RAPESEED OILS AS SOURCES OF ESSENTIAL FATTY ACIDS

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Although the high erucic acid content of the older varieties of rapeseed oil undoubtedly accounts for many of the problems associated with these oils, the relatively low linoleate-linolenate ratio in the oil has led to speculation that marginal essential fatty acid deficiency might occur when rapeseed oil is the sole source of dietary fat. This premise is based on the known competition between these acids for the elongation-desaturation enzymes in the tissues, which favours the W3 (linolenic) over the W6 (linoleic) acid. Rapeseed oils were therefore evaluated as sources of essential fatty acids (EFA) by monitoring physiological and biochemical parameters believed to reflect the EFA status of the animal. Erythrocyte hemolysis, mitochondrial swelling and glutamic dehydrogenase activity, testicular histology and tissue fatty acid profiles were the parameters evaluated and the relationship of dietary fatty acids to cardiac necrosis was also investigated.

EXPERIMENTAL

Male weanling Wistar rats were fed purified diets containing 20 % fat (by weight). Safflower oil, soybean oil, hydrogenated coconut oil, high erucic acid rapeseed oil (HEAR, B.campestris), low erucic acid rapeseed oil (LEAR, B.napus, CV Tower), "soybean high erucic acid oil" (SBHEAO) and "low linolenic-high erucic acid oil" (LLHEAO) were the fats investigated. SBHEAO and LLHEAO contained as much erucic acid as HEAR (26-30 %) but the 18:2/18:3 ratio of the former was similar to that of rapeseed oil (approx. 6) and the latter contained little linolenic acid.

Erythrocytes were hemolyzed in isotonic (0.3M) thiourea and the time required to hemolyze 75 % of the cells was recorded from the decrease in absorbance at 650nm. Mitochondrial swelling was assessed in 0.24M sucrose containing 20mM TRIS-HCL and 2mM K_2HPO_4; the change in absorbance at 520nm was recorded with time. After rupture of liver mitochondria by repeated freezing and thawing and removal of sediment by centrifugation glutamic dehydrogenase was assayed in the supernatant by measuring the reduction of NAD to NADH spectrophotometrically.

Rat testicular and cardiac sections were examined histologically after staining with hematoxylin and eosin and the incidence and severity of any lesions recorded. Fatty acid analyses were performed by gas-liquid chromatography of methyl esters prepared from chloroform-methanol extracts of the various tissues. Cholesterol was determined colorimetrically using the ferric chloride reagent and phosphorus was assayed by formation of molybdenum blue.

In a second study, rats were fed purified diets containing 20 % corn oil, lard-corn oil, olive oil or peanut oil, and the preceding oils admixed with 15 % linseed oil, to yield dietary fats containing approximately 10 % linolenic acid. Cardiac tissue was examined histologically as before.

RESULTS AND DISCUSSION

Hemolysis of erythrocytes in the non-electrolyte, thiourea, was more rapid

with cells from EFA-deficient rats than with those from rats fed the safflower oil control diet (Table 1), confirming our earlier observations. Cells from rats fed soybean oil, SBHEAO or the rapeseed oils were more stable than cells from rats fed safflower oil and those from rats fed LLHEAO had the greatest hemolysis time.

TABLE 1

THIOUREA-INDUCED HEMOLYSIS IN ERYTHROCYTES FROM RATS FED DIFFERENT DIETARY FATS

Dietary fat	75 % Hemolysis Time (secs)	Dietary fat	75 % Hemolysis Time (secs)
Hydrogenated coconut oil	32.0 ± 0.94*	HEAR	41.8 ± 0.8
Safflower oil	36.1 ± 1.20	SBHEAO	40.7 ± 1.16
Soybean oil	41.0 ± 0.91	LLHEAO	47.1 ± 1.27
		LEAR	42.8 ± 1.38

* Mean of 5 rats ± SEM

Erythrocytes from rats fed hydrogenated coconut oil were rich in 20:3W9 (14.7 %), whereas cells from the remaining rats contained little of this acid. Cells from the EFA-deficient rats were low in the W6 acids, particularly 20:4W6 (11.5 %) which was relatively high in the soybean and safflower groups (20.3 and 22.5 %). Dietary rapeseed oil resulted in slightly lower levels (14-19 %) of 20:4W6 in erythrocytes. Red cell erucic acid ranged from 2 to 5 % in the high erucic acid groups with the LLHEAO group containing the highest level. A complex curvilinear relationship appeared to exist between erythrocyte hemolysis and W6 or W9 fatty acids, with maximum stability associated with intermediate values for either of these two groups of acids and lower cell stability if either W6 or W9 acids were excessive (safflower and coconut oil groups).

Dietary rapeseed oils did not evoke erythrocyte hemolysis characteristics resembling that observed in EFA-deficient rats. On the contrary, diets containing these fats appeared to promote stabilization of the cells in the presence of the non-electrolyte relative to the situation observed with the control safflower oil.

As observed by other workers, swelling of mitochondria from EFA-deficient rats exceeded that of organelles from control (safflower oil) animals (Table 2). Mitochondria from rats fed safflower, soybean, HEAR and SBHEAO had similar swelling characteristics, whereas those from rats fed LEAR or LLHEAO were more susceptible to swelling and resembled mitochondria from EFA-deficient rats.

No simple relationship between mitochondrial swelling and fatty acid composition was evident.

