

Olive extract supplement decreases pain and improves daily activities in adults with osteoarthritis and decreases plasma homocysteine in those with rheumatoid arthritis[☆]

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Abstract

The aim of this study was to determine the effects of a polyphenolic-rich olive extract on pain, inflammation, loss of mobility, and patients' general quality of life associated with osteoarthritis (OA) and rheumatoid arthritis (RA). In this double-blind, randomized, placebo-controlled study, the efficacy of freeze-dried olive vegetation water (OVW) on subjects with a diagnosis of OA or RA was evaluated. The treatment group received 400 mg of freeze-dried OVW per day for 8 weeks. Subjects were assessed using the Health Assessment Questionnaire–Disability Index. In addition, the Disease Activity Score With 28-Joint Count was used to evaluate joint pain and inflammation, and the Profile of Moods State questionnaire for overall well-being. During the same time intervals, serum samples were taken for clinical and biochemical tests. The RA subjects in the OVW treatment group showed significant decreases in serum homocysteine levels after 8 weeks of treatment. There was no significant change in any other clinical marker, including markers of kidney and liver function, at any time during the study, suggesting that the supplement was safe. There was significant improvement by treatment group for the Health Assessment Questionnaire–Disability Index. Subjects with OA in the OVW treatment group showed significant improvement in the Disease Activity Score With 28-Joint Count index. Thus, after a relatively short treatment with OVW, subjects with OA and RA reported decreased pain and improvement in activities of daily living. In addition, subjects with RA had a statistically significant decrease in serum homocysteine. High homocysteine levels in patients with RA have been associated with higher rates of mortality from cardiovascular events.

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1. Introduction

Arthritis, an inflammatory disorder of the joints, is the most common chronic illness in older adults. It is one of the

oldest of human diseases and impacts more than 38 million individuals in the United States, or 1 of 7 Americans [1]. Rheumatoid arthritis (RA), a systemic disease that results in inflammation of the lining of the joints, and osteoarthritis (OA), a degeneration of cartilage in the joints, are associated with chronic pain, swelling, stiffness, and fatigue. As a result, muscles, ligaments, and tendons may weaken, resulting in coordination and posture deterioration. A person with arthritis is less active; and as exercise level declines, the

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risks of cardiovascular disease (CVD) and fractures increase. There is no known treatment that can reverse this degeneration process.

Inflammation is thought to be the catalyst for the resulting cartilage and bone damage in both OA and RA [2–4]. It is well documented that inflammation triggers the overexpression of many proteolytic enzymes including the matrix metalloproteinases (MMPs) whose substrates include many of the structural proteins of cartilage including collagen, proteoglycan, and other noncollagenous proteins that make up the extracellular matrix of joint cartilage. Patients with OA and RA have increased levels of a variety of MMPs and proinflammatory cytokines in plasma and in the synovial fluid of affected joints [5–8].

The most effective medications on the market today are for RA and include the cyclooxygenase-2 inhibitors and nonsteroidal anti-inflammatory drugs; however, negative side effects have resulted in the recall of some of these types of medications by the Food and Drug Administration and discontinued use. In addition, the first generation of MMP inhibitors showed poor bioavailability, caused musculoskeletal inflammation and pain, and lacked efficacy [9]. This has created an unfulfilled need for a safe and effective medication capable of attenuating inflammation, decreasing pain, and preventing degeneration of cartilage, particularly as in OA.

Interestingly, diet and moderate exercise have been shown to decrease the pain and improve the quality of life for some arthritis patients. Case controlled studies have shown that consumption of foods of the Mediterranean diet, particularly fish, fresh vegetables, olives, and olive oil, may independently protect against or diminish the effects of RA [10,11]. Patients with RA subjected to a Mediterranean intervention diet showed a reduction in inflammation, an increase in physical activity, and improved vitality [12]. Follow-up studies have shown that compounds unique to foods of this type of diet are responsible for the disease modification, specifically, fatty acids in the fish and olive oil, and polyphenols in the fruits and olives.

Recently, a natural supplement derived from the aqueous fraction of the olive (olive vegetation water, OVW) was shown to have potent antioxidant and anti-inflammatory activities [13,14]. The olive supplement, rich in simple and polyphenolics, was demonstrated to be safe at concentrations as high as 5 g/kg [13].

The objective of this study was to determine whether OVW may be effective in decreasing pain and inflammation in patients with OA or RA, thereby improving quality of life. The present trial was an 8-week study conducted to evaluate the efficacy and safety of an OVW nutritional supplement administered daily to patients with active OA or RA. The primary end point was improvement in function of daily activities as measured by the Health Assessment Questionnaire–Disability Index (HAQ-DI; [15]).

