Dietary level of maize oil affects growth and lipid composition of Walker 256 carcinosarcoma

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(*Received 4 January 1993 – Revised 24 March 1993 – Accepted 1 April 1993*)

Walker 256 carcinosarcoma cells (1×10^4) were injected into the right thigh muscle of Sprague–Dawley rats (125 g) consuming isoenergetic (200 g fat/kg) diets containing 20, 100 and 200 g maize oil/kg and 180, 100 or 0 g hydrogenated lard/kg respectively. Ten rats from each dietary regimen were killed every 4th day. Tumours grew rapidly from day 0 to day 8 post-transplant regardless of dietary regimen. However, after 8 d more tumours regressed and there were fewer deaths in animals fed on 200 g maize oil/kg compared with animals fed on 20 or 100 g maize oil/kg. Linoleic acid (LA) levels were higher in phospholipids (PL) of growing tumours than in regressing tumours whereas arachidonic acid levels in PL were lower in growing tumours indicating a possible alteration in the desaturation and elongation of LA. Serum prostaglandin E_2 levels were slightly lower in rats with regressing tumours than in rats with growing tumours.

Linoleic acid: Cancer: Lipids: Walker 256 carcinosarcoma: Growth

Walker 256 carcinosarcoma was discovered in 1928 as a spontaneous mammary tumour (Morrison, 1972). This tumour cell line has been used in many studies because it is easily transplanted, is species specific for rats and grows rapidly in the host animal. A palpable tumour usually develops within 4 d post-transplant and it can grow to a mean diameter of 20–30 mm within 8 d (Cho-Chung, 1974; Morrison, 1972; Krause *et al.* 1979; Owen, 1982; Varani & Perone, 1985). If tumour growth is left unchecked the tumour can kill the animal within 1–3 weeks (Kwong *et al.* 1984). The growth and/or regression of Walker 256 carcinosarcoma is dependent on the number of cells injected into the recipient animal (Kwong *et al.* 1984). Injection of 1×10^4 tumour cells into the thigh of rats resulted in a logarithmic growth pattern for the first 8 d post-transplant followed by regression of a non-palpable state by the 14th day post-transplantation. In contrast, injection of 2×10^4 tumour cells resulted in a similar growth pattern with no sign of regression of the tumour after 14 d.

The Walker 256 carcinosarcoma grows rapidly, is quite invasive, and its growth is affected by nutrients. Dills *et al.* (1984) showed a direct relationship between the level of glucose in the diet and tumour growth, reflecting the limiting level of glycolysis in the rapidly dividing tumour cells. Cho-Chung and co-workers (Cho-Chung & Gullino, 1973, 1974; Cho-Chung & Berghoffer, 1974; Cho-Chung & Clair, 1977) demonstrated that growth could be arrested by administration of dibutyryl cyclic AMP in certain instances.

Kwong *et al.* (1984) observed regression of tumours in rats fed on a semi-purified diet containing 200 g maize oil (MO)/kg after 9 d post-transplant (1×10^4 cells initially) while tumours of rats fed on a diet containing only the corresponding fatty acids (derived from MO) continued to grow, suggesting that components of the non-saponifiable fraction of maize oil might be involved in tumour regression.

https://doi.org/10.1079/BJN19940134 Published online by Cambridge University Press

Other researchers have shown that the type of fat consumed by experimental animals affects the growth of tumours (Gammal *et al.* 1967; Hillyard & Abraham, 1979; Cohen *et al.* 1986) and certain unsaturated fatty acids are more effective than saturated fats in enhancing mammary tumorigenesis (Hopkins & Carroll, 1979; Hopkins *et al.* 1981). According to Brenner (1984), this may be related to eicosanoid synthesis, especially prostaglandin E_2 (PGE_2), from arachidonic acid (AA). PGE_2, an immunosuppressant, is present in large amounts in human malignant tumour tissue (Doll *et al.* 1966; Karmali, 1980) and this can suppress normal immune responses. The Walker 256 carcinosarcoma produces PGE_2 in ascites fluid (Varani & Perone, 1985). Thus, the consumption of high levels of dietary linoleic acid (LA), a precursor of AA, may facilitate tumour growth by enhancing PGE_2 synthesis.

