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# The effects of rose hip (*Rosa canina*) on plasma antioxidative activity and C-reactive protein in patients with rheumatoid arthritis and normal controls: A prospective cohort study

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ABSTRACT

*Objectives:* Rose hip (*Rosa canina*) has been used as an herbal remedy against a wide range of ailments including inflammatory disorders. The anti-inflammatory and antioxidant properties of rose hips have been evaluated *in vitro* and active constituents have been isolated. Rose hip contains antioxidant nutrients and an anti-inflammatory galactolipid. Rheumatoid arthritis (RA) is an inflammatory disease where activated cells release reactive oxygen substances. Thus it could be relevant to investigate if rose hip had an anti-inflammatory and/or antioxidant effect in this situation.
*Methods:* In this open case–control study 20 female patients with RA and 10 female controls were given 10.5 g rose hip powder daily (Litozin®) for 28 days. Blood samples were analysed at baseline and follow-up for the capacity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase and the inflammatory marker C-reactive protein (CRP). The participants kept a food diary for the first 3 days and the last 3 days of the intervention period. The RA-patients completed The Stanford Health Assessment Questionnaire at baseline and follow-up.
*Results:* CRP-concentrations of both patients and healthy controls did not change. Nor was any effect found on the activity of antioxidant enzymes. There was no difference in food intake at baseline, but in the last week the RA-group reduced their energy intake.
*Conclusions:* 10.5 g Litozin® in 28 days had neither effect on clinical symptoms or laboratory measurements in patients with RA or healthy controls. This is in contrast to previous intervention studies with rose hip powder that found a reduction in the concentration of CRP. The results of the present study indicate that a daily amount of approximately 10 g rose hip powder for one month has no anti-inflammatory and/or antioxidant effect.



## Introduction

Patients with chronic painful inflammatory diseases such as rheumatoid arthritis (RA) often seek alternative therapy. Rose hips and in particular those of dog rose (*Rosa canina* L., Rosaceae) have traditionally been used for the prevention and therapy of infections, and inflammatory diseases (Kharazmi and Winther 1999; Winther et al. 1999; Rein et al. 2004; Willich et al. 2010). Inflammation is associated with increased generation of reactive oxygen-and nitrogen species (ROS/RNS), which is thought to play an important role in the inflammatory process and to contribute to tissue damage. Antioxidant nutrients such as vitamin C, vitamin E, carotenoids, polyphenols and antioxidant enzymes play a significant role in the protection against the damaging effects of ROS/RNS. Important antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (Halliwell and Gutteridge 1999). A rose-hip powder (Litozin®) has shown to reduce symptoms associated with rheumatoid inflammation in clinical trials and extracts of this powder has shown strong anti-inflammatory activities (Kharazmi and Winther 1999; Warholm et al. 2003; Rein et al. 2004) as well as high total antioxidant capacity (TAC) *in vitro* (Daels-Rakotoarison et al. 2002;

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Wenzig et al. 2008). Evidence from *in vitro* studies indicates that rose hip powder exert anti-inflammatory properties via reduced chemotaxis of peripheral blood neutrophils and monocytes in healthy subjects, and a reduction in C-reactive protein (CRP) is seen after 4 weeks supplementation in patients with osteoarthritis (OA) (Winther et al. 1999; Kharazmi and Winther 1999). A specific galactolipid (1,2-di-*O*-$\alpha$-linolenoyl-3-*O*-$\beta$-D-galactopyranosyl-*sn*-glycerol; GOPO) isolated from rose hip powder by bioassay-guided fractionation and has been shown *in vitro* to reduce chemotaxis of peripheral blood polymorphonuclear leukocytes and monocytes, and therefore could play an important role as a mediator of the pain reducing property of rose hips (Larsen et al. 2003). Rose hips also contain high amounts of vitamin C, minerals, carotenoids, polyphenols and fatty acids that may contribute to the antioxidant and/or anti-inflammatory activities (Jäger et al. 2007; Wenzig et al. 2008). These considerations have been confirmed in other *in vitro* studies, where extracts of rose hips were shown to have potent anti-inflammatory activities and to inhibit cyclooxygenase (COX)-1 and -2 (Jäger et al. 2007; Wenzig et al. 2008). The inhibition of COX was found to be related to particular unsaturated fatty acids but also other constituents such as triterpenoic acids were found to contribute to the COX inhibitive activity (Jäger et al. 2007; Wenzig et al. 2008).

