Supporting Information

Changes in glycogen structure over feeding cycle sheds new light on blood-glucose control

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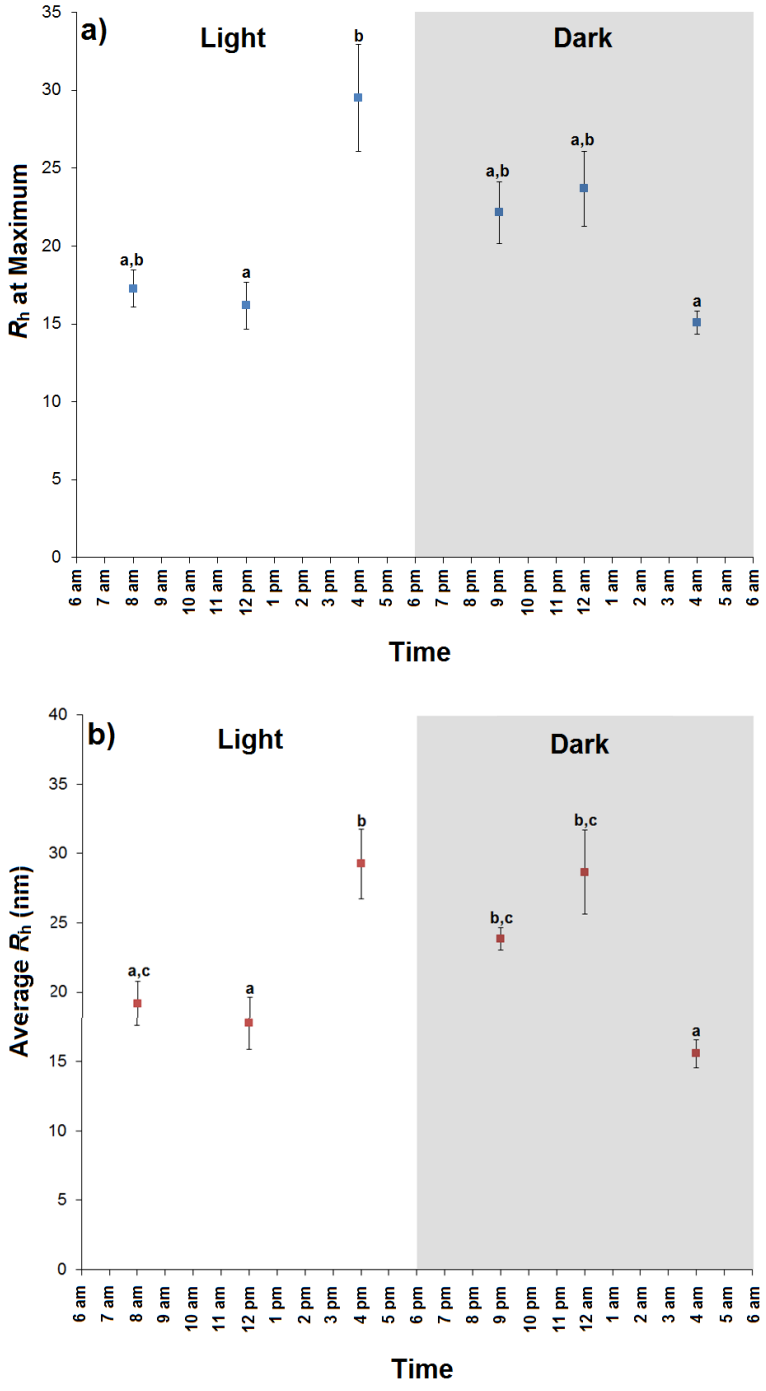

Figure S1. The mean hydrodynamic radius (R_h) at which the maximum occurs (a) and the average hydrodynamic radius (b) (calculated as previously)[1] for SEC weight distributions of glycogen at various times during a light/dark cycle. Values shown are the mean ± S.E.M. of 3–9 mice. Different letters indicate statistical significance ($p < 0.05$).

