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University of Turku

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Vaccinium and Other Superfruits
MECC Maastricht
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Health Effects of Sea Buckthorn Berries;

Investigations at the University of Turku, Finland

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Natural sea buckthorn in the whole Eurasia

Multi-disciplinary, international research active, including health issues



Picking of sea buckthorn
in northern Finland
(Kittilä Tepasto)
in February 2010,
A Finnish variety
(*H. rhamnoides* ssp.
rhamnoides)



Picking of sea buckthorn
in Lhasa, Tibet,
September 2011,
A natural
H. gyantsensis



Natural *H. rhamnoides*
ssp. *rhamnoides*, Pyhämaa,
southern Finland

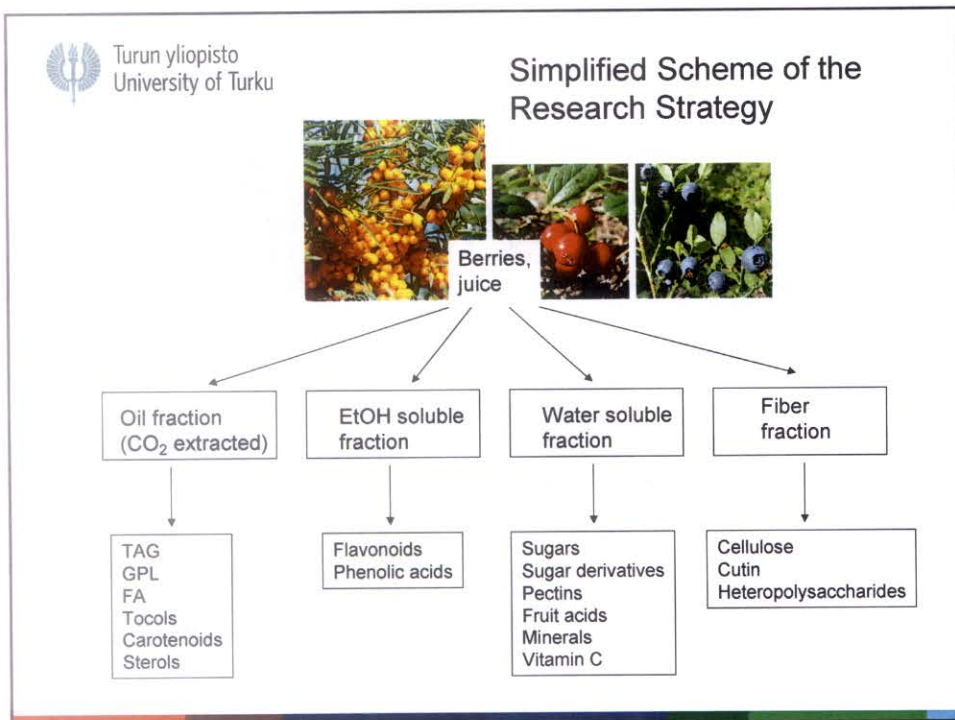
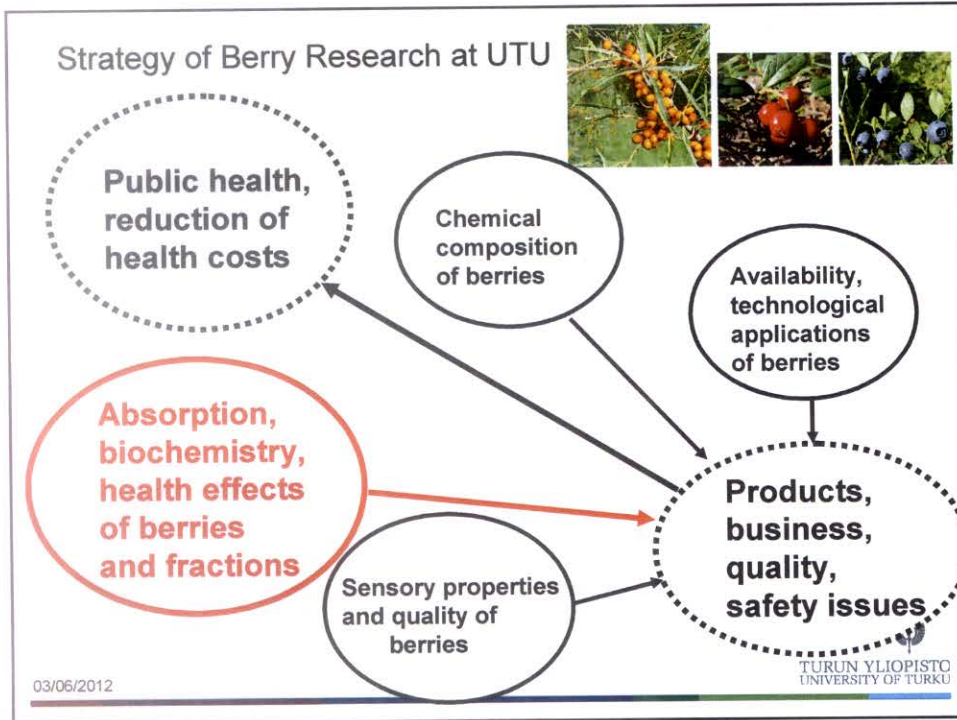


