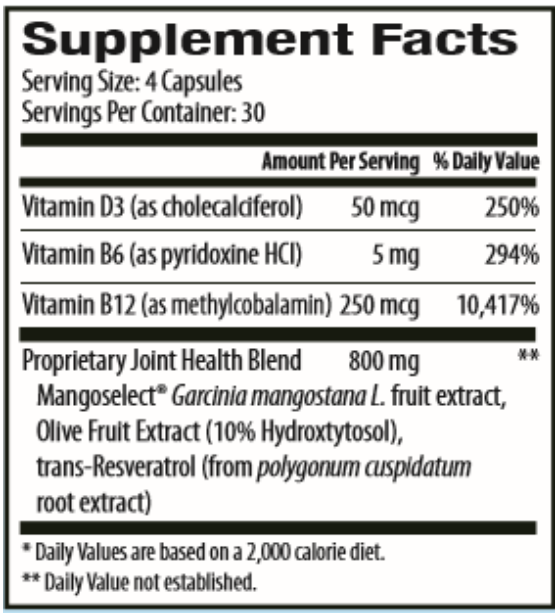
Advanced Knee Discovery™

USP: Three powerful nutraceuticals, plus three critical vitamins to help promote joint health, and knee joint comfort, with benefits in only 5 days, and additional benefits by 4 weeks. Formulation is NON-GMO and Halal Certified.†

Bottle Image:

Supplement Facts/ Product Formulation:



Supplement Facts

Serving Size: 4 Capsules
Servings Per Container: 30

	Amount Per Serving	% Daily Value
Vitamin D3 (as cholecalciferol)	50 mcg	250%
Vitamin B6 (as pyridoxine HCl)	5 mg	294%
Vitamin B12 (as methylcobalamin)	250 mcg	10,417%
Proprietary Joint Health Blend	800 mg	**
Mangoselect® *Garcinia mangostana L.* fruit extract, Olive Fruit Extract (10% Hydroxytosol), trans-Resveratrol (from *polygonum cuspidatum* root extract)		

* Daily Values are based on a 2,000 calorie diet.
** Daily Value not established.

Other ingredients: Bovine gelatin (capsule), rice flour, and magnesium stearate

Claims Below:

Claim	Substantiation
• Formulated with powerful nutraceuticals for promoting joint health, with benefits in only 5 days, and significant additional benefits by 4 weeks.†	Mangosteen study 1-6 Hydroxytyrosol study 1
• Formulated with powerful nutraceuticals to help you maintain your mobility and independence as you age.†	Mangosteen study 1-6 Hydroxytyrosol study 1-2 Resveratrol study 1 Vitamin D study 1-2
• Formulated with evidence-based nutraceuticals clinically-tested to help promote joint comfort and flexibility.†	Mangosteen study 1-6 Hydroxytyrosol study 1 Vitamin D study 1
• Contains clinically-tested MangoSelect mangosteen extract.†	Mangosteen study 1-6
• MangoSelect is clinically tested to help reduce temporary pain associated with physical overexertion by 31%.†	Mangosteen study 1
• May help reduce temporary inflammation associated with physical overexertion.†	Mangosteen study 2, 4, 6 Resveratrol study 1-2
• MangoSelect is clinically tested to support joint and connective tissue comfort in just 5 days.†	Mangosteen study 1
• Hydroxytyrosol may help promote healthy joint cartilage.†	Hydroxytyrosol study 2
• Revolutionary new product with MangoSelect that supports joint and connective tissue comfort in just 5 days.†	Mangosteen study 1

• MangoSelect is clinically tested to supports physical movement and flexibility in just 5 days.†	Mangosteen study 1
• MangoSelect is clinically tested to provide significant results in just 5 days!†	Mangosteen study 1
• MangoSelect is clinically tested to be effective in athletes *and* in the elderly in just 5 days.†	Mangosteen study 1
• Hydroxytyrosol is clinically tested to help promote knee joint comfort by 51% while walking.	Hydroxytyrosol study 1
• Hydroxytyrosol is clinically tested to help promote knee joint comfort by 36% while going up and down stairs.†	Hydroxytyrosol study 1
• Hydroxytyrosol is clinically tested to help promote knee joint comfort by 60% while sleeping.†	Hydroxytyrosol study 1
• MangoSelect, hydroxytyrosol and resveratrol provide antioxidant protection against damaging free radicals.†	Mangosteen study 3, 5 Resveratrol study 1 Hydroxytyrosol study 3
• Vitamin B12 helps body make energy from foods.†	Vitamin B12 study 1
• Vitamin B12 helps promote a healthy mood.†	Vitamin B12 study 2-4
• Vitamin D may help promote knee joint comfort.†	Vitamin D study 1
• Research suggests that maintaining normal levels of vitamin B6 may be important for healthy joint function.†	Vitamin B6 study 1-3