The net-result of a meta-analysis of rose hip powder from *Rosa canina* for symptomatic treatment of OA was a small but relevant reduction of pain and a significant reduction of the use of medication (Christensen et al. 2008).

Drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and biologic agents are used for the treatment of symptoms in RA, but patients seek complementary-alternative therapy for various reasons. Some do not respond successfully, some suffer serious side effects from the traditional medication, and some try to maximize the effect by a combination of traditional medicine and alternative medicine. If alternative therapy exerts positive effects that can be validated, they will be of interest in the traditional treatment of patients with RA.

The objectives of the present study were to investigate if intervention with rose hip powder from *Rosa canina* (i) affect the inflammatory marker CRP level in RA patients and controls, (ii) affect functional status and pain evaluated by the Health Assessment Questionnaire (HAQ) in RA patients, and (iii) affect the level of antioxidant enzyme activity in RA patients and/or controls.

## Materials and methods

### *Subjects*

Twenty consecutive, female patients with RA as defined by the American Rheumatism Association (ARA) were recruited from the outpatient clinic, Department of Rheumatology, the Parker Institute, Frederiksberg Hospital. The inclusion criteria for the RA-group were stable RA disease activity and unchanged pharmacological treatment for at least 3 months. Patients with other systemic, immunologic, or inflammatory diseases such as reactive arthritis, MB Bechterew, fibromyalgia or psoriatic arthritis were not included in the trial. Ten healthy, age-matched female controls were recruited from volunteering hospital staff members. Persons with body mass index > 30 kg/m$^2$, plant allergy, odd food habits, and vegetarians were not included.

### *Intervention*

A standardised rose hip powder of *Rosa canina* (Litozin®) made from the seeds and husks of the fruits from a subtype of *Rosa canina* was used in this study. This common wild-growing hedgerow rose is marked as a food supplement with antioxidant properties and has been on the Scandinavian market for a decade (Warholm et al. 2003; Rein et al. 2004; Willich et al. 2010). The powder used was from a single batch.

The plants used for the preparation of the rose hip powder in Litozin® are grown in standardised fields in Denmark (Langeland) according to good agriculture practice (GAP). The rose hips are harvested when they are mature and brought immediately to freezing facilities. The selection of rose hips for production of the powder is made by laser technique and the temperature never exceeds 40 °C (Rein et al. 2004). The standardised rose hip powder used in this study is assumed to be of the same quality, and hence contain the same amounts of GOPO, vitamins, minerals and other constituents (Larsen et al. 2003; Rein et al. 2004) as the rose hip powder used in other clinical trials (Winther et al. 1999; Kharazmi and Winther 1999; Warholm et al. 2003; Rein et al. 2004; Willich et al. 2010) because (i) the same subtype of *R. canina* has been used, (ii) the plants have been grown in the same fields and (iii) the production of the rose hip powder has been performed under the same standardised conditions as described above.

### *Study design*

The study was designed as an open 4-week prospective cohort study, carried out between February and April 2008. Patients and controls received 10.5 g rose hip powder daily (2 × 7 capsules) for 28 days. Each capsule contained 750 mg rose hip powder. The capsules were taken two times a day along with a meal. During the study period, the patients and controls were instructed not to change exercise habits, medication, consumption of medicine, food, drinks and vitamin- and minerals or dietary supplements. All participants filled out a form with these information at baseline and at follow up. The study was approved by the Regional Ethical Committee and in accordance with the Declaration of Helsinki. The volunteers all gave their oral and written informed consent.

### *Dietary assessments*

For the calculation of dietary intake of energy and nutrients, the participants completed a 2 × 3 day food dairy (first and last three days of the intervention period). To estimate the portion size standard household measurements were used. The average estimated intake was calculated using the Dankost 3000 calculation program (Dansk Catering Center, Herlev, Denmark) based on the food-composition database of the Danish Veterinary and Food Administration. The use of any kind of supplementation (e.g., fish oils, vitamins, minerals, etc.) prior to the study was recorded. The intake of GOPO was estimated after analyses of some of the most popular types of fruits, vegetables, cereals, and potato (Larsen and Christensen 2007).

### *Outcome measures*

At study entry and at the end of the intervention period the following items were assessed: HAQ, a 100 mm VAS for pain assessment, a 100 mm VAS for assessment of disease severity (patients global). At the same time, blood samples were drawn for analyzing the plasma concentration of CRP and antioxidant enzymes activity: SOD, GPx, GR and catalase. Compliance was assessed by counting the total number of returned capsules, and the participants were instructed to report any adverse event.

