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Low-erucic acid rapeseed oil and platelet function

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Platelet function and CHD

The classical risk factors – elevated serum cholesterol, high blood pressure and tobacco smoking – only partly predict coronary heart disease (CHD) risk [1, 2]. Imbalance in the function of hemostatic factors may account for part of the morbidity. Hemostasis is the delicate process which regulates the fluidity of blood. It is the result of interplay of four components: platelets, blood coagulation system, fibrinolytic system, and endothelium. These form a network by interacting with each other. The physiological role of this system is to arrest bleeding and promote wound healing. However, imbalance in hemostasis may contribute to atherosclerosis and thrombosis.

Platelets are the smallest cellular particles in blood. They form the primary plug which arrests bleeding from a wounded blood vessel. In this process platelets are activated by adhesion to exposed subendothelial molecules. Activated platelets change their shape, spread on the site of injury, and release their granule contents. Platelet-platelet interactions lead to formation of platelet aggregates which temporarily seal the break in the blood vessel [3–5]. Eicosanoids synthesized in platelets as well as molecules secreted from platelet granules have several effects on cells in the platelet environment (e.g. endothelial cells, monocytes and other platelets) and on the blood coagulation and fibrinolysis cascades [3, 6]. Platelets may participate in atherosclerosis by several mechanisms and play a central role in thrombosis [see 6–8]. The importance of platelet function in the development and outcome of human CHD and stroke is demonstrated by the effectiveness of antiplatelet drugs like aspirin and antagonists of the platelet fibrinogen receptor in both primary and secondary prevention of thrombotic events [6, 8].

Dietary fatty acids and platelet function

The importance of diet, and in particular dietary fatty acid (FA) composition, in the development of CHD has been realized decades ago. Diet FA composition is not only an important determinant of serum cholesterol concentrations, but it also influences hemostatic factors of which platelets have been studied most frequently.

The mechanisms by which dietary FAs can modulate platelet function may be either direct or indirect. Platelets are surrounded by plasma membrane and they have intensive intracellular membrane systems like the dense tubular system. The phospholipid FA composition in these membranes can be modified by dietary FAs. Phospholipid FA composition may have effects on the physicochemical properties of the cell membranes which can cause alterations in receptor activities and intracellular signalling. The function of receptor proteins and enzymes linked to the membrane are modified by membrane FAs [9, 10]. Phospholipid FAs are also the precursor pool for eicosanoids. Thromboxane A_2 (TXA_2), synthesized from arachidonic acid (C20:4 n-6) by cyclooxygenase, is one of the most important activators of platelet aggregation and its synthesis can be modified by dietary FAs, either due to an altered precursor pool, competition for or inhibition of cyclooxygenase [11]. Alterations in the precursor FA pool may also affect platelet function *via* lipoxygenase products [12]. Indirectly dietary FAs can affect platelets *via* alterations in serum lipoprotein concentrations [6].

In dietary studies the different aspects of platelet function and activation have been studied by several ways, summarized in Fig. 1. Of all tests, *ex vivo* platelet aggregation has been used most frequently.

After the reports showing low *ex vivo* platelet aggregability and long bleeding times in Greenland Eskimos [13], many diet-related hemostatic studies have been centered on the effects of fish oils and long-chain n-3 FAs eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA) on platelet function. A general outcome of the fish or fish oil studies has been a prolongation of bleeding time and decreased platelet TXA_2 production, findings interpreted to indicate decreased thrombosis tendency. The results from *ex vivo* platelet aggregation studies are controversial. However, in the majority of the well-controlled studies platelet aggregation in response to various activators was either decreased or not changed by

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fish oils or long-chain n-3 FAs [14, 15]. Less information is available on the effects of the FAs found in vegetable oils on platelet function.

Rapeseed oil and platelet function

Very low-erucic acid rapeseed oil (erucic acid <1% of FAs) or canola oil are low in saturated fatty acids (SFA), rich in oleic acid (C18:1 n-9, OA), and contain substantial amounts of both the essential FAs linoleic (C18:2 n-6, LA) and α-linolenic acid (C18:3 n-3, ALA). When rapeseed oil replaces other fat sources in the diet, the total dietary FA intake is modified accordingly. Based on epidemiological data [16–18] and experimental studies focusing on lipoprotein metabolism [19, 20] this kind of dietary FA composition is considered to prevent CHD.

