BENEFICIAL HEALTH EFFECTS OF RAPESEED OIL ON HEMOSTASIS

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Abstract

In the hemostatic system, fibrinogen is the main protein affecting blood coagulation, and elevated levels, found in prothrombotic and proinflammatory states, are associated with increased risk of several chronic diseases.

Our study has recently shown that elevated plasma fibrinogen caused by inadequate □-linolenic acid (18:3n-3, ALA) intake can be reduced by rapeseed oil. Forty-two subjects, who had average cholesterol and fibrinogen levels of 5 mmol/L and 2.6 g/L, respectively, replaced fat by rapeseed (spring turnip rape) oil for six weeks.

At the study entry, total plasma ALA had a hyperbolic relationship with long-chain n-6 polyunsaturated fatty acids (LC n-6 PUFA) indicating that increases in ALA inhibit the formation of LC n-6 PUFA from linoleic acid (18:2n-6, LA). Most of the highest fibrinogen values were found at the lowest range of plasma phospholipid (PL) ALA, often with low LA.

Fat substitution covered about a quarter of the fat intake, and ALA intake increased from 0.8 to 1.7 g/day, on average. Effective reduction of saturated fatty acids (SaFA) led to a marked fall in cholesterol levels. At six weeks, all the fibrinogen values originally higher than the mean fell by 1 g/L (n = 14), on average, along with the dietary LA/ALA ratio from 7 to 4.

At elevated fibrinogen levels, ALA exhibited a specific behaviour at three weeks, i.e it did not raise docosahexaenoic acid (22:6n-3, DHA) but reduced arachidonic acid (20:4n-6, AA). Diminished actions of thrombotic n-6 eicosanoids could thus lead to reduced fibrinogen. Expected ALA conversion to DHA was found at lower fibrinogen levels only.

ALA efficiently competed with LA for delta-6-desaturase, resulting in a decrease in LC n-6 PUFA, particularly AA. At elevated fibrinogen levels, ALA also inhibited DHA which was even decreased along with AA. That the AA/DHA ratio remained stable during the study, suggests that the ratio of these LC PUFA is of primary importance for hemostatic balance.

The results demonstrate that elevated fibrinogen caused by imbalances in linoleic and □-linolenic acid intake can be lowered by easily implemented fat replacement by rapeseed oil. During the reducing process of fibrinogen, the unique competitive function of □-linolenic acid that regulates both long-chain n-6 and n-3 PUFA levels also favourably affect hemostasis.

Introduction

Hemostasis involves a complex system of factors that normally form and degrade blood clots (Lefevre et al., 2004). Elevated fibrinogen levels, in turn, promote platelet aggregation and thrombus formation, and associate, for example, with increased risk of coronary heart disease (CHD) and stroke (Danesh et al., 2005), diabetes (Klein et al., 2003), and Alzheimer disease and dementia (van Oijen et al., 2005).

We have recently shown that elevated fibrinogen can be due to simple imbalances in essential fatty acid intake and that it can be reduced by canola-type rapeseed oil (Seppänen-Laakso et al., 2010). The study aimed at investigating the effects of rapeseed oil on serum lipids, plasma fibrinogen, LDL oxidation and plasma fatty acids in healthy subjects. This presentation focuses on fatty acid composition of rapeseed oil and specific competitive and metabolic features during fat replacement.

Subjects and methods

Study design. The volunteers were obtained from the registry of the Helsinki University Hospital, from the staff of the hospital, and through an advertisement in a newspaper. From the 42 healthy subjects (35 women, 7 men, aged 16–62 years), 32 did not consume fish in their habitual diets. They formed groups A (n=16) and B (n=16), and were compared with group C (n=10) who were consuming 1–2 fish meals/week. The study lasted for 6 weeks in a parallel design. Zero erucic-acid spring turnip rape oils (cold-pressed or ordinary) contained 5–6% SaFA, 60–62% MUFA, 21–23% LA, 11–12%□ALA and 25–30 mg □-tocopherol/100 g.

