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Effects of feeding mustard oil diets on the collagen biosynthesis in various tissues of male rats*)

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Summary

30 rats were divided into 3 groups to study the effects of erucic-acid-rich mustard oil (MO) and MO plus fish oil and carnitine (MOFOC) diet as compared to groundnut oil (GNO) diet on the collagen biosynthesis in various tissues. Changes in collagen content and monoamine oxidase (MAO) of hearts, aorta, skeletal muscles, lungs and skin were determined after 24, 60 and 80 days of feeding, respectively. Incorporation of U-^{14}C-proline into collagen of these tissues was also studied. MO diets increased the incorporation of U-^{14}C-proline into total and acid-insoluble collagen in heart, skeletal muscles and lungs but this increase was of lower magnitude in heart and skeletal muscles as compared with MOFOC diet. Total and acid-insoluble collagen contents in all these tissues, except skin, were increased by MO diet and MOFOC diet retarded this increase. Similar trends were observed in the activities of MAO. The results suggest that the MO diet increases both the biosynthesis and maturation of collagen and MOFOC diet retards this trend. The response of various tissues being variable in this respect.

Zusammenfassung

Zur Untersuchung der Wirkungen von Diätformen mit erukasäurehaltigem Senföl (MO), MO + Fischöl und Carnitin (MOFOC) und als Vergleich mit Erdnußöl (GNO) auf die Kollagenbiosynthese in verschiedenen Geweben wurden 3 Gruppen von je 10 Ratten gebildet. Änderungen im Kollagengehalt und in der Monoaminoxidase (MAO) von Herz, Aorta, Skelettmuskulatur, Lungen und Haut wurden nach 24, 60 und 80 Tagen Fütterung untersucht. Der Einbau von U-^{14}C-Prolin in das Kollagen dieser Gewebe wurde ebenfalls untersucht. MO-Diäten erhöhten den Einbau von U-^{14}C-Prolin in das gesamte und säureunlösliche Kollagen von Herz-, Skelettmuskeln und Lungen, aber bei Herz- und Skelettmuskeln war die Erhöhung geringer als die bei den Tieren auf MOFOC-Diät. Das gesamte und säureunlösliche Kollagen in allen untersuchten Geweben mit Ausnahme der Haut wurde durch MO-Diät erhöht, während diese Zunahme durch MOFOC-Diät verzögert wurde. Ähnliche Trends wurden bei der Bestimmung der MAO-Aktivität beobachtet. Die Ergebnisse legen nahe, daß die MO-Diät die Biosynthese und Reifung des Kollagens steigert und MOFOC-Diäten diesen Vorgang verzögern. Das Ansprechen der verschiedenen Gewebe war unterschiedlich.

Key words: mustard oil, carnitine, collagen, mitochondrial monoamine oxidase

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The feeding of mustard *(Brassica juncea)* oil, rich in erucic acid (Δ^{13}-cis-docosenoic acid), produces lipidosis (3–5, 11, 18) and fibrosis in heart (1, 2, 8, 12, 16) of laboratory animals. A reduction in the erucic-acid-induced cardiac lipidosis has been reported by supplementing erucic-acid-rich diets with fish oil or fish oil plus carnitine (9, 10, 19). The present work was carried out to study the levels and extent of collagen biosynthesis under these feeding regimens, as no studies appear to have been carried out to determine the quantitative changes in the collagen content of various tissues.

Materials and methods

30 albino rats were divided into 3 groups of 10 animals each and maintained on experimental diets (table 1). The food and water were given *ad libitum*. After 24 days, 3 rats from each group were injected (i.p.) 3 times with U-^{14}C-proline (Sp. act. 100 mCi/mmole) at 2 hrs intervals, before sacrifice, to study the biosynthesis of collagen. Again after 60 days 5 rats from each group were killed to determine the levels of various types of collagen. The remaining 2 rats from each group were killed after 80 days of feeding the experimental diets to determine the activity of monoamine oxidase (6) in mitochondria isolated (14) from various tissues. U-^{14}C-proline was obtained from BARC, Trombay (India), and the radioactivity was measured in Packard 3330 liquid scintillation spectrometer, using scintillation fluid of Bray (7). The salt-soluble, acid-soluble, and acid-insoluble collagen were fractionated (17) by using 0.45 M NaCl, 1 M NaCl, and 0.5 M citrate (pH 3.7), respectively. The hydroxyproline content determined by the method of Steegeman and Stadler (21) was multiplied by 7.46 to get the collagen content. The protein content was determined (12) and statistical analysis was also carried out (20).