In contrast, dietary *n*-3 polyunsaturated fatty acids (PUFA) apparently reduce mammary tumour growth (Doll *et al.* 1966; Berg, 1975; Bang *et al.* 1976; Nielsen & Hansen, 1980). The *n*-3 fatty acids, in particular eicosapentaenoic acid (EPA), inhibit PGE_2 synthesis and this may be related to their antitumour effects (Lokesh & Kinsella, 1988). Karmali *et al.* (1984) demonstrated that dietary fish oil decreased PGE_2, $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and thromboxane B_2 (TXB_2), and reduced the size of R3230AC transplantable mammary tumour. However, in trials with Walker 256 carcinosarcoma we found no inhibition of growth or regression of tumours in rats fed on 200 g fish oil/kg (J. M. Black, M. C. Nesheim and J. E. Kinsella, unpublished results). In contrast, preliminary studies revealed that tumours showed a dramatic regression after 9 d in rats fed on 200 g MO/kg. This was associated with a reduction in AA levels in tumours, suggesting that Δ-6 desaturase was inhibited, thereby reducing AA and subsequently PGE_2 production.

To examine this possibility in more detail we conducted experiments to ascertain the effects of three levels of MO in an isoenergetic diet, on the growth, fatty acid composition of phospholipids, prostaglandin E_2, and regression of Walker 256 carcinosarcoma.

MATERIALS AND METHODS

Tumour

Walker 256 carcinosarcoma was obtained from EG&G Mason Research Institute (Worcester, MA, USA) and was propagated by trocar injection into the right thigh of a Sprague–Dawley rat weighing approximately 125 g. Uniformly viable cells were routinely prepared according to the procedure of Kwong *et al.* (1984). At 8 d after injection of 2×10^4 cells the tumour was removed and homogenized in sterile saline (9 g NaCl/l) to disperse the cells which were recovered, as described by Kwong *et al.* (1984). Tumour cell number was determined by haemocytometer and the cells were aseptically injected by syringe into rats (approximately 125 g) for further propagation. Tumour propagation was carried through three generations before starting experiments (Kwong *et al.* 1984).

Animals

Sprague–Dawley rats (Blue Spruce Farms, Altamont, NY, USA); *n* 150, 50–75 g initial weight) were fed on standard rat laboratory ration (Prolab Agway, Syracuse, NY, USA) for 2 d before feeding experimental diets. The rats were then randomly assigned to the three dietary regimens. Animals were housed individually in stainless-steel cages. Food and water were provided *ad lib.* and a 12 h light–dark cycle was maintained in the room. After 1 week the rats were injected in the thigh with 1×10^4 viable Walker 256 carcinosarcoma cells as described by Kwong *et al.* (1984). Animal weight, food consumption and tumour growth were recorded daily as described (Kwong *et al.* 1984). Ten animals from each dietary group were killed every 4 d from day 0 to day 16 following injection of the tumour cells.

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Diets

The three experimental diets (Table 1) were based on AIN-76 purified diets (American Institute of Nutrition, 1977). Total dietary fat was 200 g/kg diet. Dietary fat was composed of 20, 100 or 200 g MO (Mazola Oil; CPC International, Englewood Cliffs, NJ, USA)/kg and 180, 100 or 0 g hydrogenated lard (Kraft, Inc., Chicago, IL, USA)/kg respectively. The hydrogenated lard contained less than 20 g linoleic acid (LA)/kg. Saturated, monounsaturated and *n*-6 diunsaturated fatty acids comprised approximately 99% of the fatty acid composition (Table 2). LA contributed 5·6, 13·2 and 23·2% energy in the 20, 100 and 200 g MO/kg diets respectively.

