Practice Assignment – Tutorial 2

2014-09-23

Things to remember before starting (tips)

- Read and understand the experimental design/methods carefully
- Read and understand the tables/figures carefully
- Often there are hints in the methods
- Everything included is intentional
- Try to understand the concept under the studies

Study 1

Six week old rats were fed standard chow for 7 days and then, following an overnight fast, were force fed either a low starch, long chain triglyceride diet (LCT) (70% fat as corn oil), a low starch, medium chain triglyceride diet (MCT) (70% fat as medium chain triglycerides), or a high carbohydrate diet (HC) (70% carbohydrate) for 12 hours. For the rats on the HC diet, carbohydrate was provided either as sucrose or α -methylglucoside, a non-metabolizable sugar. Following sacrifice, jejunal lactase mRNA levels were measured, and lactase activity.

Experimental Design

Study 1

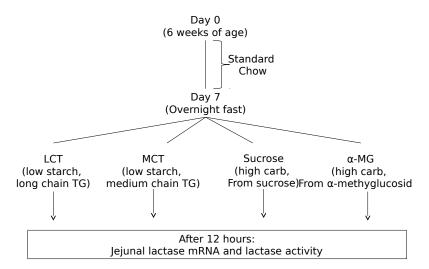
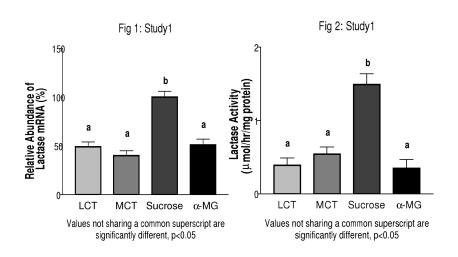


Figure 1 & 2: Study 1

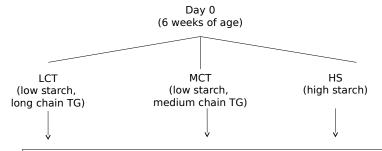


Study 2

Six week old rats were fed either the LCT diet, the MCT diet, or a high starch diet (HS) (70% of energy as starch) for 7 days. In order to estimate the absolute rate of synthesis of lactase, the rats were given an i.v. infusion of (³H)-phenylalanine 30 minutes prior to sacrifice. The jejunum was then removed and the incorporation of the (³H)-phenylalanine was measured by standard methods. Lactase activity and mRNA levels were also measured.

Experimental Design

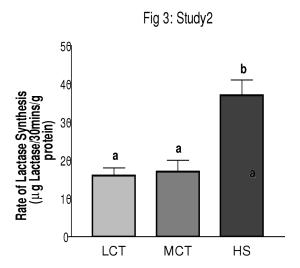
Study 2



After 7 days:

i.v. infusion of [H3]-phenylalanine 30 minutes prior to sacrifice Measurements: rate of lactase synthesis, lactase activity, lactase mRNA

Figure 3: Study 2



Values not sharing a common superscript are significantly different, p<0.01

Table 1: Study 2

Variable Measured	LCT	MCT	HS
Lactase Activity (µmol/hr/mg protein)	0.45 ± 0.04^{a}	0.64 ± 0.05^{b}	1.49 ± 0.08°
Relative abundance of lactase mRNA (%)	54 ± 3°	55 ± 2 ^a	100 ^b

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Or shorthand:

- Lactase mRNA (fig. 1) \rightarrow LCT = MCT = α -MG < Sucrose
- Lactase activity (fig. 2) \rightarrow LCT = MCT = α -MG \lt Sucrose

Question 1b

Q: Describe the effects of the diets in study 2 on lactase rate of synthesis, activity, and mRNA levels (3/20).

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E.g. A: Rats on a HS diet had signif. greater rate of lactase synthesis (Figure 3), had greater lactase activity and greater relative abundance of lactase mRNA (Table 1) vs the other groups. Both lipid groups had similar lactase synthesis (fig 3) and abundance of mRNA (table 1). The MCT group had a signficantly greater lactase activity than LCT group, but less than HS group (table 1).

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Or shorthand:

- Lactase synthesis (fig. 3) \rightarrow LCT = MCT \lt HS
- Lactase activity (tab. 1) \rightarrow LCT < MCT < Sucrose
- % lactase mRNA (tab. 1) \rightarrow LCT = MCT \lt Sucrose

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Break it down:

 Acute sucrose (glucose and fructose) ingestion (study 1) appears to increase lactase mRNA synthesis (fig 1) and activity (fig 2), while the lipids and non-metabolizable CHO do not

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Break it down:

- Acute sucrose (glucose and fructose) ingestion (study 1) appears to increase lactase mRNA synthesis (fig 1) and activity (fig 2), while the lipids and non-metabolizable CHO do not
- Chronic HS (glucose) diet (study 2) appears to increase lactase enzyme protein synthesis (fig 3) and activity (table 1), and mRNA abundance (table 1)

Question 2, con't

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- Chronic MCT diet (study 2) appears to increase lactase activity (table 1), though not as much as HS
- Therefore, HS (likely glucose), has an effect on lactase at the transcriptional and post-transcriptional level both acutely and chronically, likely mediating its effects at the gene level
- While chronic MCT has a post-translational effect on lactase

Question 2, con't

E.g. A: Lactase mRNA expression and enzyme activity are regulated acutely by metabolizable sugar (sucrose). Chronic intake of metabolizable CHO (starch) regulates lactase mRNA, enzyme production, and activity. Both surcose and starch contain glucose, since glucose initiates an insulin response, either glucose or insulin may be mediating the lactase effect. Chronic exposure to MCT diets stimulate lactase activity, but not acute exposure, but does not stimulate mRNA expression or enzyme production. Therefore, MCT effects lactase activity post-translationally

Final tips and comments

- Sometimes its ok to **not** have an explanation without further data (eg. MCT and lactase activity)
 - Science and research is often like that describe the finding, but you don't need to explain it for the assignments and exams
- Keep things simple simplicity is better for you and for us who mark the assignment/exams