



The *in vitro* and *ex vivo* antithrombotic properties and nutritional profile of an ovine Irish yoghurt drink

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S9. Functional Foods, Nutrition and Health



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Background & Aims

- **Platelet function** is linked with thrombosis and cardiovascular disease.
- Foods in the Mediterranean diet (yogurt, fish, olive oil) contain bioactive **polar lipids** (PL) proven to combat inflammation.
- **Fermented dairy** products have enhanced anti-thrombotic and anti-inflammatory properties.
- **Sheep's milk**: Nutrient rich, more digestible, sustainable.



- Assess the **effect of ovine yogurt enriched with PL on platelet sensitivity** and antithrombotic effect of polar lipid fractions in human plasma.

The effect of ovine milk fermentation on the antithrombotic properties of polar lipids



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ABSTRACT

The effect of fermentation on the antithrombotic properties of polar lipids in ovine milk has been assessed through the production of yoghurts. The total lipids (TL), total neutral lipids (TNL), and total polar lipids (TPL) were extracted. The fatty acid profiles of all yoghurt polar lipids were analysed by GC-MS. The levels of MUFA increased in the fatty acids of the polar lipids, but there was a reduction in PUFA as milk was fermented to yoghurt. The bioactivity of each lipid extract was assessed against platelet-activating factor (PAF) induced platelet aggregation. All yoghurt polar lipids exhibited potent antithrombotic activities with IC₅₀ values ranging from 45 to 77 µg. Shotgun metagenomics determined the species-level microbial composition and functional potential of the yoghurts. Yoghurts containing *L. acidophilus* seem to correlate with greater bioactivity. Several phospholipid biosynthetic genes have been identified in the most antithrombotic yoghurts. This study has demonstrated that fermentation enhances the antithrombotic properties of yoghurt polar lipids against PAF.

PAF and thrombin inhibition by dairy PL

- Platelet-activating factor (**PAF**) is an inflammatory phospholipid agonist that activates platelets through its GPCR transmembrane receptor (**PAF-R**).
- Thrombin is a serine protease agonist that binds GPCR receptors **PAR1** and **PAR4**.
- PL with structural similarity to PAF or thrombin can competitively bind their receptors → Inhibition of platelet activation.