The vitamin E level was held constant in all diets by adjusting the 20 and 100 g MO/kg diets with α-tocopherol (Sigma, St Louis, MO, USA). Fresh diets were fed to the animals daily and uneaten food was discarded. Fresh diets were kept in sealed containers, flushed with N_2 and stored at $-30°$ until used.

Analytical procedures

Animals were anaesthetized with diethyl ether, the peritoneum was opened and blood was drawn from the dorsal aorta. Serum was separated from erythrocytes by centrifugation and 0·5 ml serum was extracted for prostaglandin analyses as described previously (Bruckner *et al.* 1983; Lokesh *et al.* 1986). Tumour growth and size was analysed as previously described in detail (Dills *et al.* 1984; Kwong *et al.* 1984). The tumour tissue from the right thigh and the semi-membranosus muscle from the contralateral thigh were then excised from each animal. Representative samples of both the viable tumour and the muscle (0·5 g) were homogenized and extracted for prostaglandin analyses. All samples were immediately frozen in liquid N_2 and stored at $-70°$ until analysed.

Prostaglandin analysis

Serum, tumour and muscle were extracted in three volumes ethyl acetate and quantified by radioimmunoassay (Lokesh *et al.* 1986). The PGE_2 antiserum had a cross reactivity of less than 1% with TXB_2, 6-keto $PGF_{1\alpha}$, hydroxyeicosatetraenoic acid and AA.

Lipid extraction and quantification

Total lipids (TL) in diets and tissues were extracted using a modified hexane–propan-2-ol technique (Emilson & Sundler, 1985). Tissue samples were homogenized in 50 mM-Tris, pH 7·6, containing 1 mM-EDTA before extraction. Following extraction, the organic phase containing the lipid was dried under vacuum and the total amount of lipid per g sample was determined gravimetrically. Neutral lipids (NL) were separated from phospholipids (PL) using BondElute aminopropyl microcolumns (Analytichem International, Harbor City, CA, USA). The phospholipid classes were separated by TLC using a solvent system of chloroform–methanol–acetic acid–water (50:40:8:2, by vol.; Swanson *et al.* 1987) and PL classes were identified using PL standards (Sigma). Bands corresponding to PL standards were scraped and lipids were eluted from the silica gel with toluene. All fractions were saponified (Christie, 1982), fatty acids were converted to fatty acid methyl esters (FAME) by diazomethane (Lokesh & Kinsella, 1985), and separated and quantified by GLC (5880A gas chromatograph; Hewlett Packard, Avondale, PA, USA) using a 60 m × 0·75 mm SP-2330 glass capillary column (Supelco, Bellefonte, PA, USA). Flow-rate was set at 6·0 ml/min and oven temperature was programmed at 5°/min from 140 to 240°. H_2 was used for makeup gas at 20 ml/min. FAME were identified by comparison of retention times with authentic FA standards (Lokesh & Kinsella, 1985).

The α- and γ-tocopherol levels were determined in both MO and lard by a modified

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Table 1. *Composition (g/kg) of diets fed to experimental rats for 23 d*

Diet...	1	2	3
Vitamin-free casein	200	200	200
DL-Methionine	3	3	3
Maize starch	150	150	150
Sucrose	350	350	350
Cellulose (Alphacel)	50	50	50
Mineral mix (AIN-76A)	35	35	35
Vitamin mix (AIN-76A)	10	10	10
Choline chloride	2	2	2
α-Tocopherol (mg)	3	3	3
BHT (mg)	5	5	5
Maize oil	20	100	200
Lard	180	100	0

BHT, butylated hydroxytoluene.