†These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

I. Substantiation Studies

Mangosteen background

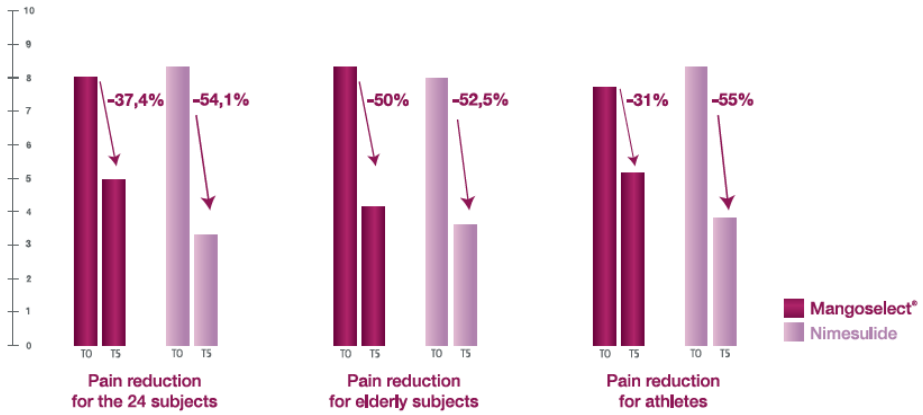
Mangosteen (*Garcinia mangostana* L., Clusiaceae) is a tropical fruit known as "the queen of fruits". Mangosteen has been used as a traditional medicine in Southeast Asia and possesses biologically active compounds, including phenolic acids, xanthones, tannins, and anthocyannins. Currently, mangosteen is popular as a botanical dietary supplement.

Mangosteen has antioxidant, antibacterial, antifungal, anti-inflammatory, and anti-HIV activities in vitro. Mangosteen also exhibits central nervous system depressant, anti-inflammatory, and antiulcer activities in vivo.[1]

In Southeast Asia, mangosteen fruit shell is a traditional folk medicine used for the treatment of diarrhea, sprains, typhoid, ulcers, skin infections, and is used as an anti-inflammatory and for sterilization. In China, G. mangostana—also named as Shan Zhu, mountain bamboo imported from Thailand, Malaysia and other Southeast Asian countries—was one of the most popular fruits, called the "Queen of Fruits" for its sweetness and juiciness as well as its importance in enhancing a person's health.[2]

Mangosteen study 1

A human clinical study[3] was been carried out on 24 subjects (9 elderly individuals and 15 athletes) who had acute (n=15) or chronic (n=9) soft tissue conditions; namely, osteoarthritic pain, inflammation of the tendons, and post-traumatic inflammation. They were randomly split into two groups of 12 to receive either the active extract (600 mg/d) or a positive control (100 mg/d Nimesulide) for 5 days. The use of Visual Analog Scales (VAS) in assessment of pain has been well validated. The scale ranges from 0 (no pain) to 10 (intense pain).



Pain has been measured both at baseline and after 5 days of supplementation. Results were that after 5 days of treatment, a significant ($p<0.00001$) overall pain reduction for the 24 subjects (both groups) was noted. Pain in joint and connective tissues was reduced by -37.4% ($p<0.05$) with MangoSelect®, and by -54% ($p<0.05$) with Nimesulide.
For the elderly subjects, the pain relief was as good with MangoSelect (50% reduction) as compared to Nimesulide (52.5%). For the 15 athletes, MangoSelect® helped reducing pain by -31% ($p<0.05$) whereas Nimesulide by -55%.

Mangosteen study 2

Systemic inflammation was initiated in 25 mice with the injection of bacterial lipopolysaccharide (LPS). Various treatments were then given to alleviate the inflammation: MangoSelect®, Nimesulide and Dexamethasone. TNF which is the main inflammatory mediator was measured after 90 minutes. TNF not only directly affects tissues where it is released, it also turns on COX-2 enzyme that induces the production of further inflammatory mediators.

The results were that treatment with MangoSelect® significant decreased blood TNF-alpha level ($p<0.05$) by 69%. As expected the glucocorticoid (Dexamethasone) significantly decreased the level of blood TNFalpha by 83% as compared to the control animals (only LPS) ($p<0.005$).[4]

Mangosteen study 3

Oxidative damage is involved in many chronic diseases including those cited as the major causes of death in Western societies such as cardiovascular disorders and cancer. Antioxidants may prevent these degenerative processes by various mechanisms including the scavenging of free radicals. Intake of antioxidant supplements is associated with preventing oxidative damages. This study investigated the absorption and antioxidant effects of a xanthone-rich mangosteen liquid in healthy human volunteers after the acute consumption of 59 mL of the supplement.

The liquid contained mangosteen, aloe vera, green tea, and multivitamins. Results indicated that R-mangostin and vitamins B2 and B5 were bioavailable, with observed Cmax at tmax of around 1 h. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased

with a maximum effect of 18% after 2 h, and the increased antioxidant level lasted at least 4 h. Overall, this study demonstrated the bioavailability of antioxidants from a xanthone-rich mangosteen product and its in vivo antioxidant effects.[5]

Mangosteen study 4

We investigated[6] the effect of gamma-mangostin purified from the fruit hull of the medicinal plant Garcinia mangostana on spontaneous prostaglandin E(2) (PGE(2)) genase release and inducible cyclooxy-2 (COX-2) gene expression in C6 rat glioma cells. An 18-h treatment with gamma-mangostin potently inhibited spontaneous PGE(2) release in a concentration-dependent manner with the IC(50) value of approximately 2 microM, without affecting the cell viability even at 30 microM.

By immunoblotting and reverse-transcription polymerase chain reaction, we showed that gamma-mangostin concentration-dependently inhibited lipopolysaccharide (LPS)-induced expression of COX-2 protein and its mRNA, but not those of constitutive COX-1 cyclooxygenase.