2. Methods and materials

2.1. Subjects and study design

Male and female volunteers with a diagnosis of OA or RA were recruited for this study. The experimental design was a randomized, double-blind, placebo-controlled trial. Upon entry into the study, subjects were randomly assigned to either group A or group B. The treatment code was not broken during the course of the study. Group A received placebo, and group B received the polyphenol-rich OVW nutritional supplement. The capsules were prepared by a third party, so that all personnel involved in the project were blinded to the treatment groups until after completion of the statistical analysis. Capsules were an opaque white to prevent distinction of placebo from supplement.

Subjects were instructed to take 2 capsules twice per day and were instructed to return the supplement bottles after 1 month, at which time they were given 2 additional bottles for the remainder of the study. All subjects were given a supplement log sheet and were instructed to record the number of pills taken each day and to record any missed doses.

For the baseline visit, subjects were asked to bring their current medications and other supplements so that a record could be made of concomitant drugs and supplements taken during the study. The subjects were instructed to contact the laboratory if there were any changes in their medications or in their disease activity during the study.

Measurements, as described below, were obtained at baseline (visit [V] 1), at 1 week (V2), and at 2 weeks (V3) to examine acute effects, and at 4 weeks (V4) and at 8 weeks (V5) to examine more chronic effects. All procedures were approved by the Arizona State University's Institutional Review Board.

The primary end point was the HAQ-DI, considered the most sensitive device for measuring the effect of a supplement or treatment [15]. The HAQ determines, through questionnaires, the degree of difficulty the patient experiences in performing physical functions of daily living. A score showing an improvement of 20% or greater was considered significant. Secondary efficacy end points were disease activity as measured by the Physician Assessment and the Disease Activity Score With 28-Joint Count (DAS-28). The DAS-28 measurement combines the Physician Assessment of disease; joint size, tenderness, and inflammation; patient assessment of disease; and plasma C-reactive protein (CRP) levels (measure of systemic inflammation). Safety was assessed by clinical and biochemical tests, physical examinations, and any adverse events reported by patients.

2.2. Study subjects

2.2.1. Disposition of the subjects

Three hundred two OA and RA patients were screened, with 105 patients able to meet the entry criteria. The patients

were randomized into 2 treatment groups: placebo (n = 54) and 400 mg olive pulp nutritional supplement per day (n = 51). Of the 105 patients enrolled, 90 completed the 60-day study. The 90 subjects that completed the study were distributed as follows: group A (placebo), 14 RAs and 33 OAs; group B (supplement treatment), 13 RAs and 30 OAs.

2.2.2. Demographics and characteristics of the patients

There were no statistically significant differences in the demographic and baseline characteristics between patients in the 2 treatment groups (Table 1). The age range of the study patients was 55 to 75 years. All subjects had been diagnosed with active disease OA or RA, and were currently on medications. Subjects were excluded from participating in the study if they had renal or hepatic disease, severe CVD, fibromyalgia, or cancer, or if they were being medicated with high-dose steroids or any experimental therapeutics.

All subjects were thoroughly informed of the details of the study, had an opportunity to ask questions, and signed an informed consent form. All subjects had the opportunity to voluntarily withdraw from the study at any time without prejudicing treatment.

2.3. Study compound

Manzanilla olive fruit were de-pitted; and the pit-less olive pulp was pressed to yield a liquid phase mixture composed of olive oil, vegetation water, and solids by a mechanical device. Solids were removed from the liquid phase mixture by centrifugation. The oil and aqueous fractions were separated by centrifugation, and the aqueous phase was collected. The aqueous olive fraction was treated with citric acid (1%) for 6 months. The olive water fraction was freeze dried, yielding a golden brown crystalline product containing at least 6% simple phenols and polyphenols. The freeze-dried product was processed into vegetarian-based capsules, each containing 100 mg of freeze-dried product. The capsules were given to the OVW treatment group.

Table 1
Description of subjects by treatment group

Description	Placebo control (n = 47), mean (SEM)	OVW treatment (n = 43), mean (SEM)
Weight (lb)	161.2 ± 4.6	181.8 ± 5.5
Body mass index	26.1 ± 0.6	29.6 ± 0.9
% Fat	37.1 ± 1.3	41.4 ± 1.3
Heart rate	71.2 ± 1.4	69.8 ± 1.8
Blood pressure (systolic)	129.6 ± 2.5	132 ± 2.8
Blood pressure (diastolic)	76.2 ± 1.3	78.0 ± 1.7
Hematocrit (%)	40.5 ± 0.6	40.7 ± 0.5
Hemoglobin (g/dL)	13.8 ± 0.2	14.0 ± 0.2
Smokers	4	4
Glucosamine users	19	18

Control and treatment subjects were compared using an independent *t* test. The 2 groups were not statistically different for any of the tests, nor were they statistically different by glucosamine or tobacco use.

