

## Supplementary Materials for

Unhealthy diets induce distinct and regional effects on intestinal inflammatory signalling pathways and long-lasting metabolic dysfunction in rats

Nogueira *et al*

Corresponding author: brunofonseca@ff.up.pt

**Includes:** Tables 1 to 3; Figs 1 to 3

Table S1. Statistics of energy and nutrient intake in rats fed with chow (CD), high-sugar (HSD) and Western (WD) diets.

Daily intake	CD		HSD		WD		p-value		
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	CD vs HSD	CD vs WD	HSD vs WD
Energy intake (Kcal)	63.86	3.71	54.45	1.34	120.29	30.74	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$
Protein energy intake (%)	25.95	0.19	13.62	0.48	3.90	0.42	$3.53 \times 10^{-4}$	$3.53 \times 10^{-4}$	$3.74 \times 10^{-4}$
Total carbohydrate energy intake (%)	64.58	0.26	81.42	0.65	22.96	3.22	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$
Carbohydrate intake (Kcal)	37.95	2.3	17.09	0.79	12.12	3.00	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$1.36 \times 10^{-3}$
Simple sugar intake (Kcal)	3.30	0.18	27.24	1.04	15.14	3.48	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$
Total fat energy intake (%)	9.47	0.07	4.96	0.17	73.14	3.58	$3.36 \times 10^{-4}$	$3.36 \times 10^{-4}$	$3.74 \times 10^{-4}$

Unsaturated fat intake (Kcal)	5.03	0.28	2.25	0.10	29.27	7.86	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$
Saturated fat intake (Kcal)	1.01	0.06	0.45	0.02	59.16	17.44	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$	$3.74 \times 10^{-4}$

Note: *p*-value of Mann-Whitney U test. *n* = 9 per group.

Table S2. Pearson's partial correlations analysis between adiposity index and nutrient intake.

Parameter	Daily intake	Pearson's r	p-value	Significant correlations, one-tailed
Adiposity index	Simple sugar intake (g)	0.56**	$1.57 \times 10^{-3}$	** $p < 0.01$
	Total fat energy intake (%)	0.64***	$2.08 \times 10^{-4}$	*** $p < 0.001$
	Unsaturated fat intake (g)	0.57**	$1.18 \times 10^{-3}$	** $p < 0.01$
	Saturated fat intake (g)	0.62***	$3.80 \times 10^{-4}$	*** $p < 0.001$
	Energy intake (Kcal)	0.48**	$6.20 \times 10^{-3}$	** $p < 0.01$
	Protein energy intake (%)	-0.85***	$1.53 \times 10^{-8}$	*** $p < 0.001$
	Total carbohydrate energy intake (%)	-0.49**	$5.40 \times 10^{-3}$	** $p < 0.01$
	Carbohydrate intake (g)	-0.86***	$1.11 \times 10^{-8}$	*** $p < 0.001$
	Dietary fibre intake (g)	-0.86***	$6.21 \times 10^{-9}$	*** $p < 0.001$

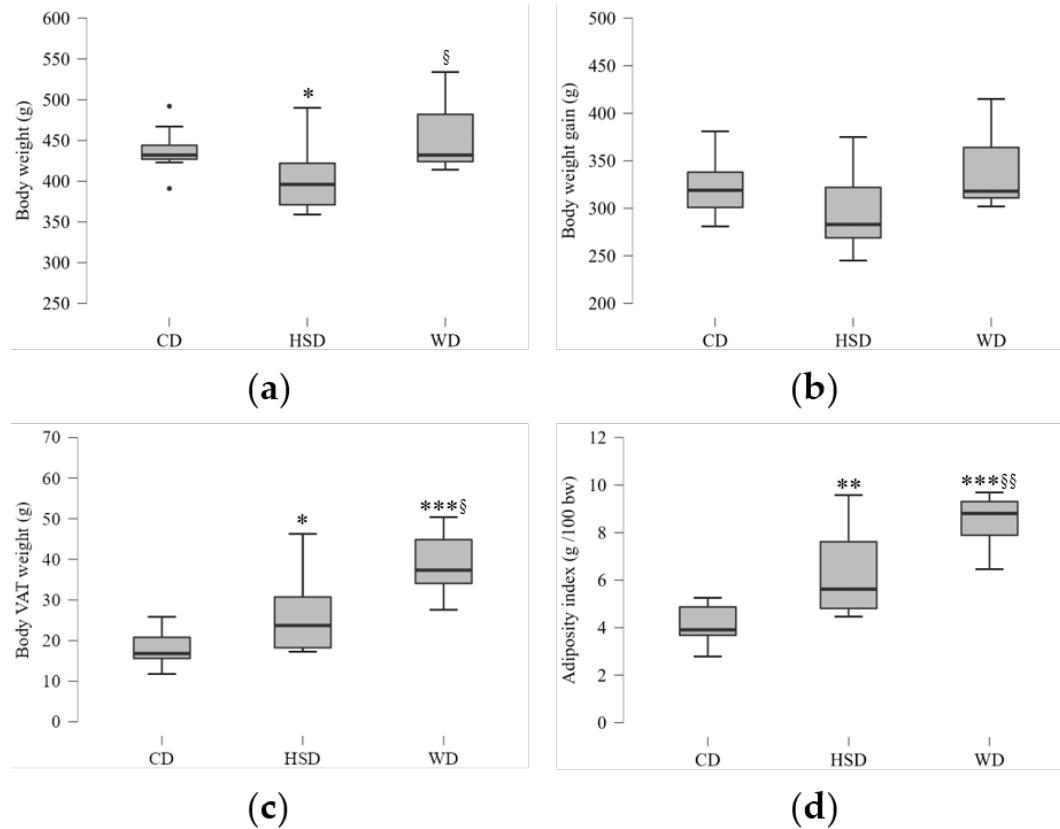
Note: Conditioned on variables: Body weight grain. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , one-tailed