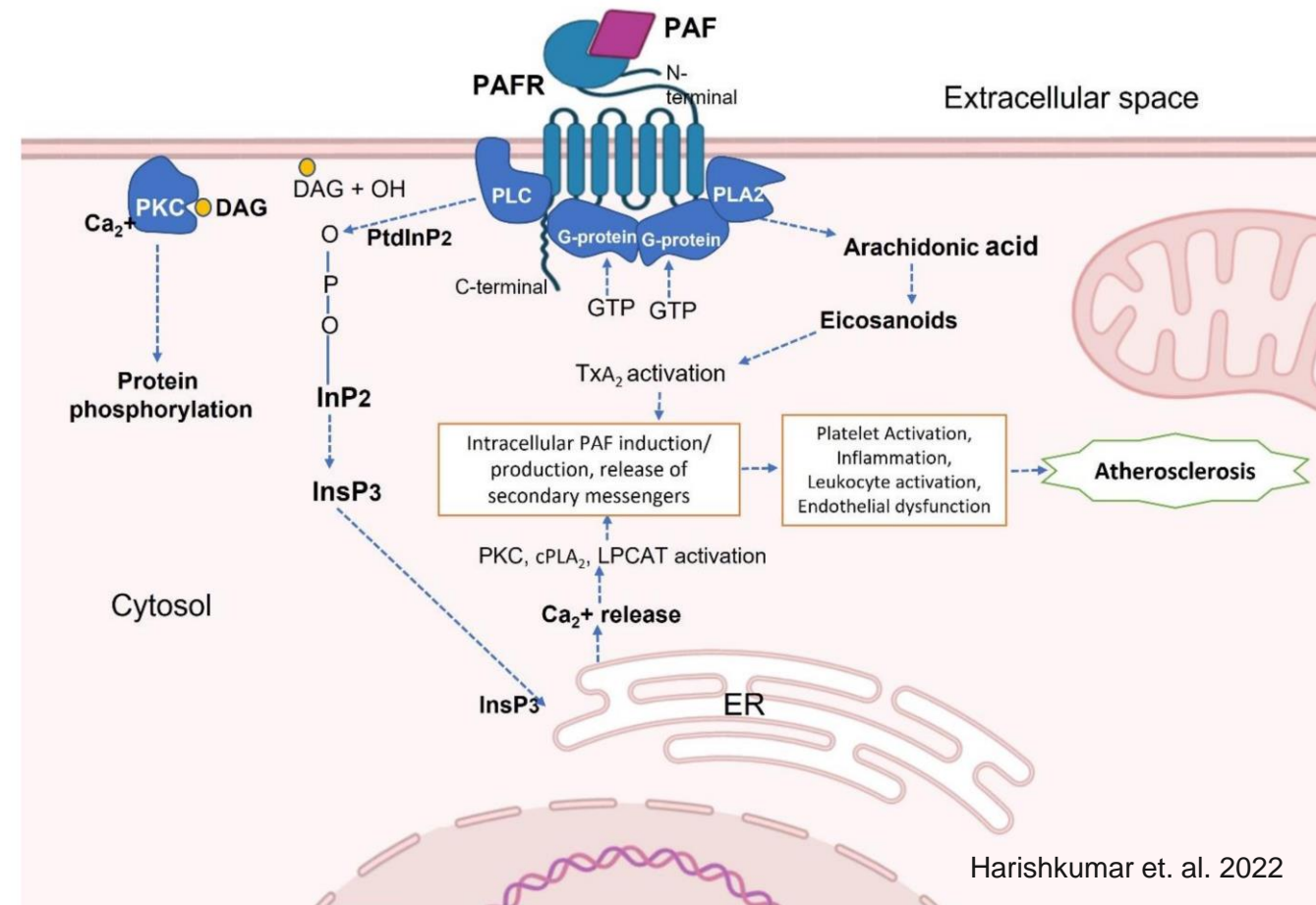
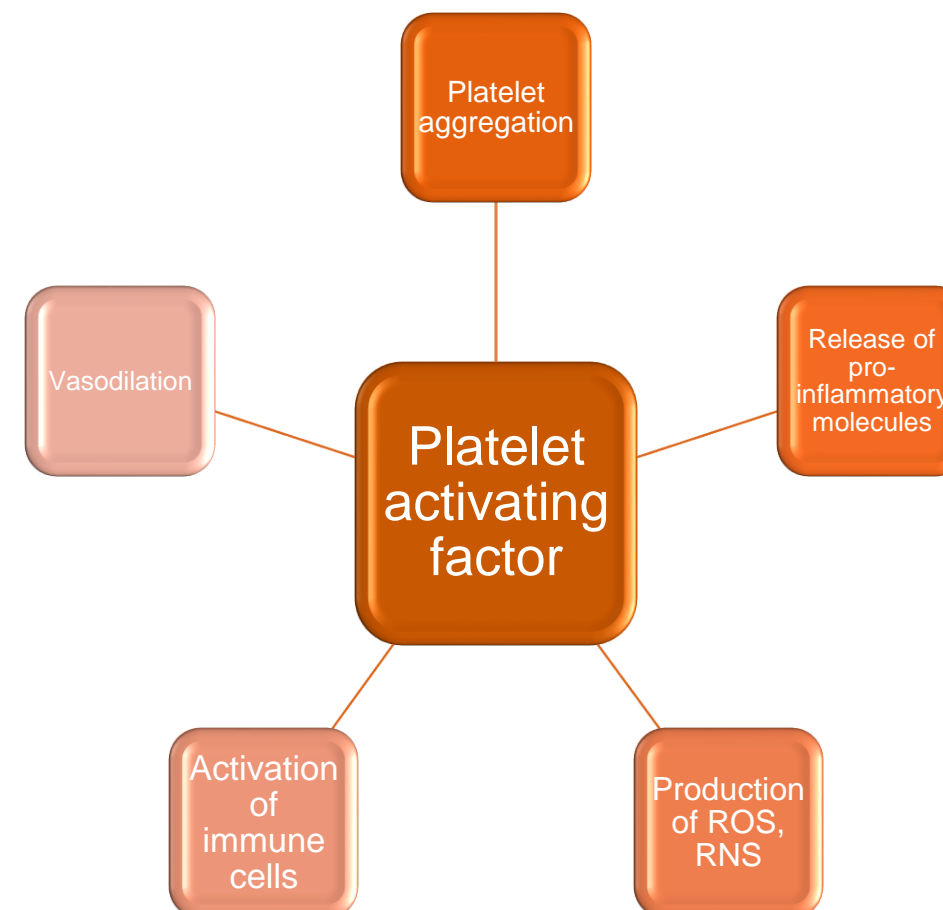