2.4. Assessment of disease

2.4.1. Health Assessment Questionnaire–Disability Index

Quality-of-life changes were measured using the HAQ-DI, a self-reporting questionnaire that evaluates 9 categories of functional activity [16]. Patients were questioned on difficulty experienced in performing specific activities of daily living on a scale of 0 to 3, with 0 = no difficulty and 3 = extreme difficulty. Scoring of the HAQ-DI also takes into consideration the use of aids or devices to assist in these activities. The HAQ-DI includes the Visual Analog Scale, in which patients rate their level of disability using a scale from 0 to 100.

2.4.2. Physician Assessment

The Physician Assessment consists of grading joints (in motion) for tenderness and pain, swelling of joints, and the physician's assessment of arthritis severity. Standard questionnaires are designed to determine the degree of disease-associated inflammation and were administered by a board-certified physician. Questions included the number of tender and swollen joints, patient's rating of disease activity, and physician's assessment of disease activity.

2.4.3. Profile of Moods State

The emotional state of the individual was determined by use of the Profile of Moods State (POMS), a questionnaire that measures 6 specific mood states including anger, confusion, depression, fatigue, vigor, and tension [17]. Subjects were asked to rate their feelings from 0 to 4 when presented with each of 60 words associated with one of the 6 mood states. For accuracy, each questionnaire was graded twice by 2 different technicians. The final score for each evaluation was determined by summing the scores for each mood, with the one positive mood, vigor, added as a negative. A high score translates to a more negative mood.

2.4.4. Disease Activity Score With 28-Joint Count

The DAS-28 is a composite index that includes the scores from 28-joint counts for tenderness and swelling, the Visual Analog Scale from the HAQ-DI, and the plasma levels of CRP or the erythrocyte sedimentation rate (ESR) [18]. The current literature suggests that CRP elevation reflects disease progression more closely than ESR [19,20]; thus, the DAS-28 was calculated using CRP values.

2.5. Laboratory methods

2.5.1. Clinical and biochemical tests

Blood was drawn from each participant (4 vacutainer tubes) at each visit by a qualified phlebotomist or research nurse. One tube of plasma and 2 tubes of blood were sent to Sonora Quest Laboratory (Tempe, Ariz) for biochemical and clinical testing that included analysis of lipids, liver and kidney functions, ESR, and homocysteine. The remaining plasma tube was centrifuged at 3000 rpm for 15 minutes at 10°C. Plasma samples were aliquotted into microcentrifuge tubes and stored at –80°C until assayed. Samples were

distributed into measured aliquots to avoid multiple freeze-thaw cycles. Samples used for measurements were thawed only once.

2.5.2. CRP and salivary cortisol assays

Plasma levels of CRP were measured using a high-sensitivity ELISA assay (MP Biomedical, Orangeburg, NY). The manufacturer's protocol was adjusted by diluting all samples at 1:500. The detectable range of the assay is approximately 0.01 to 100 mg/mL. The intraassay and interassay coefficients of variation were <15% and <10%, respectively.

Salivary cortisol levels were analyzed using a coated-tube radioimmunoassay from a commercial kit ([21]; ICN Pharmaceuticals, Costa Mesa, Calif). All participants were given 9 salivettes (Sarstedt Inc, Rommelsdorf, Germany) together with verbal and written instructions for the sampling procedure. Sampling was done at baseline (time 0) and at the end of the trial (week 8). Participants were instructed to refrigerate the salivettes after the sample was collected. The salivettes were returned to the laboratory at the next visit, at which time they were centrifuged at 3000 rpm for 30 minutes at 4°C; and the saliva sample was transferred to a microcentrifuge tube and stored at -80°C until analysis.

2.5.3. Measurement of serum metalloproteinases and cytokines

Plasma levels of MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13 and the cytokines interleukin (IL) 1 β , IL-6, IL-8, tumor necrosis factor α , granulocyte-macrophage colony-stimulating factor, and IL-17 were measured using multianalyte profiling kits (R&D Systems, Minneapolis Minn; BioSource International, Camarillo Calif) in conjunction with the Luminex 100 analyzer (Luminex Corp, Austin, Tex). Matrix metalloproteinases have been shown to degrade a variety of matrix proteins and have been implicated in the pathogenesis of RA [22,23]. Elevated levels of MMP-3 and MMP-1 were demonstrated in synovial fluid and serum of RAs [8]. For the present studies, MMP-2, MMP-3, and MMP-9 levels were determined by ELISA.

