Practice Assignment

Tutorial 7

Tips: Some brief background

What is glucose-6-phosphatase (G6Pase)?

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Glycogenolysis

- Glucose-6-phosphate –(G6Pase)–> glucose

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Glycogenolysis

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What is para-Nitrophenyl phosphate (PNPase)?

- In the study, PNPase is a control that does not get influenced by refeeding

Tips: Eugylcemic hyperinsulinemic clamp

Eu = constant, hyper = increased

1. Infuse a set amount of insulin into vein
2. Infuse glucose into vein and adjust glucose infusion to maintain blood glucose at normal levels (approx. 5mM)
3. Measure how much glucose had to be infused to keep blood glucose normal

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What are clamps trying to measure/determine?

Assess insulin resistance...less glucose infused –> more insulin resistant (cellular uptake less) at any given insulin level.

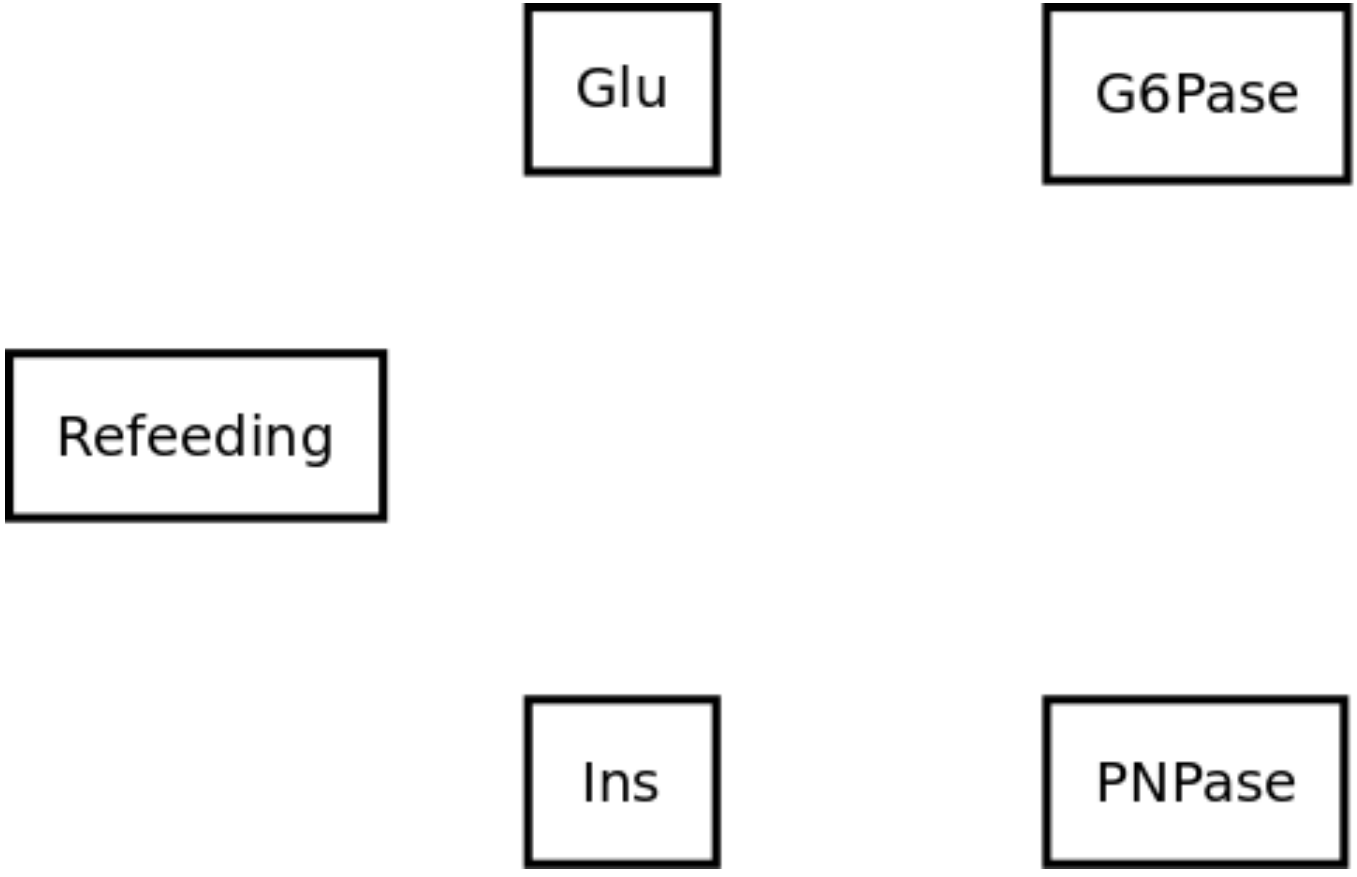
Study 1

- The objective of this study was to determine the effect of refeeding food-deprived rats on hepatic glucose-6-phosphatase (G6Pase) activity.
- Rats were food-deprived for 48hrs and then given free access to food for 0, 90, 180 or 360 minutes.
- At each time point, plasma was obtained for glucose and insulin measurements and liver samples removed for enzyme determination. In addition to G6Pase, paprnitrophenyl-phosphatase (PNPase) activity was monitored as a control for the G6Pase measurement.

Study 1, con't

- To determine whether changes in glucose or insulin were mediating the effects of refeeding on hepatic G6Pase activity, a euglycemic, hyperinsulinemic clamp experiment was performed.
- Food-deprived rats were fitted with catheters in the carotid artery and jugular vein for blood sampling (artery) and glucose and insulin infusions (vein).
- Rats were perfused with predetermined doses of insulin and then variable doses of glucose were administered in order to maintain normal blood glucose levels.
- Fifty minutes after commencement of the infusion, liver samples were removed for G6Pase determination.