*Blood collection and biochemical analysis*

Fasted blood (20 ml) was drawn (at baseline and endpoint) into evacuated tubes with EDTA anticoagulant (Vacutainer: Becton Dickenson Lot no 367525). Fasted blood samples were stored on ice for a maximum of 1 h prior to centrifugation (4 °C, 1500 × *g*, 10 min). Plasma and leukocytes were removed, and erythrocytes were washed twice in NaCl 0.9%, lysated with MilliQ-water, and stored at −80 °C until analyzing. GPx, catalase, SOD and GR activities were measured in erythrocyte lysates by previously published procedures (Wheeler et al. 1990) on a Cobas Mira Plus+ analyzer (Roche Diagnostic System, Basel, Schweiz).

The enzymatic activities were calculated relative to the amount of hemoglobin. All samples were run in duplicate. Laboratory controls were: SOD: RANDOX RANSOD KONTROL Lot nr 228RD; GPx: RANDOX RANSEL KONTROL Lot nr 291RS; GR: RANDOX GLUTATHIONE REDUCTASE KONTROL Lot nr. 110GR in supplement to the internal standard.

*CRP*: Blood samples (1.4 ml) were collected in sterile tubes and allowed to stand at room temperature for a minimum of 15 min before centrifugation (20 °C, 2500 × *g*, 10 min). CRP was determined in serum on an ABX Pentra 400 (Horiba ABX, France) with a high sensitive latex-enhanced immunonephelometric assay (CRP CP from ABX Pentra).

*Statistics*

The primary statistical analyses were based on observed mean differences (i.e. changes in scores, denoted with a $\Delta$). In order to compare groups we used two-sample *t* tests (or the Mann–Whitney test when appropriate) – testing for potential differences between changes in the RA- and the control group, respectively after 28 days intervention. Testing the statistical hypothesis $H_0$: $\Delta$healthy control = $\Delta$RA. As this was designed and prespecified as an explorative analysis, we applied the paired test statistics as well: one-sample *t*-test and Wilcoxon's test for matched pairs were used to compare baseline findings with those after 28 days intervention in each group separately.

All data were handled as recommended by STROBE for estimating epidemiologic data (http://www.strobe-statement.org). Data are given as mean ± standard deviation (SD) unless otherwise stated. Any *P*-value associated with a two-sided hypothesis equal to or <5% was regarded as statistically significant *per se*.

## Results

The base-line characteristics of RA patients and controls are presented in Table 1. None of the potential prognostic factors in RA such as age and body mass index were significantly different ($P > 0.47$). The same applied for the use of anticonception, vitamins and mineral supplements, dietary supplements and use of alternative medications. There were only few smokers in both groups, and the frequency of exercise was similar in the two groups. All participants succeeded in maintaining their usual medication, intake of vitamin- and dietary supplements or exercise habits during the intervention period. Two RA patients but no controls dropped-out from the study. One RA patient dropped out before the intervention, and one after two weeks because of mild abdominal symptoms. The effects of *Rosa canina* powder (Litozin®) on plasma CRP, erythrocyte antioxidant enzyme activity and HAQ are shown in Table 2.

Baseline plasma levels of the inflammation marker CRP did not differ significantly between the two groups. Intervention with Litozin® had no effect on CRP in RA patients or controls. At follow-up there was no significant change in CRP between the two groups ($P = 0.156$). Furthermore, no difference was found between the of antioxidant enzyme activity at baseline in the two groups (Table 1). None of the four erythrocyte antioxidant enzyme activities were affected by intervention with Litozin® (Table 2). *Rosa canina* powder also had no significant effect on HAQ, VAS pain or VAS patient global in the RA-group (Table 2).

The average habitual dietary intakes in the two groups are shown in Table 1. At baseline, there were no significant differences between the consumption of specific food groups that influence intake of antioxidant nutrients such as fruits, vegetables, cereals, legumes or the anti-inflammatory n-3 PUFA such as fish. The mean daily intake of energy, protein, vitamin A, vitamin C, vitamin E, selenium, and galactolipid (GOPO) was the same in the two groups at baseline and at end of the intervention (Tables 1 and 2). The RA-groups reduced their intake of energy significantly more that the control-group from baseline to follow-up ($P = 0.029$) (Table 2).

*Compliance*

Compliance was calculated from the amount of returned capsules. Compliance was 98.8 ± 1.4% for the RA group and 99.3 ± 1.3% for the control group.

