PIVOTAL ASSESSMENT OF THE EFFECTS OF BIOACTIVES ON HEALTH AND WELLBEING. FROM HUMAN GENOMA TO FOOD INDUSTRY.

DIETARY BIOACTIVE COMPOUNDS AS POSSIBLE AGENTS FOR THE REGULATION OF LIPID METABOLISM

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INTRODUCTION

Metabolic syndrome (MS) is a risk factor for cardiovascular disease and mortality, and its incidence is increasing worldwide as a consequence of the continued obesity epidemic [1]. The liver plays a central role in whole body lipid homeostasis and dyslipidemia is a component of MS and an important independent risk factor for insulin resistance [2]. The modulation of lipid metabolism by food bioactives represents a possible approach to the nutritional prevention of MS. Indeed many naturally-occurring compounds in dietary plants and animal products are being studied intensively for their possible contribution toward reducing the risk of many diet related-diseases. **In this study, an array of assays was developed to detect the influence of bioactive compounds on lipid composition and metabolism in hepatic cultured cells and to understand their cross-talk at the cellular level and their role in the aetiology of MS.**

METHODS

Three model bioactives were tested: i. **docosahexaenoic acid (DHA**), a long-chain omega-3 fatty acid; ii. **propionate (PRO),** a short chain fatty acid deriving from the colonic microbiota fermentation of beta-glucans; iii. **protocatechuic acid (PCA**), the main *in vivo* metabolite of anthocyanins. PRO and PCA were supplemented alone or in combination with DHA to investigate their possible synergistic, antagonistic or neutral effects. Once established non-cytotoxic concentrations within the physiological range (DHA 50 µM, PA 70 µM, PCA 20 µM), the selected bioactives or their combinations were supplemented to HepG2 cells for 6 and 24 h and their effect on **fatty acids composition and content** (GC), **lipid accumulation** (Oil Red O assay), **cell triglycerides content**, **extracellular and intracellular cholesterol concentration**, and **apolipoprotein secretion** (commercial kits) was evaluated.

RESULTS

PRO and PCA supplementation for 24 h could reduce the fatty acid content of cultured liver cells, with slight modification in the fatty acid profile. On the contrary DHA supplementation did not affect the total fatty acid content, while it significantly increased the fatty acid pattern and the UI of cell lipids (**Table 1**).

Cell treatment with PRO and PCA for 24h caused a significant reduction in lipid accumulation, whereas cell treatment with DHA alone or in combination with PCA caused a significant increase in lipid accumulation compared to C cells (**Fig. 1**).

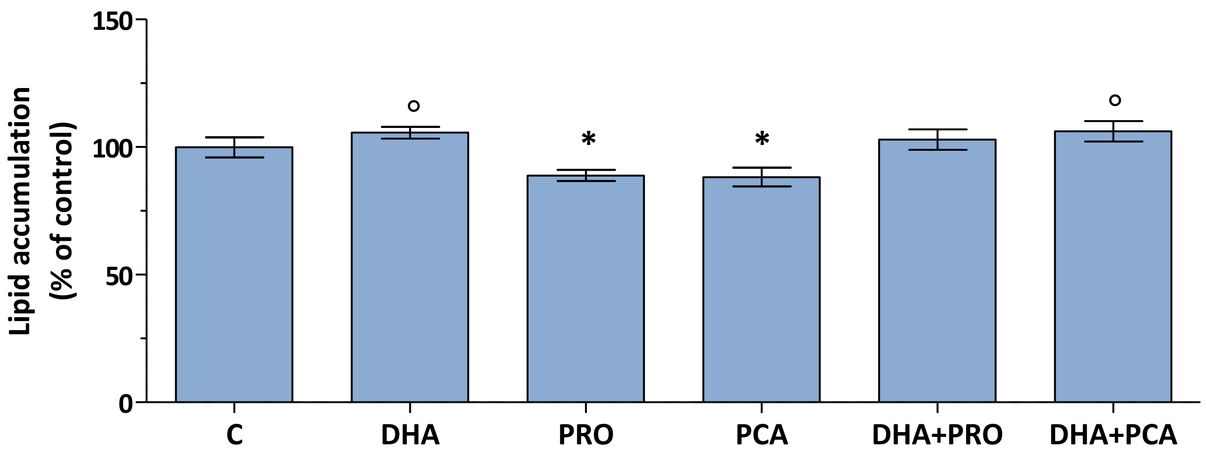

*Fig. 1. Lipid accumulation after 24 h supplementation. Data are expressed as the percentage of the value obtained in C. Statistical analysis was by the one-way ANOVA using Dunnett's post-test (° p<0.01; * p<0.001).*

