Herring Milt: A Potential Functional Ingredient for Obesity and Type-2 Diabetes

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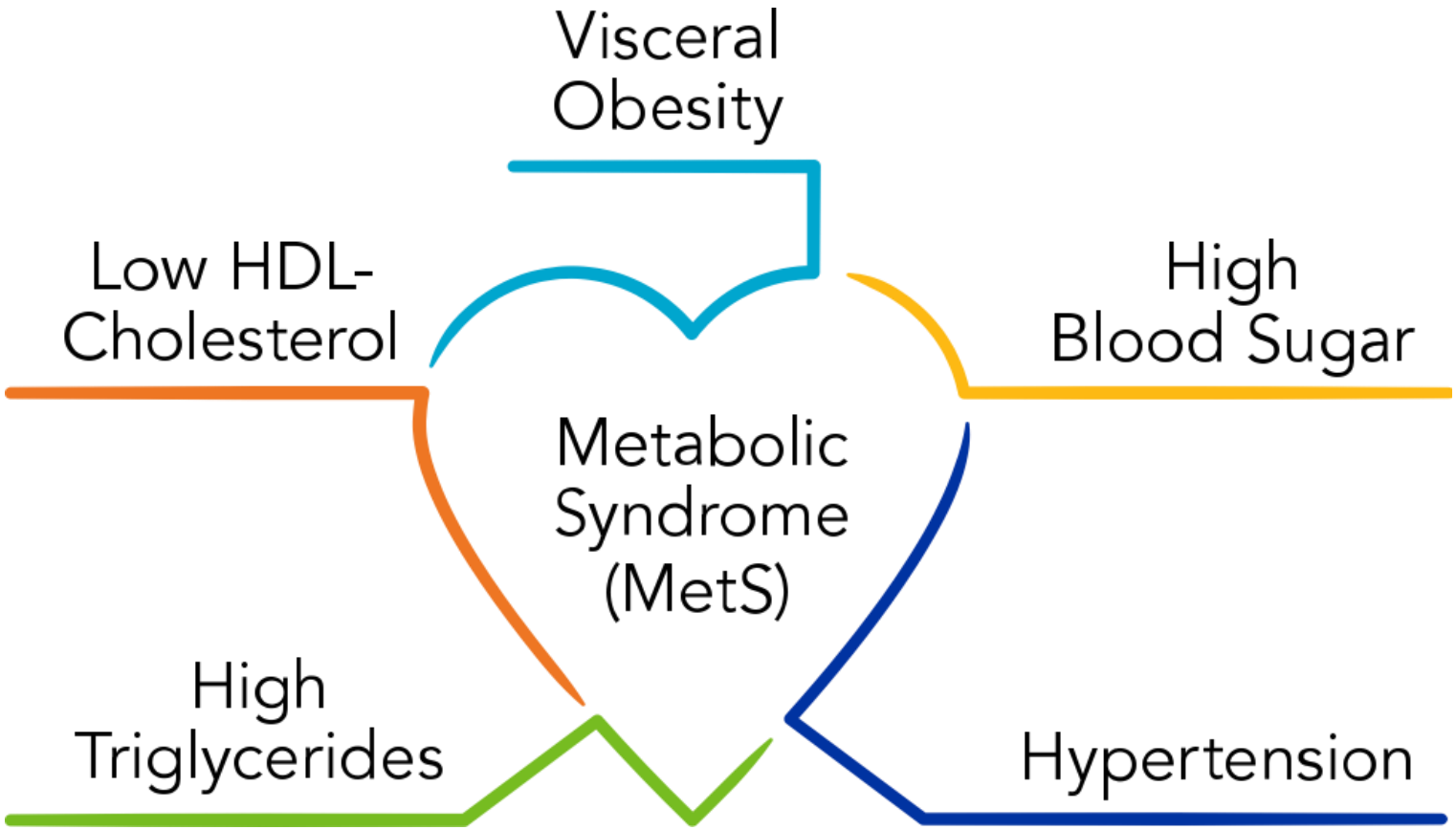
Metabolic syndrome



https://www.metabolicsyndromecanada.ca/about-metabolic-syndrome

Key facts

- Heart disease has remained the number one cause of death globally for the last 20 years. The number of deaths increased by > 2 million since 2000 to nearly 9 million in 2019.
- Currently, more than 1 billion adults are overweight – of which at least 300 million are clinically obese.
- Childhood obesity is already epidemic in some areas and on the rise in others.
- Deaths from diabetes increased by 70% globally between 2000 and 2019.

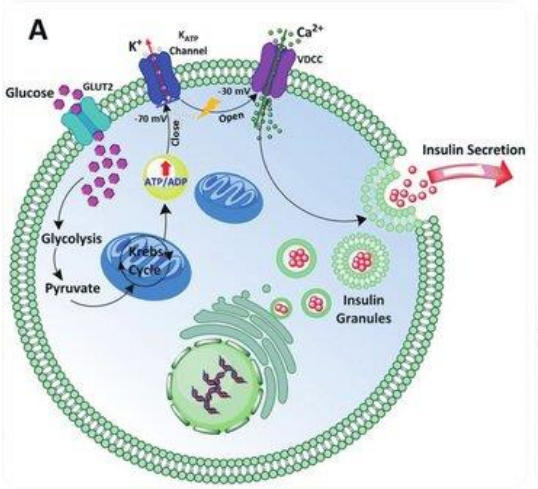
WHO, 2020

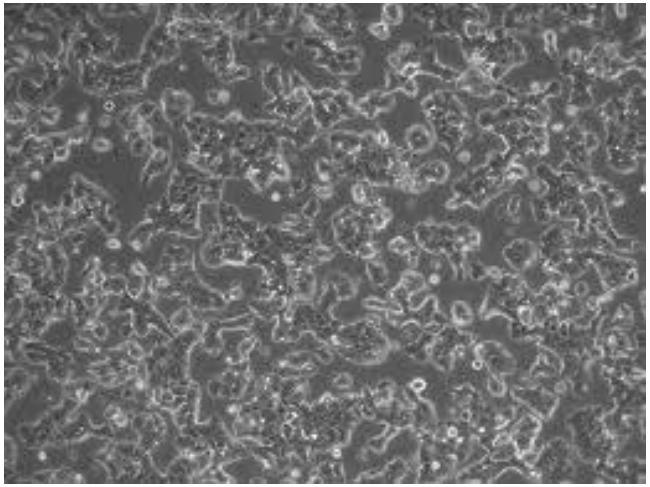
Valorization of marine biomass and marine-based by-products

