DIET AND ARTHRITIS

A report prepared by scientists at MRC Human Nutrition Research
at the request of The Arthritic Association

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A. Executive Summary

The Arthritic Association recommends a diet that is rich in natural foods, particularly raw fruits, vegetables and whole grains in order to combat arthritis. The rationale for this type of diet for patients with arthritis was considered in a detailed review of the scientific literature.

A key aim of the Arthritic Association diet is to increase potassium intake. Data from two UK national nutrition surveys of adults below 65 years of age in 1985/6 and of older adults >65 y in 1994/5 were analysed to determine the nutrient intake associated with diets rich in fruits, nuts, vegetables and whole grains.

Key findings from the literature review

- A relationship between diet and rheumatic disease could occur through two possible mechanisms that are not mutually exclusive (a) nutritional factors might alter immune and inflammatory responses and thus modify manifestations of rheumatic diseases; and (b) food related antigens might provoke hypersensitivity responses leading to rheumatological symptoms.

- Only a few studies have reported food intake in patients with RA or OA. \Of those that did, patients with RA were found to eat more servings of sweet, fatty foods and fewer servings of vegetables and fruits daily than healthy controls (page 9) and lower intakes (and/or biochemical markers of status) of n-3 fatty acid, dietary fibre, β-carotene, vitamin B_6, vitamin B_{12}, folate, vitamin C, vitamin E, calcium (Ca), zinc (Zn), magnesium (Mg) and selenium (Se) relative to healthy subjects. Higher intakes of saturated fatty acids and sodium were reported in RA patients than healthy subjects. Information on copper (Cu) intake is conflicting.

- No published papers have specifically addressed potassium intake or status in relation to arthritic disease.

- No association between incident knee OA and micronutrient intake has been reported but progression of knee OA appears to be inversely related to vitamin C intake, and possibly β-carotene, vitamin E and vitamin D intake and status.

- OA, but not RA, is associated with obesity and obesity is frequently linked to a high fat intake. However in ecological studies total fat intake is not consistently related to arthritis and is unlikely to be a significant independent risk factor (other than via the association with obesity).

- Several well-controlled dietary supplementation studies have shown that fish oils, which are rich in n-3 PUFA, can ameliorate some of the symptoms suffered by patients with RA. The required dose for benefit has been suggested as 3-6g n-3 PUFA/day, with benefits occurring only after about 12 weeks. Such n-3 PUFA intake may reduce the need for non-steroidal anti-inflammatory drugs, and further work is required to determine the optimal doses and diet/drug interactions.

- The seed oils of evening primrose (EPO), blackcurrants and borage plants, contain relatively large amounts of gamma-linolenic acid (GLA). GLA may have an anti-inflammatory action by decreasing the production of potent pro-inflammatory products of arachidonic acid (AA).

- Patients with RA who are prescribed methotrexate (MTX) (a folate antagonist) may benefit from folic acid supplementation, so long as vitamin B_{12} status is adequate.

- Vitamin B_6 status is reduced in patients with RA, but in a case-control study oral vitamin B_6 showed no reduction in RA symptoms. There is currently no basis to recommend B_6 supplements.

- Beneficial effects of high intakes of dietary antioxidants, especially vitamin E have been reported in patients with OA and RA. High intakes of most antioxidants can be achieved through the consumption of at least five portions of fruits and vegetables each day, especially when a variety of these foods are included in the diet.

- Zinc status is reduced in patients with RA. Clinical studies of zinc supplementation have shown conflicting results and zinc supplements are not indicated for therapeutic use. However, a diet containing adequate amounts of zinc is prudent. The main sources of zinc are high protein foods such meat and dairy products.

- Foods most frequently reported to improve arthritic symptoms are fish (at least one portion per week), vegetables and oils especially fish and olive oils. Milk and other dairy products, red meat, cereal products, citrus fruits, chocolate, spices, and alcohol have been implicated in the aggravation of symptoms.

- An inverse relationship has been observed between vegetable consumption (raw and cooked) and arthritis. This may be due to higher intakes of antioxidants, vitamin K, some minerals or a reflection of other lifestyle attributes in high vegetable consumers.

- Consumption of olive oil is inversely associated with the development of RA and intervention studies have demonstrated some improvements in arthritis symptoms with increased consumption of olive oil. Olive oil contains a high proportion of oleic acid, and its metabolite eicosatrienoic acid (ETA) may exert an anti-inflammatory effect via a mechanism similar to that of fish oil.

- Evidence is growing that a proportion of patients with RA may have a food intolerance. However to date, it has been estimated that probably less than 5% of patients with rheumatic disease have an immunological sensitivity to foods (pages 45-50).

Key findings from the dietary analyses

- Individuals in the highest quintile of fruit, nuts, vegetables and whole grain intake (FNVW) have an overall dietary profile which is closer to the recommended dietary intake for good health than the general population.

- A diet rich in FNVW is associated with a higher intake of the antioxidant vitamins, (C, E and carotenoids) as well as the minerals potassium, calcium, zinc and magnesium.

- A diet rich in FNVW is associated with higher intakes of oily fish. However consumption is still much lower than recommended for good health.

- Consumers of a diet rich in FNVW have a higher energy intake, but no increased likelihood of being obese. This suggests that activity levels are also higher. They are also more likely to be non-smokers and employed in non-manual occupations. These demographic differences are also likely to be associated with better health than the general population.

Areas for potential future research and education

- Case control studies of the precise nutrient intake of individuals adhering to the Arthritic Association dietary programme or other patients with arthritis.

- Randomised controlled clinical trials to consider in detail the effects of components of the Arthritic Association recommended diet which have not yet been formally tested.

- Educational campaigns to inform patients with arthritis of the benefits of a diet containing oily fish and/or the use of long chain n-3 PUFA supplements.

- Initiatives to highlight the benefits of a diet rich in FNVW for good health, especially the prevention of heart disease and cancer.

B. A review of the scientific literature on diet and arthritis

1. Objective

1.1 To undertake an extensive review of the scientific literature to ascertain the current state of knowledge regarding the relationship between specific nutrients and/or certain food groups and the management of osteo- and rheumatoid arthritis.

2. Review

2.1 The database MEDLINE was searched for entries in which diet, specific foods and nutrients and arthritis were mentioned to identify relevant literature. Limits were set for the search which included English language papers from 1 Jan 1991 to May 2001, and with an abstract available. Original scientific papers, reviews, and abstracts of articles were used to select material pertinent to the objectives of this review. Other papers were identified from papers obtained, especially from reviews. Given the broad scope of the review the volume of material available prohibits a more comprehensive data extraction procedure.

2.2 Tables summarising papers providing evidence of the effect of diets, specific foods and nutrients on rheumatoid and osteoarthritis were prepared.

2.3 The tables provide information on:

- type of study (e.g. review, outcome evaluation (RCT, non-randomised trial, one group pre-test and post-test design), survey, case control, laboratory, animal)
- country where study was carried out
- aims and objectives;
- Study population (NB There were very few studies identified for osteoarthritis compared with rheumatoid arthritis)
- Key findings reported by authors (these will vary according to type of study but will include all relevant outcomes in terms of evaluation measures).

2.4 A summary of the findings for each nutrient, food or specific diets for which relevant papers were found has been prepared for the report.

3. Characteristics of rheumatoid arthritis

3.1 Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disease resulting in joint inflammation that is manifested by swelling, pain, functional impairment, morning stiffness, osteoporosis and muscle wasting. Erosion of bone occurs commonly in the joints of the hands and feet. It is characterised by infiltration of activated T lymphocytes, macrophages and plasma cells (B lymphocytes that have differentiated into antibody secreting cells) into the synovium (the tissue lining the joints), and the initiation of a chronic inflammatory state that involves overproduction of pro-inflammatory cytokines and a dysregulated T-helper-1-type response. Eicosanoids synthesised from arachidonic acid and cytokines cause progressive destruction of cartilage and bone (Calder and Zurier 2001). This is most likely due to cytokine- and eicosanoid-mediated induction of destructive enzymes such as matrix metalloproteinases. RA is also characterised by signs of systemic inflammation, such as elevated plasma concentrations of some cytokines (e.g. interleukin-6 (IL-6) and interleukin-1 (IL-1)), acute-phase proteins, and so-called rheumatoid factors). Cell types involved in the chronic inflammatory process include synoviocytes, lymphocytes, monocytes, macrophages and polymorphonuclear (PMN) leucocytes. The PMN leucocytes dominate the synovial fluid and their proposed functions are phagocytocic and digestion of several kinds of material. The phagocytosis stimulates (1) the release of lysosomal proteinases, which may digest collagen and activate other biologically active mediators; (2) oxygen free radicals, which directly produce cellular injury; and (3) oxidation of arachidonic acid, which generates pro-inflammatory by-products of the cyclooxygenase and lipoxygenase pathways (Tarp 1995).

3.2 As yet the cause(s) of RA are unknown. Worldwide, RA affects approximately 1% of the adult population (Martin 1998). It can strike at any age, yet typically occurs between 40 and 60 years of age. Approximately three females are affected for every male. The auto-antibody rheumatoid factor is found in 70-80% of patients (Harris 1990). Pharmacological therapies for RA

include non-steroidal anti-inflammatory drugs (NSAIDs), slow-acting anti-rheumatic drugs (gold, methotrexate, sulphasalazine, penicillamine, cyclosporin and antimalarials), and corticosteroids (Scott, Shipley et al. 1998). Conventional treatments aim to reduce patients' pain and joint inflammation, to minimize loss of function and alter the course of the disease by decreasing the progression of joint damage. Management of RA however, is often ineffective and pharmacological therapies have the potential to cause side effects (Sarzi-Puttini, Comi et al. 2000). Patients frequently self-prescribe, and complementary therapies including diet modification are widely used (Struthers, Scott et al. 1983). The outcome of recent research strongly suggests that dietary intervention should be considered as a component of therapy. However, careful studies are required to assess dietary therapies because RA relapses and remits spontaneously and because RA patients have a high placebo-response rate (Darlington and Ramsey 1993).

3.3 As yet the RA is associated with several nutritional abnormalities, including loss of body cell mass, lean body mass, and muscle mass, as well as elevated resting energy expenditure (Roubenoff, Roubenoff et al. 1990),(Roubenoff, Roubenoff et al. 1994). The alterations in energy expenditure and body composition appear to be driven by elevated production of the inflammatory cytokines IL-1β and TNFα, a process which has been termed 'rheumatoid cachexia' (Roubenoff, Roubenoff et al. 1994).

4. Characteristics of osteoarthritis

4.1 Osteoarthritis (OA) is caused by loss of cartilage in the synovial joints. Subsequent compensatory attempts made at bone repair result in reformation (remodelling) at the ends of the bones. OA is not a passive process of joint wear and tear but rather a metabolically active process with anabolic and catabolic activity occurring simultaneously. Structural changes visible on radiography include narrowing of the joint space, osteophyte production or the growth of small bony projections on joint surfaces, and bone reformation around the joint. The pain and disability associated with OA arise from the secondary effects rather than the primary disease. These effects may include synovitis, joint capsule distension, bony proliferation, and damage to surrounding articular structures (Kee 2000).

4.2 OA is more common in women than men and it is a major health problem in the elderly. Clinical OA of the hips and knees affects 10-20% of the over-65s in the UK (Scott, Shipley et al. 1998). It is not curable, but therapies can control symptoms, especially joint pain, minimize disability, and reduce progression of joint damage. There is increasing evidence that avoiding obesity may prevent the development of OA. Treatments include analgesics, NSAIDs, local steroid injections, surgical joint replacement (Scott, Shipley et al. 1998) nutriceuticals, and patient education (Felson, Lawrence et al. 2000). Increasingly, it is common for treatment of OA to combine one or more oral agents with exercise and other biomechanical techniques.

4.3 Evidence suggests that pathophysiologic processes in bone are important determinants of outcome in OA of the knee (McAlindon, Felson et al 1996a).Cartilage in patients with OA is biochemically different from cartilage in age-matched controls (Sack 1995). Compared with healthy cartilage, cartilage in OA contains more water, less karatan sulphate than in normal cartilage, and normal link protein. Structural changes in the proteoglycan macromolecules are key features of OA and together with ultrastructural changes in collagen fibres, cause deterioration in the function of cartilage. The chondrocyte is likely to be an important participant in this process. It has both anabolic and catabolic functions. In contrast to RA where the synovium is the primary source of proteolytic enzymes, in OA the chondrocyte seems to be the principal producer of these substances. The following cytokines are likely to be important in the pathogenesis of OA; IL-1, interferon gamma, TNFα, IL-6, transforming growth factor beta, and insulin-like growth factor (Sack 1995).

4.4 There is evidence that synovium-generated IL-1, by inducing nitric oxide (NO) synthase and thus inhibiting chondrocyte synthesis of aggrecan and type II collagen, is crucial to the pathogenesis of OA (McCarty and Russell 1999). Niacinamide and other inhibitors of ADP-ribosylation have been shown to suppress cytokine-mediated induction of NO-synthase in a number of types of cells. McCarty and Russell (1999), therefore, speculated that niacinamide will have a comparable effect in IL-1-exposed chondrocytes, blunting the anti-anabolic impact of IL-1. Other nutrients reported to be useful in OA may

likewise intervene in the activity or synthesis of IL-1. Supplemental glucosamine can be expected to stimulate synovial synthesis of hyaluronic acid; hyaluronic acid suppresses the anti-catobolic effect of IL-1 in chondrocyte cell cultures (McCarty and Russell 1999). S-adenosylmethionine (SAM), also used as a therapy for OA, upregulates the proteoglycan synthesis of chondrocytes, possibly because it functions physiologically as a signal of sulphur availability. IL-1 is likely to decrease SAM levels in chondrocytes; supplemental SAM may compensate for this deficit (McCarty and Russell 1999). Non-toxic nutritional regimens, by intervening at multiple points in the signal transduction pathways that promote the synthesis and mediate the activity of IL-1, may provide an alternative to NSAIDs in the treatment and perhaps prevention of OA (McCarty and Russell 1999).

5. Characteristics of Juvenile Rheumatoid Arthritis

5.1 Juvenile Rheumatoid Arthritis (JRA) is the most common paediatric rheumatic disease and one of the more common chronic diseases of childhood. It is divided into three subtypes: systemic (arthritis in association with rheumatoid rash and intermittent fever), polyarticular (arthritis in more than four joints), pauciarticular (arthritis in four joints or fewer). There is an increased risk for malnutrition in JRA patients leading to growth retardation and protein-energy malnutrition (PEM). PEM has been described in up to 50% of JRA patients and growth failure is another well known complication of JRA (Henderson and Lovell 1991). They frequently develop deficiencies of somatic and visceral protein stores but have above normal fat stores. These changes in body composition may be related to reductions in physical activity.

5.2 Poor appetite occurs most commonly in periods of increased articular inflammation (Henderson and Lovell 1991). Although many factors are contributory, inflammatory cytokines (such as IL-1 and TNF-α) have been demonstrated to cause profound anorexia in experimental animals and humans and are present in elevated amounts in JRA patients (Henderson and Lovell 1991). Weight loss in the presence of inflammation results in over 50% of the weight lost coming from lean body mass (such as skeletal muscle) (Mascioli and Blackburn 1985) as reported in (Henderson and Lovell 1991). In a random sample of 33 JRA patients, all had a caloric intake 50% lower than

their estimated needs (Henderson and Lovell 1991). This type of anorexia makes efforts to meet the nutritional needs of children with JRA difficult. However, the provision of adequate nutrition is an essential factor in ensuring appropriate growth in JRA patients.

5.3 Anaemia is present in JRA and its severity is usually correlated with the level of underlying inflammation (Cassidy 1985) which can result in sequestration of iron from the reticuloendothelial cells. This anaemia is commonly a moderate to severe normocytic hypochromic anaemia with a haemoglobin range between 4 and 11 g/dl, low serum iron, low iron binding capacity, and normal or elevated haemosiderin levels (Cassidy and Petty 1990). Iron deficiency anaemia may also develop because of poor dietary intake, gastrointestinal blood loss secondary to medications, or preferential uptake of iron by synovial tissue in inflammed joints (Giodano, Floravanti et al. 1984). Henderson and Lovell conclude that the desirability of supplemental iron therapy remains questionable, however, because of the demonstrated association between the amount of iron deposited in the synovial tissue and the degree of erosive joint damage (Henderson and Lovell 1991). Supplemental iron therapy for patients with JRA should be instituted with caution and with detailed clinical follow-up to assess its effect on synovial inflammation.

5.4 Demineralization of bone is another frequent finding in JRA. This is thought to be associated with increased bone resorption due to cytokine action, immobility and steroid treatment. Corticosteroids can create negative calcium balance, which contributes to the development of osteopenia. There have been reports about hypercalciuria accompanying JRA. However, Nemcova et al measured basic biochemical indices of bone metabolism in 12 patients with JRA. In spite of decreased BMD, no significant hypercalciuria was found (Nemcova, Kutiled et al. 1994).

5.5 In recognition of the multiple diet-related issues Henderson and Lovell recommend that paediatric dietitians should be part of the interdisciplinary team involved in the care of JRA patients (Henderson and Lovell 1991).

6. Dietary patterns

6.1 Few studies have reported on dietary patterns in either RA or OA patients. Morgan et al reported on the dietary pattern of 79 patients participating in a one-year study (to evaluate the effectiveness of folic acid supplementation in lessening the toxicity of low-dose weekly methotrexate (MTX)) compared to typical intake among adults in the US and the Dietary Pyramid Guidelines (Morgan, Anderson et al. 1997). The mean daily intake for patients was 5.4 servings of sweets, fats and oils, 1 serving of milk, 2 servings of meat, 1.9 servings of vegetables, 0.8 servings of fruits, and 4.1 servings of breads, cereals, rice and pasta. The mean intake for all of the food groups, with the exception of fats, oils and sweets, is below the recommended levels of the Food Guide Pyramid, and the mean intake of 5.4 servings from the fats, oils and sweets group is 1.5 times that of the average US population.

7. Nutrient intakes and nutritional status of RA and OA patients

7.1 Several papers have looked at the nutrient intake or nutrient/nutritional status of RA patients (Mody, Brown et al. 1989), (Van de Laar, Nieuwenhuis et al. 1990), (Kalla, Brown et al. 1992), (Rauma, Neonen et al. 1993), (Kremer and Bigaouette 1996b), (Hansen, Nielsen et al. 1996), (Stone, Doube et al. 1997), (Morgan, Anderson et al. 1997), and also OA patients (Cleland, Hill et al. 1995), (McAlindon, Felson et al. 1996a), (McAlindon, Jacques et al. 1996b), (McAlindon and Felson 1997). Most of them have noted impaired nutritional status or inadequate nutrient intake patterns. In a study of Danish RA patients nutritional status together with disease activity parameters were recorded. At baseline, the patients had *'significant reductions in total energy, vitamin D, vitamin E and n-3 fatty acids'*. (Hansen, Nielsen et al. 1996).

7.2 Reported macronutrient intakes include the study of Morgan et al. Among 79 RA patients in the USA 46% of total calories were from carbohydrate, 38% from fat, and 16% from protein (Morgan, Anderson et al. 1997). Thirteen percent of fat calories were from saturated fatty acids (SFAs), 15% from monounsaturated fatty acids (MUFAs), and 8% from polyunsaturated fatty acids (PUFAs). In another USA study by Kremer and Bigaouette on 41 RA patients, both men and women consumed significantly less energy from carbohydrates (women 47.4% vs 55% RDA, p=0.0001; men = 48.9%, p=

0.025) and more energy from fat (women = 36.8% *vs* 30% RDA. P = 0.001 and men =35.2%, p = 0.02). Women consumed significantly more SFA and MUFA than the RDA (p= 0.02 and p = 0.04 respectively) while men consumed significantly less PUFA (p= 0.0001). Fibre intake was also considerably less than that recommended (p= 00001) (Kremer and Bigaouette 1996b). New Zealand RA patients were reported to have on average 50% of total energy as carbohydrate, 31% as total fat, 17% as protein and 1% as alcohol. The contribution of SFA to total energy was 16%, of MUFA was 11% and of PUFA was 5% (Stone, Doube et al. 1997).

7.3 For micronutrients, Morgan *et al* found that more than 15% of the patients had blood vitamin levels for vitamin B_{12}, pyridoxine, vitamin C, β-carotene, plasma folate, and vitamin E below normal (Morgan, Anderson et al. 1997). The proportions were highest for β-carotene and plasma folate levels (42% and 47% below normal levels respectively). On average, the study patients consumed less than 67% of the RDI (generally considered to be a deficient intake) for the following nutrients: folate, vitamin B_{12}, vitamin E, calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu), and zinc (Zn). Pyridoxine intake was 68% of the RDI. Kremer and Bigaouette reported that on average nutrient intake in the 41 RA patients in the USA was deficient in pyridoxine, Zn, Cu, and Mg (Kremer and Bigaouette 1996b). In an USA prospective case-control study, serum concentrations of α-tocopherol (vitamin E), β-carotene and retinol preceding the diagnosis of RA were measured 2 to 15 years before the disease developed. RA cases had lower serum concentrations of α-tocopherol, β-carotene and retinol than their matched controls. The difference for β-carotene (-29%) was statistically significant.

7.4 Stone et al reported that RA patients in New Zealand had inadequate intakes of Ca, folic acid, vitamin E, Zn, and Se (Stone, Doube et al. 1997). The diet of the New Zealand RA patients contained satisfactory levels of protein, Fe, thiamin, niacin, vitamin B_6, vitamin B_{12}, and vitamin C. There were borderline deficiencies of vitamin A, phosphorus, and riboflavin. Sodium (Na) intake was high, even excluding added table salt.

7.5 Rauma *et al* collected dietary intake information from 43 Finnish RA patients prior to embarking on a strict vegan diet (Rauma, Neonen et al. 1993). These patients had lower than recommended intakes of Fe, Zn and niacin, and their energy intake was low compared to mean daily energy intake of the healthy Finnish population of the same age.

7.6 Elsewhere, a difference in vitamin A metabolism or intake between patients with RA and controls was reported by Fairney et al (Fairney, Patel et al. 1988), low dietary intakes of vitamin E in RA patients were also reported in a paper by Martin (Martin 1998), and vitamin C levels have been shown to be low in RA patients (Oldroyd and Dawes 1985).

7.7 Many minerals and trace elements appear to be low in the diets or body stores of RA patients. In addition to the above, low dietary intakes of Zn and Mg have been reported. (Martin 1998), Selenium (Se) and Zn serum levels have been reported to be decreased in RA patients (Mangge, Hermann et al. 1999), (Honkanen, Konttinen et al. 1991), (Tarp 1995).

7.8 In a study by Honkanen et al, low serum Zn and high serum Cu were explained by disease activity parameters (Honkanen, Konttinen et al. 1991). They suggested that IL-1 causes both changes by i) increasing the metallothionein-mediated hepatic uptake to serum Zn and ii) upregulating ceruloplasmin (acute phase reactant) gene and synthesis in liver and subsequently the level of ceruloplasmin-Cu complexes in the blood. Cu absorption was diminished by Zn intake. Cu- and Zn-dependent erythrocyte SOD was increased in RA patients.

7.9 Poor nutrient intake (Van de Laar, Nieuwenhuis et al. 1990), (Morgan, Hine et al. 1993), (Kowsari, Finnie et al. 1983), (Bigaouette, Timchalk et al. 1987), (Stone, Doube et al. 1997) and the anti-nutrient effects of drug therapy (Morgan, Baggott et al. 1987), (Roubenoff, Roubenoff et al. 1990), (Morgan, Baggott et al. 1994), (Tarp 1995) can have a detrimental effect on nutritional status and on health. Nutritional assessment of patients with RA using anthropometric measurements such as skin fold thickness and upper arm muscle circumference found a 26% prevalence of patients classified as

malnourished (Helliwell, Coombes et al. 1984). Hypermetabolism with elevated resting energy expenditure and loss of body cell mass probably accounts for this phenomenon (Rouebenoff et al, 1994) as reported in (Kremer and Bigaouette 1996b). The hypermetabolic state associated with active RA makes consideration of dietary intake and nutritional status important. Malnourished patients have been reported to have more severe functional impairments and worse medical outcomes than well-nourished patients (Mody, Brown et al. 1989), (Collins, Dunn et al. 1987), (Helliwell, Coombes et al. 1984). Patients with RA may require increased intake of several nutrients to maintain a steady state. For example, protein requirements may rise in response to increased metabolic rate or there may be an increased antioxidant requirement to combat chronic inflammatory disease (Stone, Doube et al. 1997). However, others have found no relationship between the biochemical indicators of nutritional status (measured as albumin, thyroxine-binding pre-albumin, and retinol-binding globulin), and the indicators of disease activity (ESR, CRP level, and plasma viscosity) (Kalla, Brown et al. 1992). Statistical analysis with correlation matrices showed that calcium, folic acid, protein and iron intake were not related to functional disability or disease activity in a cohort of New Zealand RA patients (Stone, Doube et al. 1997).

7.10 The Framingham Knee OA Cohort Study showed that obesity was associated with symptomatic knee OA even when obesity pre-dated radiographic evidence by 20 years (Felson, Anderson et al. 1987). This finding suggests that excess energy intake over energy expenditure is associated with OA in certain joints. Weight loss of approx. 5 kg will reduce a person's risk for the development of knee OA over the subsequent 10 years by 50%.

7.11 Cleland *et al* have reported that apart from caloric excess, there is no evidence for any specific dietary factors which causes or aggravates OA (Cleland, Hill et al. 1995). There was no significant association of incident knee OA with any micronutrient intake in the Framingham cohort, but for progression of radiographic knee OA, there was a three fold reduction in risk for those in the middle and highest tertiles for vitamin C intake (adjusted OR for highest v. lowest tertile =0.3; 95% CI 0.1-0.6). Reduction in risk for progression was also seen for β-carotene intake (OR 0.4 for highest v. lowest tertile of intakes;

95% CI 0.2-0.9) and vitamin E intake (OR 0.7 for highest v. lowest tertile of intakes; 95% CI 0.3-1.6) but was less consistent i.e. β-carotene association diminished considerably after adjustment for vitamin C, and the vitamin E effect was seen only in men. No significant associations were seen for the non-antioxidant nutrients. Risk for progression increased 3-fold in participants in the middle and lower tertiles for both vitamin D intake (OR for lower v. upper, 4.0 95% CI, 1.4-11.6) and serum levels of vitamin D (OR for lower v. highest, 2.9; 95% CI 1.0-8.2). Incident OA of the knee occurring after baseline was not consistently related to either intake or serum levels of vitamin D.

7.12 Considerable, scientifically based evidence indicates that nutrition and nutritional status exert a profound effect on the immune and inflammatory responses as well as on the disease activity of RA

8. Total fat and saturated fat

8.1 There is evidence from studies of dietary intake in RA patients that the proportion of dietary energy from total fat and saturated fatty acids is above the recommended levels for good health (see paragraph 7.2.1) (Morgan, Anderson et al. 1997), (Kremer and Bigaouette 1996b). However, there does not appear to be a consistent pattern of total fat consumption in RA patients between countries so it seems unlikely that the contribution of total fat to total energy intake is a significant factor in RA other than to increase the risk of obesity and to affect other health parameters.

8.2 As in healthy controls in Western-style populations the proportion of energy intake contributed by saturated fatty acids is greater than that recommended for good health and this is often associated with lower than recommended proportions of MUFAs and PUFAs in the diet. General healthy eating advice to reduce SFA intake should apply to RA and OA patients too.

8.3 Evidence for a direct effect of dietary fat intake and the incidence or progression of OA has proved inconclusive. In some animal models of OA a diet high in saturated fat increases the severity of the disease, but in others this effect has been hard to reproduce (Sack 1995).

9. Long chain polyunsaturated and monounsaturated fatty acids

9.1 There is much interest, as indicated by the number of papers in the scientific journals, in the role of PUFAs in the pathophysiology of RA and in their use in the treatment of RA patients. Clinical studies with diets containing different proportions of PUFAs have clearly demonstrated an anti-inflammatory effect, although the mechanism is still being debated. The effects of PUFAs on production of eicosanoids, cytokine levels, and on oxidative stress are all generating interest.

9.2 The polyunsaturated fatty acids (PUFA) can be considered as three sub-groups, the n-3, n-6 and n-9 groups, the inter-relationships between which are summarised in Figure 1. The modern Western diet contains excess (n-6) fatty acids and a low level of n-3 fatty acids (Cleland and James 1997), (Whelan 1996), (Simopoulos 1999), (Darlington and Stone 2001). Humans evolved consuming a diet that contained about equal amounts of n-3 and n-6 essential fatty acids. Over the past 100-150 y there has been an enormous increase in the consumption of n-6 fatty acids due to the increased intake of vegetable oils. Today in Western diets, the ratio of n-6 to n-3 fatty acids ranges from around 20-30:1 instead of the traditional 1-2:1.

9.3 Dietary n-6 and n-3 fatty acids are modulators of the lipid content of membrane phospholipids, precursors of eicosanoids. Eicosanoids mediate inflammation, cytokine synthesis, and cell communication (Volker, Fitzgerald et al. 2000b). Oxygenase and lipoxygenase enzymes metabolise the n-3 and n-6 PUFAs in competition, so that a high proportion of n-6 compounds leads to a relative deficiency of the products of n-3 metabolism. This is seen in the generation of different eicosanoids, see Fig. 1. Linoleic acid (LA) (18:2n-6) can be elongated to γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA) and to arachidonic acid (AA). Because of the high proportion of linoleic acid to α-linolenic (ALA) or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in Western diets, the membranes of most cells contain large amounts of AA, compared to DGLA and EPA. AA is usually the principal precursor for eicosanoid synthesis resulting in the 2-series prostaglandins (PGs) and thromboxanes (TXs), and the 4-series leukotrienes (see fig. 2), which have pro-inflammatory biological actions (James, Gibson et al. 2000).

In general n-6 PUFAs enhance, and n-3 PUFAs and MUFAs suppress cytokine mediated aspects of inflammation. In addition, n-6 PUFAs and cholesterol enhance and n-3 PUFAs suppress cytokine production. DGLA and EPA, which is found in fish oil, are able to decrease the production of AA-derived eicosanoids and to decrease the production of pro-inflammatory cytokines TNF-α, IL-1β, interleukin-6, and reactive oxygen species, and the reactivity of lymphocytes (Calder and Zurier 2001).

9.4 Eicosanoids may mediate much of the early pathology of inflammatory joint disease, such as swelling, pain, and leucocyte infiltration, cytokines have been implicated in the late, destructive phase of the disease, which includes cartilage loss and bone resorption (James, Gibson et al. 2000). The cytokines IL-1β and TNF-α have pro-inflammatory cellular actions stimulating the production of collagenases and increasing the expression of adhesion molecules necessary for leucocyte infiltration. Many anti-inflammatory pharmacotherapies are directed at inhibiting the production of these inflammatory mediators and hence possibilities exist for therapies that incorporate n-3 and n-9 dietary fatty acids (James, Gibson et al. 2000). Some of the studies demonstrating that inclusion of n-3 fatty acids in the diet can suppress the production of TNF-α and IL-1β are reviewed below. The n-3 fatty acids are precursors of eicosanoid synthesis of the 3-series prostaglandins and thromboxanes and the 5-series leukotrienes with lower inflammatory actions. The n-9 eicosatrienoic acid (ETA) metabolised from oleic acid has an inhibitory effect on the production of LTB_4 from AA. Increasing dietary n-3 and n-9 fatty acids can shift the balance of eicosanoids produced to a less inflammatory mixture (James, Gibson et al. 2000).

9.5 The non-essential n-9 PUFAs include the mono-unsaturated fats found in olive oil. When present in the diet these can replace n-6 PUFA in several aspects of cell metabolism and can reduce the competition between n-6 and n-3 PUFA, which results in the increased use and incorporation of n-3 fatty acids into cell membranes (Darlington and Stone 2001). Changes in the fatty acid composition of cell membranes leads to a variety of biochemical effects. However, recent studies suggest that changes in bulk membrane fluidity are

unlikely to underlie the substantial modulatory effects of fats on cytokine biology (Grimble 1998).

10. Fish oil and n-3 fatty acid supplements

10.1 The rationale for fish oil supplementation is that dietary (n-6) and (n-3) fatty acids are modulators of the lipid content of membrane phospholipids, precursors of eicosanoids. Fish oils, n-3 EPA and DHA may modulate cytokine biology by a number of mechanisms closely linked to membrane phospholipid composition.

10.2 Sixteen papers were identified between 1991 and 2001 which reviewed the evidence for the mechanisms and outcomes of the manipulation of the balance of dietary PUFAs and/or dietary supplementation with n-3 fatty acids and/or fish oils most of which are listed in Table 1. These covered clinical studies of n-3 fatty acid supplementation, (Kremer 1991), (James and Cleland 1997), (Ariza-Ariza, Mestanza-Peralta et al. 1998), (Belch and Muir 1998), (Simopoulos 1999), (Kremer 2000), animal and human ex vivo studies which have looked at the evidence that dietary fatty acids can alter the membrane phospholipid composition, and modify the generation of cytokines and eicosanoids (Sperling 1991), (Sperling 1995), (Calder 1997), (Grimble and Tappia 1998), (Watkins and Seifert 2000), (James, Gibson et al. 2000), (Calder and Zurier 2001), (Darlington and Stone 2001)

10.2 The reviewers reached the following conclusions:

- Overall, the relationship between PUFA, eicosanoids and cytokines is emerging as an area of great interest and potential clinical relevance (Darlington and Stone 2001).

- Dietary manipulation of fatty acid levels do produce changes in the generation of eicosanoid hormones and cytokines, and can modify their cellular actions (Darlington and Stone 2001), (Calder 1997), (Calder and Zurier 2001), (James, Gibson et al. 2000), (Grimble and Tappia 1998), (DeLuca, Rothman et al. 1995), (Sperling 1995), (Sperling 1991).

- There is increasing evidence that these compounds play an important role in the disease process underlying arthritis and other inflammatory disorders. Dietary control of fatty acid intake would be expected to modify the disease process and provide a useful adjunctive strategy in the treatment of these disorders (Calder and Zurier 2001), (Darlington and Stone 2001), (Kremer 2000).

- The effects described in the clinical, animal and ex vivo human studies suggest that n-3 PUFAs may be useful for chronic inflammatory disorders including RA (Darlington and Stone 2001), (Calder and Zurier 2001) and should be included as part of a therapeutic approach to RA (Calder and Zurier 2001).

- Supplementation with n-3 fatty acids has been associated with improvement in some clinical outcome measures in RA. The benefit most often observed with fish-oil supplementation in human studies is a reduction in the number of tender joints on physical examination, although some authors reported improvement in the Ritchie Articular Index and in morning stiffness (Kremer 2000).