Results

The incorporation of U-^{14}C-proline (table 2) was maximum into the acid-insoluble collagen as compared to the soluble collagen fractions from heart, skeletal muscles, lungs, and skin of rats getting the 3 diets. The

Table 1. Components (g%) of groundnut oil (GNO), mustard oil (MO)* and MO + fish oil + carnitine (MOFOC) diets.

Dietary component	GNO	MO	MOFOC
Wheat flour	50	50	50
Gram (crushed)	6	6	6
Skim milk powder	20	20	20
Groundnut oil	20	–	–
Mustard oil*)	–	20	17.5
Fish oil	–	–	2.5
Carnitine	–	–	0.1
Yeast powder	2	2	2
Common salt	0.5	0.5	0.5
Mineral mixture	0.5	0.5	0.5
Vitamin mixture**)	1	1	1

*) 47.2% erucic acid content.
**) One gram supplied 3000 I.U. Vit. A and 300 I.U. Vit. D^2.

Table 2. Effects of feeding GNO, MO, MOFOC diets, for 24 days, on the incorporation of U-^{14}C-proline (mean ± S.D. p_{moles}/mg proteins) into collagen fractions of various tissues.

Tissue Diet	NaCl-soluble collagen 0.45 M salt	 1.0 M salt	Acid-soluble collagen	Acid-insoluble collagen
Heart				
GNO	0.067 ± 0.03	1.12 ± 0.69	1.03 ± 0.29	4.45 ± 1.10
MO	0.050 ± 0.02	1.53 ± 0.37	0.99 ± 0.21	11.37 ± 4.21
MOFOC	0.020 ± 0.01	1.68 ± 0.15	1.16 ± 0.20	6.78 ± 0.79
Aorta				
GNO	0.067 ± 0.03	1.61 ± 0.25	0.89 ± 0.20	1.14 ± 0.67
MO	0.085 ± 0.05	2.69 ± 0.97	1.19 ± 0.52	0.82 ± 0.06
MOFOC	0.122 ± 0.07	1.79 ± 0.30	0.71 ± 0.48	0.59 ± 0.06
Skeletal muscles				
GNO	0.059 ± 0.02	0.908 ± 0.07	1.48 ± 0.31	5.63 ± 1.03
MO	0.028 ± 0.01	0.996 ± 0.04	1.13 ± 0.23	15.86 ± 9.52
MOFOC	0.036 ± 0.02	0.830 ± 0.07	0.74 ± 0.25	11.04 ± 2.11
Lungs				
GNO	0.048 ± 0.01	1.28 ± 0.43	2.75 ± 0.58	6.20 ± 0.50
MO	0.072 ± 0.00	1.44 ± 0.10	2.71 ± 0.43	16.59 ± 1.99
MOFOC	0.120 ± 0.04	1.32 ± 0.09	2.88 ± 0.26	17.40 ± 9.12
Skin				
GNO	0.11 ± 0.03	1.43 ± 0.45	1.29 ± 0.12	10.87 ± 3.31
MO	0.10 ± 0.03	2.58 ± 1.01	1.71 ± 0.16	9.60 ± 3.30
MOFOC	0.08 ± 0.03	4.14 ± 0.90	1.49 ± 0.66	8.61 ± 2.32

incorporation into acid-soluble collagen of lungs, salt-soluble collagen of aorta and skin were also considerable. Feeding of mustard oil (MO) diet, as compared to groundnut oil (GNO) diet, substantially increased the incorporation into acid-insoluble collagen of heart, skeletal muscles and lungs. Supplementation of MO diet with fish oil plus carnitine (MOFOC) diet decreased the extent of this increase in incorporation in heart and skeletal muscles. No such effect was observed in lungs. In skin the incorporation of U-^{14}C-proline into acid-insoluble collagen was decreased both by MO and MOFOC diets, and in aorta the incorporation into salt-soluble (1.0 M NaCl) collagen was maximum with MO diet. On all the 3 diets the incorporation of U-^{14}C-proline into salt-soluble collagens of heart, aorta and skin was higher than that into the acid-soluble collagen and was increased by MO and MOFOC diets (table 2).