Because LPS is known to stimulate inhibitor kappaB (IkappaB) kinase (IKK)-mediated phosphorylation of IkappaB followed by its degradation, which in turn induces nuclear factor (NF)-kappaB nuclear translocation leading to transcriptional activation of COX-2 gene, the effect of gamma-mangostin on the IKK/IkappaB cascade controlling the NF-kappaB activation was examined. An in vitro IKK assay using IKK protein immunoprecipitated from C6 cell extract showed that this compound inhibited IKK activity in a concentration-dependent manner, with the IC(50) value of approximately 10 microM.

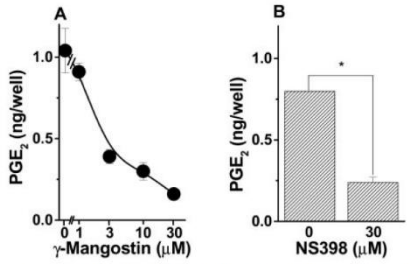


Fig. 2. Concentration-dependent inhibition of endogenous PGE_2 release from C6 cells by γ-mangostin (A) and the effect of NS398 on endogenous PGE_2 release from C6 cells (B). A and B, C6 cells were incubated with the indicated concentrations of γ-mangostin or 30 μM NS398 for 18 h. The released PGE_2 into the culture medium was determined by radioimmunoassay. Each point represents the mean ± S.E.M. (n = 3). *, $P < 0.05$ compared with the value for cells treated with 0.1% DMSO (as vehicle control).

Consistently gamma-mangostin was also observed to decrease the LPS-induced IkappaB degradation and phosphorylation in a concentration-dependent manner, as assayed by immunoblotting.

Furthermore, luciferase reporter assays showed that gamma-mangostin reduced the LPS-inducible activation of NF-kappaB- and human COX-2 gene promoter region-dependent transcription. gamma-Mangostin also inhibited rat carrageenan-induced paw edema.

These results suggest that gamma-mangostin directly inhibits IKK activity and thereby prevents COX-2 gene transcription, an NF-kappaB target gene, probably to decrease the inflammatory agent-stimulated PGE(2) production in vivo, and is a new useful lead compound for anti-inflammatory drug development.

Mangosteen study 5

Mangosteen (Garcinia mangostana L.) is a tropical tree native to Southeast Asia that produces a fruit whose pericarp contains a family of tricyclic isoprenylated polyphenols referred to as xanthones. Numerous in vitro studies have shown that these xanthones possess anti-oxidant, anti-proliferative, pro-apoptotic, anti-inflammatory and anti-carcinogenic activities.

Aggressive marketing of such health promoting benefits has resulted in mangosteen's classification as a "superfruit". This has led to sales of mangosteen containing beverages in USA alone exceeding $200 million in 2008 despite very limited animal and human studies. This review will (a) critically address

recent reports of in vivo studies on the bioavailability and metabolism of mangosteen xanthones, (b) update the in vitro and in vivo data on anti-cancer and anti-inflammatory activities of mangosteen xanthones, and (c) suggest needed areas of inquiry regarding the absorption, metabolism and efficacy of mangosteen xanthones.[7]

Note: The following study was conducted using a proprietary mangosteen juice blend, whose primary ingredient was identified as being mangosteen whole fruit puree. Since the New Vitality product uses an extract rather than a juice, this study does not directly support product claims. However, the study acknowledges that "alpha- and gamma-mangostins from G. mangostana are identified as bioactive substances with anti-inflammatory effects." Since these same compounds are present in standardized amounts in the extract, however, this study does lend support to the anti-inflammatory effects of the extract.

Mangosteen study 6

The ability to reduce inflammation in overweight and obese individuals may be valuable in preventing the progression to metabolic syndrome with associated risks for heart disease and diabetes.

Table 2: HS-CRP

Group	Baseline	8 weeks	Change
Placebo	13.10 ± 5.2	14.00 ± 8.5	+ 0.90 ± 9.5
6 oz Xango	21.30 ± 16.8	14.65 ± 8.8	- 6.65 ± 11.7
12 oz Xango	18.33 ± 9.0	11.67 ± 4.2	- 6.66 ± 5.8
18 oz Xango	12.00 ± 5.6	10.67 ± 5.6	-1.33 ± 3.0

Mean HS-CRP levels plus/minus standard deviations at baseline, after 8 weeks and the change over time. None of the changes from baseline were statistically significant. However the comparison of change from baseline to 8 weeks between the 18 oz group and the placebo group was significant (p = 0.019).

The purpose of this 8 week randomized, double-blind, placebo-controlled study with a pre-study 2 week washout period[8] was to evaluate the effect of multiple dosages of a proprietary Mangosteen Juice blend on indicators of inflammation and antioxidant levels in obese patients with elevated C-reactive protein (CRP) levels.

The study included four groups including placebo and three difference doses of the test product, XanGo Juice: 3, 6 or 9 oz twice daily. The primary outcome measure of this study was high-sensitivity (HS)-CRP. Secondary outcome measures included other biochemical indicators of inflammation, anthropomorphic measures and a safety evaluation. The results were that one hundred twenty two (122) persons were screened for the study, 44 were randomized and 40 completed the study. HS-CRP measurements dropped after 8 weeks treatment compared to baseline in all 3 dose groups and increased in the placebo group.

The changes from baseline were not significant but the comparison of change from baseline was significant for the 18 oz group when compared to placebo (p = 0.02). Other markers of inflammation (inflammatory cytokines) and a marker for lipid peroxidation (F2 isoprostane) did not show any significant differences when compared with placebo.

