Effects of extra virgin olive oil oleocanthal and oleacein content on platelet reactivity in healthy adults

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Introduction

- Cardiovascular disease (CVD) is a chronic inflammatory disease thought to be propagated by platelet-dependent processes.
- CVD risk reduction strategies include anti-platelet therapeutics and flavonoid-rich food consumption.
- Extra virgin olive oil (EVOO) contains >30 phenolic compounds with antioxidant, anti-inflammatory and platelet modulating properties including various tyrosols, and the secoiridoids oleocanthal and oleacein **(Figure 1)**. The relative abundances of these phenolics vary among olive varieties.
- Oleocanthal is an *in vitro* cyclooxygenase (COX) inhibitor [1] and thus may have anti-platelet activity *in vivo*, while oleacein is a 5-lipoxygenase inhibitor with anti-inflammatory properties [2].
- By comparing postprandial platelet reactivity following acute intake of total phenolic matched but oleocanthal-rich or -poor EVOOs, we will **test the hypothesis** that:
  1. **Platelet responses track oleocanthal doses, but not the total phenolic content of the consumed EVOOs.**
  2. **Reduction in COX-dependent oxylipins will correlate with platelet inhibition.**

Methods

Experimental Oil Selection and Characterization:

- EVOO samples were obtained from olives harvested between November 2014 and January 2015. Oils were initially screened by quantitative ^{1}H NMR (qNMR) to determine their oleocanthal, oleacein, oleuropeinaglycon and ligstrosideaglcon content and choose the samples for clinical study [3].
- EVOO total phenolic contents of selected oils were determined by the Folin-Ciocalteu method [4] and the non-secoiridoid phenolic compounds were measured using HPLC-UV. Briefly, phenolics were resolved by reverse phase LC and identified by published relative retention times. Concentrations were estimated using a calibration curve established with the absorbance of *p*-hydroxyphenylacetic acid (IS), and normalized to the IS response.
- EVOOs chosen for inclusion in the study were an oil of Mediterranean origin obtained from a supermarket in California (Oil A), an Arbequina variety oil provided by Corto Company from California (Oil B), and a Koroneiki variety oil from Kalamata, Greece (Oil C).

Clinical Studies and Plasma Analyses:

- Subjects (n=9) healthy males (20-40 yrs) were enrolled in a randomized, blinded, controlled crossover design to consume 40 mL of three EVOOs containing varying levels of oleocanthal and oleacein but matched in total phenolics (Oils A, B and C) with Ibuprofen (400 mg *p.o.*) as positive control on the fourth study visit.
- Plasma was collected by venipuncture immediately before and two hours after EVOO or Ibuprofen consumption.
- Platelet-rich plasma was stimulated by 1 µg/mL or 3 µg/mL collagen and:
  - Subjected to optical platelet aggregometry using a modified version of the method by Born and Cross [5]. Only low dose results are presented.
  - Analyzed by LC-MS/MS to quantify oxygenated lipids (i.e. oxylipins) derived from cyclooxygenase, lipoxygenase, and cytochrome P450 dependent metabolism of polyunsaturated fatty acids [6].

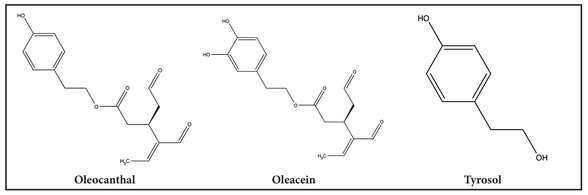

Figure 1. EVOO phenolics used as experimental oil primary selectors.

Table 1. EVOO phenolic profiles quantified by ^{1}H NMR.

Phenolic	Concentration (mg/kg)		
	Oil A	Oil B	Oil C
Tyrosol	189 ± 10	< 10	< 10
Dialdehydic Form of Oleuropein Aglycone (DAFOA)	< 10	< 10	< 10
Dialdehydic Form of Ligstroside Aglycone (DAFLA)	< 10	< 10	< 10
Aldehydic Form of Oleuropein Aglycone (AFOA)	25 ± 2	5 ± 1	23 ± 2
Aldehydic Form of Ligstroside Aglycone (AFLA)	21 ± 2	5 ± 1	10 ± 1
Oleocanthal	< 10	172 ± 8	310 ± 15
Oleacein	< 10	312 ± 15	150 ± 8
D1 Index [a]	–	484	460
D2 Index [b]	–	1.8	0.48

[a] D1 Index refers to the sum of an oil's oleocanthal and oleacein concentrations

[b] D2 Index = oleocanthal/oleacein ratio

Table 2. EVOO phenolic relative abundance determined by HPLC-UV.

Phenolic	Relative Abundance [a]		
	Oil A	Oil B	Oil C
Olive Variety	Mediterranean	Aberquina	Koroneiki
Tyrosol	19	1	3
Hydroxytyrosol	13	3	5
Vanillic Acid	0	3	1
p-Coumaric Acid	1	5	0
Ferullic Acid	0	0	0
1-Acetoxypinoresinol	5	22	8
Luteolin	2	4	2
Apigenin	1	1	1

[a] The reported relative abundance is the internal standard normalized absorbance of the individual compound divided by the sum of normalized phenolics in all three oils

Table 3. Regression modeling of platelet aggregation and oxylipin production inhibition as a function of oleocanthal, oleacein and tyrosol dose (mg/kg body weight) in all subjects (n = 9 subjects x 3 treatments = 27).