- Some studies have shown NSAID sparing effects of n-3 supplements. Further studies are needed to determine if they might represent an alternative to NSAIDs in certain circumstances (James, Gibson et al. 2000), (Ariza-Ariza, Mestanza-Peralta et al. 1998), (Calder and Zurier 2001), (Kremer 2000), (James, Gibson et al. 2000), (DeLuca, Rothman et al. 1995).

- The minimum doses and the duration required to bring about clinical improvement are not known (Calder and Zurier 2001). Darlington and Stone (2001) conclude that if fish oil is to be taken or used in clinical trials, the lowest possible effective dose should be used i.e. equivalent to EPA 500-750 mg/d (Darlington and Stone 2001). However, Kremer concludes “on the basis of the totality of the data, it is recommended that patients consume dietary supplements containing 3-6 g n-3 fatty acids

daily for 12 weeks or more" (Kremer 2000). Further work to determine dosage, duration, background diet and drug therapy is required.

- The effectiveness of these fatty acids might have been underestimated, because in most studies patients have continued with existing drug therapies, and with one exception, because the intake of n-6 fatty acids in the diet has not been modified. It is possible that DGLA and n-3 fatty acids might be more effectively incorporated into immune cells if n-6 fatty acid intake is lowered (Calder and Zurier 2001).

- Further studies on the long term consequences of alterations in n-3:n-6 balance in favour of the n-3 PUFA need to be carried out. Such an alteration could lead to detrimental immunological and haematological effects (Darlington and Stone 2001).

- Improvements usually are not observed until after at least 12 weeks of continuous use and appear to increase with extended treatment intervals of 18 to 24 weeks (Kremer 1991).

10.4 Table 2 lists the papers of experimental studies of RA and PUFAs reviewed for this report. Study objectives, methods and outcomes along with conclusions or authors comments are all summarised.

10.4.1 Studies in healthy subjects, patients and experimental animals clearly demonstrate that unsaturated fats modulate pro-inflammatory cytokine biology (Sanders and Hinds 1992), (Hinds and Sanders 1993), (Karsten, Schafer et al. 1994), (Yaqoob and Calder 1995), (Whitehouse, Macrides et al. 1997), (Hughes and Pinder 2000), (Volker, Fitzgerald et al. 2000a), (Curtis, Hughes et al. 2000). A study to investigate the effects of feeding mice lipids with different fatty acid composition upon the ability of stimulated macrophages to produce inflammatory mediators concluded that the most potent effect is caused by fish oil consumption (Yaqoob and Calder 1995). A second study investigated the effect of increasing levels of dietary fish oil rich in EPA and DHA on lymphocyte phospholipid fatty acid composition and cell-mediated immunity in the mouse (Hinds and Sanders 1993). The immune response was suppressed by 160 g fish oil/kg diet, but not by lower doses. The authors

concluded that moderate intakes of fish oil are not immunosuppressive. An Australian research group studied the effect of two diets with different ratios of EPA and DHA in rats and compared them with beef tallow and safflower based diets (Volker, Fitzgerald et al. 2000a). Macrophage phospholipids revealed cellular incorporation of EPA and DHA from the fish-oil-based diets which modified lipid and peptide mediators of inflammation. They concluded that the ratio of EPA/DHA can be fine tuned to optimize the immunoregulatory effect.

10.4.2 Other studies looked at the effect of dietary n-3 fats and other fatty acids on cytokine inflammatory mediators on lymphocytes. Karsten et al (1994) found that palmitic, stearic, oleic and linoleic acid are physiological regulators of cytokine release in human peripheral lymphocytes (Karsten, Schafer et al. 1994). They suggest that modulation of free fatty acids (FFA) ratios may be an effective means for the fine-tuning of the immune system. As secretory mechanisms of cytokines appear to exhibit substrate specificity for FFA, the release of individual cytokines may be selectively influenced by FFA. In a study on the effects of different classes of fatty acids on the expression and activity of cartilage aggrecanases, cytokines (IL-1β and TNF-α) and cyclo-oxygenases, COX-1 and COX-2 (Curtis, Hughes et al. 2000), the results show that incorporation of n-3 fatty acids (but not other PUFAs or SFAs) into articular cartilage chondrocyte membranes results in a dose-dependent reduction in: (i) the expression and activity of proteoglycan degrading enzymes (aggrecanases) and (ii) the expression of cytokines (IL-1β) and TNF-α) and COX-2, but not cyclo-oxygenase COX-1.

10.4.3 Hughes and Pinder (2000) investigated the combined effect in vitro of EPA and DHA, when provided in the same ratio as is commonly found in fish-oil supplement capsules (3:2), on the expression of functionally associated surface molecules on human monocytes. In addition, they carried out an *in vitro* assay of antigen presentation to investigate whether changes in the expression of surface molecules were associated with an alteration in antigen-presenting function (Hughes and Pinder 2000). The authors concluded that the results of these studies and those of previous studies support the hypothesis that n-3 PUFAs suppress cell-mediated immune responses, at least in part by inhibiting the function of antigen-presenting cells.

10.4.4 Two studies on healthy volunteers found that plasma vitamin E (α-tocopherol) concentrations fell below the normal range when fish oil was fed and concluded that further studies are needed to investigate the extent to which fish oil increases requirements for antioxidant nutrients (Meyandi, Natiello et al. 1991b), (Sanders and Hinds 1992).

10.5 Many dietary intervention studies have been carried out to determine the efficacy of fish oil in RA. A number of studies found increases in plasma levels of n-3 fatty acids and a substantial cellular incorporation of n-3 fatty acids (Volker, Fitzgerald et al. 2000b), (Mantzioris, Cleland et al. 2000), (Fahrer, Hoeflin et al. 1991), (Lau, Morley et al. 1993), (Skoldstam, Borjesson et al. 1992), (Meyandi, Natiello et al. 1991b), (Sperling, Weinblatt et al. 1987) following intervention studies of 12 weeks or more.

10.6 Several human intervention studies appear to show that n-3 PUFA generally reduces the synthesis of IL-1β and TNFα by mononuclear cells stimulated *in vitro* or reduce other inflammatory mediators (Sperling, Weinblatt et al. 1987), (Kremer, Lawrence et al. 1990), (Meyandi, Endres et al. 1991a), (Cleland, French et al. 1988), (Espersen, Grunnet et al. 1992), (Grimble 1998), (Mantzioris, Cleland et al. 2000).

10.7 In a meta-analysis of randomized, double-blind, placebo controlled studies, Fortin et al found that dietary fish oil supplementation for 3 months significantly reduced tender joint count and morning stiffness compared with heterogeneous dietary control oils (Fortin, Lew et al. 1995). In a review of six clinical studies carried out with coincidental laboratory and immune parameters described, Kremer (1991) reported that these six controlled, blinded studies have established that dietary supplementation with n-3 (fish oil) fatty acids is associated with reproducible clinical benefits in RA patients but improvements are usually not observed until after at least 12 weeks of continuous supplementation (Kremer 1991). The improvements appear to increase with extended treatment intervals. The author stated that further work is required on the formulation and dosage of fish oils.

10.7.1 In a double-blind controlled study, Kremer *et al* (1985) compared two diets, the experimental diet was high in PUFA and low in SFA, with a daily supplement of 1.8 g EPA and the control diet with higher SFA and lower PUFA with a paraffin wax placebo (Kremer, Michalek et al. 1985). Although results favoured the experimental diet group at 12 weeks of supplementation for morning stiffness and number of tender joints, the experimental group had deteriorated significantly at 1-2 months after the intervention ended. They concluded that any benefit from the experimental diet was transient. The plasma concentrations of AA and LA (the precursors of PGE2 inflammatory prostaglandins) changed little in either group. In a later double-blind randomised parallel study in RA patients, Kremer *et al* (1990) compared different doses of n-3 fatty acids over a period of 24 weeks (Kremer, Lawrence et al. 1990). The low dose contained 27 mg/kg EPA and 18 mg/kg DHA and the high dose contained 54 mg/kg EPA and 36 mg/kg DH. A third group were given 6.8 g oleic acid. Significant improvements from baseline in the number of tender joints were noted in the low-dose group at 24 weeks ($p=0.05$) and in the high-dose group at 18 weeks ($p=0.04$) and 24 weeks ($p=0.02$). Significant decreases from baseline in the number of swollen joints were noted in the low- and high-dose groups from week 12. A total of 5 of 45 clinical measures were significantly changed from baseline in the olive oil group, 8 of 45 in the low-dose fish oil group, and 21of 45 in the high-dose fish oil. The authors conclude that the clinical benefits of dietary supplementation with n-3 fatty acids are more commonly observed in patients consuming higher dosages of fish oil for time intervals that are longer than those previously studied. Dietary supplementation with olive oil is also associated with certain changes in immune function, which require further investigation (Kremer, Lawrence et al. 1990).

10.7.2 Others using quite different dosages of fish oil or EPA and DHA also reported improvements in clinical variables. One of the highest doses, 18 g fish oil/d for 12 weeks, was tested by Cleland *et al* (Cleland, French et al. 1988). They reported improvements in tender joint score and grip strength at 12 weeks in the fish oil group. Geusens *et al* reported that daily supplementation with 2.6 g of n-3 PUFAs results in significant clinical benefit measured by patients and doctors following 12 months of supplementation (Geusens, Wouters et al.

1994). In a study using 3.6 g n-3 PUFA compared to placebo control for 12 weeks, the clinical status of the patients was improved in the fish oil group, but not in the placebo group, judged by Ritchie's articular index ($p<0.02$) (Espersen, Grunnet et al. 1992).

10.7.3 A more recent study by Volker *et al* (2000) with 50 RA patients whose background diet was naturally low (<10g/d) in n-6 fatty acids supplemented fish oil containing 60% n-3 fatty acids at a rate of 40mg/kg body weight or a control capsule of 50/50 corn/olive oil (Volker, Fitzgerald et al. 2000b). Analysis of 9 clinical variables indicated there was a significant difference ($p<0.02$) between control and treatment groups after 15 weeks (but not after 4 and 8 weeks). Five subjects in the treatment group and 3 in the control group met the American College of Rheumatology 20% improvement criteria. They conclude that fish oil supplementation at a dose of 40mg/kg body weight/day, with a background diet containing <10g/d at n-6, results in improvements in clinical status in patients with RA.

10.8 Two intervention studies using fish or foods rich in n-3 PUFA have been identified (Fahrer, Hoeflin et al. 1991), (Mantzioris, Cleland et al. 2000). In the earliest study, a diet rich in fish was examined to see if it had a similar effect on membrane and plasma lipids as a dietary fish oil supplement using healthy volunteers (Fahrer, Hoeflin et al. 1991). The intervention study lasted 8 weeks. The participants were allocated to the 3 treatment groups according to their dietary preferences: a fish diet n=16 (the participants were instructed to eat 700g fish per week and keep a record of fish consumption); fish oil diet n=12 (the participants were instructed not to eat fish but to take 15 capsules corresponding to 7.5 g of fish oil with 2.1 g EPA and 1 g DHA/d); regular diet n = 14 (the participants were instructed to follow their usual dietary habits, but to avoid any form of fish during the 8 weeks). The relative amounts of both EPA and DHA in the platelet-rich plasma increased significantly in the fish oil group and in the group with the fish diet compared to the baseline value and to the control group; no change was seen in the control group. The researchers concluded that four to six meals (approx. 700 g) of fish per week without any other dietary changes can induce similar changes in lipids as a supplement of fish oil. In the recent open intervention study with healthy male volunteers, a

diet incorporating foods rich in n-3 fatty acids consumed for 4 weeks was examined for an effect on tissue concentrations of EPA and in suppressing the production of inflammatory mediators (Mantzioris, Cleland et al. 2000). Analyses of dietary records indicated that intake of EPA plus DHA averaged 1.8 g/d and intake of ALA averaged 9.0 g/d. These intakes led to an average 3-fold increase in EPA in plasma, platelet, and mononuclear cell phospholipids. TBX_2, PGE_2, IL-1β synthesis decreased by 36%, 26% and 20% ($p<0.05$), respectively. It would appear that a diet containing foods enriched in γ-linolenic acid (cooking oil, margarine, salad dressing and mayonnaise) and EPA and DHA (sausages and savoury dip) and foods naturally rich in n-3 PUFA, such as flaxseed meal and fish can produce similar effects to fish oil supplements and may be better tolerated by RA patients. A case control study found that consumption of baked and broiled fish was associated with a decreased risk of incidence of RA which the authors claimed support the hypothesis that n-3 fatty acids may help prevent RA (Shapiro, Koepsell et al. 1996).

10.9 Several studies have been carried out to assess the extent to which the use of fish oil or EPA and DHA supplements and/or evening primrose oil (EPO) supplements could reduce the dosage of NSAIDs used by RA patients. In a recent multicentre trial by Scotia Pharmaceuticals reported by Darlington and Stone (2001) but cited as unpublished, 402 RA patients were given 2-3 g/d EPO with much smaller doses of EPA and DHA. No information on the duration of this study was given, however, according to Darlington and Stone the trial yielded no support for the use of EPO as a NSAID sparing agent (Darlington and Stone 2001). An earlier UK study by Belch et al (1988) using a double blind, placebo controlled trial to determine whether EPO or an EPO/fish oil combination could be substituted for NSAID therapy without deterioration in clinical symptoms found that, at the end of 12 months supplementation, patients receiving EPO and EPO/fish oil had significantly reduced their NSAIDs. However, after 3 months follow-up receiving placebo, those who received EPO or EPO/fish oil supplements had relapsed. The dosage for the EPO group was = 540 mg GLA and that for the EPO/fish oil group was 240 mg EPA and 450 mg GLA.

10.10 Other studies using fish oil showed NSAID sparing effects. 43 Swedish RA patients were randomised into two groups, one taking 10 g fish oil/d and the other taking a placebo inactive oil for 6 months (Skoldstam, Borjesson et al. 1992). Patients in the fish oil group reported a significantly decreased consumption of NSAIDs at 3 and 6 months and the status of global arthritic activity improved at 3 months in the physician's assessment. Control patients reported an increased global arthritic activity at 6 months. No change was found in patient assessment of pain, duration of morning stiffness or functional capacity. In a larger study by Lau et al (1993) RA patients were supplemented with 10 Maxepa (fish oil) capsules/d (equivalent to 1.7 g EPA/d and 1.1g DHA/d) for 15 months (Lau, Morley et al. 1993). There was a significant reduction in NSAID usage in patients on Maxepa when compared with placebo from 3 months. This effect reached its maximum at month 12 and persisted to month 15 ($p<0.001$, ANOVA). No statistically significant trend in any of the clinical and laboratory assessments of RA was observed within and between the two groups. The authors conclude that Maxepa fish oil, containing EPA and DHA, has NSAID sparing effects when given over 1 year to patients with mild RA. A third study on the long-term effect of supplementation with n-3 fatty acids (three groups: 1.3 g n-3 PUFA; 2.6 g n-3 PUFA including 1.7 g EPA and placebo olive oil) for 12 months found a significant improvement in the patient's global evaluation and in the physician's assessment of pain in those taking 2.6 g/day of n-3 PUFAs (Geusens, Wouters et al. 1994).

11. Plant oils

11.1 Many plant oils are abundant in n-6 linoleic acid (LA), e.g. soy, corn, safflower and sunflower oils. LA desaturates and elongates to GLA, DGLA and AA (see fig. 1). In contrast only few plants contain n-3 α-linolenic acid (ALA) but it is present in leafy green vegetables and in flaxseeds, rapeseeds and canola oils. ALA desaturates and elongates in the human body to EPA and DHA and by itself may have beneficial effects on health and in the control of chronic diseases.

11.2 Certain plant seed oils, notably those extracted from the seeds of evening primrose (EPO), blackcurrants and borage plants, contain relatively large

amounts of GLA which can be converted to DGLA (see fig. 1), the fatty acid precursor of PGE_1. In humans the delta 5 desaturase that converts DGLA to AA is "sluggish" (Callegari and Zurier 1991). Although GLA is readily converted to DGLA, concentrations of AA do not increase appreciably; therefore DGLA competes with AA for oxidative enzymes, reducing production of cyclooxygenase products derived from AA. In addition DGLA cannot be converted to inflammatory leukotrienes by 5-lipoxygenase. DGLA therefore should have anti-inflammatory actions because of its capacity to reduce synthesis of oxygenation products of AA that are more potent mediators of inflammation. Olive oil, another plant oil, is a rich source of n-9 oleic acid. Oleic acid is an n-9 MUFA that is converted to eicosatrienoic acid (20:3n-9; ETA) by desaturation and elongation. ETA is converted to LTA_3 which is a potent inhibitor of LTB_4 synthesis. Thus oleic acid, which is abundant in olive oil, and its metabolite ETA may exert an anti-inflammatory effect through a mechanism similar to that of fish oil, which contains EPA, that acts competitively with n-6 fatty acids. Because ETA is substantially less unsaturated than EPA, it may have greater chemical stability, which would be an advantage for use as a dietary constituent or supplement (James, Gibson et al. 1993).

11.3.1 Several human trials have taken place to determine if oils rich in GLA or oleic acid are anti-inflammatory in RA. A study in Denmark which measured the disease activity in patients with RA when supplemented with PGE_1 precursors, cis-linoleic acid and GLA plus a multi vitamin/mineral capsule given together for 12 weeks found no effect on plasma or urine PGE_1, cAMP and cGMP or on clinical measures or the patient's estimate of pain (Hansen, Lerche et al. 1983). A 12 week trial in the USA on a small number of RA patients, Pullmann-Mooar *et al* (1990) using GLA (1.1 g/d) from borage seeds found significant biochemical changes (Pullman-Mooar, Laposata et al. 1990). GLA supplementation resulted in increased proportions of DGLA in circulating mononuclear cells. The ratios of DGLA to AA and DGLA to stearic acid increased significantly in these cells and there were significant reductions in PGE_2, LTB_4, and LTC_4 (AA oxygenation products) produced by stimulated monocytes. A UK trial supplemented RA patients with GLA in blackcurrant seed oil (BCO) (525 mg GLA/d) for 12 weeks to determine the effect on the secretion of cytokines IL-1β, TNFα and IL-6 and of PGE_2 from cultured

peripheral blood monocytes of healthy subjects and RA patients (Watson, Byars et al. 1993). A significant improvement in morning stiffness was noted in RA patients receiving BCO. The production from the cultured monocytes of the cytokines, IL-1β, TNFα and IL-6 as well as the prostaglandin PGE_2 was markedly altered in subjects given BCO.

11.3.2 A much longer study by Belch *et al* (1988) supplemented patients with EPO (540 mg GLA/d) or EPO/fish oil combination (240 mg EPA and 450 mg GLA/d) for 12 months and compared these with placebo controls. The results showed a significant subjective improvement for the two treatments with a reduction in NSAID use (Belch, Absell et al. 1988). A 24-week supplementation trial with GLA (1.4 g/d) from borage seed oil was used to assess the efficacy and side effects of GLA (Leventhal, Boyce et al. 1993). Treatment with GLA resulted in clinically important reductions in the signs and symptoms of disease activity in patients with RA ($p<0.05$). Patients given a placebo showed no change or worsening of disease. GLA reduced the number of tender joints by 36%, the tender joint score by 45%, swollen joint count by 28%, and swollen joint score by 41%. The placebo group had no significant improvement in clinical measures. Overall clinical responses (significant change in four measures) were also better in the treatment group ($p<0.05$).

11.4 Studies on olive oil are limited. Linos *et al* carried out two case-control studies in Greece and found that in both studies olive oil consumption was inversely associated with development of RA (Linos, Kaklamanis et al. 1991; Linos, Kaklamani et al. 1999). In an intervention study where olive oil (6 g/d) was used as the placebo, with EPO (540 mg GLA/d) as the experimental supplement to determine if EPO enabled RA patients to reduce or stop their NSAID, it was shown that equal numbers of patients on both EPO and olive oil treatment reduced their dose of NSAID (Brzeski, Madhok et al. 1991). Patients on olive oil were found to have significant reduction in pain and articular index at 6 months so the authors concluded that olive oil may have unrecognized benefits and should, itself, be investigated.

12. Folate and other B vitamins

12.1 Low dietary intakes of vitamin B_6 (pyridoxine) and folate have been consistently reported by different investigators (Stone, Doube et al. 1997; Martin 1998). Methotrexate (MTX), an anti-rheumatic drug, is a known folate antagonist (Morgan, Baggott et al. 1994), (Dijkmans 1995), (Ortiz, Shea et al. 2001). Dose response-related toxic effects have been reported in 30% to 90% of patients given MTX. Some side effects of MTX administration, such as gastrointestinal intolerance, mimic complicated folate deficiency. Folate deficiency occurs frequently in patients with RA and folate stores are decreased in RA patients on MTX, suggesting that impaired folate status is related to MTX toxicity (Morgan, Baggott et al. 1994). Morgan et al carried out a randomized, placebo controlled, double-blind study to determine the effect of two different weekly doses of folic acid on the toxicity and efficacy of low-dose MTX therapy for RA (Morgan, Baggott et al. 1994). They concluded that folic acid (an inexpensive vitamin) is safe in a broad range of doses (from 5 mg to 27.5 mg per week) and protects patients with RA who are taking MTX from toxicity while preserving the efficacy of MTX. They also concluded that this data suggests that an intake of one multi-vitamin pill containing 400 μg of folic acid per day may also modulate MTX toxicity.

12.2 An updated Cochrane Library review of the effects of folic acid and folinic acid (a one-carbon-substituted, fully reduced folate) in reducing the mucosal and gastrointestinal and haematologic side effects of low-dose MTX in RA patients and to determine whether or not folate supplements alters MTX efficacy was published in 2001 (Ortiz, Shea et al. 2001). Seven trials met their inclusion criteria, in which 147 patients were treated with folate supplementation (80 with folinic acid and 67 with folic acid). A 79% reduction in mucosal and GI side effects was observed for folic acid [OR = 0.21 (95% CI, 0.10- 0.44)]. For folinic acid, a clinically but non-statistically significant reduction of 43% was found [OR = 0.57 (95% CI 0.28 to 1.15)]. No major differences were observed between low and high doses of folic or folinic acid. Haematologic side effects were not analyzed, since details of haematologic side effects were not provided. No consistent differences in disease activity parameters were observed when comparing placebo and folic or folinic acid at low or high doses, although patients on high dose folinic acid

had an increase in the number of tender joints, but not swollen joints. The reviewers concluded that the results support the protective effect of folate supplementation in reducing MTX side effects related to the oral and GI systems (Ortiz, Shea et al. 2001). It can be concluded, therefore, that RA patients using MTX therapy should also be taking a folate supplement. Because large doses of folic acid can mask and exacerbate vitamin B_{12} deficiency, vitamin B_{12} status should be assured before folic acid supplementation (Morgan, Baggott et al. 1994).

12.3 Low vitamin B_6 levels in the circulation have been found in RA patients (Roubenoff, Roubenoff et al. 1995). Vitamin B_6 is an important regulator of protein and energy metabolism, since it is a cofactor in more than 100 enzymatic reactions involving amino acids. Thus, altered availability of B_6 could have an important effect on the balance of protein synthesis and degradation. Pyridoxal-5-phosphate (PLP) is the metabolically active form of vitamin B_6. In a study to compare vitamin B_6 levels in RA patients with healthy control subjects, Roubenoff et al found that although plasma folate and vitamin B_{12} concentrations and erythrocyte B_6 activity coefficients were similar in the patients and controls, plasma levels of PLP were lower in the RA patient group ($p< 0.004$) (Roubenoff, Roubenoff et al. 1995). In a multivariate analyses, PLP was inversely associated with TNFα production by peripheral blood mononuclear cells (PBMC) ($p<0.001$), after adjustment for age, pain score, ESR, and use of NSAIDs. They concluded that PLP levels are reduced in patients with RA and that this reduction is associated with TNFα production by PBMC (Roubenoff, Roubenoff et al. 1995). They also concluded that further investigations are needed to determine whether low plasma PLP reflects intracellular PLP deficiency or whether there is a redistribution, rather than an absolute decline in the amount, of PLP in RA patients. Oral vitamin B_6 has been shown to be of no therapeutic benefit in treating the symptoms of RA and thus the cause and clinical significance of apparent vitamin B_6 deficiency in RA remain uncertain.

12.4 Roubenoff et al have since looked at homocysteine (Hcy) metabolism in RA patients because of the role of PLP in methyl group transfer in the interconversion of methionine and Hcy (Roubenoff, Dellaripa et al. 1997).

Since circulating levels of PLP in RA patients is low, it is possible that homocysteine (Hcy) metabolism may also be abnormal in RA patients. They found that elevated levels of total Hcy occur commonly in patients with RA, which may explain some of the increased cardiovascular mortality seen in such patients. Their data also suggest that MTX affects Hcy metabolism in RA but not in the direction expected. They found that MTX therapy did not lead to increased total Hcy levels which is consistent with the findings of Morgan et al (Morgan, Baggott et al. 1991). Roubenoff et al advise that larger studies of the distribution of PLP status in RA are now warranted. They also recommend, although treatment with vitamin B_6 is not helpful for the clinical symptoms of RA, the possibility that this vitamin in conjunction with folate and vitamin B_{12} should be prescribed in moderate dosages as a nutritional supplement in at least a subgroup of patients with RA should be explored. The potential toxicity of large doses of vitamin B_6 is well documented. Therefore clinical trials are required before the safety and effectiveness of vitamin B_6 supplementation can be established.

12.5 Glutathione is a major endogenous antioxidant and is important for lymphocyte replication. Vitamin B_6 and riboflavin participate in the maintenance of glutathione status. Vitamin B_6 acts as a cofactor in the synthesis of cysteine (the rate limiting precursor for glutathione biosynthesis) and riboflavin is a cofactor for glutathione reductase. Deficiencies in B_6 and riboflavin (and vitamin E) reduce cell numbers in lymphoid tissues of experimental animals and produce functional abnormalities in the cell mediated immune response (Grimble 1998). In humans dietary supplementation with ascorbic acid, tocopherols and vitamin B_6 enhances a number of aspects of lymphocyte function (Grimble 1998). Until more is known about the role of vitamin B_6 or PLP in RA, it is advisable to urge RA patients to meet the Dietary Reference Value (DRV) for dietary intake of vitamin B_6 (Department of Health 1991) but not to take supplements without dietetic and medical supervision.

13. Sodium and potassium

13.1 A high salt intake may exacerbate Ca deficiency at the proximal tubule, where Na and Ca absorption are linked. Although there are no studies addressing

this, many disabled people rely heavily on prepackaged food and meals, much of which is high in sodium and this may contribute to intakes higher than the recommended level.

13.2 No published papers were found which specifically highlighted potassium (K) intake or status in relation to arthritic disease.

14. Calcium and vitamin D

14.1 Morgan et al reported on average a low intake of calcium (68% patients with intakes ≤67% RDI) in a 1-year study of 79 RA patients (Morgan, Anderson et al. 1997). Low dietary intakes of vitamin D in RA patients are reported by different investigators (Martin 1998). Increased Ca and vitamin D intakes may protect against bone loss during steroid treatment for RA (Gulko and Mulloy 1996).

14.2 Patients with RA are vulnerable to steroid-induced and disease-associated osteoporosis because corticosteroids impair calcium absorption (Reid, Veale et al. 1994). Use of corticosteroids can lead to loss of bone mineral density and higher risk for vertebral fractures. Corticosteroid-induced osteoporosis occurs, in part, because intestinal Ca absorption is inhibited. Evidence indicates that bone loss occurs rapidly within the first 6 to 12 months of corticosteroid therapy and then slows (Adachi, Bensen et al. 1998). The role of prolonged inadequate calcium intake on the acceleration of osteoporosis is well known. $1,25(OH)_2D_3$ stimulates intestinal calcium absorption and mobilizes stem cells to mobilize calcium stores from bone. Noncalcemic tissues that possess receptors for $1,25(OH)_2D_3$ respond to the hormone in a variety of ways. Of great interest is that $1,25(OH)_2D_3$ is a potent antiproliferative and prodifferentiation mediator. As a result, $1,25(OH)_2D_3$ and its analogs have wide clinical application in such diverse clinical disorders as RA and OA.

14.3 To assess the effects of calcium and vitamin D_3 supplementation on BMD of patients with RA, Buckley *et al* carried out a 2-year randomized, double-blind, placebo-controlled trial on 96 RA patients, 65 of whom were receiving treatment with corticosteroids (mean dosage, 5.6 mg/d). Calcium carbonate (1000 mg/d) and vitamin D_3 (500 IU/d) or placebo were supplemented.

Calcium and vitamin D_3 prevented loss of BMD in the lumbar spine and trochanter in patients with RA who were treated with low-dose corticosteroids, but did not improve BMD at any site in patients who were not receiving corticosteroids (Buckley, Leib et al. 1996). In another double-blind, placebo controlled trial to determine the efficacy and safety of vitamin D (50,000 units/week) and calcium (1,000 mg/d) in the prevention of corticosteroid-induced osteoporosis with a 3-year follow-up (Adachi, Bensen et al. 1996), the researchers concluded that vitamin D and calcium may help prevent the early loss of bone seen in the lumbar spine as measured by densitometry of the lumbar spine. But, long-term vitamin D and calcium in those undergoing extended therapy with corticosteroids did not appear to be beneficial. Adachi and Ioannidis in a more recent paper reviewing the evidence for calcium and vitamin D supplementation in corticosteroid-induced bone loss conclude that calcium prophylaxis alone, when patients start corticosteroids, is associated with rapid rates of spinal bone loss and offers only partial protection from corticosteroid-induced spinal bone loss (Adachi and Ioannidid 1999). They recommend that at most, Ca and vitamin D therapy should only be considered adjunctive therapy in the treatment or prevention of corticosteroid-induced bone loss and should be administered in combination with other treatments (Adachi and Ioannidid 1999). They suggest that activated vitamin D may be of greater benefit.

15. Antioxidants

15.1 The generation of reactive oxygen species (ROS) (free radicals) is an important factor in the development and maintenance of rheumatoid arthritis. One source of free radicals is nitric oxide produced within the synoviocytes and chondrocytes and giving rise to the highly toxic radical peroxynitrite. Several cytokines, including TNFα are involved in the formation of free radicals, partly by increasing the activity of nitric oxide synthase. Nitric oxide may mediate some of the deleterious effects of cytokines on bone resorption.

15.2 ROS produced during the inflammatory response enhances pro-inflammatory cytokine production by activation of nuclear factor kappa B. The interaction between ROS and cytokines has the potential to damage the host but can be

held in check by the antioxidant defences. Nutrient intake directly and indirectly influences antioxidant defence (Grimble 1998).

15.3 Antioxidant defences limit the extent of tissue damage exerted by oxidative molecules released during the inflammatory response. The defences include enzymes which detoxify oxidants (superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHpx) and reductase), glutathione (a tripeptide), vitamins C and E (tocopherols), carotenoids and a number of plant components (catechins from tea and tannins from tea and red wine) and uric acid. Glutathione synthesis and the activities of SOD, catalase, GSHpx and reductase are increased by pro-inflammatory cytokines (Grimble 1994). However the enhancement of antioxidant defences may not be able to completely protect the subject from tissue damage during the inflammatory process. There is therefore the need to improve antioxidant defences. The ability of the subject to do this will depend on previous and current intakes of nutrients and nutritional status.

15.4 Synthesis of acute phase proteins and glutathione is influenced by protein intake and sulphur amino acid adequacy. The intake of Cu, Zn and Se influences the activity of antioxidant enzymes. In addition to the protein components of the oxidative defence system, which are synthesised *de novo*, a number of other nutrients that have antioxidant properties, for example, vitamin C (ascorbic acid), vitamin E (tocopherols) and β-carotene are important dietary nutrients in this system. Vitamins E and C and glutathione are intimately linked in antioxidant defence. For example, when oxidant molecules interact with cell membranes, the oxidised form of vitamin E is restored to the antioxidant form by reduction by ascorbic acid. Dehydroascorbic acid formed in the process is converted to ascorbic acid by interaction with the reduced form of glutathione (GSH). Subsequently, oxidised glutathione formed in the reaction is reconverted to the GSH by glutathione reductase (Grimble 1998).

15.5 Vitamin B_6 and riboflavin, which have no antioxidant properties per se, also contribute to antioxidant defences indirectly. Vitamin B_6 is a co-factor in the metabolic pathway for the biosynthesis of cysteine. Cellular cysteine is rate

limiting for glutathione synthesis. Riboflavin is a cofactor for glutathione reductase (Grimble 1998).

15.6 Adequate tissue concentrations of all these antioxidants are important especially where greater oxidant stress such as in RA and OA occurs. In a study of over 1400 people in Finland, 14 developed RA and their antioxidant status, measured by a combination of β-carotene, vitamin E and selenium, was significantly lower than that of other subjects (Heliovaara, Knekt et al. 1994). Beneficial effects of high intakes of dietary antioxidants have been reported especially in OA (McAlindon, Jacques et al. 1996b).

15.7 Data from the Framingham Knee OA Cohort Study (McAlindon, Jacques et al. 1996b) did not support the hypothesis that diets rich in antioxidant micronutrients reduced the risk of incident knee OA but they did suggest that antioxidants might protect people with established disease from disease progression. There was a three-fold reduction in risk for those in the middle and highest tertiles for vitamin C intake (adjusted OR 0.3, 95% CI 0.1, 0.6). Those in the highest tertiles for vitamin C intake also had reduced risk for developing knee pain (adjusted OR 0.3; 95% CI 0.1, 0.8). Reduction in risk of progression was also seen for β-carotene and vitamin E but less consistently since β-carotene association diminished substantially after adjustment for vitamin C, and the vitamin E effect was seen only in men (described in (Darlington and Stone 2001)).

15.8 *Vitamin E*: Low dietary intakes of vitamin E in RA patients have been reported (Kajanachumpol, Vanichapuntu et al. 2000), (Martin 1998), (Heliovaara, Knekt et al. 1994) and in JRA patients (Ashour, Salem et al. 2000). Vitamin E (α-tocopherol) is the major lipid soluble antioxidant. It protects the unsaturated double bonds of fatty acids in phospholipid bilayers from oxidation by scavenging free radicals and is oxidised in the process. It is also an important cofactor for phagocytosis, and T-cells, which are highly susceptible to membrane peroxidation contain high amounts of intracellular vitamin E (Mangge, Hermann et al. 1999). It also blocks AA formation from phospholipids and inhibits lipoxygenase activity, resulting in a mild anti-inflammatory effect (Darlington and Stone 2001). As a result, vitamin E

exerts modulatory effects on both inflammatory and immune components of the immune function. In general vitamin E deficiency and low tissue vitamin E content enhances components of the inflammatory response and suppresses components of the immune response. Dietary vitamin E supplementation brings about the opposite effect (Grimble 1998).