However, between group comparisons IL-12p70 resulted in significant differences for all three juice doses in comparison to placebo at 8 weeks. Levels of IL-12p70 were comparatively decreased in all juice groups; 6 oz (p = 0.0420, 12 oz (p = 0.0120 and 18 oz (p = 0.006) There was a trend towards a decrease in BMI in the juice groups.

There were no side effects reported in any of the groups and none of the laboratory or EKG safety assessments indicated clinically significant changes for any subject. In conclusion, in this pilot, dose-finding study, a proprietary mangosteen juice blend (XanGo Juice) reduced CRP levels (increased change

from baseline) compared to placebo for those taking the highest dose of 18 oz per day. Further studies with a larger population are required to confirm and further define the benefits of this juice. The juice was administered safely.

Note: Of the following study summaries, the first two identify inflammation markers associated with physical overexertion; in this case an ironman triathlon race and multi-day relay trail running. Likewise, four of the following studies demonstrate that specific nutraceuticals/nutraceutical combinations are effective at reducing some of those same inflammation markers in different populations, including a normal weight, healthy population. The table below synopsizes these results. Collectively, these studies help serve as the basis for the inflammatory claims for resveratrol.

Increase of Inflammatory Markers with Physical Overexertion. Decrease of Inflammatory Markers with Nutraceuticals.						
Population	Ironman triathlon race[*]	Multi-day relay trail running[†]	Normal weight healthy[‡]	Hypertensive w/Type-2 diabetes[§]	Knee osteoarthritis[**]	Stable angina pectoris[††]
Nutraceutical	n/a	n/a	Resveratrol	Grapeseed ext + Resveratrol	Calcium fructoborate	Calcium fructoborate + Resveratrol
Daily Dose	n/a	n/a	40 mg	293 mg + 16 mg	216 mg	112 mg + 20 mg
ALT	↑(P<0.001)	n/a	n/a	n.s.	n/a	n/a
AST	↑(P<0.001)	n/a	n/a	n.s.	n/a	n/a
Mb	↑(P<0.001)	n/a	n/a	n/a	n/a	n/a
CRP	↑(P<0.001)	n/a	↓(P<0.05)*†	n.s.	↓(P<0.0102)*	↓(P<0.02)*
SAA	↑(P<0.001)	n/a	n/a	n.s.	n/a	n/a
IL-6	n/a	↑(P=0.0002)	↓(P<0.05)*†	↓ (P<0.00)*	n/a	n/a
TNF-α	n/a	↑(P=0.002)	↓(P<0.05)*†	n.s.	n/a	n/a

*Compared to baseline. †Compared to placebo.

Inflammation markers associated with physical overexertion study 1

Researchers investigated[9] the effects of an Ironman triathlon race on markers of muscle damage, inflammation and heat shock protein 70 (HSP70). Nine well-trained male triathletes (mean +/- SD age 34 +/- 5 years; VO(2peak) 66.4 ml kg(-1) min(-1)) participated in the 2004 Western Australia Ironman triathlon race (3.8 km swim, 180 km cycle, 42.2 km run). Plasma samples were analyzed before (Pre), immediately after (Post) and 1 day after race (1 day post). The results were that all inflammatory markers (ALT, AST, Mb, CRP and SAA) were significantly elevated 1 day post:

[*] *EJAP.2006.98.525*
[†] *EJAP.2012.112.1839*
[‡] *JCEM.2010.95.E1*
[§] *PharmR.2013.72.69*
[**] *AJBS.2012.10.5099*
[††] *Nutrition.2013.29.178*

Table 3 Changes in blood markers of muscle damage and inflammation before (Pre), immediately (Post) and 1 day after race (1 day post)

Variable (units)	Normal range	Pre	Post	1 day post	*P* value
CK (U/l)	50–230	210 (115)	2,436 (1,363)*	5,834 (3,075)‡	<0.001
CK-MM (U/l)	44–225	200 (111)	2,302 (1,137)*	5,606 (2,973)*	<0.01
CK-MB (U/l)	0–14	8 (3)	126 (105)*	227 (114)*	<0.05
ALD (U/l)	2.7–7.5	4.4 (1.2)	14.3 (6.6)*	29.8 (14.7)‡	<0.001
LDH (U/l)	120–245	180 (24)	285 (66)†	351 (111)‡	<0.001
ALT (U/l)	5–45	14 (4)	17 (6)	40 (17)‡	<0.001
AST (U/l)	10–40	29 (7)	93 (33)	249 (116)‡	<0.001
Mb (ng/ml)	12–90	21 (6)	2,998 (1,509)*	487 (249)‡	<0.001
CRP (mg/dl)	<0.45	0.10 (0.12)	0.24 (0.09)	3.15 (1.32)‡	<0.001
SAA (μg/ml)	<8	4.8 (5.1)	23.0 (10.1)	230 ± (101)‡	<0.001

Mean (SD) values of nine athletes are shown

*Significantly different from pre-exercise $P < 0.05$; †significantly different from pre-exercise $P < 0.01$; ‡significantly different from pre-exercise $P < 0.001$

CK creatine kinase, *MM* skeletal muscle-derived, *MB* heart-derived, *ALD* aldolase, *LDH* lactate dehydrogenase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Mb* myoglobin, *CRP* C-reactive protein, *SAA* serum amyloid A

Inflammation markers associated with physical overexertion study 2

Researchers investigated[10] the effects of multi-day relay trail running on muscle soreness and damage, and systemic immune, inflammatory, and oxidative responses. 16 male and 4 female athletes ran 894 km in 47 stages over 95 h, with mean (SD) 6.4 (1.0) stages per athlete and 19.0 (1.7) km per stage. Plasma samples were analyzed before the run (Pre run) and after the run (Post run). The results were that the two inflammatory markers tested (IL-6 and TNF-α) were significantly elevated Post run:

Table 2 Effect of ultra-endurance trail running on markers of immune function, inflammation, and muscle damage

Parameter	Pre run	Post run	Outcome			
	Mean (SD)	Mean (SD)	Effect size	Effect size 95% CL±	Qualified effect[a]	*p*
Hemoglobin (g L^{-1})	149.1 (9.3)	139.6 (10.5)	−1.02	0.47	Moderate	0.0003
Mean cell hemoglobin concentration (g L^{-1})	345 (4)	347 (5)	0.50	0.61	Small	0.10
Hematocrit	0.43 (0.03)	0.40 (0.03)	−1.00	0.13	Moderate	0.0002
Leukocytes ($\times 10^9$ L^{-1})	5.7 (1.5)	9.0 (2.3)	2.20	0.095	Very large	$<10^{-5}$
Lymphocytes ($\times 10^9$ L^{-1})	1.93 (0.56)	1.89 (0.56)	−0.07	0.58	Unclear	0.80
Lymphocytes (fractional %)	0.34 (0.09)	0.21 (0.07)	−0.14	0.067	Trivial	0.0004
Neutrophils ($\times 10^9$ L^{-1})	3.26 (1.13)	6.37 (2.09)	2.75	1.1	Very large	$<10^{-5}$
Neutrophils (fractional %)	0.57 (0.10)	0.70 (0.07)	1.30	0.65	Large	0.0006
Creatine kinase (iU)	214 (134)	2,339 (2281)	15.86	9.1	Extremely large	0.002
Sodium (mmol L^{-1})	139.9 (1.5)	139.2 (1.7)	−0.47	0.78	Small	0.22
Interleukin-6 (pg L^{-1})	0.78 (0.75)	4.1 (2.8)	4.37	1.9	Extremely large	0.0002
TNF- α (pg L^{-1})	0.48 (0.14)	0.70 (0.22)	1.57	0.9	Large	0.002
Serum ORAC total antioxidant capacity[b]	570 (162)	612 (188)	0.26	0.51	Small	0.30

[a] Qualifiers of effect size: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, extremely large >4.0

[b] Total antioxidant capacity as determined by the oxygen radical absorbance capacity assay and quantified against Trolox standards

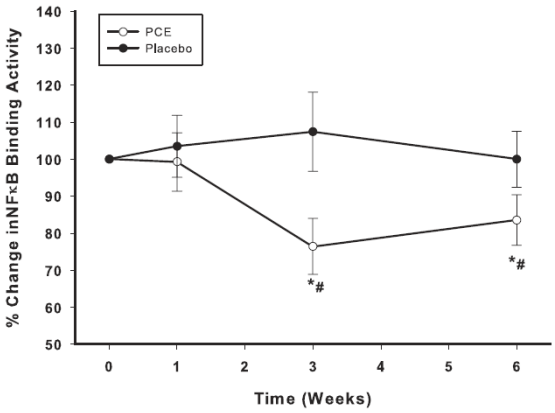
Resveratrol study 1

A randomized, placebo-controlled study[11] was conducted to investigate the effect of a Polygonum cuspidatum extract (PCE) containing resveratrol on oxidative and inflammatory stress in normal subjects. Two groups (10 each) of normal-weight healthy subjects were randomized to placebo or PCE containing 40 mg resveratrol daily for 6 wk. Fasting blood samples were obtained prior to and after treatment at 1, 3, and 6 wk. Mononuclear cells were prepared for reactive oxygen species generation,

RNA isolation, nuclear extract, and total cell homogenate preparation. Indices of oxidative and inflammatory stress, suppressor of cytokine signaling-3, phosphotyrosine phosphatase-1B, jun-N-terminal kinase-1, and inhibitor of kappaB-kinase-beta were measured by RT-PCR and Western blotting.

The results showed that the extract induced a significant reduction in reactive oxygen species generation, the expression of p47(phox), intranuclear nuclear factor-kappaB binding[‡‡] (25±7% below the baseline ($P < 0.05$), and the expression of jun-N-terminal kinase-1, inhibitor of kappaB-kinase-beta, phosphotyrosine phosphatase-1B, and suppressor of cytokine signaling-3 in mononuclear cells when compared with the baseline and the placebo. PCE intake also significantly suppressed plasma concentrations of TNF-alpha, IL-6, and C-reactive protein from baseline and compared to placebo ($P < 0.05$ in all cases).

There was no change in these indices in the control group given placebo. In conclusion, The PCE-containing resveratrol has a comprehensive suppressive effect on oxidative and inflammatory stress.



Resveratrol study 2

Exercise can lead to acute oxidative stress, which can result in oxidative damage and induce inflammation. Resveratrol may reduce the levels of inflammatory cytokines. Thus, we investigated[12] the effects of this compound on the plasma levels of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) in male professional basketball players.

Twenty healthy male professional basketball players were randomized into two groups (10 each). For 6 weeks, they received daily either 200 mg of polygonum cuspidatum extract (PCE) standardized to contain 20% trans-resveratrol equivalent to 40 mg trans-resveratrol or placebo. Indices of inflammation were measured before and after 6 weeks of supplementation.