2.6. Statistical analysis

Statistical analyses on results were conducted using SPSS for Windows (Chicago, Ill) or GraphPad Prism, version 4 (San Diego, Calif) software programs. Patient changes over time were compared using analysis of variance or paired *t* tests (2 visits; ie, V1 vs V5) for biochemical markers (homocysteine and CRP) or Mann-Whitney for POMS (GraphPad Prism, version 4). Regression models were used to determine the impact of disease on improvement and the percentage improvement in placebo control and OVW treatment groups as measured by the HAQ-DI and DAS-28 assessment devices (SPSS for Windows). Significance of statistical tests was $P < .05$.

3. Results

Descriptive characteristics of the subjects in each treatment group (placebo control and OVW treatment) are given in Table 1. Group A was determined to be the placebo group, and Group B was determined to be the OVW supplement group.

3.1. Safety and tolerance

Subjects were in the age range of 55 to 75 years and were taking a variety of medications; therefore, function tests represent safety of olive pulp extract combined with the patient's other medications. Overall, the participants tolerated placebo and supplement well, with only 2 participants, one from each group (placebo and supplement), complaining of heartburn at V2. This problem was alleviated when the participants took the placebo or supplement with food.

All subjects underwent kidney and liver function tests at baseline (before starting trial) and after 8 weeks on placebo or supplement. These tests included serum blood urea nitrogen and creatinine for kidney function, and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin for liver function. No adverse changes were reported; and in fact, some subjects with abnormal levels before the study showed improvement with time.

3.2. Health Assessment Questionnaire–Disability Index

After 8 weeks of treatment, there were statistically significant improvements over baseline as measured by 9 specific activities of daily living in the supplement group (OVW) vs the placebo group (Table 2). Although not as significant as the OVW group, the placebo group also showed improvement over time. The OVW supplement–

Table 2

Comparison in OA patients of HAQ-DI activities of daily living results for the placebo control and OVW treatment groups

Activity	Greater improvement in treated vs placebo groups	Greater improvement in placebo vs treated groups
Dressing	4	0
Arising	3	1
Eating	1	3
Walking	3	1
Hygiene	4	0
Reach	3	1
Grip	4	0
Activity	3	1
Length	3	1

Values represent improvement for each of the follow-up visits (1 week, 2 weeks, 4 weeks, and 8 weeks); for example, “4” signifies improvement at 4 of the 4 follow-up visits. Patient scored each of the daily living activities on a scale of 1 to 4; the lower the score, the better the general feeling. Each of the follow-up visits was compared with V1 (baseline). If the patient registered a lower score on a follow-up visit relative to their baseline score, it was counted as an improvement. The OVW and placebo groups were analyzed by Student *t* test; $n = 31$ (OVW treated) and $n = 28$ (placebo).

treated group showed greater improvement in 8 of the 9 activities when compared with placebo (Table 2). In addition, when subjects were compared using disease diagnosis as the variable, those with OA showed statistically significant improvement in their HAQ-DI score after 8 weeks of treatment (Table 3), whereas in the RA group, although there was statistically significant improvement in the treatment group (comparing week 0 with week 8), it was not statistically greater than that observed in the placebo group.

Changes in HAQ-DI score of >20% are notable, bearing in mind that an absolute change of 0.22 or more in the HAQ-DI represents a change in disability that physicians note as clinically relevant and patients with RA perceive as a difference in functional status. For the patients in this clinical trial, >75% of patients with >20% improvement had an absolute change in their HAQ-DI score of 0.25 or more; and for most of those patients, their HAQ-DI score was ≥ 0.5 .

3.3. Physician assessment of disease

After 8 weeks of treatment, there was a significant improvement in both the placebo and supplement-treated groups. However, the improvement in the OVW supplement-treated group was not significantly different from that in the placebo group; and there was no difference in disease diagnosis (OA vs RA).

3.4. Profile of moods state

The POMS scores were seen to decrease over the 8-week period for both the OVW supplement and placebo groups, with no significant difference between groups (Fig. 1).

3.5. Disease Activity Score With 28-Joint Count

After 8 weeks of treatment, there was a significant improvement (>20%) in OVW supplement-treated OA patients vs placebo-treated OA patients (Table 3). Interestingly, for the RA group, placebo-treated patients reported greater improvement than OVW-treated patients (Table 3), suggesting a strong placebo effect that may or may not be masking the efficacy of the treatment.