	C	50 µM DHA	70 µM PRO	20 µM PCA	50 µM DHA + 70 µM PRO	50 µM DHA+ 20 µM PCA
3:0	0.68 ± 0.3	1.12 ± 1.15	0.66 ± 0.19	1.17 ± 1.24	1.18 ± 1.48	0.71 ± 0.48
14:0	3.11 ± 0.15	3.37 ± 0.82	3.54 ± 0.98	3.76 ± 0.66	3.85 ± 0.36	3.68 ± 0.4
16:0	29.85 ± 1.18	28.45 ± 2.13	29.06 ± 1.89	29.62 ± 0.99	28.09 ± 1.02	28.34 ± 0.52
16:1n-7	4.62 ± 0.83	3.89 ± 0.43	5.7 ± 1.05	5.62 ± 1.01	4.24 ± 0.21	4.05 ± 0.32
18:0	27.1 ± 5.36	21.6 ± 3.97	18.15±3.63*	17.48±1.72°	17.65±2.4#	17.4 ± 3.49°
18:1n-9	17.16 ± 2.47	14.48±1.25#	21.41 ± 0.8°	21.08±0.83°	15.53 ± 0.8	15.45 ± 1.04
18:1n-7	10.88 ± 1.63	7.78 ± 0.94*	13.63±0.24	13.68±0.43*	8.42 ± 0.47°	8.2 ± 0.6°
18:2n-6	0.82 ± 0.18	0.41 ± 0.05*	0.96 ± 0.08	0.94 ± 0.13	0.44 ± 0.02*	0.41 ± 0.02*
18:3n-3	0.86 ± 0.16	0.66 ± 0.06#	1.05 ± 0.09#	1.06 ± 0.11#	0.68 ± 0.05	0.7 ± 0.06
20:4n-6	1.6 ± 0.26	1.39 ± 0.39	2.15 ± 0.79	2.12 ± 0.11	1.87 ± 0.36	1.98 ± 0.46
20:5n-3	0.69 ± 0.6	0.38 ± 0.44	0.71 ± 0.84	0.64 ± 0.82	0.25 ± 0.42	0.63 ± 0.48
22:6n-3	2.62 ± 0.81	16.48 ± 3.01*	2.99 ± 0.96	2.83 ± 0.75	17.81 ± 1.11*	18.44 ± 0.82*
UI	57.87±11.98	131.35±21.71*	70.17±7.21	68.48±8.98	142.43±7.55*	148.31±8.35*
n-3/n-6	1.73 ± 0.65	10.2 ± 3.57*	1.68 ± 0.98	1.49 ± 0.59	8.24 ± 1.04*	8.49 ± 1.63*
Σ PUFA	6.6 ± 1.58	19.31 ± 3.38*	7.86 ± 1.33	7.59 ± 1.49	21.05 ± 1.16*	22.16 ± 1.27*
Total content (µg/10^6 cells)	52.83 ± 7.2	51.09 ± 6.78	32.73±4.84*	31.56±6.67*	50.24±7.78	56.88±12.36

*Table 1. Fatty acids composition and content after 24 h supplementation. Statistical analysis was by the one-way ANOVA using Dunnett's post-test (# p<0.05; ° p<0.01; * p<0.001). UI=unsaturation index.*

After 24 h supplementation a significant decrease in cholesterol content was detected in DHA supplemented cells compared to C (**Fig. 2**).

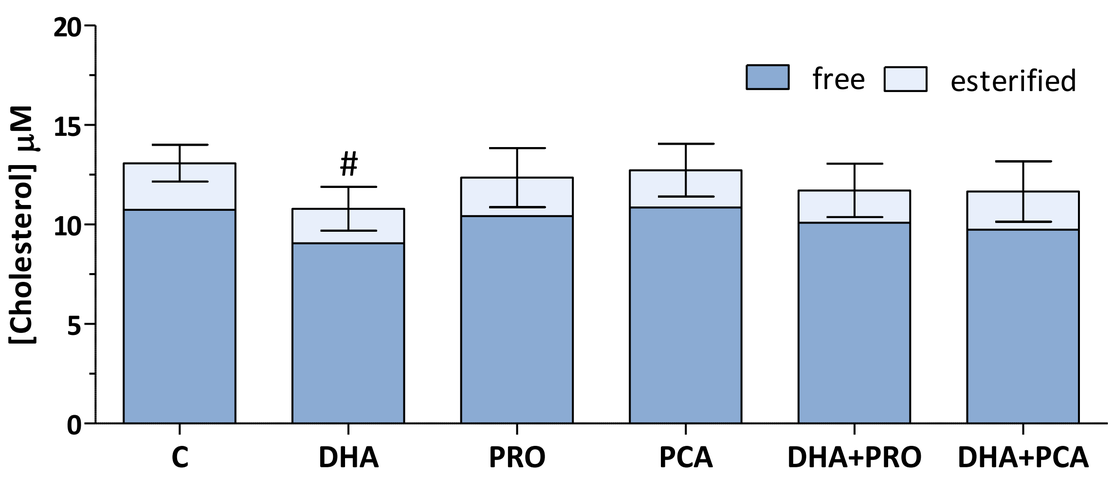

Fig. 2. Cholesterol concentration after 24 h supplementation. Statistical analysis was by the one-way ANOVA using Dunnett's post-test (# p<0.05).

The main results are summarized in **Table 2**.