Natural *H. rhamnoides* ssp.
mongolica, Buryatia, Russia



Natural *H. rhamnoides* ssp.
sinensis, Quinghai, China

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Examples of Berries Investigated at UTU



Empetrum nigrum



Sorbus acuparia



*Chamaepericlymenum
suecicum*



*Hippophae
rhamnoides*



Rosa dumalis



Rubus saxatilis



Rubus arcticus



Ribes nigrum



*Vaccinium
vitis-idaea*



Fragaria vesca



Rubus avium
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Ribes rubrum



Ribes alpinum



Rubus idaeus



Vaccinium myrtillus



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Examples of Health Effects of Sea Buckthorn (SB) Results from the University of Turku

1. Berries (sea buckthorn, bilberry and lingonberry) reduce the risk of type 2 diabetes
2. SB oil has high antioxidative capacity
3. The bioactive compounds of SB are bio-available in the gut
4. SB oil enhances absorption of flavonoids
5. SB lowers the postprandial insulin peak
6. SB alleviates the common inflammation (lowers CRP)
7. SB juice may reduce the risk of CHD
8. SB oil relieves the dry eye symptoms
9. SB oil relieves symptoms of atopic dermatitis
10. SB oil reduces and retards platelet aggregation



The northernmost SB
plantation in the World;
Kittilä, Tepasto,
University of Turku



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1. Hypothesis: Industrial Berry Products May Reduce the Risk of Type 2 Diabetes and Metabolic Syndrome



Berry powders



61 women, parallel design
20 weeks intervention
2 groups, difference c.a. 120 g of berries a day
Blood samples before, during, after

Berry seed oils (CO₂-extr)



Dried sweetened berries



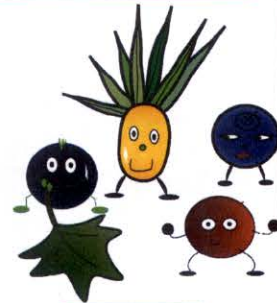
Berry juices



Lingonberry rye bread



Frozen berries



black currant
bilberry
lingonberry
sea buckthorn

Eur J Clin Nutr
2010, 64, 614-621

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1. Result of the Four Berries Research: Industrial berry products lowered the ALAT -values. May indicate reduction of the risk of metabolic syndrome and of type 2 diabetes

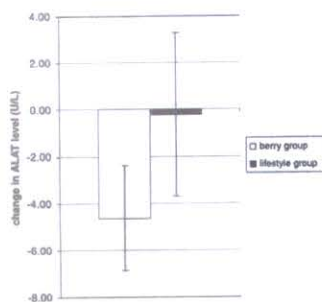


Figure 1 Changes in plasma alanine aminotransferase (ALAT) levels in berry group (-4.6, P<0.001) and in control group (-0.2, P>0.05) during the intervention period.

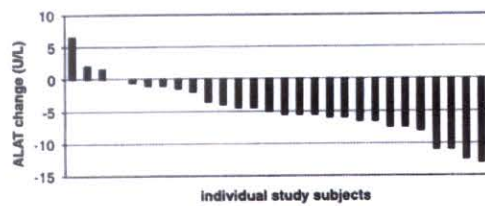


Figure 3 Changes in alanine aminotransferase (ALAT) levels during intervention period of individual study subjects in berry group.



