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HEPATIC PROTEOME IS INFLUENCED BY DIETARY PROTEINS: MEAT *vs.* PLANT BASED MEAT ANALOGUES

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I. INTRODUCTION

Red meat has been harshly criticized in recent years owing to the alleged negative impact on health.[1] Certain consumers decide to replace red meat with plant-based alternatives produced from soy (tofu) and wheat gluten (seitan). According to previous research, the intake of these vegan ultraprocessed foods (UPFs) lead to dysbiosis, oxidative stress, inflammatory responses and adiposity.[2][3] In this paper, we aim to unveil some molecular mechanisms by looking at hepatic metabolome and proteome of *Wistar* rats fed with beef and the vegan UPFs.

II. MATERIALS AND METHODS

In compliance with the Helsinki Declaration and the regional Ethics Panel of Animal Experimentation (EXP-20200904), twenty-one *Wistar* rats were involved in the study. Three different diets (differed in protein source) were formulated from basal fed (14% of protein) with cooked beef (B), gluten wheat (i.e., seitan, S) and tofu (T), respectively, to reach high isoprotein (30%) and isocaloric diets, supplied *ad libitum* to the animals (n=7 per group) for 10 weeks. Euthanasia by exsanguination via cardiac puncture was applied to the rats at the end of the study, and the liver of the animals were dissected and suitably stored at -80ºC until respective analyses were carried out. Both metabolites and proteins were extracted from the hepatic tissues, and untargeted metabolomic and proteomic analyses were carried out using a Dionex UltiMate 3000 RSLC system coupled to a Q-Exactive high-resolution mass spectrometer (Thermo Fisher Scientific) as previously described.[2][4] Pathway analysis was carried out using hepatic metabolomic results in the MetaboAnalyst 6.0 web-based platform, and the results supported a target search through proteomic statistically pair-comparisons results.

III. RESULTS AND DISCUSSION

The consumption of vegan UPFs for 10 weeks promoted changes in energy-related metabolic pathways, such as glycolysis, pyruvate or citrate cycle pathways, among others (Figure 1). This might be related to the increased oxidative stress showed in previous studies in the gut of the animals provided with the plant-based diets,[2][3] and is in accordance with previous studies that linked altered energy metabolism with altered lipid hepatic deposition and metabolic diseases.[5] The proteomic results strengthen the above-mentioned metabolomic results. Only an example of the multiple altered amounts of hepatic proteins resulted from the proteomic analysis is shown in Table 1.

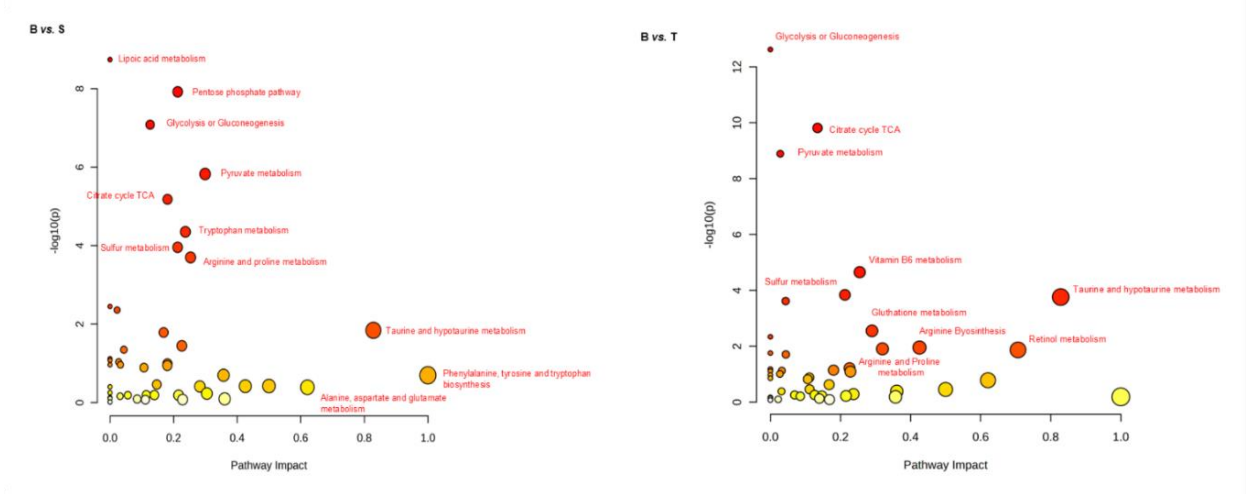


Figure 1. Enrichment pathway analysis plots comparing metabolites from B vs. S (left) and B vs. T (right)[1].

[1]The metabolome view in Figure 1 shows all matched pathways according to the *p* values from pathway enrichment analysis and the pathway impact values from a pathway topology analysis performed using MetaboAnalyst 6.0. About node colors, ranging from yellow to red, mean varying levels of statistical significance. Node sizes indicate the relative pathway impact.

Table 1. Glycolytic hepatic proteins levels changed after a 10-weeks consumption of different protein-source diets (i.e., beef (B), seitan (S) and tofu (T)).

Protein name	Gene name	FASTA accesion number	Fold change	*p value*	Fold change	*p value*
Glycolysis or Gluconeogenesis			B *vs.* S		B *vs.* T	
Aldehyde dehydrogenase 3 family, member A2	*Aldh3a2*	P30839	0.66	***	-	-
Aldehyde dehydrogenase 9 family, member 1	*Aldh9a1*	A0A0G2JSI1	1.53	***	1.20	***
Aldehyde dehydrogenase 1 family, member B1, mitoch	*Aldh1b1*	G3V7I5	1.68	***	0.76	*
Alpha-aminoadipic semialdehyde dehydrogenase	*Aldh7a1*	Q64057	1.53	***	1.33	**
Aldehyde dehydrogenase, mitochondrial	*Aldh2*	P11884	1.46	***	4.40	***
Dihydrolipoyl dehydrogenase, mitocondrial	*Dld*	Q6P6R2	1.59	***	1.47	***
Pyruvate dehydrogenase E1 component subunit beta, mitoch	*Pdhb*	P49432	0.91	**	0.78	***
L-lactate dehydrogenase A chain	*Ldha*	P04642	1.66	***	1.50	***
Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	*Pck1*	P07379	1.66	***	1.22	*
Fructose-1,6-bisphosphatase 1	*Fbp1*	F1LRT1	1.27	***	-	-
Fructose-bisphosphate aldolase A	*Aldoa*	P05065	1.55	**	1.32	*
Fructose-bisphosphate aldolase	*Aldob*	Q66HT1	1.32	***	2.67	*
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	P04797	1.36	***	3.94	**
Glucose-6-phosphate isomerase	*Gpi*	Q6P6V0	1.62	***	1.22	*
Phosphoglycerate kinase 1	*Pgk1*	P16617	1.35	***	1.14	**

The relevance of the modified quantities of glycolytic proteins such as aldehyde dehydrogenase family of proteins, related also with lipid metabolism and protection against oxidative stress, deserve special attention as key molecular regulators of the hepatic negative changes associated with UPFS consumption previously highlighted by our group.[3][4]

IV. CONCLUSION

The impact of dietary proteins on the liver indicates that protein composition and structure, potentially affected by processing degree and intensity, encodes protein functionality and health outcomes, which is manifested in hepatic function. These results strengthen the allegations made by the World Health Organization (WHO) on the potential health risks of the sustained intake of plant-based UPFs.

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