Dependent Variable	R^2	Int	Oleocanthal			Oleacein			Tyrosol		
		(S.E.)	B (S.E.)	β	*p*	B (S.E.)	β	*p*	B (S.E.)	β	*p*
Δ Max Platelet Aggregation	0.487	1.19 (0.40)	-8.22 (2.09)	-42.9	0.0007	-4.30 (1.86)	-20.0	0.0298	-6.68 (2.47)	-41.2	0.0127
Δ [Oxylipins] [a]	0.375	1.25 (0.44)	-4.65 (2.26)	-23.9	0.0516	-7.28 (2.02)	-33.4	0.0015	-7.35 (2.69)	-44.9	0.0118

[a] Sum of changes in cyclooxygenase- and lipoxygenase-derived oxylipins with known roles in platelet aggregation: thromboxane B2; prostaglandin E2; 11-, 12- and 15-hydroxyeicosatetraenoic acid

Results

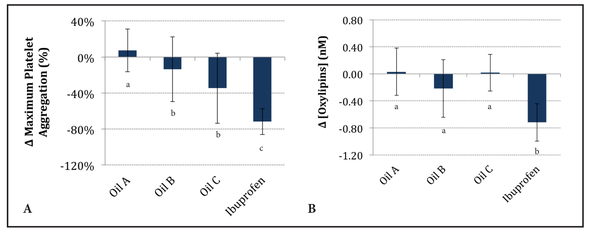

Figure 2. Effects of tested EVOOs on maximum platelet aggregation (**A**) and oxylipins associated with platelet function (**B**) in healthy male subjects. Oils B and C and Ibuprofen all decreased maximum platelet aggregation compared to Oil A, and Ibuprofen decreased oxylipin concentrations compared to all oils ($p < 0.05$, repeated measures ANOVA).

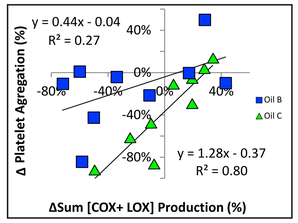

Figure 3. Correlations between inhibition of collegen-stimulated platelet aggregation and oxylipin production differ in effective oils. Platelet aggregation and oxylipin production inhibition are significantly correlated in Oil C, but not Oil B.

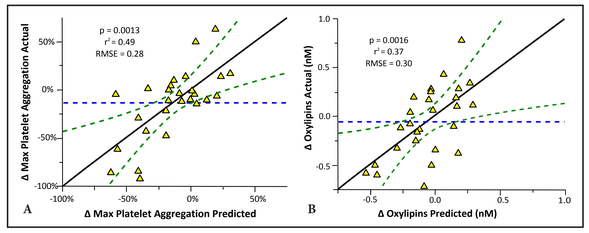

Figure 4. Oleocanthal, oleacein and tyrosol concentrations combined provide the best prediction of observed platelet aggregation (**A**) and oxylipin inhibition (**B**), respectively. As seen in Table 3, inhibition of platelet aggregation and oxylipins best correlates to the mg/kg body weight dose of oleocanthal, oleacein and tyrosol consumed by subjects in Oils A, B and C, though oleocanthal appeared to be a more potent effector of platelet aggregation while oleacien was an apparently more potent effector of oxylipin production.

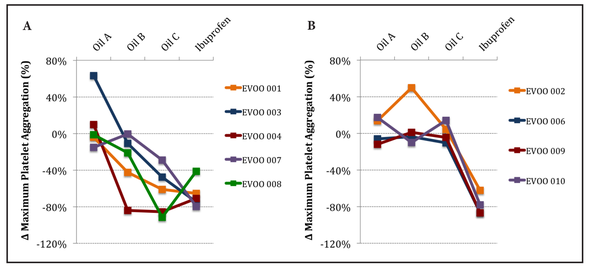

Figure 5. Subjects could be stratified into "responders" and "non-responders" based on their response to Oils B and C. Out of nine subjects sampled, five subjects ("responders") demonstrated changes in collagen-stimulated maximum platelet aggregation > -25% two hours following consumption of Oils B and C **(A)** whereas four subjects showed no change from baseline in maximum platelet aggregation two hours following consumption of Oils B and C (**B**).

Conclusions

- The impact of EVOO consumption on collagen-stimulated platelet activity varies with changing phenolic composition, not total phenolic content.
- Oleocanthal-rich EVOOs were more potent effectors of platelet aggregation than oxylipin production, and while less potent, oleacien and tyrosol appear to contribute to this effect.
- Oleocanthal-rich EVOO effects on platelet aggregation appear to be cyclooxygenase-independent, suggesting an up-stream effect possibly associated with calcium mobilization or blockade of the physical aggregation process.
- While intra-individual variability in platelet reactivity is common, the arbitrary selection of a single postprandial sampling time and low dose of oleocanthal and oleacein contained within complex mixtures limits the power of this pilot study and may contribute to the "responder" effect.
- Future studies will consider time course, dose-response, and a greater variety of source oils to determine optimal dose, sampling time, and phenolic associations with EVOO-dependent platelet effects.
- These findings may suggest that EVOO variety may be an important factor associated with the health benefits of these products, which may ultimately have economic impacts on EVOO producing regions.

References

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