Fig. 1 Mechanism of PAF-R activation and PAF-mediated signalling pathway (Harishkumar et. al. 2022)





Project overview

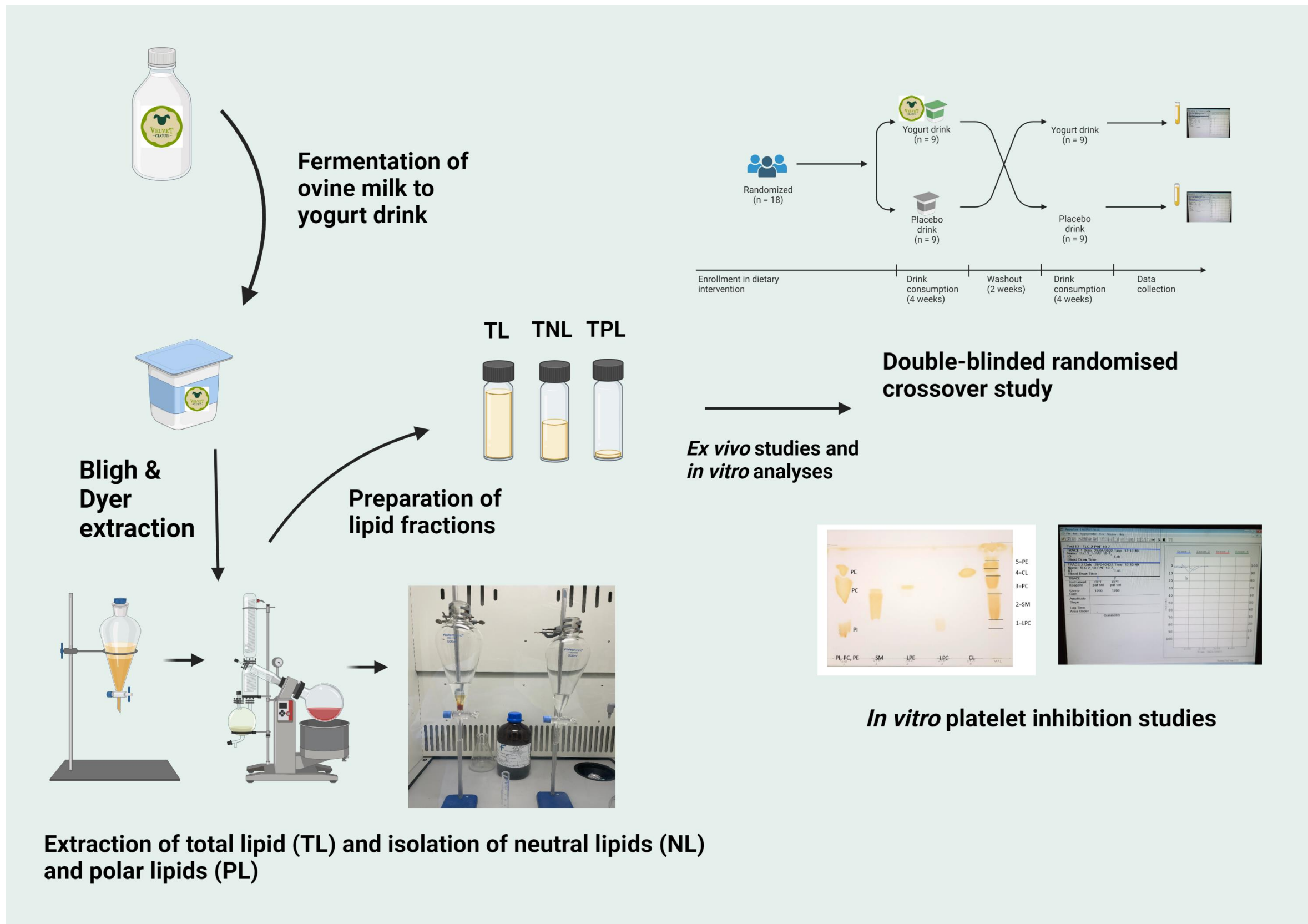


Fig. 2 Graphical abstract and project overview.