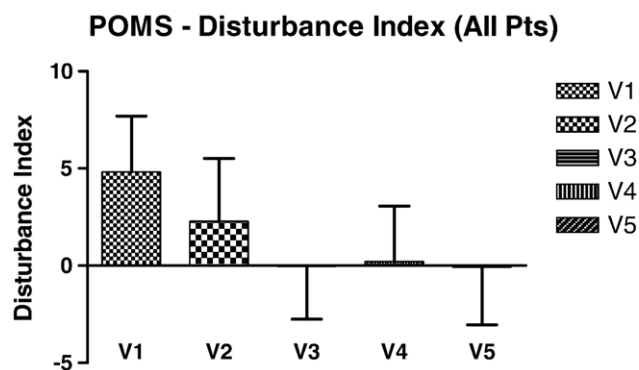


Fig. 1. Comparison of disturbance index for all subjects (OA and RA, placebo and OVW) from visit to visit: baseline (V1), week 1 (V2), week 2 (V3), week 4 (V4), and week 8 (V5). Higher scores indicate greater mood disturbance. Patient responses suggest a better mood profile on the last visit compared with the first visit (baseline). Data from nonparametric Mann-Whitney test are presented as means \pm SEM; $P < .05$ for V1 vs V5.

3.6. Biochemical markers

Changes in the hormones cortisol and prolactin, hormones that have been shown in our earlier studies to change with disease activity, were evaluated. The results show no significant change in plasma levels of cortisol, nor were there changes in the ratio of cortisol to prolactin as had been hypothesized. In addition, there was no significant change in diurnal salivary cortisol levels as indicated by an analysis of values across time and by an analysis of the areas under the curve.

There was a statistically significant decrease in plasma homocysteine levels in OVW supplement-treated RA patients vs placebo-treated RAs, as seen by comparison of values, from V1 to V5 (Fig. 2, $P < .015$).

Significant changes in CRP from V1 to V5 were detected, and there was a diagnosis by time interaction. Upon further analysis of the subgroups, OA and RA, the data indicate that the significant difference in CRP levels is attributed to a decrease in CRP in the RA group between V1 and V5 (Fig. 3). C-reactive protein was highest in RA patients (vs OA patients) and in individuals with CVD (based on their medical records and their disease history).

Table 3

Comparison of self-reported improvement (HAQ-DI) or physician-evaluated improvement (DAS-28) in placebo control and OVW-treated OA and RA patients

Diagnosis	OA		RA		OA		RA	
	Placebo	OVW	Placebo	OVW	Placebo	OVW	Placebo	OVW
Test	HAQ-DI	HAQ-DI	HAQ-DI	HAQ-DI	DAS-28	DAS-28	DAS-28	DAS-28
No improvement (frequency)	58% (19)	24% (7)	23% (3)	15% (3)	30% (10)	7% (2)	8% (1)	31% (4)
0%-20% improvement (frequency)	3% (1)	7% (2)	15% (2)	8% (1)	24% (8)	21% (6)	23% (3)	23% (3)
>20% improvement (frequency)	39% (13)	69% (20)	62% (18)	77% (10)	45% (15)	72% (21)	69% (9)	46% (6)
<i>P</i>		<.05		.24		.058		.117

Control and OVW interventions were compared using a logistic regression model. Improvement by HAQ-DI and DAS-28 was disease dependent; thus, improvement was calculated independently for OA and RA patients. For each measurement, the score for week 8 was subtracted from the baseline score. Baseline HAQ-DI scores ranged from 0.125 to 2.75; baseline DAS-28 scores ranged from 1.03 to 6.27.

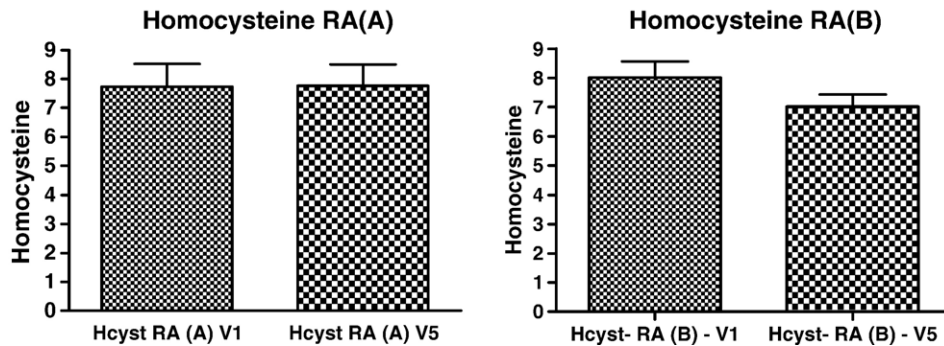


Fig. 2. Comparison of homocysteine levels in placebo (A) and OVW-treated (B) RA subjects at baseline (V1) and week 8 (V5). Data from paired *t* test are presented as means \pm SEM; $n = 14$ (placebo) and $n = 13$ (OVW); $P < .01$ for OVW treated at V1 vs V5.