Concept map



Question 1a

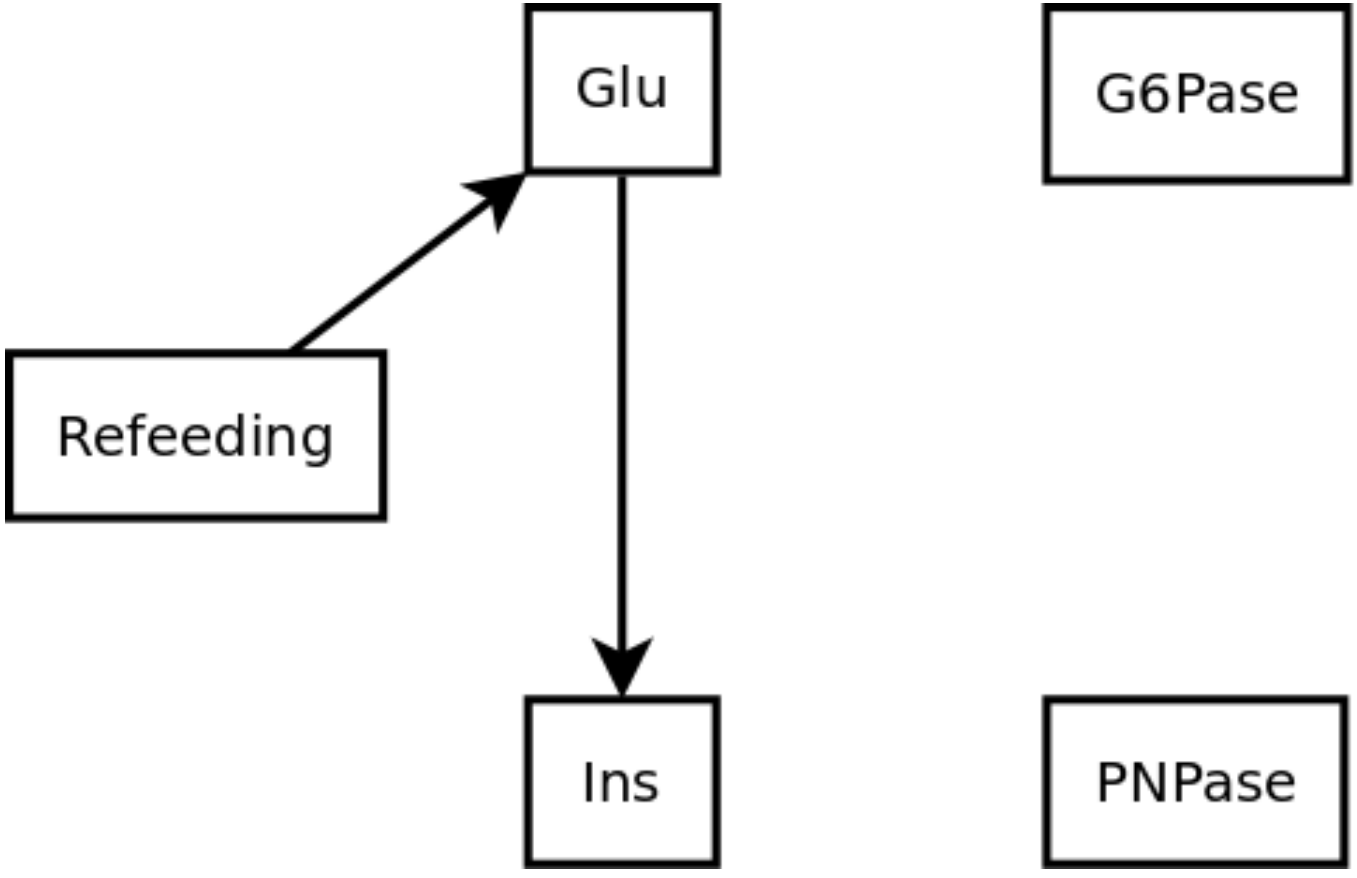
Describe the differences in blood glucose and insulin associated with refeeding the food-deprived rats (Table 1) (2).

Table 1:Changes in plasma glucose and insulin levels in previously unfed rats in the course of refeeding

Time	Glu (mmol/L)	Ins (pmol/L)
0	6.3+/-0.6	83+/-14
90	11.1+/-0.7*	366+/-54
180	10.1+/-1.1*	331+/-56*
360	9.2+/-0.8*	345+/-41*

Note: Values are means +/- SEM. * $p<0.05$ vs time zero (0).

Concept map



Question 1b

Describe the effects of refeeding on G6Pase activity (Figure 1) (1).