There was a significant reduction in plasma levels of TNF-a and IL-6 after 6 weeks of supplementation; while no change was observed in these markers in the control group. Present study shows that 6 weeks of PCE

Table 2

Variables	Group	Baseline	After intervention	*P* value[c]
TNF-α (pg/mL)	Resveratrol	9.73±0.25	9.31±0.2	0.001
	Placebo	9.78±0.15	9.83±0.25	0.322
	P value[b]	0.597	0.001	
IL-6 (pg/mL)	Resveratrol	75±8.3	70.8±7.27	0.001
	Placebo	79.5±8.21	77.7±7.79	0.179
	P value	0.239	0.048	

TNF-α=Tumor necrosis factor-α, IL-6=Interleukin-6. [a]Data are presented as mean±standard deviation. [b]To test for statistical difference between the two study groups independent-samples *T* test was used. [c]To test for statistical difference between two intervals within a group paired-samples *T* test was used

Tumor necrosis factor-α and interleukin-6 levels of participants during the study[a]

[‡‡] Nuclear factor-kappaB is the major proinflammatory transcription factor.

containing resveratrol supplementation reduces the inflammation in male professional basketball players.

Hydroxytyrosol study 1

Hydroxytyrosol is mainly found in olive leaves after hydrolysis of oleuropein and has anti-oxidant, anti-bacterial, and anti-inflammatory properties. The aim of this study[13] was to investigate the effect of hydroxytyrosol for alleviating the pain in patients with gonarthrosis.

We conducted a 4-weekdouble-blind clinical trial in which olive leaf extract (yielding 10 mg hydroxytryrosol) or placebo was administered to 25 men and women with early-stage knee OA with gonarthrosis (early-stage knee OA). Evaluation at 2 and 4 weeks with a specific knee function scale: Japanese Orthopaedic Association (JOA) score and a VAS (visual analog scale) for pain.

Results were that the group administered hydroxytyrosol showed significant improvement in the JOA score and VAS compared to the placebo group. Compared to baseline, JOA scores showed significant improvement in: pain while walking (improved 11%), pain while going up and down stairs (improved 36%). In the VAS, compared to placebo, there was a 51% improvement in pain while walking on a flat place, and a 60% improvement in pain while sleeping compared to placebo.

Hydroxytyrosol study 2

Hydroxytyrosol (HT), a phenolic compound mainly derived from olives, has been proposed as a nutraceutical useful in prevention or treatment of degenerative diseases. In the present study[14] we have evaluated the ability of HT to counteract the appearance of osteoarthritis (OA) features in human chondrocytes.

Pre-treatment of monolayer cultures of chondrocytes with HT was effective in preventing accumulation of reactive oxidant species (ROS), DNA damage and cell death induced by H2O2 exposure, as well as the increase in the mRNA level of pro-inflammatory, matrix-degrading and hypertrophy marker genes, such as iNOS, COX-2, MMP-13, RUNX-2 and VEGF. HT alone slightly enhanced ROS production, but did not enhance cell damage and death or the expression of OA-related genes.

Moreover HT was tested in an in vitro model of OA, i.e. three-dimensional micromass cultures of chondrocytes stimulated with growth-related oncogene α (GROα), a chemokine involved in OA pathogenesis and known to promote hypertrophy and terminal differentiation of chondrocytes. In micromass constructs, HT pre-treatment inhibited the increases in caspase activity and the level of the messengers for iNOS, COX-2, MMP-13, RUNX-2 and VEGF elicited by GROα.

In addition, HT significantly increased the level of SIRT-1 mRNA in the presence of GROα. In conclusion, the present study shows that HT reduces oxidative stress and damage, exerts pro-survival and anti-apoptotic actions and favourably influences the expression of critical OA-related genes in human chondrocytes treated with stressors promoting OA-like features.

Hydroxytyrosol study 3

In vitro and animal studies show that polyphenols from olives have potent antioxidant activities; 50% of the phenolic compounds contained in olives and virgin olive oil are hydroxytyrosol and derivatives thereof. Hydroxytyrosol is the major olive polyphenol consumed and well absorbed in humans. It is considered to have the highest antioxidant potency compared to the other olive polyphenols

Review of the human intervention studies showed that olive polyphenols decreased the levels of oxidized-LDL in plasma and positively affected several biomarkers of oxidative damage. The antioxidant effects of olive polyphenols on low-density lipoprotein (LDL) oxidation are observed after a dietary intake of about 10 mg per day.

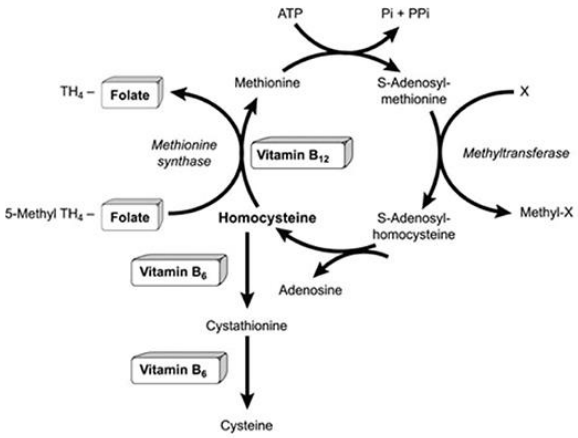
The overall evidence from in vitro assays, and animal and human studies support the antioxidant effect of olive polyphenols. However, further larger human studies are needed to clarify the effect of olive polyphenols on markers of oxidative stress, particularly DNA damage and plasma isoprostane levels.[15]

Vitamin B12 study 1

Once converted into the coenzyme methylcobalamin, vitamin B12 is required for the function of the folate-dependent enzyme, methionine synthase. This enzyme is required for the synthesis of the amino acid, methionine, from homocysteine. Methionine in turn is required for the synthesis of S-adenosylmethionine, a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA, RNA, and proteins.