15.8.1 Clinical studies with RA patients reported a mild analgesic effect of vitamin E at doses in the region of 1200 mg/day compared to placebo (Abate, Yang et al. 2000), (Edmonds, Winyard et al. 1997), (Miehle 1997), (Sangha and Stucki 1998), (Wittenborg, Paetersen et al. 1998). However, the efficacy of NSAIDs could not be achieved, and the laboratory parameters of inflammation remained unchanged (Edmonds, Winyard et al. 1997), (Miehle 1997) (see Table 3). A double-blind, placebo controlled study of 50 patients with OA randomly assigned to two groups and treated for 6 weeks with 400 IU vitamin E or a placebo preparation, found that vitamin E was superior to placebo with respect to relief of pain and the need for analgesic treatment ($p<0.05$ to $p<0.01$). Improvement of mobility was better in the group treated with vitamin E, however, this result was not statistically significant (Blankenhorn 1986).

15.8.2 There is increasing evidence suggesting that chondrocyte death may contribute to the progression of OA. The induction of cell death in human OA chondrocytes by nitric oxide is related to the production of PGE_2 via the induction of COX-2 (Notoya, Jovanovic et al. 2000), (Abate, Yang et al. 2000). An *in vitro* study by Tiku at al examined the evidence linking chondrocyte lipid peroxidation to cartilage matrix protein (collagen) degradation and the effect of antioxidants on matrix degradation (Tiku, Shah et al. 2000). They found that vitamin E, at physiological concentrations, significantly diminished the release of labelled matrix by activated chondrocytes. They concluded that the study provided *in vitro* evidence linking chondrocyte lipid peroxidation to cartilage matrix protein oxidation and degradation and suggest that vitamin E has a preventive role. These observations indicate that chondrocyte lipid peroxidation may have a role in the pathogenesis of OA.

15.8.3 Combined supplementation with vitamins C and E appears to be more immunopotentiating than supplementation with either vitamin alone in healthy adults (Jeng, Yang et al. 1996). However, clinical studies combining vitamin E with other antioxidants have not reported significant improvements in clinical parameters. Following two 6 month double-blind controlled trials with a physiological dose of Se, vitamins A, C and E, the researchers reported no significant improvement in clinical parameters (Petersson, Majberger et al. 1991), (Hill and Bird 1990) although one study found a non-significant improvement in some clinical parameters in both treated and control groups (Hill and Bird 1990).

15.9 *Vitamin C (ascorbic acid)*: Vitamin C is an intra- and extracellular scavenger of free radicals (Whiteman and Halliwell 1996). Vitamin C levels have been shown to be low in RA patients (Oldroyd and Dawes 1985) and in JRA patients (Ashour, Salem et al. 2000), however, a supplementation study failed to show any beneficial effect for the synovial inflammatory process (Mangge, Hermann et al. 1999). An animal study using 50 mg/kg body weight of vitamin C for 4-, 7- and 21-days in arthritic rats found improvements after 21-day administration which the researchers interpreted as suggesting a beneficial role for vitamin C in the treatment of arthritis (Eldin, Hamdy et al. 1992). A more recent animal study has demonstrated that the infiltration of inflammatory cells into the synovial tissues was markedly decreased by ascorbic acid (Sakai, Hirano et al. 1999). The increase in SOD activities produced by adjuvant injection were significantly reduced in both the synovium and RBC at ascorbic acid doses of 1.0 and 2.0 g/kg body weight. The decrease in SOD activity could be one of the mechanisms underlying the suppressive effects of large-dose ascorbic acid on the development of arthritis in this animal model, inhibiting the damaging ROS.

15.9.1 Vitamin C also has non-antioxidant effects. Ascorbate stimulates procollagen secretion and vitamin C deficiency is associated with defective connective tissue. Vitamin C is also required for the hydroxylation of proline and lysine in the production of collagen (Mangge, Hermann et al. 1999). It is also thought to be necessary for glycosaminoglycan synthesis (Merry, Grootveld et al. 1991) as described in (Darlington and Stone 2001). No studies were found

reporting on vitamin C levels and collagen degradation or glycosaminoglycan synthesis in arthritis patients in this review.

15.10 *β-carotene:* β-carotene when supplemented in the diet of healthy adults, has been shown to significantly increase the percentages of monocytes expressing the major histo compatability class II molecule, HLA-DR, and the adhesion molecules, intracellular adhesion molecule-1 and leucocyte function-associated molecule (Hughes, Wright et al. 1997). β-carotene may also quench singlet oxygen which may reduce the free radical burden and protect lipids from peroxidation (Darlington and Stone 2001).

15.11 *Selenium:* A major review has recently considered the effects of selenium in RA (Tarp 1995). The collective body of evidence suggests that Se deficiency in animals generally leads to a less responsive immune system, that nutritional Se adequacy and moderate Se supplementation ensure a viable and responsive immune system in both animals and humans and that toxic doses of Se produce immunosuppression. Se has three major functions in the cells of the immune system: (1) reduction of organic and inorganic peroxides formed in the general metabolism and from drugs or other initiators of free radical reactions; (2) modulation of radicals from the phagocytic cell resulting from NADPH oxidase activity; and (3) metabolism of endogenously formed hydroperoxides generated in the AA cascade (Spallholz, Boyland et al. 1990), (Tarp 1995). The major function of Se and GSHpx in the immune cells, and especially those that elicit NADPH oxidase activity, would be to control excessive production of peroxidative substrates (Tarp 1995). Owing to the antioxidant function of GSHpx, Se may influence the inflammation in RA. Adequate Se nutrition may down-regulate cytokine signalling (McCarty and Russell 1999).

15.11.1 Studies from different parts of the world have evaluated Se status in patients with arthritis and low concentrations of Se in serum or plasma in patients with RA have been reported (Aaseth, Haugen et al. 1998), (Tarp, Hansen et al. 1987). Tarp sites 15 reports and Se concentrations were generally lower in the RA patients than in the controls, although many individuals were within the normal range (Tarp 1995). The depression in serum Se was found in several

studies to be related to clinical indices and biochemical parameters of inflammation (Tarp, Overvad et al. 1985), (Honkanen, Konttinen et al. 1991). In a prospective study, 28 RA patients were followed for a mean period of 7.3 years and during the course of the disease, serum Se fluctuated in most of the patients. Concentrations were low in periods of high disease activity and normal in periods of low activity (Tarp 1995).

15.11.2 The hypothesis that the Se level drops in response to inflammation is supported by other observations. Se levels decrease during acute bacterial infections; Se levels are related to dearrangement in plasma proteins; Se levels in plasma and liver decreased in a study of arthritis induced in rats (reported in (Tarp 1995)).

15.11.3 Heliovaara et al followed a Finnish cohort of 1419 persons for 20 years, in which 14 individuals developed RA (Heliovaara, Knekt et al. 1994), see Table 3. A non-significant increased risk of developing RA was found among the subjects in the lowest tertile for serum Se (P=0.11). Adding the results for other antioxidants, their findings supported the hypothesis that antioxidants provide protection against RA. Tarp et al found no relationship in a Danish cohort between the course of the disease and the initial low SE level (Tarp 1995).

15.11.4 Se supplementation has been shown to have anti-inflammatory properties in several models of inflammation and Se deficiency, in turn, has been reported to enhance adjuvant-induced arthritis in rats. There have been few supplementation studies evaluating the biochemical and clinical responses of RA patients and even fewer evaluating the responses in OA. Tarp et al supplemented 6 patients with severe RA and 6 controls with 256 μg of Se in Se-enriched yeast for 6 months (Tarp, Hansen et al. 1987). The reduced Se levels in plasma and red blood cells were promptly corrected and both groups reached a plateau around 200 μg/L after 7 weeks of supplementation. In red blood cells the Se concentration rose in both groups to a level of around 500 μg/L. A similar response in serum and red blood cells was seen in 20 patients supplemented in the same manner (Tarp, Overvad et al. 1985). Supplementation with 250 μg selenomethionine for 6 months in 9 patients

with severe RA and 8 controls induced a Se increase in serum to approximately 400 μg/L without a sign of plateauing in the patients (Tarp, Stengaard-Pedersen et al. 1992). Se supplementation produced no response in the PMN leucocytes of the RA patients, but the Se level increased significantly in the controls. This suggests that active RA affects the Se metabolism in the PMN leucocytes, probably owing to impaired incorporation into the cells during formation in the bone marrow. In the PMN leucocytes the initial lower GSHpx levels among the patients were sustained during supplementation. In the group receiving yeast Se, a slight but significant increase was found; in the selenomethionine group no changes were recorded. The lack of response in GSHpx activity seems in accordance with the lack of increase in the Se content of the PMN leucocytes.

15.11.5 Studies of the biological action of Se assessed by GSHpx activity have not yielded consistent results. In those that were focused on patients with severe, active RA, low activities of GSHpx were found in serum, RBCs and PMN leucocytes. Se supplementation increased GSHpx activity in serum, RBCs and platelets, but not in PMN leucocytes. This apparent lack of *de novo* synthesis of GSHpx in the PMN leucocytes may be explained by the inability of the leucocytes to increase the Se content, in spite of high levels of available Se. This might also explain the lack of clinical anti-arthritic effect of Se supplementation in most of the controlled trials, although some studies indicate an effect on joint pain, a semi-objective parameter of disease activity (Tarp 1995).

16. Trace elements (zinc, copper and magnesium)

16.1 RA is characterised by low serum Zn (Helliwell, Coombes et al. 1984) and high serum Cu (Honkanen et al, 1991) or ceruloplasmin-Cu complexes (Helliwell, Coombes et al. 1984). In a multiple regression both were explained by disease activity parameters. Drug treatment and Zn intake did not affect serum Zn concentration (Honkanen et al 1991). Honkanen et al suggest that IL-1 causes low serum Zn and high serum Cu by 1) increasing the metallothionen-mediated hepatic uptake to serum Zn and 2) upregulating ceruloplasmin gene and synthesis in liver and subsequently the level of ceruloplasmin-Cu complexes in blood (Honkanen ref.) (Svenson, Hallgren et

al. 1985). Biochemical tests showed that serum Cu had a good correlation with serum ceruloplasmin. Cu was found to be the only trace element sensitive to nutritional intake. Cu absorption was diminished by Zn intake. Low dietary intakes of Zn (Mangge, Hermann et al. 1999) and also Mg have been reported in RA patients by others (Martin 1998).

16.2 Zn is a component of at least 200 enzymes in the body and is essential for maintenance of the immune system and for growth and immune function (Honkanen, Konttinen et al. 1991), (Stone, Doube et al. 1997). In spite of the changes in serum Zn and Cp mentioned above, Cu- and Zn-dependent erythrocyte SOD, an antioxidant enzyme, was increased in RA (Honkanen, Konttinen et al. 1991) but was not associated with clinical or laboratory disease activity parameters. The low serum concentrations of Zn may also be explained partly by hypoalbinemia, the use of corticosteroids and NSAIDs (Milanino, Frigo et al. 1993), or may be a non-specific feature of inflammation (Stone, Doube et al. 1997). Zn is a cofactor in collagen synthesis, and low serum levels were observed to correlate with secondary osteoporosis in female RA patients, however, clinical studies investigating a therapeutic supplementation yielded contradictory results (Mangge, Hermann et al. 1999). The clinical data so far available do not support a therapeutical use of Zn, however, a diet adequate in Zn, and Mg would appear to be advisable. No data exist on supplementing a group of zinc-deficient patients with RA up to the RNI.

17. Other nutrients

17.1.1 *Iron*: An evaluation of iron status in anaemic RA patients suggested that, on the basis of serum transferrin receptor and ferritin concentrations, in approximately one third of patients with RA anaemia is due to the depletion of iron stores. Therefore, in all patients with RA iron deficiency must be considered as a potential cause of the anaemia (Punnonen, Kaipiainen-Sepranen et al. 2000).

17.1.2 Iron deficiency anaemia may develop as a result of chronic inflammation resulting in sequestration of iron from the reticulo-endothelial cells, and because of poor dietary intake, gastrointestinal blood loss secondary to

medications, or preferential uptake of iron by inflamed synovial tissue (Giodano, Floravanti et al. 1984).

17.1.3 Patients who have active RA and who receive treatment with deferioxamine, an iron chelating agent with anti-inflammatory properties, demonstrated increased haemoglobin and serum iron and an improvement in disease activity. Improvement lasted eight weeks following cessation of treatment (Marcus 1987).

17.2 *Boron*: The role of boron in nutrition and metabolism has been reviewed by Naghil and Samman who state that numerous studies suggest that boron interacts with other nutrients and plays a regulatory role in the metabolism of minerals, such as calcium, and subsequently bone metabolism (Naghil and Samman 1993). Although the mechanism of action has not been defined, it may be mediated by increasing the concentration of steroid hormones such as testosterone and beta-oestradiol. Based on a limited number of studies, increasing dietary boron results in increases in the boron concentration of all tissues. Boron is obtained from a diet rich in fruits, vegetables, nuts and legumes. Large amounts of boron are well tolerated while consistent signs of deficiency include depressed growth and a reduction in some blood indices, particularly steroid hormone concentrations. Via its effect on steroid hormones and interaction with mineral metabolism, boron may be involved in a number of clinical conditions such as arthritis. However, there is insufficient evidence and further research is required to prove a link between dietary boron intake and arthritis.

17.3 *Fluoride*: Fluoride therapy in prevention of RA induced bone loss was tested in an 18 month, randomized, double-blind, placebo-controlled trial in 38 RA patients (Adachi, Bell et al. 1997). Sodium fluoride at 40 mg/d was compared with a placebo. There was a significant percentage difference between groups ($p=0.0005$) in lumbar spine BMD after 18 months of treatment in favour of the fluoride group. No significant difference was found for any of the other sites measured. Lumbar spine BMD increased in about 80% of patients treated with fluoride (responders) compared to 44% of patients treated with placebo.

The researchers concluded that fluoride therapy was well tolerated and increased vertebral bone mass in patients with RA.

18. Foods

18.1 A wide range of foods have been associated with improvements in symptoms or worsening of arthritic symptoms. Foods most linked with improving symptoms have been reported as fish, vegetables and oils (Martin 1998), (Nenonen, Helve et al. 1998), (Garrett, Kennedy et al. 1993) while milk and dairy products, red meats, cereals, flour products and wheat gluten, citrus fruits, chocolate, spices, and alcohol have been implicated in the aggravation of symptoms (Panush, Stroud et al. 1986), (Buchannan, Preston et al. 1991), (Van da Laar and van der Korst 1992a), (Darlington and Ramsey 1993), (Garrett, Kennedy et al. 1993), (Kavanagh, Workman et al. 1995), (Danao-Camara and Shintani 1999).

18.2 Foods associated with lower incidence or improvements of RA

18.2.1 *Fish*: Fish oils have been found to improve clinical symptoms of RA, however, few studies have examined the effect of fish in the diet on the risk of developing RA or on improvements in RA. A hospital-based case-control study of self-reported diet in Greece found that increased consumption of fish and of olive oil was associated with a reduced prevalence of RA (Linos, Kaklamanis et al. 1991), however, multiple logistic analysis found that only the association with olive oil consumption remained significant. A more recent study by the same group did not find that fish consumption was significantly associated with the incidence of RA (Linos, Kaklamani et al. 1999), however they did find an association between olive oil and cooked vegetables and the incidence of RA. An association with decreased risk of RA and the consumption of broiled and baked fish was found in a population-based, case-control study in the USA (Shapiro, Koepsell et al. 1996). The adjusted OR for 1-2 servings and ≥2 servings of broiled or baked fish/week, compared with <1 serving, were 0.78 [95% CI = 0.53-1.14] and 0.57 [95% CI = 0.35-0.93]. The case for fish became stronger when the analysis was restricted to cases positive for rheumatoid factor.

18.2.2 Two intervention studies using fish or foods rich in n-3 PUFA have been identified (Fahrer, Hoeflin et al. 1991), (Mantzioris, Cleland et al. 2000) see paragraph 10.9. In the earliest study, a diet rich in fish was examined to see if it had a similar effect on membrane and plasma lipids as a dietary fish oil supplement using healthy volunteers (Fahrer, Hoeflin et al. 1991). The researchers concluded that 4 to 6 meals (approx. 700 g) of fish per week without any other dietary changes can induce similar changes in lipids as a supplement of fish oil. In the recent open intervention study with healthy male volunteers, a diet incorporating foods rich in n-3 fatty acids including fish consumed for 4 weeks was examined for an effect on tissue concentrations of EPA and in suppressing the production of inflammatory mediators (Mantzioris, Cleland et al. 2000). The authors concluded that a diet containing foods enriched in γ-linolenic acid (cooking oil, margarine, salad dressing and mayonnaise) and EPA and DHA (sausages and savoury dip) and foods naturally rich in n-3 PUFA, such as flaxseed meal and fish can produce similar effects to fish oil supplements and may be better tolerated by RA patients. A diet rich in fish might be both preventive of and lead to improvement of symptoms in RA. Eating fish more than once a week or eating about 700g per week could be recommended to patients who might potentially benefit from n-3 fatty acids and who enjoy eating fish.

18.2.3 *Vegetables:* A case-control study in Greece was identified which reported the consumption of vegetables in relation to RA (Linos, Kaklamani et al. 1999). Risk of developing RA was inversely and significantly associated with cooked vegetables (OR for lowest vs. highest quartile of consumption: 0.39; 95% CI 0.20-0.77). Those in the highest quartile consumed 2.9 servings/day. The OR for developing RA also appeared to be reduced when consumption of raw vegetables increased, but this was not significant. The results of a multiple logistic regression analysis model controlled for the effect of several confounding factors showed that both cooked vegetables (OR = 0.24 for the top quartile) and olive oil (OR = 0.38 for the top quartile) consumption had an independent effect on risk of developing RA, whereas no other food group appeared to play a role of comparable significance. A large cross sectional study (1993 Italian Household Multipurpose Survey) was used to analyze the relationship between vegetable consumption and prevalence of chronic

diseases in Italy (La Vecchia, Decarli et al. 1998). An inverse relationship was observed between vegetable consumption and arthritis (OR= 0.84). Since plants are rich natural sources of antioxidants, and RA subjects consuming diets high in fruits and vegetables (as in the living foods vegan diet) have been found to have increased levels of serum α- and β-carotenes, lycopene and lutein, as well as vitamin C and E (adjusted to cholesterol) (Hanninen, Kaartinen et al. 2000), the observed association between incidence of RA and cooked vegetables may be due to the antioxidant effect, however this still remains to be elucidated.

18.2.4 *Fruits:* Only a very small number of studies mentioning fruit intake were identified. Some of these are covered under the paragraph on vegan diets. In the USA population-based, case-control study carried out by Shapiro et al (1996), there was a small decreased risk of developing RA with increased fruit consumption but this was not significant, highest vs. lowest quartile of consumption, aOR =0.88 [95% CI, 0.61-1.26].

18.2.5 *Oils:* Two case-control studies carried out in Greece have reported that consumption of olive oil is inversely associated with a reduction in the development of RA. In the first study, results of univariate analysis revealed that RA cases consumed significantly less olive oil (Linos, Kaklamanis et al. 1991). In a later study to confirm this finding, the researchers estimated lifelong consumption of olive oil based on adherence to the Greek Orthodox Lent. The risk of developing RA decreased significantly with increased lifelong consumption of olive oil (chi-square: 4.28; p = 0.03). In addition, persons in the highest quartile of olive oil exposure had an OR of 0.39 [95%CI, 0.19-0.82] when compared with the lowest quartile of consumption. There is also evidence from intervention studies that olive oil may be effective in relieving arthritis symptoms, see paragraph 11.4. Olive oil contains a high proportion of oleic acid, and its metabolite ETA may exert an anti-inflammatory effect through a mechanism similar to that of fish oil, see paragraph 9.4, which might explain the benefits of consuming olive oil described above. Information regarding fish oil is covered in chapter 9.

18.3 *Foods associated with aggravation of symptoms in RA*

18.3.1 Food allergy or intolerance has been suggested as a pathogenic factor in some arthritis patients. Patients with RA often claim that their symptoms are alleviated by special diets or by simple elimination of certain constituents of the daily diet. Evidence for the role of food intolerance in RA is inconclusive, derived mainly from anecdotal reports. In cases where the behaviour of the arthropathy satisfies classical medical criteria for causation, i.e. exacerbation with challenge, remission with removal, and reactivation with rechallenge, then food allergy may be identified. However, these cases are sporadic and fairly rare (Van da Laar and van der Korst 1992a), (Panush 1991).

18.3.2 A relation between diet and rheumatic disease could occur through two possible mechanisms that are not mutually exclusive:

i) nutritional factors might alter immune and inflammatory responses and thus modify manifestations of rheumatic diseases; and

ii) food related antigens might provoke hypersensitivity responses leading to rheumatological symptoms (Panush 1991).

The possible food-antigens are predominantly proteins. Elevated antibody activity against dietary antigens has been found in RA patients, but without association with the clinical course (Kjeldsen-Kragh, Hvatum et al. 1995a). Panush speculated that probably less than 5% of rheumatic disease patients may have immunologic sensitivity to foods (Panush 1991). When patients have been subjected to controlled challenge studies, some have been identified as having worsened symptoms with specific foods which suggests a possible role for food allergy in at least some patients who have rheumatic disease.

18.3.3 Dietary therapy can be divided into two types: elimination therapy, in which foods are removed from the diet, and supplementation therapy, in which foods or food components are added. The most studied dietary supplement is fish oil, which can partially improve RA (Fortin, Lew et al. 1995). Also botanical lipids (Callegari and Zurier 1991), Se (Tarp 1995), vitamin E (Edmonds, Winyard et al. 1997), and fish (Shapiro, Koepsell et al. 1996) have been studied with different outcome.

19. Elimination diets

19.1 Elimination therapy is based on the premise that if a food antigen has a role to play in the pathogenesis of a disease then its elimination from the diet should result in an objective improvement. There is evidence both from case reports (Parke and Hughes 1981), (Williams 1981) and controlled studies (Darlington and Ramsey 1993), (Kjeldsen-Kragh, Haugen et al. 1991) that an individualized diet where offending foods are identified and removed from the diet can cause an improvement in RA. Elimination diets have been used with success in RA patients (Kjeldsen-Kragh 1999). Fasting followed by a one-year vegetarian diet has given a prolonged improvement to a number of patients with RA (Kjeldsen-Kragh, Haugen et al. 1991), (Muller, Wilhelmi de Toledo et al. 2001).

19.2 Müller et al recently carried out a systematic review of trials of fasting followed by vegetarian diets (Muller, Wilhelmi de Toledo et al. 2001). Only four controlled studies were found to have investigated the effects of fasting and subsequent diets for at least three months. The pooling of these studies showed a statistically and clinically significant beneficial long-term effect. , Fasting alone reduces objective as well as subjective indices of disease activity in most patients with, but most patients relapse after reintroduction of an omnivorous diet (Uden, Trang et al. 1983), (Hafstrom, Ringertz et al. 1988), (Skoldstam and Magnusson 1991), (Muller, Wilhelmi de Toledo et al. 2001). This suggests that fasting without further diet therapy is of limited therapeutical use. Some fasts are subtotal fasts as in the Norwegian study, i.e. a limited number of drinks and or foods are allowed which comprised herbal teas, garlic, vegetable broth, decocted potatoes and parsley, and the juices of carrots, beets and celery (Kjeldsen-Kragh, Haugen et al. 1991), The energy intake in this subtotal fast varied between 0.8 and 1.3 MJ/d which is effectively an elimination diet. Fasting must always be under medical supervision, as should any serious change in diet, such as a ‘living food’ vegan diet described below.

19.3 The mechanism by which fasting leads to improvement is not yet fully confirmed. Subjective improvement in pain and stiffness starts within three to five days of the initiation of the fast, and is sustained for its duration. Joint

inflammation indices as well as acute phase reactants decrease. Lymphocytes are affected by fasting. Antigen specific B cell responses improve. Suppressor cell activity, usually depressed in RA patients, normalizes with fasting (Danao-Camara and Shintani 1999). Neutrophilic functions are also influenced by food deprivation. Release of pro-inflammatory LTB_4 from neutrophils goes down, as does the ability to generate cytotoxins *in vitro* (Hafstrom, Ringertz et al. 1988). Levels of LA and ALA are unchanged; their metabolites, AA and EPA increase. Such a profile can be produced by impaired activity of phospholipase and 5-lipoxygenase, enzymes involved in the metabolism of AA to LTB_4. Hafstrom *et al* have postulated stimulus-response decoupling of neutrophil metabolism, reduced ability to generate cytotoxins, reduced leukotriene formation as mechanisms of the anti-inflammatory effect of food deprivation (Hafstrom, Ringertz et al. 1988).

19.4 Fasting also alters intestinal permeability. Polyethylene glycol molecules penetrate intestinal mucosa less well during fasting; this reverses with refeeding of a lactovegetarian diet (Sundqvist, Lindstrom et al. 1982). If inflammatory arthritis is an allergic or hyperimmune reaction to foreign antigens, the decreased penetration of immunostimulants may explain the temporary relief experienced by patients on a fast (Danao-Camara and Shintani 1999).

19.5 Trials using exclusion diets and elemental diets are listed in Table 5. Panush *et al* (Panush, Carter et al. 1983) (Panush 1991) were one of the first groups to have carried out a 10–week, double-blind, clinical trial of an exclusion diet (the Dong diet) for rheumatoid arthritis. The experimental diet excluded certain food items (dairy products, red meat, citrus fruits, tomatoes, herbs, spices, alcohol and coffee) and the placebo diet included these food items but excluded other foods. Although there was no statistical difference between the experimental and placebo diet groups, two patients in the experimental diet group improved considerably and experienced recurrence of symptoms when they deviated from it. Further clinical study found that one of these patients experienced disease exacerbation with dairy products and immunological studies confirmed a sensitivity to milk.

19.6 In 1986, Darlington et al (Darlington, Ramsey et al. 1986) published the results of a single-blinded, placebo controlled study of 6 weeks dietary manipulation therapy with 53 RA patients. During the first week the patients were only allowed to eat foods they were unlikely to be intolerant to (the paper does not describe these). Other food items were then introduced one at a time to see whether any symptoms were elicited by the dietary challenge. Foods producing symptoms were then excluded from the diet. Both objective and subjective variables improved significantly in the exclusion diet group than the placebo diet group. However, a sub-group experienced even more significant improvement and these were termed 'good responders'. In 1987 this same group investigated the possibility that weight loss, which occurs on elimination diets had been responsible for the improvement of the dietary treatment (Darlington and Ramsey 1993). Correlation coefficients were calculated between weight loss and variables which improved significantly during the diet but no significant correlations were found, suggesting that weight loss does not play a causal role in the improvement of symptoms in RA patients on dietary manipulation.

19.7 A further elimination study was carried out with 48 RA patients over 6 weeks by Darlington and Ramsey in 1987, reported in (Darlington and Ramsey 1993). Forty-one patients identified foods producing symptoms. Cereal foods comprised four of the top seven symptom-inducing foods. Darlington and Ramsey carried out a prospective, blind challenge study on 15 RA patients which began with an exclusion diet, followed by reintroduction of foods, after which three symptomatic foods were selected for each patient to challenge that patient (Darlington and Ramsey 1993). Results suggested that, although RA patients improved on dietary manipulation, three weeks of blind food challenge rapidly produced deteriorating trends in subjective and objective symptoms.

19.8 Darlington and Ramsey (1993) (Darlington and Ramsey 1993) also describe a study by Wojtulewski (Wojtulewski 1987) in which he treated 41 RA patients for 4 weeks to an elimination diet. Twenty three patients improved and of these 10 patients were found to give positive reactions to challenges with four food groups. In an earlier study involving the same author (Bourne, Kumar et

al. 1985) six patients with coeliac disease in whom arthritis was prominent at diagnosis were found to improve with a gluten-free diet.

19.9 O'Farrelly et al. (O'Farrelly, Melcher et al. 1988), (O'Farrelly, Price et al. 1989) investigated 93 RA patients of for humoral sensitization to gliadin and compared their small intestine biopsy specimens with those from controls. 44 patients (47%) had raised levels of IgG to gliadin and, of these, 38 (86%) were also positive for IgA RF. The authors concluded that the gut may play a more important role in the immuno-pathogenesis of some cases of RA than of others, and that the former might be identified by raised levels of IgA RF and wheat protein IgG.

19.10 Panush suggests that most of these studies have not been rigorously and completely controlled and should not be interpreted yet as definitive (Panush 1991). Since then further studies using food in capsules to provide a blinded challenge have been carried out. Panush reports that 3 of 16 patients convincingly demonstrated subjective and objective rheumatoligic symptoms following double-blinded, encapsulated food challenges; they were virtually asymptomatic on elemental nutrition (Panush 1990), (Panush 1991).

19.11 In 1992, van de Laar and van der Korst (Van da Laar and van der Korst 1992a) described a double-blind, randomized, controlled trial of two types of artificial elementary diet, one diet was allergen free, the other allergen restricted, containing only lactoproteins and yellow azo dyes in 94 RA patients. A subgroup of nine patients (three on the allergen restricted and six on the allergen free diets) showed favourable responses, followed by marked disease exacerbation during rechallenge (van de Laar, Aalbers et al. 1992b). The authors suggested that serious consideration should be given to the theory that food intolerance influences the activity of seropositive RA at least in some patients.

19.12 In a cross-over study, Gianfranceschi et al (Gianfranceschi, Fasani et al. 1996) compared the clinical effects of two different normocaloric diets, one with no suspect foods admitted and the other diet was a well balanced diet. Patients were first tested for foods with a low tolerance using a dynamometric

challenge test (described in the paper). Diet A excluded all foods identified in this test. After Diet A, patients had 42% less joint pain ($p<0.005$). Results from Diet B were not significant. Diet A also resulted in a 40% reduction in morning stiffness but not Diet B. Both diets slightly reduced the number of swollen joints, but the results were not significant. The authors concluded that diet and avoidance of selected foods appear to be useful in RA management, and the dynamometric challenge test represents a practical clinical tool for establishing the best diet. They also state that a drop in tolerance to foods is strictly individual so that a diet for the relief of RA symptoms cannot be a standard one, but should be selected according to individual food hypersensitivities.

19.13 Darlington and Ramsey (1993) give possible reasons for improvement of patients on elimination dietary therapy (Darlington and Ramsey 1993). These may be the result of a number of different mechanisms, acting singly or in combination, for example, placebo response, suppression of Type I reaction, weight loss, reduced gastrointestinal permeability and bacterial antigens, secretory IgA deficiency and lectins.

20. Elemental diets

20.1 Elemental diets provide food in its simplest formulation: protein as amino acids or oligopeptides, carbohydrate as glucose or small saccharides, and fat as medium-chain triglycerides. Such a diet is considered hypoallergenic. Elemental diets can improve some disease parameters such as grip strength and Ritchie articular score in RA patients, but return to normal diet often results in relapse of symptoms (Holst-Jensen, Pfeiffer-Jensen et al. 1998), (Van da Laar and van der Korst 1992a), (Kavanagh, Workman et al. 1995), (Haugen, Kjeldsen-Kragh et al. 1994). Certain individuals respond better than others.

20.2 Two trials of elemental diets were identified. Both were randomized controlled trials with RA patients (see Table 4). In the first, 47 RA patients were randomly allocated to elemental diet group or control group (Kavanagh, Workman et al. 1995). The intervention (elimination phase) lasted for 4 weeks followed by a period of food reintroduction. There was a high default

rate with only 38% of the patients completing the study. Following the elemental diet there was a statistically significant improvement in the diet group in grip strength and Ritchie articular score but not in other variables. The improvement in grip strength disappeared with the reintroduction of an individualized diet, i.e. foods which did not cause a worsening of symptoms. In the second study, a commercially available elemental peptide diet was given to 15 RA patients for 4 weeks, followed by the introduction of normal food with a follow-up period of 6 months (Holst-Jensen, Pfeiffer-Jensen et al. 1998). The outcome was compared with those in a control group of 15 patients who maintained their usual diet. The elemental diet made statistically significant improvements at the end of the diet period in 2 of the subjective efficacy variables, average level of pain during the last week and Health Assessment questionnaire (HAQ) score. These effects had disappeared at 3 months of follow-up. In both studies weight loss was considerable during the intervention. Since both studies produced only transient improvements in disease parameters and the drop out rate was significant, it might be concluded that elemental diets are not a treatment of choice in unselected RA patients. The elemental diet might be beneficial to a subset of patients in whom a food allergy or intolerance seems to be an aggravating factor.

21. Vegetarian Diets

21.1 Both vegan and vegetarian diets have been shown to relieve symptoms in RA patients (Kjeldsen-Kragh, Haugen et al. 1991), (Peltonen, Nenonen et al. 1997) see Table 5. When fasting was followed by an individually adjusted vegetarian diet for 12 months in a Norwegian cohort of RA patients, RA improved in a proportion of the patients (Kjeldsen-Kragh, Haugen et al. 1991). Fifty three RA patients were randomised into a diet and control group in this single-blind trial. The diet group was allocated to a 4-week stay at a health farm. After an initial 7-10 day subtotal fast, they were put on a strict gluten-free vegan diet for 3.5 months followed by an individually adjusted lacto-vegetarian diet for 9 month (see list of foods allowed in Table 7). As with other individually adjusted diets, the patients introduced a new food every second day. If they experienced increased pain, stiffness, or joint swelling within 2-48 h, the food was omitted from the diet for ≥7 d before being reintroduced. If symptoms exacerbated again, the food item was excluded

from the diet for the rest of the study. The control group stayed for 4 weeks at a convalescent home, but ate an ordinary diet (omnivorous) throughout the entire study period. After 4 weeks at the health farm the diet group showed a significant improvement in all clinical variables and most laboratory variables measured compared with the controls who showed improvement in only one clinical variable. The benefits in the diet group were still present after one year. However, 10 of the diet group showed substantial improvement in 3 or more of the core variables and were classified as diet group responders. The remaining diet group were classified as diet non-responders. This classification is used in further studies carried out on this cohort (see below).

21.2 One year after the patients completed the trial, they were re-examined. Compared with baseline, the improvements measured were significantly greater in the diet responders than in diet non-responders and omnivores (Kjeldsen-Kragh, Haugen et al. 1994a). At the time of the follow-up examination all of the diet responders, but only half of the diet non-responders, were still following the diets they had consumed during the trial. The beneficial effect could not be explained by patients' psychological characteristics (Kjeldsen-Kragh, M. et al. 1994b), antibody activity against food antigens (Kjeldsen-Kragh, Hvatum et al. 1995a), or changes in concentration of prostaglandin and leukotriene precursors (Haugen, Kjeldsen-Kragh et al. 1994). However, the faecal flora differed significantly between samples collected at time points at which there was substantial clinical improvement and time points at which there were no or only minor improvements (Peltonen, Kjeldsen-Kragh et al. 1994). In a recent Finnish RA dietary intervention using an uncooked vegan diet described below, Peltonen et al found significant diet-associated changes in faecal flora. In this trial they also found significant differences in the gut microflora between diet responders and non-responders, but no attempts were made to identify which bacterial species were responsible for these differences (Peltonen, Nenonen et al. 1997).