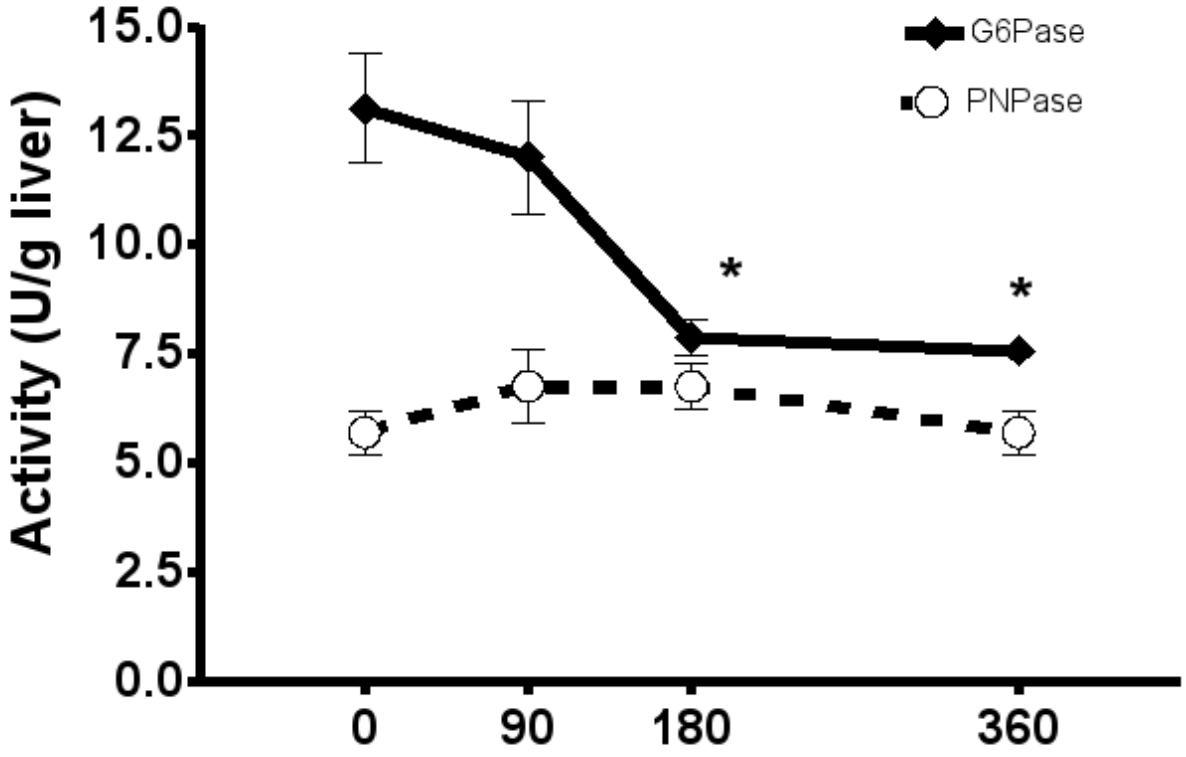
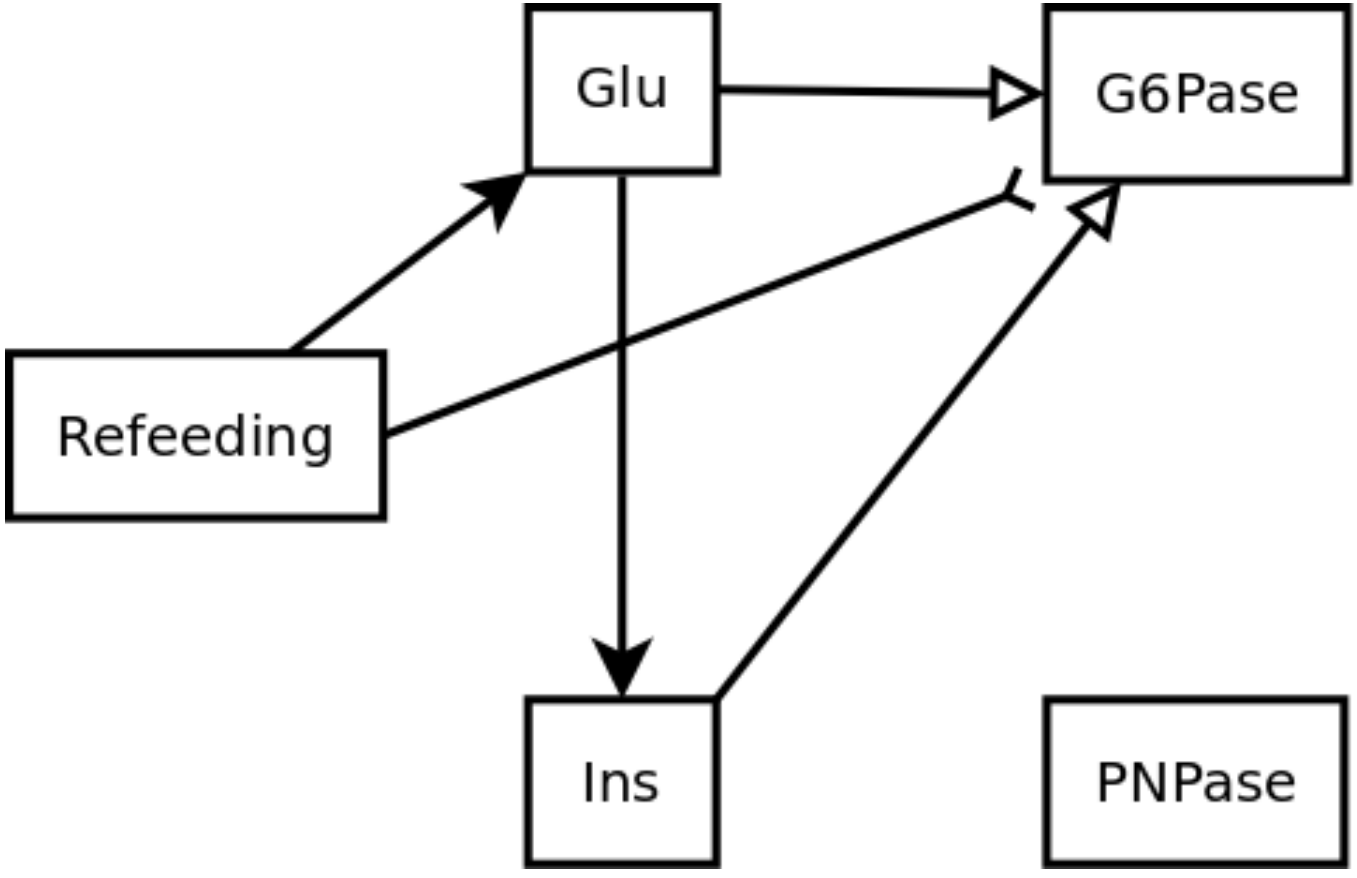


Figure 1. Time course of glucose-6-phosphatase activity during refeeding of previously unfed rats. Values are means±SEM, n=6/group. *Significantly different from unfed value ($p<0.01$).