## Discussion

Intervention with Litozin® had no effect on CRP level in the RA or control group in the present study. This result is contrary to the studies by Kharazmi and Winther (1999) and Winther et al. (1999) where an initial intervention with 45 g Litozin® a day significantly reduced CRP levels in OA patients (Winther et al. 1999) and healthy participants (Winther et al. 1999; Kharazmi and Winther 1999). 45 g Litozin® is a much higher dose than used in this study, and baseline CRP levels were also higher in the two previous studies than in the present study. This could explain the differences in outcome, and indicate that a dose of approximately 10 g rose hip powder a day is insufficient. Another confounder could be the low CRP level in our RA patients. At baseline there were no significant difference in CRP level between the RA group and the control group, which could indicate that the patients were well treated. A low CRP level at baseline means a reduced possibility for a further decline.

The amount of GOPO in the used Litozin® was not analysed, but according to previous investigations, the estimated total amount of this bioactive galactolipid in 10 g standardised *Rosa canina* powder is approximately 2.5 mg (Larsen et al. 2003). The standardised rose hip powder investigated in the study of Larsen et al. (2003) originate from the same subtype of *Rosa canina* as the standardised rose hip powder of Litozin® capsules used in this and other studies (Kharazmi and Winther 1999; Winther et al. 1999; Warholm et al. 2003; Rein et al. 2004; Willich et al. 2010). Because the rose hip powder in Litozin® are produced from the fruits of *Rosa canina* plants grown according to GAP on standardised fields, at the same locality in Denmark, and by standard procedures, it is reasonable to assume that the content of GOPO, vitamins, and other constituents in this standardised rose hip powder is approximately the same despite of the production year. This is also supported by previous investigations of batches of fresh prepared Litozin® rose hip powder between 2002 and 2007 for the content of GOPO by analytical HPLC (Larsen and Christensen 2007). According to these investigations a rather constant level of GOPO between 2.4 and 2.8 mg GOPO in 10 g Litozin® rose hip powder was found (unpublished results). In this study, the average estimated intake of GOPO and related galactolipids from food was 50 mg a day. It seems that 10 g Litozin® contribute with a small amount of GOPO compared with intake from normal food. GOPO is a combination of galactose, glycerol and α-linolenic acid, and is probably digested in the gastro-intestinal tract before absorption. Animal studies show that

**Table 1**
Baseline characteristics for 19 patients with rheumatoid arthritis (RA) and 10 healthy controls.

| | RA ($n$ = 19)Mean ± SD | Control ($n$ = 10)Mean ± SD | Difference RA and control*P* values |
|---|---|---|---|
| *Subject characteristics* | | | |
| Age (years) | 59.8 ± 13.7 | 55.6 ± 14.7 | 0.465 |
| BMI (kg/km$^2$) | 23.8 ± 3 | 24.2 ± 2.6 | |
| Anticonception (%) | 0 | 1 (10) | 0.690 |
| Exercise, h/week | 5.3 ± 2.6 | 3.6 ± 2.3 | 0.083 |
| Vitamins and minerals, *n* (%) | 12 (63) | 5 (50) | |
| Herbal remedies, *n* (%) | 11 (58) | 3 (33) | |
| Complementary and alternative medicine, *n* (%) | 0 (0) | 0 (0) | |
| Vegetarian, *n* (%) | 0 (0) | 0 (0) | |
| Smoker, *n* (%) | 2 (11) | 1 (10) | |
| *Clinical data* | | | |
| HAQ disability | 0.6 ± 0.47 | | |
| VAS, pain | 2.8 ± 1.5 | | |
| VAS, global | 3.3 ± 1.6 | | |
| *Biochemical data* | | | |
| CRP (mg/l) | 4.2 ± 5.4 | 2.1 ± 2.8 | 0.179 |
| Catalase, U/g Hb | 12.6 ± 2.5 | 12.2 ± 2 | 0.613 |
| GR, U/g Hb | 12.1 ± 1.6 | 11.7 ± 1.3 | 0.503 |
| GPx, U/g Hb | 35.1 ± 5.7 | 32.3 ± 6.2 | 0.255 |
| SOD, U/g Hb | 1195 ± 322 | 1325 ± 141 | 0.143 |
| *Characteristics of diet* | | | |
| Energy intake, kJ/d | 7342 ± 1266 | 7422 ± 294 | 0.876 |
| Energy intake, kJ/kg | 114 ± 28 | 112 ± 18 | 0.820 |
| EI/BEE$_{est}$ | 1.2 ± 0.2 | 1.3 ± 0.17 | 0.109 |
| Protein, g/d | 68 ± 14 | 70 ± 15 | 0.741 |
| Protein, g/kg | 1.1 ± 0.3 | 1.1 ± 0.2 | 0.972 |
| Vitamin A, RE/d | 2244 ± 3555 | 2365 ± 2166 | 0.912 |
| Vitamin C, mg/d | 164 ± 103 | 149 ± 107 | 0.714 |
| Vitamin E, a-TE/d | 7.1 ± 2.4 | 7.3 ± 2.4 | 0.875 |
| Selenium, μg/d | 36.4 ± 10.6 | 37.6 ± 10.8 | 0.777 |
| Fruit (g/d) | 256 ± 8 | 250 ± 144 | 0.902 |
| Vegetable, g/d | 210 ± 122 | 236 ± 105 | 0.572 |
| Fish, g/d | 27 ± 31 | 43 ± 47 | 0.354 |
| Galactolipid (GOPO), mg/d | 58.7 ± 42.5 | 44.2 ± 28.8 | 0.292 |