21.3 The effects of an uncooked vegan diet, rich in lactobacilli, fibre and antioxidants was tested in Finnish chronic RA patients (Peltonen, Nenonen et al. 1997), (Nenonen, Helve et al. 1998), (Hanninen, Kaartinen et al. 2000),

(Agren, Tvrzicka et al. 2001). Forty three patients were randomized into a diet and a control group. The diet group (22 patients) were provided with all the raw food components of their diet daily whereas the control group continued with their normal diet. The diet comprised Berries, fruits, vegetables and roots, nuts, germinated seeds and sprouts. The diet did not contain any animal products or added salt. The items may be soaked, sprouted (seeds and grains), fermented, blended or dehydrated. Caffeine-containing drinks, chocolate, alcohol and tobacco smoking were prohibited in both groups. The dietary intervention was planned for 3 months, however, half of the patients on the vegan diet experienced adverse effects (nausea, diarrhoea or difficulties with the taste) and terminated the diet after 2 months but they were included in the outcome measures. The diet group experienced subjective relief of rheumatic symptoms during the intervention, with 5 patients being classified as high responders as a result of their significantly improved disease indices. The positive subjective effect experienced by the diet group was not discernible in the objective measures of disease activity when analysed separately but a composite index of disease activity did find a statistically significant connection between compliance with the diet and decrease in disease activity. A return to an omnivorous diet aggravated symptoms. A limited 'living food', vegan diet does not appear to be an acceptable therapy for more than a few weeks for many RA patients because of the difficulties experienced.

21.4 The patients consuming .the vegan diet showed highly increased levels of serum α- and β-carotenes, lycopene and lutein (Hanninen, Kaartinen et al. 2000). Also the increases of vitamin C and vitamin E (adjusted to cholesterol) were statistically significant. The polyphenolic flavonoids, quercetin, myricetin and kaempherol were all much higher than in the omnivorous controls, and the urinary excretion of polyphenols like enterodiol and enterolactone were much increased in subjects eating the vegan diet (Hanninen, Kaartinen et al. 2000). Serum total cholesterol, phospholipid, cholestanol and lathosterol concentrations were significantly decreased by the vegan diet without changing their ratios (Agren, Tvrzicka et al. 2001). The levels of serum plant sterols was also changed with the concentration of campesterol decreasing and that of sitosterol increasing. This effect resulted in a significantly greater sitosterol:campesterol value in the vegan diet group

than in the control group which suggests that a strict uncooked vegan diet changes the relative absorption rates of these sterols and/or their clearance. In conclusion, this extreme vegan diet with high lactobacilli, fibre, antioxidant and polyphenolic content, with no cholesterol but high in plant sterols and rich in unsaturated fatty acids appears to be beneficial to a proportion of RA patients but many patients find it unacceptable for more than short periods of time.

22. All diets

22.1 Approximately 150 studies have found dietary influences on RA (Panush 1997), (Henderson and Panush 1999), the level of scientific methodology for most of the studies is such that generally they are not accepted by the medical establishment (Kjeldsen-Kragh 1999). From the few studies which did meet high scientific standards it appears that diet affects the severity of RA symptoms (Muller, Wilhelmi de Toledo et al. 2001), rather than its prevalence (Grant 2000). However, there is evidence that a diet high in vegetables, fish and olive oil may reduce the incidence or development of RA.

22.2 More randomised long-term studies are needed to confirm the benefits of specific diets, especially vegetarian diets, for patients with RA by methodologically convincing data. However, there are considerable methodological problems associated with clinical trials aimed at evaluating the effects of a particular diet. Compliance is more difficult to verify than drug trials and it is extremely difficult to implement a double-blind protocol in such trials.

23. Dietary supplements

23.1 Overall, there is a growing scientific rationale for the use of dietary supplements of fish oils and antioxidants, as adjuncts in the treatment of inflammatory disorders such as RA and OA (Darlington and Stone 2001).

C. An analysis of nutrient intakes among UK consumers following the principles of the Arthritic Association diet

1. Background

Since 1966 the Arthritic Association has recommended adherence to the Home Treatment programme for individuals suffering from arthritis. This programme incorporates dietary advice, exercise and the use of specific preparations based on homeopathic and other natural agents. The programme is designed to be used in conjunction with, and not instead of, the advice of medical practitioners.

The recommended diet is rich in fruits, vegetables, nuts and wholegrain foods with the aim of providing a micro-nutrient rich and "energising" diet. There is a particular focus on potassium-rich foods, claiming that: "potassium deficiency can produce rapid calcification of the arteries, teeth, muscles and joints." However the overall impact of this type of dietary pattern on nutrient intakes is unclear.

Considerable scientific research into the links between diet and health has been conducted since the Home Treatment programme was initiated. There is now good evidence that dietary factors play an important role in the aetiology of many chronic diseases. There is also some evidence for a role of diet specifically in relation to arthritis which has been reviewed in section A of this report. This analysis will consider the intake of key nutrients in those consuming a diet rich in FNVW as recommended in the Home Treatment plan with respect both to the management of arthritis and the prevention of other chronic diseases.

2. Objective

Firstly, to examine the nutrient intake of subjects who consume a diet rich in fruit, nuts, vegetables and wholegrains, in line with the Arthritic Association Home Treatment programme. Secondly, to examine the typical consumption of oily fish and olive oil based spreads since these foods have been identified as potentially valuable in relieving the symptoms of arthritis.

3. Methods

3.1 Surveys

Data from two nationally representative samples of UK adults were analysed to estimate habitual dietary intake of key nutrients. The *Dietary and Nutritional Survey of British Adults* (Gregory *et al*, 1990) was conducted between October 1986 and September 1987. It collected dietary, biochemical, socio-economic and lifestyle data from randomly selected participants aged 16-64y from England, Scotland and Wales. In total, 2197 individuals completed 7-d weighed dietary records. Data from those individuals who did not provide full information on demographic and lifestyle factors, or those individuals who were unwell with eating affected were excluded from this analysis and thus 2040 diaries were analysed for this report. The *National Diet and Nutrition Survey of people aged 65 years and over* (Finch *et al.* 1998) was conducted from October 1994 to September 1995. Food consumption data were collected using 4-d dietary records in a random sample of 2626 adults aged 65 years and over, living either in private dwellings or in institutions. For these analyses, only data from free-living participants (i.e. those living in private dwellings) who provided full demographic data and were not unwell with eating affected, were considered, giving a total of 1152 individuals.

3.2 Dietary variables

In order to identify a sub-group of the population with an eating pattern most closely resembling the Arthritic Association's Home Treatment programme, a new compound intake variable was generated that summed the intake of fresh fruit, vegetables, nuts and wholegrain foods (FNVW). Based on this index the populations were divided into quintiles. Consumers in the lowest, middle and highest fifth were selected for comparative analysis of key nutrient intakes. The top fifth of consumers were considered to be those with a dietary pattern which approximated to that Arthritic Association's recommended eating plan.

3.3 Nutrient intakes and food consumption analyses

Intakes of the following nutrients were determined for each group:

- Macronutrients: Total energy , protein, fat, carbohydrate, alcohol
- Micronutrients and trace elements: vitamin C, vitamin E, carotenoids, sodium, potssium, calcium, zinc, magnesium

- Cis n-3 polyunsaturated fatty acids (n-3 PUFA) from oily fish and olive oil/olive oil based spreads (NB. Olive oil consumption was analysed in both surveys, but information on olive oil based spreads was only available in the 1995-6 survey).

Socio-demographic information was also analysed to identify any differences between the three dietary groups:

- Body Mass Index (BMI, kg/m^2)
- Region: England, Scotland and Wales
- Smoking behaviour: smokers vs. non-smokers
- Occupational social class: non-manual vs. manual employment

3.4 *Statistical analysis*

Food consumption data (supplied by *The Data Archive*, Essex, UK) for the two surveys were collated and analysed using Excel (Microsoft Corp., USA) and SPSS (SPSS Inc., USA) software programs. Combined mean daily consumption of fruit, nuts, fruit juice, vegetables and vegetable products (excluding potatoes and potato products) and wholegrain foods (i.e. those containing at least 51% wholegrain ingredients by weight) was calculated and separated into fifths for men and women separately, for each survey.

The dietary analysis compared the intake of consumers in the lowest, middle and highest fifths of FNVW. Nutrient intake data were expressed per unit of energy intake (in MJ) and then further analysed with age as a continuous covariate. Post-hoc (Bonferroni) tests examined pair-wise differences between the three groups of consumers. Mantel-Haenszel tests were performed to test for linear associations with FNVW consumption.

Smoking habit, occupational social class and nutrient intakes were compared between the three groups. Chi-squared tests were performed to identify differences between the discontinuous variables; smokers versus non-smokers and non-manual versus manual employment groups. For BMI, logistic regression was used with adjustment for age. Statistical significance was defined as $p<0.05$ throughout.

4. Results

4.1 *FNVW consumption*

The intake of FNVW in the population is generally low. However consumption in the highest quintile of the population was disproportionately greater than in the remainder of the group suggesting a different dietary pattern in this subgroup (Figure 2). It is interesting to note that despite differences in the age of the individuals studied and the 10y time difference between the two surveys, the mean intakes in each fifth are very similar, with the exception of substantially greater consumption of FNVW in the younger men in the highest quintile.

4.2 *Nutrient and food analysis*

4.2.1 Energy and macronutrients

Table 9 shows the energy intake and proportion of macronutrients in the diet for each group of subjects. Total energy intake was significantly greater among high FNVW consumers than those in the mid and lower quintile. However it should be noted that this is without adjustment for body size of physical activity.

In women, among high FNVW consumers there was a lower intake of fat and higher intake of carbohydrate compared to those in the middle and lower quintiles of FNVW intake. Protein intake was higher in the high FNVW consumers (except for men > 65y). In all groups the consumption of alcohol was greater in the high FNVW consumers.

4.2.2 Micronutrients and trace elements

Table 10 illustrates the intake of a variety of micronutrients and trace elements, adjusted for age and total energy intake. Overall there were significantly higher intakes of most micronutrients studied in the high FNVW consumers, indicating a higher nutrient density of the diet. Potassium intakes in all groups were higher than those likely to be associated with potassium deficiency. However the high FNVW consumers had the highest intake of potassium. The intake of sodium in all groups was high, and above recommended intakes for good health, but was not significantly related to FNVW consumption.

4.2.3 Cis n-3 polyunsaturated fatty acid

Reported intakes of cis n-3 PUFA were low in both surveys (1986-7 = 0.18 g/MJ/d, 1994-5 = 0.2 g/MJ/d). There was a slight trend towards higher intakes of cis n-3 PUFA in high FNVW consumers but this was only significant among men.

4.2.4 Consumption of oily fish and olive oil

Table 11 shows the percentage of each FNVW group who consumed oily fish and olive oil or olive oil based spreads within the survey period. Although the proportion of oily fish consumers increased significantly with increasing consumption of FNVW, only about half the population consumed oily fish even in the high FNVW group. Only a tiny proportion of the individuals consumed olive oil or olive oil based products. On average less than one gram of olive oil-based food was consumed per person per day.

4.3 *Socio-demographic and lifestyle factors*

The socio-demographic and lifestyle characteristics of each group of FNVW consumers are outlined in Table 12. The proportion of individuals with a BMI of over 30kg/m^2 (indicating obesity) is not significantly related to FNVW consumption.

There were significant differences in the pattern of FNVW consumption by region in the younger adults. When analysed in more detail, it is apparent that in Scotland there was a significantly higher proportion of low FNVW consumers than high FNVW consumers (P<0.01, M-H trend) and likewise in Welsh women (P=0.04, M-H trend). In the older population there were fewer regional differences, but a significantly higher proportion of low FNVW consumers in Wales compared to high FNVW consumers (P=0.04 M-H trend).

Smoking behaviour and manual employment were significantly inversely associated with FNVW consumption. This was observed for both sexes in both survey populations.

5. Discussion

The analysis shows clearly that individuals who consume a diet rich in FNVW have a different nutrient intake to low FNVW consumers and are closer to meeting dietary guidelines for good health. However this group also have other characteristics which have previously been shown to be linked to improved health outcomes. High FNVW consumers tend to have higher energy intakes but body weight was not significantly different suggesting that physical activity in this group was also higher than in other sectors of the population. This group are less likely to smoke and to have a manual occupation, suggesting a higher educational level in these individuals. In general there are fewer low FNVW consumers and more high FNVW consumers in England than in Scotland or Wales.

There have been no specific recommendations for overall protein, fat or carbohydrate intake for patients with arthritis. However the macronutrient composition of the diet among high FNVW consumers was closer to meeting government recommendations than for lower FNVW groups. But fat intake was still higher, and carbohydrate intake lower, than the recommended 35 and 50% energy respectively. Protein intake in all groups was satisfactory. Alcohol intake was higher than recommended in all groups except women >65y.

Increased intake of a variety of micronutrients have been linked to potential benefits to those suffering from arthritis (see section A). The intake of a range of micronutrients was higher in the high FNVW consumers, reflecting the high nutrient density of these foods. Although there is no specific evidence of a benefit to patients with arthritis, a high potassium intake is a key feature of the Arthritic Association diet programme. This analysis confirms that a diet rich in FNVW leads to a significantly higher intake of potassium. There was no difference in sodium intake, suggesting that sodium consumption is not related to consumption of FNVW. This is important because the average intake of sodium is approximately twice the recommended amount.

A review of the scientific literature pertaining to diet and arthritis suggests a specific beneficial role for oily fish, rich in cis n-3 PUFA and olive oil (see section A). This analysis has shown that intakes of these foods are low throughout the population and are not strongly linked to FNVW consumption.

This analysis confirms that patients following the Arthritic Association Home Treatment programme, rich in fruits, nuts, vegetables and wholegrains, would be expected to achieve a dietary pattern which is closer to the government recommended intakes than observed in the general population. Such a diet is associated with a reduced risk of heart disease and some types of cancer. In addition this dietary pattern is associated with a higher intake of a number of micronutrients which have been linked to a reduction in the severity of symptoms in patients with arthritis. Further benefits would be predicted from increases in oily fish well above current intakes and by a substitution of olive oil for saturated fats.

D. Conclusions

1. Overview of the principal findings

The Arthritic Association Home Treatment programme recommends a diet rich in fruits, vegetables, nuts and wholegrains. Although more tightly focussed this is consistent with current dietary guidelines for the whole population which advocate a reduction in fat intake, increase in carbohydrate, especially from wholegrain sources, at least 5 portions (about 400g) of fruit and vegetables per day and a reduction in salt. Using data from nationally representative dietary surveys this analysis has examined the impact of fruit, nut, vegetable and wholegrain consumption on overall nutrient intake and the extent to which high FNVW consumers are meeting the broader dietary guidelines for good health and the intake of nutrients reported in the scientific literature to be linked to arthritis. This analysis has shown that diets rich in FNVW are associated with lower intakes in fat and higher intakes of carbohydrate than the population average. This dietary programme is therefore consistent with current government recommendations for good health and the prevention of diseases such as coronary heart disease and cancer.

Individuals consuming diets rich in FNVW have higher intakes of a range of micronutrients including the antioxidant vitamins; vitamin C, vitamin, vitamin E, and carotenoids. Antioxidants are believed to offer important protection against oxidative tissue damage and this may contribute to reductions in the risk of chronic diseases. A review of the scientific literature has indicated that increased intakes of antioxidants can also alleviate some of the symptoms of arthritis through either antioxidant mechanisms or alternative pathways.

Individuals consuming higher intakes of FNVW have higher intakes of calcium and the trace elements zinc and magnesium reflecting other differences in dietary intake. Together this implies a higher nutrient density. Nutrient density becomes particularly important with age as energy intake tends to decline, yet there is need to maintain the intake of micronutrients. This analysis has considered only a limited range of nutrients which may be particularly pertinent to arthritis and where adequate dietary information is available. Diets rich in FNVW are also likely to provide greater than

average intakes of vitamin K. Vitamin K is an important factor in controlling calcification in soft tissue, including blood vessels.

A fundamental tenet of the Arthritic Association diet is to increase potassium intake and this is successfully achieved with a diet rich in FNVW. However there is no mechanistic basis to suggest a role for potassium in the management of arthritis, no specific observational studies of potassium intake in patients with arthritis and no intervention studies of potassium supplementation. A diet rich in potassium may be valuable in reducing the potassium to sodium ratio of the diet, which may be linked to other health benefits, especially with respect of blood pressure. However further improvements in this area could also be achieved by reductions in sodium intake.

The scientific review identified a number of studies which suggest improvements in the symptoms of arthritis in individuals consuming a diet with modified fat composition. Specifically, increases in cis n-3 PUFA and olive oil may be beneficial. The dietary survey data shows that intakes of cis n-3 PUFA and olive oil are low and there is good evidence of the benefits of increasing the proportion of mono- and polyunsaturated fats in the diet for the whole population, since they may reduce the risk of cardiovascular disease. The intake of olive oil could be increased, without increasing fat intake, by the substitution of olive oil or olive oil based spreads for other fats, especially saturated fat or n-6 PUFA. Long chain n-3 PUFA intake can be increased by the regular consumption of oily fish. Current government recommendations suggest at least one portion of fish (approximately 100 g) per week to reduce the risk of cardiovascular disease. However the literature reviewed suggests that substantially higher intakes (up to 700 g per week) may be necessary to alleviate the symptoms of arthritis. Moreover, given the high levels of n-3 required for benefit and the reduced n-3 content of most farmed fish in comparison with wild varieties, some people may wish to consider n-3 supplements. Some fat spreads, especially those based on soya or rapeseed oil, also provide n-3 PUFA in the form of alpha linolenic acid. However the efficiency of conversion of alpha-linolenic acid to the metabolically active long chain derivatives, EPA and DHA is unclear. Further research is necessary to identify the precise dose of olive oil or n-3 PUFA for optimal health benefit and to evaluate the relative roles of olive oil and n-3 PUFA, in combination with non-steroidal anti-inflammatory drugs to control the inflammatory process in patients with arthritis.

2. Potential areas for future Arthritic Association activities

2.1 *Research*

2.1.1 Case-control studies of nutrient intake

There is scope for studies to document the precise nutrient intake of patients with arthritis who follow the Arthritic Association recommended diet in comparison with other patients with arthritis (matched for age, gender and severity of symptoms) and healthy controls.

2.1.2 Randomised controlled clinical trials (RCTs)

Randomised controlled clinical trials to consider in detail the effects of components of the Arthritic Association recommended diet which have not yet been formally tested may be justified. The literature review has identified limited evidence of the efficacy of a number of micronutrients, but in most cases this needs to be confirmed in an RCT. There is currently no evidence of the efficacy of increases in potassium intake and this could also be tested in an RCT. In the first instance such trials may be simplified by using capsules to provide single nutrients in a double-blind manner. However even with this approach the complexity of a RCT such not be underestimated. It will require large numbers of subjects, good compliance to the supplementation programme, detailed and objective measurements of arthritic symptoms and an adequate duration of intervention, which is likely to be at least 3 months and ideally longer. The costs of such a trial will depend on the precise protocol but is likely to be several hundred thousand pounds.

2.1.3 Mechanistic research studies

There are still many unanswered questions in relation to the mechanistic basis for the action of various nutrients in the alleviation of the symptoms of arthritis and/or prevention of the disease. However real progress in this area is likely to require sustained and substantial investment in an ongoing programme of research from basic laboratory studies to clinical trials and stretching over a minimum of a 5y period.

2.2 *Educational initiatives*

2.2.1 To increase n-3 PUFA intake

Educational campaigns to inform patients with arthritis of the benefits of a diet containing oily fish and/or the use of long chain n-3 PUFA supplements may offer significant benefits. This strategy offers the opportunity to make an immediate contribution to improving the diet of patients with arthritis since intakes of n-3 PUFA in the population are low and there is good evidence of benefits of increased n-3 PUFA intake in alleviating symptoms of arthritis and reducing the risk of cardiovascular disease.

2.2.2 To highlight the benefits of a diet rich in FNVW

Current government dietary guidelines emphasise the need to increase the proportion of carbohydrate in diet, especially wholegrains and to increase fruit and vegetable intake to at least 5 portions a day. A diet rich in fruit, vegetables and wholegrains is associated with a decreased risk of heart disease and some cancers. Since patients with arthritis are also at increased risk of these diseases it would be valuable to highlight these additional benefits of the Arthritic Association Home Treatment dietary programme.

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APPENDIX 1

Table 1 Summary of the findings of published reviews on dietary fatty acids, especially n-3 and n-6 fatty acids

Paper title, reference and year of publication	Review objectives	Summary of review findings	Conclusions
Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. (Darlington and Stone 2001), 2001	To summarise some of the key findings in the area of antioxidant therapy and the evidence that dietary fatty acids can modify the generation of cytokines and eicosonoids in ways which can influence patient symptoms and the course of RA. [The evidence for antioxidant therapy is covered elsewhere]	The ratio of n-6:n-3 PUFAs in modern Western diets is approx. 25:1, whereas it was nearer to 2:1 in pre-industrialised societies. Oxygenases metabolise the n-3 and n-6-PUFA in competition, so that a high proportion of n-6 compounds leads to a relative deficiency of the products of n-3 metabolism. The clinical work with diets containing different proportions of PUFA has clearly demonstrated an anti-inflammatory effect, although the mechanism remains the subject of debate. At least two hypothese are prominent, i) relates to the effects of PUFA on cytokine levels, and ii) deals with the effects on oxidative stress. The evidence for both is reviewed using human and animal mechanistic studies and clinical studies using EPA, DHA and fish oil. Two studies were cited as showing a fall in plasma α-tocopherol levels, with an increase in lipid peroxides, following EPA and DHA supplementation. This resulted in the recommendation that encapsulated fish oil should include α-tocopherol/vitamin E (3mg/g fish oil), alone or with the additional antioxidants, to prevent oxidation in the capsules and *in vivo* after ingestion. Studies using marine oils (oily extracts of green-lipped mussel), vegetable oils (including olive oil and evening primrose oil (EPO)) alone or in combination with EPA were also reviewed. Most of these studies describe anti-inflammatory effects.	Fish oil supplements, rich in n-3 PUFA such as EPA have been claimed as beneficial in RA, possibly by suppression of the immune system and its cytokine repertoire. Some other oils of marine origin and a range of vegetable oils (e.g. olive oil and EPO) have indirect anti-inflammatory actions, probably mediated via PGE_1. The long term consequences of alterations in n-3:n-6 balance in favour of the n-3 PUFA is incompletely understood in humans but it could lead to detrimental immunological and haematological effects. If fish oil is to be taken or used in clinical trials, therefore, the lowest possible effective dose should be used i.e. equivalent to EPA 500-750 mg/d. More studies are clearly required to investigate the safety of long-term supplementation with fish oil in man. Overall, the relationship between PUFA, eicosonoids and cytokines is emerging as an area of potential clinical relevance. The results obtained to date are often inconsistent and vary with the nature of the experimental preparation or model, the concentrations of fatty acids used, and whether they are tested acutely or chronically. However, dietary manipulation of fatty acid levels do produce changes in the generation of eicosonoid hormones and cytokines, and can modify their cellular actions. Dietary control of fatty acid intake would be expected to modify the disease process and provide a useful adjunctive strategy in the treatment of these disorders.

Polyunsaturated fatty acids and rheumatoid arthritis (Calder and Zurier 2001) 2001	To discuss the effects of γ-linolenic acid (GLA; 18:3n-6) and its derivative DGLA, and of the long chain n-3 PUFA EPA and its derivative DHA (22:6n-3) on eicosonoid and cytokine production and lymphocyte reactivity, and the applications of these effects to the treatment of RA. Recent in-vitro and animal feeding studies of relevance are highlighted and the results of a recent clinical trial of n-3 PUFA in RA are described.	The production of arachidonic acid (AA)-derived pro-inflammatory eicosanoids such as PGE_2, LTB_4 and 5-HETE is decreased by GLA or by the long-chain n-3 PUFAs found in fish oil. In addition, these fatty acids decrease the production of pro-inflammatory cytokines and reactive oxygen species, and decrease lymphocyte reactivity. Trials of GLA and fish oil in RA have shown significant improvements in a variety of clinical outcomes. There have been no detailed studies of the dose response relationship between GLA or EPA plus DHA and clinical improvement in RA. The potential role of α-linolenic acid (ALA), the precursor of EPA, in RA has not been investigated. For ALA to be effective, it would probably need to be converted to EPA. In humans this conversion is inefficient, meaning that large doses of ALA would be required to effect immunological responses.	The effects described in the findings, suggest that GLA and n-3 PUFAs may be useful for chronic inflammatory disorders such as RA. These fatty acids should be included as part of a normal therapeutic approach to RA. The effectiveness of these fatty acids might have been underestimated, because in most studies patients have continued with existing drug therapies, and with one exception, because the intake of n-6 fatty acids in the diet has not been modified. It is possible that DGLA and n-3 fatty acids might be more effectively incorporated into immune cells if n-6 fatty acid intake is lowered. The minimum doses required to bring about clinical improvement are not known. It is unclear whether there would be added benefit in RA by using a combination of GLA and fish oil.
n-3 fatty acid supplements in rheumatoid arthritis (Kremer 2000) 2000	Review of animal and human studies.	Not all animal studies of dietary supplementation with n-3 fatty acids have resulted in beneficial effects: type II collagen-induced arthritis worsened in animals fed fish oil compared with those fed beef tallow. Recent studies of purified EPA and DHA indicate that the mixture of these 2 major n-3 fatty acid constituents of fish oil may be more effective than either fatty acid by itself. The benefit most often observed with fish-oil supplementation in human studies is an improvement in the number of tender joints on physical examination, although some authors reported improvement in the Ritchie Articular Index and in morning stiffness. The overall clinical response to fish-oil supplements in these investigations is modest. Improvements from	On the basis of the totality of the data, it is recommended that patients consume dietary supplements containing 3-6 g n-3 fatty acids daily for ≥12 weeks. The dietary supplement should not replace the standard therapeutic medical regimen, but be added to it. However, there are many forms of arthritis and clinical studies demonstrating efficacy have been performed only in patients with RA. After taking n-3 fatty acid dietary supplements for 3-4 months, patients may try reducing their NSAID dose under the supervision of a physician.

		baseline in RA patients consuming fish oil are often not significantly different from improvements in patients receiving other dietary fatty acids interventions. The issue of an ideal control fatty acid (placebo) to compare with fish oil in the study of autoimmune inflammatory disease has not yet been settled. Investigations into whether dietary fish-oil supplements can affect NSAID use indicate that selected individuals with RA can discontinue or reduce NSAID therapy while consuming n-3 fatty acid supplements.	
Dietary polyunsaturated fatty acids and inflammatory mediator production (James, Gibson et al. 2000) 2000	A review of the evidence that n-3 and n-9 fatty acids inhibit the production of inflammatory mediators, e.g. n-6 eicosanoids, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), cytokines, IL-1β and TNF-α.	The proinflammatory eicosanoids PGE_2 and LTB_4 are derived from the n-6 fatty acid AA, which is maintained at high cellular concentrations by the high n-6 and low n-3 fatty acid content of the modern Western diet. Flaxseed oil contains the 18-C n-3 ALA, which can be converted after ingestion to the 20-C n-3 EPA. Fish oil contains both EPA and DHA. EPA can act as a competitive inhibitor of AA conversion to PGE_2 and LTB_4, and decreased synthesis of one or both of these eicosanoids has been observed after inclusion of flaxseed oil or fish oil in the diet. In addition inclusion of 20-C n-9 fatty acid eicosatrienoic acid (ETA) in the diet also results in decreased synthesis of LTB_4. Studies of healthy volunteers and RA patients have shown ≤90% inhibition of pro-inflammatory cytokine production after dietary supplementation with fish oil. Use of flaxseed oil in domestic food preparation also reduced production of pro-inflammatory cytokines.	A beneficial clinical effect of dietary supplementation with fish oil on RA was observed in at least 11 double blind, placebo controlled studies. In the studies in which drug use was examined, there was partial sparing of NSAIDs use. Common features of the clinical studies that may have moderated the size of the effects observed were: 1) all studies were conducted against unmodified Western diets, i.e. diets high in linoleic acid (LA), and 2) the usual anti-inflammatory and anti-rheumatic medications were used in addition to fish oil in amounts likely to confer maximum suppression of their common molecular and cellular targets. The first issue could be addressed by substituting vegetable oils rich in LA and, preferably, by also using oils with substantial ALA content. The second issue could be addressed by optimizing the possible additive effects of drug-diet combinations: (i)Anti-inflammatory drug use could be decreased in some patients with RA in concert with increased fish oil concentration if both the drug and fish oil are exerting their therapeutic effects through the same molecular actions, e.g. inhibition of PGE_2 and TXA_2 production. (ii) treatments that aim to suppress cytokine concentrations, i.e. there may be an opportunity for beneficial additive effects with fish-oil supplementation or any other dietary approach to

			increasing intake of n-3 fats. Investigation of potentially beneficial interactions will require greater knowledge of the dose-response effects for both the drug and the dietary intake of n-3 PUFAs.
Conjugated linoleic acid and bone biology (Watkins and Seifert 2000) 2000	Tutorial review summarising findings of conjugated linoleic acid (CLA) on bone modelling in rats and effects on cellular functions of osteoblasts and chondrocytes.	CLAs occur naturally in ruminant food products (beef, lamb and dairy). Recent investigations with growing rats given butter fat and supplements of CLA demonstrated an increased rate of bone formation and reduced *ex vivo* bone PGE_2 production, respectively. The supplements of CLA isomers resulted in their enrichment in lipids of various bone compartments of animals. The effects of CLA on bone biology in rats (IGF action and cytokines) appear to be dependent on the level of n-6 and n-3 fatty acids in the diet; however, these studies generally showed that CLA decreased *ex vivo* PGE_2 production and in osteoblast-like cultures.	Excess production of PGE_2 is linked to arthritis and is associated with bone and proteoglycan loss. Anti-inflammatory diets, including nutraceutical applications of CLA, may be beneficial in moderating cyclooxygenase 2 (COX-2) activity or expression (influencing PGE_2 biosynthesis) and might help to reduce RA secondary osteoporosis. The rat experiments indicate that CLA isomers possess anti-inflammatory activity in bone by moderating prostanoid formation.
Health benefits of docosahexaenoic acid (DHA) (Horrocks and Yeo 1999) 1999	A review of the role of DHA on diseases such as hypertension, arthritis, atherosclerosis, depression, adult-onset diabetes mellitus, myocardial infarction, thrombosis, and some cancers.	Much evidence supports the conclusion that an increased intake of linoleic acid and an elevated ratio of n-6 to n-3 fatty acids is a major risk factor for diseases for which anti-inflammatory drugs are effective. The effects of dietary fatty acids on inflammation were studied in rats. Inflammatory swellings were reduced when diets rich in EPA and DHA were given compared to control groups. Swelling was correlated with the proportion of AA in the PUFAs.	A high ratio of linoleic acid to linolenic acid causes a depletion of the longer chain n-3 fatty acids, including DHA, by competing for enzymes necessary for desaturation and elongation. The WHO and others are now recommending a ratio of between 3:1 and 4:1 for n-6 to n-3 fatty acids. DHA and EPA possess anti-inflammatory properties and may alter lymphocyte, monocyte, and macrophage functions. Increased levels of DHA and EPA in the diet of persons with RA should alleviate the pain and inflammation in their joints.
Essential fatty acids in health and chronic disease. (Simopoulos 1999) 1999	A review of the evolutionary aspects of the diet, the biological effects of n-6 and n-3 fatty acids, and the effects of dietary α-linolenic acid (ALA) compared with long chain n-3 derivatives on coronary heart disease and	Human beings evolved consuming a diet that contained about equal amounts of n-3 and n-6 essential fatty acids. Today, in Western diets, the ratio of n-6 to n-3 fatty acids ranges from approx. 20-30:1 instead of the traditional range of 1-2:1. EPA and DHA lead to 1) decreased production of PGE_2 metabolites; 2) decreased formation of LTB_4, an inducer of inflammation; 3) increased	Over the past 100-150 y there has been an enormous increase in the consumption of n-6 fatty acids compared to n-3 fatty acids due to the increased intake of vegetable oils. n-3 fatty acids have anti-inflammatory properties. These beneficial effects of n-3 fatty acids have been shown in the secondary prevention in some patients

	diabetes. (Reference also made to RA).	concentrations of LTB_5, a weak inducer of inflammation and chemotactic agent. Most of the studies on n-3 fatty acids were carried out with fish oils (EPA and DHA), however, ALA, found in green leafy vegetables, flaxseed, rapeseed, and walnuts, desaturates and elongates in the human body to EPA and DHA and by itself may have benefical effects in health and in the control of chronic diseases.	with RA and other inflammatory diseases.
Modulation of pro-inflammaotry cytokine biology by unsaturated fatty acids (Grimble and Tappia 1998) 1998	To consider: - The influence of n-6 PUFAs and total unsaturated fatty acid intake on cytokine production and actions; - Mechanisms whereby fats may modulate production and actions of cytokines	A number of trials have demonstrated the anti-inflammatory effects of fish oils, which are rich in n-3 PUFAs, in RA. Animal studies indicate that a range of fats can modulate pro-inflammatory cytokine production and actions. Fats rich in n-6 PUFAs enhance IL1 production and tissue responsiveness to cytokines, fats rich in n-3 PUFAs have the opposite effect, MUFAs decrease tissue responsiveness to cytokines and IL6 production is enhanced by total unsaturated fatty acid intake.	Cytokine biology can be modulated by anti-inflammatory drugs, recombinant cytokine receptor antagonists and nutrients. Among the nutrients, fats have a large potential for modulating cytokine biology. TNF induced IL1 and IL6 production relate in a positive curvilinear fashion to linoleic acid (LA) intake; LTB_4 production relates positively with dietary LA intake over a range of moderate intakes and is suppressed at high intakes, while PGE_2 production is enhanced. None of these mechanisms, acting alone, can explain the positive effect of dietary LA intake on pro-inflammatory cytokine production, however, each may be involved in the modulatory effects observed.
n-6 and n-3 essential fatty acids in rheumatoid arthritis and other rheumatic conditions. (Belch and Muir 1998) 1998	A review of the literature reporting studies evaluating essential fatty acid (EFA) treatment in RA and conditions associated with RA.	In one of the first studies, EPO was evaluated, the dose selected was 700 mg LA acid and 70 mg GLA/kg daily. It is now known that this dose is unlikely to produce benefit, and this study was negative. Later studies have used greater doses (540 mg GLA/d or 450 mg GLA + 240 mg EPA/d) and showed a decreased requirement of NSAIDs. A recent study used 2.8 g GLA as the free fatty acid/d. This treatment resulted in a statistically significant reduction in the signs and symptoms of RA disease activity. In at least two studies the time period evaluated was only 3 months, and it is likely that a period of 4-6	The literature is difficult to review because of the tendency for inappropriate study design. This is not necessarily the fault of the investigator(s) concerned, but merely reflects the state of the art as it was when these studies were carried out. Dietary manipulation of EFA or supplementation with therapeutic doses may be effective as a treatment for rheumatological diseases. The assessment of their effects is, however, poorly studied to date, with inconclusive results, particularly in the field of Raynaud's phenomenon and Sjögren's syndrome. More convincing evidence exists in support of EFA

		months is required for therapeutic benefit to become apparent. A further problem is the selection of an active placebo e.g. olive oil. Early studies of EPA treatment in RA patients show clinical improvement in the EPA-treated groups. Doses used varied but 2.6 g n-3 EFA produced a significant clinical benefit. A mixture of EPO and EPA was also effective in decreasing NSAID usage over 12-month study period.	usage in RA. A recently published study where higher doses of GLA was evaluated is particularly interesting.
Omega-3 fatty acids in rheumatoid arthritis: An overview. (Ariza-Ariza, Mestanza-Peralta et al. 1998) 1998	To review background, pharmacological properties, mechanisms of action, and published clinical experience using n-3 fatty acids in RA.	n-3 fatty acids are superior with respect to placebo in improving some outcome measures, and decrease the long-term requirements for NSAIDs. Some of these effects are statistically significant, but their clinical significance remain to be established.	Treatment with n-3 fatty acids has been associated with improvement in some outcome measures in RA. Studies are needed to determine if they might represent an alternative to NSAIDs in certain circumstances.
n-3 polyunsaturated fatty acids and cytokine production in health and disease (Calder 1997). 1997	A review of the production of cytokines in health and disease	AA-derived eicosanoids modulate the production of pro-inflammatory and immunoregulatory cytokines. Overproduction of these cytokines is associated with both septic shock and chronic inflammatory diseases. The n-3 PUFAs, EPA and DHA suppress the production of AA-derived eicosanoids and EPA is a substrate for the synthesis of an alternative family of eicosanoids. Animal feeding studies have provided a great deal of evidence that feeding plant or fish oils rich in n-3 PUFAs does alter the ex vivo production of TNF, IL-1, IL-6 and IL-2, but many contradictory observations have been made. Human studies provide more consistent data: several studies in both healthy adults and RA patients that supplementation of the diet with EPA and/or DHA reduces *ex vivo* production of IL-1, IL-6 and IL-2 by peripheral blood mononuclear cells. Animal studies also indicate that dietary fish oil reduces the response to endotoxin and to pro-inflammatory cytokines.	The dietary fats which are rich in n-3 PUFAs have the potential to alter cytokine production. The beneficial effects of dietary n-3 PUFAs may be of use as a therapy for acute and chronic inflammation and for disorders which involve an inappropriately activated immune response.
Dietary n-3 fatty acids and therapy for rheumatoid arthritis	To examine the potential for dietary n-3 fats to be	There is consistent evidence from double-blind, placebo controlled, clinical trials that dietary n-3	There are many overlapping biochemical effects of n-3 fatty acids and anti-inflammatory drugs that could