Concept map



Question 2

Using the data provided, discuss the relative contributions of hepatic and dietary carbohydrate to plasma glucose levels observed over the time course monitored (7).

Possible answer:

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Possible answer:

- G-6-Pase activity decreases significantly by 180 & 360 minutes, while blood glucose & insulin levels continue to rise during this time. We would expect this since a supply of glucose from the food reduces the amount that the body needs to supply endogenously from the liver

Question 2, con't

- Illustrates the shift from the metabolic state seen in the post-absorptive or fasted state, to the metabolic state of a fed animal

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- Illustrates the shift from the metabolic state seen in the post-absorptive or fasted state, to the metabolic state of a fed animal

- PNPase is just a housekeeping enzyme.

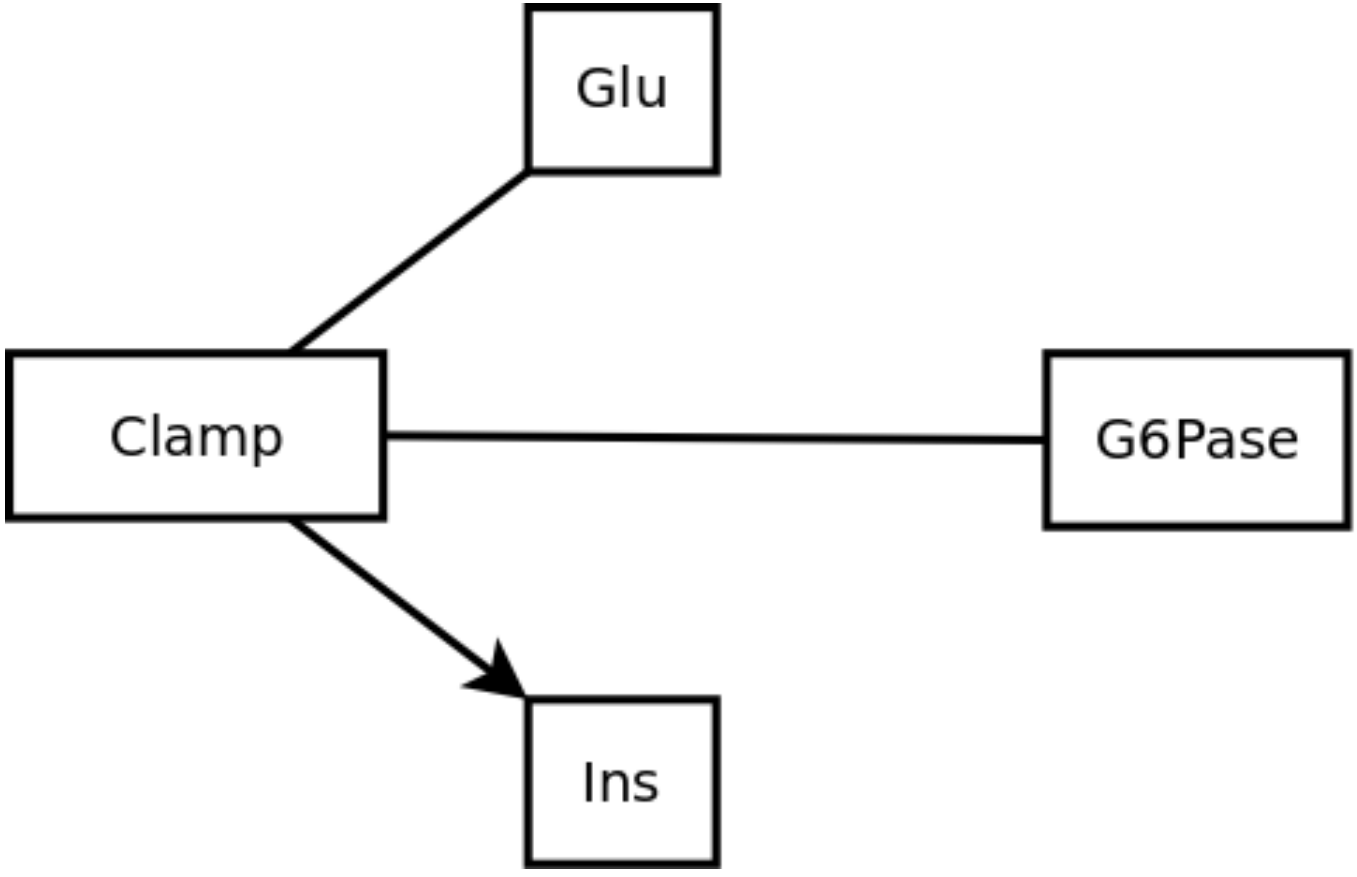
Question 3

Describe the major outcomes of the euglycemic clamp study (Table 2) (2).

Table 2:Effect of insulin infusion on glucose-6-phosphatase activity in unfed rats with euglycemic clamps. (Note: Glu/Ins in = glucose/insulin perfused into the blood).