*Abbreviations*: SD: standard deviation; d; day; EI; energy intake; BEE; estimated basal energy expenditure; CRP; C-reactive protein; Hgb; hemoglobin; GR; glutathione reductase; GPx; glutathione peroxidase; SOD; superoxide dismutase; HAQ; Health Assessment Questionnaire; VAS; visual analog scale.

pancreas preparations hydrolyzed galactolipids into free fatty acids and water-soluble galactose-containing compounds (Andersson et al. 1996), and that no radiolabeled intact galactolipid appeared in chyle lipids in rats (Ohlsson et al. 1998). It is further suggested that galactosylglycerols will be fermented into galactose and glycerol in colon (Sugawara and Miyazawa 2000). A human *in vitro* study showed that pancreas juice and duodenal content hydrolyze galactolipids and liberate free fatty acids (Andersson et al. 1995). An

**Table 2**
Difference from baseline to follow-up inside the groups and between the groups, for 19 patients with rheumatoid arthritis (RA) and 10 healthy controls.

| Variable difference Δ | RA ($n$ = 19)Mean ± SD | RA ($n$ = 19)*P*-value | Control ($n$ = 10)Mean ± SD | Control ($n$ = 10)*P*-value | Difference RA and control *P*-value |
|---|---|---|---|---|---|
| *Biochemical data* | | | | | |
| ΔCRP, mg/l | 1.0 ± 5.6 | 0.636 | −1.3 ± 2.8 | 0.838 | 0.156 |
| ΔCatalase, U/g Hgb | −0.4 ± 1.9 | 0.993 | 0.2 ± 1.0 | 0.838 | 0.322 |
| ΔGR, U/g Hgb | 0.0 ± 0.9 | 0.928 | 0.1 ± 0.5 | 0.881 | 0.769 |
| ΔGPx, U/g Hgb | −0.2 ± 3.1 | 0.918 | 0.0 ± 2.2 | 1.000 | 0.240 |
| ΔSOD, U/g Hgb | −0.6 ± 92.8 | 0.918 | −10.8 ± 121.2 | 0.853 | 0.819 |
| *Clinical data* | | | | | |
| ΔHAQ | −0.1 ± 0.17 | 0.424 | | | |
| ΔVAS, pain | −0.1 ± 2.2 | 0.871 | | | |
| ΔVAS, patient global | 0.7 ± 1.58 | 0.182 | | | |
| *Characteristics of diet* | | | | | |
| ΔEnergy intake, kJ/d | −824 ± 1171 | 0.058 | 162 ± 1026 | 0.836 | 0.029 |
| ΔEnergy intake, kJ/kg | −13.2 ± 18.6 | 0.123 | 4.3 | 0.696 | 0.014 |
| ΔEI/BEE$_{est}$ | −0.1 ± 0.23 | 0.105 | 0.05 ± 0.19 | 0.648 | 0.039 |
| ΔProtein, g/d | −6.4 ± 14.7 | 0.129 | 1.9 ± 18.2 | 0.843 | 0.231 |
| ΔProtein, g/kg | −0.1 ± 0.2 | 0.238 | 0.0 ± 0.3 | 0.795 | 0.505 |
| ΔVit, A, RE/d | −954.7 ± 3219 | 0.251 | −978 ± 1974 | 0.202 | 0.981 |
| ΔVit, C, mg/d | −14.2 ± 78.1 | 0.626 | 6.1 ± 91.8 | 0.887 | 0.561 |
| ΔVit, E, a-TE/d | 0.2 ± 4 | 0.248 | −0.1 ± 3 | 0.932 | 0.852 |
| ΔSelenium, μg/d | 4.8 ± 32.7 | 0.537 | −5.8 ± 13.2 | 0.325 | 0.227 |
| ΔFruit, g/d | −18.3 ± 127 | 0.645 | 31.8 ± 122 | 0.594 | 0.314 |
| ΔVegetable, g/d | −8.2 ± 103 | 0.818 | 1.8 ± 87 | 0.963 | 0.786 |
| ΔFish, g/d | 17.7 ± 32.7 | 0.086 | −19.9 ± 51.4 | 0.317 | 0.056 |
| Δgalactolipid GOPO, mg/d | −3.76 ± 59.6 | 0.632 | −0.22 ± 26.9 | 0.208 | 0.831 |