Cytokines were evaluated initially to determine whether there were changes in the balance of pro- and anti-inflammatory cytokines. The values for the cytokines in the plasma were relatively low, but were higher in RA patients, as reflective of their underlying autoimmune disease. There were no significant changes in the cytokines when comparing baseline with week 8 using analysis of variance and as determined by paired *t* test analysis. The inability to detect changes in cytokines could be due to low levels in the plasma and/or to sensitivity limits of the assays.

There was a slight increase in both MMP-2 and MMP-3 levels in the placebo-treated group that was not observed in the OVW treatment group; however, for all of the MMPs measured, there was no statistically significant differences between placebo- and OVW supplement-treated subjects.

4. Discussion

In the current study, an olive extract supplement given to patients with OA and RA decreased pain and inflammation associated with OA, improved quality of life, and decreased biochemical markers of systemic inflammation in patients with RA.

Previous studies had shown that diet can positively effect patients with RA; specifically, an intervention diet rich in

foods common to the Mediterranean diet reduced pain and increased physical activity [12]. In the current study, diet intervention with an olive extract had a similar effect in OA patients. The OVW supplement-treated OA group achieved statistically greater improvement as measured by HAQ-DI than the supplement-treated RA patient group. Similar decreases in HAQ-DI have been shown in other studies to be a measure of significant improvement in health-related quality of life and disability [24,25].

The differential improvement between OA and RA patients may be related to the difference in mechanism of the 2 diseases or may be related to the number of RA patients participating in the trial; that is, increasing the number of RA patients may have increased the statistical significance of improvement. If the difference is related to the difference in disease, the greater effectiveness of the OVW in OA patients suggests a mechanism of action related to pathways associated with localized joint inflammation rather than systemic inflammation (as in RA). These changes are important given that the dose of supplement was moderate (4 capsules per day) and that the study itself was relatively short in duration (8 weeks).

The placebo-treated group also showed considerable improvement at 8 weeks relative to baseline. The placebo effect is not unexpected and is well documented in the

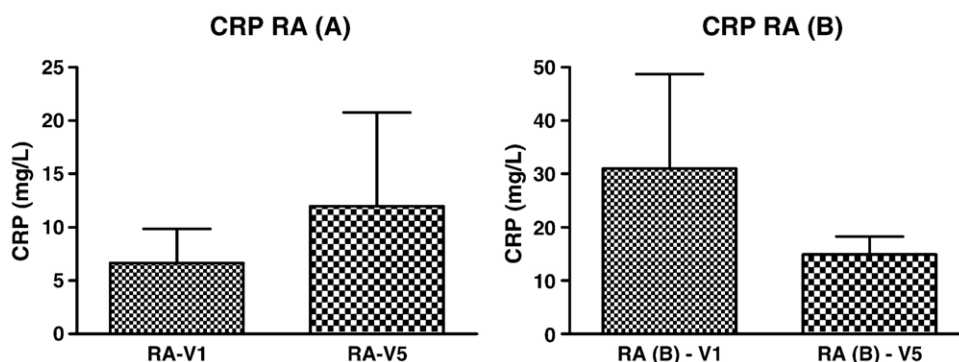


Fig. 3. Comparison of CRP levels in placebo (A) and OVW-treated (B) RA subjects at baseline (V1) and week 8 (V5). Data from paired *t* test are presented as means \pm SEM; $n = 14$ (placebo) and $n = 13$ (OVW); $P = .1995$ for OVW at V1 vs V5.

literature. Factors contributing to the placebo effect include patient's expectations of improved health; patient's interaction with laboratory staff and other participants, which many subjects do not have because of lack of activity secondary to their arthritic disease; and patient's general anxiousness or anxiety at the start of the study. In fact, the latter effect was documented in changes observed in the patient's mood as reported in their POMS. It has been reported by several other laboratories that there is a significant inverse correlation of pain state and positive mood [26].

C-reactive protein was significantly decreased by OVW supplement, and this effect was most notable in patients with RA. C-reactive protein has long been a measure of inflammation in patients with RA, and studies have shown a correlation in CRP levels to disease activity [27]. Recent studies have shown activation of the complement system in the pathogenesis of RA [28]; thus, a reduction in CRP levels supports a reduction in RA disease activity.

