Supporting Information for Molecular structural differences between diabetic/obese and healthy glycogen

Mitchell A. Sullivan, Jiong Li, Chuanzhou Li, Francisco Vilaplana, David Stapleton, Angus A. Gray-Weale, Stirling Bowen, Ling Zheng and Robert G. Gilbert

Experimental Procedures

Glycogen Extraction and Purification

The procedure for liver-glycogen extraction and purification used here has been described previously in the literature.[1] The livers from mice were homogenized in five volumes of glycogen isolation buffer, an inhibitor of glucosidase activity (50 mM Tris, pH 8, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 5 mM sodium pyrophosphate, and mini-complete protease inhibitors (Roche Applied Science, Indianapolis, IN)). The samples were centrifuged at 6000 *g* for 10 min at 4 ^{o}C with the resulting supernatant centrifuged further at 50 000 g for 30 min at 4 ^{o}C. The pellets were resuspended in 1.5 mL of glycogen isolation buffer and layered over a 22.5 mL, stepwise sucrose gradient (25%, 50% and 75% in glycogen isolation buffer). These samples were centrifuged at 300 000 g for 2 h at 4 ^{o}C. The glycogen fraction pelleted through all three sucrose layers whilst the microsomal layer only penetrated to the 25–50% sucrose fraction. The supernatant was discarded and the pellets were resuspended in 1 mL of deionized water and the samples were lyophilized (freeze-dried). 1 mL of 80% ethanol was added to glycogen, samples were centrifuged at 4000 *g* for 10 min at 10 ^{o}C and the supernatant was discarded. This step was repeated a further two times. Pellets were resuspended in 1 mL of deionized water and the samples were lyophilized.

Dissolution of Glycogen for SEC

Extensive work has been carried out recently on the development of a procedure to avoid the loss and degradation of starch upon dissolution.[2, 3] This procedure was employed here for the dissolution of glycogen in order to avoid similar detriment. Purified mouse-liver glycogen was directly dissolved in the SEC eluent of DMSO (HPLC grade, Sigma-Aldrich) with 0.5 wt % LiBr (ReagentPlus) on a thermomixer at 80 ^{o}C for 6 h at a concentration of 0.5 g L^{-1}.

SEC of Glycogen

Utilizing a procedure described in the literature,[4] samples were injected into an Agilent 1100 Series SEC system (PSS GmbH. Mainz, Germany) using a GRAM preColumn, 30 and 3000 columns (PSS GmbH. Mainz, Germany) in series, in a column oven at 80 ^{o}C. The flow rate of the chromatography was carried out at 0.3 mL min^{-1} which is expected to result in minimal shear scission of the glycogen.[5] The system included multi-angle laser light scattering (MALLS) (BIC-MwA7000, Brookhaven Instrument Corp., New York, USA) detection followed by detection from a refractive index detector (RID) (Shimadzu RID-10A, Shimadzu Corp., Japan). Pullulan standards (PSS GmbH, Mainz, Germany), with a MW range of 342 Da to 2.35 × 10^{6} Da were directly dissolved into eluent and run through the system to generate a universal calibration curve, allowing the determination of the hydrodynamic volume from the elution volume. The Mark-Houwink parameters for pullulan in DMSO/LiBr (0.5 wt %) at 80 ^{o}C are K = 2.427 × 10^{-4} dL g^{-1} and α = 0.6804 (Kramer and Kilz, PSS, Mainz, private communication).

Debranching of Glycogen

The procedure for debranching starch[6] is used here for glycogen. 0.9 mL of deionized water was added to glycogen (1–2 mg). Samples were heated at 100 ^{o}C for 15 min before cooling to room temperature. 0.1 M of sodium acetate buffer (0.1 mL, pH 3.5) was added with isoamylase solution (25 μL, 5 U) to the dispersions. The mixtures were incubated in a water bath at 37 ^{o}C for 3 h. The samples were then lyophilized.

Labeling Glycogen Chains

Using a proven labeling procedure,[7] 5 μL of 0.2 M APTS in aqueous glacial acetic acid (15%) and 5 μL of freshly prepared 1 M aqueous sodium cyanoborohydride was added to 1 mg of debranched glycogen. The same reactants were also added to 0.5 mg of maltohexaose to act as an internal marker. The mixtures were incubated at 40 ^{o}C for 15 h and diluted by a factor of 100 with water.