Feeding of MO diet for 24 days (table 3) and 60 days (table 4) increased the total collagen content in heart, skeletal muscles, aorta and lungs as compared to the GNO diet. MOFOC diet suppressed this increase induced by MO diet, especially after feeding for 60 days. In skin the MO and MOFOC diets decreased the total collagen content.

After 60 days, the MO diet increased the acid-insoluble collagen content in heart, lungs, aorta and skeletal muscles (table 4). This was observed in heart and skeletal muscles even after 24 days of feeding MO diet (table 3).

Table 3. Effects of feeding GNO, MO, MOFOC diets, for 24 days, on the levels (mean ± S.D. μg/mg tissue proteins) of collagen fractions of various tissues.

Tissue Diet	NaCl-soluble collagen 0.45 M salt	 1.0 M salt	Acid-soluble collagen	Acid-insoluble collagen
Heart				
GNO	4.89 ± 0.41	3.74 ± 1.55	2.46 ± 1.30	16.12 ± 7.67
MO	4.14 ± 1.61	4.33 ± 0.75	1.23 ± 0.46	20.81 ± 0.96
MOFOC	5.65 ± 1.08	2.74 ± 1.14	2.51 ± 0.36	11.68 ± 2.30
Aorta				
GNO	26.70 ± 16.25	0.72 ± 0.33	0.50 ± 0.35	28.22 ± 15.72
MO	67.81 ± 15.08	2.73 ± 0.39	1.32 ± 0.23	72.52 ± 13.31
MOFOC	49.89 ± 12.59	1.52 ± 0.65	2.12 ± 0.18	53.75 ± 13.48
Skeletal muscles				
GNO	10.96 ± 6.55	2.59 ± 0.98	10.74 ± 9.63	25.02 ± 9.76
MO	8.21 ± 2.54	2.98 ± 0.84	2.02 ± 0.60	43.21 ± 7.76
MOFOC	8.70 ± 2.31	1.76 ± 0.33	3.76 ± 0.90	22.83 ± 5.66
Lungs				
GNO	4.06 ± 1.12	1.96 ± 0.33	3.18 ± 0.19	10.77 ± 1.72
MO	20.27 ± 3.51	1.48 ± 0.18	4.08 ± 1.46	27.04 ± 2.76
MOFOC	13.12 ± 4.83	2.30 ± 1.30	3.89 ± 1.76	21.99 ± 6.69
Skin				
GNO	9.08 ± 2.02	2.92 ± 1.20	1.41 ± 0.84	25.60 ± 16.94
MO	8.05 ± 3.31	1.95 ± 0.05	1.68 ± 0.34	19.12 ± 3.77
MOFOC	10.02 ± 1.86	1.62 ± 0.25	2.13 ± 0.64	17.78 ± 2.32

MOFOC diet retarded this increase in acid-insoluble collagen. Both MO and MOFOC diets decreased this acid-insoluble collagen content in skin (tables 3, 4). After 24 days of feeding, MO diet decreased the acid-soluble collagen content of heart and skeletal muscles (table 3), whereas in lungs and skin it decreased after 60 days of feeding (table 4). The salt-soluble collagen content increased in aorta both with MO and MOFOC diets, but the increase was of lower magnitude with MOFOC diet (tables 3, 4).

Feeding of MO diet for 80 days considerably increased the activity of monoamine oxidase (MAO) in all the tissues except skin, whereas MOFOC diet decreased the activity of MAO in all the tissues except aorta (table 5).

Discussion

The results (tables 3, 4) indicate that the increase in the levels of total collagen in heart, aorta, lungs and skeletal muscles of rats, induced by MO diet, is depressed with MOFOC diet. Similar trends observed in the activities of mitochondrial MAO in these tissues (table 5) indicate that MO diet influences the accumulation of collagen through its effect on MAO. Since MAO is involved in the maturation of collagen (15), the MO diet may enhance the maturation of collagen.

The salt-soluble, acid-soluble and acid-insoluble collagen represent newly formed collagen, degraded collagen and mature collagen, respec-

Table 4. Effect of feeding GNO, MO, MOFOC diets, for 60 days, on the levels (mean ± S.D. μg/mg tissue protein) of collagen fractions.

