APPENDIX A - SUPPLEMENTAL FIGURES AND TABLES:

Oleocanthal-rich extra virgin olive oil demonstrates acute anti-platelet effects in healthy men in a randomized trial

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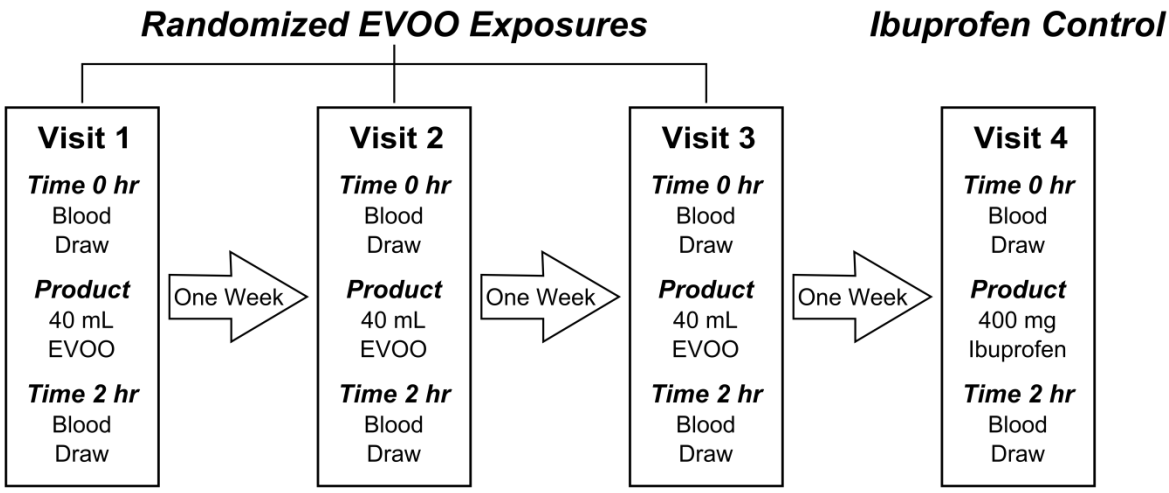

Fig. A.1. Schematic of the blinded randomized controlled crossover study testing the acute effects of ingesting three unique EVOOs on platelet aggregation. All EVOOs were matched in total phenols, but varied in their tyrosol, oleocanthal and oleacein content. Study participants (n = 9) were healthy men, aged 25 ± 4 yr with BMI of 25.5 ± 4.1 kg/m^2. Throughout the study, participants were asked to refrain from consuming olive products and non-steroidal anti-inflammatory drugs, and 24h before each study visit, participants were asked to maintain a low flavonoid diet. Plasma collected at each of the two timepoints was subjected to optical platelet aggregometry and oxylipin analysis.