Eur J Clin Nutr
2010, 64, 614-621

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2. Hypothesis:

SB Oils amongst Northern berry oils show exceptional antioxidative activities

Berries

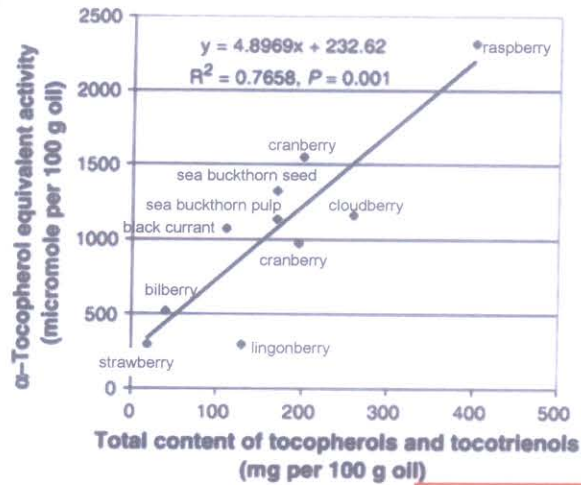
- bilberries, lingonberries, cranberries, crowberries, cloudberrries, sea buckthorn, raspberries, blackcurrants, redcurrants, snowball, rowanberries, strawberries

Isolation of oils

- Dried, milled seeds extracted with SF CO₂, 50 °C, 350 bar

Analyses

- Tocols by HPLC-DAD, FAs as Me-esters by GC, **peroxyl radicals by oxidation of luminol (ref. α-tocopherol)**, peroxidation inhibition by t-BuOOH –induced peroxidation in rat liver microsomes (*in vitro*), LDL oxidation inhibition (*in vitro*), LDL incorporation (*in vitro*), superoxide anion scavenging activity (by xanthine oxidase), DNA oxidation in rat liver homogenate by UV



Peroxy radical scavenging activity (corresponding to lipophilic ORAC). Dots represent individual seed oils.

Food Res Int
2011 44 2009-2017



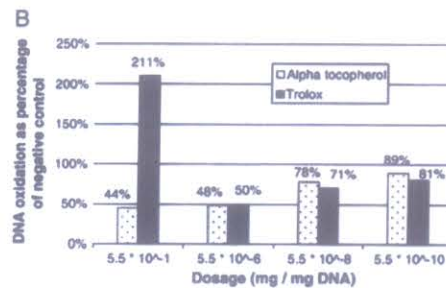
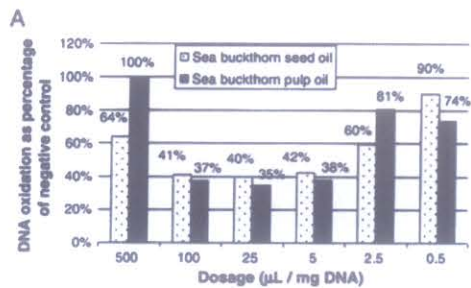
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2. Results:

SB oils protect purified DNA from oxidation *in vitro* (A).

References tocopherol and trolox (B)

Three replica on three different days. Coefficient of variation 9 % within day and 12 % between-day.



Food Res Int
2011 44 2009-2017



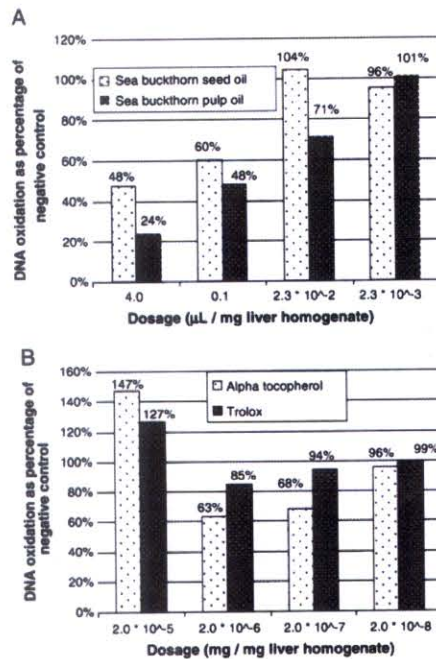
2. Results:

SB oils protect purified rat liver homogenate from oxidation *in vitro* (A).

References tocopherol and trolox (B)

Three replica on three different days. Coefficient of variation 11 % within day and 14 % between-day.