Table S3. Pearson's partial correlation analysis between serum biochemical levels and nutrient intake.

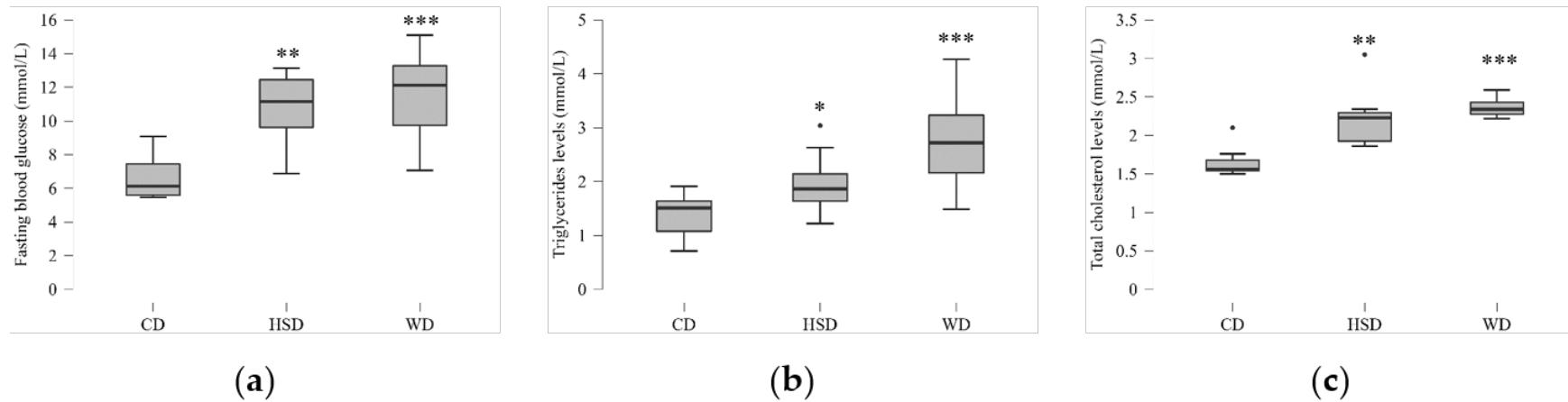
Serum parameter	Daily intake	Pearson's r	p-value	Significant correlations, one-tailed
Fasting blood glucose (mmol/L)	Simple sugar intake (g)	0.49**	$7.16 \times 10^{-3}$	** $p < 0.01$
	Total fat energy intake (%)	0.20	0.17	Ns
	Unsaturated fat intake (g)	0.24	0.13	Ns
	Saturated fat intake (g)	0.31	0.07	Ns
	Energy intake (Kcal)	0.24	0.87	Ns
	Protein energy intake (%)	-0.63***	$4.63 \times 10^{-4}$	*** $p < 0.001$
	Total carbohydrate energy intake (%)	-0.05	0.40	Ns
	Carbohydrate intake (g)	-0.63***	$4.30 \times 10^{-4}$	*** $p < 0.001$
	Dietary fibre intake (g)	-0.64***	$3.54 \times 10^{-4}$	*** $p < 0.001$
Triglyceride serum levels (mmol/L)	Simple sugar intake (g)	0.18	0.20	Ns
	Total fat energy intake (%)	0.34*	0.05	* $p < 0.05$
	Unsaturated fat intake (g)	0.34	0.05	Ns
	Saturated fat intake (g)	0.38*	0.04	* $p < 0.05$
	Energy intake (Kcal)	0.32	0.06	Ns
	Total carbohydrate energy intake (%)	-0.25	0.12	Ns
	Protein energy intake (%)	-0.49**	$7.24 \times 10^{-3}$	** $p < 0.01$
	Carbohydrate intake (g)	-0.35*	0.04	* $p < 0.05$
	Dietary fibre intake (g)	-0.43*	0.02	* $p < 0.05$