Dietary data. The daily energy and nutrient intakes were calculated from 3-day food records, kept just before fat substitution and after 3 weeks' of fat substitution, and analysed using the Nutrica programme. The portions of oils used were also recorded.

Blood sampling. Fasted plasma samples for lipid, fibrinogen and fatty acid analyses were taken before fat substitution, at 3 weeks, and at the end of the study (6 weeks). Blood samples for LDL oxidation studies were taken at the baseline and at 6 weeks.

Copper-induced oxidation and radical scavenging capacity of LDL. In studying the LDL oxidation susceptibility *in vitro*, the lagtime of oxidation, the rate of the propagation phase (oxrate) and the maximum level of conjugated dienes (MaxCD) were measured. In addition, the total peroxyl radical scavenging capacity of LDL (TRAP) was determined.

Plasma fatty acids. Total plasma and plasma phospholipid (PL) fatty acid compositions were analysed by gas chromatography (Seppänen-Laakso et al., 2002).

Statistical methods. Baseline comparisons were made by ANOVA or by the unpaired t-test, while paired t-test or ANOVA were used to study the changes within the groups. Pearson correlation and regression analyses were done to study the relationships between plasma fatty acids and lipids, lipid oxidation markers and fibrinogen.

Results and Discussion

Plasma lipids and lipid oxidation. Before fat substitution, the subjects normally used margarine, cheese and/or butter on bread in their habitual diets. There were no between-group differences in nutrient intakes, serum lipid and plasma fibrinogen levels, or the lagtime of LDL oxidation and oxrate (Seppänen-Laakso et al., 2010).

RSO substitution (mean dose 16 ml/d) significantly changed dietary fat composition, *i.e.* decreased SaFA and increased ALA, LA and MUFA. Individual data showed that the fall of 2-7%-units in total plasma SaFA was related to that in serum cholesterol (0.5-1.0 mmol/L).

The effects on LDL oxidation were minor, and the lagtime shortened in group B only (3.6 min; $p < 0.01$), which could be also enhanced by increases in AA and DHA due to LA and ALA metabolism, respectively. During fish oil supplementation, the reduction of lagtime usually indicates clearly higher oxidation susceptibility of LDL (Pedersen et al., 2003).

Competitive interactions. Already early animal studies on liver lipids reported that the metabolism of the main unsaturated fatty acids favor the substrates in the order linolenate > linoleate > oleate (Mohrhauer and Holman 1963). In human fatty acid metabolism, ALA and LA metabolism also occur in the order of their unsaturation degree, rather than abundance. The prior conversion of ALA during RSO substitution can appear as clearly higher increases in LC n-3 PUFA compared to LC n-6 PUFA (Seppänen-Laakso et al., 1992, 2002).