The Lyon Diet Heart study was a secondary prevention trial in which the diet of the experimental group was changed in the direction of a Mediterranean diet (reviewed in this special issue, see p. 490). The dietary modifications were carried out by increasing the consumption of vegetables, fruit, legumes and bread and by replacing saturated fats by a canola oil-based margarine and using olive and canola oils for salads and cooking [21, 22]. The survival curves for cardiac death of the experimental and control groups separated early (under 1 year) in favor of the experimental group. The fast protective effect of the experimental diet is most probably not due to decreased atherogenesis, but rather due to alterations in factors associated with thrombosis or cardiac arrhythmias. Plasma ALA was inversely associated with myocardial infarction and cardiac death in the study [21, 22]. Although the results indicate that rapeseed oil can have favorable effects, one has to keep in mind that the experimental and control groups also differed in their intake of bread, legumes, fruit, and vegetables.

In addition to FAs, rapeseed oil contains also other compounds which may affect platelets, e.g. phytosterols [23] and γ-tocopherol [24]. Thus far, however, the human studies related to platelet function have been focused on FAs. When the specific effects of oils or FAs are studied, one has to be careful not to change the total amount of fat or other nutrients in the diet. Thus, the studies focusing on the effects of certain edible oils or FAs are principally comparisons between oils or FAs and no "absolute" effects can be studied. In the case of rapeseed oil the comparisons have most often been carried out with sunflower oil. No comparisons e.g. with another rich source of OA, olive oil, have been published thus far.

Rapeseed oil *vs.* sunflower/safflower oil

One of the earliest interventions on the effects of vegetable oils on platelet function is a study of *Renaud* et al. [25] in which the SFA-rich habitual diet of *Moselle* farmers was modified by replacing butter and cream with either margarine based on sunflower oil or rapeseed oil for one year. Platelet aggregation in response to ADP, collagen

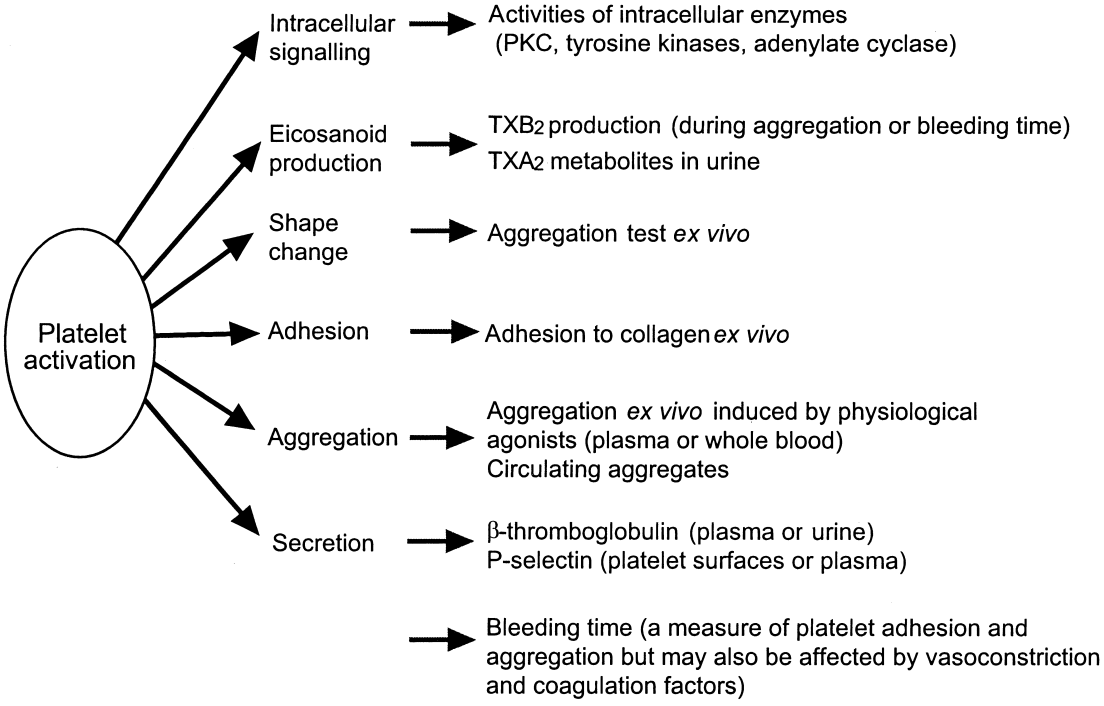

Fig. 1. Events related to platelet activation and measurements of platelet activation/function used in dietary studies.

and thrombin decreased in both groups. The dietary modifications tended to decrease total fat intake from the habitual level, which may have had some effect on the results [25].