In addition to CRP, OVW supplement-treated patients had lowered levels of homocysteine relative to placebo-treated patients. This effect was statistically significant in RA patients. High levels of homocysteine are often found in patients with RA and are thought to account, in part, for the high rate of cardiovascular mortality [29–31]. The mechanisms responsible for high homocysteine levels in RA are not clear; however, vitamin B supplementation has been shown to decrease homocysteine levels in RA patients [32]. Regression analysis showed that the reduction in homocysteine resulted in a concomitant decrease in inflammatory variables. And in another study, RA patients had accelerated catabolism of vitamin B6 [33].

Moreover, a correlation between CRP and homocysteine has been shown [32]. This correlation however is not a cause and effect relation; but rather, both are independent risk factors for CVD [34]. It is thought that homocysteine has an oxidant stress effect on the vasculature that involves auto-oxidation of its sulfhydryl group generating superoxide radicals, which in turn consume nitrous oxide to form peroxynitrite. A reduction in both CRP and homocysteine would, in theory, be beneficial to the cardiovascular health of those taking the OVW supplement.

To begin to dissect the mechanism of action of OVW, we evaluated levels of specific cytokines during the treatment course. We began by evaluating plasma samples of study participants who had the most improved HAQ-DI scores. These patients were designated "responders," and samples from these subjects were used to explore changes in pro- and anti-inflammatory cytokine markers as a measure of supplement action. Olive polyphenols have been reported to provide protection against oxidative stress-related damage, particularly reactive oxygen species-induced damage; and reactive oxygen species have been shown to regulate MMP gene expression. A decrease in plasma MMP levels was found in OVW supplement-treated responders; however, the decrease was not significantly different from that in placebo-treated subjects. Thus, although we did not

observe any relationship between plasma cytokine levels and OVW treatment, diminishment of pain and improved mobility suggest a beneficial reduction in inflammation inducers, possibly more localized to the site of inflammation, perhaps in the synovial fluid.

In summary, an 8-week treatment with olive extract improved daily living activities in patients with OA and significantly reduced plasma CRP and homocysteine levels in patients with RA. Thus, introducing a natural and safe food extract into the diet may have the same or better beneficial effects as an intervention diet. Unlike current medications that have been found to place users at risk for CVD, OVW acts both as an anti-inflammatory and cardioprotective agent.

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References

- [1] Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998; 41(5):778–99.
- [2] Chikanza I, Fernandes L. Novel strategies for the treatment of osteoarthritis. *Expert Opin Investig Drugs* 2000;9(7):1499–510.
- [3] Brennan FM, Feldmann M. Cytokines in autoimmunity. *Curr Opin Immunol* 1992;4(6):754–9.
- [4] Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001;344(12):907–16.
- [5] Koolwijk P, Miltenburg AM, van Erck MG, Oudshoorn M, Niedbala MJ, Breedveld FC, et al. Activated gelatinase-B (MMP-9) and urokinase-type plasminogen activator in synovial fluids of patients with arthritis. Correlation with clinical and experimental variables of inflammation. *J Rheumatol* 1995;22(3):385–93.
- [6] Kontinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, et al. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. *Ann Rheum Dis* 1999;58(11):691–7.
- [7] Tchetcherikov I, Ronday HK, Van El B, Kiers GH, Verzijl N, TeKoppele JM, et al. MMP profile in paired serum and synovial fluid samples of patients with rheumatoid arthritis. *Ann Rheum Dis* 2004;63(7):881–3.
- [8] Mahmoud RK, El-Ansary AK, El-Eishi HH, Kamal HM, El-Saeed NH. Matrix metalloproteinases MMP-3 and MMP-1 levels in sera and synovial fluids in patients with rheumatoid arthritis and osteoarthritis. *Ital J Biochem* 2005;54(3–4):248–57.
- [9] Skiles JW, Gonnella NC, Jeng AY. The design, structure, and clinical update of small molecular weight matrix metalloproteinase inhibitors. *Curr Med Chem* 2004;11(22):2911–77.
- [10] Linos A, Kaklamani VG, Kaklamani E, Koumantaki Y, Giziaki E, Papazoglou S, et al. Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? *Am J Clin Nutr* 1999;70(6): 1077–82.
- [11] Linos A, Kaklamanis E, Kontomerkos A, Koumantaki Y, Gazi S, Vaiopoulos G, et al. The effect of olive oil and fish consumption on rheumatoid arthritis—a case control study. *Scand J Rheumatol* 1991; 20(6):419–26.

