthesis of OAA. +2
- Digesting all the protein makes it harder to catch food or escape predation – and more energy is stored compactly in fat. +2

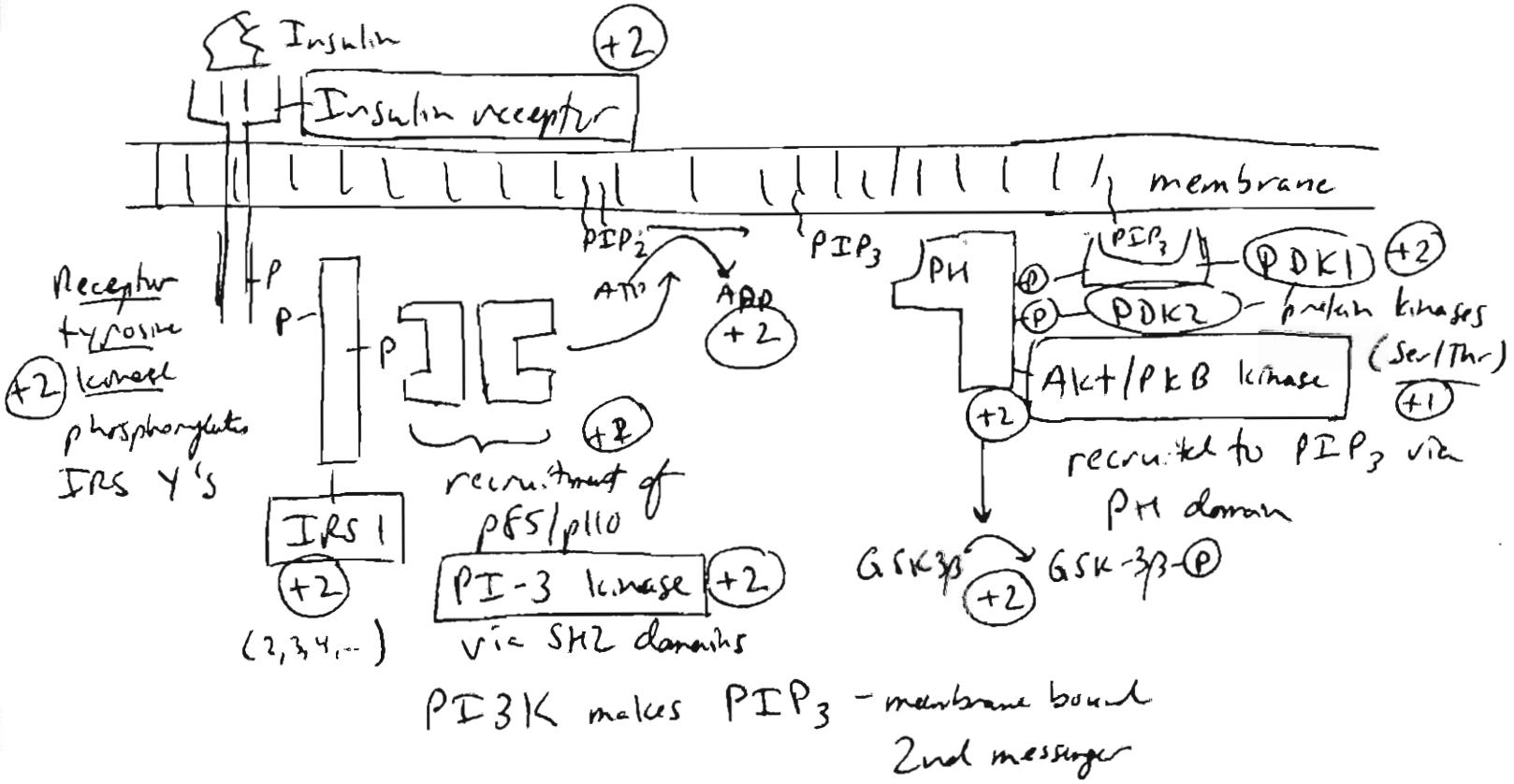
(d; 4 pts) Why is the production of ketone bodies elevated in starving people and some diabetics?