Aberrant methylation of DNA and proteins, which causes alterations in chromatin structure and gene expression, are a common feature of cancer cells. Inadequate function of methionine synthase can lead to an accumulation of homocysteine, which has been associated with increased risk of cardiovascular disease (Figure 1).[16]

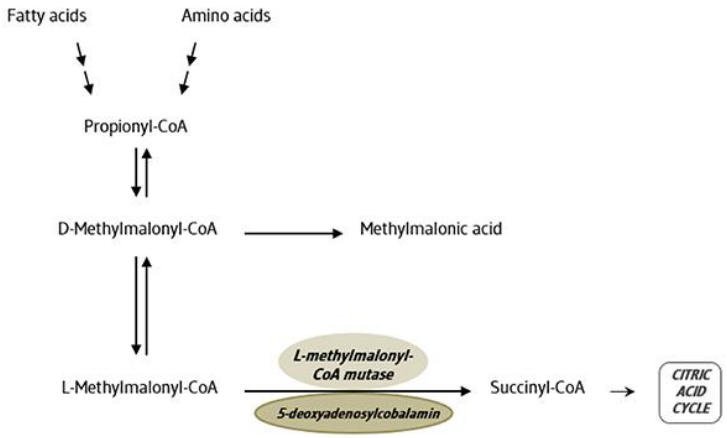
Figure 1. Vitamin B_{12} and Homocysteine Metabolism



Methionine synthase is a vitamin B_{12}-dependent enzyme that catalyzes the formation of methionine from homocysteine using 5-methyltetrahydrofolate (5-methyl TH_4), a folate derivative, as a methyl donor. Another pathway catalyzed by betaine homocysteine methyltransferase also remethylates homocysteine to methionine using betaine as a methyl donor (not shown here). Methionine, in the form of S-adenosylmethionine, is required for most biological methylation reactions, including DNA methylation.

Once converted to the coenzyme 5-Deoxyadenosylcobalamin, vitamin B12 is required by the enzyme that catalyzes the conversion of L-methylmalonyl-coenzyme A to succinyl-coenzyme A (succinyl-CoA), which then enters the citric acid cycle (Figure 2). Succinyl-CoA plays an important role in the production of energy from lipids and proteins and is also required for the synthesis of hemoglobin, the oxygen-carrying pigment in red blood cells.[1]

Figure 2. Metabolic Pathway Requiring 5-deoxyadenosylcobalamin



CoA, coenzyme A.

Vitamin B12 study 2

The classic neurological and psychiatric features associated with vitamin B12 deficiency have been well described and are the subject of many excellent review articles. The advent of sensitive diagnostic tests, including homocysteine and methylmalonic acid assays, has revealed a surprisingly high prevalence of a more subtle 'subclinical' form of B12 deficiency, particularly within the elderly. This is often associated with cognitive impairment and dementia, including Alzheimer's disease. Metabolic evidence of B12 deficiency is also reported in association with other neurodegenerative disorders including vascular dementia, Parkinson's disease and multiple sclerosis.

These conditions are all associated with chronic neuro-inflammation and oxidative stress. It is possible that these clinical associations reflect compromised vitamin B12 metabolism due to such stress. Physicians are also increasingly aware of considerable inter-individual variation in the clinical response to B12 replacement therapy. Further research is needed to determine to what extent this is attributable to genetic determinants of vitamin B12 absorption, distribution and cellular uptake.[17]

Vitamin B12 study 3

Approximately 6-8% of all persons aged >65 years have Alzheimer disease and the prevalence of the disease is increasing. Any intervention strategy aimed at decreasing risks or delaying the onset of the disease will therefore have a substantial effect on health care costs. Nutrition seems to be one of the factors that may play a protective role in Alzheimer disease.

Many studies suggest that oxidative stress and the accumulation of free radicals are involved in the pathophysiology of the disease. Several studies have shown the existence of a correlation between cognitive skills and the serum concentrations of folate, vitamin B-12, vitamin B-6, and, more recently, homocysteine. One study found lower vitamin B_{12} levels in the cerebrospinal fluid of patients with Alzheimer's disease than in patients with other types of dementia, though blood levels of vitamin B-12 did not differ.

However, nutritional factors have to be studied not alone but with the other factors related to Alzheimer disease: genetics, estrogen, anti-inflammatory drug use, and socioeconomic variables. The objective of this article was to review recent studies in this field.[18]

Vitamin B12 study 4

The neurologic symptoms of vitamin B12 deficiency include numbness and tingling of the hands and, more commonly, the feet; difficulty walking; memory loss; disorientation; and dementia with or without mood changes.[19]

Vitamin D study 1

We conducted a meta-analysis[20] of RCTs to evaluate the effects of vitamin D supplementation in the prevention of symptom and structural progression of knee OA. PubMed, Embase, and Web of Science databases were searched to identify relevant studies. Outcomes included Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain, function, stiffness, tibial cartilage volume, and serum vitamin D3 levels, and adverse events.

Results were expressed as weight mean difference (WMD) with 95% confidence interval (CI), and risk ratio (RR) with 95%CI. Four RCTs involving 1136 patients were included in this study. Pooled estimates suggested that vitamin D supplementation was associated with a significant reduction in WOMAC pain, and WOMAC function, but not in WOMAC stiffness.