*Abbreviations*: SD: standard deviation; d: day; EI: energy intake; BEE: estimated basal energy expenditure; CRP: C-reactive protein; Hgb: hemoglobin; GR: glutathione reductase; GPx: glutathione peroxidase; SOD: superoxide dismutase; HAQ: Health Assessment Questionnaire; VAS: visual analog scale.

unpublished *in vivo* study with 10 volunteers that had undergone ileostomy surgery, was given a triglyceride emulsion containing 2 g galactolipid–phospholipid mixture to a drink. There were almost no intact galactolipid in the intestinal content and it was concluded that humans efficiently hydrolyse galactolipids.

Rosehip contains fatty acids such as palmitic, linoleic, and α-linolenic acids, which are known to affect inflammatory processes. Intervention with 10 g Litozin® contributes with 0.4 g fat, and even if all of it was PUFA, it is hard to believe that such a small amount could have any biologic or clinical effect when the daily dietary intake was around 80 g in total fat and 20 g PUFA (data not shown). Jäger et al. (2007) have suggested that PUFA are the active components in rose hip acting via the COX system. However, Wenzig et al. (2008) found that rose hip fatty acids only play a minor role in the anti-inflammatory process, and concluded that unidentified constituents or a synergistic effect is causing the observed anti-inflammatory effect of rose hip.

*Antioxidant enzyme*

No effect on antioxidant enzyme activity was detected in erythrocytes from RA patients or controls in this study. To the best of our knowledge, an effect of *Rosa canina* on antioxidant enzyme activity has never been published, whereas two previous studies have measured the effect of 10 ml extract of *Rosa roxburghii* on antioxidant activity in healthy humans. The study of van Rensburg et al. (2005) was small-scale, short-term, and intervention had no effect on GR, GPx or SOD. Yong-Xing et al. (1997) showed an effect on SOD and catalase activity after two months intervention in 120 healthy participants. Other intervention studies of varying length with for example spinach (Castenmiller et al. 1999), parsley (Nielsen et al. 1999) or grape fruit (Young et al. 2000) have observed varied effects on enzyme activity in healthy human subjects.

A high production of ROS/RNS as a result of inflammation might result in different levels in antioxidant enzyme activities in healthy persons and RA patients. This was not the case in this study. Previous studies have shown inconsistent results with regard to the difference in antioxidant enzyme activities in RA patients compared to healthy controls. Some studies have shown higher levels of SOD activity (Cimen et al. 2000; Taysi et al. 2002) some lower SOD activity (Kiziltunc et al. 1998; Vipartene et al. 2006) and some the same level of activity (Kiziltunc et al. 1998). The same pattern applies to GPx where some studies show low activity (Taysi et al. 2002; Kamanli et al. 2004; Vipartene et al. 2006) and some similar levels (Cimen et al. 2000) in RA patients and healthy controls. Seven et al. (2008) and Kamanli et al. (2004) found a correlation between high degree of RA-disease activity and low antioxidant enzyme activity. Contrary to this finding Cimen et al. (2000) found a higher degree of RA-inflammation and SOD activity but an unchanged activity of GPx and catalase. So far there is no consensus on the assumption that RA patients have an affected and weakened antioxidant enzyme activity. In this study, blood samples were taken 12–14 h after the consumption of rosehip and an antioxidative and/or anti-inflammatory effect may not have been detected if bioactive components have been cleared from the organism within this period. A rat study showed that injected radioactive galactolipid (10 mg) was rapidly cleared from plasma manly by the liver. $T_{1/2}$ from plasma was 1–5 min and less than 6% of intact galactolipid was left after 4 h in liver and plasma together (Blom et al. 1996).