+1 Ketone bodies are essentially soluble forms of acetyl CoA. They are produced when a) there is no way to make blood +2 glucose or b) the body thinks there isn't any blood +1 glucose.

Score for page: ____

2. Insulin Signal transduction (50 pts):

(a; 20 pts) Sketch the pathway leading from insulin binding to the phosphorylation of glycogen synthase kinase 3β (GSK3β). Identify the catalytic activity of each protein that has one.



- Akt/PKB is phosphorylated by PDK1 and PDK2 to release it into the cytoplasm (+1)
- It phosphorylates GSK 3β to inactivate it, and ∴ allow activation of glycogen synthase by dephosphorylation.

Score for page: ____

(b; 8 pts) We have emphasized that turning signals off is just as important as turning them on. For each of the following signal transduction proteins, identify how the activity of the protein itself is down-regulated and also how its downstream signal is turned off.

Example: Protein kinase A (PKA): Decrease in [cAMP] leads to reassociation of inhibitory R subunit. Phosphorylation of target proteins like phosphorylase kinase is reversed by protein phosphatase I.

(i) PI3K:

- protein tyrosine phosphatase (+2) removes (P) from IRS-Y residues — PI3K is no longer recruited, regulatory subunit inhibits catalytic subunit. [or: Ser/Thr (P) of IRS → ↓ of response]
- PIP_3 2nd messenger is dephosphorylated by PTEN and SHIP2 to regenerate less active PIP_2 (+2)

(ii) GPCRs that signal to Adenylyl Cyclase:

- GPCR's stop signalling if agonist leaves, and they are phosphorylated and endocytosed (+2)
  - by e.g. βARK
- cAMP is hydrolyzed to AMP by a phosphodiesterase (+2)

or - G protein converts GTP → GDP and shuts off, G_α stops stimulating AC

(c; 8 pts) How can the elevated free fatty acids and/or continual insulin signalling found in some obese people eventually lead to insulin resistance and Type II diabetes?

[If you know the whole answer, let the rest of us know, but in...]

+2 for either

- elevated FFA → inflammatory response, in part from ER stress due to ↑ in protein synthesis possibly.

- This leads to activation of the JNK kinase, which

+2 phosphorylates IRS proteins on Ser/Thr residues.

- This targets IRS proteins for ubiquitination/proteolysis, which shuts down insulin response and can lead to ↑ β-cell apoptosis in the pancreas. (+2) (+2)

Score for page: ____

(d; 8 pts) We read a paper on genome-wide association studies used to identify genes linked to Type II diabetes. In general, how are such screens done? Perhaps surprisingly, none of the proteins we covered up to this point of this exam was identified in the screens. Why not? What might explain the existence in the population of some alleles that predispose their owners to diabetes?

- They compared genotypes using SNP analysis of diabetics vs.
+3 matched control patients, and looked for haplotypes that correlated with increased risk for Type II diabetes. Then they looked for genes ~~linked to~~ near the SNP's.
- Mutant alleles in vital proteins like PI3K are strongly
+3 selected against - they aren't in the population at high frequency! We see alleles with less deleterious effects.
- There may be "thrifty genes" that lead to more efficient
+2 energy storage in lean times (for example FTO).