Vitamin D supplementation increased the serum vitamin D3 level, but had no effect on tibial cartilage volume. Subgroup analysis showed that, a daily supplement of more than 2000 IU vitamin D significantly decreased the WOMAC pain and WOMAC function.

There was no significant difference in incidence of adverse events between the vitamin D and placebo groups. In conclusion, vitamin D supplementation was effective in improving the WOMAC pain and function in patients with knee OA. However, it had no beneficial effect on the prevention of tibial cartilage loss.

Vitamin D study 2

Vitamin D is a potent regulator of calcium homeostasis and may have immunomodulatory effects. The influence of vitamin D on human autoimmune disease has not been well defined. The purpose of this study[21] was to evaluate the association of dietary and supplemental vitamin D intake with rheumatoid arthritis (RA) incidence. We analyzed data from a prospective cohort study of 29,368 women of ages 55-69 years without a history of RA at study baseline in 1986.

Diet was ascertained using a self-administered, 127-item validated food frequency questionnaire that included supplemental vitamin D use. Risk ratios (RRs) and 95% confidence intervals (95% CIs) were estimated using Cox proportional hazards regression, adjusting for potential confounders. Results showed that, through 11 years of follow-up, 152 cases of RA were validated against medical records.

Greater intake (highest versus lowest tertile) of vitamin D was inversely associated with risk of RA (RR 0.67, 95% CI 0.44-1.00, P for trend = 0.05). Inverse associations were apparent for both dietary (RR 0.72, 95% CI 0.46-1.14, P for trend = 0.16) and supplemental (RR 0.66, 95% CI 0.43-1.00, P for trend = 0.03) vitamin D. No individual food item high in vitamin D content and/or calcium was strongly associated with RA risk, but a composite measure of milk products was suggestive of an inverse association with risk of RA (RR 0.66, 95% CI 0.42-1.01, P for trend = 0.06). In conclusion, greater intake of vitamin D may be associated with a lower risk of RA in older women, although this finding is hypothesis generating.

Vitamin B6 study 1

Abnormalities of tryptophan metabolism have been reported in patients with rheumatoid arthritis (RA) and it has been suggested that these abnormalities are the result of disordered vitamin B6 metabolism. Fasting serum pyridoxal, assayed by an automated microbiological system, was found to be below normal in 35 out of 42 patients with RA while a similar abnormality was found in 8 out of 35 patients with osteoarthrosis (OA).

Within the RA group the abnormality could not be related to the age, sex, or drug therapy of individuals but of the 8 patients with OA and a low serum pyridoxal, 7 were receiving indomethacin either alone or in conjunction with aspirin.[22]

Vitamin B6 study 2

A vitamin B6-deficiency-induced disorder in avian articular cartilage resembling osteoarthritis has been further characterized. We measured several parameters of proteoglycan (PG) metabolism, i.e., fixed charge density and sulfated glycosaminoglycans (S-GAG) content in PN-deficient versus control articular cartilage and synovial fluid from the knee joint.

Statistically significant changes were: 1) decreased content and increased extractability of total sulfated PGs from articular cartilage with guanidine HCl; 2) elevation of S-GAG concentration in synovial fluid; 3) increased plasma cystathionine (sulfur amino acid) levels. PG synthesis as assessed by 35SO4 incorporation into S-GAGs was not impaired. A lack of cartilage swelling in 0.15 M saline and the normal water content indicated that although disturbed, the collagen network was not disrupted.

This finding was in agreement with a previous microscopic study that revealed no fissures in the articular cartilage. Previous findings of a normal aggregating PG size-distribution and absence of elevated metalloproteases made a disturbance of aggregating PG metabolism unlikely. Escape into the synovial fluid of small PGs, normally bound to articular collagen, was believed to result from an alteration in collagen molecular organization that could be secondary to elevated circulating SH-compounds.[23]

Vitamin B6 study 3

A short period of fasting leads, in the mouse, to usually reversible damage to chondrocytes and in patients with rheumatoid arthritis often to a temporary improvement. Slight hypo-alimentation and a low-caloric diet reduce the spontaneous development of osteo-arthritis in the mouse, whereas a high-caloric diet promotes the disease.

In man, mice, and, in particular, fattened animals, obesity is often associated with forms of osteo-arthritis. In such cases, it may be assumed that metabolic damage to cartilage is involved as well as damage due to weight-bearing forces. Elderly people, i.e., persons with a predisposition to osteo-arthritis, often suffer from a generalized vitamin deficiency. Vitamins E, B2, and C have been shown to exert an inhibitory effect on osteo-arthritis in animals, and it has been found that supplementation therapy, particularly with vitamin E and the combination of vitamins B1, B6, and B12, can exert a beneficial effect on the symptomatology of human degenerative joint disease.

Mineral deficits in calcium, zinc and selenium (Kashin-Beck disease; endemic osteo-arthritis deformans) can provoke skeletal damage in humans and animals.

On the other hand, calcium, iron, and copper have been reported to give rise to storage diseases, in some cases with involvement of articular cartilage. There have been indications that chondrotoxic damage may result from food contaminants. So far very little is known about the influence of phytopharmacodynamic substances (other than derivatives of rutin and rhein) on osteo-arthritis. The large gaps in our knowledge of the chondrotropic properties of the constituents of food and common stimulants underline the need for further investigations.[24]

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