*Health Assessment Questionnaire (HAQ)*

RA-patients are monitored with different kinds of subjective self-reported instruments, where HAQ is widely used for determining functional status and pain. *Rosa canina* (Litozin®) had no effect on HAQ-scores in this study. A study by Willich et al. (2010) investigating the effect of 5 g *Rosa canina* powder a day in RA-patients, showed effect on HAQ after 3 months but effect was first significant after 6 month intervention on Physician's Global Scale and Health-related Quality of Life instruments. This might indicate a late onset of clinical effect and that 28 days are too short time to gain benefits. Besides, HAQ may not be the right instrument for short-term studies. Petri et al. (2003) compared different kinds of instruments and found that HAQ reflects effects later than other questionnaires.

It is difficult to judge the external validity of the findings in this study. The female patients, with good disease control and interest and energy to participate, were not a representative sample of a RA population and the controls were all educated and working in the health area. It should therefore be considered that while *Rosa canina* powder proved ineffective in this short-term and small-scale study of well treated RA patients and healthy controls, their effect in a larger study on individuals with higher risk of endogenous oxidative stress (such as cigarette smokers, elderly people, and RA with a higher degree of inflammation) remains to be investigated.

In conclusion, the short-term supplementation with 10.5 g *Rosa canina* powder a day did not influence the inflammatory marker CRP, the erythrocyte levels of the antioxidant enzymes SOD, GPx, GR, catalase or the self-reported HAQ in well treated female RA patients or healthy female controls.

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## References

Andersson, L., Bratt, C., Arnoldsson, K.C., Herslöf, B., Olsson, N.U., Sternby, B., Nilsson, A., 1995. Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents. J. Lipid Res. 36, 1392–1400.

Andersson, L., Carriere, F., Lowe, M.E., Nilsson, Å., Verger, R., 1996. Pancreatic lipase-related protein 2 but not classical pancreatic lipase hydrolyzes galactolipids. Biochem. Biophys. Acta 1302, 236–240.

Blom, M., Andersson, L., Carlsson, A., Herslöf, B., Zhou, L., Nilsson, Å., 1996. Pharmacokinetics, tissue distribution and metabolism of intravenously administered digalactosyldiacylglycerol and monogalactosyldiacylglycerol in the rat. J. Liposome Res. 6, 737–753.

Castenmiller, J.J.M., Lauridsen, S.T., Dragsted, L.O., van het Hof, K.H., Linssen, J.P.H., West, C.E., 1999. β-Carotene does not change markers of enzymatic and non enzymatic antioxidant activity in human blood. J. Nutr. 129, 2162–2169.

Christensen, R., Bartels, E.M., Altman, R.D., Astrup, A., Bliddal, H., 2008. Does the hip powder of *Rosa canina* (rosehip) reduce pain in osteoarthritis patients? – a meta-analysis of randomized controlled trials. Osteoarthritis Cartilage 16, 965–972.

Cimen, M.Y.B., Cimen, O.B., Kacmaz, B., Oztyrk, H.S., Yorgancioglu, R., Durak, I., 2000. Oxidant/antioxidant status of erythrocytes from patients with rheumatoid arthritis. Clin. Rheumatol. 19, 275–277.

Daels-Rakotoarison, D.A., Gressier, B., Trotin, F., Brunet, C., Luyckx, M., Dine, T., Bailleul, F., Cazin, M., Cazin, J.C., 2002. Effect of *Rosa canina* fruit extract on neutrophil respiratory burst. Phytother. Res. 16, 157–161.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine , 3rd ed. Oxford University Press, UK, pp. 134–181.

Jäger, A.K., Eldeen, I.M.S., van Staden, J., 2007. COX-1 and-2 activity of rose hip. Phytother. Res. 21, 1251–1252.

Kamanli, A., Naziroglu, M., Aydilek, N., 2004. Plasma lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. Cell Biochem. Funct. 22, 53–57.

Kharazmi, A., Winther, K., 1999. Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils *in vitro* and reduces certain inflammatory parameters *in vivo*. Inflammopharmacology 7, 377–386.

Kiziltunc, A., Coqalqil, S., Cerrahoglu, L., 1998. Carnitine and antioxidant levels in patients with rheumatoid arthritis. Scand. J. Rheumatol. 27, 441–445.