Table 2. *Fatty acid composition (mol/100 mol) of dietary fats fed to experimental rats for 23 d**

(Values are means with their standard errors for three analyses in each group)

Diet...	1		2		3	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14:0	1·1	0·1	0·7	0·1	0·0	
16:0	25·1	0·1	19·4	0·1	12·2	0·1
16:1	2·7	0·1	1·8	0·1	0·0	
18:0	13·8	0·2	8·5	0·1	1·9	0·1
18:1	41·7	0·2	35·2	0·1	27·1	0·1
18:2*n*6	14·1	0·2	33·3	0·1	58·0	0·1
18:3*n*3	0·6	0·1	0·7	0·1	0·8	0·1
20:4*n*6	0·1	0·1	0·0		0·0	

* For details of composition of diets, see Table 1.

HPLC method using a Waters Model M-45 solvent delivery system, a Whatman Partisphere c18 cartridge column and a Hewlett Packard 1040 Photodiode Array spectrophotometric detector (Chow & Omaye, 1983). A methanol–water (95:5, v/v) solvent system separated α- and γ-tocopherol and their esters. Total amounts of vitamin E were adjusted to α-tocopherol by giving a potency value of 0·1 α-tocopherol = 1·0 γ-tocopherol.

Statistical analysis

Changes in the concentrations of fatty acids, prostaglandins and vitamin E over time were analysed by analysis of variance (Snedecor & Cochran, 1980). Means for each group were tested by Duncan's multiple-range test. Statistical analysis was performed using SAS Version 5 (SAS Institute Inc., 1985).

RESULTS

Animal weight gain and food consumption did not vary significantly ($P > 0·05$) among the groups over the experimental period. At approximately day 4 post-transplant, food consumption by all animals decreased to a minimum and then slowly increased from days

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12 to 16. However, when the tumour weight was subtracted from total animal weight there was no gain in animal weight from day 4 post-transplant to day 8, indicating that during this period the tumour was growing at the expense of the host.

Tumour growth

Palpable tumours were detectable around 4 d post-transplant in all 150 rats. Examination postmortem showed that the tumour had migrated above the femur into the semimembranosus muscle and as the tumour grew it appeared to consume the surrounding muscle. On the 8th day post-transplant the tumour had developed a necrotic core while the outer layer was highly vascularized and viable. Between 8 and 12 d post-transplant the tumour had metastasized in some rats and lymph nodes were swollen. These animals showed no regression of the tumour. In rats in which tumour regression occurred the tumour mass appeared smaller, the viable outer layer thinner and regrowth of muscle tissue was apparent by 12 d post-transplant. Rats with regressing tumours recovered almost completely by the 16th day post-transplant and only a small necrotic mass was observed on examination post-mortem. The tumours that did not regress had a large necrotic core and a viable vascularized outer layer, which was weighed and recorded (Fig. 1).

Fifteen animals (10% of total) died from tumour growth during the study (Table 3). The first death occurred 8 d post-transplant with eight deaths occurring 9 d post-transplant. The remaining five deaths occurred during the last 2 d of the study. Of the deaths, 40% occurred in each of the groups of animals consuming diets with 20 and 100 g MO/kg while 20% of the deaths occurred in the group consuming diets with 200 g MO/kg.

Effect of diet

No difference was observed in the growth rate of the tumour between diets for the first 8 d post-transplant; however, after day 8 tumour growth behaviour differed with each dietary regimen (Fig. 1). The average tumour weight of rats fed on 20 and 200 g MO/kg tended to decrease after 8 d post-transplant while the average tumour weight of rats fed on 100 g MO/kg tended to increase. Since each dietary group contained animals with growing and regressing tumours, the data were analysed according to tumour growth from day 8 to day 16 (the end of the study). Tumours that continued to grow were classified as growing tumours while tumours that became smaller after day 8 were classified as regressing tumours. Most of the regressing tumours were observed in rats fed on the 200 g MO/kg diet. Of the total tumours, 24% regressed in rats consuming 200 g MO/kg as compared with only 8% of all tumours in animals consuming 20 g MO/kg, while tumours from rats fed on 100 g MO/kg exhibited an intermediate response.