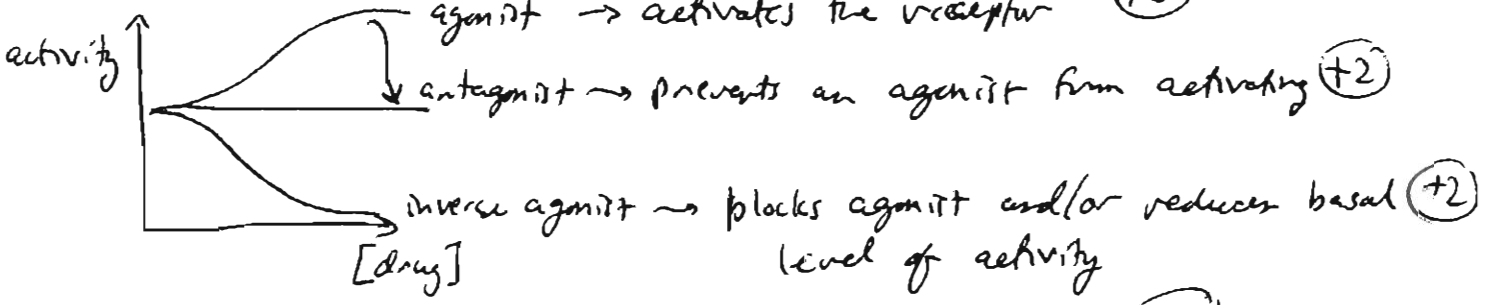
(e; 6 pts) Very briefly discuss an ethical issue surrounding the substantial research investment in Type II diabetes on the part of the pharmaceutical industry.

- Many possible answers - +6 for anything thoughtful
  - We are looking for drugs when lifestyle change would be more effective and cheaper.
  - Resources are diverted from AIDS, malaria, etc.
  - But many people have genetic predispositions, and eating well is expensive
  - Do we choose not to ~~help~~ help someone with a treatable disease??
  - Propriety of seeking to treat long-term incurable disease of relatively wealthy people?

Score for page: ____

3. G-Proteins and G-protein Coupled Receptors (25 pts):

(a; 10 pts) Define GPCR agonists, inverse agonists, and antagonists. Why is it often difficult to tell the difference experimentally between an inverse agonist and an antagonist?



(+2) (+2) (+2)

- We often don't know what the agonists are in the body, so if a drug to activity we don't know whether it blocked intrinsic activity or blocked access of an unknown agonist. (+2)

(b; 15 pts) Give three reasons that GPCR structures have been so difficult to obtain. What experimental trick enabled the recent structures, and why did the advance, ironically, make the resulting structures less interesting?

+2 – GPCR's are hard to express and purify in large quantities.

+2 – They are membrane proteins, ∴ hard to crystallize

+3 – They have floppy intra + extracellular loops.

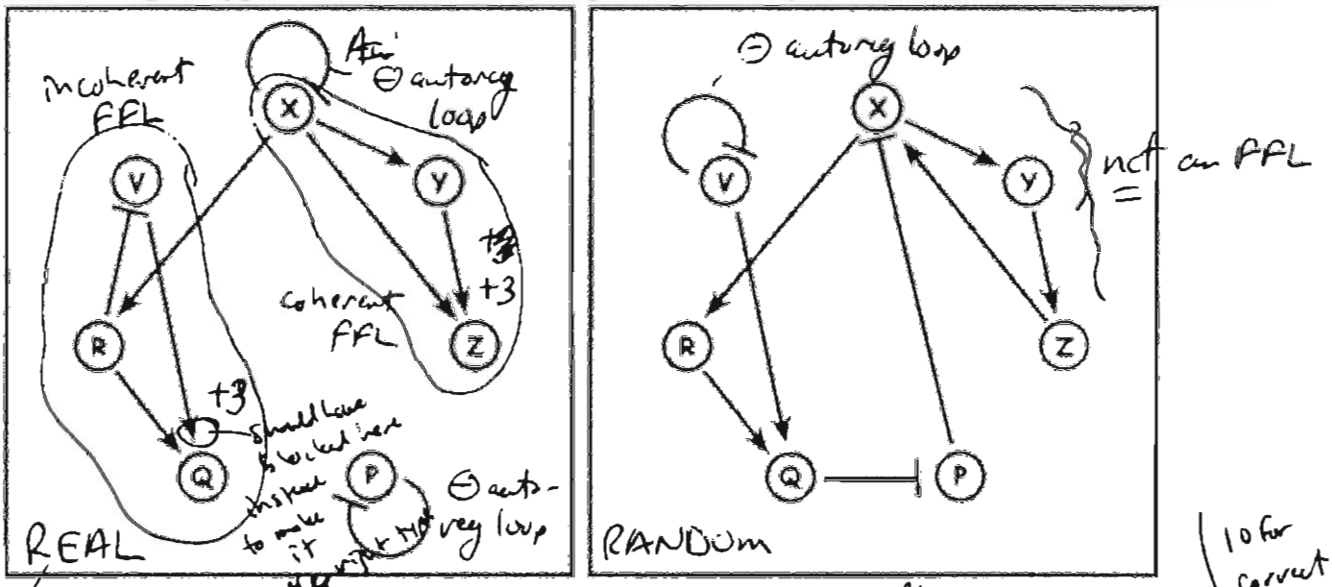
+4 – Recent structures ~~either~~ used GPCR's that had floppy loops either engineered out (replaced by T4 lysozyme) or bound to an antibody to ↓ flexibility.