(James and Cleland 1997). 1997	components of therapy for RA. The potential for use of n-3 fats was evaluated within a dietary framework rather than a quasi-pharmaceutical framework.	fats, supplied as fish oil, can have beneficial effects in RA. The beneficial effects appear modest, but their size and extent may have been moderated by common trial design factors such as high n-6 PUFA diets and concurrent anti-inflammatory drug use. Mechanisms for the clinical effects of n-3 fats in RA may involve their ability to suppress production of inflammatory mediators, including n-6 eicosanoids and proinflammatory cytokines. Suppression of n-6 eicosanoid and cytokine production will be possible using foodstuffs that are rich in n-3 fats and poor in n-6 fats.	explain the clinical actions of n-3 fats in RA. They suggest that there is the potential for complementation between drug therapy and food choices that increase intake of n-3 fats and decrease intake of n-6 fats. There is potential for drug sparing effects. Future studies with n-3 fats in RA need to address the fat composition of the background diet and the issue of concurrent drug use.
Rheumatoid arthritis and the balance of dietary n-6 and n-3 essential fatty acids. Editorial (Cleland and James 1997). 1997	Editorial on the evidence to form health messages based on the use of n-3 fatty acids in the relief of RA symptoms.	Briefly summarised the evidence for a change in the balance of dietary fatty acids to alleviate the symptoms of RA.	Sufficient evidence exists to form the basis for positive health messages that can potentially reduce unwanted inflammation. The nub of this advice is to choose foods that provide substantial amounts of n-3 fatty acids (fish, products based on n-3 rich seeds and vegetables) and to avoid foods that are very rich in n-6 fatty acids (products based on staple PUFA oils, certain nuts). Variety in n-3 rich and/or n-6 poor foodstuffs and ingredients has increased in recent years to make these dietary changes simple and practical. Fish oil supplements can be added for extra effect.
Marine and botanical lipids as immunomodulatory and therapeutic agents in the treatment of rheumatoid arthritis (DeLuca, Rothman et al. 1995). 1995	A review of the evidence that supports the use of marine and botanical lipids as immunomodulatory and anti-inflammatory agents in the treatment of RA	Studies in animals and humans indicate that changes in dietary intake of essential fatty acids alter cell membrane fatty acids, the eicosanoid profile of stimulated cells, and inflammatory responses. Clinical and experimental evidence is accumulating to support the use of marine and botanical lipids as anti-inflammatory and immunomodulating agents. Effects of these lipids on multiple events in cell activation have shifted attention to very early events that generate and propogate the inflammatory response – at the plasma membrane.	The clinical efficacy of marine and botanical lipids (possibly comparable to anti-inflammaotry doses of NSAIDs) and their relative freedom from serious adverse effects may make them useful adjunctive agents in the treatment of RA.

		Some of these effects are unique to GLA and DGLA.	
Eicosanoids in rheumatoid arthritis (Sperling 1995). 1995	A review of the literature on eicosanoid production and its modification in RA patients.	Eicosanoids are potent mediators in the cellular environment. They have different effects depending on tissue or organ, the PUFA content of the diet of the individual, and the net effect of local microenvironmental factors – as eicosanoids, cytokines and hormones modulate each others' effects through a complex, network of interactions. In general, eicosanoids have significant net proinflammatory effects.	Changes in the dietary PUFA composition to increased intake of marine n-3 fatty acids and/or DGLA may favourably modulate eicosanoid synthesis towards less inflammatory or anti-inflammatory eicosanoids and may ameliorate disease activity in RA.
Dietary omega-3 fatty acids: Effects on lipid mediators of inflammation and rheumatoid arthritis (Sperling 1991). 1992	A review of the science behind the effects of n-3 fatty acids on lipid mediators of inflammation and RA.	Similar findings to the review above.	The data now available indicate that dietary supplementation with n-3 PUFAs may inhibit (at least partially) three pathways of the synthesis of lipid mediators of inflammation: the platelet-activating factor (PAF) synthesis pathway, the cyclooxygenase pathway, and the 5-lipoxygenase pathway. In addition selected cellular functions of PMN may be modulated by dietary fish oil. It is quite probable that effects of dietary supplementation with n-3 fatty acids will vary with the duration, dose, and composition of n-3 fatty acids preparation, with background medical therapy, and or the presence of and degree of activity of the underlying inflammatory disease state.
Clinical studies of Omega-3 fatty acid supplementation in patients who have rheumatoid arthritis (Kremer 1991) 1991	This paper addresses mainly the results of clinical studies with coincidental laboratory, inflammatory, and immune parameters described in association with these investigations.	A review of 6 clinical studies all using fish oil supplements produced these results: – three claimed significant improvement in tender joints accompanied by improvement in either morning stiffness, interval to fatigue onset or grip strength; one claimed significant improvement in joint pain index, one in improvements in swollen joints and morning stiffness and the largest study claimed multiple significant clinical outcomes. The observed improvements in clinical disease parameters usually do not occur until after at least	Six controlled, blinded studies have established that dietary supplementation with n-3 (fish oil) fatty acids is associated with reproducible clinical benefits in patients who have rheumatoid arthritis. Improvements usually are not observed until after at least 12 weeks of continuous use and appear to increase with extended treatment intervals of 18 to 24 weeks. Because of the observed prolonged suppression of IL1 after ingestion of fish oil is stopped in normal volunteers and because of the sustained improvements seen in a crossover investigation after

		12 weeks of dietary supplementation with fish oil despite the fact that significant alterations of luekotriene metabolism occur as early as 6 weeks in neutrophils and monocytes in RA patients who consume fish oil.	discontinuing these supplements, the crossover format is not appropriate to study the clinical effects of fish oil. Future investigations will need to establish whether it is practical to use fish oil as part of the standard therapeutic approach for RA and in what dosage(s), the formulation of n-3 fatty acids and duration.
Botanical lipids: Potential role in modulation of immunologic responses and inflammatory reactions. (Callegari and Zurier 1991) 1991	To consider a novel, safe approach to treatment of RA.	-Evidence from experiments *in vitro* and *in vivo* in small animals and in humans suggests that other (than in fish oils) novel fatty acids may be safe and effective anti-inflammatory and immunomodulatory agents, e.g. certain plant seed oils, notably those extracted from seeds of evening primrose and borage plants. These contain relatively large amounts of GLA which can be converted to DGLA – the fatty acid precursor of $PGE_{1.}$ DGLA competes with AA for oxidative enzymes, reducing production of cyclo-oxygenase products derived from AA. In addition DGLA cannot be converted to inflammatory leukotrienes by 5-lipoxygenase. -GLA enrichment of diet markedly suppresses acute and chronic inflammation as well as joint tissue injury in several animal experiments. -Enrichment of cells in culture with DGLA leads to increased production of PGE_1 during subsequent stimulation of cells. -GLA in human studies has been shown to reduce pain and the need for NSAIDs in RA patients	The potential capacity of particular fatty acids to regulate cell activation and immune responses is exciting to consider at the clinical, cellular, and molecular levels. A better understanding of how fatty acids modulate function of cells involved in host defence might lead to development of novel, benign therapy for diseases such as RA. Therapeutic strategies may be achieved more readily as new sources of novel fatty acids become available.

Table 2 Experimental studies of rheumatoid arthritis and dietary fatty acids, n-3, n-6, and n-9 polyunsaturated fatty acids, fish oil and plant oils

Study type, Country Paper reference	Study objective	Study population	Study methods	Outcomes	Conclusion or authors comments
Meta-analysis or collective reviews					
Meta-analysis. (Fortin, Lew et al. 1995)	To validate the results of a meta-analysis showing the efficacy of fish oil in RA with the results of a re-analysis of the complete primary data set.	395 individual patients from 10 published trials	Medline search for studies which met the criteria plus contacting authorities in the field. Inclusion criteria included (1) double-blind, placebo-controlled study, (2) use of at least one of seven predetermined outcome measures, (3) results reported for both placebo and treatment groups at baseline and follow-up, (4) randomisation, and (5) parallel or cross-over design. Papers were scored for quality. For the re-analysis of the primary data, the same variables were abstracted for the 395 individual patients randomised.	The meta-analysis demonstrated that dietary fish oil supplementation for 3 months significantly reduced tender joint count (rate difference [RD] [95% CI] = -2.9 [-3.8 to –2.1] [p= 0.001]) and morning stiffness (RD [95%CI] = -25.9 [-44.3 to –7.5] [p<0.01] as compared with heterogeneous dietary control oils. The re-analysis of the primary data confirmed a significant reduction in tender joint count (p=0.001) and in morning stiffness (p<0.02) in the parallel analysis that ignored interaction terms. The analyses that included an interaction term between site and treatment again confirmed a significant reduction in tender joint count. The results for morning stiffness were similar to the meta-analysis, but did not quite reach statistical significance.	Use of fish oil improved the number of tender joints and duration of morning stiffness at 3 months as analyzed by both meta- and mega-analysis. The fuller mega-analysis confirmed the results of the meta-analysis. The advantages of mega-analysis were the ability to: (1) analyze the homogeneity of the patient populations, (2) make clinically sensible adjustments in the form of the comparison, and (3) examine subsets of the data.
Review of clinical studies available in 1990. (Kremer 1991)	This paper addresses mainly results of clinical studies with coincidental laboratory inflammatory, and immune parameters described in association with these investigations	Information from 6 studies carried out by the author's group and others between 1985 and 1990.	In the 6 studies: The patient numbers ranged from 12 to 49. All were prospective, 5 were double-blinded and 1 was blinded; 3 were placebo controlled and 2 were cross-over controlled. Duration ranged from 6 to 24 weeks. Placebos used: 1 = paraffin wax, 3	Results: 3 claimed significant improvement in tender joints accompanied by improvement in either morning stiffness, interval to fatigue onset or grip strength; 1 claimed significant improvement in joint pain index, 1 improvements in swollen joints and morning stiffness and the largest study claimed multiple significant outcomes.	6 controlled, blinded studies have established that dietary supplementation with n-3 (fish oil) fatty acids is associated with reproducible clinical benefits in RA patients. Improvements usually are not observed until after at least 12 weeks of continuous

			= olive oil, 1= coconut oil.		use and appear to increase with extended treatment intervals. Further work is required on the formulation and dosage of fish oils.
Animal studies					
Animal study UK (Yaqoob and Calder 1995)	To investigate the effects of feeding mice lipids with different fatty acid composition upon the ability of stimulated macrophages to produce inflammatory mediators	Weanling mice	5 groups of mice were fed for 8 weeks on one each of a low fat diet (LF; 2.5% by wt) or on diets containing 20% by weight of hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SFO), or fish oil. Peritoneal macrophages were isolated. Production of superoxide, hydrogen peroxide, nitric oxide (NO), cytokine and eicosanoids were measured in stimulated macrophages.	Macrophages from fish oil fed mice produced less PGE_2, 6-keto-$PGF_{1\alpha}$, TXB_2, and IL-6 in response to lipopolysaccharide (LPS) stimulation than those fed each of the other diets. Macrophages from mice fed the OO, SFO or fish oil diets produced less TNFα in response to LPS stimulation than those from mice fed the LF or HCO diets. There was no effect of dietary lipid manipulation on the production of IL-1 by LPS-stimulated macrophages. Macrophages fed the fish oil diet produced more superoxide and hydrogen peroxide in response to phorbol ester stimulation than those from mice fed the other diets. LPS-stimulated NO production was greater from macrophages from OO-, SFO-, and fish oil-fed mice than from those fed LF- and HCO- diets.	The nature of the lipid consumed in the diet has significant effects upon the production of a variety of inflammatory mediators by macrophages. The most potent effect is caused by fish oil consumption. Possible mechanisms by which dietary fatty acids, particularly n-3 fatty acids, could affect mediator production by macrophages are discussed in the paper.
Animal study UK (Hinds and Sanders 1993)	To study the effect of increasing levels of dietary fish oil rich in EPA and DHA on lymphocyte phospholipid fatty acid composition and cell-mediated immunity in the mouse	Mice	Animals were fed 25, 50, 100 and 160 g fish oil/kg diet and compared with a control of no added fish oil. A popliteal lymph node assay technique was used to study cell mediated immunity. The proportion of 20:5n-3 and 22:6n-3 were measured in spleen leucocyte phospholipid.	The proportion of 20:5n-3 in spleen leucocyte phospholipid increased from 0.14 in the controls to 3.8, 7.2, 8.5 and 9.4% with the increasing doses. The proportion of 22:6n-3 increased from 5.1 in the controls to 12.1, 12.2, 12.8 and 12.9% respectively.	The immune response was suppressed by 160 g fish oil/kg diet, but not by lower doses. The authors concluded that moderate intakes of fish oil are not immunosuppressive.
Animal study	To study the effect of two diets with	Weanling female (SPF)	4 semi-purified 20% fat diets, based on either BT or SFO or DHA and	Footpad inflammation, reported as percentage change (adjusted for	The course of SCW-induced arthritis can be altered by

Australia (Volker, Fitzgerald et al. 2000a).	different ratios of EPA and DHA and to compare and contrast them with beef tallow (BT) and safflower oil (SFO)-based diets.	Lew/SSN rats aged 3 wk, weighing between 30 to 50 g. n= 5-7 for each experimental diet	EPA were provided. The DHA and EPA ratios of the n-3 fatty acid-based diets were 1.1 and 3.4 respectively. BT and SFO were used as control diets. The effect of pre-feeding diets differing in EPA and DHA ratios prior to the induction of arthritis in rats was examined. Rats were fed diets for 5 wk before arthritis induction and 5 wk post-arthritis induction. Footpad thickness, hock circumference, plasma and macrophage fatty acids and histological assessment were compared.	growth) was greatest for rats fed the BT-based diet, intermediate in those fed the SFO-based diet and least for the rats fed the DHA- and EPA-based diets ($p<0.05$). Macrophage phospholipids revealed cellular incorporation of EPA and DHA from the fish-oil based diets which modified lipid and peptide mediators of inflammation. Histological sections of rat hocks ranked by severity of arthritis-related changes suggested that the SFO- and EPA-based diets were more successful in ameliorating the destructive arthritic phase in the hock joints than the BT- and DHA-based diets ($p=0.09$) in this model of arthritis.	diet-induced changes in macrophage fatty acid composition. The EPA-based diet is more effective in suppression of inflammation than the DHA-based diet. However, the total PUFA content of the diet and the balance of n-6 to n-3 fatty acids are important in determining the overall effect. The ratio of EPA/DHA can be fine tuned to optimize the immunoregulatory effect.
Animal study using a lipid fraction from green-lipped mussel Australia (Whitehouse, Macrides et al. 1997)	Evaluation of Lyprinol, a lipid-rich extract prepared from mussel powder as an anti-inflammatory agent	Wistar and Dark Agouti rats	Lyprinol was given therapeutically and prophylactically to rats developing either adjuvant-induced polyarthritis or collagen(II) – induced autoallergic arthritis.	Lyprinol showed little or no activity in acute irritation assays indicating that it is not mimicking rapid-acting NSAIDs. Incorporating Lyprinol into arthritigenic adjuvants effectively prevented arthritis development as a dose of 5 mg/rat. Lyprinol subfractions inhibited LTB4 biosynthesis by stimulated human polymorphonuclear leukocytes in vitro, and PGE2 production by activated human macrophages in vitro. Much of this anti-inflammatory activity was associated with PUFA and natural antioxidants.	These data show Lyprinol to be a reproducible, relatively stable, source of bioactive lipids with much greater potency than plant/marine oils currently used as nutritional supplements to ameliorate signs of inflammation
Molecular studies					
Human study UK (Sanders and Hinds	To study the influence of a fish oil high in DHA on plasma lipoprotein and vitamin E	9 healthy male volunteers	Subjects consumed a daily fish oil supplement providing 2.1 g DHA and 0.8 g EPA for 6 weeks. The proportion of EPA and DHA in plasma, erythrocytes, leucocytes	The proportion of EPA and DHA in plasma, erythrocytes, leucocytes and platelet phospholipids was increased by the supplement. Platelet aggregation and thromboxane B2	Further studies are needed to investigate the extent to which fish oil increases the requirement for antioxidant nutrients.

1992)	concentrations and haemostatic function in healthy adults		and platelet phospholipids was measured as were the lipoprotein fractions, platelet aggregation, and thromboxane B2 production.	production induced by collagen were partially inhibited. Statistically significant increases in leucocyte and monocyte counts occurred with the supplement. Plasma α-tocopherol concentrations fell below the normal range.	
Molecular study Using human peripheral lymphocytes Germany (Karsten, Schafer et al. 1994)	To investigate the effects of palmitic, stearic, oleic, and linoleic acid on mitogen-induced DNA synthesis, on production of IL-1β, IL-2, IFN-gamma, TNF-α, and expression of the IL-2 receptor in human peripheral lymphocytes	Lymphocytes from healthy human blood donors	Human peripheral lymphocytes from venous blood from healthy blood donors were used. Palmitic (C_{16}), stearic (C_{18}), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) FFA were administered either singly or in combination over a wide range of concentrations and cytokine production was measured.	Cytokine production became affected by all FFA tested. Palmitic acid enhanced the release of IFN-γ at concentrations that diminished TNF-α production. SFAs were significantly more potent than unsaturated Fas in affecting cytokine production. IFN-γ secretion was significantly more stimulated or inhibited by the various FFA compared with the other cytokines. IL-2R expression correlated with the production of IL-2. When tested in combination, stimulatory as well as inhibitory effects of the individual FFA became attenuated.	The authors suggest that palmitic, stearic, oleic, linoleic acid are physiological regulators of cytokine release in human peripheral lymphocytes. Modulation of FFA ratios may be an effective means for the fine tuning of the immune system. As secretory mechanisms of cytokines appear to exhibit substrate specificity for FFA, the release of individual cytokines may be selectively influenced by FFA.
Molecular study (chondrocytes *in vitro)* UK (Curtis, Hughes et al. 2000)	To determine a molecular basis for potential therapeutic properties associated with dietary intake of fish oils. Investigation of the effects of different classes of fatty acids on the expression and activity of cartilage aggrecanases, cytokines (IL-1α and TNF-α) and cyclo-oxygenases, COX-1 and COX-2.	Bovine chondrocytes	Articular cartilage chondrocytes were exposed in culture to fatty acids at concentrations which cover the typical range (50-70 μg/ml) for free fatty acid levels in human plasma. Cultures were maintained for 8 h in the absence or presence of n-3 PUFAs (linolenic, EPA or DHA), 1 n-6 PUFA (18:2 LA), 1 SFA (16:0 palmitic), or 1 MUFA (18:1 oleic). The chondrocyte membranes were analysed for fatty acids and chondrocyte metabolism and phenotype were analysed.	The results show that incorporation of n-3 fatty acids (but not other PUFAs or SFAs) into articular cartilage chondrocyte membranes results in a dose-dependent reduction in: (i) the expression and activity of proteoglycan degrading enzymes (aggrecanases) and (ii) the expression of inflammation-inducible cytokines (IL-1α) and TNF-α) and COX-2, but not the constitutively expressed cyclo-oxygenase COX-1.	The findings in these *in vitro* studies provide evidence that n-3 fatty acid supplementation can specifically affect regulatory mechanisms involved in chondrocyte gene transcription and thus further advocate a beneficial role for dietary fish oil supplementation in alleviation of several of the physiological parameters that cause and propogate arthritic disease.
Molecular study	To investigate the	Monocytes from	Using an *in vitro* system,	No significant reductions in the	The results of these studies

(human monocytes *in vitro)* UK (Hughes and Pinder 2000)	combined effect of EPA and DHA, when provided in the same ratio commonly found in fish-oil supplements (3:2), on the expression of functionally associated surface molecules on human monocytes. In addition to investigate whether changes in the expression of surface molecules were associated with an alteration in antigen-presenting function.	healthy, non-smoking, adult volunteers. No subjects had been consuming fish oil supplements or were regular consumers of oily fish.	monoclonal antibodies were used to investigate the modulatory effects of the fatty acids on cell surface antigen expression: anti-HLA-DR, anti-HLA-DP, and anti-HLA-DQ; and anti-CD54 (ICAM-1), anti-CD11a (LFA-1), and anti-CD58 (LFA-3). Monocytes were cultured in the presence or absence of a combination of EPA and DHA (3:2) for 48h. The percentages of monocytes expressing the various surface molecules after incubation were measured as was lymphocyte proliferation.	percentages of monocytes expressing the various surface molecules after incubation with EPA and DHA were seen in the MHC class II molecules (HLA-DR, HLA-DP, HLA-DQ), but there were significant decreases in the percentages of ICAM-1-positive cells ($p<0.05$) and LFA-3-positive cells ($p<0.05$). There were significant decreases in the percentage of monocytes expressing the MHC class II molecules HLA-DR ($p<0.05$) and HLA-DP ($p<0.01$) and the adhesion molecules ICAM-1 and LFA-3 after incubation in the presence of the n-3 PUFAs and IFN-γ (i.e. stimulated monocytes). The ability of activated monocytes to present antigen to autologous lymphocytes was significantly reduced after culture with the combined n-3 PUFAs.	and those of previous studies support the hypothesis that n-3 PUFAs suppress cell-mediated immune responses, at least in part by inhibiting the function of antigen-presenting cells.
Experimental study (human) Spain (Navarro, Esteve et al. 2000)	To assess the fatty acid pattern in plasma and synovial fluid in RA and to determine clinical factors related to possible abnormalities.	39 consecutive RA patients (24 female, 15 male; median age 64 ± 3.4 y) were included in the study.	Synovial fluid samples were obtained from 9 patients. Disease activity was assessed using the Ritchie Articular Index and erythrocyte sedimentation rate (ESR). Fatty acids were assayed with GLC.	Decreased levels of EPA ($p<0.0001$) and total n-3 PUFAs ($p<0.05$) were observed in plasma and in joint fluid, respectively. An increase of the substrates of delta-5-desaturase (C20:3n6 and C20:2n6) and decrease of their products (C20:4n6 and C22:4n6) was observed in plasma total lipids and phospholipids. The long chain MUFAs (C20:1n9, C22:1n9, C24:1n9) were increased in the joint fluid and in plasma phospholipids. Patients with active disease showed a mild decrease of several SFAs, n-3, and n-6 PUFAs. Minor abnormalities or no changes in fatty acid profile were found related to use of steroids, NSAIDs, and gold salts, or malnutrition.	The fatty acid pattern found in RA (decreased levels of n-3 PUFAs) may explain the beneficial effect of fish oil. Changes in n-6 PUFAs suggest that delta-5-desaturation is decreased and this might facilitate the anti-inflammatory effect of botanical lipids in RA.

Human trials					
Randomised , double-blind, placebo-controlled trial. Australia (Volker, Fitzgerald et al. 2000b)	To determine the efficacy of fish oil derived n-3 fatty acid supplementation (3-6 capsules/day) in subjects with RA whose n-6 fatty acid intake in the background diet was <10 g/day, compared to olive/corn oil capsule supplement over a 15-week period.	50 subjects with RA whose background diet was naturally low (<10g/d) in n-6 fatty acids.	A placebo controlled, double-blind, non-crossover, randomised 15 week study to determine the effect of supplementation on clinical variables in the RA subjects. Fish oil containing 60% n-3 fatty acids was supplemented at a rate of 40mg/kg body weight. The control capsule was 50/50 corn/olive oil. Clinical evaluations at baseline, and at 4, 8 and 15 weeks. Plasma and monocyte lipids were analyzed at baseline and 15 weeks.	Analysis of 9 clinical variables indicated there was a significant difference ($p<0.02$) between control and treatment groups after 15 weeks (but not after 4 and 8 weeks). Five subjects in the treatment group and 3 in the control group met the American College of Rheumatology 20% improvement criteria. Dietary supplementation resulted in a significant increase in EPA in plasma and monocyte lipids in the supplemented group.	The findings suggest that fish oil supplementation that delivers n-3 fatty acids at a dose of 40mg/kg body weight/day, with dietary n-6 fatty acid intake <10g/day in the background diet, results in substantial cellular incorporation of n-3 fatty acids and improvements in clinical status in patients with RA.
Randomized, controlled double-blind study Italy (Sarzi-Puttini, Comi et al. 2000)	To examine the clinical and laboratory effects of two different diet regimens on RA patients	50 patients with active RA	24-week double-blind RCT of 2 different dietary regimen (an experimental diet high in PUFAs, low in SFAs with hypoallergenic foods vs. a control well-balanced diet). The primary end points of the study were 20% and 50% improvement in disease activity according to composite symptoms (Paulus index) of arthritis. Other end points were the other measures of disease activity at 12 and 24 weeks of diet treatment.	The 2 groups were comparable at inclusion. Rate of drop-out was low. No differences in % of patients with global 20 or 50% response between experimental and control group were found after the 24-week of diet treatment. The experimental diet group did better for all the variables considered but only four variables (Ritchie's index, tender and swollen joints, and ESR) reached a statistical difference by multivariate analysis. Adjusting these data for weight variations, the number of tender joints ($p=0.014$) and ESR ($P=0.025$) were still statistically significant.	Dietary manipulation, either by modifying food supplements or by reducing weight, may give some clinical benefit although no significant improvement over a well balanced diet can be observed assessing the results with a composite index.
Human intervention study (non-randomized, non-controlled and non-blinded) Australia (Mantzioris, Cleland et al. 2000)	To examine the effectiveness of a diet that incorporates foods rich in n-3 fatty acids in elevating tissue concentrations of EPA and in suppressing the production of inflammatory	15 healthy male volunteers (mean age 37.7 ± 6.5 y) without a history of hyperlipidemia or inflammatory disease.	Foods that were enriched in ALA (cooking oil, margarine, salad dressing and mayonnaise) and EPA and DHA (sausages and savoury dip) and with foods naturally rich in n-3 PUFA, such as flaxseed meal and fish were added to the diet at home for 4 wk. A target value for EPA of 1.5% of total fatty acids in the mononuclear cells was set,	Analyses of dietary records indicated that intake of EPA plus DHA averaged 1.8 g/d and intake of αLA averaged 9.0 g/d. These intakes led to an average 3-fold increase in EPA in plasma, platelet, and mononuclear cell phospholipids. TBX_2, PGE_2, IL-1β synthesis decreased by 36%, 26% and 20% ($p<0.05$), respectively.	This study suggests that foods that are strategically or naturally enriched in n-3 fatty acids can be used to achieve desired biochemical effects without the ingestion of supplements or a change in dietary habits. A wide range of n-3 enriched foods could be developed to

	mediators.		measured after 2 w. Subjects kept diet diaries. Fatty acid intakes, cellular and plasma fatty acid concentrations, and monocyte-derived eicosanoid and cytokine production were measured.		support large-scale programs on the basis of the therapeutic and disease-preventive effects of n-3 fatty acids.
Human intervention study Switzerland (Fahrer, Hoeflin et al. 1991)	To measure the effect on the membrane lipid composition of a diet rich in fish, and compare it with a diet supplemented by a medicinal preparation of fish oil, without any other dietary recommendations.	42 healthy volunteers (21 female, 21 male) recruited by 2 rheumatology units in Switzerland. Mean age 37 y (range 23-59 y)	The intervention study lasted 8 weeks. The participants were allocated to the 3 treatment groups according to their dietary preferences. Fish diet (group A) n=16: the participants were instructed to eat 700g fish per week and keep a record of fish consumption; Fish oil diet (group B) n=12: were instructed not to eat fish but to take 15 capsules corresponding to 7.5 g of fish oil with 2.1 g EPA and 1 g DHA per day. Regular diet (group C) n= 14: were instructed to follow their usual dietary habits, but to avoid any form of fish during the 8 weeks. Outcome measures were the lipid composition of platelet- rich plasma, the serum cholesterol and triglycerides before the study and after at 1 and 2 months of the diet.	The fish diet group (group A) consumed a median of 740 g (range 636 – 1,000) of the recommended fish per week for the 8 weeks of the study. The relative amounts of both EPA and DHA in the platelet-rich plasma increased significantly in the fish oil group and in the group with the fish diet compared to the baseline value and to the control group; no change was seen in the control group. The effect on triglycerides, which were low at the beginning of the study, was minor and no change in cholesterol was seen.	Four to 6 meals of fish per week without any other dietary changes can induce similar changes in lipids as a supplement of fish oil. A diet rich in fish could be recommended to patients who might potentially benefit from n-3 fatty acids and who don't mind eating fish.
Case Control Study USA (Shapiro, Koepsell et al. 1996)	To investigate the hypothesis that n-3 fatty acids, as measured by fish consumption, protect against RA.	324 incident RA cases and 1245 controls.	A FFQ was used to measure dietary intake during a 1-year period 5 years before first physicians visit for joint symptoms.	Consumption of broiled or baked fish, but not of other types of fish, was associated with a decreased risk of RA. The aOR for 1-<2 servings and ≥2 servings of broiled or baked fish/week, compared with <1 serving, were 0.78 [95% CI = 0.53-1.14] and 0.57 [95% CI = 0.35-0.93]. The case for fish became stronger when the analysis was restricted to cases positive for rheumatoid factor.	These results support the hypothesis that omega-3 fatty acids may help prevent RA.
Randomised	To study the long-	90 RA patients	12 month study.	Significant improvement in the	Daily supplementation with

double blind controlled trial Belgium (Geusens, Wouters et al. 1994)	term effects of supplementation with n-3 fatty acids in patients with active RA.	(3 treatment groups). All patients had active disease, and had been receiving a stable dosage of NSAIDs and/or DMARDs for at least 3 months prior to study entry.	Patients were randomly assigned to 1of the following 3 daily regimes: 6 capsules containing 1 g olive oil each (placebo), or 3 capsules containing 1 g of fish oil each (1.3 g n-3 PUFA) plus 3 placebo capsules, or 6 capsules containing 1 g of fish oil each (2.6 g of n-3 PUFA including 1.7 g EPA). Evaluations were made at 0, 3, 6, 9, and 12 months.	patient's global evaluation and in the physician's assessment of pain was observed only in those taking 2.6 g/day of n-3 PUFAs. The proportions of patients who improved and of those who were able to reduce their concomitant anti-rheumatic medications were significantly greater with 2.6 g/d of n-3 PUFAs.	2.6 g of n-3 PUFAs results in significant clinical benefit and may reduce the need for concomitant antirheumatic medication. These findings confirm the positive results obtained in previous short-term studies and extend these observations to the long-term follow-up of 1 year of treatment.
Randomised, double-blind, controlled trial. UK (Lau, Morley et al. 1993)	To investigate the effects of Maxepa (contains EPA 171 mg/capsule and DHA 114 mg/capsule) on NSAID usage in patients with RA over a 15-month period.	64 RA patients (45 female and 19 male) mean age 51 ± 12.7 y. All had stable active RA, all required NSAIDs but none required DMARDs.	Patients received either 10 Maxepa (n=32) or air-filled placebo capsules (n=32) per day for 12 months. All then received placebo capsules for a further 3 months. Patients were reviewed at 3-monthly intervals. NSAID requirement at entry visit for each patient was assigned as 100%. Patients were instructed to slowly reduce their NSAID dosage providing there was no worsening of their symptoms. Clinical and laboratory parameters of RA activity were also measured.	There was a significant reduction in NSAID usage in patients on Maxepa when compared with placebo from 3 months [mean (95% CI for mean) requirement – 71.1 (55.9-86.2)% and 89.7 (73.7-105.7)% respectively]. This effect reached its maximum at month 12 [40.6 (24.5-56.6)% and 84.1 (62.7-105.5)% respectively] and persisted to month 15 [44.7 (27.6-61.8)% and 85.8 (60.5-111.1)% respectively ($p<0.001$, ANOVA). No statistically significant or trend of changes in any of the clinical and laboratory variables of RA was observed within and between the two groups. In the MAXEPA treatment group EPA levels were significantly elevated at months 6 and 12 and returned to baseline levels at 15 months. In the same group DHA was significantly elevated at 12 month and this persisted to 15 month.	Authors conclude that the findings in this study suggest that Maxepa fish oil, containing EPA and DHA, has NSAID sparing effects when given over 1 y to patients with mild RA. This also suggests that Maxepa has anti-inflammatory properties similar to those of NSAIDs.
Prospective randomised, double-blind, controlled trial	As part of a clinical study, the researchers examined the possible effects of dietary	32 patients with active RA	A prospective, randomised double-blind study with two groups RA patients. They were given a daily dietary supplement of either 3.6 g n-3 PUFA (18 patients) or placebo	The IL-1β concentration in plasma was reduced significantly after 12 weeks of dietary supplementation with fish oil ($p<0.03$). No significant difference was observed in the	The researchers conclude that dietary supplementation with n-3 fatty acids results in significantly reduced plasma IL-1β levels in patients with