Preliminary kinetics studies

Materials

Potassium phosphate, ethylenediaminetetraacetic acid (EDTA), magnesium chloride, α-D glucose 1,6-diphosphate, β-nicotinamide adenine dinucleotide phosphate (β-NAPD), glucose-6-phosphate dehydrogenase, phosphoglucomutase, glycogen phosphorylase b from rabbit muscle and adenosine 5′-monophosphate (AMP) were purchased from Sigma-Aldrich. Diabetic C57BL/6j-db/db and wild-type female mice were sacrificed at either ~1.5 months ("young") or ~3 months ("old"). Glycogen extraction was performed using established methods.[2,3] Glycogen was extracted from the livers of eight mice with 4 non-diabetic (3 old and 1 young) and 4 diabetic (2 old and 2 young). The important factor is that there are a range of glycogen sizes to test the dependence of size on the initial rate of phosphorylase degradation; this range of sizes was achieved (see Figure S2).

Preparation of Digestion Buffer

A solution containing 500 mM potassium phosphate, 300 mM magnesium chloride and 100 mM EDTA (adjusted to pH 6.8) was prepared. The digestion buffer was obtained by mixing this solution (1.5 mL) with deionized water (10 mL), NADP 0.08 mg mL^{-1} (1 mL) and α-D glucose 1,6-diphosphate 0.08 mg mL^{-1} (30 μL).

Glycogen Phosphorylase Assay

The phosphorylase assays were performed on mouse-liver glycogen using a similar assay to a past study.[4] 0.8 mg of mouse-liver glycogen was dissolved into 0.2 mL of digestion buffer. Samples were then dissolved in a thermomixer at 80 °C and 350 rpm for 4 h. Samples were then diluted to 0.1125 mg mL^{-1} with the digestion buffer. .

A microplate reader (BMG FLUOstar OPTIMA) was used to measure the absorbance of β-NADPH, which is produced during the reaction. The following reagents were placed into each well: 180 μL glycogen solution, 6 μL of glycogen phophorylase (0.75 U mL^{-1}), 6 μL of

phosphoglucomutase (10 U mL^{-1}), 6 µL glucose-6-phosphate dehydrogenase (10 U mL^{-1}) and 2 µL of 100 mM 5 ′ AMP. Absorbance of the samples at 37 °C was measured at 340 nm every 90 s. A standard curve of known concentrations of β-NADPH was used. Initial rates for the reaction were obtained from the linear region of a plot of product concentration against time.

