Hypothalamic neural networks in control of glucose homeostasis
Yi, C.X.

Citation for published version (APA):
Floating rhythms in plasma glucose concentration and glucose appearance -a technical note-

Introduction
Although the daily rhythm in plasma glucose concentration is not determined by food intake but by the central biological clock located in the suprachiasmatic nucleus (SCN), the rhythmic physiology and behavior of animals can be influenced by changing feeding conditions, as evidenced by food anticipation studies. Most of the studies on day-night fluctuations of glucose metabolism have been done by measuring plasma glucose concentration. The plasma glucose pool is determined by input – rate of glucose appearance (Ra) and output – rate of glucose disappearance (Rd). Any influence on either of these two fluxes will affect the final plasma glucose concentration. The main contributions to Ra come from endogenous glucose production and the direct glucose intake by feeding. Rd is the sum of the insulin-dependent (mainly muscle and adipose tissue) and insulin-independent (mainly liver and brain) glucose uptake. In the present study we aimed to map the Ra along the day/night-cycle under 3 different experimental conditions, including groups of animals that were: 1) fed ad libitum (with food pellets on floor of the cage); 2) fasted for 24-hours; 3) trained to eat only during the 12-h dark period (food pellets available in food hoppers). The reason to include the third group was due to the consideration, that in studies of glucose metabolism, food is always removed from the rats’ home cage either in advance or temporarily, to avoid feeding derived glucose entering the circulation. In our lab, we observed a slight increase in food seeking behavior when the rats notice that food has been removed from the cage. Despite the fact that rats don’t eat big meals when food is present in the cage during this time of the day, i.e., the light, thus sleep, period for rats. Therefore, when we observed that the endogenous glucose production (EGP) was continuously decreasing shortly after the food was removed (as shown in the following results), we reasoned this could be an immediate response of the animal in order to save energy, as the animals did not know of course when the food would become available again. Remarkably, previously we observed a similar response when we were studying daily changes in plasma glucagon concentrations, i.e., upon removal of food during the light period there was an immediate decrease in plasma glucagon concentrations (Ruiter et al., unpublished observations). Group 3 animals thus were adapted to the presence of food only during the dark period (and its absence during the light period) for at least 10 days before we did the actual experiment.
Experimental preparation

Silicon catheters were inserted into the right jugular vein and left carotid artery for intravenous (i.v.) infusions and blood sampling, as described in previous chapters. 

Group-1: Ad libitum, food present inside the cage all the time.

Group-2: Fasting, food was removed at ZT0 of the experimental day till the end of the blood sampling (totally 28 hours).

Group-3: Food availability restricted to the dark period.

For Ra measurement, a tracer dilution technique was applied, in which the enrichment (tracer/tracee ration) of [6,6-D2] glucose (Cambridge Isotope Laboratories, Cambridge, USA) was measured, with continuous intravenous infusion. A primed (8.0 μmol in 5 min)-continuous (16.6 μmol/h) [6,6-D2] glucose intravenous infusion started at ZT2 (ZT0=7am) (t=0), blood samples were taken at t=-5 min for background [6,6-D2] glucose enrichment measurement, and at ZT3.5 for determining enrichment after the equilibration state, from ZT3.5, hourly sampling was performed, in group1-2 till ZT2.5 the next day, and in group 3 till ZT14.5 of the first day. The volume of each blood sampling was limited to 0.1ml.

Results

1. The plasma glucose concentration of all three groups showed a significant fluctuation along the light/dark-cycle. In ad libitum fed animals plasma glucose concentration slowly increased from ZT3.5 onwards till a peak was reached at ZT12.5 in the dark period. In fasting animals plasma glucose concentrations also slowly increased from ZT3.5 onwards, but only until ZT 9.5, i.e, the peak was reached 2.5 hours before the start of the dark period. In the dark-fed animals, plasma glucose concentrations at ZT3.5 were very much comparable to those of the ad libitum fed animals. These animals also showed a slow increase from ZT3.5 onwards, and reached peak levels at ZT13.5 in the dark period. However, the peak level of the dark-fed animals was much lower than that of the ad libitum fed animals. In fact, during the light period the increase did not reach significance (Fig. 1).

2. Because in group 1, glucose appearance probably is not only derived from the endogenous production (as it is in groups 2 and 3), i.e., despite the low feeding activity during the light period we can not exclude that some of the glucose is coming from food intake in these animals, we used Ra instead of EGP for all groups.

As can be clearly observed in Fig. 2, the pattern of Ra does not follow closely the pattern of plasma glucose concentrations. In the ad libitum rats, the peak of Ra was found at ZT16.5. In the fasted animals of group 2, Ra is continuously decreasing. Remarkably the starting point is virtually equal for all 3 groups, i.e., despite the decreased plasma glucose concentrations at this time Ra is not affected yet. The Ra pattern in the dark-fed animals of group 3 closely follows the pattern of the fasting rats, with a
continuous decrease during the light period. At the start of the dark period, however, a strong increase in Ra is observed due the onset of feeding. A comparison of Figs. 1 and 2 shows that the Ra increase at ZT12.5, is followed by an increase in plasma glucose concentrations at 13.5.

Figure 1 Plasma glucose concentration along the light/dark-cycle.

Figure 2 Rate of glucose appearance (Ra) along the light/dark-cycle.
3. Because the differences between plasma glucose concentrations and Ra might be caused by a different glucose disappearance (i.e., Rd), we also calculated the metabolic clearance rate (MCR). But as there were no major differences between the patterns of MCR and Ra, apparently no profound changes in Rd occurred during the different experiments (Fig. 3).

4. Total food intake during this 25.5 hours sampling period did not differ between group 1 and group 3 (22.6 ± 2.2 g vs. 25.4 ± 1.3 g, p=0.30).

**Conclusion**

We conclude that: 1) different patterns of energy intake will influence the effects of the SCN timing information on glucose homeostasis, i.e., all 3 groups showed a different peak time in their daily plasma glucose rhythm; 2) a short-time training of the animals to adapt them to feeding in the dark period does not change the daily patterns of Ra and MCR as compared to acutely fasted animals, but it does affect the daily rhythm in plasma glucose concentration, i.e., trained rats do not show the steep decrease in plasma glucose concentrations before the end of the light period as observed in fasted animals. Whether after a longer training period with only feeding in the dark period, a closer approximation of the *ad libitum* situation can be accomplished will need further testing.