In smaller-scale controlled settings the effects of rapeseed/canola oil diets rich in OA + ALA and sunflower or safflower oil rich in LA on platelet function have been compared in three studies (Fig. 2) [26–28]. In the cross-over study of *McDonald* et al. [26] canola oil increased bleeding time and sunflower oil decreased the concentration of the metabolite of the vasoconstrictive eicosanoid TXA_2, thromboxane B_2 (TXB_2) in blood collected during the determination of bleeding time in comparison with a high-SFA baseline diet. The effects of the oil diets did not differ. *Kwon* and colleagues [27] carried out a parallel dietary study with canola and safflower oil and found that both diets slightly decreased platelet aggregation to collagen in whole blood. The effects of the diets did not differ

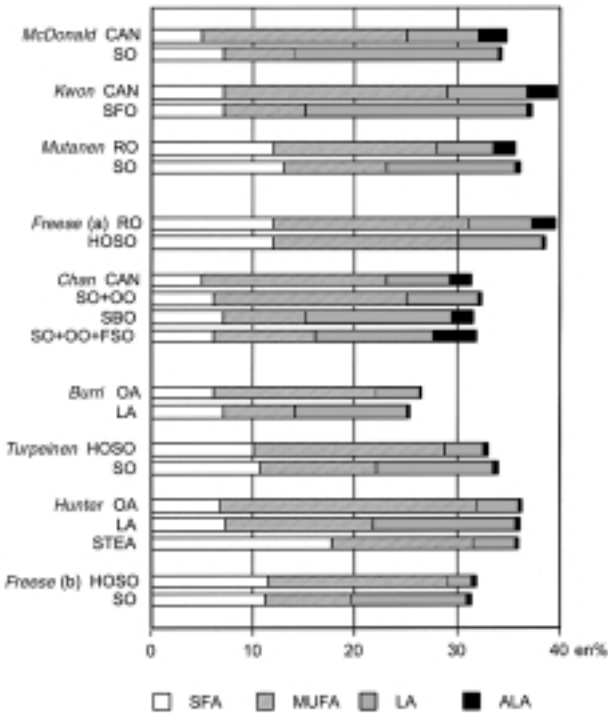

Fig. 2. Dietary fatty acid composition (% of energy) of experimental diets in platelet function studies comparing low-erucic acid rapeseed/canola oil and other vegetable oils or relevant fatty acids. Data partly calculated by the author. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, OA – oleic acid, LA – linoleic acid, ALA – α-linolenic acid, CAN – canola oil, SO – sunflower oil, SFO – safflower oil, RO – rapeseed oil, HOSO – high-OA sunflower oil, OO – olive oil, SBO – soybean oil, FSO – flaxseed oil. References: *McDonald* [26], *Kwon* [27], *Mutanen* [28], *Freese* (a) [36], *Chan* [35], *Burri* [30], *Turpeinen* [31], *Hunter* [29], *Freese* (b) (unpublished results).

at the end of the 8-week study period. In our highly-controlled cross-over dietary intervention study [28], a diet with rapeseed oil did not differ from that with sunflower oil in its effects on platelet aggregation to ADP. Both diets increased platelet aggregation to ADP and collagen as well as TXB_2 production in collagen-activated platelets in comparison with baseline dairy fat diet [28]. These three studies suggest thus that rapeseed/canola oil diets rich in OA + ALA and sunflower/safflower oil diets rich in LA do not differ from each other in their effects on platelet function.

Rapeseed oil *vs.* saturated fatty acids

Although it is feasible to think that increased consumption of rapeseed oil could replace SFA in the diet, controlled studies focusing on such a comparison have not been carried out. The comparisons between the run-in diets rich in SFA and the rapeseed oil diets in the above-mentioned studies give conflicting results by indicating decreased [26, 27] or increased [28] platelet activation after the experimental oil diets. The differences in the SFA intakes during the experimental diets (5–7 *vs.* 12% of energy, en%) may partly explain the discrepancy [26–28]. Also the fact that the studies were designed to compare the two oils instead of SFA and oils may complicate the results.