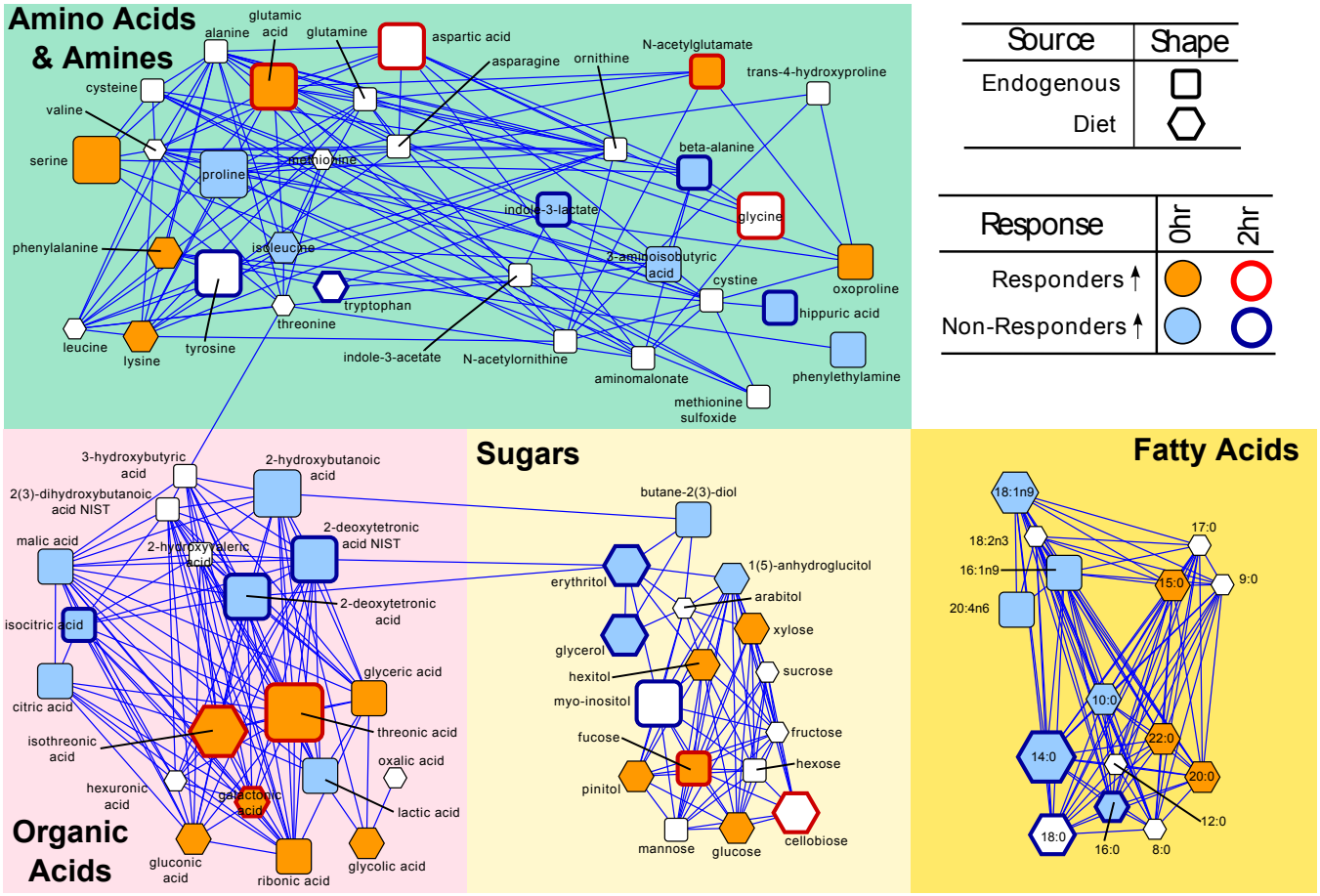

Fig. A.2. Biochemical network map showing detected changes in metabolites associated with primary metabolism in subjects before and after EVOO consumption. Metabolites are clustered and connected based on known biochemical pathways and structural similarity. Subjects that "responded" to EVOO intake had increased baseline and postprandial plasma concentrations of carbohydrates and sugar acids suggesting a diet rich in soy, fruits and vegetables. Subjects that did not "respond" to EVOO intake had increased baseline and postprandial plasma concentrations of non-esterified fatty acids (NEFA) and the citric acid cycle metabolites malic acid, isocitric acid and citric acid suggesting a diet rich in fats and oils. Increases in metabolites associated with "responders" were characterized by Variable Importance in Projection (VIP) scores > 1 based on a Partial Least Squares-Discriminant Analysis (PLS-DA) of the metabolite data whereas increases in "non-responders" were characterized by VIP scores < -1 in the same PLS-DA.

Table A.1

Table A.1
Selected baseline characteristics of study participants assessed at the screening visit (n = 9).

Parameter	Value
Age (years)	26 ± 4
BMI (kg/m^2)	25.5 ± 4.1
Blood Glucose (mg/dL)	93 ± 11
Cholesterol (mg/dL)	187 ± 35
HDL (mg/dL)	52 ± 18
LDL (mg/dL)	113 ± 34
Triglycerides (mg/dL)	117 ± 59
White Blood Cell Count ($x10^3/mm^3$)	6 ± 1.6
Red Blood Cell Count ($x10^3/mm^3$)	5 ± 0.4
Platelet Count ($x10^3/mm^3$)	222 ± 51
Mean Platelet Volume (μm^3)	9 ± 1.4

Data reported as mean ± SD

Table A.2

Table A.2
Range-scaled changes in oxylipins screened for and detected in the 1 μg/mL collagen-stimulated plasma of subjects (n = 9) following consumption of extra-virgin olive oils.

