

Supplemental Information

Dietary protein to carbohydrate ratio and caloric restriction: comparing metabolic outcomes in mice

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Supplemental Figures

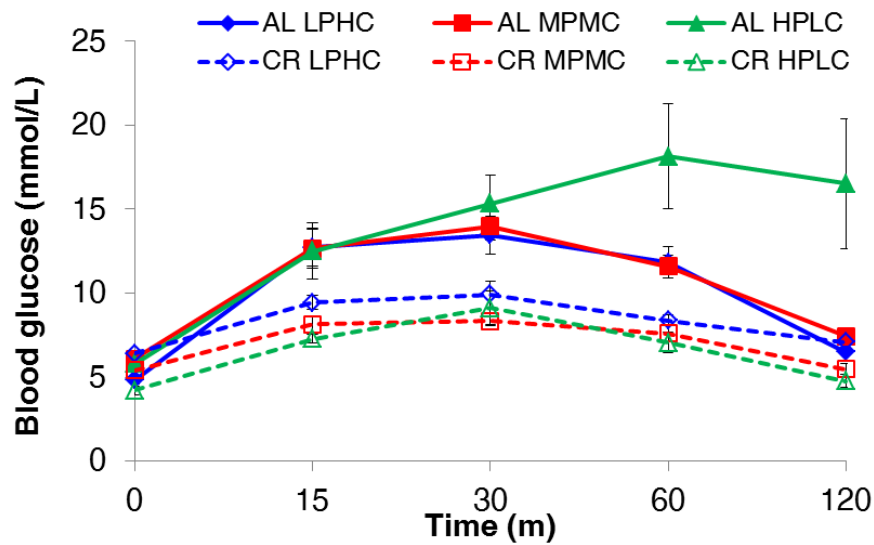


Figure S1, related to Figure 2 and Table S3 \pm SEM. Glucose tolerance test results showing changing blood glucose levels over time and analysed using repeated measures ANOVA. (AL *ad libitum*; CR caloric restricted; HPLC high ratio of protein to carbohydrate; MPMC medium protein to carbohydrate ratio; LPHC low protein to carbohydrate ratio). See also Table S3.

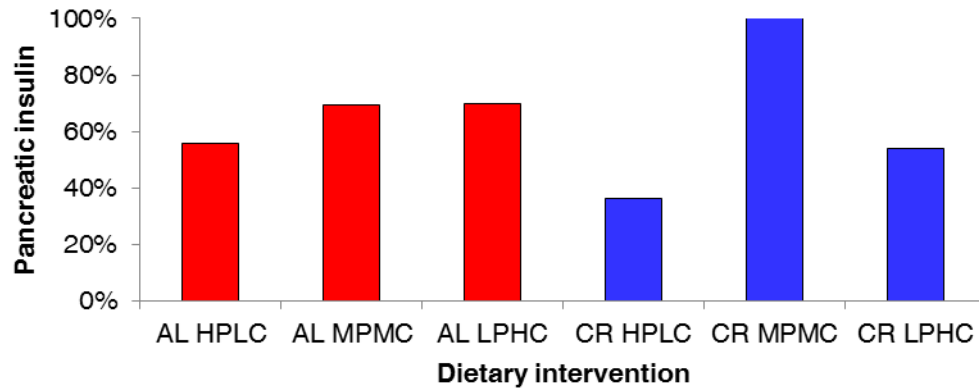


Figure S2, related to Figure 3. Pancreatic insulin staining. The effect of diet on pancreatic β -cell staining. Percentages indicate the proportion of samples with high intensity staining. (AL *ad libitum*; CR caloric restricted; HPLC high ratio of protein to carbohydrate; MPMC medium protein to carbohydrate ratio; LPHC low protein to carbohydrate ratio).

Supplemental Tables

Table S1, related to experimental procedures. Experimental diets. Ingredients of low (P5%, LPHC), medium (P33%, MPMC), and high (P60%, HPLC) protein to carbohydrate diets fed to mice on both a mass (*g/kg*) and energy (*kcal/kg*) basis.