Denmark (Espersen, Grunnet et al. 1992)	supplementation with n-3 PUFAs on the plasma levels of IL-1β, TNF-α and complement activation in patients with RA.		(14 patients) for 12 weeks. The patients were allowed to continue ongoing treatment with NSAIDs, corticosteroids and slow acting antirheumatic drugs. The cytokines were measured in plasma before and after treatment with fish oil or placebo.	placebo group. The TNF-α activity in plasma did not change significantly (p=0.167). No significant changes were observed in the degree of complement activation.	RA. Even though the cytokine levels were low, the anti-inflammatory effect of n-3 fatty acids could in part be explained by their ability to decrease cytokine production.
Randomised, controlled, double-blind trial Sweden (Skoldstam, Borjesson et al. 1992)	To document possible clinical and biochemical effects of fish oil over six months in a randomised, controlled, double-blind study of parallel groups of patients with RA. In addition, an investigation into whether the intake of oil capsules affected the nutrient intake compared to that before treatment was undertaken.	46 patients (34 females, 12 males), mean age 57 y with RA as defined by the ARA.	Two groups of patients were given either 10 capsules of fish oil (10 g/d) or a placebo inactive oil (a mixture of maize oil, olive oil and peppermint oil) (10 g/d) in a randomised, controlled, double blind study. The fish oil contained 37% n-3 PUFA (18% EPA, 12% DHA). Patients were evaluated at 0, 3 and 6 months.	43 patients completed the study, 22 taking fish oil and 21 taking placebo capsules. 3 dropped out because their disease became more active. The nutrient intake in the two groups was essentially similar. In the fish oil group, the percentage of n-3 fatty acids in serum phosphatidylcholine increased by 9.6 (range 2.6-16.1). Patients in the fish oil group reported a significantly decreased consumption of NSAID at 3 and 6 month and the status of global arthritic activity improved at 3 months in physician's assessment. Control patients reported an increased global arthritic activity at 6 months. No change was found in patient assessment of pain, duration of morning stiffness or functional capacity. Essentially no change occurred in biochemical markers or inflammation.	The researchers concluded that fish oil has small anti-inflammatory effects with at most a NSAID-saving potential. The value of prolonged supplementation remains to be evaluated.
Randomized, Double-blind, non-cross over study Australia (Cleland, French et al. 1988)	To evaluate the clinical and biochemical effects of dietary fish oil supplements in RA patients receiving their usual medication and given dietary advice designed to achieve a	60 patients with active RA	Patients were randomly allocated to fish oil (18 g/day; 18x1g Maxepa capsules) or comparative olive oil (18 g/day; 18x1 g capsules) treatment groups. Duration: 12 weeks 7 patients in each group dropped out	An improvement in tender joint score and grip strength was seen at 12 weeks in the fish oil treated group but not in the olive oil treated group. The more subjective measures of mean duration of morning stiffness and analogue pain score improved to a similar extent in both groups, although statistical significance was only achieved in paired analyses in	The findings of this and previous studies reporting the effects of dietary fish oil supplements in RA provide a basis for recommending this approach as an adjunct to conventional therapy for patients who are seeking to reduce disease activity by dietary means.

	total daily fat intake of 60 g/day.			the olive oil treated group. Production of leukotriene B4 by isolate neutrophils stimulated in vitro was reduced by 30% in the fish oil treated group.	[No discussion or conclusion was reached regarding the olive oil group].
Prospective, double-blind, controlled study USA (Kremer, Michalek et al. 1985)	To examine the effect of manipulation of dietary fatty acids on RA by comparing a diet high in PUFA and low in SFA, with a supplement of EPA, and the typical American diet high in SFA.	37 patients with active RA, all taking anti-rheumatic slow acting drug and a NSAID.	17 patients took an experimental diet high in PUFA and low in SFA, with a daily supplement of EPA (1.8 g). 20 patients took a control diet with a lower PUFA:SFA ratio and a placebo (paraffin wax) supplement. All patients received a daily multivitamin/mineral tablet containing iron and vitamin E. Duration: 12 weeks supplementation plus 1-2 months follow-up period Clinical and laboratory measurements were made at 0, 4, 8 and 12 weeks and at follow-up.	Results favoured the experimental diet group at 12 weeks for morning stiffness and number of tender joints. On follow-up evaluation at 1-2 months the experimental group had deteriorated significantly in patient and physician global evaluation of disease activity, pain assessment, and number of tender joints. The control group had improved in morning stiffness and number of tender joints on follow-up.	Any benefit derived from the experimental diet was transient. The plasma concentrations of arachidonic acid and linoleic acid (the precursors of the PGE2 inflammatory prostaglandins) changed little in either group.
Open dietary intervention USA (Meyandi, Endres et al. 1991a)	To investigate the effect of supplementation with 2.4 g of n-3 fatty acid/d for up to 3 months on cytokine production and lymphocyte proliferation of young and older women	6 healthy young women (23-33y) and 6 post menopausal healthy older women (51-68y)	Subjects supplemented their diets with 2.4 g of n-3 fatty acids for 3 months. Blood was collected at 0, 1, 2 and 3 months of supplementation.	The n-3 fatty acid supplementation reduced total interleukin IL-1β synthesis by 48% in young women but by 90% in older women; TNFα was reduced by 58% in young and 70% in older women. IL-6 was reduced in young women by 30% and by 60% in older women. Older women produced IL-2 and had lower mitogenic responses to phytohemagglutinin (PHA) than young women prior to n-3 fatty acid supplementation. The n-3 fatty acid supplementation reduced IL-2 production in both groups; however, this reduction was significant only in older women. The PHA-stimulated mitogenic response was significantly reduced by n-3 fatty acids in older women (36%)	Long-term n-3 fatty acid supplementation reduced cytokine production in young women and cytokine production and T-cell mitogenesis in older women. The reduction was more dramatic on older women than younger women. Although n-3 fatty acid-induced reduction in cytokine production may have beneficial anti-inflammatory effects, its suppression of IL-2 production and lymphocyte proliferation in older women may not be desirable.

Open dietary intervention USA (Meyandi, Natiello et al. 1991b)	To investigate the potential change of plasma lipid peroxides in young and older healthy women following long-term supplementation with commercially available fish oil capsules	15 young (22-35 y) and 10 older (51-71 y) healthy women	Each subject's usual diet was supplemented with n-3 fatty aciids contained in 6 capsules of Pro-Mega (commercially available fish oil supplement) daily for 12 weeks. This supplement equated to 1680 mg EPA, 720 mg DHA, 600 mg other fatty acids and 6 IU of vitamin E daily. Blood samples collected at 0, 1, 2 and 3 months of supplementation.	Older women had a significantly higher increase in plasma EPA and DHA than younger women. The decrease in the AA:EPA ratio was greater in the older women. Plasma TGs decreased significantly, and the ratio of PUFA:SFA was significantly ($p<0.01$) increased. Plasma vitamin E levels did not change significantly after supplementation, however, after 3 months supplementation by young women, plasma vitamin E was significantly lower than after 1 month. The vitamin E:TG ratio was significantly increased and vitamin E:(EPA+DHA) significantly decreased. All women showed a significant increase in plasma lipid peroxide up to month 2 of supplementation, but after 2 month older women had significantly higher lipid peroxide levels than young women. The lipid peroxide:TG ratio which declined by 3 months was still significantly higher than at baseline.	These data indicate that although long-term fish oil supplementation may be beneficial in reducing plasma total TH, susceptibility of plasma lipids to free radical attack is potentiated. The decrease in plasma vitamin E:(EPA=DHA) ratio and increase in plasma lipid peroxide, particularly in older subjects, indicates that vitamin E content of fish oil capsules may not be sufficient to provide adequate antioxidant protection.
Prospective, double-blind, randomised, parallel trial USA (Kremer, Lawrence et al. 1990)	To determine if different doses of n-3 fatty acid dietary supplements (fish oil) ingested over a period of 24 weeks results in some dose-related clinical benefits and changes in production of leukotrienes and cytokines.	49 patients with active RA randomised for age, sex, use of DMARDs, and disease severity.	Three groups of RA patients completed a 24 week, prospective, double-blind, randomised study of dietary supplementation with 2 different dosages of fish oil (27 mg/kg EPA and 18 mg/kg DHA/low-dose, n=20; 54 mg/kg EPA and 36 mg/kg DHA/high-dose, n= 17) and 1 dosage of olive oil (6.8 g oleic acid, n=12). Clinical evaluations were performed at baseline and every 6 weeks, and immunologic variables were measured at baseline and after 24 weeks of study.	Significant improvements from baseline in the number of tender joints were noted in the low-dose group at 24 weeks ($p=0.05$) and in the high-dose group at 18 weeks ($p=0.04$) and 24 weeks ($p=0.02$). Significant decreases from baseline in the number of swollen joints were noted in the low- and high-dose groups from week 12. A total of 5 of 45 clinical measures were significantly changed from baseline in the olive oil group, 8 of 45 in the low-dose fish oil group, and 21of 45 in the high-dose fish oil group during the study ($p=0.0002$).	The authors conclude that the clinical benefits of dietary supplementation with n-3 fatty acids are more commonly observed in patients consuming higher dosages of fish oil for time intervals that are longer than those previously studied. Dietary supplementation with olive oil is also associated with certain changes in immune function, which require further investigation.

				Neutrophil LTB4 production decreased by 19% from baseline in the low-dose fish oil group (p=0.0003) and 20% in the high-dose group (p=0.03), while macrophage IL-1 production decreased by 38.5% in the olive oil group (p not significant), 40.6% in the low-dose group (p=0.06), and 54.7% in the high-dose group (p=0.0005).	
Human dietary intervention study (not blinded or placebo controlled) US (Sperling, Weinblatt et al. 1987)	To determine whether the potential antiinflammatory effects of dietary supplementation with fish oil would be manifest in the biochemical and functional responses of leukocytes from patients with active RA	14 active RA patients enrolled, 2 patients withdrew early from the study, so results based on 12 subjects	Subjects supplemented their usual diet with 20x1 mg capsules of Max-EPA (commercially available fish oil capsule) daily for 6 weeks. (3.6 g EPA and 2.4 g DHA and 180 kcal are provided daily from this dose) Clinical and laboratory assessments at 0 and 6 weeks	The ratio of AA to EPA in the patients' neutrophil cellular lipids decreased from 81:1 to 2.7:1, and the mean generation of LTB_4 (with calcium ionophore stimulation) significantly declined by 33%. The mean neutrophil chemotaxis to LTB_4 significantly increased toward the normal range at week 6. The generation of 5-lipoxygenase products by stimulated monocytes was not significantly suppressed, but a significant decline (37%) in platelet-activating factor generation was noted at week 6.	The modulation of such measures of leukocyte inflammatory potential suggests that fish oil supplementation may have an antiinflammatory effect.
Plant oils					
Multicentre double-blind, randomised, placebo controlled parallel group trial Scotia-Pharmaceuticals 1999 as reported by Darlington & Stone (Darlington and Stone 2001)	To assess the extent to which the use of EPO supplements could reduce the dosage of NSAIDs used by RA patients	402 RA patients	RA patients were randomized to treatment with EPO 2-3 g/d or to sunflower oil as placebo. Each test capsule of EPO contained approximately 280 mg GLA, 45 mg EPA and 30 mg DHA.	No results were given, only conclusions,	The trial yielded no support for the use of EPO as a NSAID-sparing agent in RA.
Case control study Greece	To investigate the possible effect of diet on the clinical	168 cases (32 males and 136 females) who	An interview based, case control study of RA. Patients and controls were interviewed with regard to a	Results of univariate analysis revealed that RA cases consumed significantly less olive oil and fish	The authors conclude that olive oil consumption and adherence to Orthodox lent

(Linos, Kaklamanis et al. 1991)	expression of RA	met the ARA criteria for definite or classical RA and 137 controls, group matched to the cases by sex and age.	variety of socio-economic, medical and dietary factors. The dietary questionnaire included lifelong consumption of more than 100 different food items and adherence to the dietary restrictions traditional in Orthodox lent.	and adhered more rarely to the dietary restrictions traditional in Orthodox lent than controls. Multiple logistic analysis found that only the association with olive oil consumption and lent adherence remained significant. An increase in olive oil consumption by two times per week, resulted in a RR = 0.49 (95% CI, 0.30-0.81) for development of RA.	may have a protective effect on the development and/or the severity of RA. This hypothesis needs verification.
Case control study Greece (Linos, Kaklamani et al. 1999)	To examine the relation between dietary factors and risk of RA in persons from southern Greece. To try to confirm the hypothesis generated by their previously published study (see above) that olive oil consumption may have a protective effect on the development and/or the severity of RA.	145 RA patients and 188 control subjects	The subjects provided information on demographic and socio-economic variables, prior medical and family history, and present disease status. Subjects responded to an interviewer-administered, validated, FFQ that assessed the consumption of >100 food items. The ORs and linear trend were calculated for the development of RA in relation to consumption of olive oil, fish, vegetables, and a series of food groups classified in quartiles.	Risk of developing RA was inversely and significantly associated only with cooked vegetables (OR: 0.39) and olive oil (OR: 0.39) by univariate analysis. A significant trend was observed with increasing olive oil (chi-square: 4.28; p= 0.03) and cooked vegetable (chi-square: 10.48; p=0.001) consumption. Multiple logistic regression analysis models confirmed the independent and inverse association between olive oil or cooked vegetable consumption and risk of RA (OR: 0.38 and 0.24, respectively).	Consumption of both cooked vegetables and olive oil was inversely and independently associated with risk of RA in this population. Further research is needed to elucidate the underlying mechanisms of this finding, which may include the antioxidant properties or the high n-9 fatty acid content of the olive oil. There was no association with fish consumption.
Uncontrolled dietary intervention study Denmark (Hansen, Lerche et al. 1983)	To analyse the disease activity in patients with RA during a 12 week-treatment with cis-linoleic acid and GLA, given together with the co-factors Zn, ascorbic acid, niacin, and pyridoxine.	20 patients with active RA	Each patient received 2 EPO capsules and 2 vitamin/mineral supplement capsules 4 times per day for 12 weeks. EPO capsules contained 0.6 ml seed oil of evening primrose (72% cis-linoleic; 9% GLA; 10% oleic; 9% other fatty acids) together with 13.6 IE vitamin E. Vitamin/mineral capsules contained 125 mg ascorbic acid, 25 mg niacin, 25 mg pyridoxine, 5 mg Zn sulphate. Clinical assessments were made	There was a slight fall in skin reactivity to UV light during the treatment, but no effect on plasma or urine concentrations of PGE_1, cAMP or cGMP. There was no effect of the treatment on ESR, P-fibrinogen, number of tender joints, number of swollen joints, the duration of morning stiffness, or on the patient's estimation of pain.	A 12 week treatment with the PGE1 precursors cis-linoleic acid and GLA and the co-factors ascorbic acid, niacin, pyridoxine and Zn sulphate had no detectable clinical effect in active RA.

			every 2 weeks.		
Double-blind, placebo controlled study UK (Belch, Absell et al. 1988)	To determine whether EPO or an EPO/fish oil combination containing EPA could be substituted for NSAID therapy without deterioration in clinical symptoms.	49 active RA patients, all on NSAIDs	16 patients received EPO treatment = 540 mg GLA/day (EPO gp), 15 patients received 240 mg EPA and 450 mg GLA/day (EPO/fish oil gp), and 18 patients an inert oil/liquid paraffin (placebo gp). All capsules contained vitamin E as an antioxidant (120 mg/d)/ 12 month treatment period followed by 3 months placebo (without vitamin E) for all groups. NSAIDs were monitored monthly. Clinical assessments and blood samples were made at 0, 3, 6, 12 and 15 months.	Results at 12 months showed a significant subjective improvement for EPO and EPO/fish oil compared with placebo. By 12 months patients receiving EPO and EPO/fish oil had significantly reduced their NSAIDs. After 3 months receiving placebo, those receiving active treatment had relapsed. Despite the decrease in NSAIDs, measures of disease activity did not worsen.	It is suggested that EPO and EPO/fish oil produce a subjective improvement and allow some patients to reduce or stop treatment with NSAIDs. There is no evidence, however, that they act as disease modifying agents.
Double blind controlled study Finland (Jantti, Nikkari et al. 1989)	To report on the changes in serum lipids and fatty acids in a double blind study using EPO and olive oil	18 patients with active RA	The serum concentrations of lipids and composition of fatty acids after overnight fasting were studied in 18 patients with RA treated for 12 weeks with either 20 ml of EPO containing 9% of GLA or olive oil (OO).	The serum concentrations of oleic acid, EPA, and apolipoprotein B decreased and those of linoleic acid, GLA, DGLA, and AA increased during treatment with EPO. During OO treatment the serum concentration of EPA decreased and those of HDL-cholesterol and apolipoprotein A-I increased slightly.	The decrease in serum EPA and the increase in AA concentrations induced by EPO may not be favourable effects in patients with RA in the light of roles of these fatty acids as precursors of eicosanoids.
Open uncontrolled human study USA (Pullman-Mooar, Laposata et al. 1990)	To determine whether the potential antiinflammatory effects of GLA (in borage seed oil) administration would be reflected in biochemcial responses.	7 patients with active RA and 7 normal controls	Both patients and controls were given borage seed oil (9 capsules/day = 1.1 g/d of GLA) for 12 weeks. Clinical and biochemical assessments took place at 0, 6 and 12 weeks	GLA administration resulted in increased proportions of DGLA in circulating mononuclear cells. The ratios of DGLA to AA and DGLA to stearic acid increased significantly in these cells. Significant reductions in PGE_2, LTB_4, and LTC_4 produced by stimulated monocytes were seen after 12 weeks of GLA supplementation.	The antiinflammatory effect of GLA administration observed in animal models, and the apparent clinical improvement experienced by 6 of 7 RA patients given borage seed oil in this open, uncontrolled study may be due in part to reduced generation of AA oxygenation products.
Randomized, double-blind, placebo controlled study	To determine if evening primrose oil (EPO) supplements enable RA patients to reduce or stop their	40 RA patients , all with upper gastrointestinal lesions due to NSAIDs	19 patients received active therapy (EPO 6 g/d = 540 mg/d GLA) in capsules. EPO capsules contained 10 mg α-tocopherol as antioxidant. 21 patients received placebo (olive	No patient stopped NSAIDs therapy but 3 patients in each group reduced their dose. There was a significant reduction in morning stiffness with GLA at 3	Whilst GLA might produce mild improvement in RA, olive oil may itself have hitherto unrecognized benefits.

UK (Brzeski, Madhok et al. 1991)	dose of NSAIDs and thus reduce the side effects of NSAIDs		oil 6 g/d) in identical capsules. Duration: 6-month Assessments at 0, 3 and 6 months: use of NSAIDs and analgesia, subjective and objective clinical evaluations	months and reduction in pain and articular index at 6 months with olive oil.	Olive oil can no longer be used as a placebo control and should itself be investigated.
Randomized, double-blind, placebo controlled trial USA (Leventhal, Boyce et al. 1993)	To assess the clinical efficacy and side effects of GLA, a plant-seed derived essential fatty acid that suppresses inflammation and joint tissue injury in animal models	37 patients with RA and active synovitis	Treatment with 1.4 g/d GLA in borage seed oil or cotton seed oil (placebo) for 24 weeks. Assessment: Physicians and patients global assessment of disease activity	Treatment with GLA resulted in clinically important reduction in the signs and symptoms of disease activity in patients with RA ($p<0.05$). Patients given a placebo showed no change or worsening of disease. GLA reduced the number of tender joints by 36%, the tender joint score by 45%, swollen joint count by 28%, and swollen joint score by 41%. Placebo group had no significant improvement in clinical measures. Overall clinical responses (significant change in four measures) were also better in the treatment group ($p<0.05$).	GLA in doses used in this study is well-tolerated and effective treatment for active RA. GLA is available worldwide as a component of EPO, BCO and borage seed oils. It is usually taken in far lower doses than used in this trial. Further controlled trials of its use in RA are warranted.
Randomized, double-blind, placebo controlled trial UK (Watson, Byars et al. 1993)	To investigate the effects of dietary supplementation with blackcurrant seed oil (BCO), a particularly rich source of GLA, on the secretion of cytokines IL-1β, TNFα and IL-6 and of PGE_2 from cultured peripheral blood monocytes of healthy subjects and RA patients.	30 RA patients (25 female and 5 male) receiving only NSAIDs. Controls = 20 healthy student volunteers (10 male, 10 female) BCO treatment gp = 20 patients and 10 students Placebo gp = 10 patients and 10 students	BCO treatment gp received 6x500 mg capsules/d = 525 mg GLA/d Placebo gp received 6x500 mg sunflower seed oil capsules/d Study duration = 12 weeks with all subjects taking capsules for first 6 weeks only. Clinical evaluations were carried out and blood samples were taken at 0, 6 and 12 weeks. Monocytes were isolated from all subjects and cultured in the presence of lipopolysaccharide.	A significant improvement in morning stiffness was noted in RA patients receiving BCO. The production from the cultured monocytes of the cytokines, IL-1β, TNFα and IL-6 as well as the protaglandin PGE2 was markedly altered in subjects given BCO.	The results suggest that the numerous beneficial effects of PUFAs in inflammatory diseases such as RA may be due to a reduction in the secretion of the inflammatory cytokines IL-1β and TNFα via redirection of eicosanoid metabolism although the possibility cannot be excluded that the PUFAs may be altering cytokine release directly through an effect on monocyte membranes.

Table 3 Papers on antioxidants in RA

Study type, Country Paper reference	Study population	Daily dose and Treatment period or methodology	Outcomes
Human laboratory study Thailand (Kajanachumpol, Vanichapuntu et al. 2000)	91 RA patients and 26 healthy subjects.	Plasma lipid peroxide products, malondialdehyde (MDA) and conjugated dienes (CD) and plasma vitamins C and E were measured.	RA patients had increased plasma CD but not MDA and decreased plasma vitamin E, when expressed per unit of cholesterol and triglyceride i.e. RA patients had increased oxidant stress that might play a role in tissue damage and inflammation process.
Nested case-control study Finland (Heliovaara, Knekt et al. 1994)	Cases = 14 subjects in a large Finnish cohort of men and women who developed RA during a median follow-up of 20 years. Controls = 28 cohort subjects age and sex matched	Serum α-tocopherol, β-carotene and selenium concentrations were measured from stored serum samples. An antioxidant index was calculated as the product of the molar concentrations of these 3 micronutrients.	Elevated risks of RA were observed at low levels of α-tocopherol, β-carotene and selenium but none of these associations were statistically significant A significant association was observed with a low anti-oxidant index (p for trend = 0.03), the RR of RA between the lowest and highest tertiles of its distribution being 8.3 (95% CI, 1.0-71.0). Conclusion: The results of the present study are in line with the hypothesis that a low antioxidant level is a risk factor for RA.
Human laboratory study Egypt (Ashour, Salem et al. 2000)	Children with JRA	Measurement of enzymatic and non-enzymatic antioxidant status in a children with JRA and controls.	Plasma concentrations of albumin, ceruloplasmin, vitamin C, vitamin E, erythrocyte SOD and whole blood glutathione peroxidase activities were all significantly decreased in the presence of JRA compared to those without JRA. Vitamin A and retinol binding protein remained unaffected by JRA.
Human laboratory study UK (Fairney, Patel et al. 1988)	33 patients with classical or definite RA according to the standard ARA criteria, and 24 patients with OA.	Random venous blood samples were collected from all the subjects plus synovial fluid samples from 18 of the RA and 18 of the OA patients.	Serum retinol values in RA were lower than in matched controls ($p<0.01$), and in OA patients ($p<0.001$). Retinol was present in the synovial fluid and the mean values for RA and OA were similar (0.92 µmol/l and 1.16 µmol/l) and represented 49% and 40% of the serum values respectively. There was a close relationship between the amount of retinol in serum and synovial fluid in RA patients ($r=0.77$, $p<0.001$) but this correlation was not present in OA patients ($r=0.37$, NS). The serum RBP values in RA were lower than those in matched controls but in OA they were not different from normal. However, the serum RBP values were higher in OA than RA ($p<0.001$). The synovial fluid RBP showed a close relationship to that in serum in RA ($r=0.8$, $p<0.001$) but this was not found in OA ($r=0.31$, $p<0.2$). The mean serum osteocalcin was higher than normal in both RA and OA ($p<0.001$ and $p<0.005$) and the values were not related to retinol or RBP. These findings suggest that there may be a difference in vitamin A metabolism or intake between OA and RA patients.
Randomized double-blind	85 hospitalized RA	High dose vitamin E (3 x 400 mg/d)	Both groups demonstrated significant improvement in all assessed clinical

comparison Sweden (Wittenborg, Paetersen et al. 1998)	patients	versus diclofenac sodium Duration – 3 weeks	parameters (i.e. morning stiffness, Ritchie articular index, pain). Physician and patient global assessments considered both treatments to be similarly effective .
Molecular Germany (Abate, Yang et al. 2000)	Macrophage cell line (J774. 1A)	Exploring the synergistic effect of aspirin and vitamin E on the expression and activity of cyclooxygenase-2 in macrophage cells. Vitamin E (100-300 μM) and aspirin (1-100 μM)	PGE2 formation was significantly reduced by aspirin or vitamin E. When combined with vitamin E, aspirin-dependent inhibition of PGE2 formation was increased from 59% to 95% of control. Induced cyclooxygenase-2 protein and mRNA expression were virtually abolished by the combined treatment of aspirin and vitamin E, and the 2 agents alone were modestly effective. Vitamin C did not mimic vitamin E under these conditions, suggesting that redox-independent mechanisms underlie the action of vitamin E. Anti-inflammatory therapy might be successful with lower aspirin doses when combined with vitamin E. .
Review Germany (Miehle 1997)		Review of the scientific evidence for the use of vitamin E in active arthroses	The results of the double-blind studies and clinical empiricism support the following hypothesis: the pathogenic substrate free oxygen radicals increases quantitatively from activated arthrosis to chronic polyarthritis. This could explain the graded differentiated antioxidative effects of α-tocopherol.
Review Germany (Sangha and Stucki 1998)		A review of current and past studies about vitamin E in the treatment of rheumatic diseases.	As early as the sixties, first case reports have described beneficial effects of vitamin E in the therapy of OA. However, most of the following studies were not conducted properly, thus not allowing valid inferences about the efficacy of vitamin E. Newer studies with sound methodology have shown a beneficial effect in rheumatic diseases, mainly in the reduction of pain.
Prospective, placebo-controlled, double-blind trial UK (Edmonds, Winyard et al. 1997)	42 patients with active RA. Supplementary group n=20 Placebo group n=22	600 mg α-tocopherol twice/day (2x2 capsules) or placebo Duration 12 weeks	All laboratory measures of inflammatory activity and oxidative modification were unchanged. The clinical indices of inflammation were not influenced by the treatment when compared with placebo. The pain parameters were significantly decreased after vitamin E treatment when compared with placebo.
Molecular study USA (Tiku, Shah et al. 2000)	Cultured primary articular chondrocytes	In vitro model to study the role of chondrocyte-derived ROS in cartilage matrix protein degradation	The findings suggested a chondrocyte-dependent mechanism of matrix degradation. Antioxidant enzymes such as catalase or SOD did not influence matrix release but vitamin E at physiological concentrations, significantly diminished the release of labelled matrix by activated chondrocytes. The fact that vitamin E is a chain-breaking antioxidant indicates that the mechanism of matrix degradation and release is mediated by the lipid

			peroxidation process. This study suggests that vitamin E has a preventive role in cartilage matrix protein (collagen) oxidation and degradation.
Animal study Japan (Sakai, Hirano et al. 1999)	Rats (9 per group) with induced arthritis	Ascorbic acid at doses of 0.5, 1.0, and 2.0 g/kg body weight injected twice each week for 3 weeks	The arthritic control rats showed significant increases in paw volume and arthritis score from day 11. These changes were dose-dependently reduced by ascorbic acid administration. The infiltration of inflammatory cells into the synovial tissues was markedly decreased by ascorbic acid. The increase in SOD activities produced by adjuvant injection were significantly reduced in both the synovium and RBC at ascorbic acid doses of 1.0 and 2.0 g/kg body weight. The decrease in SOD activity could be one of the mechanisms underlying the suppressive effects of large-dose ascorbic acid on the development of arthritis in this animal model, inhibiting the damaging ROS.
Molecular studies UK (Whiteman and Halliwell 1996)	Cells	Laboratory study to compare the abilities of several biological antioxidants to protect against peroxynitrite-dependent inactivation of alpha 1-antiproteinase, and to inhibit tyrosine nitration upon addition of peroxynitrite.	GSH and ascorbate protected efficiently in both systems. Uric acid inhibited tyrosine nitration but not alpha 1-antiproteinase inactivation. The possibility that ascorbic acid is an important scavenger of reactive nitrogen species in vivo is discussed.
Animal study Egypt (Eldin, Hamdy et al. 1992)	Rats with induced arthritis.	Administration of vitamin C (50 mg/kg body weight) to acute phase (4 days after inoculation) and chronic phase (21 to 29 days after inoculation) arthritic rats.	Results showed that prolonged administration of vitamin C (21 days) but not 4- and 7-day administration increased the lowered serum sulphydryl (SH-groups) to prearthritic values while it decreased the elevated level of blood glutathione (GSH) of arthritic rats. The results showed also a slight significant increase in the level of RBC SOD activity upon 7-day treatment with vitamin C. 4-, 7- and 21-day treatment with vitamin C produced no significant change in the elevated levels of serum ceruloplasmin and alpha 2-macroglobulin of arthritic rats. 7- and 21-day administration of vitamin C improved the lowered A/G ratio in these animals. The improvements after 21-day dosage of vitamin C suggests a beneficial role for it in the treatment of arthritis.
Double blind trial Denmark (Tarp, Overvad et al. 1985)	40 patients with active RA	256 µg yeast Se 6 months duration	No clinical or biochemical response on parameters of inflammation or in terms of the patients' self-assessment. Significant increases in serum and RBC levels were found in the Se group
Double-blind, controlled trial UK	OA patients	Selenium-ace, an over the counter formulation containing Se with 3 vitamins (A,C and E) 6 months	No significant improvement although there was a non-significant trend to improvement in some clinical parameters in both groups.