Larsen, E., Christensen, L.P., 2007. Common vegetables and fruits a source of 1,2-di-*O*-α-linolenoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol, a potential anti-inflammatory and anti-tumor agent. J. Food Lipids 14, 272–279.

Larsen, E., Kharazmi, A., Christensen, L.P., Christensen, S.B., 2003. An antiinflammatory galactolipid from rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils *in vitro*. J. Nat. Prod. 66, 994–995.

Nielsen, S.E., Young, F.J., Daneshvar, B., Lauridsen, S.T., Knuthsen, P., Sandström, B., Dragsted, L.O., 1999. Effect of parsley (*Petroselinum crispum*) intake on urinary

apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subject. Br. J. Nutr. 81, 447–455.
Ohlsson, L., Blom, M., Bohlinder, K., Carlsson, A., Nilsson, Å., 1998. Orally fed digalactosyldiacylglycerol is degraded during absorption in intact and lymphatic duct cannulated rats. J. Nutr. 128, 239–245.
Petri, A., Dreyer, L., Danneskiold-Samsøe, B., Terslev, L., Bliddal, H., 2003. TNF-alpha-blocker-treatment of arthritis – four years follow-up. J. Danish Med. Assoc. 165, 4649–4652.
Rein, E., Kharazmi, A., Winther, K., 2004. A herbal remedy Hyben vital (stand. powder of a subspecies of *Rosa canina* fruits), reduces pain and improves general wellbeing in patients with osteoarthritis – a double-blind, placebo-controlled, randomized trial. Phytomedicine 11, 383–391.
Seven, A., Güzel, S., Aslan, M., Hamuryudan, V., 2008. Lipid, protein DNA oxidation and antioxidant status in rheumatoid arthritis. Clin. Biochem. 41, 538–543.
Sugawara, T., Miyazawa, T., 2000. Digestion of plant monogalactosyldiacylglycerol and digalactosyldiacylglycerol in rat alimentary canal. J. Nutr. Biochem. 11, 147–152.
Taysi, S., Potalt, F., Gul, M., Sari, R.A., Bakan, E., 2002. Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol. Int. 21, 200–204.
van Rensburg, C.J., Erasmus, E., Loots, T., Oosthuizen, W., Jerling, J.C., Kruger, H.S., Louw, R., Brits, M., van der Westhuizen, F.H., 2005. *Rosa roxburghii* supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state. Eur. J. Nutr. 44, 452–457.
Vipartene, D., Iasiulevichute, L., Butkene, B., Valiukene, K., Keturkene, A., Redaitene, E., 2006. Pro- and antioxidant blood system in patients with rheumatoid arthritis and systemic lupus erythematosus. Ter. Arkh. 78, 10–14.
Warholm, O., Skaar, S., Hedman, E., Mølmen, H.M., Eik, L., 2003. The effects of a standardized herbal remedy made from a subtype of *Rosa canina* in patients with osteoarthritis: a double-blind, randomized, placebocontrolled clinical trial. Cur. Ther. Res. 64, 21–31.
Wenzig, E.M., Widowitz, U., Kunert, O., Chrubasik, S., Bucar, F., Knauder, E., Bauer, R., 2008. Phytochemical composition and *in vitro* pharmacological activity of two rose hip (*Rosa canina* L.) preparations. Phytomedicine 15, 826–835.
Wheeler, C.R., Salzman, J.A., Elsayed, N.M., Omaye, S.T., Korte, D.W., 1990. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Anal. Biochem. 184, 193–199.
Willich, S.N., Rossnagel, K., Roll, S., Wagner, A., Mune, O., Erlendson, J., Kharazmi, A., Sörensen, H., Winther, K., 2010. Rose hip herbal remedy in patients with rheumatoid arthritis – a randomized controlled trial. Phytomedicine 17, 87–93.
Winther, K., Rein, E., Kharazmi, A., 1999. The anti-inflammatory properties of rosehip. Inflammopharmacology 7, 63–68.
Young, J.F., Dragsted, L.O., Daneshvar, B., Lauridsen, S.T., Hansen, M., Sandström, B., 2000. The effect of grape-skin extract on oxidative status. Br. J. Nutr. 84, 505–513.
Yong-Xing, M., Yue, Z., Chuan-Fu, W., Zan-Shun, W., Su-Ying, C., Hei-Hua, S., Gan, J.M., Zhang, J.G., Gu, Q., He, L., 1997. The aging retarding effect of "Long-Life CiLi". Mech. Ageing Dev. 96, 171–180.