	6h					24h				
	DHA	PRO	PCA	DHA + PRO	DHA + PCA	DHA	PRO	PCA	DHA + PRO	DHA + PCA
Fatty acids content	-	-	-	-	-	-	-40%	-40%	-	-
Lipid accumulation	+15%	-	-	-	-	+5%	-12%	-12%	-	+6%
Intracellular cholesterol	-	-	-	-	-	-13%	-	-	-	-
Intracellular TG content	+7%	-	-	+8%	+12%	-	-	-	-	-
Extracellular cholesterol	-		-38%	-	-33%	-	-29%	-41%	-	-44%

Table 2. % modification compared to C cells. Only statistically significant differences are reported.

ApoAI secretion was reduced in all tested conditions after 24 h (**Fig. 3A**), and in PCA supplemented cell after 6 h supplementation (data not shown). ApoB was significantly increased in DHA+PRO and in DHA+PCA supplemented cells after 6 h supplementation (data not shown), whereas it was significantly decreased in cells exposed to PRO after 24 h supplementation (**Fig. 3B**).

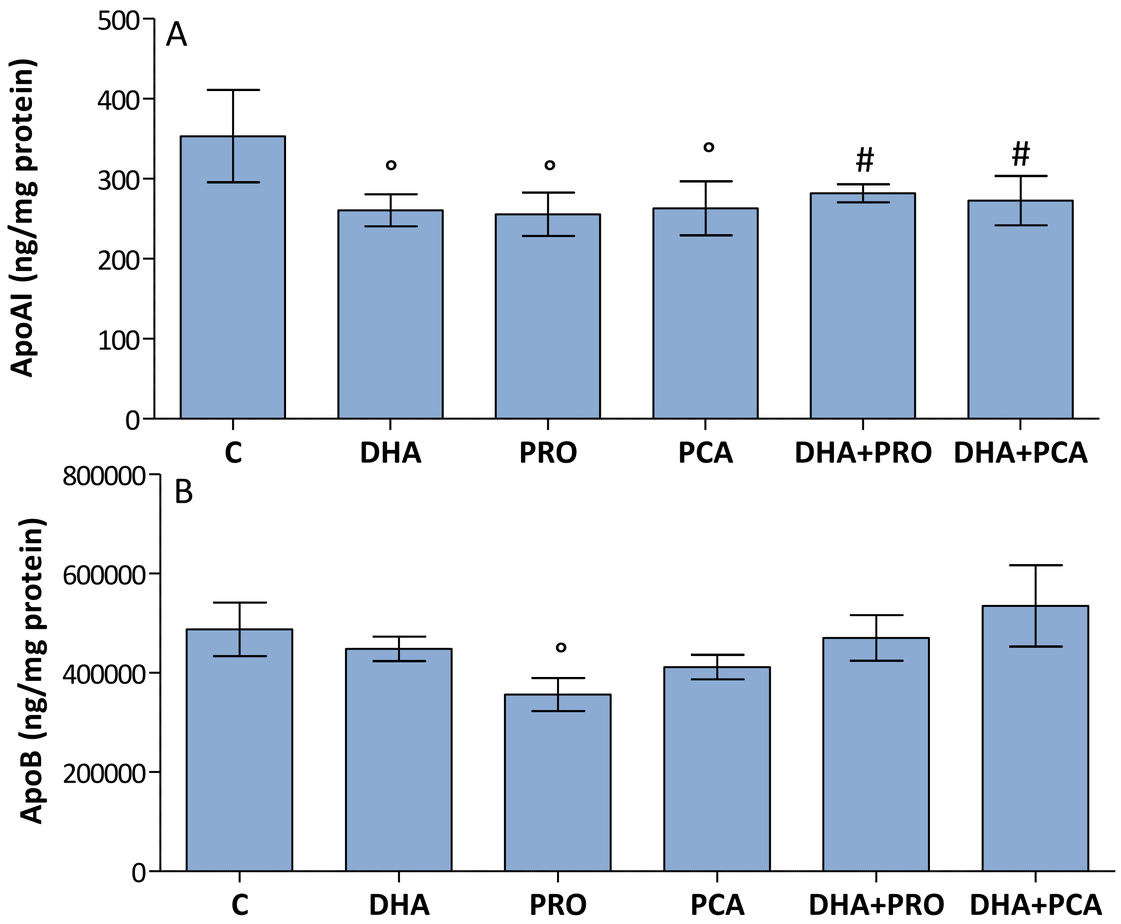

Fig. 3. ApoAI (A) and ApoB (B) concentration in the media after 24h supplementation. Statistical analysis was carried out by the one-way ANOVA with Dunnett's test for comparison with C (p<0.05, ** p<0.01).*

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Future in depth investigations will elucidate molecular mechanism of the selected bioactives in the liver using proper omics techniques.

Identifying bioactives, establishing their mechanisms of actions and health effects are all active areas of scientific inquiry and, through industrial exploitation, can lead to potential societal benefit. In particular the approach set in this study appears essential for the developing of new nutritional strategies in the prevention and treatment of MS.

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The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 311876: Pathway-27 - Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry