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Prophylactic and therapeutic effects of n-3 polyunsaturated fatty acids, capsaicin, and curcumin on adjuvant induced arthritis in rats

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The prophylactic and therapeutic effects of dietary n-3 polyunsaturated fatty acids and antiinflammatory spice principles—curcumin and capsaicin on adjuvant induced arthritis in rats were studied. Rats fed codliver oil (1 mL/day/rat or 8 wt % in the diet) rich in n-3 fatty acids were found to have a decreased incidence of adjuvant induced arthritis as compared with those observed in coconut oil- or groundnut oil-fed animals. The inflammation in animals which developed adjuvant arthritis in codliver oil-fed animals was also significantly lower than that observed in the other two groups. Additional feeding of spice principles—capsaicin (from red pepper) (5 mg/kg bw/day) or curcumin (from turmeric) (30 mg/kg bw/day) along with dietary lipids delayed the onset of the disease and also lowered the extent of inflammation in arthritic rats. In addition, feeding of the codliver oil-containing diets to rats which have already developed arthritis arrested further progression of the disease. Curcumin and capsaicin feeding to arthritic rats also lowered paw inflammation. This beneficial effect of spice principles was observed irrespective of the nature of the dietary lipids fed to the rats. These studies indicated that the dietary n-3 polyunsaturated fatty acids, capsaicin, and curcumin can decrease the incidence, delay the onset and reduce the extent of inflammation of adjuvant-induced arthritis in rats. (J. Nutr. Biochem. 8:397–407, 1997)

Keywords: n-3 fatty acids; capsaicin; curcumin; inflammation; arthritis

Introduction

Rheumatoid arthritis is an autoinflammatory disease of unknown etiology.[1] The levels of circulating immune complexes are very high in patients with rheumatoid arthritis.[2] These antigen–antibody complexes are not cleared rapidly from the system, but accumulate in the joints.[2] This inturn triggers an inflammatory response at the joints, causing the erosion and destruction of the synovial membrane and the underlying cartilage.[1] Such uncontrolled inflammatory reactions can lead to the destruction of the joint.[1]

Dietary long chain n-3 polyunsaturated fatty acids (PUFAs) are shown to have antiinflammatory effects.[3,4] Fish oil rich in n-3 PUFAs such as eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), was shown to lower the production of proinflammatory mediators such as eicosanoids, interleukin-1, tumor necrosis factor, reactive oxygen species, and nitric oxide.[5–9] Dietary n-3 fatty acids were also shown to suppress autoimmune diseases such as systemic lupus erythematosus, glomerulonephritis, and immune complex-induced vasculopathy.[4,10,11] Patients with inflammatory bowel disease given 3.24 gm of EPA and 2.16 gm DHA/day for 4 months had reduced levels of LTB_4 in their rectal dialysate.[11] Dietary fish oil administered to rheumatoid arthritic patients for 3 months, lowered the classical symptoms of arthritis.[12,13]

Some of the spices used in Asian diets have antiinflammatory properties. Curcumin (from turmeric) is used widely

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in traditional Indian medicine as an anti-inflammant.[14] Curcumin has also been demonstrated to lower the release of proinflammatory eicosanoids.[15,16] Curcumin was shown to alleviate the swelling, morning stiffness, and other symptoms in arthritic patients.[14] Orally administered curcumin also lowered carrageenan-induced acute inflammation in rats.[17] Topical application of capsaicin (from red pepper) has been reported to alleviate the painful osteoarthritis of the hands.[18] In addition, earlier studies from our laboratory have shown that dietary curcumin and capsaicin lower the generation of proinflammatory mediators such as reactive oxygen species and nitric oxide released by macrophages.[9]

All these studies indicate a therapeutic influence of dietary n-3 PUFAs and spice principles on inflammation by lowering the inflammatory mediators. However, little attention has been focused on the role of dietary n-3 fatty acids and anti-inflammatory spice principles for prophylactic uses in chronic inflammatory diseases such as rheumatoid arthritis. The present study was undertaken to evaluate the possible prophylactic uses of dietary n-3 fatty acids and spice principles—capsaicin (from red pepper) and curcumin (from turmeric) in a chronic inflammatory model viz. the adjuvant-induced arthritis in rats. The effects of combination of spice principles with various dietary lipids on the induction and progression of arthritis in rats was also investigated. In addition, we investigated the therapeutic effects of these antiinflammatory spice principles alone and in combination with different dietary lipids on adjuvant-induced arthritis in rats.

Methods and Materials

Attenuated cultures of mycobacterium tuberculosis (H37Rv) were obtained from the National Tuberculosis Institute, Bangalore, India. Capsaicin (95% pure) and Boron trifluoride in methanol, were purchased from Sigma Chemical Co., MO USA. Curcumin (99% pure) was procured from Flavors and Essences Pvt. Ltd., Mysore, India. Medicinal grade codliver oil was a generous gift from Dr. Baldur Hjaltasan, Lysi, Iceland. Pure (refined) coconut oil and groundnut oil were purchased from the local market. Standard fatty acid methyl esters were obtained from Nuchek. Prep, MN USA. All other chemicals and solvents used were of the analytical grade.

Animals

Wistar rats [OUTB-wistar, IND-cft (2c)] were used for all the experiments.

Preparation of adjuvant

Attenuated cultures of Mycobacteria were delipidated by extraction with 5 mL of diethyl ether:alcohol (1:1 v/v) for 60 min. The extraction was repeated twice. The delipidated pellet was air dried and powdered using a glass homogeniser. The finely powdered material was suspended in paraffin oil (10 mg/mL) and stored at 0–5°C.[19] This adjuvant was used within a period of 1 to 2 weeks of preparation.

Induction and monitoring of arthritis

Rats weighing 70 to 80 gm were injected intradermally with 50 μL of the mycobacterial adjuvant at the base of their tails.[19] On the 11th day after adjuvant injection, the animals developed visual symptoms of arthritis (i.e., edema of the paws). The onset and progression of inflammation was monitored by measuring their paw thickness using a Mitutoyo dial caliper. Based on the extent of inflammation, rats were assigned arthritic scores as follows:

Increase in paw thickness (mm)	Arthritic score
0.1 to 1	+
1.1 to 2	++
2.1 to 3	+++
3.1 to 4	++++
4.1 and above	+++++

Prophylactic studies on arthritis with dietary lipids and spice principles

Three groups of adult female rats (230–250 g) were maintained on AIN [American Institute of Nutrition] purified diets[20] containing 8 wt % coconut oil or groundnut oil or codliver oil. Two wt % of groundnut oil was added to all the diets to supplement essential fatty acids. The basal composition of the diets was as follows: Sucrose (60%), AIN 76 vitamin mix (1%), methionine (0.3%), and choline chloride (0.2%).[20] The fatty acid analysis of the diets are summarized in Table 1. Twenty five grams per day of fresh diet was given to each rat daily. After 2 weeks the rats were caged with male rats for breeding. The pregnant rats were segregated and continued to be fed with purified diets. The pups born of these rats in each dietary group were weaned after 21 days and subdivided into three groups of 20 animals each. The subgroups were administered 0.5 mL of groundnut oil alone or the oil containing capsaicin (5 mg/kg bw/day) or curcumin (30 mg/kg bw/day), respectively. When these rats gained 70 to 80 gm of body weight, they were injected with adjuvants. Feeding the spice principles by gavage was continued till the termination of the experiment.

Table 1 Fatty acid composition of dietary lipids (mol %)

Fatty acid	Coconut oil diet	Groundnut oil diet	Codliver oil diet
10:0	5.81	n.d.	n.d.
12:0	44.55	0.84	0.48
14:0	15.98	n.d.	1.60
16:0	9.93	15.97	13.45
16:1	n.d.	n.d.	8.16
18:0	2.42	2.80	3.36
18:1	13.10	43.98	30.69
18:2	8.20	32.77	13.05
18:3	n.d.	0.28	2.68
20:0	n.d.	0.84	0.56
20:1	n.d.	0.56	5.88
20:4	n.d.	1.96	0.28
20:5	n.d.	n.d.	7.44
22:1	n.d.	n.d.	3.08
22:5	n.d.	n.d.	0.56
22:6	n.d.	n.d.	7.72
Saturated fatty acids (S)	78.7	20.5	19.5
Polyunsaturated fatty acids (P)	8.2	35.0	31.7
P/S ratio	0.1	1.71	1.63

n.d., Not detected; Addition of capsaicin and curcumin did not alter the fatty acid composition of the diets.

Table 2 Effect of dietary lipids on the serum fatty acid composition of mothers and pups (mol %)

Fatty acid	Coconut oil group		Groundnut oil group		Codliver oil group	
	Mothers	Pups	Mothers	Pups	Mothers	Pups
12:0	5.67 ± 3.49	n.d.	0.66 ± 0.02	n.d.	n.d.	n.d.
14:0	3.35 ± 0.91	3.49 ± 1.94	1.06 ± 0.03	1.43 ± 0.13	1.04 ± 0.82	1.01 ± 0.03
16:0	22.91 ± 3.05	21.71 ± 0.55	19.01 ± 1.81	19.11 ± 1.22	22.38 ± 1.80	22.40 ± 0.62
16:1	3.67 ± 1.45	2.93 ± 0.99	5.09 ± 1.24	5.15 ± 1.22	5.61 ± 0.60	5.58 ± 0.45
18:0	10.17 ± 2.31	9.31 ± 1.44	8.02 ± 1.63	8.53 ± 0.94	8.12 ± 0.97	8.04 ± 1.03
18:1	24.94 ± 8.20	28.76 ± 1.82	36.77 ± 3.68	37.09 ± 4.10	27.09 ± 3.43	28.05 ± 2.94
18:2	17.81 ± 7.46	18.37 ± 5.79	15.56 ± 3.72	15.59 ± 2.75	10.68 ± 1.80	10.35 ± 1.09
18:3	n.d.	1.83 ± 0.39	0.59 ± 0.26	n.d.	n.d.	n.d.
20:0	0.90 ± 0.32	0.84 ± 0.02	n.d.	n.d.	n.d.	n.d.
20:1	n.d.	n.d.	n.d.	n.d.	1.80 ± 1.14	1.56 ± 1.16
20:4	9.35 ± 3.80	9.39 ± 2.94	9.41 ± 1.33	9.95 ± 1.25	3.57 ± 1.43	4.53 ± 1.19
20:5	n.d.	n.d.	1.47 ± 0.38	1.59 ± 0.39	8.09 ± 3.62	7.02 ± 1.33
22:1	n.d.	n.d.	0.24 ± 0.15	0.31 ± 0.11	2.72 ± 0.38	2.52 ± 0.55
22:5	n.d.	n.d.	n.d.	n.d.	1.64 ± 0.26	1.52 ± 0.14
22:6	1.37 ± 0.02	1.43 ± 0.01	2.06 ± 0.14	1.63 ± 0.15	7.25 ± 0.35	7.00 ± 0.15
Saturated fatty acids (S)	43	35.35	28.75	29.07	31.54	31.45
Monounsaturated fatty acids	28.61	31.69	42.10	42.55	37.22	37.71
Polyunsaturated fatty acids (P)	28.53	31.02	29.09	28.76	31.23	30.42
P/S ratio	0.66	0.87	1.01	0.99	0.99	0.97

n.d., Not detected; Serum samples were drawn from the mothers immediately after parturition and from the pups 21 days after birth. Results are expressed as mean ± SD of six samples.

Therapeutic studies on arthritis by administration of oils and spice principles

Rats (80 gm) on normal lab diet were injected with mycobacterium containing adjuvant to induce arthritis. Rats with confirmed arthritis were divided into nine groups of 5 animals each with fair distribution of different arthritic scores in each group. They were administered the following combinations of dietary oils (1 mL) or spice principles—capsaicin (5 mg/kg bw/d) and curcumin (30 mg/kg bw/day) in 1 mL oil by gavage for 15 days: (1) coconut oil (CO); (2) CO + capsaicin; (3) CO + curcumin; (4) groundnut oil (GNO); (5) GNO + capsaicin; (6) GNO + curcumin; (7) codliver oil (CLO); (8) CLO + capsaicin; (9) CLO + curcumin. The rats were fed with the laboratory diet ad libitum through out the treatment regimen. The extent of paw inflammation was constantly monitored during the 15 days of treatment.

Therapeutic studies on arthritis modulated by dietary lipids and spice principles

Rats (80 gm) on laboratory diet were made arthritic by injecting mycobacterium containing adjuvants. Arthritic rats with different scores were divided into three groups of 15 animals each with representation of different arthritic scores in each group. They were switched from laboratory diets to AIN purified diets containing 8 wt% coconut oil or groundnut oil or codliver oil, for 2 months. The diets were also supplemented with 2% groundnut oil. During the last 2 weeks of feeding, the rats in each dietary lipid group were divided into three subgroups and administered 1 mL of GNO alone or in combination with capsaicin (5 mg/kg bw/day) or curcumin (30 mg/kg bw/day), respectively. The paw thickness was routinely monitored throughout the study.

Isolation of serum

Rats were anaesthetized by ether inhalation and 2 mL of blood was drawn from each rat by retro-orbital puncture. Blood was allowed to clot at room temperature. Serum was separated by centrifugation at 600 g for 15 min and used for lipid extraction.

Fatty acid analysis

Lipids were extracted from 0.8 mL of serum by Bligh and Dyer's method.[21] The lipid extract was saponified and methylated.[22] The fatty acid methyl esters were separated on a permabond-FFAP capillary column (25 m × 0.25 mm ID) in Shimadzu 14B GC. The conditions used for the resolution of fatty acid methyl esters were as follows: carrier gas: 1 bar H_2; temperature gradient: 200–240°C @ 6°C/min. The resolved fatty acid methyl esters were detected by FID, integrated, and analyzed on an online Shimadzu CR6A chromatopac. They were identified by comparing retention times with that of authentic standards.

Results

Modification of serum fatty acid composition

The fatty acid composition of serum lipids in both mothers and pups were altered by dietary lipids. The most significant changes were observed in PUFA composition of rats fed codliver oil (*Table 2*). 18:2 n-6 was decreased by 36 to 40% in the serum lipids of animals fed codliver oil as compared with that observed in animals fed coconut oil ($P < 0.05$) or groundnut oil diets ($P < 0.01$). Similarly, 20:4 n-6 levels were decreased by 62% in animals fed codliver oil ($P < 0.05$). This was compensated by a significant increase in n-3 PUFA, 20:5 ($P < 0.001$) and 22:6 ($P < 0.01$) in serum lipids of animals fed codliver oil. The total n-3 PUFA in animals fed codliver oil was enhanced by 380% as compared with that observed in animals fed groundnut oil diets and by 1180% as compared with animals fed coconut oil diets. Similar trends were observed in fatty acid compo-

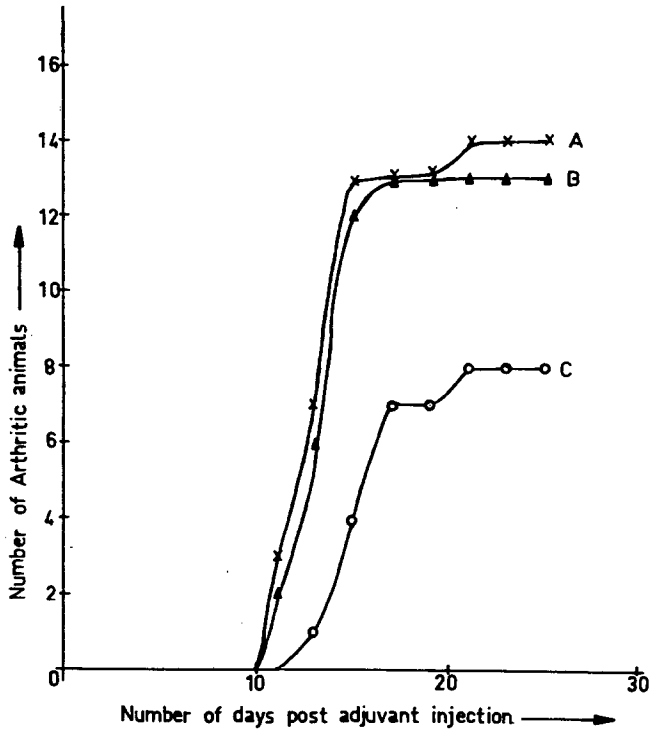

Figure 1 Effect of dietary lipids on the induction of arthritis. Pups born to mothers maintained on the dietary lipids—A: Coconut oil; B: Groundnut oil; C: Codliver oil were continued to be fed with the dietary lipids and injected with adjuvants. Out of 20 pups/group, the number of animals which developed arthritis as observed by visible paw inflammation have been represented.

sitional changes in pups born to these mother. Inspite of 16 to 17 fold differences in the P/S ratio of dietary lipids between groundnut oil or codliver oil diets as compared with coconut oil diets, the P/S ratio observed in serum lipids were not significantly different in all the three groups of animals (*Table 2*). Therefore the presence of n-3 PUFA in codliver oil significantly altered the nature of PUFA, but not the overall P/S ratios of lipids accumulated in serum.

Incidence of arthritis as affected by dietary lipids and spice principles

The number of animals which developed arthritis after injecting adjuvants in the group fed codliver oil was significantly lower than that observed in the group of animals fed coconut oil or groundnut oil (*Figure 1*). Thus, a maximum of only 40% of animals fed codliver oil developed arthritis after 21 days of injecting adjuvants (*Figure 1*). However, 70% of animals on coconut oil diet and 65% of animals on groundnut oil diet developed arthritis by this period. The onset of arthritis was also delayed significantly by feeding codliver oil. Thus, by day 11, 15% and 10% of animals on coconut oil and groundnut oil diets became arthritic, whereas none of the animals on codliver oil diet showed any visible symptoms of arthritis. By day 13, 35% and 30% of animals on coconut oil and groundnut oil diets, respectively, became arthritic, whereas only 1 out of 20 animals in codliver oil diet developed arthritis. By day 21, 65 to 70% of animals on coconut oil or groundnut oil diets became arthritic, whereas only 35 to 40% of animals on codliver oil diets showed symptoms of arthritis. No further increase in the incidence of arthritis in animals from any of the dietary groups were observed beyond 21 days after injecting adjuvants.

The arthritic scores in codliver oil fed animals were also significantly lower as compared with that observed in groups of animals fed coconut oil or groundnut oil diets (*Table 3*). Thus, by day 25 after injecting adjuvants, 50% of the animals on coconut oil or groundnut oil diets showed an arthritic score of >4, whereas only 25% of animals on codliver oil diet showed an arthritic score of >4. Therefore, codliver oil feeding reduces the incidence of the disease, delays the onset, and minimizes the extent of inflammation in arthritic animals.

Oral feeding of the spice principles capsaicin (5 mg/kg body weight) and curcumin (30 mg/kg body weight) to rats on specific dietary lipids had further beneficial effects on arthritis. The most significant effects of spice principle feeding is in delaying the progression of arthritis. This effect

Table 3 Prophylactic effect of dietary lipids on paw edema in arthritic rats

Dietary group	No. of days after adjuvant injection	Arthritic score					Total number of arthritic rats*
		+1	+2	+3	+4	+5	
Coconut oil	11	2	1				3
	13	2	2	3			7
	15	4	2	4	3		13
	25	1	3	—	2	8	14
Groundnut oil	11	1	1				2
	13	2	2	2			6
	15	4	3	1	2	2	12
	25	1	2	—	2	8	13
Codliver oil	11	—					—
	13	1					1
	15	2	2				4
	25	—	2	1	2	3	8

Twenty rats in each dietary lipid group were injected with adjuvant. Paw thickness (mm) was monitored routinely and rats were assigned arthritic scores as described under Methods and materials.
*Values represent the number of arthritic rats with the respective arthritic score.

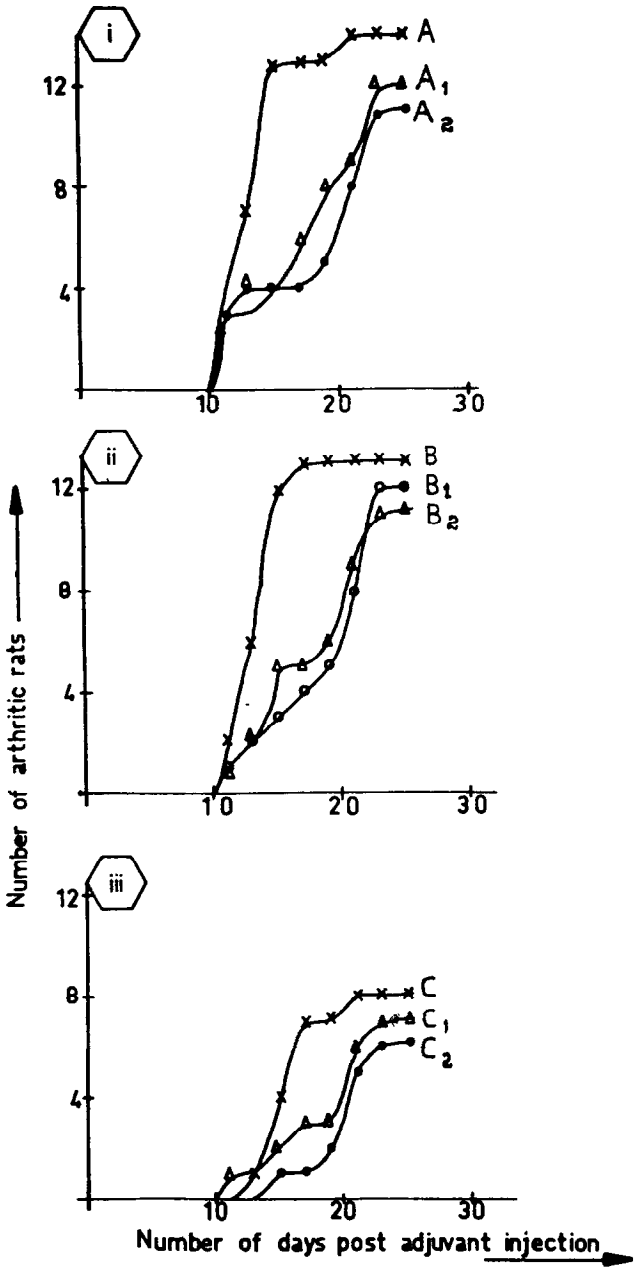

Figure 2 Effect of oral feeding of capsaicin and curcumin on the induction of arthritis in the dietary lipid groups of animals. Pups born to mothers maintained on the dietary lipids—A: coconut oil (CO); B: groundnut oil (GNO) and C: codliver oil (CLO) were additionally force fed with capsaicin and curcumin as described under materials and methods. The number of animals which developed arthritis in the dietary groups—A_1: CO + capsaicin; A_2: CO + curcumin; B_1: GNO + capsaicin; B_2: GNO + curcumin; C_1: CLO + capsaicin and C_2: CLO + curcumin, have been represented.

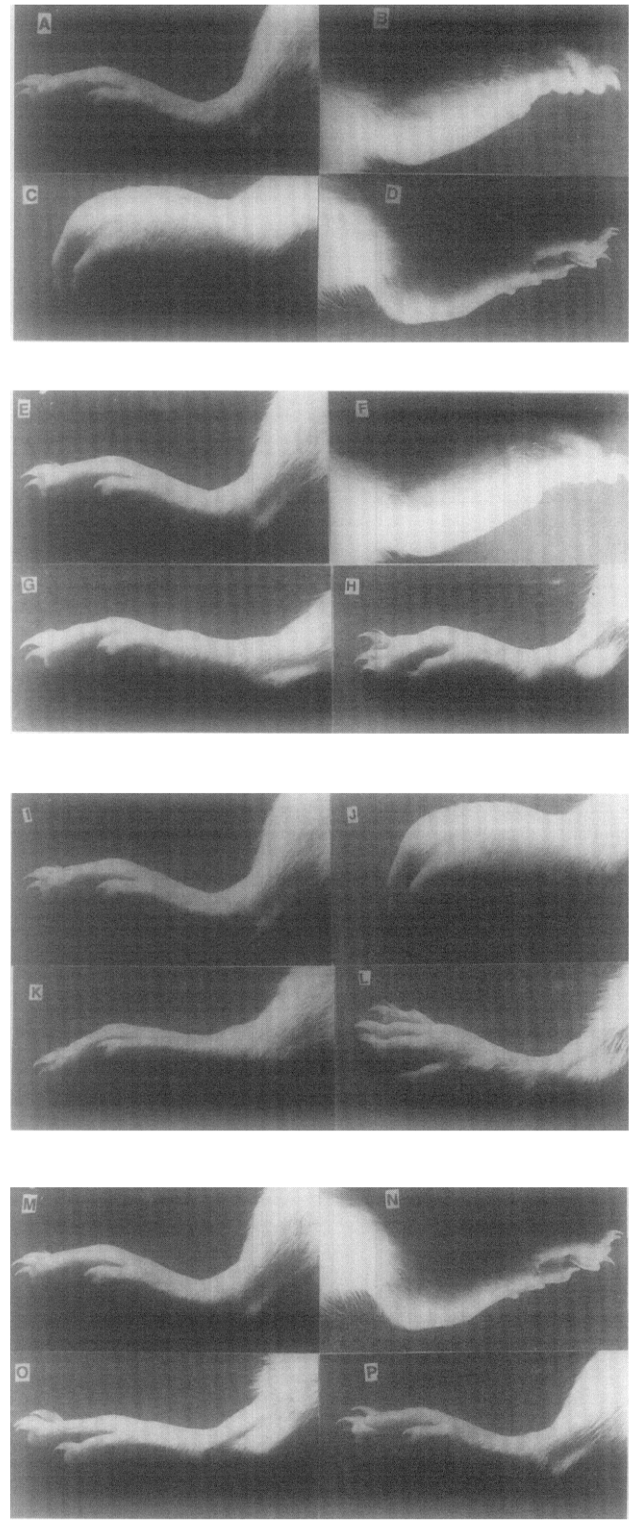

Figure 3 Prophylactic effect of dietary lipids and spice principles on the paw inflammation in arthritic rats. Pups born to mothers maintained on the dietary lipids: B, F: coconut oil (CO); C, J: groundnut oil (GNO); D, N: codliver oil (CLO) were additionally force fed with spice principles in 1 ml GNO – G: CO + capsaicin, H: CO + curcumin, K: GNO + capsaicin, L: GNO + curcumin, O: CLO + capsaicin and P: CLO + curcumin. The visual inflammation on day 25 post adjuvant injection in the different groups have been depicted. A, E, I, and M represent paws of control animals.

was observed irrespective of the nature of the dietary lipids fed to animals. Thus, by day 15, 65%, 60% and 20% of the animals fed coconut oil, groundnut oil, or codliver oil, respectively, developed adjuvant-induced arthritis (*Figure 1*; Table 3). However, only 20%, 15%, and 10% of animals fed capsaicin along with coconut oil, groundnut oil, and codliver oil diets, respectively, developed adjuvant-induced

Table 4 Effect of capsaicin and curcumin on paw edema in arthritic rats

Dietary group	No. of days after adjuvant injection	Arthritic score +1	+2	+3	+4	+5	Total number of arthritic rats*
Coconut oil	11	2	1				3
	13	2	2	3			7
	15	4	2	4	3		13
	25	1	3	—	2	8	14
Coconut oil	11	2	1				2
+ curcumin	13	2	1				3
	15	3	1				4
	25	—	1	2	3	6	12
Coconut oil	11	3					3
+ capsaicin	13	1	3				4
	15	—	3	1			4
	25	2	1	1	1	6	11
Groundnut oil	11	1	1				2
	13	2	2	2			6
	15	4	3	1	2	2	12
	25	1	2	—	2	8	13
Groundnut oil	11	2					2
+ curcumin	13	1	2				3
	15	3	1	1			5
	25	1	3	1	1	5	11
Groundnut oil	11	1					1
+ capsaicin	13	2					2
	15	2	1				3
	25	2	2	1	—	7	12
Codliver oil	11	—					—
	13	1					1
	15	2	2				4
	25	—	2	1	2	3	8
Codliver oil	11	1					1
+ curcumin	13	1					1
	15	2					2
	25	1	2	2	1	1	7
Codliver oil	11						—
+capsaicin	13						—
	15	1					1
	25	—	3	2	1	—	6

*Values represent the number of arthritic rats with the respective arthritic score.

arthritis (*Figure 2*). Similarly, curcumin feeding reduced the development of arthritis to 20%, 25%, and 10% in animals on coconut oil, groundnut oil, or codliver oil diets, respectively, (*Figure 2*). Thus, spice principles can significantly delay the onset of arthritis irrespective of the nature of the dietary lipids fed to animals. However, the spice principles had very little effect on the overall incidence of arthritis in experimental animals when the chronic stage of the disease was manifested. The total number of animals which ultimately became arthritic was dependent on the nature of dietary lipids fed to the animals. Spice principles, however, reduced the arthritic score even on day 25 after injecting adjuvants to animals. Thus, the animals which developed arthritic score of >4 were decreased after curcumin feeding by 30%, 30%, and 80% in animals fed coconut oil, groundnut oil, and codliver oil, respectively (*Table 4*). Thus, spice principles can delay the progression of the disease and also reduce the extent of inflammation in arthritic animals. The paw inflammation of arthritic animals fed different dietary lipids and lipids in combination with spice principles is illustrated in *Figure 3*.

Effect of lipids and spice principles on inflammation in arthritic rats

A. Effect of lipids given orally in the form of oils. Because dietary n-3 fatty acids and spice principles reduced the extent of inflammation during the development of arthritis, the therapeutic effects of these dietary constituents on the inflammation in animals which have already developed arthritis was also investigated. After 21 days of injecting adjuvants, arthritic rats were additionally given by intubation, 1 mL of coconut oil (saturated fat), groundnut oil (n-6 PUFA), or codliver oil (n-3 PUFA) or 1 mL of these oils containing capsaicin (5 mg/kg body weight) or curcumin (30 mg/kg body weight) per day. The inflammation in animals receiving a single dose of oils per day showed differential responses (*Figure 4*). Whereas the inflammation in arthritic animals fed coconut oil or groundnut oil continued to increase, the inflammation in animals receiving codliver oil was decreased significantly (*Figure 4*). However, when animals were given capsaicin or curcumin along with the oils, the inflammation in all the groups of animals were reduced irrespective of the nature of the oils given to

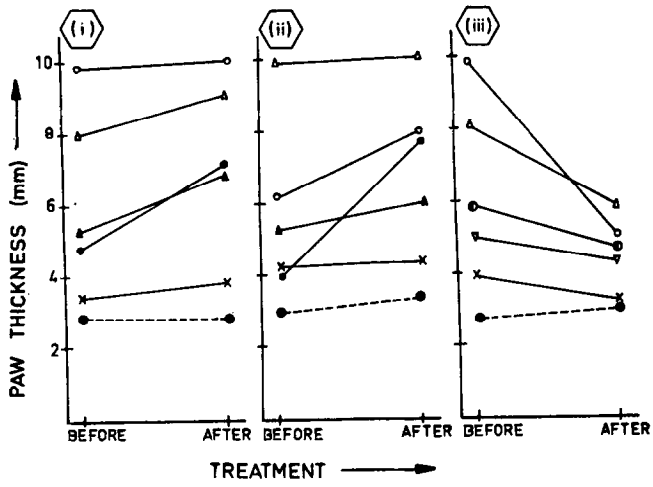

Figure 4 Effect of oral feeding of lipids on paw inflammation in arthritic rats. Rats maintained on normal laboratory chow were induced with arthritis as described in materials and methods. On the 21st day, rats were divided into 3 groups of five rats each and fed with 1 mL of (i) coconut oil, (ii) groundnut oil, (iii) codliver oil, for 15 days. Paw swelling as measured before and after the force feeding regimen has been represented. (●----●) represents the paw thickness in control rats which were not injected with adjuvants but received equivalent volume of paraffin oil.

the animals (*Figure 5*). Whereas the inflammation in rats fed coconut oil alone increased by a mean value of 15.5%, administration of capsaicin or curcumin in coconut oil lowered inflammation by 10.6% and 28.1%, respectively (*inset, Figure 5a*). In the arthritic animals given groundnut oil alone, the paw inflammation increased by a mean value of 32.4%. However, paw inflammation in the animals administered groundnut oil containing capsaicin or curcumin was lowered by 27.2% and 28.7% (*inset, Figure 5b*). Oral feeding of codliver oil resulted in decreasing the paw swelling in arthritic rats by 19.1%. Animals fed codliver oil and capsaicin or curcumin also showed a decrease in paw inflammation by 15.5% and 13.4%, respectively (*inset, Figure 5c*).

B. Effect of lipids supplemented in the diet. When the lipids were administered to the animals by mixing in the diet, a similar effect on inflammation in arthritic rats was observed. Based on the diets consumed by the animals, it was calculated that these animals received 1.2 mL of oils (coconut oil, groundnut oil, or codliver oil), which is comparable with that received by animals given oils by oral route (1 mL). Whereas the inflammation in coconut oil- and groundnut oil-fed animals continued to increase, the inflammation in animals fed codliver oil were arrested at the initial level (*Figure 6*). However, when these animals were given either capsaicin or curcumin, again a decrease in the inflammation was observed in all the groups of animals irrespective of the nature of the dietary lipids (*Figure 7*). The paw inflammation in arthritic rats fed coconut oil containing diets showed an increase in paw inflammation up to a mean of 4.2% by 15 days (inset, *Figure 7a*). Administration of capsaicin or curcumin lowered paw inflammation in coconut oil-fed rats by 5.7% and 7.3%, respectively (inset, *Figure 7a*). In the groundnut oil-fed animals, the paw inflammation increased by 8.9% (inset, *Figure 7b*). Feeding capsaicin or curcumin to arthritic rats on groundnut oil-containing diets lowered paw inflammation by 7% and 16%, respectively (inset, *Figure 7b*). Codliver oil feeding lowered the paw inflammation by 5.3% in arthritic rats (inset, *Figure 7c*). Further administration of capsaicin or curcumin lowered paw inflammation in codliver oil-fed animals by 10.6% and 15.2%, respectively, (inset, *Figure 7c*).

The extent of decrease in inflammation in arthritic rats given spice principles was dependent on the initial arthritic scores of the animals. Whereas the animals with higher arthritic scores showed a greater percentage of decrease in inflammation, the animals with lower arthritic scores showed lesser percentage of reduction in inflammation after giving spice principles (*Figures 5* and *7*). However, irrespective of the arthritic scores in animals before the administration of spice principles, the visible paw inflammation in all the arthritic animals receiving either capsaicin or curcumin seemed to be completely ameliorated. This beneficial effect of capsaicin and curcumin was observed in all the animals irrespective of the nature of the lipids fed to arthritic animals.

When the animals were examined by x-ray, it was noticed that codliver oil feeding could arrest the further progression of the disease in the joints, but was not able to reverse the damage that has already occurred (data not shown).

Discussion

This study demonstrates the prophylactic as well as therapeutic influence of dietary n-3 PUFA and spice principles (capsaicin and curcumin) on inflammatory responses in arthritic animals. Many epidemiological studies involving Eskimos, Japanese, and Dutch have shown that those who consume high proportion of n-3 fatty acids have lowered the incidence of inflammatory diseases such as asthma, systemic lupus erythematosus, and type 1 diabetes mellitus.[23–25] Consumption of fish/fish oils on a regular basis reduce the incidence of cardiovascular diseases.[26] Dietary n-3 fatty acids also decrease the rapid proliferation of tumor cells in experimental animals.[27] A moderate improvement in clinical symptoms of arthritis have been observed after feeding of n-3 PUFA for 14 weeks.[28] However, the possible prophylactic effects of fish oils on arthritis have not received much attention. In the current investigation, we demonstrated that the pups born to a mother on a fish oil diet when continued to be fed on the same diet shows lower incidence of arthritis after injecting the adjuvants as compared with those brought up on coconut oil- or groundnut oil-containing diets. The total PUFA content of groundnut oil and fish oil are comparable. However, groundnut oil is enriched in n-6 PUFA, whereas fish oil contains n-3 PUFA. Therefore, it is the nature of the PUFA, but not their levels that seems to be responsible for the reduced incidence of adjuvant-induced arthritis observed in fish oil-fed animals. In addition, the extent of inflammation observed in codliver oil-fed animals which developed arthritis was significantly lower compared with that observed in coconut oil- and groundnut oil-fed animals. Therefore, n-3 PUFA consump-

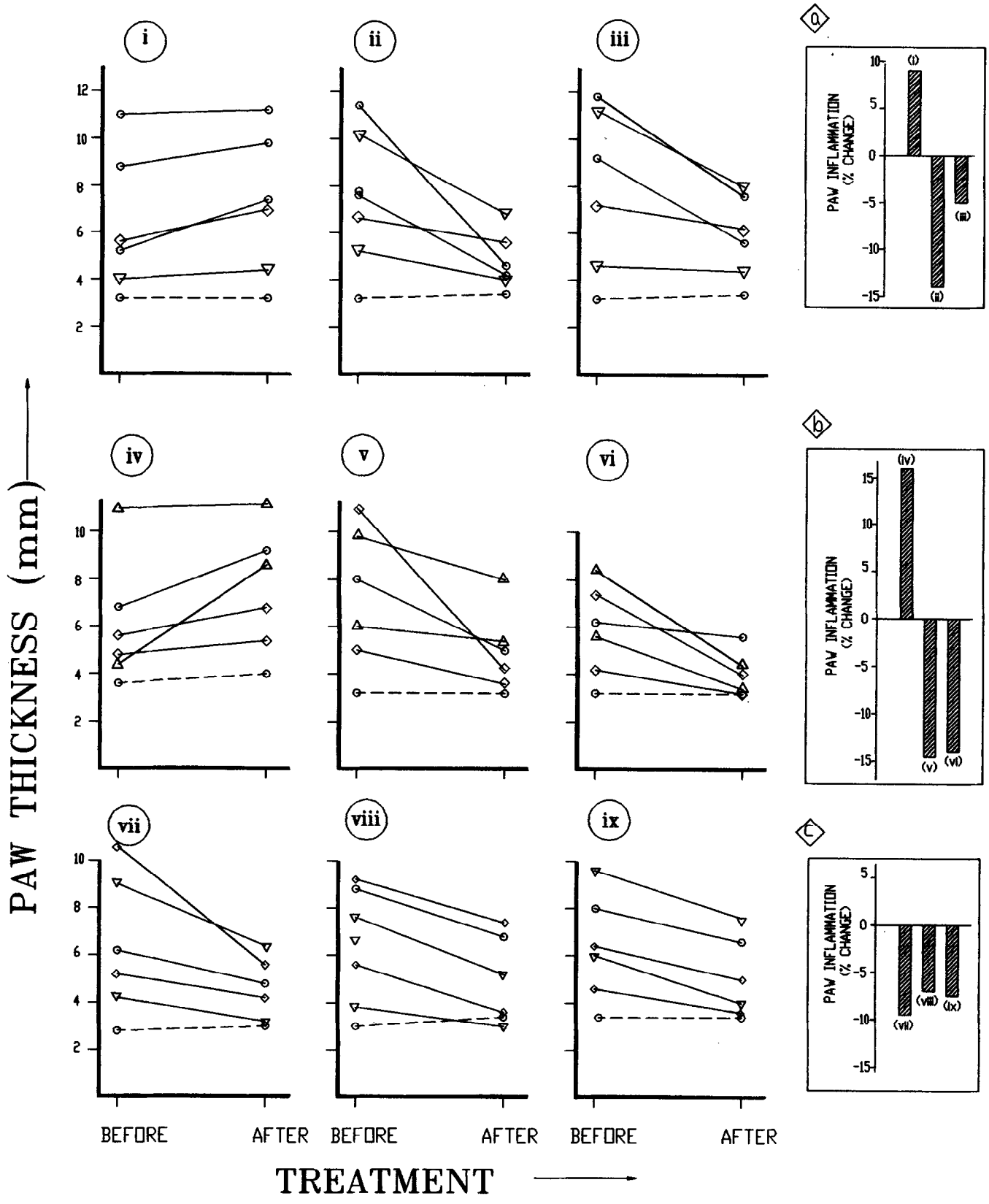

Figure 5 Effect of spice principles on paw inflammation in arthritic rats given different oils by intubation. Five arthritic rats/group were maintained on normal laboratory chow and fed with 1 ml of dietary oil in combination with spice principles for 15 days. The different dietary groups are: (i) coconut oil (CO); (ii) CO + curcumin; (iii) CO + capsaicin; (iv) groundnut oil (GNO); (v) GNO + curcumin; (vi) GNO + capsaicin; (vii) codliver oil (CLO); (viii) CLO + curcumin; (ix) CLO + capsaicin. The paw thickness before and after feeding have been recorded. (○----○) represents paw thickness of normal rats which were not injected with adjuvants but received equivalent volume of paraffin oil. *Insets a, b,* and *c* indicate the change in the paw inflammation of the arthritic animals of different dietary groups.

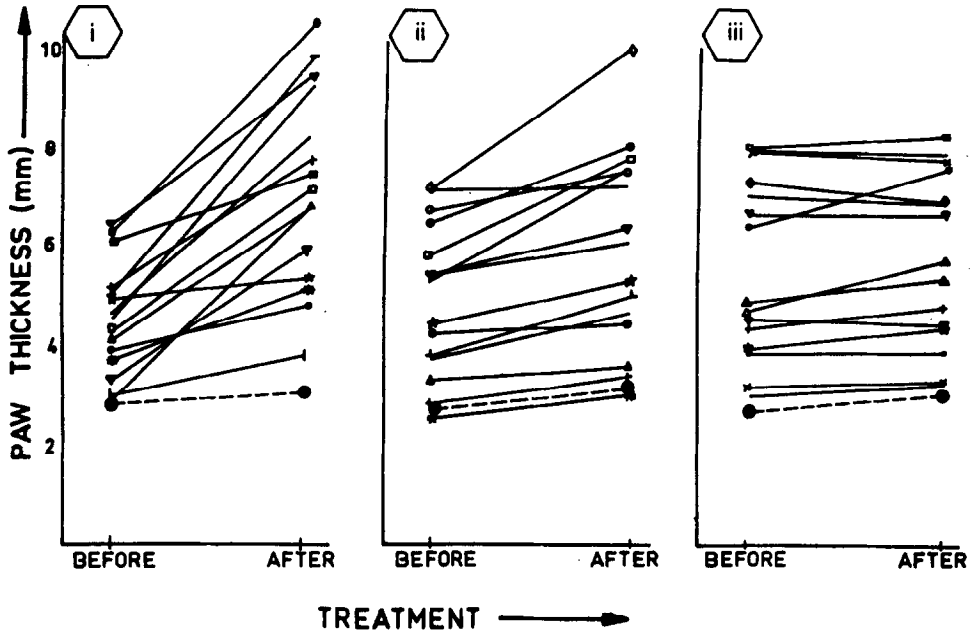

Figure 6 Effect of dietary lipids on adjuvant induced arthritic rats. On day 21 after adjuvant injection 20 arthritic rats/group were maintained on purified diets containing 8% of (i) coconut oil, (ii) groundnut oil, and (iii) codliver oil, for 2 months. Paw thickness before and after feeding the purified diets are shown. (●----●) represents paw thickness of normal rats which were not injected with adjuvants but received equivalent volume of paraffin oil.

tion not only reduced the incidence of inducible arthritis, but also lowered the extent of inflammation in arthritic animals. Codliver oil additionally had a therapeutic effect in lowering the inflammation in arthritic animals. This beneficial effect of codliver oil was observed irrespective of the mode of administration (oral administration or as dietary supplementation). Subsequent x-ray analysis confirmed that codliver oil feeding arrested the erosion of joints in arthritic animals but could not repair the damage that has already occurred (data not shown).

Dietary n-3 PUFA are known to reduce the levels of arachidonic acid metabolites and lower the formation of proinflammatory compounds like prostaglandins and leukotrienes.[5,29,30] Fish oil feeding also reduced proinflammatory cytokines like interleukin-1, tumor necrosis factor and platelet activating factor.[8,31] Nitric oxide and superoxide anions play an important role in the initiation and development of adjuvant arthritis in rats.[32] Our earlier studies demonstrated that peritoneal macrophages from rats fed fish oil secreted lower levels of reactive oxygen species and nitric oxide.[9] Fish oil feeding also inhibits chemotaxis and prevents the migration of leukocytes to the site of inflammation.[31] Therefore fish oil feeding can reduce inflammation by altering the levels of a number of inflammatory mediators.

In addition to n-3 PUFA, the spice principles curcumin and capsaicin also exhibited beneficial effects in modulating arthritis. Administration of spice principles delayed the onset and severity of adjuvant-induced arthritis. This effect was observed irrespective of the nature of dietary lipids fed to animals. Therefore the beneficial effect of spice principles on arthritis is mediated independent of the lipid effect. The antiinflammatory effects of spice principles were comparable with that of known antiinflammatory drugs such as aspirin, indomethacin, and piroxicam (unpublished results). The overall incidence of arthritis in animals were however strictly affected by the nature of dietary lipids.

The therapeutic potentials of spices/spice principles are known for a long time in Indian medicine.[14] Their antimicrobial and antiinflammatory properties have been exploited over centuries.[14] Curcumin and volatile oils of tumeric were shown to reduce edema in rat paw injected with carrageenan and also shown to possess antiarthritic properties.[17,33] Curcumin given to osteoarthritic and rheumatoid patients for 3 months at doses ranging from 1,000 to 2,500 mg/day showed significant reduction of clinical symptoms.[14] These studies are reported to be in phase II clinical trials.[14] Spice principles have also been shown to inhibit the formation of many proinflammatory compounds. Curcumin inhibits lipid peroxidation and the formation of prostaglandins and leukotrienes.[15,16,34] Curcumin and capsaicin are shown to lower the formation of superoxide anions, hydrogen peroxide, and nitric oxide in activated macrophages.[9] These effects of spice principles may enable them to act as good antiinflammatory compounds. Spices are nontoxic, nonmutagenic with good antioxidant and antiinflammatory properties.[35] They are promising viable alternatives for drugs in the treatment of arthritis.

In conclusion, our studies demonstrate that arthritis in an experimental animal model like rats can be modulated by a combination of dietary n-3 PUFA and spice principles like curcumin and capsaicin to reduce the incidence, delay the onset of the disease, and also lower the severity of inflammation.

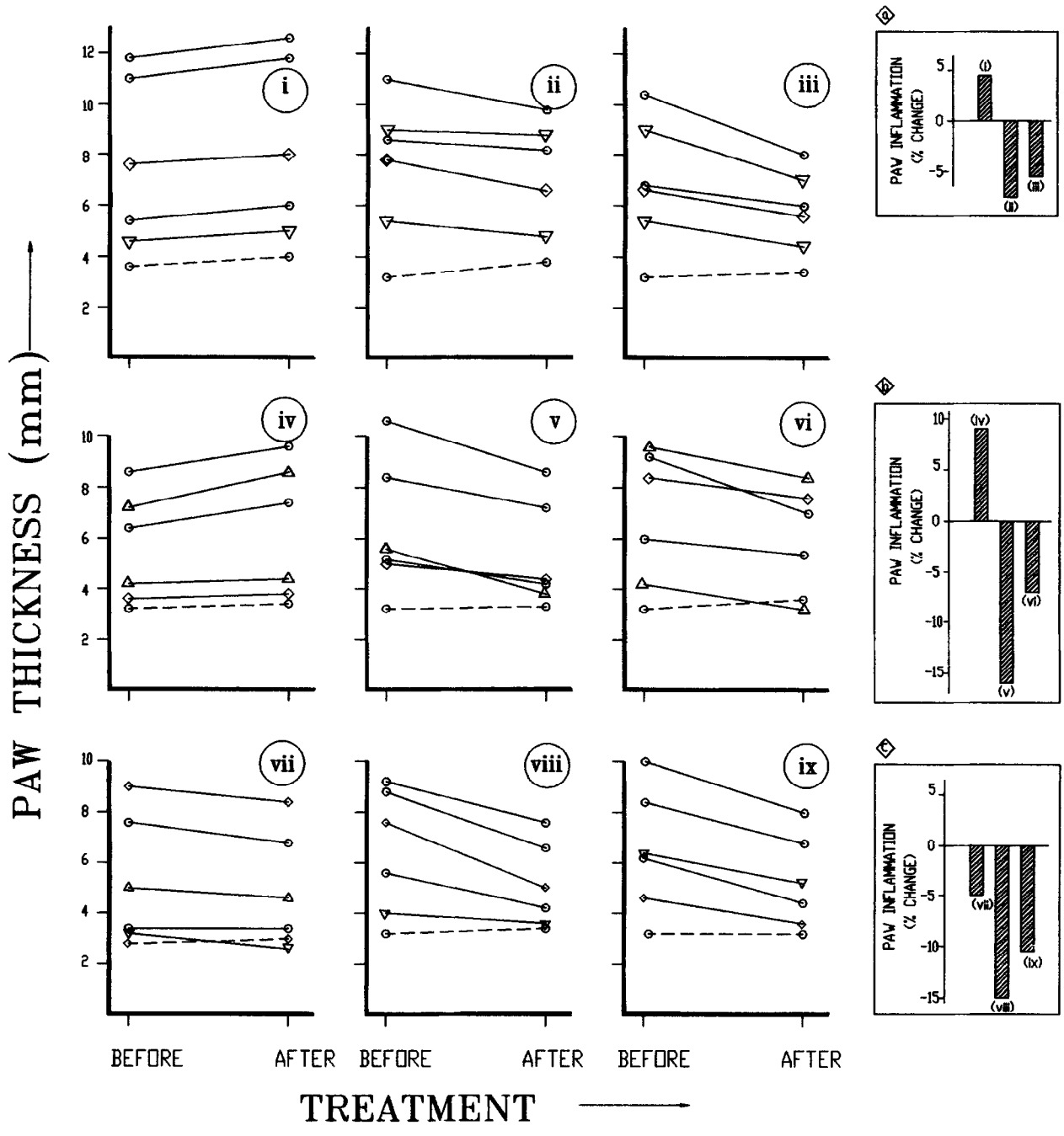

Figure 7 Effect of spice principles given in combination with different dietary lipids on arthritic rats. Arthritic rats maintained on (i) coconut oil (CO), (iv) groundnut oil (GNO) and (vii) codliver oil (CLO) for 2 months were additionally fed with (ii), (v) and (viii) curcumin (30 mg/kg bw/day) and (iii), (vi) and (ix) capsaicin (5 mg/kg bw/day) in 1 ml of respective oils during the last two weeks of feeding. Paw thickness was monitored before and after the treatment regimen of the last 15 days of feeding. (○----○) represents the paw thickness of normal rats which were not injected with adjuvants but received equivalent volume of paraffin oil. Insets a), b) and c) indicate the change in the paw inflammation of the arthritic animals in different dietary groups.

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