- Collection, preservation and processing
- Extraction and fractionation
- Chemical analysis/characterization
- In vitro cell based assays
  - Glucose-stimulated insulin release in pancreatic beta cells
  - Glucose update in peripheral tissues (muscle, fat cells)
  - Anti-obesity activity
  - Anti-inflammation
  - Antioxidant activity
  - Neuroprotection/ anti-ageing

In vitro screening of marine-based extracts for glucose-stimulated insulin secretion

- INS-1E rat insulinoma cells were used as the *in vitro* assay model.
- Cytotoxicity was measured using the MTT assay.
- Extracts were screened for glucose stimulated insulin secretion (GSIS) using ELISA kit.
- 82 extracts/samples were tested.





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The 1st screening

The first screening conditions

- 3 doses (10, 50, 100 μg/mL) of each product
- GSIS at 25 mM of glucose

Results

- 10 : Toxic, not tested
- 38 : 0- 20% of increase
- 32 : ≥ 20% of increase
- 2 :Toxic, but have ≥ 20% of increase

The 2nd screening

Screening conditions

32 candidates (>20% increase of GSIS) were tested at 2 doses (50, 100 μg/ml)
GSIS at 5.5, 11, and 25 mM of glucose, respectively
40% increase of GSIS was set as the cut-off.

Results

Number of Sample	≥ 40 % increase in insulin secretion	Number of Sample	≥ 40 % increase in insulin secretion
1	1-2W	8	1-5 W
2	1-5A	9	1-5 H
3	1-5 M	10	3-2 W
4	2-1 P+ A	11	3-3 R
5	2-4 M	12	3-3 f ®
6	1-4 f ®	13	5-3 P
7	1-4 R		

The 3rd screening on fractionation prepared from the lead candidates in the 2nd screening

Sample code	Origin	Form
YW-0046	Shrimp oil	Liquid
1-2 W	Sea cucumber - internal organs	Dry powder
1-5 W	Sea cucumber - dry flower	Dry powder
1-5 H	Sea cucumber - dry flower	Dry powder
1-5 A	Sea cucumber - dry flower	Dry powder
1-5 M	Sea cucumber - dry flower	Dry powder
2-1 P+A	Herring - milt / hydrolysis	Dry powder
3-2 W	Crab - hepatopancreas	Dry powder
5-3 P	Sardine - cutting / hydrolysis	Dry powder

Preparation of herring milt protein hydrolysate (HPH)

Herring milt → (Bioreactor, Alcalase) → HPH

Protein	Lipids	EPA	DHA	Arginine
70.8	10.8	8.0	10.8	29

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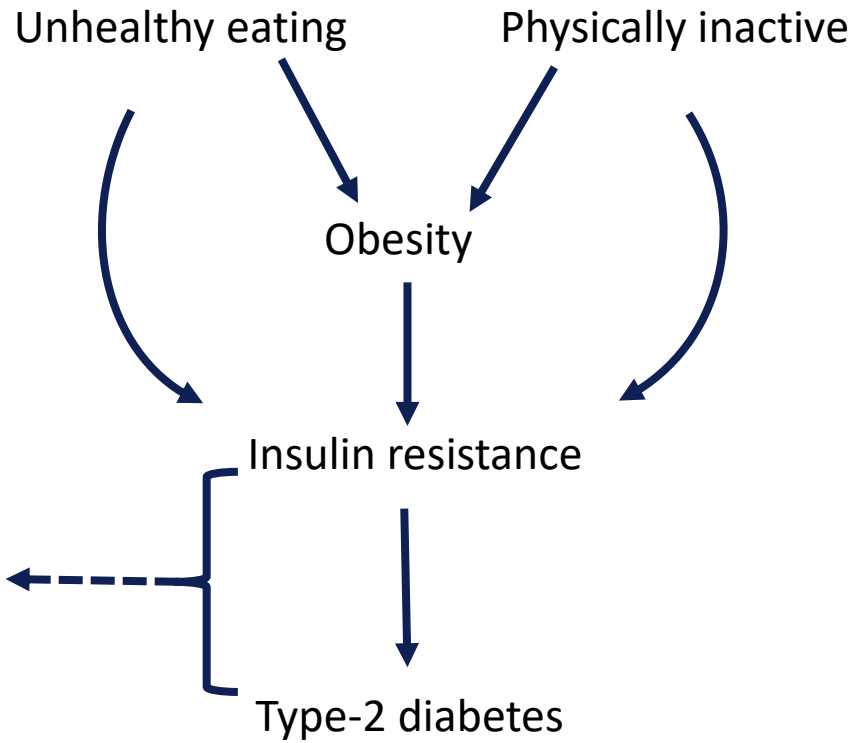
Diet composition

Ingredient#	LFC	HFC	HPH15	HPH35	HPH70
			Diet composition		
Casein (80 Mesh)	200	200	170	130	60
L-Cystine	3	3	3	3	3
Corn Starch	315	0	0	0	0
Maltodextrin 10	35	125	125	125	125
Sucrose	350	68.8	68.8	68.8	68.8
Herring Milt Hydrolysate	0	0.00	42.4	98.9	197.7
Cellulose, BW200	50	50	50	50	50
Lard (96%)	20	245	240.4	234.3	223.6
Soybean Oil	25	25	25	25	25
Mineral Mix S10026	10	10	10	10	10
DiCalcium Phosphate	13	13	13	13	13
Calcium Carbonate	5.5	5.5	5.5	5.5	5.5
Potassium Citrate, 1 H2O	16.5	16.5	16.5	16.5	16.5
Vitamin Mix V10001	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2
FD&C Blue Dye 1	0.05	0.05	0.05	0.05	0.05
Total (g)	1055	774	782	792	810
			Calorie information		
Protein (% kcal)	20	20	20	20	20
Fat (% kcal)	10	60	60	60	60
Carbohydrate (% kcal)	70	20	20	20	20
Energy density (kcal/g)	3.82	5.21	5.21	5.21	5.21