In the intervention of *Hunter* et al. [29] controlled experimental diets rich in stearic acid, OA or LA did not differ in respect of platelet aggregation, membrane fluidity or the binding of anti-aggregatory eicosanoid, prostaglandin E_2 to platelet membranes.

Oleic acid *vs.* linoleic acid

Because rapeseed oil is a good source of OA while many other vegetable oils are good sources of LA, it is of interest to compare the effects of these two main unsaturated FAs in our diet. In controlled settings these kind of studies can be carried out e.g. by using high-OA and high-LA sunflower oils which contain very small amounts of ALA. Three published and one unpublished (*R. Freese*, unpublished results) study show that controlled high-OA and high-LA diets both low in ALA do not differ in their effects on platelet function [29–31]. The fatty acid composition of these diets is presented in Fig. 2.

α-Linolenic acid

Recent findings from two cohort studies [32, 33] support the hypothesis that ALA may exert a protective role on its own with regard to myocardial infarction or coronary mortality. The correlation analyses of the Lyon Diet Heart study [34] also indicate that plasma ALA is associated with protective effects. The effects of ALA on platelet func-

tion have been investigated using various experimental designs.

The effects of ALA in rapeseed oil on platelet function have been examined in two intervention studies. *Chan* et al. [35] studied the effects of isocaloric diets with different amounts and ratios of LA to ALA on bleeding time and on the formation of TXB_2 in blood lost during the determination of bleeding time (bleeding time blood collected). The ratio of LA to ALA varied in the diets from 2.7 to 27.4. No significant differences in bleeding time or TXB_2 formation were seen between the diets. However, when comparing two diets with similar OA intake (for composition see Fig. 2), bleeding time tended to be longer (5.3 *vs.* 5.0 min) and TXB_2 production lower (3.8 *vs.* 5.0 μg/l) after the canola oil diet which contained more ALA than a diet based on sunflower oil + olive oil [35]. We used rapeseed oil and high-OA sunflower oil in a partly controlled crossover study to compare the effects of two high-OA diets differing only in their LA to ALA ratio (2.8 an 28, respectively) (Fig. 2) [36]. In this study, platelet aggregation at the end of the rapeseed oil diet did not differ from that at baseline (habitual diet), but sunflower oil diet enhanced aggregation to ADP, collagen and thrombin. Platelet aggregation responses were lower during the rapeseed oil diet than during the sunflower oil diet [36]. These results indicate that dietary LA to ALA ratio or the absolute intakes of ALA and LA affect platelet function if intakes of SFAs and OA are kept constant.

A controlled long-term study carried out in 1960's and 70's showed lower platelet *ex vivo* aggregation at the end of a long-term high-polyunsaturated fat (PUFA) diet (16 en% LA) in comparison with a high-SFA diet (4 en% LA) [37, 38]. The treatment effect has been attributed mainly to LA [38] although the high-PUFA diet was carried out by replacing habitual milk and margarine with soybean oil-filled skim-milk and soybean oil-based margarine and was thus also rich in ALA [39, 40].

The effects of ALA on hemostatic factors have also been investigated by supplementing linseed or flaxseed oil to subjects whose dietary intake has been controlled. In such studies the intake of ALA can be increased to a clearly higher level than in rapeseed oil-based mixed diets. *Kelley* et al. [41] saw no changes in bleeding time when a low-fat (23 en%) baseline diet was supplemented with flaxseed oil (ALA 6.3 en%). Flaxseed or sunflower oil (40 g/day) was supplemented to a basal low-fat diet in a study of 10 male subjects [42]. The flaxseed oil supplementation increased n-3 FA intake (mainly ALA) from 0.4 to 8.5 en% and sunflower oil supplementation increased n-6 FA intake (mainly LA) from 2.7 to 11.7 en%. Collagen-induced aggregation in whole blood (cell-counting method) decreased in the flaxseed oil group, but ADP-induced aggregation remained unchanged. Sunflower oil had no effects on platelet aggregation. Unfortunately, comparisons between the two oils were not reported [42]. In a parallel intervention among vegetarian men moderate (2.0 en%) or high (6.3 en%) intake of ALA after a low-ALA diet (0.6–0.8 en%) were not associated with differences in platelet aggregation or urinary 11-dehydro-TXB_2 excretion [43].