	LPHC P5%		MPMC P33%		HPLC P60%	
	g/kg	Kcal/kg	g/kg	Kcal/kg	g/kg	Kcal/kg
Casein	54.68	195.75	356.43	1276.02	654.28	2342.32
Methionine	0.80	3.20	5.30	21.20	9.80	39.20
Sucrose	66.27	265.08	65.47	261.88	66.07	264.28
Cornstarch	649.82	2339.36	317.68	1143.70	42.14	151.71
Dextrinised cornstarch	99.46	377.95	98.05	372.59	99.05	376.39
Soya bean oil	88.37	795.33	87.26	785.34	88.06	792.54

Table S2, related to Figures 1-4. Statistical results. Comparison of each outcome was performed first by ANOVA. If ANOVA was significant, a post hoc Fisher LSD test was performed. (AL *ad libitum*, CR caloric restricted, HPLC high protein to carbohydrate, MPMC medium protein to carbohydrate, LPHC low protein to carbohydrate). Of particular note is that the AL LPHC did not have any differences with the CR diets for insulin, HOMA, triglycerides (Trig) and HDLc, but was different in terms of RER, energy expenditure (EE), body weight and body fat (shaded area). AUC indicates the area under the curve for glucose tolerance tests. P-values for significant comparisons are shown.

	Body mass	Insulin	HOMA	Trig	HDLc	RER	EE	% Body fat	AUC
<i>ANOVA P-value</i>	<0.001	<0.001	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	<0.001
AL LPHC vs CR HPLC	<0.001					<0.001	<0.001	0.027	0.041
AL LPHC vs CR MPMC	0.004					<0.001	<0.001	0.001	0.012
AL LPHC vs CR LPHC	0.005					<0.001	<0.001	0.002	0.014
AL HPLC vs CR HPLC	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001		<0.001
AL HPLC vs CR MPMC	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	0.011	<0.001
AL HPLC vs CR LPHC	<0.001	<0.001	<0.001	<0.001		<0.001		0.017	<0.001
AL HPLC vs AL MPMC	0.048			<0.001	0.001	0.010	<0.001		<0.001
AL HPLC vs AL LPHC		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		0.005
AL MPMC vs CR HPLC	<0.001	<0.001	<0.001	0.025		<0.001		0.005	
AL MPMC vs CR MPMC	<0.001	<0.001	<0.001	0.003		<0.001		<0.001	
AL MPMC vs CR LPHC	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	
AL MPMC vs AL LPHC	<0.001	<0.001	0.004	<0.001		<0.001	<0.001		
CR HPLC vs CR MPMC									
CR HPLC vs CR LPHC							<0.001		
CR MPMC vs CR LPHC							<0.001		

Table S3, related to Figures 2 and Figure S1. Repeated measures analysis for glucose tolerance tests. (AL *ad libitum*, CR caloric restricted, HPLC high protein to carbohydrate, MPMC medium protein to carbohydrate, LPHC low protein to carbohydrate). Basal blood glucose levels did not differ between groups. The AL HPLC group (shaded area) showed rising blood glucose levels over time and were the only group that did not return to basal levels at 120 m. P-values for significant comparisons are shown.

<i>ANOVA P-value Diet x Time</i>	<0.001*				
Time (m)	0	15	30	60	120
AL HPLC vs CR HPLC		0.025	0.004	<0.001	<0.001
AL HPLC vs CR MPMC			0.001	<0.001	<0.001
AL HPLC vs CR LPHC			0.028	<0.001	<0.001
AL HPLC vs AL MPMC				0.002	<0.001
AL HPLC vs AL LPHC				0.003	<0.001
AL HPLC vs CR HPLC		0.025	0.004	<0.001	<0.001
AL LPHC vs CR HPLC		0.020			
AL LPHC vs CR MPMC			0.040		
AL LPHC vs CR LPHC					
AL MPMC vs CR HPLC		0.020	0.042		
AL MPMC vs CR MPMC			0.018		
AL MPMC vs CR LPHC					
AL MPMC vs AL LPHC					
CR HPLC vs CR MPMC					
CR HPLC vs CR LPHC					
CR MPMC vs CR LPHC					