Organelles from EFA-deficient rats were low in 20:4W6 in contrast to those fed LEAR but they exhibited similar swelling tendencies. Mitochondria from rats fed HEAR swelled significantly less than those from LEAR fed rats and yet the 20:4W6 contents were similar. Erucic acid usually accounted for less than 2 % of the total fatty acids even when high erucic oils were

TABLE 2

SWELLING OF RAT LIVER MITOCHONDRIA IN PHOSPHATE BUFFER

Dietary fat	Relative swelling[1]	Dietary fat	Relative swelling
Hydrogenated coconut oil	58.3 ± 0.92	HEAR	50.2 ± 0.55
Safflower oil	53.2 ± 1.74	SBHEAO	51.7 ± 1.51
Soybean oil	53.3 ± 2.82	LLHEAO	56.4 ± 2.23
		LEAR	59.3 ± 0.72

[1] Mean of 5 rats ± SEM

[2] Expressed as % decrease in initial absorbance over a 25 minute period

fed. There did appear to be a positive linear correlation between the susceptibility of the mitochondria to swell and the magnitude of the cholesterol: lipid phosphorus ratio in the particle which is consistent with cholesterol promotin increased membrane permeability.

Low erucic rapeseed oil and LLHEAO did behave in a similar manner to coconut oil with respect to promoting mitochondrial swelling but this was not true of the high erucic oils.

Mitochondrial glutamic dehydrogenase proved to be of limited use in assessing EFA-status of the experimental animals since the activity recorded for the deficient rats did not differ significantly from those observed with the safflower and soybean oil fed control animals; the HEAR group also yielded similar results to these three groups. Significantly lower activity was noted with the SBHEAO and LEAR rats.

After 12 weeks on experimental diets, mean testicular weights (absolute) did not differ among the dietary groups. Because of the lower body weight, the relative testicular weight of rats fed coconut oil was significantly greater than the control groups, whereas those of the rapeseed oils did not differ from the controls. There was no histological evidence for testicular degeneration in rats fed the rapeseed oils.

Feeding oils rich in erucic acid for 25 weeks induced severe cardiac necrosis in male Wistar rats (Table 3). LEAR resulted in a lower lesion incidence-severity rating than the high erucic groups and soybean oil rated just below LEAR. Addition of linoleic acid to HEAR failed to alleviate cardiac necrosis (SBHEAO) but complete omission of 18:3 from high erucic oil (LLHEAO) resulted in a substantial reduction in lesion-incidence severity rating. Lesion incidence was low in the EFA-deficient group, indicating that such a deficiency was not the cause of cardiac necrosis with rapeseed oils.

The possible role of linolenic acid in the etiology of cardiac necrosis in rats fed rapeseed oil was investigated by adding linseed oil to control oils not considered to be cardiotoxic. This process failed to increase lesion incidence substantially in rats fed the fats for 17 weeks (Table 4).

TABLE 3

INCIDENCE-SEVERITY RATINGS[1] OF CARDIAC LESIONS IN RATS FED DIFFERENT DIETARY FATS

Dietary fat	Fresh necrosis	Old necrosis	Dietary fat	Fresh necrosis	Old necrosis
Coconut oil (14)	14	10	HEAR (14)	50	69
Safflower oil (15)	2	11	SBHEAO (15)	49	60
Soybean oil (14)	24	29	LLHEAO (14)	21	38
			LEAR (14)	36	36

Severity: 1 - very mild; 2 - mild; 3 - moderately severe. Ratings expressed as % of maximum possible rating for group. Numbers of animals in group given in parentheses.

TABLE 4

LINOLENIC ACID AND CARDIAC NECROSIS IN RATS[1]

Dietary fat	Incidence severity	Dietary fat	Incidence severity
Corn oil (20)	0	Corn-Linseed (19)	7
Corn-Lard (20)	7	Corn-Lard-Linseed (20)	7
Olive oil (20)	15	Olive-Linseed (19)	19
Peanut oil (20)	20	Peanut-Linseed (20)	7
LEAR (20)	20	Soybean oil (19)	11

[1] see footnotes, Table 3

Indeed, addition of linseed oil to peanut oil reduced cardiac necrosis. The relatively high ratings of olive and peanut oil were unexpected, and are not readily explained. The involvement of linolenic acid in the etiology of necrosis was not confirmed.

In a subsequent study, the effects of adding a commercial antioxidant to rapeseed oil were investigated. After 17 weeks, the two LEAR oils investigated (var. Tower and var. Candle), resulted in approximately twice the incidence severity ratings of the control oils (Table 5). However, addition of 0.05 % antioxidant to the Candle oil limited lesion induction to that of the control oils, raising the possibility that autoxidation of dietary fat may play a role in cardiac necrosis. Rapeseed oils with their high linolenic and total unsaturated fatty acid content would be particularly susceptible to oxidative deterioration. This possibility is under investigation.

TABLE 5

ANTIOXIDANT AND CARDIAC NECROSIS IN RATS

Dietary fat	Incidence severity[1]
Corn oil (15)	11
Soybean oil (15)	13
LEAR, var. Tower (15)	38
LEAR, var. Candle (15)	31
LEAR, var. Candle and antioxidant[2] (15)	16
HEAR (14)	48

[1] see footnotes, Table 3

[2] 0.05 % of oil, contains 20 % BHA, 20 % BHT, 10 % glyceryl monocitrate, 50 % vegetable oil carrier

There was no consistent support for over or covert essential fatty acid deficiency in rats fed rapeseed oil.