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Inhibitory effect of the dietary lipid di-homo-gamma linolenic acid on platelet reactivity

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The platelet lipid membrane provides the substrates for downstream eicosanoid production that in turn may alter platelet reactivity. The lipid composition of the membrane is strongly influenced by diet. We therefore examined the effect of the saturated fatty acid, stearic acid (SA), and the omega-6 fatty acids linoleic acid (LA) and di-homo-gamma linolenic acid (DGLA), on platelet aggregation.

Blood was collected by venepuncture into sodium citrate (3.2% w/v), from healthy volunteers who had provided written consent (St Thomas's Hospital Research Ethics Committee, reference 07/Q0702/24). Platelets were isolated by sequential centrifugation and washing steps before final suspension in modified HEPES buffer. Samples were incubated with SA, LA or DGLA (10μM) for ten minutes. Aggregation, measured by 96-well Optimul or traditional light transmission aggregometry was then tested using, as platelet agonists, either collagen (0.1-30μg/ml) or thrombin receptor-activating peptide (TRAP) 6 (0.1-30μM).

Concentration response curves were constructed and log EC_{50} values calculated for collagen and TRAP-6 induced aggregation in presence of vehicle or fatty acid (Table 1). SA caused no differences in response to either agonist, whereas LA, and more

Table 1: **Log EC_{50} values (mean ±SEM, n=4)**	Collagen		TRAP-6	
	Vehicle	Fatty acid (10μM)	Vehicle	Fatty acid (10μM)
SA	-6.1±0.2	-6.2±0.1	-6.2±0.2	-6.2±0.2
LA	-6.0±0.2	-5.3±0.3*	-6.5±0.2	-5.8±0.3*
DGLA	-6.0±0.2	-5.1±0.1*	-6.0±0.2	-5.2±0.2*

strongly DGLA, reduced aggregation to both (*$p<0.05$ by two-way ANOVA).

The inhibitory effects of LA and DGLA on aggregation were confirmed using traditional light transmission aggregometry. In response to collagen (10μg/ml),

compared to vehicle (71±2% maximum aggregation) LA did not demonstrate significant inhibition (65±2%; $p>0.05$) whereas DGLA did (41±5%, $p<0.05$). The inhibitory effects of LA and DGLA were more pronounced when TRAP-6 (3μM) was used as the agonist (vehicle, 62±7%; LA, 39±11%; and DGLA, 22±12%; $p<0.05$, $n=4$ for both). DGLA but not LA, compared to vehicle, demonstrated an inhibitory effect even when aggregation was induced by an increased TRAP-6 concentration (20μM; 51±3% vs 73±2%; $p<0.05$, $n=4$).

In conclusion, the two omega-6 fatty acids investigated inhibited platelet aggregation, whereas SA was without effect. DGLA had the greatest inhibitory effect but more work is required to identify the mechanistic pathway. Considering the significance of dietary fatty acid consumption on the constituents of the platelet phospholipid membrane, these data indicate the potential therapeutic benefits that could arise from diet modification. In particular, a DGLA-rich diet may help to reduce platelet reactivity.