DOSE-DEPENDENT EFFECTS OF ALPHA-LINOLENATE ON RAT PLATELET COMPOSITION, AGGREGATION AND THROMBOXANE PRODUCTION

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Diets rich in very long chain n-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, appear to be cardioprotective. One of the mechanisms is believed to be via a reduction in the amount of arachidonic acid in the platelet membrane. Arachidonic acid can be converted to thromboxane A_2 a potent promoter of platelet aggregation. However, in Australia it may not be possible for the population at large to consume large amounts of foods which are rich in these n-3 fatty acids. Instead, the population may be able to increase its intake of the precursor fatty acid, alpha-linolenic acid (C 18:3 n-3). The aim of this work was to assess the effects of varying amounts of alpha-linolenate on platelet fatty acid composition, the production of thromboxane A_2 and the aggregation of platelets using a rat model.

Eight week old Sprague Dawley rats were housed in metabolic cages and fed a diet with 30% total energy as fat with a P:M:S ratio of 1:1:1. Rats were assigned to receive one of four different levels of alpha-linolenate (n=9 per diet). The diets were made by the addition of varying amounts of palm stearin, palm olein, sunflower, canola and linseed oils to rat chow. The final fatty acid composition of the diets were 3.7, 6.7, 14.3 and 22.2% total fatty acids as alpha-linolenate.

After consuming the diets for four weeks the rats were anaethetised and their blood withdrawn from the abdominal aorta. The platelets were harvested for analysis of fatty acid composition by gas chromatography. Platelet-rich plasma (PRP) was used to measure aggregation induced by three different concentrations of collagen i.e. 0.5, 2.0 and 4.0 μg/mL, using the optical method and measuring maximum aggregation at six minutes after injection of the collagen. The concentration of thromboxane in the PRP following collagen-induced aggregation was determined by radioimmunoassay for thromboxane B_2, the stable metabolite.

Increasing alpha-linolenate in the diets meant that dietary linoleate was decreased so that the n-3:n-6 ratios were 1:7, 1:3, 1:1 and 1.4:1. The platelets fatty acid composition demonstrated dietary ratio-dependent changes. The platelet eicosapentaenoic acid increased and the arachidonic acid content decreased as the dietary ratio of n-3 increased. (Table 1)

Fatty acid	Dietary n-3:n-6 fatty acid ratio			
	1:7	1:3	1:1	1.4:1
	% Total fatty acids			
Eicosapentaenoic acid	1.0± 0.0	1.2± 0.1	2.5± 0.2	2.6± 0.1
Arachidonic acid	15.1± 0.6	15.3± 0.6	13.6± 0.3	11.5± 0.6

The lowest platelet concentration of arachidonic acid was associated with less thromboxane production i.e. differences were found between the 1.4:1 diet and the 1:3 and 1:1 diets at the highest collagen dose and between the 1:3 diet and the 1.4:1 diet at 2μg collagen. No differences were detected for the 0.5μg collagen dose. Platelet aggregation was less on the 1.4:1 diet compared to the 1:3 and 1:1 diets at 2μg and 0.5μg. No differences were detected when 4μg collagen was used but this is probably because it is a dose which results in supramaximal aggregation. The most surprising finding was that platelet aggregation and thromboxane production were as low on the 1:7 as the 1.4:1 diet.

In conclusion, alpha-linolenate rich diets will lead to platelet enrichment with eicosapentaenoic acid and decreases in arachidonic acid. At the highest dietary ratio the reduction in arachidonic acid was greatest and thromboxane production least. However, changes in platelet fatty acids failed to explain all the observed differences in platelet aggregation and thromboxane production.

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