Ins in	Glu in	Glu start	Glu end	Ins	G6Pase
Saline	———	6.4 ± 0.2	6.8 ± 0.2	97 ± 7	12.0 ± 0.8
240	11 ± 1	6.4 ± 0.1	7.1 ± 0.2	221 ± 8	10 ± 0.4
480	18 ± 2	6.7 ± 0.5	6.8 ± 0.2	324 ± 29	11.3 ± 0.7
960	35 ± 1	6.0 ± 0.4	6.9 ± 0.5	704 ± 94	12.1 ± 1.2
2880	50 ± 2	5.9 ± 0.2	6.0 ± 0.2	>1400	14.6 ± 0.8
28800	49 ± 2	5.9 ± 0.4	6.5 ± 0.8	>1400	13.6 ± 0.3

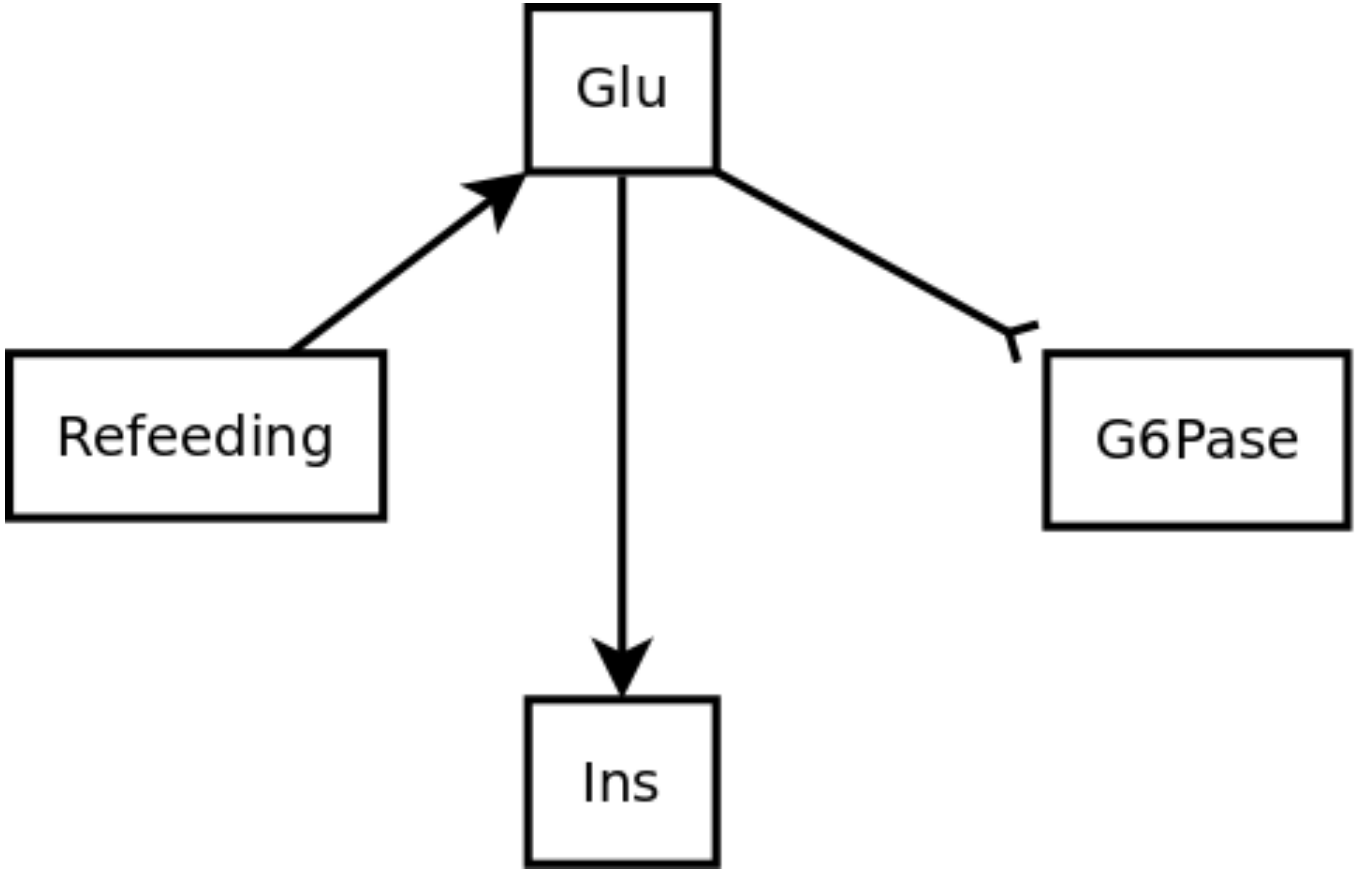
Concept map — Glu or Ins on G6Pase?



Question 4

Discuss whether G6Pase activity is predominantly regulated by dietary carbohydrate or hormonal factors (8). (Hint: Does insulin (hormone) or glucose (diet) change G6Pase?)

Concept map



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Possible answer:

- Feeding increased glucose & insulin (Table 1)
- After the increase in glucose and insulin, G6Pase activity drops (Figure 1)
- This drop in G6Pase is likely due to changes in glucose since changes in insulin have no effect on G6Pase activity

Tip: Brief background

What is phosphofructokinase-I (PFK-I)?

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What is phosphofructokinase-I (PFK-I)?