Food Res Int
2011, 44, 2009-2017



3. Hypothesis:

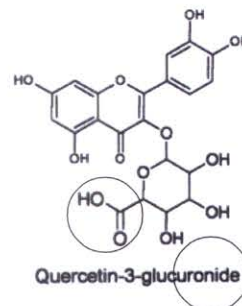
Flavonoid glycosides of SB and lingonberry are bioavailable

Study design:

- Postprandial trial, four subjects
- Sea buckthorn breakfast (300 g) with vanilla yoghurt
- Blood, urine, feces samples before the trial
- Blood samples at 1, 2, 4, 8 h (-70 °C)
- Urine samples for 24 h (-70 °C) and feces samples for 48 h (lyophilized)
- uHPLC-MS/MS (C_{18} , ESI, MRM) analysis of flavonols

SB flavonoids:

- I-3-rut 2.2 mg/100g
- I-3-gluc 0.88
- Q-3-gluc 0.55
- I-3-gal 0.22
- Kae-3-rut 0.08
- Q-3-gal 0.04



The major flavonol derivative in urine after sea buckthorn meal



J Agric Food Chem
2010, 58, 620-627

3. Result:

Metabolites of SB and lingonberry flavonoids are absorbed, reflect SB flavonoids and are mainly converted to glucuronides

- Isorhamnetin and quercetin glucuronides found in plasma after SB meal
- In urine 5 % glycosides, 95 % glucuronides
- In feces glycosides only

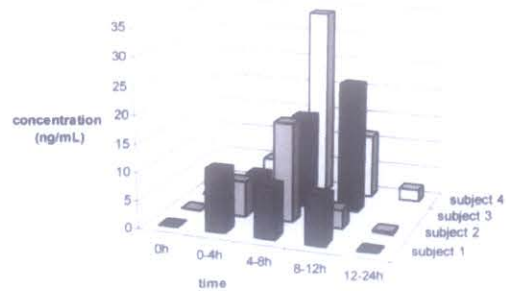


Figure 7. Concentrations of isorhamnetin-3-glucoside in urine samples of the four study subjects after the ingestion of sea buckthorn.

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J Agric Food Chem
2010, 58, 620-627

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4. Hypothesis:

SB oil may increase the bioavailability SB flavonoids

Aim:

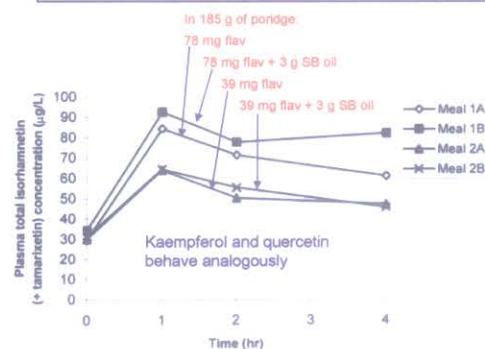
- to investigate absorption of SB flavonoids and to see the effect of SB oil on absorption

Study design:

- 22 healthy males, divided in 2 groups
- Double-blind, placebo-controlled, crossover
- Group a) 78 mg flavonols in oat meal porridge (185 g)
- Group b) 39 mg flavonols in oat meal porridge (185 g)
- 3 g SB oil randomly mixed in one of the two meals of each subject
- Blood drawn for flavonol analyses at 0, 1, 2 and 4 h
- Plasma levels of quercetin, kaempferol and isorhamnetin determined after hydrolysis

4. Result:

3 g of SB of oil in porridge increases the absorption of SB isorhamnetin, kaempferol and quercetin glycosides significantly



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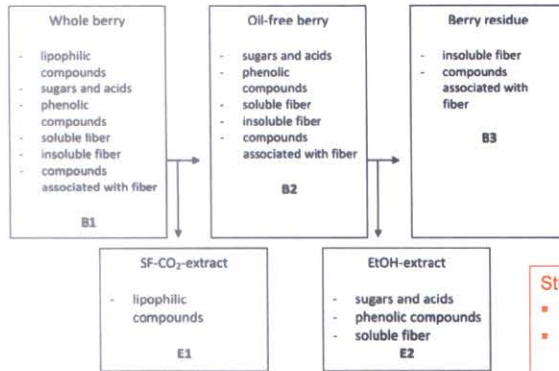
J Agric Food Chem
2006, 54, 7364-7369

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5. Hypothesis:

SB berry and EtOH fraction may affect in postprandial glucose and insulin levels



Study design:

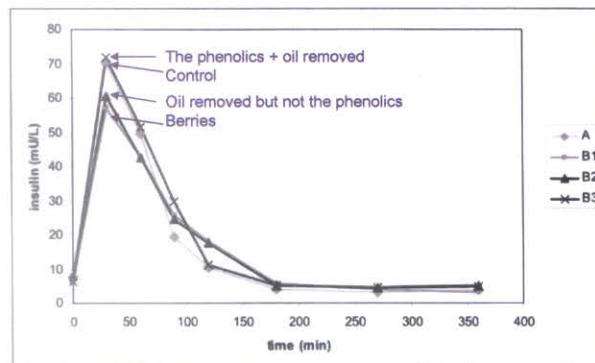
- 10 healthy males
- 200 g fat-free, lactose-free yoghurt + 50 g glucose as base and control
- Study meals included: B1 = berries 40 g; B2 = CO₂ extracted berries 32.4 g; B3 = EtOH and CO₂ extracted berries 18 g