Features of type 2 diabetes

- Glucose intolerance
- Elevation of blood glucose
- Elevation of blood insulin and leptin
- Increase of blood free fatty acids, cholesterol and triglycerides
- Decrease of blood adiponectin



Experimental design

Male C57BL/6J mice

5 wk

HFD

LFD

11 wk

11 wk

HFC

HPH15

HPH35

HPH70

LFC

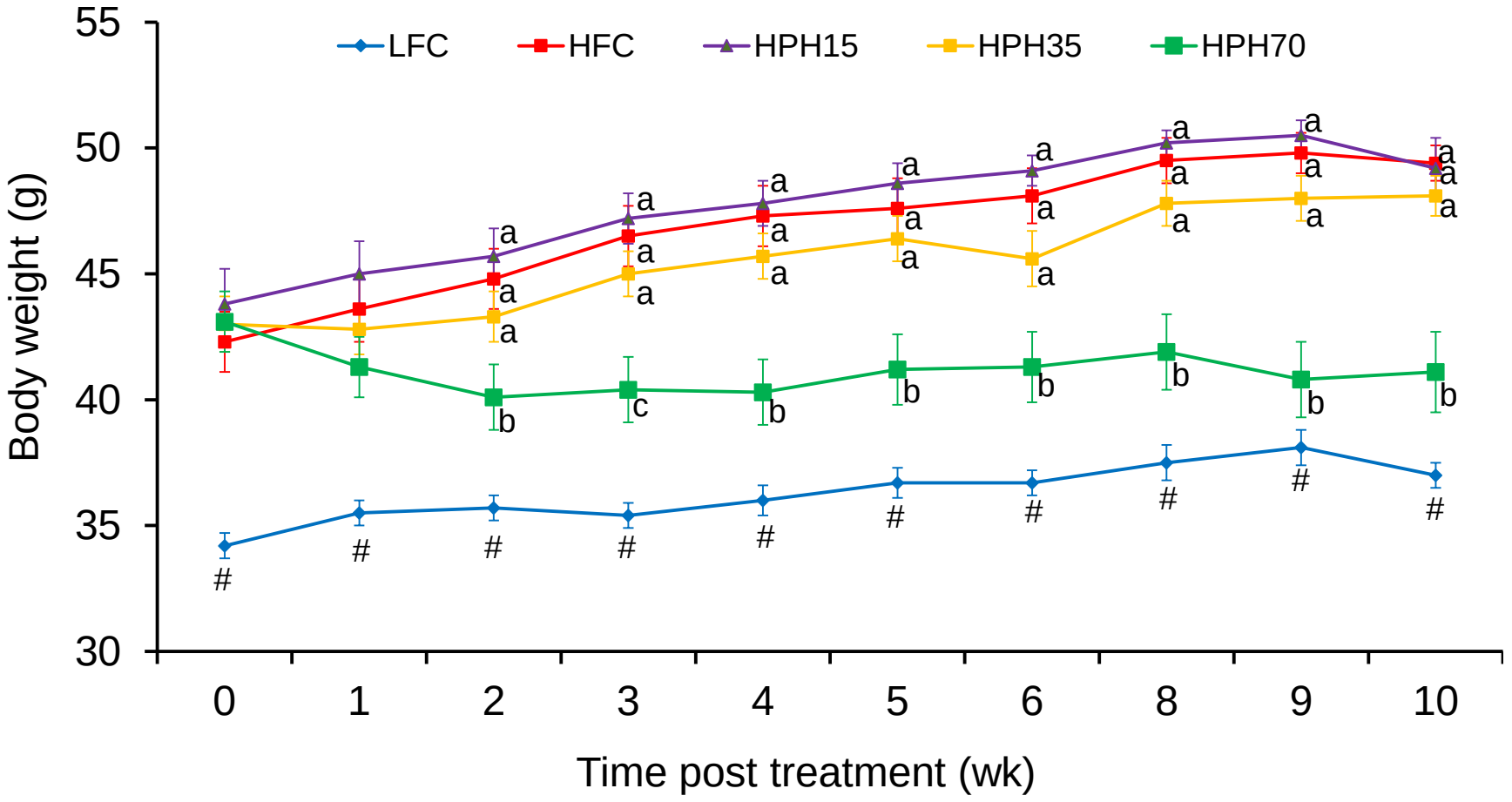
10 wk

10 wk

Food intake, body weight, semi-fasting BG, OGTT

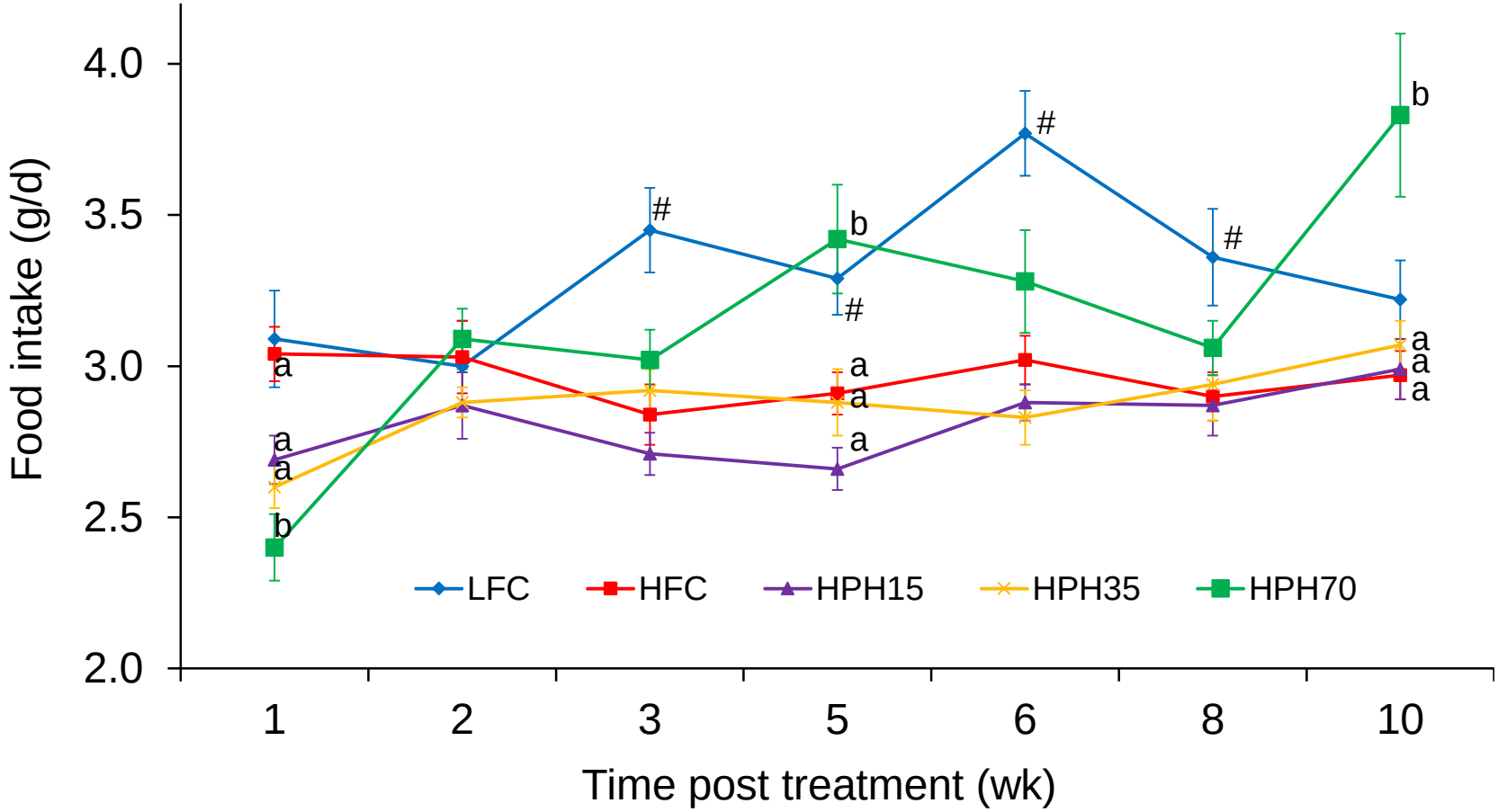
Fasting BG, insulin, leptin, adiponectin, FFA, lipids

Body weight

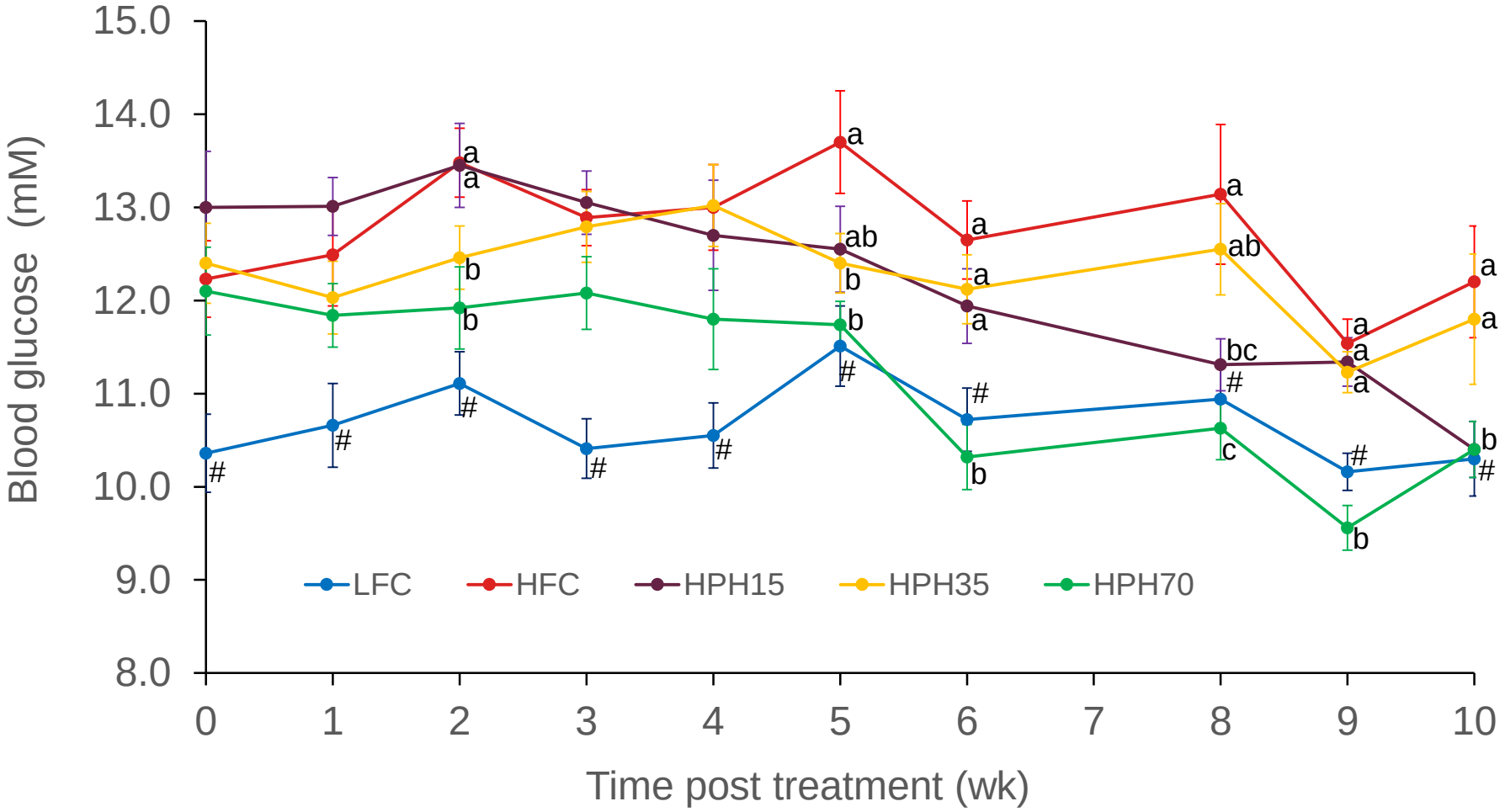


#Compared to HFC, P < 0.0001; [a,b]values labeled with different letters differ, p < 0.05