Oxylipin	Fatty Acid Precursor	Change in Concentration (nM)			*P*
		$D2_i0$	$D2_i2$	$D2_i0.5$	
Alcohols					
9-HODE	C18:2n6	0.518 ± 0.593 ^{A}	0.00729 ± 0.375 ^{B}	0.419 ± 0.482 ^{A,B}	**0.047**
13-HODE	C18:2n6	0.609 ± 0.606 ^{A}	0.0773 ± 0.272 ^{B}	0.478 ± 0.383 ^{A}	**0.022**
9-HOTE	C18:3n3	0.635 ± 0.633 ^{A}	0.00032 ± 0.144 ^{B}	0.36 ± 0.369 ^{A}	**0.008**
13-HOTE	C18:3n3	0.623 ± 0.628 ^{A}	0.0679 ± 0.114 ^{B}	0.401 ± 0.364 ^{A}	**0.040**
5-HETE	C20:4n6	0.121 ± 0.37 ^{A,B}	-0.188 ± 0.442 ^{B}	0.373 ± 0.605 ^{A}	**0.046**
8-HETE	C20:4n6	0.181 ± 0.304	-0.265 ± 0.542	0.35 ± 0.657	0.13
9-HETE	C20:4n6	0.0604 ± 0.605	-0.125 ± 0.256	0.187 ± 0.514	0.3
11-HETE	C20:4n6	0.0836 ± 0.452	-0.29 ± 0.621	0.0951 ± 0.394	0.2
12-HETE	C20:4n6	0.0673 ± 0.454	-0.299 ± 0.592	0.00844 ± 0.312	0.3
15-HETE	C20:4n6	0.0375 ± 0.498	-0.288 ± 0.561	0.0886 ± 0.396	0.2
20-HETE	C20:4n6	ND	ND	ND	
5-HEPE	C20:5n3	0.0479 ± 0.3	-0.0159 ± 0.408	0.351 ± 0.49	0.2
9-HEPE	C20:5n3	-0.0725 ± 0.434	-0.184 ± 0.434	0.147 ± 0.49	0.3
12-HEPE	C20:5n3	0.0302 ± 0.444	-0.205 ± 0.594	0.0951 ± 0.37	0.4
15-HEPE	C20:5n3	0.171 ± 0.426	-0.225 ± 0.498	0.274 ± 0.457	0.09
4-HDoHE	C22:6n3	ND	ND	ND	
14-HDoHE	C22:6n3	0.0411 ± 0.547	-0.191 ± 0.735	0.158 ± 0.433	0.5
17-HDoHE	C22:6n3	-0.209 ± 0.585	ND	ND	

Data points with unlike letters were significantly different at $P < 0.05$ (repeated measures ANOVA). All values reported as mean ± SD. Bold text highlights p-values < 0.05

Abbreviations: $D2_i0$, EVOO with undetectable oleocanthal and oleacein; $D2_i2$, EVOO with 2:1 oleacein/oleocanthal; $D2_i0.5$, EVOO with 1:2 oleacein/oleocanthal; ND, Not Detected.

[a] Analytes are evaluated on a semi-quantitative basis due to lack of authentic standards

[b] Concentrations calculated relative to concentrations of authentic 9,12,13-TriHOME standards

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		D2$_i$0	*D2$_i$2*	*D2$_i$0.5*	
Diols					
9,10-DiHHex [a]	C16:0	ND	ND	ND	
9,10-e-DiHO	C18:0	0.108 ± 0.459	0.00247 ± 0.593	0.282 ± 0.48	0.5
9,10-DiHOME	C18:2n6	0.119 ± 0.489	0.0884 ± 0.474	0.0868 ± 0.67	1.0
12,13-DiHOME	C18:2n6	-0.112 ± 0.34	-0.218 ± 0.45	-0.053 ± 0.571	0.7
9,10-DiHODE	C18:3n3	0.226 ± 0.582	0.0569 ± 0.451	0.0435 ± 0.658	0.6
12,13-DiHODE	C18:3n3	-0.0526 ± 0.673	0.113 ± 0.619	0.0655 ± 0.683	0.8
15,16-DiHODE	C18:3n3	-0.22 ± 0.423	-0.181 ± 0.486	-0.0504 ± 0.632	0.6
5,6-DiHETrE	C20:4n6	-0.0116 ± 0.462	0.054 ± 0.448	0.0804 ± 0.456	0.9
8,9-DiHETrE	C20:4n6	0.238 ± 0.597	0.036 ± 0.538	0.273 ± 0.468	0.3
11,12-DiHETrE	C20:4n6	0.353 ± 0.267	0.241 ± 0.578	0.288 ± 0.506	1.0
14,15-DiHETrE	C20:4n6	0.346 ± 0.398	0.0663 ± 0.728	0.31 ± 0.417	0.6
5,15-DiHETE	C20:5n3	ND	ND	ND	
8,15-DiHETE	C20:5n3	ND	ND	ND	
14,15-DiHETE	C20:5n3	-0.0772 ± 0.493	0.0471 ± 0.476	0.104 ± 0.554	0.8
17,18-DiHETE	C20:5n3	0.247 ± 0.301	0.145 ± 0.559	0.112 ± 0.447	0.8
19,20-DiHDoPA	C22:6n3	0.165 ± 0.372	0.0573 ± 0.671	0.402 ± 0.389	0.14

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Abbreviations: D2$_i$0, EVOO with undetectable oleocanthal and oleacein; D2$_i$2, EVOO with 2:1 oleacein/oleocanthal; D2$_i$0.5, EVOO with 1:2 oleacein/oleocanthal; ND, Not Detected.