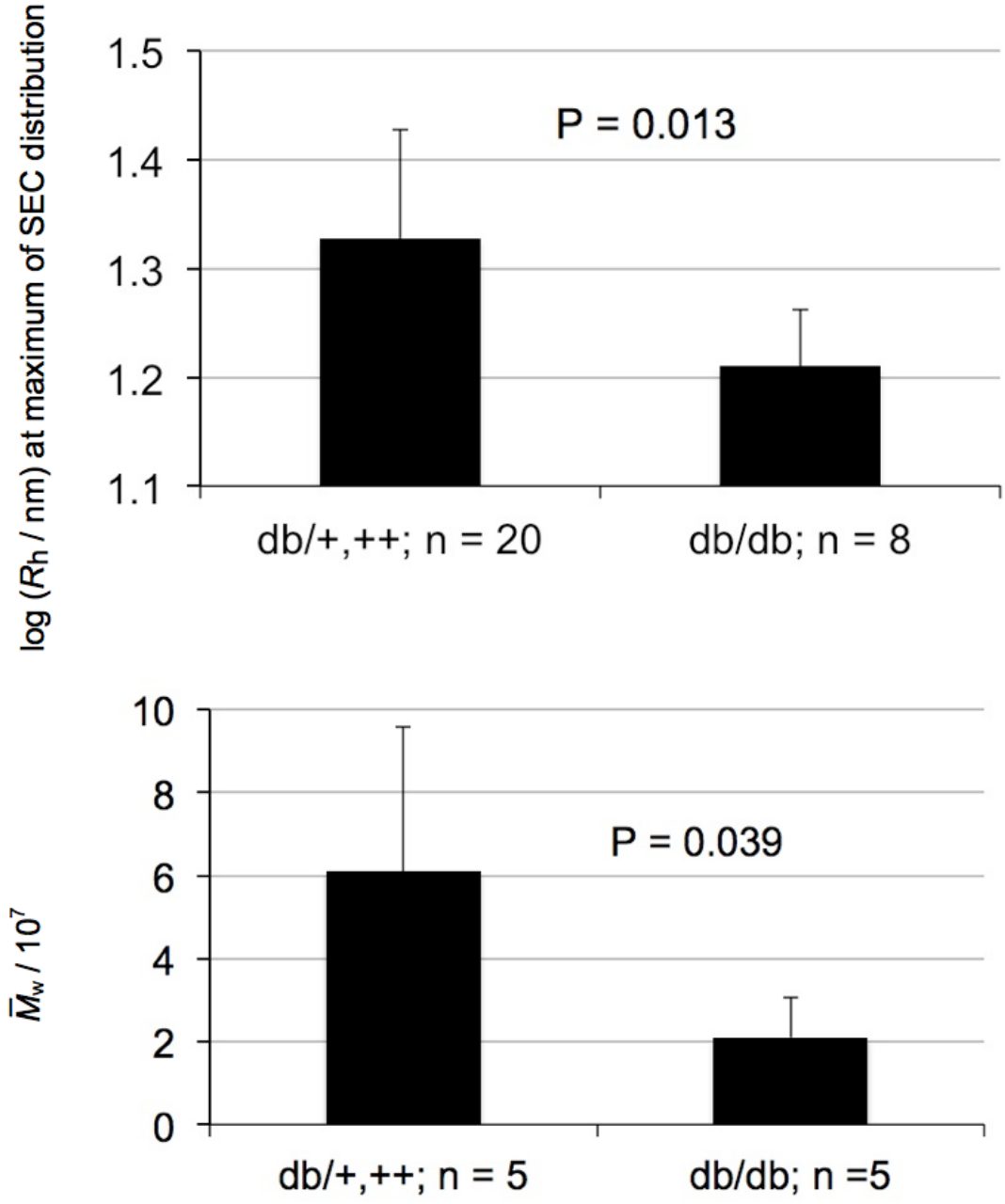
Capillary Electrophoresis

Labeled mouse glycogen were diluted by a factor of 10,000 and 20 μL of each sample was loaded into each well. Samples were injected for 3 s and then electrophoresed using 3730 buffer with EDTA (Applied Biosystems, Cheshire, UK) for a period of 5000 s at a voltage of 15 kV and a current of 400 mA using an Applied Biosystems (Applied Biosystems Division Headquarters, Foster City, CA, U.S.A) 3730 Genetic Analyzer with a 50 cm capillary array, which was equipped with an argon-ion laser at 488 nm. Data were collected using Data Collection v3.0 software and analyzed using GeneMapper® v3.7 software. The detection system was calibrated using G5 RCT dye (Applied Biosystems Division Headquarters, Foster City, CA, U.S.A).