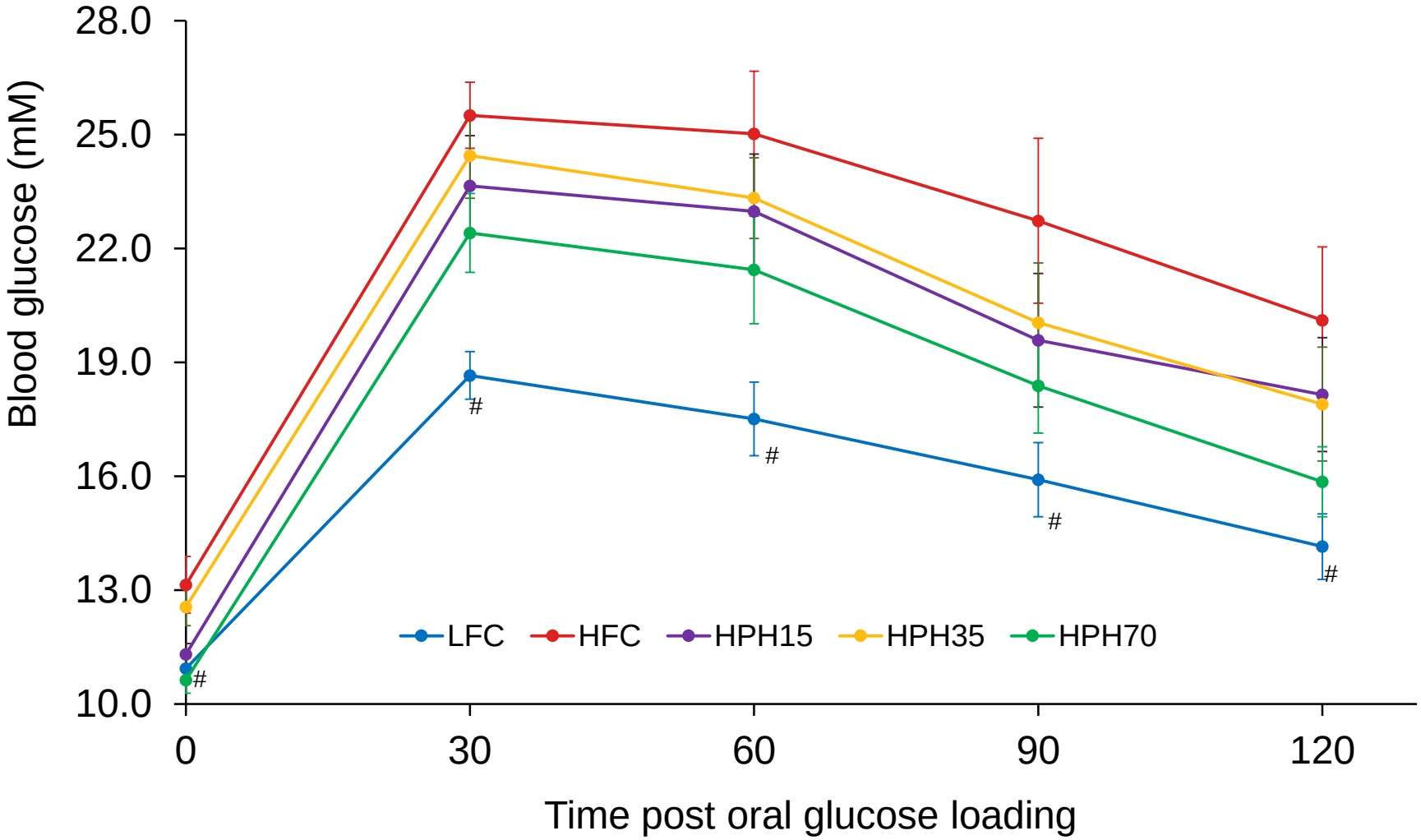
Food intake


Food intake (g/d)
4.0
3.5
3.0
2.5
2.0
1
2
3
5
6
8
10
Time post treatment (wk)
LFC
HFC
HPH15
HPH35
HPH70
#
b
a