ALA is a n-3 FA and it can be elongated to EPA in the body. It is therefore of interest to see whether ALA differs from the longer-chain n-3 FAs in its effects on platelets. When the effects of ALA from linseed oil and EPA + DHA from fish oil were compared in a controlled parallel supplementation study [44] no differences between the two treatments were seen in bleeding time, platelet aggregation to collagen or thromboxane-analogue I-BOP, or in the urinary excretion of β-thromboglobulin or 11-dehydro-TXB_2 [44]. ADP-induced aggregation was, however, enhanced during fish oil feeding, an effect not seen with linseed oil. Different results were obtained by *Adam* et al. [45] who compared the effects of ALA and EPA in formula diets with 4 en% LA in female subjects. Bleeding time lengthened and platelet aggregation to ADP and collagen decreased only with very high ALA intakes (12 or 16 en%). Similar effect was obtained already with 1.7 en% EPA.

In summary, the data concerning the effects of ALA on platelet function are contradictory. There are some indications of favorable effects but whether ALA can have an independent role or whether its possible effects are mediated *via* elongation to EPA is still not resolved.

Dietary ratio of n-6 and n-3 polyunsaturated fatty acids

As already pointed out, there are data from intervention studies that indicate that dietary LA to ALA ratio may modulate platelet function in humans [36]. These results are at least partly in line with those from *Salo* et al. [46] who studied the correlations between platelet aggregability with FAs in platelets, plasma lipids, and adipose tissue in middle-aged Finnish men. ADP-induced platelet aggregation was inversely correlated with platelet n-3 FAs, n-3 to n-6 ratio, and EPA to AA ratio while positive associations were found between platelet aggregation and LA in plasma triglycerides, cholesterol esters, and in adipose tissue. The authors concluded that the positive correlation between platelet function and LA in tissues may be due to imbalance in the dietary n-6 to n-3 ratio. Thus attention should be paid to the n-6 to n-3 ratio when SFAs are substituted with PUFAs [46].

In the Lyon Diet Heart study [22] the substitution of SFAs by fatty acids from canola and olive oil together with an in-

creased consumption of fish in the experimental group was reflected not only in plasma fatty acids but also in platelet phospholipid fatty acids. There was a significant decrease in plasma and platelet n-6 to n-3-ratio in comparison with the control group [22]. The ratio of total n-3 to n-6 FAs in the diet was inversely related to cardiovascular mortality in the Multiple Risk Factor Intervention Trial [18, 47]. These results may indicate that, at least to a certain level, increasing the relative intake of n-3 FAs may reduce coronary risk. The effects of dietary n-6 to n-3 ratio on platelet function should be studied further.

Rapeseed oil in the postprandial state

The interventions discussed thus far have studied the effects of FAs in the fasting state. It is, however, widely accepted that focusing only on the fasting state may be misleading, because alimentary lipemia may have effects on platelets and other hemostatic factors. Platelet *ex vivo* aggregation seems to be affected by a fatty meal. The direction of this change is, however, controversial [see 48].

In a comparison between high-fat (1 g/kg body weight) test meals enriched with rapeseed oil, sunflower oil and butterfat no differences in platelet aggregability were seen during the postprandial follow-up period [48]. The results are in concordance with earlier results showing similar effects on platelet function of SFA- and PUFA-rich meals [49–52].

Conclusions and future directions

The available intervention data on the effects of rapeseed oil on platelet function is yet inconclusive. Rapeseed oil seems not to differ from sunflower/safflower oil in its effects on platelets. However, when the intake of SFAs and OA are kept constant, rapeseed oil with a lower LA to ALA ratio is associated with decreased platelet function than oils higher in LA. This is particularly interesting because rapeseed oil may be used to decrease the intake ratio of n-6 to n-3 FAs in the “Western” diet and a high ratio may be associated with an increased risk of CHD.

The effects of ALA from rapeseed oil and the dietary n-6 to n-3 ratio should be studied further. Although part of the effects of ALA may be due to its elongation to EPA, the possible independent effects of ALA should also be studied in more detail. On the fat level, it would be important to compare the effects of rapeseed oil with diets high in SFAs under controlled experimental conditions. In addition, comparisons between the main OA-rich oils, rapeseed and olive oil should be carried out. Studies on the effects of the minor compounds in rapeseed oil, antioxidants and phytosterols are also needed.

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