Total cholesterol levels (mmol/L)	Simple sugar intake (g)	0.21	0.34	Ns
	Total fat energy intake (%)	0.03	0.88	Ns
	Unsaturated fat intake (g)	-0.11	0.62	Ns
	Saturated fat intake (g)	-0.06	0.77	Ns
	Energy intake (Kcal)	-0.17	0.22	Ns
	Total carbohydrate energy intake (%)	0.04	0.43	Ns
	Protein energy intake (%)	-0.28	0.10	Ns
	Carbohydrate intake (g)	-0.38*	0.04	* p < 0.05
	Dietary fibre intake (g)	-0.33	0.06	Ns

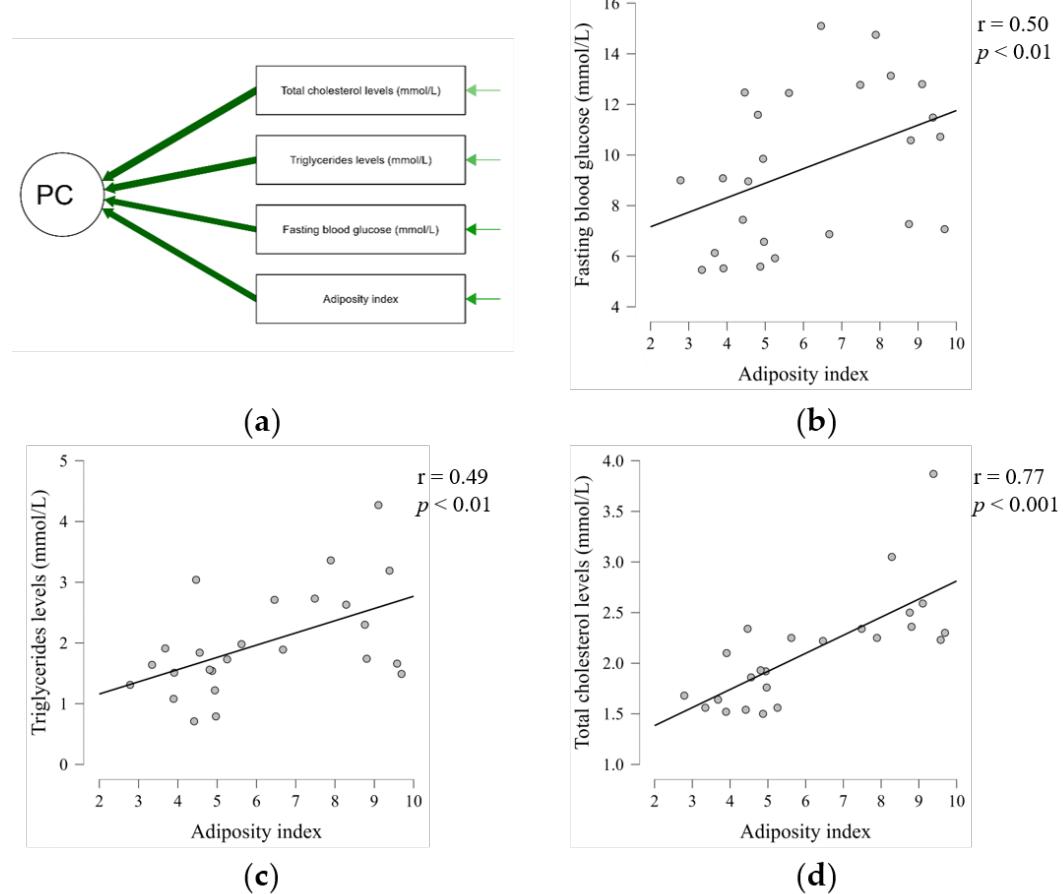
Note: Conditioned on variables: Adiposity index. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, one-tailed



**Figure S1.** Effects of dietary intervention on body weight, adipose tissue weight and adiposity. **(a)** Body weight after 14 weeks of feeding. **(b)** Total body weight gain. **(c)** Total visceral adipose tissue (VAT) weight. **(d)** Adiposity index determined as total VAT per 100 g of body weight (bw). Mean values  $\pm$  SD are plotted. Symbols indicate significant differences (one-way ANOVA) compared to CD-fed group: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and HSD-fed group: §  $p < 0.05$ , §§  $p < 0.01$  ( $n = 9$  rats per group).  $p$ -values reported for Welch statistic and using Dunn's test for multiple comparisons within each variable. CD, chow diet, HSD, high-sugar diet; WD, western diet.



**Figure S2.** Effects of dietary intervention on serum metabolic parameters of rats fed with chow (CD), high-sugar (HSD) and Western (WD) diets. Serum levels of fasting blood glucose (a), triglycerides (b) and total cholesterol (c) were significantly higher in both HSD and WD-fed rats. Mean values  $\pm$  SD are plotted. Symbol indicate significant differences (one-way ANOVA) compared to CD-fed group: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ( $n = 9$  rats per group).  $p$ -values reported for Welch statistic and using Dunn's test for multiple comparisons within each variable.



**Figure S3.** Correlation between serum metabolic parameters and adiposity index. **(a)** Path diagram of principal component (PC) used in Pearson correlation analysis. **(b)** Fasting blood glucose, **(c)** triglycerides and **(d)** total cholesterol levels correlated positively with adiposity.