Lipid composition

In order to determine whether the differences in growth and regression of the tumours were related to differences in lipid content and fatty acid composition, the lipids were analysed in detail. TL and total PL content in muscle and tumour tissue were comparable, i.e. 35 g TL/kg wet weight. The PL were approximately 15 g/100 g TL.

Choline phosphoglyceride (PC) and ethanolamine phosphoglyceride (PE) comprised greater than 90 g/100 g total PL in both muscle and tumour; the changes in fatty acid composition of muscle and tumour PC and PE were comparable (Tables 4 and 5). The LA and AA levels were the same in both PL classes, although docosahexaenoic acid (DHA) levels were much higher in PE than PC. However, the DHA and docosa-7,10,13,16,19-pentaenoic acid (DPA, *n*-3) levels in PE of tumours rapidly decreased with tumour growth.

While muscle and tumour contained the same major fatty acids, muscle PC tended to contain more unsaturated fatty acids than tumour PC. The muscle PC contained a higher

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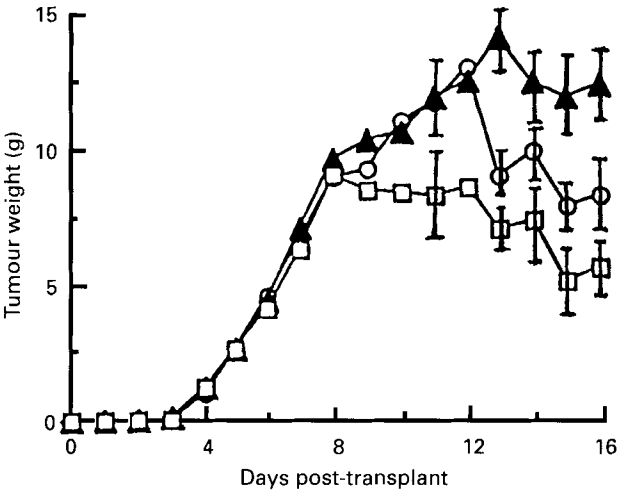

Fig. 1. Growth rate (post-transplant) of Walker 256 carcinosarcoma transplanted into the right thigh of experimental rats maintained on diets containing increasing levels (g/kg) of maize oil: (○), 20; (▲) 100; (□), 200. For details of diets and procedures, see Tables 1 and 2, and pp. 284–285. Tumour growth values (measured as described on p. 285) are daily means for each dietary group with their standard errors represented by vertical bars. $n = 150$ at day 0 but decreased by at least 30 every 4th day of post-transplant of the tumour to day 16. The decrease in n per dietary group was greater on days when rats died (see Table 3).

Table 3. *The number of animal deaths at specific days post-transplant of Walker 256 carcinosarcoma from experimental rats fed on diets containing increasing levels of maize oil (MO)**

(Values are nos. of deaths per day post-transplant of the tumour. Rats were maintained on the experimental diets 5 d before transplant of tumour into the right thigh and continued on diet until death)

Dietary fat (g MO/kg)... Period post-transplant (d)	20	100	200
8	1	0	0
9	2	4	2
10	1	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	1	1	0
15	1	1	1
Total	6	6	3

* For details of diets and procedures, see Tables 1 and 2 and p. 284.

amount of AA and DHA at day 8 post-transplant than did tumour PC. Muscle PE was more unsaturated than tumour PE because of its higher content of DHA.

The saturated fatty acids palmitic acid (PA) and stearic acid (SA) accounted for approximately 40 g/100 g PL in both muscle and tumour (values not shown). These fatty acids did not change quantitatively in PL of muscle with time or the level of dietary MO. However, PA (mg/g) decreased from 422 to 240, from 405 to 302 and from 373 to 322 in tumour tissue from animals fed on 20, 100 and 200 g MO/kg respectively. The SA in tumour tissue (mol/g) increased concomitantly from 78 to 150, 91 to 155, and from 82 to 104 respectively, with increasing dietary MO.