+4 – This means that the business end of the molecule, which interacts with the G proteins, is absent or blocked / rearranged – still don't know how the signal is sent. (There is also a strong inverse agonist present)

Score for page: ____

4. Systems Biology (35 pts):

(a; 12 pts) The sketch below shows one transcriptional regulation network that might be a real one and one randomized network with the same number and type of nodes and edges. Which is which? Identify a negative autoregulatory loop, a coherent feed-forward loop, and an incoherent feed-forward loop in the diagrams. How are randomized networks like the one below useful in determining the types of networks that are important in real systems?



+2 +3 for any ⊖ autoreg loop.

not an FFL =

+2 +3 [(+1 if motifs are identified)

10 for correct

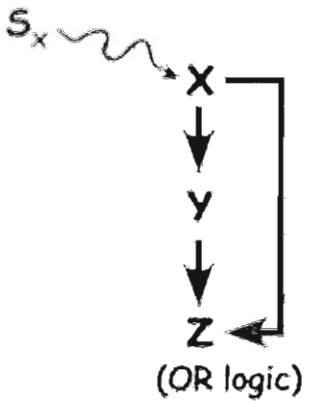
→ motifs common to real networks are seen more often

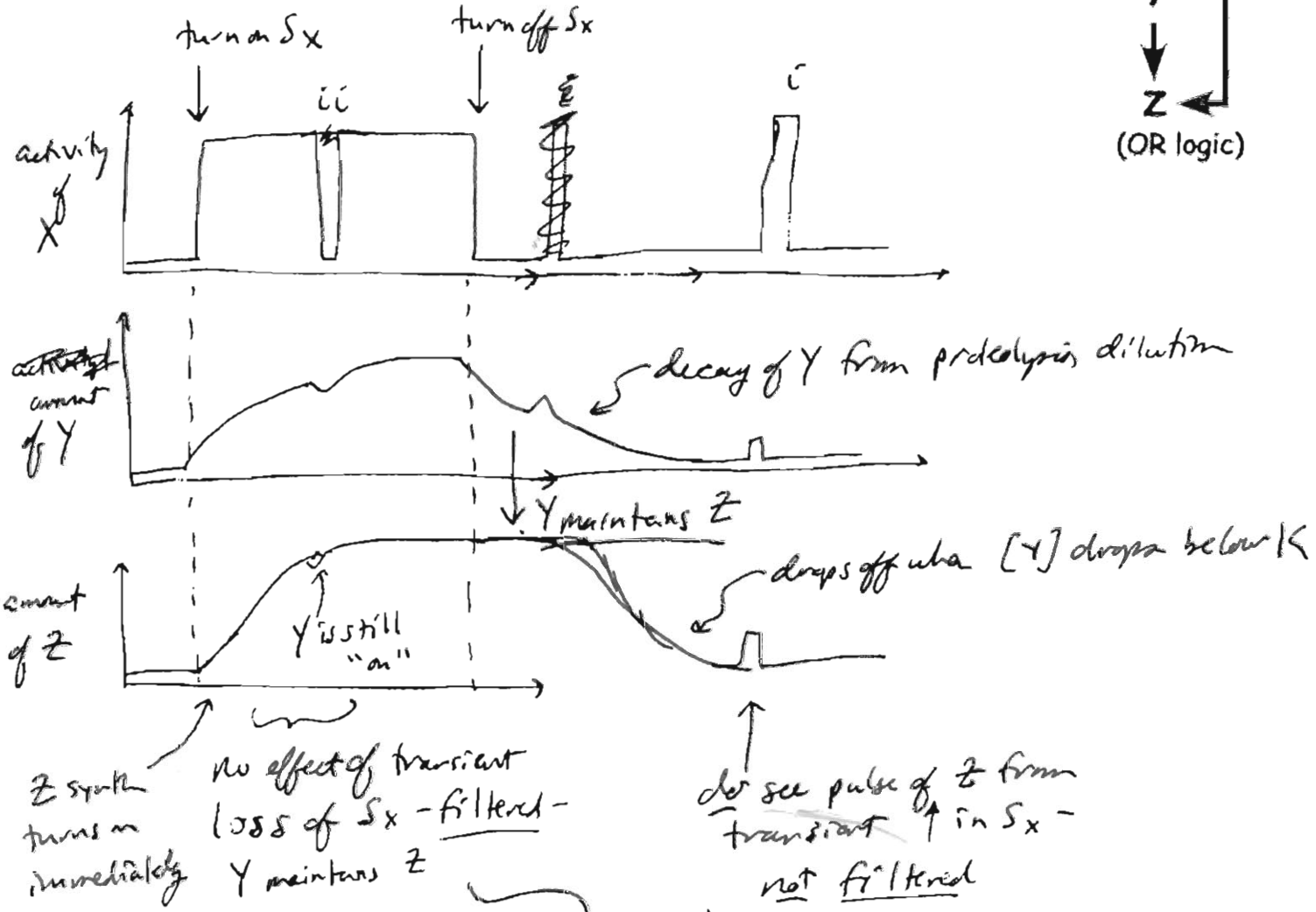
– We look at the patterns seen in real networks and ask which motifs show up more often than would be expected statistically, i.e. show up in random networks. This can be done either analytically or by simulation.

+2

Score for page: ____/____

(b; 15 pts) We have discussed the idea that a coherent feedback loop with "OR" logic at the promoter serves as a circuit with a rapid "ON" but a delayed "OFF" response to a decrease in signal S_x. Explain how this works. It also acts as a filter that rejects (i.e. prevents response to) either (i) transient increases or (ii) transient decreases in S_x activity. Which is it (i or ii), and why?





+2 for idea of time courses, +2 for relevance of protein decay/dilution

+2 for explaining fast ON of Z

+3 for slow OFF of Z

+3 for answer (ii)

+3 for explanation

Score for page: ____

(c; 8 pts) Give an everyday example of a bistable switch. What is necessary for its state to change? Why might a system that is poised to go back and forth quickly often be undesirable in biology?

+2 – A light switch

+2 – A finger (i.e. large external perturbation) +1

– Going back and forth could delay (denial) tasks that are expensive + should be completed once started, like flagellar synthesis. OR lead to futile cycles like switching back and forth between glycolysis + gluconeogenesis. +3 either

Page	Score
1	____/24
2	____/16
3	____/20
4	____/16
5	____/14
6	____/25
7	____/12
8	____/15
9	____/8
Total	____/150

Score for page: ____