(Hill and Bird 1990)			
Double blind cross over trial Sweden (Petersson, Majberger et al. 1991) as reported in (Tarp 1995)	20 RA patients with low inflammatory activity	Supplemented with Se, and vitamins A, E and C (dose not indicated) 6 months duration	No significant effects on clinical parameters
Human intervention study Finland (Jantti, Vapaatalo et al. 1991) as reported in (Tarp 1995)	28 RA patients	150 µg Se (type not disclosed) 8 weeks duration	No significant effects related to Se
Placebo controlled trial Belgium (Peretz, Neve et al. 1992)	15 patients with recent onset RA	8 patients supplemented with 200 µg yeast Se, 7 patients with placebo 3 months duration	Significant improvement in Se group resulting in reduced pain and joint score and antigenic ability compared to 7 patients receiving placebo
Human intervention study Scandinavia (Aaseth and Teigen 1993)	47 RA patients	600 µg yeast Se 8 months duration	Significant reduction in arthritic pain
Double blind, randomized controlled trial Germany (Heinle, Adam et al. 1997)	70 RA patients randomly assigned to supplementation and placebo group	200 µg sodium selenite Both groups given fish oil fatty acids at 30 mg/kg body wt. 3 months duration	Se supplemented group showed less tender and swollen joints, and morning stiffness. Se supplemented patients needed less cortisone and NSAIDs than controls. Also a decrease of laboratory indicators of inflammation (C-reactive protein, α-2-globuline, prostaglandin E2)

Table 4 Review studies/papers of nutrition and diets and RA

Paper title and reference	Review objectives	Summary of review findings	Conclusions
Vegetarian diets			
Fasting followed by vegetarian diet in patients with rheumatoid arthritis: a systematic review (Muller, Wilhelmi de Toledo et al. 2001)	To shed more light on the role of fasting in RA. A systematic review was carried out to identify all studies which report on fasting in RA, and the results of controlled trials were synthesised by a pooling of results.	31 original reports on studies in which fasting was applied in patients with RA, from which only 4 independent controlled studies evaluated the clinical consequences of fasting followed by a vegetarian diet for at least 3 months (Kjeldsen-Kragh, Haugen et al. 1991), (Skoldstam, Larsson et al. 1979),(Skoldstam 1986), (Lindberg 1973). The pooling of these studies showed a statistically significant beneficial long-term effect.	Available evidence suggests that fasting followed by vegetarian diets might be useful in the treatment of RA. There is an urgent need for more randomised long-term studies to confirm this view by methodologically convincing data. This review shows a typical research deficit which is due to difficulties in the funding of studies investigating other than drug treatments
The role of meat in the expression of rheumatoid arthritis (Grant 2000)	A short review to examine some of the data that associate meat consumption with RA and the possible factors, e.g. fat, Fe and nitrite, which may contribute to the inflammation. (A review of a number of relevant papers plus an ecological approach is used to study the links between diet and RA)	Multi-country data for prevalence of RA for females from 8 and 25 countries were compared statistically with components of national dietary supply. Fat from meat and offal for the period 2 years before the prevalence data was found to have the highest statistical association with the prevalence of RA (r^2 0.877, $p<0.001$ for 8 countries and r^2 0.670 $p<0.001$ for 15 countries). The statistical correlations for meat and offal were almost as high as those for their fat. Similar correlations were found for temporal changes in indices of effects of RA in several European countries between 1968 and 1978 as more meat was added to the national diets, although the correlations were higher for meat than fat. The review also examined some of the data that associate meat consumption with RA and the possible factors, e.g. fat, Fe and nitrite, which may contribute to the inflammation.	The primary finding of the literature review and statistical analysis is that meat and offal may be a major risk factor for the expression of RA. The fats may contribute through inflammation or free radical production. However, since the lipid profiles of meat and dairy fatty acids are similar, and dairy fat is not found to be associated with RA symptoms, the fatty acids may not play as much of a role as has been generally thought. Nitrite is another possible agent that could lead to increased inflammation. The Fe in meat may also contribute by acting as a catalyst to increase the production of free radicals. Finally other components in meat may also be involved. It is hoped that the results will lead to further case-control and clinical studies to investigate the aetiology of RA and the possible role of meat.
Rheumatoid arthritis treated with vegetarian diets	To summarise previously published results regarding dietary	Fasting followed by a vegetarian diet has a favourable influence on disease activity in some patients with RA. This effect can be explained entirely by psychobiologic factors, immuno-suppression secondary to	These findings may indicate that the beneficial effect of dietary treatment is caused by alterations in the microflora

(Kjeldsen-Kragh 1999)	treatment of RA with particular reference to the use of vegetarian dietary regimes. The results published were all taken from the same group of researchers.	energy deprivation, changes in plasma concentration or eicosanoid precursors, or changes in antibody activity against dietary antigens. Changes in disease activity were found to be associated with concurrent alterations in the faecal microflora and in the antibody activity against *P. mirabilis.*	secondary to changes in the diet. Provided that detrimental effects on nutritional status can be prevented, dietary treatment may prove to be a valuable supplement to the ordinary therapeutic treatments for RA. However, more carefully controlled trials are warranted before this treatment modality will gain wide acceptance.
The dietary treatment of inflammatory arthritis: Case reports and review of the literature (Danao-Camara and Shintani 1999)	To review the scientific literature reporting on the dietary treatment of arthritis and to provide some practical guidelines for the application of this information.	Elimination diets are variably successful. Fasting followed by a vegetarian diet can produce a sustained positive response measured clinically and by laboratory variables of inflammation. The efficacy of such an approach appears to hinge on the alteration of faecal flora. Swaying the balance of dietary fats in favour of n-3 and n-6 fatty acids has an anti-inflammatory effect, but does not appear to correct the basic immunologic processes involved in the development of the arthropathies.	Inflammatory arthritis is a true food intolerance only in a very small number of patients. No foods or food groups have been consistently identified as a cause, trigger or aggravating factor in unselected patients. Some items that have produced worsening in a few individuals are dairy products, nitrates, alcohol, simple sugars and azo dyes. Fasting clearly suppresses inflammation in the joints but should not be undertaken without medical supervision. The benefits of fasting are very difficult to sustain, but in some patients can be made to last by switching to a vegan diet. n-3 fatty acids can help suppress joint inflammation and the use of olive oil may be of some benefit.
Review studies of nutrition and diet in arthritis			
The role of nutrition and diet in rheumatoid arthritis (Martin 1998)	A review of the recent scientific investigations by the medical and related professions into the nutritional implications of RA	Low dietary intakes of Cu, Mg, Zn, vitamins D, E, and A, pyridoxine, folate and n-3 fatty acids have been consistently reported by different investigators. Evidence to date for the role of food intolerance in RA is inconclusive, derived from anecdotal reports. Milk and dairy produce, cereals, maize, wheat gluten, tartrazine and azo dyes have all been implicated in RA in recent reviews. Overall there seem to exist 'subgroups' of RA patients for whom food intolerance plays a significant role in their disease. Oil based supplements have emerged as the most promising area of	It is difficult to produce specific dietary recommendations for RA because of the lack of understanding of the nutritional requirements in RA, plus the variability in its clinical course. - In general sufferers should consume as varied a diet as possible, based on current Department of Health guidelines. Dietary counselling is

		dietary manipulation in RA. Pharmacological doses (3-10g/d) of the n-3 fatty acids found in fish oils reduce stiffness and pain in RA. The value of low doses of n-3 fatty acids is unclear; however, frequent intakes of oily fish may reduce the risk of RA or limit its severity.	important to help patients achieve this. - Self-imposed elimination diets should be avoided and suspected food intolerance tested under strict clinical supervision. Nutrient megadosing is inadvisable, although dietary supplementation with Ca, vitamin D, folic acid or multivitamins and minerals should be recommended where necessary.
Diets, dietary supplements, and nutritional therapies in rheumatic diseases (Henderson and Panush 1999)	To present the known scientific-based evidence for the use, safety, and efficacy of diets and dietary related practices subscribed to by patients with RA.	The relationships between diets, fasting, elemental nutrition, vitamins, minerals, and foods for rheumatic diseases can be summarised as follows: - Fasting had short-term antirheumatic effects. - Elemental nutrition has been inconsistently antirheumatic. - Specific diets have not been consistently beneficial for patients with rheumatic diseases. - Vitamins, minerals, or nutritional supplements have not been consistently antirheumatic. - Rare patients with rheumatic diseases have had clinical symptoms convincingly documented to be associated with food or food product sensitivity.	RA and other systemic rheumatic diseases remain illnesses of unknown cause. The possibility that food antigens induce or perpetuate symptoms in at least some patients is reasonable. The available evidence suggests that allergic reaction to foods may occur in a small subset of RA patients. Continued investigation of this issue is merited. Diets of certain nutritional content can modulate experimental and, in some circumstances, clinical inflammation. Dietary therapy for arthritis should still be considered investigational.
Review of dietary therapy for rheumatoid arthritis (Darlington and Ramsey 1993)	A clinical review of the scientific studies from the UK and abroad	Dietary therapy for RA is divided into two types: supplementation therapy and elimination therapy. Dietary supplements: Fish oils- work to date on fish oils is interesting and promising and treatment appears to be safe. Long-term toxicity studies are needed. Evening primrose oil – data so far reported appear promising but need confirmation by further work, with long-term toxicity studies. There are synergistic actions between the GLA in EPO and EPA. EPA inhibits conversion of DGLA to arachidonic acid (AA), and as a result, GLA has a greater effect in raising concentrations of DGLA. Combined administration raises the levels of two anti-inflammatory essential fatty acids, DGLA and EPA, while reducing pro-inflammatory AA. New Zealand green lipped mussel (Seatone) – the evidence to date that Seatone has significant value in RA is slight although it may have mild anti-inflammatory activity.	More research is needed. Dietary supplements not yet assessed scientifically need assessment. Fish oils need further investigation, e.g. to determine whether low-dose fish oil has any value or whether it is only useful in the concentrated form so far used in controlled studies. EPO needs to have its therapeutic value confirmed and its safety determined. Dietary elimination therapy needs investigation, with double-blind challenge. Its long-term efficacy needs further documentation and lectins and other individual dietary components need

		Selenium – Oral Se therapy appears to have limited value in the management of RA but Se-GSHpx may play some role in the complex aetiology of RA. Dietary elimination: The scientific literature on elimination diets contains many studies showing that small numbers of RA patients respond well to dietary elimination, suggesting that individualized dietary manipulation may be beneficial for certain RA patients. Blind challenge studies are essential to confirm which foods produce symptoms. Improvement of patients on dietary therapy may result from a number of mechanisms, acting singly or in combination.	to be considered in detail for effects of arthritic activity. Further work is required to investigate the link between dietary manipulation, gut flora and clinical remission.
Diet therapy for the patient with rheumatoid arthritis (Haugen, Fraser et al. 1999)	Editorial on the evidence for diet therapy for RA patients	Most clinical dietary therapy studies undertaken so far have focused on some form of dietary elimination. A percentage (25-44%) of patients show both objective and subjective improvement during a fast followed by a strict vegetarian diet. However, few patients stick to a strict diet after the study. It has been suggested that between 5 and 30% of patients with RA may have a food allergy involvement in their disease. Evidence for possible food allergy involvement is discussed along with areas for future research. Both clinical and in vitro studies have established that long chain n-3 and n-6 fatty acids inhibit T-lymphocyte function. Research suggests that manipulating the balance of dietary fatty acids in favour of increased n-3 fatty acids and decreased n-6 fatty acids may have a beneficial effect on disease activity in RA. The balance between unsaturated and saturated fatty acids may also affect lymphocyte proliferation (in vitro). Olive oil has been shown to reduce lymphocyte proliferation, natural killer cell activity, adhesion molecule expression on lymphocytes and the production of pro-inflammatory cytokines in animal models. Although studies of supplementation with a single antioxidant have not shown disease reduction in RA patients, it is still possible that patients with an inflammatory rheumatic disease will benefit from supplementation with a combination of several antioxidants or form a dietary intake that exceeds the recommended dietary allowances.	Evidence suggesting that food allergy, defined as an immunological response to food antigens or to intestinal bacterial flora, might be involved in disease pathology in most patients with RA is weak. However, it is possible that an exogenous agent like a food antigen can initiate a pathological immune process in a genetically susceptible individual. *The practical implications of the observations on the effect on RA of different dietary fatty acid patterns are currently unclear, but suggest that a diet which is high in unsaturated fatty acids and very low in saturated fatty acids may have a stronger immunosuppresive effect than that obtained by only n-3 fatty acid supplementation.* It is worth investigating whether a diet low in saturated fats, with a high content of olive oil and with n-3 supplementation, could have immunosuppressive effects in vivo and could thus be of benefit in the treatment of RA. More knowledge on the effects of dietary components upon immunological function is necessary if the potential use of dietary therapy as a tool in the treatment of RA is to be adequately

			assessed.
Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders (Darlington and Stone 2001) The fatty acids part of this review is covered under fatty acids in Table 1	Review of latest scientific evidence for the use of antioxidants and fatty acids in the improvement of disease symptoms of RA patients	Dietary antioxidants include ascorbate and the tocopherols and beneficial effects of high doses have been reported especially in OA. There is also evidence for beneficial effects of β-carotene and selenium, the latter being a component of the antioxidant enzyme glutathione peroxidase. Vitamins C and E have important non-antioxidant effects. Ascorbate stimulates procollagen secretion and vitamin C deficiency is associated with defective connective tissue. Vitamin C is needed for the vitamin C-dependent enzyme lysyl-hydroxylase for the post-translational hydroxylation of specific prolyl and lysyl residues in procollagen – actions necessary for the stabilisation of the mature collagen fibril. Vitamin C is also thought to be necessary for glycosaminoglycan synthesis. Vitamin E blocks AA formation from phospholipids and inhibits lipoxygenase activity, resulting in a mild anti-inflammatory effect. Combined supplements of vitamins E and C are more immunopotentiating than supplementation with either vitamin alone.	It may be that different effects of the antioxidants in disease processes may depend on the hydrophilicity of the antioxidant molecules concerned and the resulting pattern of tissue distribution in different tissue areas. There is a growing scientific rationale for the use of dietary supplements as adjuncts in the treatment of inflammatory disorders such as RA and OA.

Table 5 Experimental studies of elemental and exclusion diets and rheumatoid and osteoarthritis

Study type, Country Paper reference	Study objective	Study population	Study methods	Outcomes	Conclusion or authors comments
Randomized, controlled trial UK (Kavanagh, Workman et al. 1995)	To assess the role of elemental diet and subsequent food reintroduction in RA.	47 patients with definite RA randomized into 2 parallel groups: elemental diet group (n=24) and control group (n=23). Patients were allowed to take NSAIDs but patients on corticosteroids and disease-modifying anti-rheumatic drugs were excluded.	In the diet group, the intervention consisted of an elemental diet with the addition of a small number of foods (chicken, fish, rice, carrots, runner beans and bananas) to make it tolerable, followed by a period of individualized food reintroduction. Foods were removed in the event of a worsening of joint pain or stiffness. The control group were given 2 sachets elemental diet/day plus their normal diet for duration of study. The entire study lasted 24 weeks. Clinical assessment took place at baseline, 1, 4 and 24 weeks.	42 patients completed the elimination/reintroduction period and 18 patients (38%) completed the 24 weeks. After the elemental diet there was a statistically significant improvement in the diet group in grip strength (p=0.008) and Ritchie score (p=0.006) but not in ESR, CRP, thermographic joint score or functional score. The diet group lost more weight than control group and this correlated with improvement in grip strength. This improvement was not present following food reintroduction.	As the improvements took place in more subjective disease parameters and because of the difficulties in adequately blinding studies of diet in arthritis, a placebo effect must be considered. This study shows that elemental diet can cause an improvement in a number of disease parameters in RA but this is not sustained by an individualized diet. It also illustrates some of the difficulties involved in the study of diet in arthritis.
Randomized, controlled trial Denmark (Holst-Jensen, Pfeiffer-Jensen et al. 1998)	To determine whether a commercially available elemental peptide diet could improve disease activity in RA. The diet was considered as a temporary supplement to the conventional treatment.	30 patients of either sex, aged 18-75 y with confirmed RA and at least 6 months disease duration. Randomized to elemental diet group (n=15) or control group (n=15)	6 month prospective parallel controlled trial with an intervention period of 4 weeks. Diet group: all foods replaced with a commercial liquid diet. The number of cartons consumed was recorded daily. After diet intervention, normal food was reintroduced at once.	4 weeks diet made statistically significant improvements in 2 of the subjective efficacy variables: average level of pain during the last week (p=0.02) and HAQ score (p=0.03). These effects had disappeared at 3 months follow-up. No between group differences were observed at 3 months. Only 1 patient was classified	The authors concluded that the peptide diet, as a supplement to conventional treatment can produce minor to moderate, and transient improvements in some subjective and objective disease parameters. The patients have to denounce several good things in life. Only one patient (8%) had a sufficient remission. For these reasons the peptide diet is not

			Control group: maintained their usual food habits in the study period. Dietary oils were not allowed in the diet group. Clinical assessments at baseline, 4 weeks, 3 and 6 months.	as a responder. After reintroduction of normal food, there were signs of aggravation in 6 of the 7 core variables. The variable in the diet group with the most pronounced change was BMI which showed a highly significant drop at follow-up with a significant between group difference.	a treatment of choice in unselected RA patients. Food allergy or intolerance seems to be an aggravating factor in some patients with RA and the peptide diet might be beneficial to a subset of these patients.
Double-blind, placebo-controlled, randomized clinical trial USA (Panush, Carter et al. 1983), (Panush 1991)	To determine if dietary therapy is useful for RA.	26 RA patients completed the study. 11 patients on the experimental diet 15 patients on the control diet	The experimental diet (The Dong diet) eliminated dairy products, red meat, citrus fruits, tomatoes, herbs, spices, preservatives, alcohol and coffee. The placebo diet allowed these foods but excluded other foods. Trial duration: 10 weeks 183 variables were analysed.	There were no clinically important differences among rheumatologic, laboratory, immunologic, radiologic, or nutritional findings between the experimental and placebo diet groups. 6 RA patients on the placebo and 5 on the experimental diet improved by objective criteria. Improvement averaged 29% for patients on placebo and 32% for patients on experimental diets. 2 patients on the experimental diet improved significantly and continued on the diet.	The authors state that the study failed to provide evidence of objective overall clinical benefit of the Dong diet as followed by a group of patients with longstanding, progressive, active RA. However, the data are not inconsistent with the possibility that individualized dietary manipulations might be beneficial for selected patients with RA.
Single-blind, placebo controlled, clinical trial UK (Darlington, Ramsey et al. 1986)	To run a controlled trial of a dietary manipulation therapy in RA.	53 RA outpatients	6 weeks on both dietary manipulation therapy and placebo	Significantly greater benefit occurred with diet than with placebo , with significant improvement in pain by day, by night and in 24 hours, duration of morning stiffness, number of painful joints, grip strength, ESR, Hb, fibrinogen, and platelet levels. Even more significant improvements occurred in a subgroup of 'good	A proportion of the improvement was due to a placebo response, but this was not sufficient to explain the whole improvement. The authors gave as possible explanations for improvement: reduced food intolerance, reduced gastrointestinal permeability, and benefit from weight loss and from altered intake of substrates for

				responders'.	prostaglandin production.
Dietary elimination trial UK (Darlington and Ramsey 1993)	To determine if RA patients improve symptoms on an elimination diet.	48 RA patients	6 weeks of dietary elimination therapy	41 patients identified foods producing symptoms. Cereal foods comprised 4 of the top 7 symptom-inducing foods. Corn and wheat gave symptoms in more than 50% of symptomatic patients.	This was not a blinded challenge study.
Experimental study UK (O'Farrelly, Price et al. 1989) (O'Farrelly, Melcher et al. 1988)	To determine whether patients with RA were immunologically sensitised to dietary protein	93 unselected out-patients with classical or definite RA	The patients were examined for histological or other evidence of gut abnormalities. Antibodies to milk and wheat proteins were measured using an ELISA assay. 24 patients underwent jejunal biopsy.	53 patients had raised levels of IgG antibodies to one or both dietary proteins (DP). In this DP antibody positive group, 48 (90%) had raised levels of IgA RF. Only 7 (17%) of the 40 DP antibody negative patients had detectable IgA RF ($p<0.02$). There was no association between IgM RF and DP antibodies.	The authors claim that these results demonstrate that in RA, raised levels of IgA RF are associated with an increased IgG response to antigens which enter the body through the gastrointestinal tract. A breakdown in gastrointestinal tolerance to dietary antigens may play a role in the immunopathogenesis of RA in these patients who might therefore benefit from dietary manipulation. Raised levels of IgA RF and wheat protein IgG can be used to identify patients who would benefit.
"Blinded" food challenge using encapsulated food USA (Panush, Stroud et al. 1986)	To determine if whether the symptoms of a patient with inflammatory arthritis were associated with food sensitivities.	52 year old woman with 11 yrs of class I, stage I, active disease and with symptomatic exacerbations allegedly associated with meat, milk, and beans.	An unblinded food challenge, followed by an elemental diet plus challenges with lyophilized foods placed in opaque capsules. 4 milk challenges, and some placebo and other foods challenges.	An increase in symptoms was observed after an unblinded milk challenge. The elemental diet led to significant symptom improvements and the blinded milk challenges led to increased subjective and objective symptoms, peaking 24-48 hours post challenge. Placebo and other foods were without effect.	Adverse reactions to foods do occur. In this case milk caused increased symptoms in one RA patient.

Double blind controlled food challenges using encapsulated food: clinical and serological study. USA (Panush 1990)	To confirm the groups finding of a patient with rheumatoid-like arthritis with clinical and immunological milk sensitivity, to assess the prevalence of food related rheumatic symptoms, and to idnetify clinical and serological features of these patients.	16 RA patients who allegedly had food related "allergic" arthritis, including the milk sensitive patient from an earlier study.	Each patient was subjected to 19 double-blind, controlled food challenge studies.	3 patients (including the milk sensitive patient) demonstrated subjective and objective rheumatic symptoms after double blind encapsulated food challenges. The 3 were virtually asymptomatic when receiving elemental nutrition or not taking the offending foods. One was sensitive to shrimp and one to nitrates. All three were seronegative and showed increases in some immunoglobulins e.g. IgG-milk complexes.	The authors concluded that most patients alleging food induced rheumatic symptoms did not show these on blinded challenge, but some did. Probably not more than 5% of rheumatic disease patients have immunologic sensitivity to food(s). These observations suggest a role of food allergy in at least some patients with rheumatic disease.
Double-blind, controlled trial: clinical effects Netherlands (Van da Laar and van der Korst 1992a)	To determine the clinical effects of eliminating milk allergens and AZO dyes in RA patients	94 seropositive RA patients	Two types of artificial elementary diet, one diet was allergen free, the other allergen restricted, containing only lactoproteins and yellow azo dye, were fed to the patients in a double-blind, controlled trial. Study duration –12 wks. During the second 4 week period patients were randomly assigned to one of the 2 artificial diets.	Only subjective improvements were seen overall on treating the patients with artificial, elementary food. No differences were seen between the clinical effects of the two tested diets. 9 patients (3 in the allergen restricted gp, and 6 in the allergen free gp) showed favourable responses, followed by marked disease exacerbation during rechallenge. Dietary manipulation also brought about changes in objective disease activity parameters in these patients.	The authors suggested that serious consideration should be given to the theory that food intolerance influences the activity of seropositive RA at least in some patients.
Controlled clinical trial The Netherlands (van de Laar, Aalbers et al.	To determine if sero-positive RA patients demonstrate immunoallergic symptoms to certain foodstuffs.	6 RA patients who showed favourable responses with an allergen free diet from the original group of 94 (see previous study)	Following the 4 weeks of hypoallergic, artificial diet the patients were subjected to placebo-controlled rechallenges with specific foodstuffs. In 3 patients. biopsy	4 patients showed intolerance to specific foodstuffs. In two patients, both with raised serum IgE concentrations and specific IgE antibodies to certain foods, a marked reduction of mast cells in the	The authors concluded that although the number of food intolerant patients with RA remains limited and markers of allergic activity are scanty, the outcome of this study suggests an underlying

1992b)			material from synovial membrane and proximal small intestine were obtained before and during allergen free feeding.	synovial membrane and proximal small intestine was demonstrated.	immunoallergic mechanism in some patients.
Cross-over clinical trial of dietary manipulation Italy (Gianfranceschi, Fasani et al. 1996)	To determine the effects induced by a dietary approach, reestablishing the usual tolerance to foods, on three pathological features of RA (stiffness, pain and joint swelling).	12 RA patients aged 42-69 y with stable RA for an average of 10.1 y, under drug control.	Each patient underwent a dynamometric challenge test (DRIA test) to detect foods suspected of causing food intolerance. The effects of two different normocaloric diets (Diet A no suspect foods admitted and diet B well-balanced diet) for 3 months and then crossed over after a one-month wash out.	After Diet A, patients had 42% less joint pain (95% CI –58 to –25%; $p<0.005$) and 40% less morning stiffness. Results from Diet B were not significantly different from control.	The authors conclude that diet and avoidance of selected foods appear to be useful in RA management, and the DRIA test represents a practical clinical tool for establishing the best diet. The diet for the relief of RA symptoms cannot be a standard one, but should be selected according to individual food hypersensitivities.

Table 6 Experimental studies of vegetarian diets and rheumatoid arthritis

Study type, Country Paper reference	Study objective	Study population	Study methods	Outcomes	Conclusion or authors comments
Randomised, single-blind placebo-controlled trial Norway (Kjeldsen-Kragh, Haugen et al. 1991)	To assess the effect of fasting followed by one year of a vegetarian diet (individually adjusted) on RA patients	53 RA patients with active disease (45 women and 8 men) randomised to 27 in the diet group (mean age 51 years) and 26 in the control group (mean age 55 years). In the diet group, 1 patient terminated the study after 1 month and a further 3 patients after 4 months. 23 patients continued on the lactovegetarian diet for more than 3 months. In the control group, 1 patient terminated the study after 1 month and a further 4 patients after 4 months in the study.	The dietary group (n=27) were allocated to a 4-week stay at a health farm. After an initial 7-10 day subtotal fast, they were put on an individually adjusted gluten-free vegan diet for 3.5 months. The food was then gradually changed to an individually adjusted lactovegetarian diet for the remainder of the 13-month study. The control group (n=26) stayed for 4 weeks at a convalescent home, but ate an ordinary diet throughout the whole study period. Clinical examinations (listed under results) were performed 0, 1, 4, 7, 10, and 13 months and blood and urine samples were taken. 24-h dietary recalls were carried out at these times and at 1.5, 5.5, 8.8, and 11.5 m in both groups.	After 4 weeks at the health farm the experimental diet group showed a significant improvement in number of tender joints, Ritchie's articular index, number of swollen joints, pain score, duration of morning stiffness, grip strength, ESR, C-reactive protein, white blood cell count, and a health assessment questionnaire score. In the control group, only pain score improved significantly. The benefits in the diet group were still present after one year.	Evaluation of the whole course showed significant advantages for the diet group in all measured indices. The authors concluded "This dietary regimen seems to be a useful supplement to conventional medical treatment of rheumatoid arthritis".
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Haugen, Kjeldsen-	To determine the fatty acid profiles of the plasma phospholipids during the vegetarian diets and by examining whether the changes of the fatty acid concentrations were associated with	RA patients with active disease – see above. Patients in the diet group were divided into 'responders' and 'non-responders' using the disease improvement index based on clinical	The intervention is described above. 5 patients in each group took cod-liver oil supplementation daily throughout the study. Blood was drawn and dietary and clinical assessments (see below) were carried out at inclusion,	The concentrations of fatty acids 20:3n-6 and 20:4n-6 were significantly reduced after 3.5 months with a vegan diet ($p<0.0001$) and $p<0.01$ respectively), but the concentration increased to baseline values with a lactovegetarian diet. The	The changes in the fatty acid profiles in plasma phospholipids as a result of a vegan and lactovegetarian diet were extensive in patients with RA. However, the clinical improvement could not be attributed to these

Kragh et al. 1994)	concomitant changes in subjective and/or objective variables of disease activity. The concentrations of serum lipid peroxidation products were also measured for the same purpose.	variables listed in next column. To be characterised as diet responders the patients had to have improved substantially in at least 3 of these core variables at each of the last 3 clinical examinations.	after 1 month, after 4 months, and at the time point at which the patients left the study. Clinical assessments (variables): no. of swollen joints, HAQ index, pain score on a visual analogue scale, no. of tender joints, patients global assessment, and ESR (now classified as the core measures for clinical trials in RA (Tugwell and Boers 1993).	concentration of 20:5n-3 was significantly reduced after the vegan diet ($p<0.0001$) and the lactovegetarian diet periods ($p<0.01$). There was no significant difference in fatty acid concentrations between diet responders and diet non-responders after the vegan or lactovegetarian diet periods.	changes.
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Peltonen, Kjeldsen-Kragh et al. 1994)	To examine the role of faecal flora in the diet-induced improvement of RA patients.	For subjects see above.	For intervention see above. Clinical assessments see above. Stool samples were collected at each visit: before (pretest sample) and 5 times during the study period (after 1, 4, 7, 10 and 13 months). In the diet group the baseline samples represent omnivorous diet, the samples at 1 and 4 month the vegan diet, and those collected at 7,10 and 13 months, the lactovegetarian diet. GLC was used to generate bacterial cellular fatty acid profiles of the stool samples.	Patients were divided into high improvement index (HI) and low improvement index (LI). Significant alterations in the intestinal flora was observed when the patients changed from omnivorous to vegan diet. There was also a significant difference between the periods with vegan and lactovegetarian diets. The faecal flora from patients with HI and LI differed significantly from each other at 1 and 13 months during the diet.	The results show that a diet can change intestinal flora and this may somehow be beneficial in RA, perhaps by reducing intestinal inflammation and absorption of dietary and/or bacterial antigens, or in other ways. The early appearance (at 1 month) of the difference in faecal flora between Hi and LI groups suggest that the primary change would be in the faecal flora rather than in disease improvement.
Follow-up of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway	To investigate whether improvements obtained were still present one year after the trial subjects had finished the trial. Also to know to what extent the	Subjects are described above. The Diet group were defined as responders or non-responders according to whether they showed substantial	After the last clinical examination of the trial, the patients were free to change their diet or medication. A new clinical examination was carried out using the same variables as at earlier	The following variables favoured diet responders: pain score, duration of morning stiffness, HAQ index, no. of tender joints, Ritchie's articular index, no. of swollen joints, ESR, platelet count and white blood cell count.	The findings indicate that a group of patients with RA benefit from dietary manipulations and that the improvement can be sustained through a two-year period.

(Kjeldsen-Kragh, Haugen et al. 1994a)	patients in the diet group still followed the diet.	improvement in 3 or more core variables at all of the last 3 clinical examinations. 10 diet group responders, 12 diet group non-responders and 23 control group were examined one year after the end of the clinical trial	examinations.	The differences between the 3 groups were significant for all the clinical variables, except for grip strength. There was no significant difference between the groups with regard to laboratory or anthropometric variables. At the time of the follow-up examination all diet responders but only half of the diet non-responders still followed a diet.	
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Kjeldsen-Kragh, Hvatum et al. 1995a)	To compare serum antibody activity against dietary antigens in patients with RA and healthy controls, and to examine whether anti-food antibody activity fluctuated with disease activity.	As above. In addition 30 healthy controls without any history of allergy were selected from the hospital and laboratory staff.	As above. In addition serum IgG, IgA and IgM antibody activity against several food antigens was measured by an enzyme immunoassay. Abnormally high antibody activity was defined as values above the 90^{th} percentile of the measurements in 30 healthy controls. Serum IgE antibody activity was measured by a radioallergoabsorbent test.	During the trial 10 of 27 patients suspected that certain food items aggravated their arthritis symptoms. Elevated antibody activity against one or more of the dietary antigens was found in all RA patients, but these measurements could not be used to predict which food would aggravate the symptoms. Elevated IgG and IgA antibody activity against α-lactalbumin was found in a significantly larger number of RA patients than in controls. With the exception of one patient, there was no concordance between the clinical course and antibody activity against the various dietary antigens.	The results indicate that a systemic humoral immune response against food items is probably not involved in the pathogenesis of RA.
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Kjeldsen-Kragh,	To examine to what extent biochemical and immunological variables changed during the clinical trial of fasting and vegetarian diet.	See above.	See above. In addition the following biochemical and immunological variables were measured: Haemoglobin, platelet count and white blood cell count; C3-complement activation products and terminal complement complex;	There was a significant decrease in platelet count, leukocyte count, calprotectin, total IgG, IgM rheumatoid factor (RF), C3-activation products, and the complement components C3 and C4 after one month of treatment in the diet (vegetarian) group. None of the measured parameters	The results of this study are in accordance with the findings from the clinical trial, namely that dietary treatment can reduce the disease activity in some patients with RA.

Mellbye et al. 1995c)			calprotectin, TNFα, IgG, IgA, and IgM.	changed significantly during this period in the control group (omnivores). The course of 14 of 15 measured variables favoured the vegetarians compared with the omnivores, but the difference was only significant for leukocyte count, IgM RF, and the complement components C3 and C4. Most of the laboratory variables declined considerably in the vegetarians who improved according to clinical variables, indicating a substantial reduction in inflammatory activity. The leukocyte count decreased in the vegetarians irrespective of the clinical results, indicating that this decrease may be attributed to vegetarian diet *per se* and not to the reduction in disease activity.	
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Kjeldsen-Kragh, Rashid et al. 1995d)	To measure *Proteus mirabilis* and *Escherichia coli* antibody levels in patients with RA during treatment by vegetarian diet.	See above	*P mirabilis* and *E coli* antibody levels were measured by an indirect immunoflourescence technique and an enzyme immunoassay respectively in the sera of the 53 RA patients taking part in the trial.	The diet group (vegetarian diet) had a significant reduction in the mean anti-proteus titres at all time points during the study, compared with baseline values (all $p<0.05$), No significant change in titre was observed in the control group (omnivorous diet). The decrease in anti-proteus titre was greater in the diet responders compared with diet non-responders and controls. The total IgG concentration and levels of antibody against *E coli* were almost unchanged in all patient groups during the trial.	The decrease from baseline in proteus antibody levels correlated significantly ($p<0.001$) with the decrease in a modified Stoke disease activity index. The decrease *in P mirabilis* antibody levels in the diet responders and the correlation between the decrease in proteus antibody level and decrease in disease activity supports the suggestion of an aetiologic role for *P mirabilis* in RA.
Supplementary study	To examine whether	22 consecutive	Urine samples were	The growth of both bacteria in	These results show that

to the above clinical trial Norway (Kjeldsen-Kragh, Kvaavik et al. 1995b)	fasting and vegetarian diet may influence the growth of *Proteus mirabilis* and *Esherichia coli* in urine	omnivorous patients who were admitted to a health farm (same one used above) for various reasons. Exclusions were if they had received antibiotics during the last 14 days, were taking corticosteoids, or if they had diabetes, renal diseases or diseases of the urogenital system.	collected at baseline, and after 8 and 18 days from the 22 patients. The dietary regimen recommended by the health farm consisted of fasting for 7 to 10 days followed by a vegan diet.	urine samples collected after 8 days was significantly slower than in samples collected at baseline. In urine samples collected after 18 days growth was also reduced, although not significantly for *E coli*.	dietary manipulation may reduce the ability of urine to support the growth of *P mirabilis* and *E coli*.
Further analysis of the above (K-K 1991) randomised, single-blind placebo-controlled trial Norway (Kjeldsen-Kragh, Sumar et al. 1996)	To correlate changes in the proportion of IgG that lacks terminal galactose [%G(0)] with clinical changes during a 7 to 10 day period of fasting followed by 3.5 months of vegetarian/vegan diet.	The 26 RA patients in the diet group	The intervention is described above. Serum samples were stored at -20˚C until required for analysis. The disease improvement index described above was used to assess clinical improvement. %G(0) was measured in sera taken at baseline, after the fasting period and after 3.5 months.	There was no significant difference in the mean %G(0) between baseline samples and samples collected either after the fasting period or after 3.5 months of vegan diet. However, the difference between %G(0) at baseline and after the fasting period correlated significantly with the disease improvement index after the fasting period (Kendall's τ=0.29, p=0.04). After 3.5 months on a vegan diet, the correlation between the change in %G(0) and disease improvement index was no longer significant.	Although the glycosylation status of IgG may have played a role in the improvement of disease during the fasting period, it did not seem to be associated with, and therefore responsible for, the clinical improvement observed after the vegetarian diet.
Human cell study Norway (Fraser, Thoen et al. 1999)	To measure whether changes in the concentrations of circulating free fatty acids (FFAs) after a 7-day fast in RA patients would inhibit in vitro T-lymphocyte proliferation	Nine RA patients	The concentration and composition of plasma FFAs were measured in the 9 patients at the conclusion of a 7-day fast. A FFA mixture was made up based on these findings. Mitogen-induced lymphocyte proliferative responses were measured after co-culture of peripheral blood mononuclear cells (PBMC) from healthy	The FFA mixture based on the composition of RA patients plasma was 20% linoleic, 43% oleic, 10% stearic, 27% palmitic. Both concentrations of the FFA mixture and the ratio between the unsaturated and saturated fatty acids significantly influenced *in vitro* lymphocyte proliferation ($p<0.0001$). The highest concentrations of the FFA mixture increased lymphocyte	The finding that the highest concentrations of FFA mixture increased lymphocyte proliferation was unexpected. Fasting-associated increases in total plasma FFA concentrations do not inhibit, but rather enhance, *in vitro* lymphocyte proliferation. An inhibitory effect could

			individuals in the presence of increasing concentrations of the FFA mixture (from 0 to 2000 μM) and in the presence of FFA mixtures where the relative proportions of fatty acids varied.	proliferation. At equimolar concentrations (600 μM), manipulating the amounts of oleic and linoleic fatty acids relative to stearic and palmitic fatty acids had a potent inhibitory effect upon lymphocyte proliferation.	only be achieved by manipulating the balance between the unsaturated and saturated fatty acids.
Randomized, controlled clinical trial Finland (Peltonen, Nenonen et al. 1997)	To clarify the role of the faecal flora in the diet-induced decrease of RA activity.	43 consecutive adult RA patients with active disease visiting a rheumatic out-patient clinic in Helsinki. Patients randomized into 2 groups: the test group (n=22) to receive living food, a form of uncooked vegan diet rich in lactobacilli, and the control group (n=21) to continue their previous omnivorous diets. Caffeine-containing drinks, chocolate, alcohol and tobacco smoking were prohibited in both groups. No antibiotics were used during the study. All patients continued their previous medication.	The diet intervention was originally planned for 3 months. Dietary records were collected for 1 week prior to intervention and at 1 month. The test group received all the components of the diet daily and were supervised daily in the use of the diet. The disease index was based on 6 disease activity parameters. Patients with improvement of 20% or more in 5 or 6 of these parameters were assigned to high improvement (HI) index and the rest to low improvement index (LO) Blood and stool samples were collected before the intervention and at 1 month (stool samples from the test group also after the intervention period). Stool samples were analysed by direct stool sample GLC of bacterial cellular fatty acids.	18 subjects in both groups completed the study satisfactorily. 5 patients (27.8%) in the test group and none in the control group belonged to the HI category. A significant diet-induced change in the faecal flora ($p=0.001$) was observed in the test group, but not in the control group. Also, in the test group, a significant ($P=0.001$) difference was detected between the HI and LO categories at 1 month, but not in the pre-test samples. The vegan diet also induced a decrease in disease activity in some of the RA patients.	The authors conclude that a vegan diet changes the faecal microbial flora in RA patients, and changes in the faecal flora are associated with improvement in RA activity. The methods and experimental design do not allow the determination of what exactly in the faecal flora in the HI category is different from the LO category and whether the difference is linked to the disease improvement causally.
Dietary analysis of the vegan diet used in the above trial Finland	To investigate the food and nutrient intakes of RA patients on a strict uncooked vegan diet.	Patients as above	As above. 13 of the subjects in the vegan diet group followed the diet for 2 months and only five subjects followed it for 3	The RA patients had lower than recommended intakes of iron, zinc and niacin, and their energy intake was low compared to mean daily energy intake of the	The study shows that, in spite of the improved energy and nutrient content of this diet and improvements in disease