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Table 4. *Fatty acid composition (mol/100 mol) of choline phosphoglycerides extracted from Walker 256 carcinosarcoma and contralateral semimembranous muscle from experimental rats fed on different levels of maize oil (MO) from 7 d before tumour transplant for 23 d**

(Values are means with their standard errors for eight rats per group. Lipids from tumour tissue were extracted every 4 d from day 0 to day 16 post-transplant; lipids from muscle tissue were extracted from days 0, 8 and 12 post-transplant)

		Tumour						Muscle					
Dietary fat (g MO/kg)...		20		100		200		20		100		200	
Fatty acid	Period post-transplant (d)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
18:1*n*9	0	12·9^{d}	0·1	11·5^{d}	0·6	11·5^{d}	0·7	10·0^{d}	0·6	8·6^{d}	1·2	10·7	0·6
	4	15·1^{ad}	1·3	11·9^{bde}	0·3	9·9^{bd}	0·3	—	—	—	—	—	—
	8	25·1^{ae}	1·3	19·7^{bf}	1·0	14·5^{ce}	0·6	11·1^{ade}	0·9	9·8^{abde}	0·3	9·1^{b}	0·6
	12	22·9^{ae}	1·7	16·4^{bef}	2·0	11·2^{cd}	0·9	12·8^{ad}	0·5	11·5^{ae}	0·5	9·5^{b}	0·3
	16	21·7^{ae}	2·2	10·8^{bd}	0·4	11·1^{bd}	0·6	—	—	—	—	—	—
18:2*n*6	0	21·4^{a}	0·9	22·5^{abd}	1·2	28·1^{b}	2·9	23·6^{ad}	0·6	22·9^{ad}	1·6	17·2^{b}	1·7
	4	19·2^{a}	0·4	20·4^{ad}	1·2	25·0^{b}	0·7	—	—	—	—	—	—
	8	17·7^{a}	0·5	23·7^{bd}	1·3	24·5^{b}	1·8	16·4^{e}	0·9	17·1^{e}	1·0	18·4	1·0
	12	19·5	1·3	22·9^{d}	1·7	22·5	2·5	12·7^{af}	0·9	17·9^{be}	1·3	19·9^{b}	1·3
	16	16·4	3·3	12·0^{e}	1·5	21·3	2·3	—	—	—	—	—	—
20:4*n*6	0	10·0de	0·5	10·7de	0·9	11·5	1·5	12·2^{d}	0·7	8·0^{d}	2·7	13·5^{d}	1·2
	4	12·3de	0·8	13·8^{e}	0·8	12·7	0·3	—	—	—	—	—	—
	8	8·7de	0·6	9·9^{d}	0·8	10·1	1·5	17·4^{e}	0·9	17·8^{e}	0·7	17·4^{e}	1·5
	12	8·5^{ad}	0·7	10·0^{abd}	0·9	13·5^{b}	2·9	19·0^{e}	0·4	17·4^{e}	1·3	17·9^{e}	0·8
	16	13·1^{e}	3·0	23·4^{f}	0·0	15·3	2·5	—	—	—	—	—	—
22:4*n*6	0	0·5^{a}	0·3	0·9^{d}	0·3	0·9	0·5	0·4^{abd}	0·1	0·2^{ad}	0·1	0·5^{bd}	0·1
	4	0·8^{d}	0·2	1·1^{d}	0·3	0·3	0·1	—	—	—	—	—	—
	8	1·0de	0·1	1·1^{d}	0·2	1·9	1·0	0·6^{e}	0·1	0·6^{e}	0·1	0·7de	0·1
	12	1·0^{ade}	0·2	2·0^{be}	0·2	2·2^{b}	0·3	0·4^{ad}	0·0	0·6^{ae}	0·1	0·9^{be}	0·1
	16	1·6^{ae}	0·4	3·3^{bf}	0·0	1·7^{a}	0·2	—	—	—	—	—	—
22:6*n*3	0	3·32^{d}	0·49	3·48^{d}	0·96	2·4de	0·8	4·9	0·2	5·1	0·4	7·1	1·6
	4	2·48de	0·65	2·05de	0·48	3·6^{d}	1·0	—	—	—	—	—	—
	8	1·41^{ef}	0·22	1·20^{e}	0·25	1·6^{e}	0·5	6·2	0·8	5·2	0·5	4·8	0·3
	12	1·38^{ef}	0·28	1·85de	0·19	1·1^{e}	0·4	5·5	0·6	4·7	0·5	4·5	0·5
	16	1·08^{f}	0·40	0·78^{e}	0·02	1·1^{e}	0·3	—	—	—	—	—	—