- [12] Skoldstam L, Hagfors L, Johansson G. An experimental study of a Mediterranean diet intervention for patients with rheumatoid arthritis. *Ann Rheum Dis* 2003;62(3):208-14.
- [13] Christian MS, Sharper VA, Hoberman AM, Seng JE, Fu L, Covell D, et al. The toxicity profile of hydrolyzed aqueous olive pulp extract. *Drug Chem Toxicol* 2004;27(4):309-30.
- [14] Bitler CM, Viale TM, Damaj B, Crea R. Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. *J Nutr* 2005;135(6):1475-9.
- [15] Bruce B, Fries JF. The Health Assessment Questionnaire (HAQ). *Clin Exp Rheumatol* 2005;23(5 Suppl 39):S14-8.
- [16] Bruce B, Fries JF. The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation. *J Rheumatol* 2003;30(1):167-78.
- [17] Dyer III JB, Crouch JG. Effects of running and other activities on moods. *Percept Mot Skills* 1988;67(1):43-50.
- [18] van Riel PL, Fransen J. DAS28: a useful instrument to monitor infliximab treatment in patients with rheumatoid arthritis. *Arthritis Res Ther* 2005;7(5):189-90.
- [19] Walsh L, Davies P, McConkey B. Relationship between erythrocyte sedimentation rate and serum C-reactive protein in rheumatoid arthritis. *Ann Rheum Dis* 1979;38(4):362-3.
- [20] Wolfe F. Comparative usefulness of C-reactive protein and erythrocyte sedimentation rate in patients with rheumatoid arthritis. *J Rheumatol* 1997;24(8):1477-85.
- [21] Sandoval DA, Matt KS. Gender differences in the endocrine and metabolic responses to hypoxic exercise. *J Appl Physiol* 2002;92(2):504-12.
- [22] Mohammed FF, Smookler DS, Khokha R. Metalloproteinases, inflammation, and rheumatoid arthritis. *Ann Rheum Dis* 2003;62 (Suppl 2):ii43-7.
- [23] Milner JM, Cawston TE. Matrix metalloproteinase knockout studies and the potential use of matrix metalloproteinase inhibitors in the rheumatic diseases. *Curr Drug Targets Inflamm Allergy* 2005;4(3):363-75.
- [24] Kosinski M, Zhao SZ, Dedhiya S, Osterhaus JT, Ware Jr JE. Determining minimally important changes in generic and disease-specific health-related quality of life questionnaires in clinical trials of rheumatoid arthritis. *Arthritis Rheum* 2000;43(7):1478-87.
- [25] Fries JF, Ramey DR. "Arthritis specific" global health analog scales assess "generic" health related quality-of-life in patients with rheumatoid arthritis. *J Rheumatol* 1997;24(9):1697-702.
- [26] Shacham S, Reinhardt LC, Raubertas RF, Cleeland CS. Emotional states and pain: intraindividual and interindividual measures of association. *J Behav Med* 1983;6(4):405-19.
- [27] Mallya RK, deBeer FC, Berry H, Hamilton ED, Mace BE, Pepys MB. Correlation of clinical parameters of disease activity in rheumatoid arthritis with serum concentration of C-reactive protein and erythrocyte sedimentation rate. *J Rheumatol* 1982;9(2):224-8.
- [28] Molenaar ET, Voskuyl AE, Familian A, van Mierlo GJ, Dijkmans BA, Hack CE. Complement activation in patients with rheumatoid arthritis mediated in part by C-reactive protein. *Arthritis Rheum* 2001;44(5):997-1002.
- [29] Krogh Jensen M, Ekelund S, Svendsen L. Folate and homocysteine status and haemolysis in patients treated with sulphasalazine for arthritis. *Scand J Clin Lab Invest* 1996;56(5):421-9.
- [30] Landewe RB, vandenBorne BE, Breedveld FC, Dijkmans BA. Methotrexate effects in patients with rheumatoid arthritis with cardiovascular comorbidity. *Lancet* 2000;355(9215):1616-7.
- [31] Lazzarini PE, Capocchi PL, Bisogno S, Galeazzi M, Marcolongo R, Pasini FL. Reduction in plasma homocysteine level in patients with rheumatoid arthritis given pulsed glucocorticoid treatment. *Ann Rheum Dis* 2003;62(7):694-5.
- [32] Yxfeldt A, Wallberg-Jonsson S, Hultdin J, Rantapaa-Dahlqvist S. Homocysteine in patients with rheumatoid arthritis in relation to inflammation and B-vitamin treatment. *Scand J Rheumatol* 2003;32 (4):205-10.
- [33] Roubenoff R, Dellaripa P, Nadeau MR, Abad LW, Muldoon BA, Selhub J, et al. Abnormal homocysteine metabolism in rheumatoid arthritis. *Arthritis Rheum* 1997;40(4):718-22.
- [34] Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapaa-Dahlqvist S. Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2002;29(5):875-82.