Yield of yogurt lipid fractions (Bligh & Dyer method)

Table 1. Yield of the lipid fractions (TL, total lipid; PL, polar lipids; NL, neutral lipids) from raw sheep's milk, skimmed milk-derived placebo drink and full fat yogurt drink (g/100mL). Data is presented as mean \pm SD.

Samples (100 mL)	Total lipids (TL) in grams	Polar lipids (PL) in grams	Neutral lipids (NL) in grams
Raw ovine milk	9.96 \pm 0.30^a	0.16 \pm 0.01^a	8.24 \pm 0.23^a
Yogurt drink	8.17 \pm 0.22^a	0.15 \pm 0.004^a	7.75 \pm 0.19^a
Skimmed milk-derived yogurt drink (Placebo)	0.36 \pm 0.01^b	0.06 \pm 0.002^b	0.29 \pm 0.01^b

^{a,b}, Different superscripts in the same column indicate significant differences among different lipid extracts within the same lipid class ($p < 0.05$) when means are compared using a Tukey's HSD multiple comparison test.

In vitro bioactivity of ovine yogurt lipid fractions

Table 2. Inhibitory effects (IC₅₀ values) of polar lipids (PL), neutral lipids (NL), and total lipids (TL) isolated from the ovine yogurt drink against human platelet aggregation induced by PAF and TRAP-6 *in vitro*. Data is expressed as mean ± SD.

Food sample	Lipid fraction	IC ₅₀ against TRAP-6 (µg)	IC ₅₀ against PAF (µg)
Yogurt drink	YPL	313.0 ± 15.0 ^a	156.7 ± 5.9 ^a
	YNL	275.4 ± 13.6 ^b	729.2 ± 35.1 ^b
	YTL	209.6 ± 10.1 ^c	248.2 ± 12.0 ^c
Placebo (skimmed milk-derived yogurt drink)	YPL	45.8 ± 2.1 ^a	44.0 ± 2.0 ^a
	YNL	47.4 ± 2.1 ^a	47.2 ± 2.1 ^a
	YTL	59.2 ± 3.1 ^a	53.8 ± 2.9 ^a

^{a,b,c,d,e} Different superscripts in the same column indicate significant differences among different lipid extracts within the same lipid class ($p < 0.05$) when means are compared using a Tukey's HSD multiple comparison test.

In vitro bioactivity of polar lipid TLC fractions

Table 3. Antiplatelet activity of each PL subclass towards PAF-induced platelet aggregation in PRP, expressed as mean \pm SD.

Yogurt drink polar lipid fractions		EC ₅₀ (-) or IC ₅₀ against PAF (μ g)	IC ₅₀ against TRAP-6 (μ g)
1	LPC	-48.1 \pm 3.9	348.6 \pm 7.9
2	SM	1120 \pm 54	250.6 \pm 8.3
3	PC	169.5 \pm 8.3	205.3 \pm 8.9
4	CL	ND	208.0 \pm 10.8
5	PE	196.7 \pm 6.4	592.1 \pm 8.8

Abbreviations: CL, cardiolipin; LPC, lyso-phosphatidylcholine; ND, not determined; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; TRAP-6, thrombin receptor activator peptide 6