Glycolysis

- Fructose-6-Phosphate –(PFK-I)–>
  Fructose-1,6-bisphosphate –> ATP generation (glucose metabolism)

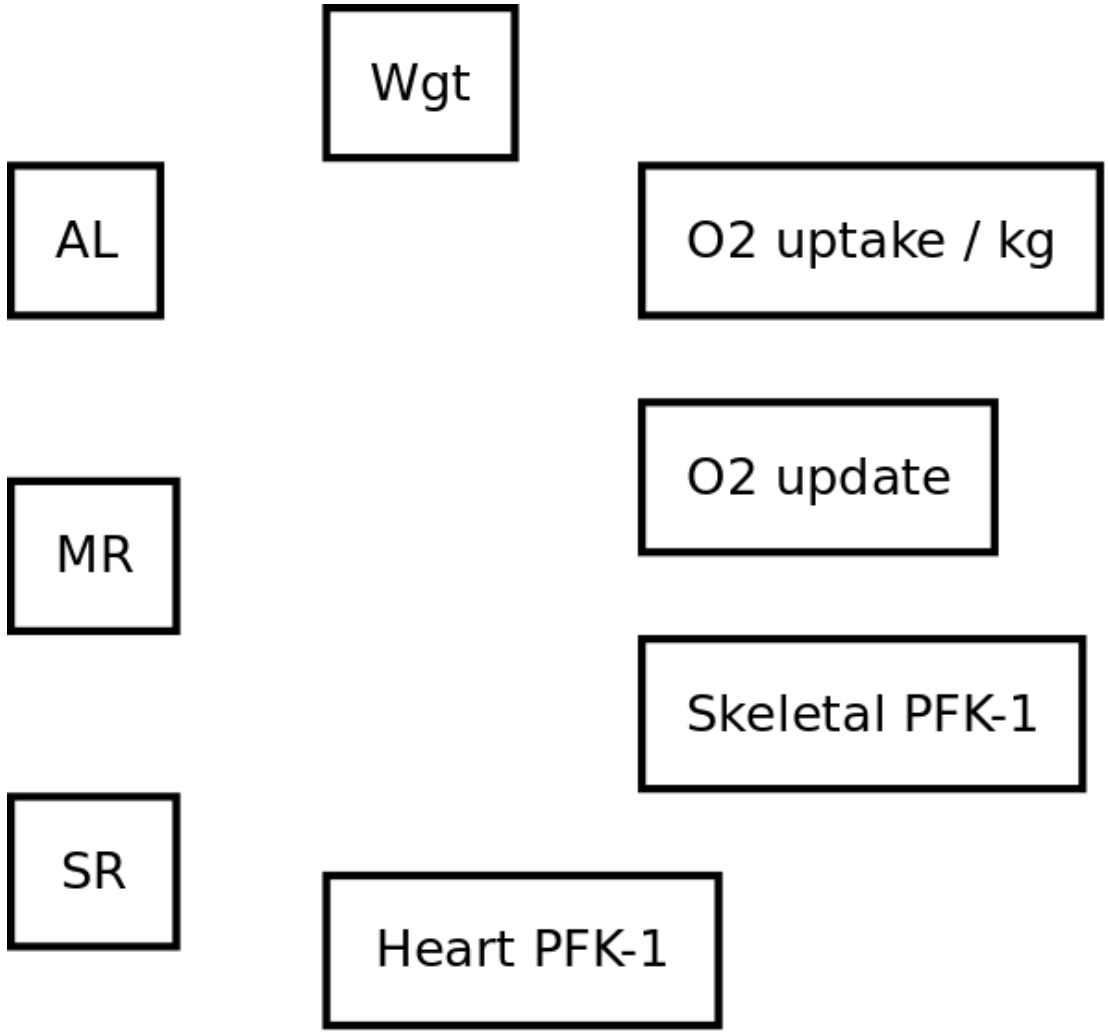
Study 2

- The purpose of this experiment was to determine the effects of dietary restriction and subsequent weight loss on phosphofructokinase-I (PFK-I) activity in selected skeletal and heart muscles.
- Fifty-five female rats were separated into 3 groups for 10 weeks of dietary restriction.
    1. AL = ad libitum fed (no restriction)
    2. MR = moderate restriction (weight reduced to 81% of AL)
    3. SR = severe restriction (weight reduced to 63% of AL).

Study 2, con't

- During the last week of the study, a measurement of the animal's resting oxygen uptake was taken in order to estimate total oxygen consumption associated with energy metabolism.
- The rats were then killed and the PFK-1 activity in the heart and various skeletal muscles was determined using standardized techniques.

Concept map



Question 1

Describe changes in body weight, and resting oxygen uptake (both in absolute and relative terms) for the AL, MR, and SR groups (Table 1, Figure 2)(3).

Table 3:Mean body weight of animals prior to and after the dietary restriction.

	Wgt (g) - AL	Wgt (g) - MR	Wgt (g) - SR
Pre-restriction	350+/-30	350+/-30	350+/-30
Post-restriction	395+/-60a	331+/-50b	253+/-38c

Mean +/- SEM. Means in the same row with different superscripts are significantly different ($p<0.05$).