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		D2$_i$0	*D2$_i$2*	*D2$_i$0.5*	
Epoxides					
9,10-EpO	C18:0	0.00691 ± 0.587	-0.0836 ± 0.486	0.0671 ± 0.686	0.8
9,10-EpOME	C18:2n6	-0.0836 ± 0.571	-0.302 ± 0.492	0.241 ± 0.428	0.07
12,13-EpOME	C18:2n6	-0.177 ± 0.464	-0.204 ± 0.377	0.092 ± 0.519	0.2
9,10-EpODE	C18:3n3	ND	0.00659 ± 0.811	ND	
12,13-EpODE	C18:3n3	0.163 ± 0.717	-0.171 ± 0.483	ND	
15,16-EpODE	C18:3n3	-0.152 ± 0.503	-0.0881 ± 0.627	0.0111 ± 0.518	0.9
8,9-EpETrE	C20:4n6	ND	ND	ND	
11,12-EpETrE	C20:4n6	-0.0993 ± 0.472	-0.0856 ± 0.636	0.589 ± 0.436	0.2
14,15-EpETrE	C20:4n6	-0.154 ± 0.737	ND	ND	
11,12-EpETE	C20:5n3	-0.441 ± 0.634	-0.0221 ± 0.669	0.248 ± 0.489	0.08
14,15-EpETE	C20:5n3	ND	ND	0.347 ± 0.509	
17,18-EpETE	C20:5n3	ND	ND	ND	
16,17-EpDPE	C22:6n3	0.164 ± 0.298	0.0547 ± 0.644	-0.015 ± 0.55	0.6
19,20-EpDPE	C22:6n3	0.0411 ± 0.783	0.197 ± 0.679	0.302 ± 0.575	0.8

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		D2$_i$0	*D2$_i$2*	*D2$_i$0.5*	
Hydroperoxides					
9-HpODE [a]	C18:2n6	-0.128 ± 0.464	0.000982 ± 0.75	-0.112 ± 0.672	0.9
13-HpODE [a]	C18:2n6	-0.192 ± 0.561	-0.304 ± 0.541	ND	0.6
5-HpETE [a]	C20:4n6	-0.18 ± 0.677	0.138 ± 0.534	0.0967 ± 0.556	0.6
12-HpETE [a]	C20:4n6	ND	0.0174 ± 0.692	ND	
15-HpETE [a]	C20:4n6	ND	0.187 ± 0.755	0.00272 ± 0.601	0.9
Ketones					
9-KODE	C18:2n6	0.189 ± 0.512	-0.0275 ± 0.302	0.431 ± 0.38	0.10
13-KODE	C18:2n6	0.363 ± 0.388 [A]	0.220 ± 0.292 [A,B]	0.742 ± 0.27 [C]	**0.044**
12,13-Ep-9-KODE	C18:3n3	ND	ND	ND	
5-KETE	C20:4n6	ND	ND	ND	
15-KETE	C20:4n6	0.161 ± 0.399	-0.15 ± 0.561	-0.033 ± 0.689	0.2
Leukotrienes					
6-trans-LTB4	C20:4n6	ND	ND	ND	
LTB4	C20:4n6	ND	ND	ND	
LTB5	C20:5n3	ND	ND	ND	
Nitrolipids					
9-Nitrooleate	C18:1n9	ND	ND	ND	
10-Nitrooleate	C18:1n9	-0.0246 ± 0.788	0.232 ± 0.452	0.412 ± 0.419	0.5
10-Nitrolinoleate	C18:2n6	0.00386 ± 0.563	-0.462 ± 0.441	ND	

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Prostanoids					
PGE1	C20:3n6	ND	ND	ND	
PGD2	C20:4n6	ND	ND	ND	
PGE2	C20:4n6	0.0769 ± 0.498	-0.231 ± 0.574	0.17 ± 0.44	0.2
15-keto PGE2	C20:4n6	ND	ND	ND	
6-keto PGF1a	C20:4n6	0.171 ± 0.576	0.093 ± 0.772	0.338 ± 0.298	0.8
PGF2a	C20:4n6	0.0941 ± 0.272	-0.311 ± 0.56	0.133 ± 0.468	0.05
15-deoxy PGJ2	C20:4n6	0.0297 ± 0.494	-0.12 ± 0.587	0.305 ± 0.441	0.05
TXB2	C20:4n6	0.0596 ± 0.457	-0.308 ± 0.616	0.0718 ± 0.373	0.2
PGE3	C20:5n3	ND	ND	ND	
PGF3a	C20:5n3	ND	ND	ND	
Triols					
Sum TriHOMEs [b]	C18:2n6	0.414 ± 0.518 [A]	-0.021 ± 0.513 [B]	0.560 ± 0.327 [A]	**0.020**
Lipoxin A4	C20:4n6	ND	ND	ND	
Resolvin D1	C20:4n6	ND	ND	ND	

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