Eur J Clin Nutr
2010 64: 1465-1471



5. Results:

SB berry and EtOH fraction affect postprandial insulin response



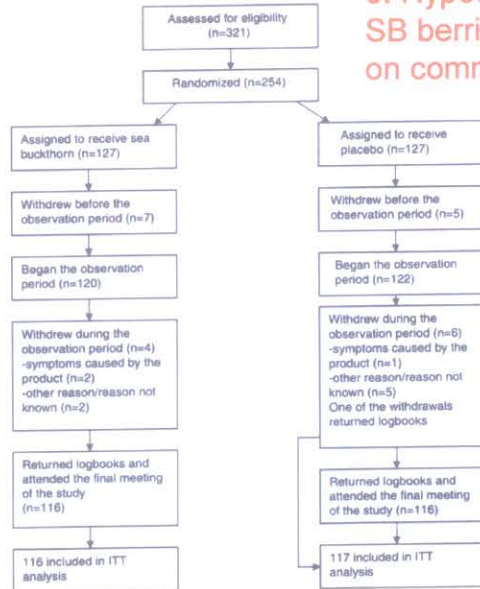
Conclusion:

- Berries suppressed insulin peak, removal of phenolics eliminated the effect
- Removal of lipids of the berries did not have any effect

Postprandial insulinemia as serum insulin values after control meal (A) and study meals (B1, B2, B3)

Eur J Clin Nutr
2010 64: 1465-1471

6. Hypothesis: SB berries may have positive effects on common cold and inflammations



Study protocol

- Effect of SB on common cold and CRP were the hypotheses
- 233 healthy volunteers (19 – 55 y men and women) consumed 28 g SB berry puree or non-effective placebo for 90 days
- SB *H. rhamnoides* ssp. *mongolica* cv. Prozcharachnaya
- DB, randomized, parallel study
- Diaries: Common cold and digestive/urinary tract infections
- Sensitive C-reactive protein (CRP) measured (inflammation marker)

Eur J Clin Nutr
2008, 62, 1123-1130

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6. Results:

A low dose of SB puree decreased the sensitive CRP concentration significantly compared to the placebo $P = 0.039$ (median change 0.059 mg/L). This also may indicate reduction of cardiovascular diseases.



Even though in this study *H. r. ssp. mongolica* was used there are old indications that the "original" Tibetan *H. gyantsensis* would be especially effective.



Eur J Clin Nutr
2008, 62, 1123-1130

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7. Hypothesis:

Sea buckthorn juice may have positive effects on coronary heart disease risk factors

Study design:

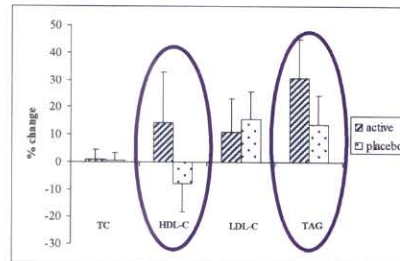
- 20 healthy male volunteers
- SB vs. placebo juice for 8 weeks, fasting venous blood samples
- SB juice supplementation contained: 462 mg Vit-V, 355 mg flavonoids, 3.2 mg α -tocopherol, 1.0 mg β -carotene
- Measured: Plasma chol, LDL-chol, platelet aggregation, ICAM-1,



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7. Results:

20 % increase in plasma HDL-chol
17 % increase in plasma TAG
Moderate decrease in LDL oxidation



TC = total cholesterol
HDL-C = HDL cholesterol
LDL-C = LDL cholesterol
TAG = triacylglycerols

J Nutr Biochem
2002, 13, 346-354

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8. Hypothesis:

Oral sea buckthorn oils relieve dry eye symptoms

Dry eye:

- Prevalence of even >20 % in elderly people
- Defining, diagnosing and treating very challenging
- Inflammation and increased osmolarity symptoms
- In aqueous-deficient dry eye lacrimal secretion reduced
- In evaporative dry eye water evaporation increased (non-stable tear film lipid layer)
- In both cases inflammation activated
- Old age, female gender and contact lenses are risk factors

Study protocol:

- 86 females, 20 – 75 years completed the study
- Double-blind, randomized, parallel study
- Age, sex and contact lens wear stratification
- 2 g oil (sea buckthorn or placebo) for three months
- Test oil: CO₂ extracted seed + pulp oil
- Placebo oil: medium-chain FA coconut and palm kernel oil
- At 0, 1 and 3 months osmolarity, tear film break-up time (TBUT), and tear film secretion measured (Schirmer)
- Symptom questionnaire

8. Results:

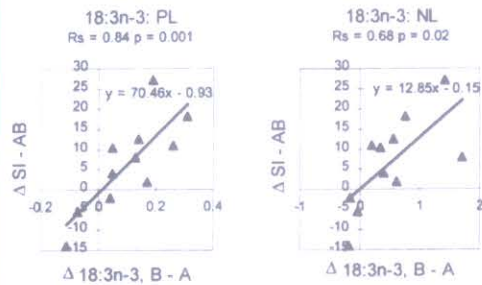
- Significantly less increase in osmolarity (autumn-winter) in SB-group (P = 0.04)
- Redness and burning lower in SB group (P = 0.04)

J Nutr
2010, 140, 1462-1468

9. Hypothesis: Oral Sea Buckthorn Oil May Relieve Atopic Dermatitis

- Study design:
- Placebo controlled, double blind, parallel study
- Sea buckthorn seed and pulp oils vs. parafin oil
- 78 atopic dermatitis patients divided in three groups
- 49 completed the study
- AD history from childhood, persistent symptoms
- Normal diet, normal medication
- Five grams of oil for four months
- Clinical examinations at 0, 1 and 4 months
- SCORAD system applied in evaluations
- Plasma samples: GPL and NL fatty acids, Chol, TAG
- Serum samples: total and specific IgE

9. Results: Common changes in plasma FAs to the "healthy" direction



Correlation between symptom improvements and increase in proportions of α-linolenic acid (18:3n-3) in plasma phospholipids (PL) and neutral lipids (NL) in sea buckthorn seed oil group after one month administration

J Nutr Biochem
1999, 10, 622-630

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9. Results:

- Reduction in SCORAD values in pulp oil and placebo groups in four months
- GPL in seed oil group: α-linolenic, linoleic and eicosapentaenoic acids increased after one month
- GPL in pulp oil group: Palmitoleic acid (16:1n-7) increased after one month
- NL in seed oil group: α-linolenic acid increased after four months
- NL in pulp oil group: palmitoleic acid increased
- Parafin oil: No changes in FAs
- Administrations had no effect on IgE, Chol or TAG

J Nutr Biochem
1999, 10, 622-630

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10. Hypothesis: SB oil may have an effect on platelet aggregation

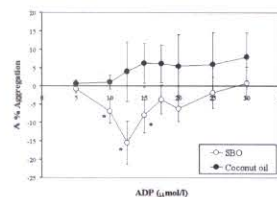
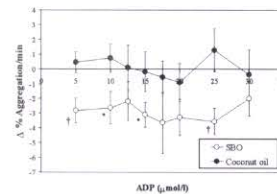
Study design:

- 12 normolipidemic, healthy men (11 completed)
- 9 exclusion criteria
- DB, randomized, controlled
- CO₂ extracted SB oil vs. fractionated coconut oil
- 4 weeks, both oils 5 g/day, randomized order
- 4-8 week wash-out
- Fasting (12 h) venous blood samples before and after treatments
- Blood in Na-citrate and centrifuged (platelet-rich plasma)
- ADP and arachidonic acid agonists → dose-response curves
- Subjects on their own control

J Nutr Biochem
2000, 11, 491-495

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10. Results: SB berry oil has an effect on platelet aggregation velocity and total aggregation in healthy men



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Yang, B.; Kalimo, K.O.; Tahvonen, R.L.; Mattila, L.M.; Katajisto, J. K., Kallio, H.P. Effect of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. *J. Nutr. Biochem.* 2000, 11, 338-340.

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Yang, B.; Kallio, H.P.; Kalimo, K.O.; Mattila, L.M.; Tahvonen, R.L.; Kallio, S.E.; Katajisto, J.K. Effects of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on fatty acid composition of plasma lipids in patients with atopic dermatitis and measurement of skin surface roughness. In: *Functional Foods – A New challenge for the food chemists*. Eds. R. Lásztity, W. Pfannhauser, L. Simon-Sarkadi, S. Tömösközi. Publishing Company of TUB, Budapest, Hungary. 1999, pp. 124-131.

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THANK YOU !



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