Statistical analysis

All results were expressed as the mean ±standard deviation. Statistical significance was evaluated with the non-parametric Kruskal-Wallis test followed by the Mann-Whitney test for three groups comparison or evaluated with the non-parametric Mann-Whitney test for two groups comparison. Differences were considered statistically significant at $P < 0.05$. Results are given in Fig. S1.

Fig. S1. Statistical results.



Details of mice

Animals were always sacrificed between 8 and 10 am. They were anaesthetized by a chloral hydrate solution (50 mg mL^{-1}), with a dosage at 500 mg per kg body weight or higher to achieve euthanasia.

Table S1: Mouse information

[a]$P < 0.05$ compared to the db/+, [b]$P < 0.05$ compared to the +/+

Age groups	Age when sacrificed (months)	Genotype (n)	Body weight (g)	Non-fasting blood glucose (mM)	Liver weight (g)
Young	1.5	db/db (4)	23.4 ± 1.4[a]	9.2 ± 1.5	1.63 ± 0.2[a]
		db/+ (8)	15.1 ± 1.6	8.5 ± 1.6	0.8 ± 0.1
	3	db/db (5)	39.0 ± 2.2[a,b]	14.9 ± 2.2[a,b]	2.6 ± 0.3[a,b]
		db/+ (6)	21.0 ± 0.9	9.2 ± 0.8	1.0 ± 0.1
		+/+ (4)	21.4 ± 0.7	10.4 ± 0.7	1.2 ± 0.1
Adult	4.5	db/db (3)	48.2 ± 1.9[a,b]	8.6 ± 1.5	3.2 ± 0.4[a,b]
		db/+ (6)	23.4 ± 1.4	8.0 ± 0.1	1.1 ± 0.1
		+/+ (5)	23.2 ± 1.6	9.3 ± 0.6	1.1 ± 0.2

Fig. S2: Identification of mice for Fig. 1. M1, M2, M3, M4, M8 are all db/db; M5/7, M9 and M10 are all db/+.

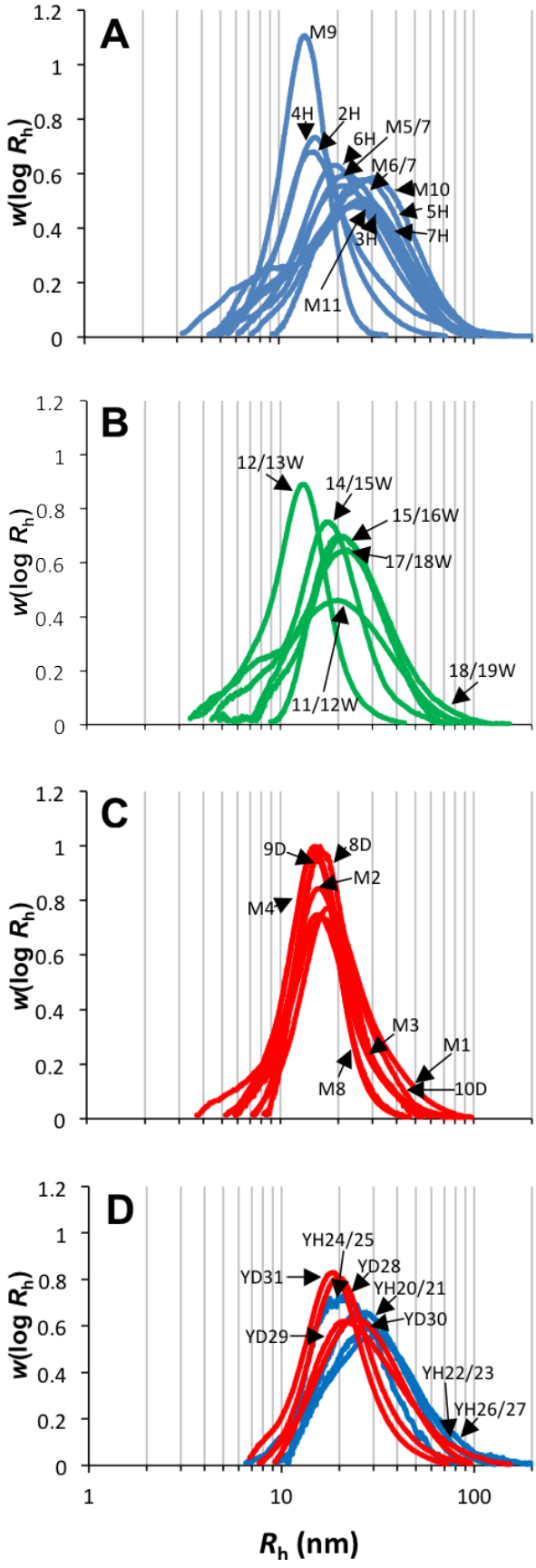


Fig. S3: Identification of mice for Fig. 2. M1, M2, M3, M4, M8 are all db/db; M5/7, M9 and M10 are all db/+.