Tissue Diet	NaCl-soluble collagen 0.45 M salt	 1.0 M salt	Acid-soluble collagen	Acid-insoluble collagen
Heart				
GNO	4.44 ± 1.09	4.61 ± 1.78	1.90 ± 1.43	14.35 ± 3.02
MO	3.50 ± 2.27	4.68 ± 1.30	2.45 ± 1.53	15.98 ± 8.46
MOFOC	5.82 ± 1.28	4.05 ± 1.49	0.64 ± 0.42	13.23 ± 3.36
Aorta				
GNO	7.56 ± 2.08	1.48 ± 0.72	0.53 ± 0.17	12.27 ± 1.55
MO	7.66 ± 2.96	7.26 ± 1.37	0.80 ± 0.57	26.14 ± 1.28
MOFOC	4.35 ± 2.01	6.39 ± 2.35	3.01 ± 0.86	15.86 ± 3.20
Skeletal muscles				
GNO	4.50 ± 0.86	1.96 ± 0.86	3.16 ± 0.74	12.98 ± 2.88
MO	3.28 ± 1.84	2.42 ± 0.49	3.72 ± 2.13	16.69 ± 2.43
MOFOC	3.48 ± 1.11	1.74 ± 0.56	3.60 ± 1.20	10.20 ± 2.38
Lungs				
GNO	7.96 ± 1.58	4.52 ± 2.15	8.96 ± 1.64	28.10 ± 17.68
MO	5.77 ± 2.59	3.55 ± 0.91	7.74 ± 2.42	34.32 ± 13.28
MOFOC	8.84 ± 2.37	0.78 ± 0.42	4.24 ± 1.17	15.21 ± 1.79
Skin				
GNO	6.43 ± 1.53	4.11 ± 2.36	5.18 ± 3.12	19.43 ± 5.38
MO	4.52 ± 1.14	3.29 ± 0.78	2.33 ± 0.35	12.34 ± 2.96
MOFOC	7.39 ± 2.35	3.66 ± 1.43	1.77 ± 0.71	14.32 ± 2.60

Table 5. Activity of monoamine oxidase (units/mg protein) in the mitochondrial preparations from tissues of rats getting GNO, MO and MOFOC diets for 80 days.

Mitochondria from	GNO	MO	MOFOC
Heart	1.54	2.66 *(72.7)*	0.99 (35.5)
Lungs	5.75	9.02 *(89.9)*	3.23 (32.0)
Skeletal muscles	3.41	4.63 *(35.8)*	2.46 (27.9)
Aorta	7.94	13.13 *(65.3)*	8.47 *(0.7)*
Skin	2.70	2.59 (0.4)	1.94 (28.2)
Liver	4.49	5.83 (29.9)	3.18 (29.2)

0.5 ml of mitochondrial preparation were incubated with 2.5×10^{-2}M benzylamine in a final volume of 4.0 ml with phosphate buffer (pH 8.0, 0.1 M) at 37 °C for 2 hours. Benzaldehyde formed was extracted with cyclohexane and read at 242 nm. Molecular extinction coefficient of benzaldehyde in cyclohexane is $1.37\times10^{4}M^{-1}$ cm^{-1} figures in parentheses represent the % change in the activity of monoamine oxidase (MAO). The figures in parentheses and in italics are for the increase in activity and others are for the decrease in activity of MAO.

tively (13, 17). On this basis, the increased levels of total and acid-insoluble collagen, and decreased levels of salt-soluble collagen in heart, lungs, and skeletal muscles indicate that the biosynthesis and maturation of collagen are increased by MO diet and depressed by MOFOC diet (tables 3, 4). The

increase in biosynthesis and maturation of collagen is substantiated by the increased incorporation of U-^{14}C-proline into total and acid-insoluble collagen in heart, lungs and skeletal muscles when MO and MOFOC diets are fed for 24 days (table 2). In skin and aorta the increased incorporation of U-^{14}C-proline into salt-soluble collagen but not into acid-insoluble collagen indicates that the collagen biosynthesis is enhanced in these tissues. The effects being of lower magnitude when MOFOC diet was fed (table 2).

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