Semi-fasting (4-6 hr) blood glucose



Oral glucose tolerance during week 8 of treatment



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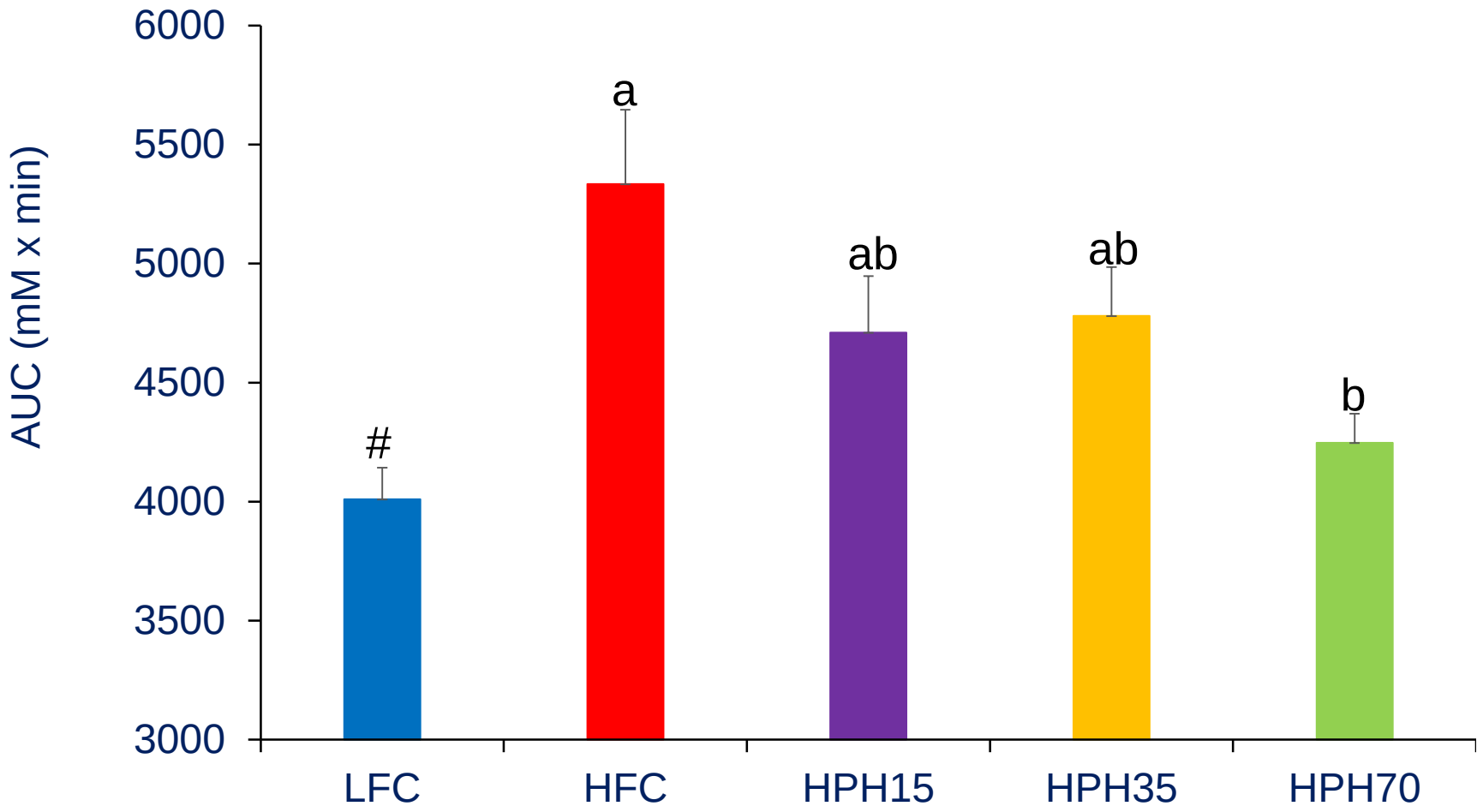
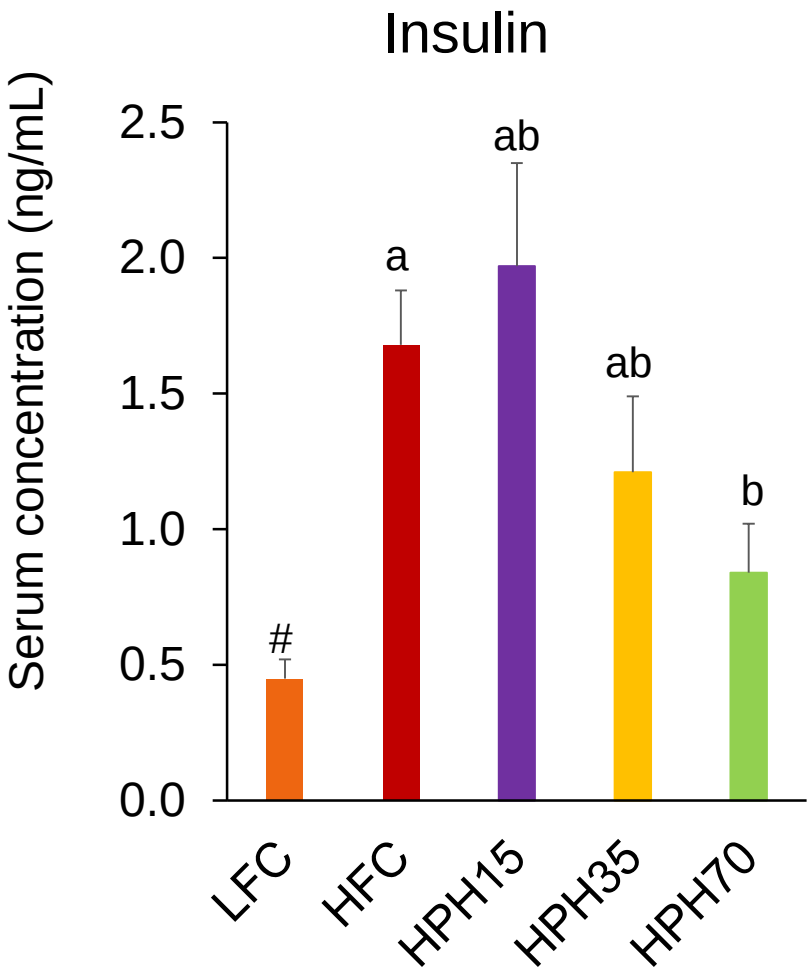
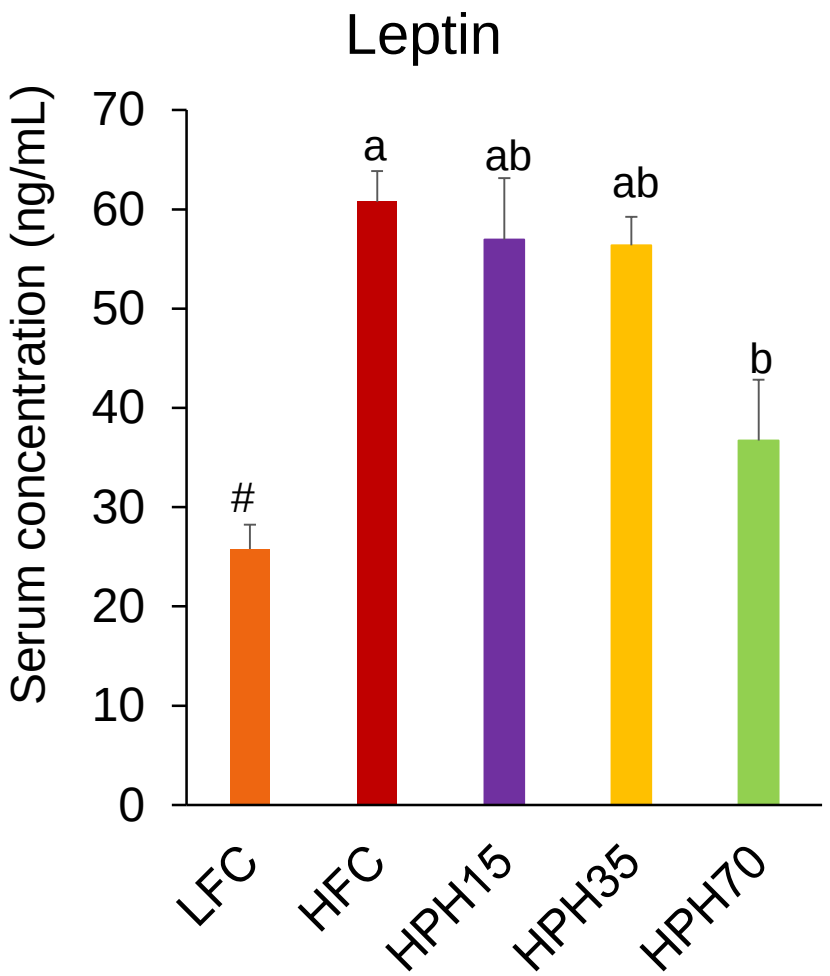
Area under the curve (AUC) of oral glucose tolerance