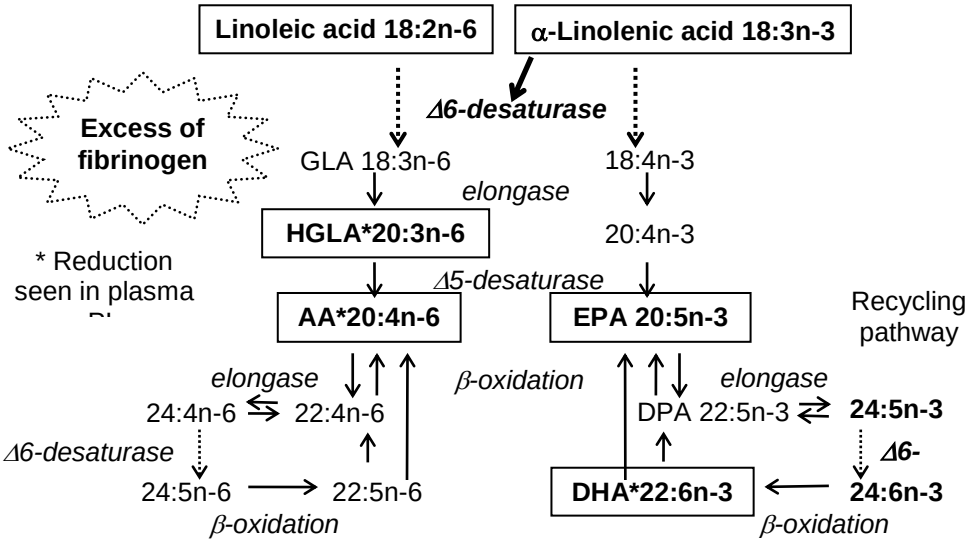
Further basic functions include competitive inhibition by dietary ALA of the conversion of LA to AA (Mohrhauer and Holman 1963), leading to decreased amounts of substrate available for eicosanoid production (Budowski and Crawford, 1985). Among the LC n-3 PUFA, the competing eicosanoid precursor, eicosapentaenoic acid (EPA), in turn, inhibits the production of n-6 eicosanoids from AA (Crawford 1983). Thus, both n-3 PUFA, C18 (ALA) and C20 (EPA), can suppress the effects of AA (Schema 1).

ALA is the first n-3 PUFA that controls LA metabolism by inhibiting the ∆6-desaturase step (Schema 1). A significant decrease in AA in plasma PL was earlier found when low ALA (2%) margarines were replaced by RSO. In this study, the decreases of 1-2%-units in PL LC n-6 PUFA, especially AA, were predominant (Seppänen-Laakso et al., 1993, 2010).

I Suppression by α-linolenic acid (18:3n-3)	**II Suppression by EPA (20:5n-3)**
↓ Linoleic acid → 18:3n-6 → 20:3n-6 → Arachidonic acid ∆6-desaturase HGLA AA, precursor of	↓ Arachidonic acid → n-6 Eicosanoids AA, 20:4n-6 incl. thromboxane A

Schema 1. Inhibition of the formation of AA and n-6 eicosanoids by n-3 PUFA.

This study shows that ALA effectively reduces AA, the precursor of n-6 eicosanoids. Diminished n-6 eicosanoid actions, in turn, could have resulted in major decreases in plasma fibrinogen levels. At elevated fibrinogen levels, on the contrary, ALA totally inhibited even its own metabolism to DHA, which also includes the final Δ6-desaturase step (Schema 2). This agrees with the *in vitro* studies showing that increases in ALA strongly inhibit the Δ6-desaturation from 24:5n-3 to 24:6n-3 in the recycling n-3 pathway (Sprecher 1992, 2000).



Schema 2. Inhibition of PUFA pathways by □-linolenic acid at elevated fibrinogen levels.

The results reveal that plasma AA and DHA levels are strictly controlled by ALA under elevated fibrinogen conditions, in particular. Such conditions found in prothrombotic and proinflammatory states may reflect a serious state in the body. Thus, the reduction of both AA and DHA can be protective in order to avoid drastic changes in the hemostatic balance. In addition, the average AA/DHA ratio remained unchanged during RSO substitution (0 - 6 w range: 2.60 - 2.65; n = 16; ns), while at lower fibrinogen levels (n = 26), the ratio decreased from 2.83 to 2.70 (3 w; $p < 0.05$) and 2.66 (6 w; $p = 0.01$), due to ALA metabolism to DHA.

Conclusions

The fatty acid composition of canola-type rapeseed oil, containing moderate amounts of linoleic and □-linolenic acids in a ratio of 2:1 combined with high oleic acid content, best meets the basic requirements of essential fatty acids in the body.

□-Linolenic acid exhibits new significant functions by initiating the reducing process of elevated fibrinogen, through effective competitive inhibition of n-6 PUFA, and, finally, by regulating LC n-6/n-3 PUFA ratio, all contributing desirable impact on hemostatic balance.

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