Question 1, con't

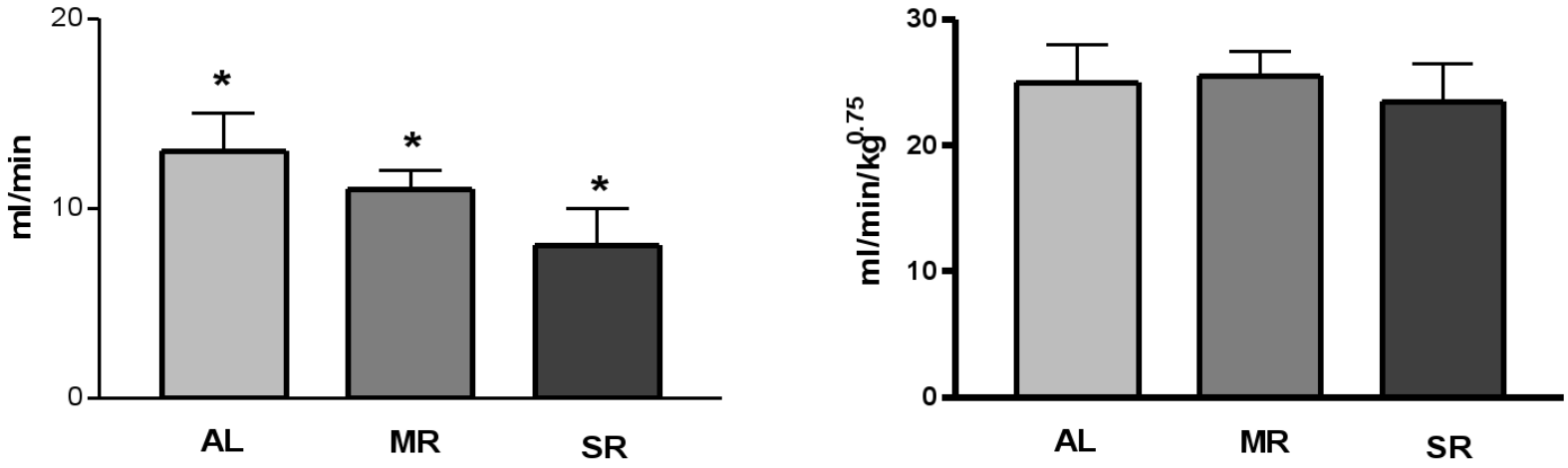
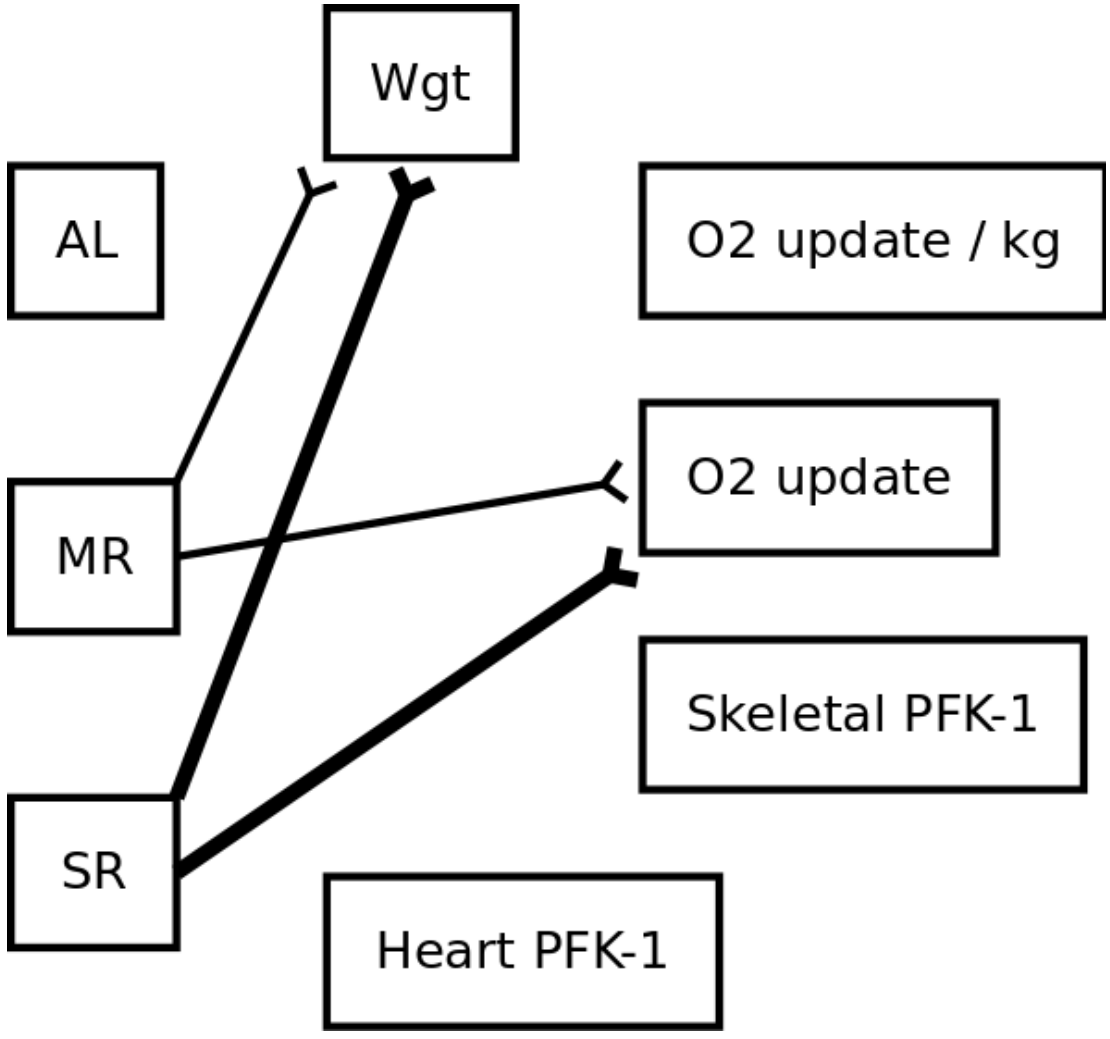


Figure 2. Resting oxygen uptake for 55 female Sprague-Dawley rats expressed in absolute (ml/min) and relative (ml/min/kg0.75) terms. * All groups are statistically different from each other, $p<0.05$.