Study design: Placebo-controlled crossover trial

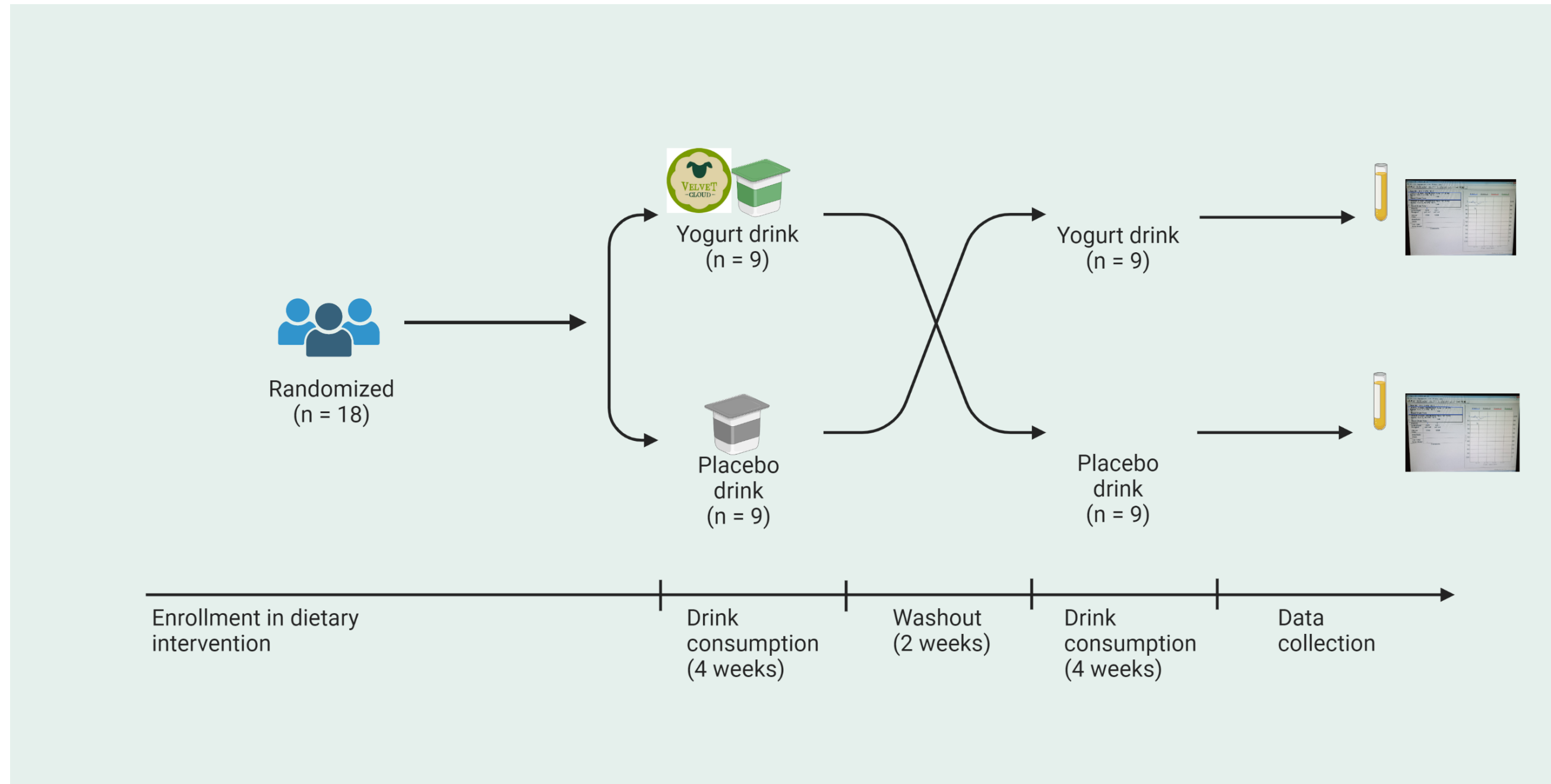


Fig. 3 Representation of the placebo-controlled crossover trial study.

Yogurt intake and platelet function: YD intake reduces platelet activation through thrombin pathway

Table 4. The effect of yogurt (YD) consumption on platelet activation by TRAP-6, expressed as the Δ EC50 (half-maximal excitatory concentration) at different timepoints.

Treatment group	Δ EC50 day 0-28	Δ EC50 day 42-70	Combined Δ EC50
YD	17.22 \pm 32.2	10.22 \pm 33.35	13.72 \pm 31.1
PD	7.45 \pm 27.5	-5.97 \pm 19.95	0.742 \pm 23.6

Conclusion and future perspectives

- **Lipid fractions** from ovine yogurt reduce platelet aggregation induced by PAF and TRAP-6.
- Consumption of PL-enriched **yogurt reduces platelet aggregation against thrombin** pathway (TRAP-6) in interim analysis, in relatively low doses of PL.
- **Larger-scale** studies required to establish the effect of yogurt intake on platelet activity.
- Intake of such lipid-enriched **functional foods** could prevent cardiovascular disease linked with systemic inflammation.



Thank you

Dr Ronan Lordan and Dr Harishkumar Rajendran and Dr Ioannis Zabetakis



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