Fasting blood insulin and leptin





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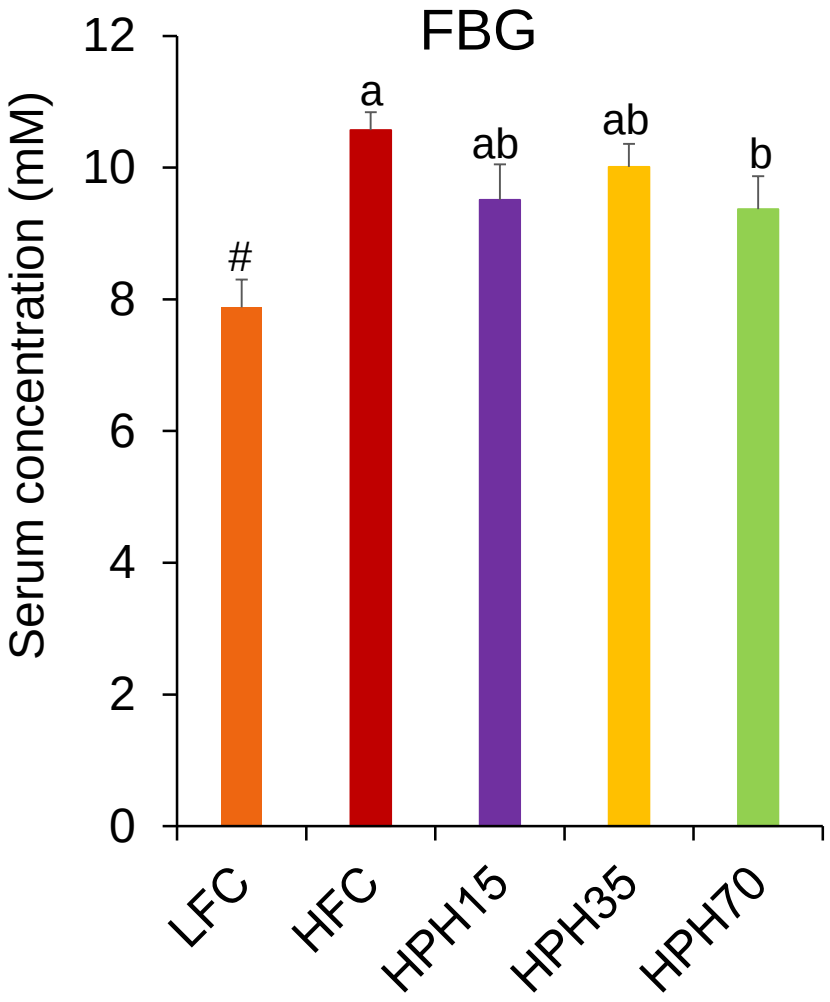
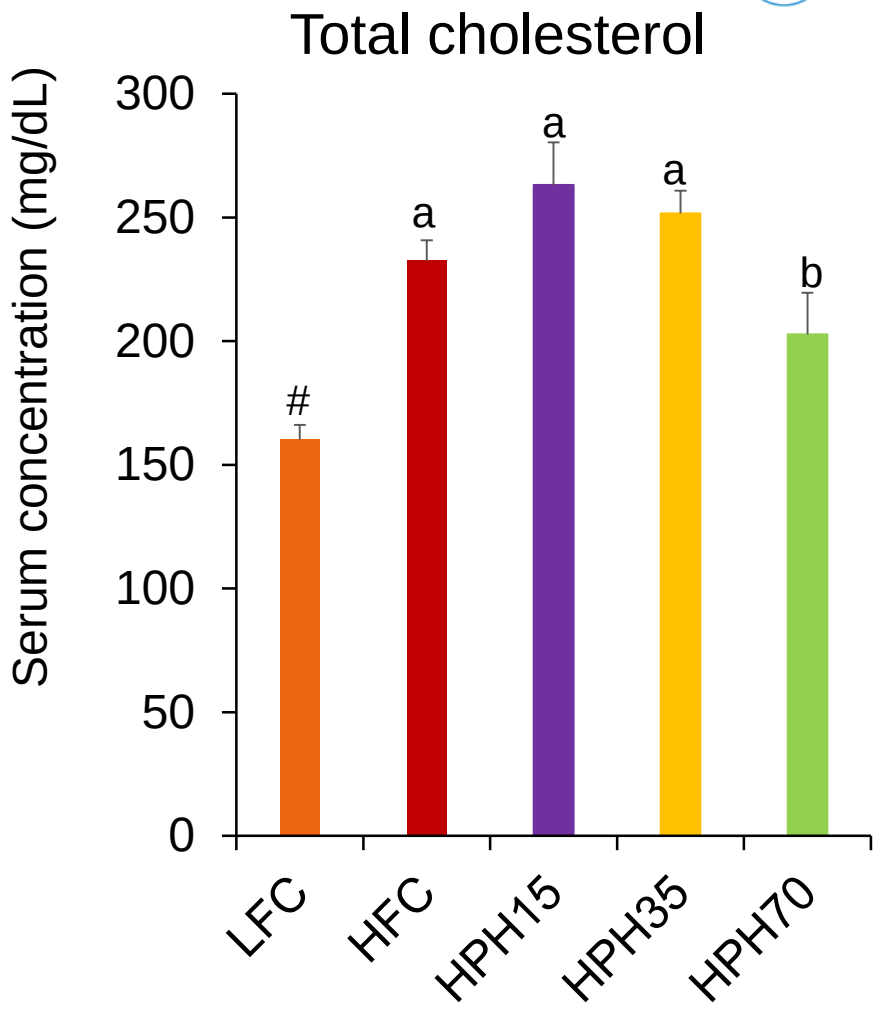
Fasting blood glucose (FBG) and total cholesterol