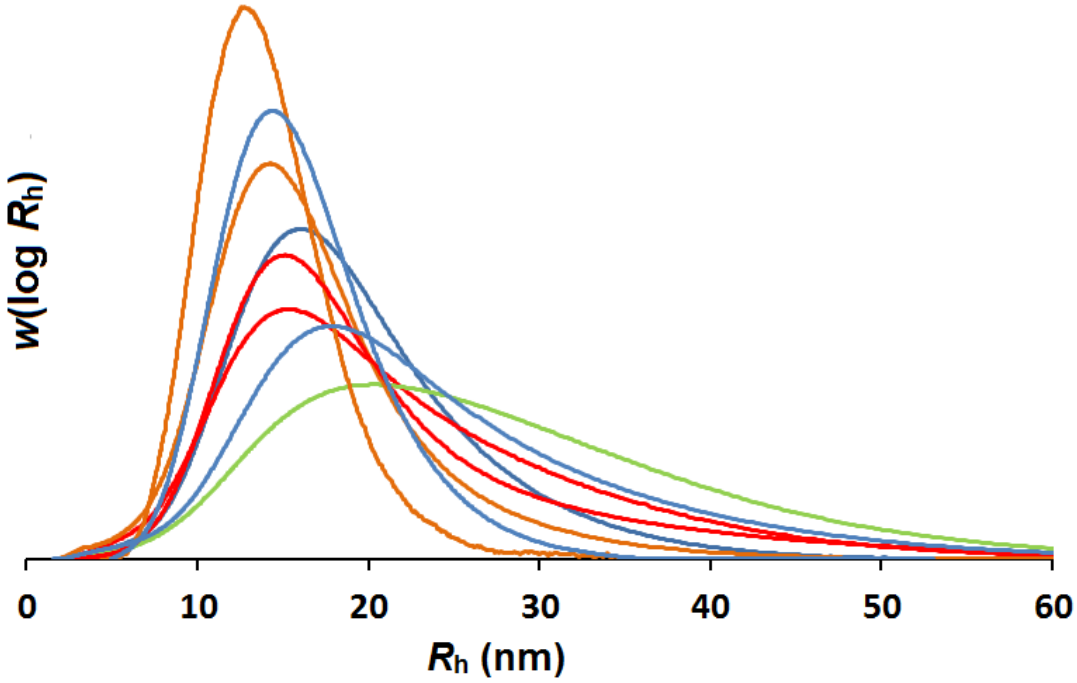

Figure S2. SEC weight distributions, $w(\log R_h)$ (normalized to equal areas) of young non-diabetic (green), old non-diabetic (blue), young diabetic (orange) and old diabetic (red) mice.

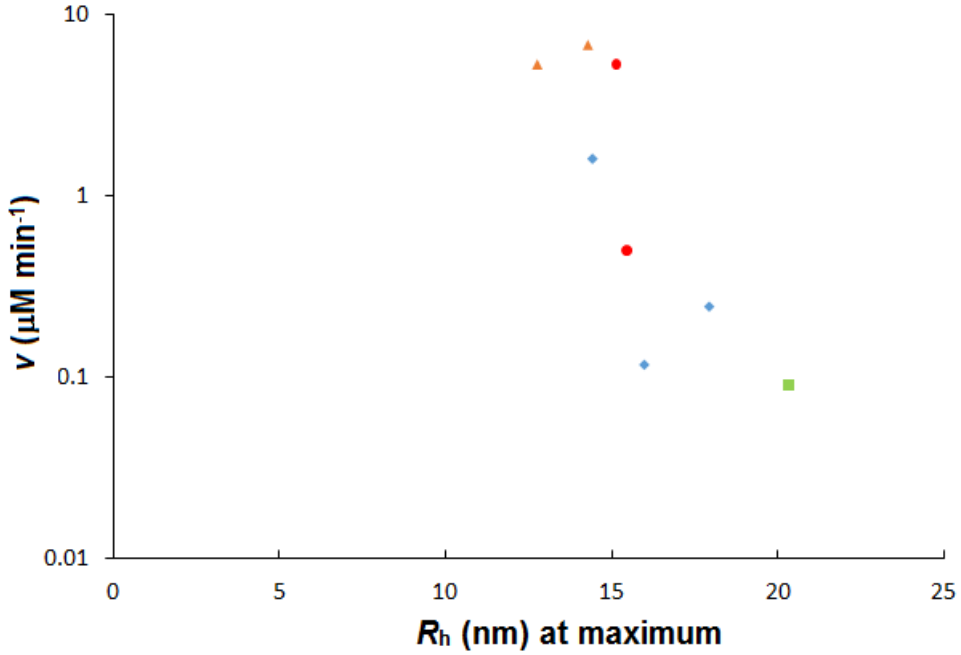

Figure S3. Initial rate *v* of glycogen phosphorylase degradation of glycogen from young non-diabetic (green square), old non-diabetic (blue diamond), young diabetic (orange triangle) and old diabetic (red circle) mice.

(1) Vilaplana, F.; Gilbert, R. G. *J. Chromatography A* **2011**, *1218*, 4434.

(2) Ryu, J.-H.; Drain, J.; Kim, J. H.; McGee, S.; Gray-Weale, A.; Waddington, L.; Parker, G. J.; Hargreaves, M.; Yoo, S.-H.; Stapleton, D. *International Journal of Biological Macromolecules* **2009**, *45*, 478.

(3) Sullivan, M. A.; Li, J.; Li, C. Z.; Vilaplana, F.; Stapleton, D.; Gray-Weale, A. A.; Bowen, S.; Zheng, L.; Gilbert, R. G. *Biomacromolecules* **2011**, *12*, 1983.

(4) Thomas, D. A.; Wright, B. E. *Journal of Biological Chemistry* **1976**, *251*, 1253.