Concept map



Question 1b

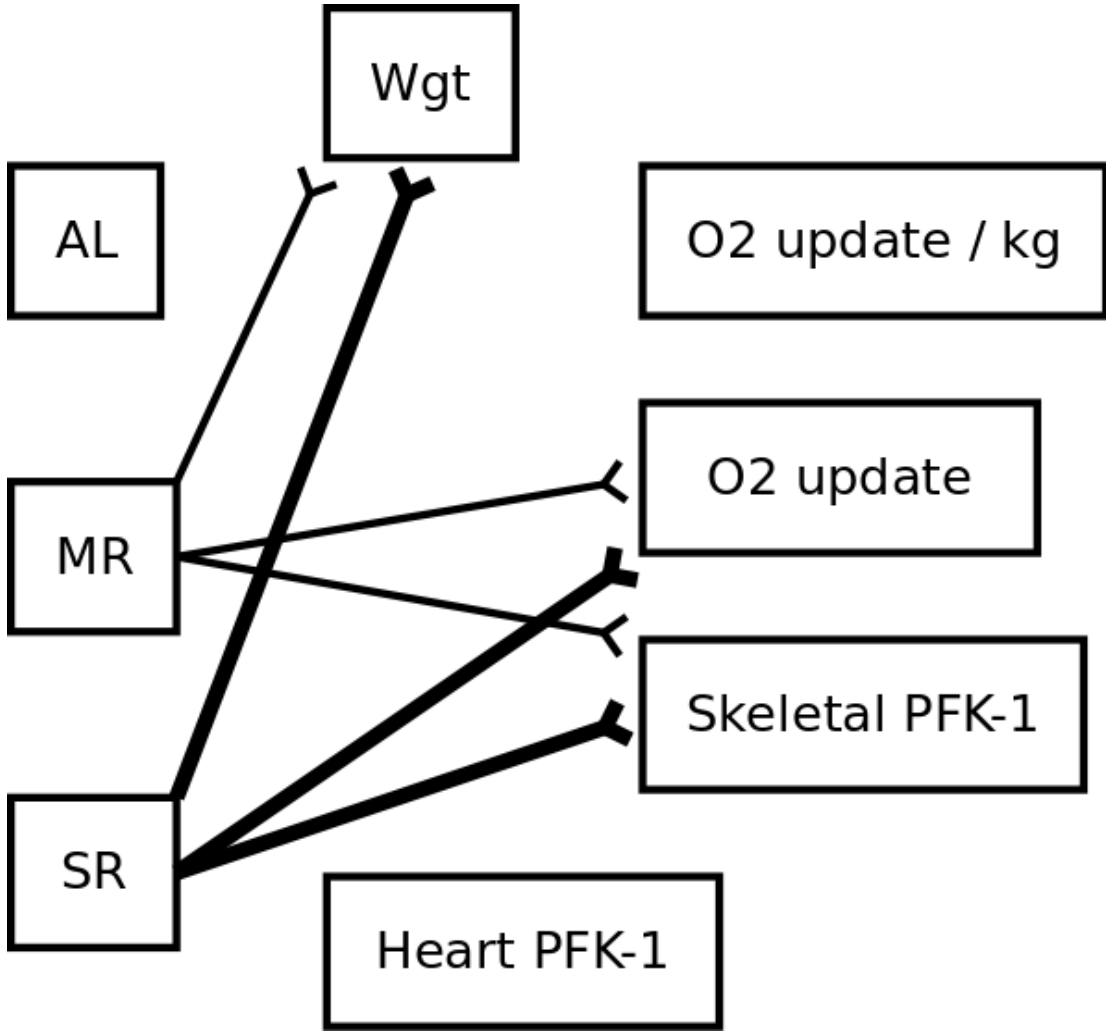
Describe changes in the activity of PFK-1 in heart and skeletal muscles caused by dietary restriction (Table 2)(2).

Table 4:Effect of dietary restriction on PFK-1 (umol/g/min) activity in skeletal and heart muscle.

	AL	MR	SR
# per group	16	20	19
Skeletal			
- Gastrocnemius	105+/-7[a]	86+/-6[b]	62+/-6[c]
- Plantaris	92+/-6[a]	86+/-5[a]	66+/-4[b]
- Soleus	25+/-2[a]	22+/-1[a]	16+/-1[b]
- Heart	64+/-3	68+/-3	66+/-3

Means +/- SEM. Means in the same row with different superscripts are significantly different, $p<0.05$

Concept map



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- Table 1: Caloric restriction causes a drop in body weight
- Figure 1: Calorically restricted rats have decreased metabolism (measured from resting O2 uptake) but this effect is totally accounted for by the loss in body weight (there is no significant difference between groups when data is normalized for body weight –> expressed in relative terms)
- Table 2: Skeletal muscle PFK-1 activity drops with caloric restriction (but not heart PFK-1)

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- Decreased energy intake leads to decreased PFK-1 activity and therefore decreased glucose use
- The animals are most likely mobilizing fat from adipose tissue for fuel and some protein

Question 3

What other enzyme/metabolites would you want to measure to verify the PFK-I enzyme effect, and why? (5)

Possible answers:

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Possible answers:

- Any enzymes/metabolites in glycolytic pathway
- Any enzymes/metabolites in beta-oxidation to confirm increased use of fat for fuel