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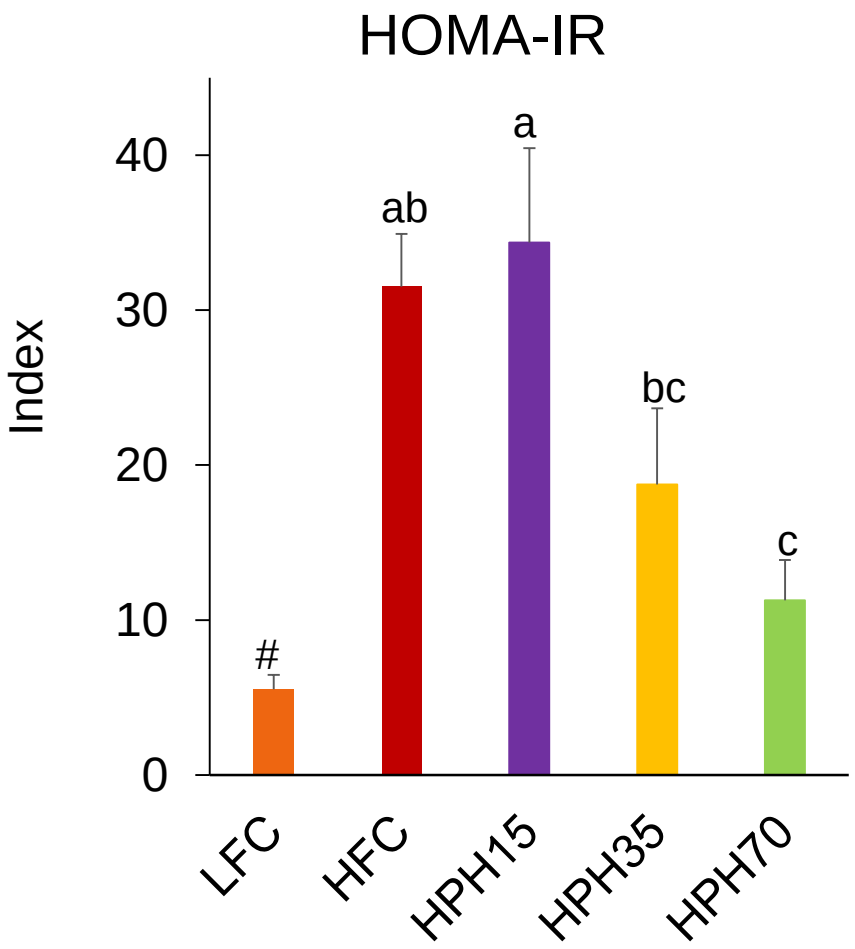
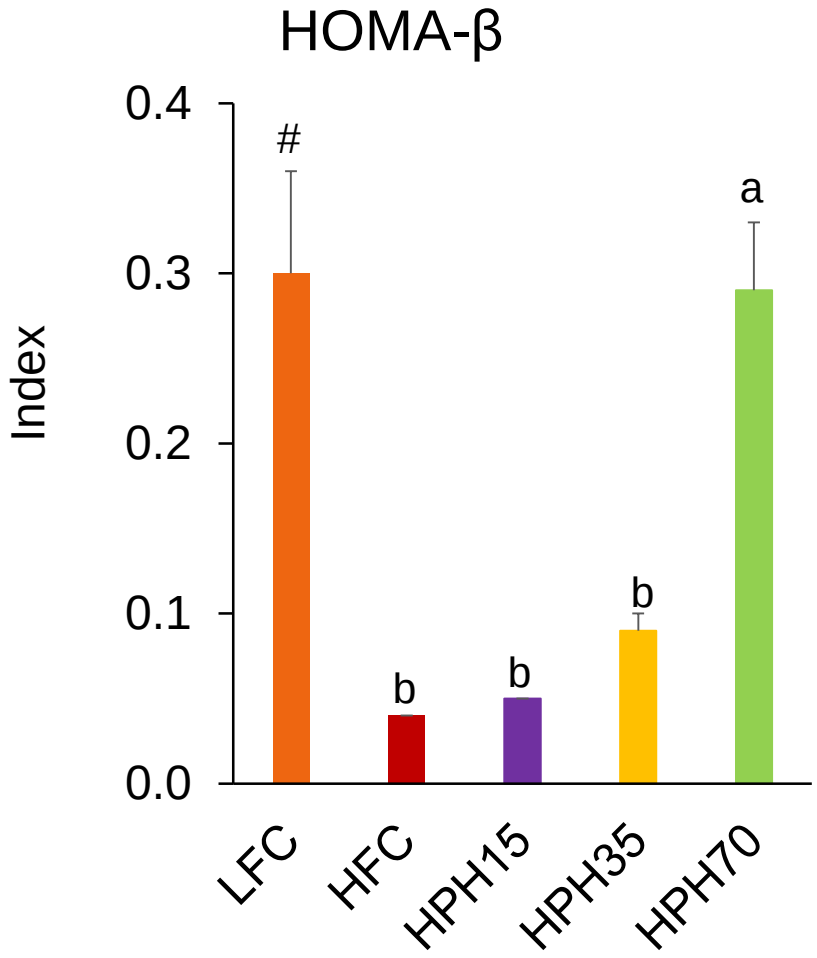
Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β)





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Blood adiponectin and free fatty acid (FFA)

	LFC	HFC	HPH15	HPH35	HPH70
Serum adiponectin (ng/mL)	10.66 ± 0.31	9.79 ± 0.26[#]	8.84 ± 0.36	9.95 ± 0.22	8.99 ± 0.40
FFA (mmol/mL)	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.01

Take home message

HPH improves insulin resistance and glucose intolerance in DIO mice

The effect might be a result of lowering weight gain, improve pancreatic beta cell function and/or peripheral tissue insulin sensitivity

Further studies are warranted to determine the responsible components (protein, peptides, AAs, FAs, antioxidants, etc.) and the underlying mechanisms

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Thank You

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