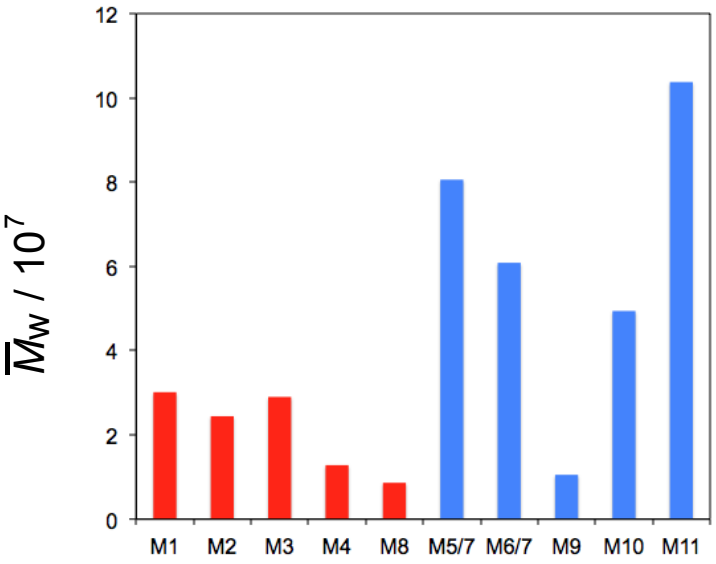
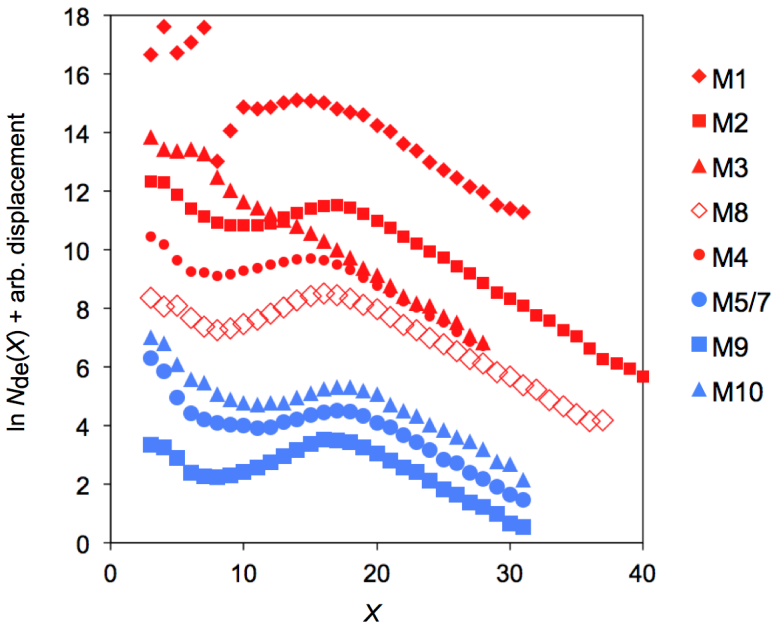


Fig. S4: Identification of mice for Fig. 3.



References

1. 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*, 478-482.

2. Schmitz, S.; Dona, A. C.; Castignolles, P.; Gilbert, R. G.; Gaborieau, M. *Macromolecular Bioscience* 2009, *9*, 506-14.

3. Syahariza, Z. A.; Li, E.; Hasjim, J. *Carbohydrate Polymers* 2010, *82*, 14-20.

4. Sullivan, M. A.; Vilaplana, F.; Cave, R. A.; Stapleton, D. I.; Gray-Weale, A. A.; Gilbert, R. G. *Biomacromolecules* 2010, *11*, 1094-1100.

5. Cave, R. A.; Seabrook, S. A.; Gidley, M. J.; Gilbert, R. G. *Biomacromolecules* 2009, *10*, 2245-53.

6. O'Shea, M. G.; Morell, M. K. *Electrophoresis* 1996, *17*, 681-6.

7. O'Shea, M. G.; Samuel, M. S.; Konik, C. M.; Morell, M. K. *Carbohydrate Research* 1998, *307*, 1-12.