(Rauma, Neonen et al. 1993)			months. 7-day diet records were collected before and during the 3 month intervention. In addition the energy-yielding nutrient content of a pooled sample representing 10 days' menu of the vegan diet were determined. Nutrient intakes were calculated with Finnish dietary analysis program.	healthy Finnish population of the same age. The uncooked vegan diet significantly increased the intakes of energy ($p<0.001$), many nutrients (Fe, Zn, K, thiamin, niacin, vitamin E) and fibre. It greatly reduced the intakes of Na and cholesterol. In spite of the increased energy intake, the diet group lost 9% body wt during the intervention period, indicating a low availability of energy from the vegan diet.	parameters, RA patients find it difficult to pursue an uncooked vegan diet. The preparation of this diet is onerous and the taste of the food is unfamiliar. It would also appear to be difficult to meet energy requirements because of the low biological availability of the energy-yielding nutrients due to the high fibre content and insufficient food processing.
Further analysis of the above (Peltonen 1997) randomized, controlled, clinical trial Finland (Nenonen, Helve et al. 1998)	To investigate subjective and objective effects of an uncooked vegan diet, rich in lactobacilli, on chronic RA, and to select possible therapeutic components of the diet for further studies.	Patients as above.	As above. The duration of the intervention was planned to be 3 months, but 8 patients out of 19 had to stop their intervention diet after 2 months because of nausea, diarrhoea or difficulties with the taste of some food items. Laboratory samples (blood, urine and faecal samples) were collected at pre-intervention (weeks –1 and 0), after the first month (weeks 4-5), at the end of the intervention period (weeks 8-9 or 12-13), and 3 months after the intervention period. There were no differences between the 2 and 3- month intervention groups, and the duration of the intervention had no effects on the clinical outcome.	The intervention group experienced subjective relief of rheumatic symptoms during the intervention. A return to an omnivorous diet aggravated symptoms. Indicators of rheumatic disease did not differ statistically between groups. The positive subjective effect experienced by the patients was not discernible in the more objective measures of disease activity. A composite index showed a higher no. of patients with 3-5 improved disease activity measures in the intervention group. Stepwise regression analysis associated a decrease in the disease activity (measured as change in the DAS (Scott, van Riel et al. 1993)) with lactobacilli–rich and chlorophyll-rich drinks, increase in fibre intake, and no need for gold, methotrexate or steroid	The results showed that an uncooked vegan diet, rich in lactobacilli, decreased subjective symptoms of RA. Large amounts of living lactobacilli consumed daily may also have positive effects on objective measures of RA.

				medication (P=0.02).	
Further analysis of the above (Peltonen 1997) randomized, controlled, clinical trial Finland (Hanninen, Kaartinen et al. 2000)	To examine the effectiveness of the living food on the symptoms of fibromyalgia and RA patients and discuss the relation of the symtoms to the antioxidants and other diet components.	As above plus 20 long term users (up to 14 years) of the 'living food' (LF) diet and their controls (n=20) as well as 33 fibromyalagic subjects divided into LF intervention and omnivorous controls.	As above plus analyses of biochemical parameters: serum carotenoids, flavonoids, and antioxidant vitamins. To estimate the activity of RA a relative activity index (RAI) which highly correlates with DAS index was created.	The subjects eating LF showed highly increased levels of β- and α- carotenes, lycopene and lutein in their sera. The increases of vitamin C and E (adjusted to cholesterol) were statistically significant. As the berry intake was 3-fold compared to controls the intake of polyphenolic compounds, quercetin, myricetin and kaempherol was much higher than in the omnivorous controls. The LF diet is rich in fibre, substrate of lignan production, and the urinary excretion of polyphenols like enterodiol and enterolactone as well as secoisolaricirecinol were much increased in subjects eating LF. The RA patients eating the LF diet reported positive subjective responses and the objective measures supported this finding. The improvement of RA was significantly correlated with the day-to-day fluctuation of subjective symptoms.	The RA patients subjectively benefited from the vegan diet rich in antioxidants, lactobacilli and fibre, and this was also seen in objective measures.
Further analysis of the above (Peltonen 1997) randomized controlled, clinical trial Finland (Agren, Tvrzicka et al. 2001)	To investigate the effects of the 'living food' diet on serum lipid and sterol concentrations in patients with RA.	Patients from the larger study described above (Peltonen, Nenonen et al. 1997). 16 subjects following the vegan diet (all females, mean age 49 (SD 7) years, BMI 25.6 (SD 4.3) kg/m^2 and 13 control subjects (1 male and 12 females, mean age 53 (SD 11) years, BMI	Serum total and LDL-cholesterol and –phospholipid concentrations were significantly decreased by the vegan diet. The levels of serum cholestanol and lathosterol also decreased, but serum cholestanol:total cholesterol and lathosterol: total cholesterol did not change. The effect of a vegan diet on	The authors concluded that a strict uncooked vegan diet decreased serum cholesterol, phospholipid, cholestanol and lathosterol concentrations without changing their ratios. The effect of a vegan diet on two major plant sterols, sitosterol and campesterol, was opposite. These results suggest that a strict uncooked vegan diet changes the relative absorption rates of these	

		22.6 (SD 2.7) kg/m^2. Fasting blood samples collected before the start of the dietary period, after 1 month and at the end of the intervention period (2 or 3 months). Serum cholesterol, HDL-cholesterol, triacylglycerol and phospholipid concentrations, and sterols were determined.	serum plant sterols was divergent as the concentration of campesterol decreased while that of sitosterol increased. This effect results in a significantly greater sitosterol:campesterol value in the vegan diet group than in the control group (1.48 (SD 0.39) v. 0.72 (SD 0.14); $p<0.001$). A higher concentration of campesterol compared with sitosterol is normal in omnivorous subjects and can be explained by lower absorption and esterification rates of sitosterol.	sterols and/or their biliary clearance.	

Table 7 Studies of nutrient intakes, including supplements, and nutritional status and OA

Paper title and reference	Review or study objectives	Summary of findings	Conclusions
Osteoarthritis. A continuing challenge. (Sack 1995)	A review	Describes the structure and function of cartilage and the changes in OA. Obesity plays a role in the occurrence and progression of the disease in the knees and also appears to be a modest predictor of OA in the hands. In some animal models a diet high in saturated fat increases the severity of the disease, but in others this effect has been hard to reproduce. Some secondary forms of OA have a presumed dietary association, e.g. Kashin-Beck disease is a noninflammatory disorder of enchondral bone growth, and proposed causes have included excesses or deficiencies of trace elements.	No conclusion regarding dietary factors although the paper considers obesity as a contributory factor to the occurrence and progression of knee OA. Author's conclusion: To meet the challenge of this disease, factors leading to its onset and progression need to be clarified and practical methods of preserving cartilage need to be devised.
Nutrition: risk factors for osteoarthritis (McAlindon and Felson 1997)	Review of literature and reference to their own study, the Framingham OA cohort study.	Overweight people are at considerably increased risk for the development of OA in their knees, and may also be more susceptible to both hip and hand joint involvement. Evidence for a direct effect of dietary fat intake has proved inconclusive. Weight reduction may reduce the risk for the development or progression of OA. The Framingham cohort study has shown that weight loss of approx. 5 kg will reduce a person's risk for the development of knee OA over the subsequent 10 years by 50%. Dietary antioxidants: Vitamins C and E and β-carotene (or other carotenoids) have been hypothesised as being protective against OA. The results of the Framingham study (see below) do not support the hypothesis that diets high in antioxidant micronutrients reduce the risk of incident knee OA, they do raise the issue about whether people with established OA might benefit from higher in intakes. Benefit from vitamin E treatment has been suggested in several small studies of OA including a 6 week double blind placebo controlled trial of 400 mg α-tocopherol in 56 OA patients. Vitamin E treated patients experienced greater improvement in every efficacy measure including pain at rest (69% better with vitamin E v. 34% better with placebo, $p<0.05$), pain on movement (62% better with vitamin E v. 27% with placebo, $p<0.01$), and use of analgesics (52% less with vitamin E v. 24% less with placebo, $p<0.01$). Non-oxidant effects of vitamins C and E - Vitamin C performs biochemical functions that might be clinically important in OA: i) through the vitamin C dependent enzyme lysylhydroxylase, vitamin C is required for the post-translational hydroxylation of specific prolyl and lysyl residues in procollagen, a	Nutritional factors can be hypothesised to influence the course of OA through a wide variety of mechanisms. Preliminary results from numerous laboratory and observational studies seem to support this possibility. On the other hand studies of such factors in relation to a slowly progressive chronic disease are limited by many considerations such as the potential for confounding, problems with outcome definitions, absence of indicators of disease activity, imprecision and misclassification in measurement of dietary variables, and by many other factors. While providing no definitive answers, they underscore the importance of nutrition as an important area for further research and pull the subject of 'diet and arthritis' into the domain of scientific research.

		modification essential for stabilisation of the mature collagen fibril. Also it acts as a carrier of sulphate groups, and seems to be required for glycosaminoglycan synthesis. Some animal and cell studies to back this up. ii) Vitamin E blocks the formation of arachidonic acid from phospholipids and inhibits lopoxygenase activity, although it has little effect on cyclooxygenase. This suggest that vitamin E could affect the modest synovial inflammation that sometimes accompanies OA. Vitamin D – suboptimal vitamin D concentrations may have adverse effects on calcium metabolism, osteoblast activity, matrix ossification, and bone density. Low tissue concentrations may impair the ability of bone to respond optimally to pathophysiological processes in OA, and predispose to disease progression. Articular cartilage, especially cartilage from OA seems to be sensitive to the effects of vitamin D, although its exact effects on matrix synthesis and degradation are unclear. The Framingham study found that low serum concentration, and low intake, of vitamin D each seemed to be associated with an increased risk of knee OA progression.	
Osteoarthritis. Manageable scourge of aging. (Kee 2000)	A review of current literature to focus the attention of health care providers on preserving function, preventing disability, and managing discomfort for those with OA	Obesity is more often associated with progressive OA of the knee than the hip. A relationship also has been found between obesity and hand OA, however, this is a finding hard to attribute to the stress of excess weight. It is likely that both joint load and systemic factors play a role. Antioxidants: The results from the Framingham study for vitamins C and E and β-carotene and for vitamin D were noted. Maintaining vitamin C and E intake is believed to be particularly critical to OA. Osteoporosis: an inverse relationship between osteoporosis and OA has been found. Cross-sectional studies have linked high bone density with OA, and both in turn are linked with obesity. It has been speculated that osteophyte production may be greater in persons with high bone mass. Less bone mass may mean less resistance (more tolerance) to forceful impact so that less damage to the cartilage occurs. Nutrition: Overall nutrition is a critical element in health maintenance and health restoration. The USA Food and Drug Administration recommendations, which substantially increase the number of fruits and vegetables to be eaten daily, are supported by research into dietary factors important to preventing OA.	There is no cure for OA but a number of potentially effective interventions can be used. OA can be managed successfully, but attentiveness to multifocal therapeutic regimens and new research findings are required.
Osteoarthritis: No cure, but many options for symptom relief.	A review of recent developments and summary of clinical	Lists risk factors for OA as being: Advanced age, female gender, excess weight, estrogen deficiency, deficiencies of vitamins C and D, physical activity and trauma.	*The authors gave some key points one of which was:* *All patients with OA should receive*

(McKinney and Ling 2000)	features and treatments currently available.	Lists nonpharmacologic therapies which include weight loss (for knee and hip arthritis). Dietary supplements: Glucosamine and chondroitin in combination are being promoted, however, credible evidence for their use is still lacking and quotes McAlindon et al's study (McAlindon, LaValley et al. 2000) which concludes that his combination has some efficacy, but its overall benefits have been exaggerated. The dietary supplement, S-adenosylmethionine (SAM) has been shown over 20 years of studies to be effective in controlling pain in OA.	*nonpharmacologic therapies, whether or not they also receive pharmacologic therapies, but the reader was left to decide which therapies.*
Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? (McAlindon, Jacques et al. 1996b)	Cumulative damage to tissues, mediated by reactive oxygen species, has been implicated as a pathway that leads to many of the degenerative changes associated with ageing. The Framingham OA cohort study was used to test the hypothesis that increased intake of antioxidant micronutrients might be associated with decreased rates of OA in knees, a common age-related disorder. The Framingham study is a USA prospective cohort study	640 participants received complete radiographic assessments of their knees at two time points. Incident and progressive OA occurred in 81 and 68 knees, respectively. There was no significant association of incident knee OA with any micronutrient , but for progression of radiographic knee OA, there was a three fold reduction in risk for those in the middle and highest tertiles for vitamin C intake (aOR for highest v. lowest tertile =0.3; 95% CI 0.1-0.6). This related predominantly to a reduced risk of cartilage loss (aOR = 0.3; 95% CI 0.1-0.8). (Mean intake of vitamin C in the 3 tertiles was 81, 152 and 430 mg/d). Those in the highest tertile for vitamin C intake also had reduced risk of developing knee pain during the course of the study (OR=0.3; 95% CI 0.1-0.9). Reduction in risk for progression was also seen for β-carotene (aOR 0.4 for highest v. lowest tertile of intakes; 95% CI 0.2-0.9) and vitamin E (aOR 0.7 for highest v. lowest tertile of intakes; 95% CI 0.3-1.6) but was less consistent i.e. β-carotene association diminished considerably after adjustment for vitamin C, and the vitamin E effect was seen only in men. No significant associations were seen for the non-antioxidant nutrients.	High intake of antioxidant micronutrients, especially vitamin C, may reduce the risk of cartilage loss and disease progression in people with OA. No effect of antioxidant micronutrients on incident OA was found. These preliminary findings warrant confirmation.
Clinical efficacy of Spondyvit® (Vitamin E) in activated arthroses. A multicenter, placebo-controlled, double blind study. (German) (Blankenhorn 1986)	A trial to determine whether vitamin E supplementation was superior to placebo in reducing pain in OA patients	50 patients with OA were randomly assigned to 2 groups and treated over a period of 6 weeks with vitamin E-capsules (daily dose 400 I.E. d-α-tocopherylacetate) or an identical placebo preparation. The results showed that vitamin E was superior to placebo with respect to the relief of pain (pain at rest, pain during movement, pressure-induced pain) and the necessity of additional analgesic treatment ($p<0.05$ to $p<0.01$). Improvement of mobility was better in the group receiving vitamin E, however, this was not statistically significant. The intensity of adverse reactions in both the vitamin E and placebo group was practically identical.	This study shows that vitamin E reduced pain in patients with OA. This may possibly lead to a reduction in standard analgesic therapy.

Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study (McAlindon, Felson et al. 1996a)	To determine whether dietary intake and serum levels of vitamin D would predict the incidence and progression of osteoarthritis of the knee in participants of the Framingham cohort study.	*556 participants had complete assessments, including knee radiography and vitamin D intakes and serum measures at two time points.* Incident OA occurred in 75 knees; progressive OA occurred in 62 knees. Risk for progression increased 3-fold in participants in the middle and lower tertiles for both vitamin D intake (OR for lowere v. upper, 4.0 95% CI, 1.4-11.6) and serum levels of vitamin D (OR for lower v. highest, 2.9; 95% CI 1.0-8.2). Low serum levels of vitamin D also predicted loss of cartilage, as assessed by loss of joint space (OR, 2.3; 95% CI 0.9-5.5) and osteophyte growth (OR, 3.1; 95% CI 1.3-7.5). Incident OA of the knee occurring after baseline was not consistently related to either intake or serum levels of vitamin D.	Low intake and low serum levels of vitamin D each appear to be associated with an increased risk for progression of OA of the knee.

Table 8

Foods allowed during the Norwegian vegetarian trial following the fasting period (Kjeldsen-Kragh, Haugen et al. 1991)

	***Strict gluten-free vegan diet (3.5 m)**	**Lacto-vegetarian diet (12 m) (Note: food items introduced one at a time and excluded if they exacerbated arthritis symptoms)**
Vegetables	All vegetables except tomatoes and cucumbers	All vegetables
Root vegetables	Potatoes, carrots, turnips, and beets	Same as vegan die
Fruits	All kinds of dried fruit, fresh pears, peaches, bananas, and melon	All kinds
Grain	Millet, buckwheat, rice, cornflower, and cornstarch	All kinds
Seeds	Sunflower seeds, linseeds, sesame seeds, and pumpkin seeds	Same as the vegan diet
Oils	All kinds	Same as the vegan diet
Lentils	All kinds	Same as the vegan diet
Dairy products	None	All kinds of milk and dairy products
Beverage	Herb teas, vegetable broths, and decocted vegetables	Same as vegan diet plus tea, fruit juices, and alcoholic beverages, except for red wine
Sweets	Honey and small amounts of brown sugar	Same as vegan diet

*Nutritional needs for vitamin D were met either by taking cod liver oil, or a vitamin D supplement, nutritional needs for calcium were met by either drinking milk made from sesame seeds or patients were encouraged to take a calcium supplements.

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Appendix 2

Table 9 Age-adjusted mean (SD) macronutrient intakes (% total energy) within each group of FNVW consumers

a) Adults 1986-7 (16-64y)

	Men				Women			
	Low	*Med*	**High**	**P**	**Low**	**Med**	**High**	**P**
Fat	37 (6)	38 (5)	37 (5)	**0.21**	40 (6)	40 (5)	37 (6)	**<0.001**
Protein [a]	13 (3)	14 (4)	15 (3)	**<0.001**	15 (4)	15 (3)	16 (4)	**<0.001**
CHO	41 (7)	42 (6)	42 (6)	**0.15**	43 (6)	42 (6)	43 (7)	**0.27**
Alcohol [b]	9 (11)	7 (7)	6 (8)	**0.007**	2 (4)	3 (4)	3 (4)	**0.04**
Energy (MJ/d)	9.4 (2.8)	10.3 (2.3)	10.9 (2.3)	**<0.0001**	6.6 (1.8)	7.3 (1.8)	7.5 (1.9)	**<0.0001**

P values from ANCOVAwith age as a continuous covariate
[a] = Significant linear trend observed ($P<0.0001$) also
[b] = Kruskal Wallis H test, men: $P=0.65$, women: $P<0.0001$

b) Elderly 1994-5 (>65y)

	Men				Women			
	Low	*Med*	**High**	**P**	**Low**	**Med**	**High**	**P**
Fat	35 (7)	35 (5)	34 (5)	**0.19**	39 (6)	36 (6)	34 (6)	**<0.001** [a]
Protein	15 (3)	15 (3)	15 (3)	**0.59**	15 (3)	16 (3)	17 (4)	**0.046** [a]
CHO	45 (7)	46 (6)	47 (7)	**0.03**	45 (6)	47 (6)	48 (6)	**<0.001**
Alcohol [b]	5 (9)	4 (6)	4 (5)	**0.28**	1 (2)	1 (3)	1 (2)	**0.34**
Energy (MJ/d)	6.9 (1.8)	8.1 (1.9)	8.9 (1.9)	**<0.0001**	5.4 (1.5)	5.9 (1.2)	6.4 (1.3)	**<0.0001**

P values from ANCOVA with age as a continuous covariate
[a] = Significant linear trend observed ($P<0.001$) also
[b] = Kruskal Wallis H test, men: $P=0.28$, women: $P=0.17$

Table 10 Mean (95% CI) nutrient intakes adjusted for age and total energy intake

a) Adults 1986-7

	Men				Women			
	Low	*Med*	**High**	**P**	**Low**	**Med**	**High**	**P**
Vitamin C (mg/MJ/d)	3.9 (3.6, 4.2)	5.9 (5.6, 6.3)	9.9 (9.2, 10.7)	**<0.0001**	4.5 (3.8, 5.0)	8.1 (7.6, 8.7)	14.9 (13.8, 16.1)	**<0.0001**
Vitamin E (mg/MJ/d)	0.77 (0.72, 0.82)	0.91 (0.87, 0.95)	1.22 (1.13, 1.32)	**<0.0001**	0.82 (0.78, 0.86)	1.04 (0.98, 1.11)	1.26 (1.17, 1.35)	**<0.0001**
Sodium (mg/MJ/d)	321 (309, 332)	315 (306, 324)	328 (318, 338)	0.18	329 (318, 340)	336 (327, 345)	331 (322, 341)	0.60
Potassium (mg/MJ/d)	283 (275, 291)	306 (299, 312)	354 (347, 363)	**<0.0001**	305 (295, 315)	332 (323, 341)	405 (392, 417)	**<0.0001**
Calcium (mg/MJ/d)	81 (78, 84)	90 (87, 93)	96 (93, 100)	**<0.0001**	91 (86, 95)	102 (99, 106)	111 (107, 115)	**<0.0001**
Carotenoids (µg/MJ/d)	103 (90, 117)	185 (167, 204)	292 (264, 322)	**<0.0001**	116 (103, 131)	229 (208, 253)	393 (356, 433)	**<0.0001**
Zinc (mg/MJ/d)	0.99 (0.95, 1.04)	1.08 (1.04, 1.11)	1.22 (1.18, 1.26)	**<0.0001**	1.1 (1.0, 1.1)	1.2 (1.1, 1.2)	1.3 (1.3, 1.3)	**<0.0001**
Magnesium (mg/MJ/d)	27 (26, 28)	30 (30, 31)	38 (37, 39)	**<0.0001**	27 (26, 28)	33 (32, 34)	40 (39, 42)	**<0.0001**

P values from ANCOVA with age and total energy intake as continuous covariates

b) Elderly 1994-5

	Men				Women			
	Low	*Med*	**High**	**P**	**Low**	**Med**	**High**	**P**
Vitamin C (mg/MJ/d)	4.4 (3.8, 5.0)	6.7 (6.0, 7.5)	11.2 (10.3, 12.3)	**<0.0001**	5.2 (4.6, 5.9)	8.3 (7.5, 9.3)	17.3 (15.8, 19.0)	**<0.0001**
Vitamin E (mg/MJ/d)	0.90 (0.81, 1.01)	0.97 (0.89, 1.06)	1.16 (1.06, 1.27)	**0.001**	0.91 (0.82, 1.01)	1.15 (1.02, 1.30)	1.34 (1.20, 1.50)	**<0.001**
Sodium (mg/MJ/d)	328 (313, 344)	320 (307, 334)	359 (342, 377)	**0.001**	340 (324, 356)	338 (322, 355)	330 (315, 346)	0.68
Potassium (mg/MJ/d)	292 (279, 305)	328 (318, 339)	371 (359, 383)	**<0.0001**	309 (296, 323)	354 (340, 368)	426 (410, 444)	**<0.0001**
Calcium (mg/MJ/d)	98 (93, 103)	102 (97, 107)	104 (99, 109)	0.31	103 (97, 110)	110 (104, 116)	121 (116, 127)	**<0.0001**
Carotenoids (µg/MJ/d)	125 (105, 149)	181 (158, 203)	256 (228, 288)	**<0.0001**	117 (101, 136)	211 (181, 246)	315 (274, 363)	**<0.0001**
Zinc (mg/MJ/d)	1.02 (0.96, 1.08)	1.07 (1.02, 1.11)	1.15 (1.10, 1.20)	**0.003**	1.03 (0,97, 1.09)	1.13 (1.08, 1.18)	1.21 (1.15, 1.28)	**<0.0001**
Magnesium (mg/MJ/d)	26 (25, 28)	31 (29, 32)	37 (36, 38)	**<0.0001**	26 (25, 27)	31 (30, 33)	38 (37, 40)	**<0.0001**

P values from ANCOVA with age as a continuous covariate

Table 11 Percentage of each FNVW group consuming oily fish and olive oil-based products

a) Adults 1986-7

FNVW group…	Men Low	Men *Med*	Men High	Men P	Women Low	Women Med	Women High	Women P
Oily fish	21	34	52	**<0.001**[a*]	19	39	52	**<0.001**[a*]
Olive oil	0	0	2.4	**0.006**[a*]	0	2.5	2.0	0.09[a]

[a] *P* Value = X^2
[b] *P* Value = Kruskal Wallis H Test
* = significant (*P*<0.05) linear M-H trend

b) Elderly 1994-5

FNVW group…	Men Low	Men *Med*	Men High	Men P	Women Low	Women Med	Women High	Women P
Oily fish	22	30	48	**<0.001**[a*]	26	25	41	**0.02**[a*]
Olive oil-based products	0.9	3.4	6.0	0.10[a]	0	1.8	2.6	0.24[a]

[a] *P* Value =Chi square test
[b] *P* Value = Kruskal Wallis H Test
* = significant (*P*<0.05) linear M-H trend

Table 12. Percentage distribution of different socio-demographic and lifestyle characteristics in each FNVW consumer group

a) Adults 1986-7

	Men				Women			
	Low	**Medium**	**High**	**P**	**Low**	**Medium**	**High**	**P**
BMI								
<20	8	7	3	0.24	13	15	6	**0.009**
20.1-25	50	46	50	0.52	52	54	60	0.68
25.1-30	31	39	41	**0.03**	22	24	24	0.44
>30.1	10	8	6	0.19	14	7	12	0.17
Region								
England	31	35	34*		31	33	36*	
Scotland	53	24	22*	**0.006**	46	42	12*	**0.006**
Wales	41	21	38		50	32	18*	
% Non-smokers	25	32	43	**<0.001**	21	37	42	**<0.001**
% Smokers	50	35	15		59	26	15	
% Non-manual	20	34	46	**<0.001**	22	35	43	**<0.001**
% Manual	43	34	23		50	31	19	

P values are from X^2 except for BMI, where logistic regression was used with age adjustment.
Smoking behaviour & occupational social class also showed significant Mentel-Haenzel (M-H) linear trends of $P<0.0001$ for both sexes.
Significant linear M-H trends ($P<0.05$) observed with region indicated by *.

b) Elderly 1994-5

	Men				Women			
	Low	**Medium**	**High**	**P**	**Low**	**Medium**	**High**	**P**
BMI								
<20	5	3	3	0.97	13	3	2	**0.02**
20.1-25	25	32	34	0.19	34	38	29	0.41
25.1-30	52	50	53	0.90	30	33	42	0.34
>30.1	18	14	10	0.17	23	26	28	0.69
Region								
England	32	33	35		33	32	35	
Scotland	34	34	31	0.37	30	43	27	0.43
Wales	53	32	16*		43	38	19	
% Non-smokers	27	34	39	**<0.001**	30	33	37	**<0.001**
% Smokers	61	29	10		51	35	14	
% Non-manual	16	40	44	**<0.001**	19	34	47	**<0.001**
% Manual	48	27	25		45	35	20	

P values are from X^2 except for BMI, where logistic regression was used
Smoking behaviour & occupational social class also showed significant M-H linear trends of $P<0.0001$ for both sexes.
Significant linear M-H trends ($P<0.05$) observed with region indicated by *.

Appendix 3

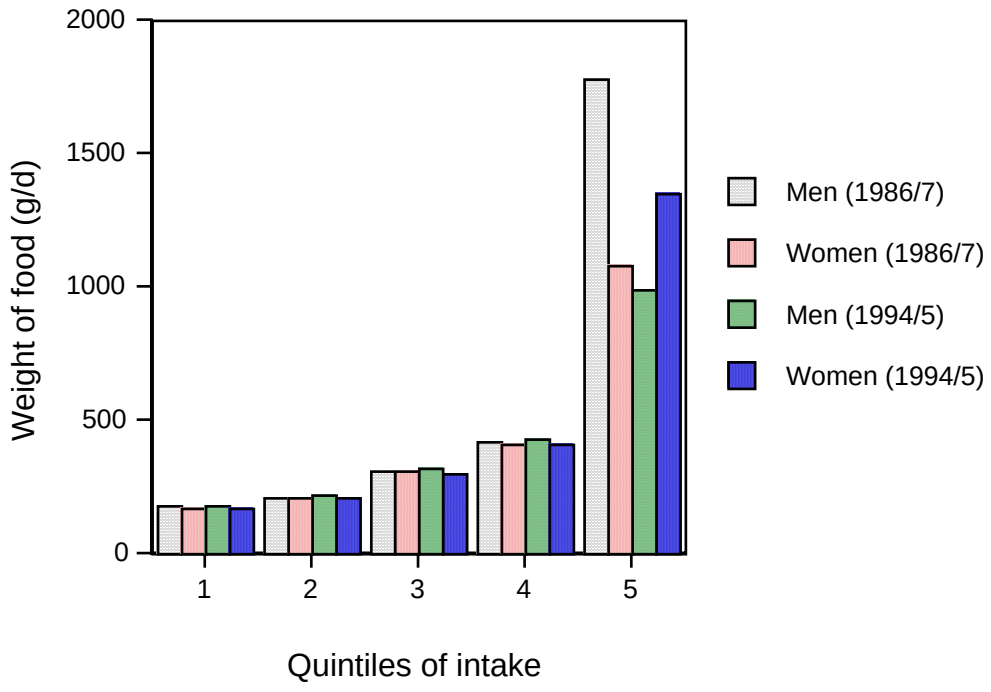
Figure 1: PUFA and MUFA metabolism

n-6	n-3	n-9
Linolenic aicd (LA) 18:2n-6	γ-linolenic acid (ALA) 18:3n-3	Oleic acid 18:1n-9
↓ Δ6-desaturase	↓ Δ6-desaturase	↓
-linolenic (GLA) 18:3n-6	Octadecatetrenoic acid (stearidonic aicd) 18:4n-3	
↓ elongase ↓	↓ elongase	↓ Δ5 desaturase
Dihomo-γ-linolenic (GLA) 20:3n-6	Eicosapentaenoic (EPA) 20:5n-3	Eicosatrienoic acid (ETA) 20:3n-9
↓ Δ5 desaturase ↓	↓ Δ5 desaturase	↓
Arachidonic (AA) 20:4n-6	Docosahexaenoic (DHA) 22:6n-3	Eiconsanoid production PGE
↓	↓	
Eicosanoid production 2-series PGs and TXs 4-series LT	Eicosanoid production 3-series PGs and TXs 5-series LTs	

Adapted from Darlington & Stone 2001

Appendix 4

Figure 2 **Mean consumption of fruit, nuts, vegetables and wholegrain foods (g/d) within each survey population**



Appendix 5

Abbreviations

AA	arachidonic acid
αLA	α-linolenic acid
anti-CD54 (ICAM-1)	intercellular adhesion molecule-1
anti-CD11a (LFA-1)	leucocyte function associated antigen-1
anti-CD58 (LFA-3)	leucocyte function associated antigen-3
anti-HLA-DP	human leucocyte antigen-DP
anti-HLA-DQ	human leucocyte antigen-DQ
anti-HLA-DR	human leucocyte antigen-DR
aOR	adjusted Odds Ratio
ARA	American Rheumatism Association
BCO	blackcurrant seed oil
BMD	bone mineral density
BT	beef tallow
cAMP	cyclic AMP
Ca	calcium
CD	conjugated dienes
cGMP	cyclic GMP
CLA	conjugated linoleic acid
COX-2	cyclooxygenase 2
CRP	C-reactive protein
Cu	copper
DAS	Disease Activity Score
DGLA	di-homo-γ-linolenic acid
DHA	docosapentaenoic acid
DMARDs	disease-modifying antirheumatic drugs
DRV	Dietary Reference Value
EFA	essential fatty acid
ELISA	enzyme linked immunosorbent assay
EPA	eicosapentaenoic acid
ETA	eicosatrienoic acid
EPO	evening primrose oil
ESR	erythrocyte sedimentation rate
Fe	iron
FFA	free fatty acid
FFQ	food frequency questionnaire
FNVW	fruit, nut, vegetable, wholegrain foods
GLA	γ-linolenic acid
GLC	gas-liquid chromatography
GSHpx	glutathione peroxidase
HAQ	Health Assessment questionnaire
HCO	hydrogenated coconut oil
Hcy	homocysteine
5-HETE	5-hydroxyeicosatetraenoic acid
IFN-γ or IFN-gamma	interferon-γ-activated
ICAM-1	intercellular adhesion molecule-1
IgA RF	immunoglobulinA rheumatoid factor
IgM RF	immunoglobulinM rheumaotid factor
IGF	insulin-like growth factor
IL-1	interleukin 1
IL-1β	interleukin 1β
IL-2	interleukin 2
IL-2R	interleukin 2 receptor(s)

IL-6	interleukin 6
LA	linoleic acid
LF	living food
LFA-1(LFA-3)	leucocyte function associated antigen-1 (3)
LT(s)	leukotriene(s)
LTB_4	leukotriene B_4
LTB_5	leukotriene B_5
MDA	malondialdehyde
MHC class II	major histocompatibility complex class II molecules
Mg	magnesium
MTX	methotrexate
MUFA	monounsaturated fatty acid
Na	sodium
NADPH	nicotinamide adenine dinucleotide phosphate
NSAID	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
OO	olive oil
OR	odds ratio
PAF	platelet-activating
PBMC	peripheral blood mononuclear cells
PEM	protein-energy malnutrition
PG(s)	prostaglandin(s)
PGE_1	prostaglandin E_1
PGE_2	prostaglandin E_2
$PGF_1\alpha$	prostaglandin $F_1\alpha$
PHA	phytohemagglutinin
PLP	pyridoxal-5-phosphate
PMN	polymorphonuclear
PUFA(s)	polyunsaturated fatty acid(s)
RA	rheumatoid arthritis
RAI	relative activity index (to estimate activity of RA disease)
RBC	red blood cells
RBP	retinol binding protein
RDA	Recommended daily amount
RDI	Recommended daily intake
RNI	Reference nutrient intake
ROS	reactive oxygen species
RR	relative risk
SAM	S-adenosylmethionine
Se	selenium
SFA	saturated fatty acid
SFO	safflower oil
TBX(s)	thromboxane(s)
TBX_2	thromboxane B_2
TGs	triglycerides
TNFα	tumor necrosis factor α
Zn	zinc