^{a,b,c} Means with different superscript letters were significantly different between diets ($P < 0·05$).
^{d,e,f} Means with different superscript letters were significantly different for period (d) on diet ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 284–286.

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Table 5. *Fatty acid composition (mol/100 mol) of ethanolamine phosphoglycerides extracted from Walker 256 carcinosarcoma and contralateral semimembranous muscle from experimental rats fed on different levels of maize oil (MO) from 7 d before tumour transplant for 23 d**

(Values are means with their standard errors for eight rats per group. Lipids from tumour tissue were extracted every 4 d from day 0 to day 16 post transplant; lipids from muscle tissue were extracted from days 0, 8 and 12 post-transplant)

		Tumour						Muscle					
Dietary fat (g MO/kg)...		20		100		200		20		100		200	
Fatty acid	Period post-transplant (d)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
18:1*n*9	0	9·5^{c}	1·5	12·0^{cd}	1·3	13·9	3·5	8·2	0·7	9·3	1·0	9·3	0·7
	4	11·3^{cd}	1·0	10·0de	0·5	11·6	0·5	—	—	—	—	—	—
	8	17·2^{d}	2·6	14·2^{d}	0·6	13·2	0·6	8·5^{ab}	0·7	9·1^{a}	0·4	7·4^{a}	0·3
	12	14·6^{acd}	0·5	10·9^{bcd}	0·5	9·1	0·6	9·5	1·8	8·0	1·3	7·9	0·3
	16	10·3^{cd}	2·1	7·9^{e}	0·0	10·2	0·4	—	—	—	—	—	—
18:2*n*6	0	8·8^{cd}	0·6	9·5	0·5	9·7^{ac}	1·9	8·0^{c}	0·7	8·4^{c}	0·3	9·5	1·1
	4	8·2^{acd}	0·4	8·6^{ac}	0·4	11·5^{bcd}	0·6	—	—	—	—	—	—
	8	10·8^{ac}	0·6	11·6^{ac}	1·5	15·7^{bd}	1·3	7·2^{cd}	0·5	8·6^{d}	0·5	7·7	0·3
	12	11·3^{c}	0·6	11·7^{c}	0·6	11·1^{cd}	2·1	5·5^{ad}	0·4	6·9^{bd}	0·5	8·3^{c}	0·5
	16	5·6^{d}	2·4	3·4^{d}	0·2	10·2^{cd}	2·1	—	—	—	—	—	—
20:4*n*6	0	14·3^{c}	0·7	14·2	0·9	14·1^{cd}	1·4	12·8^{c}	0·2	13·0^{c}	0·2	13·2^{c}	0·5
	4	16·9^{acd}	1·2	16·6^{a}	0·5	13·3^{bd}	1·3	—	—	—	—	—	—
	8	22·1de	0·9	20·0^{cd}	2·2	20·2de	1·8	15·1^{d}	0·4	16·6^{cd}	1·5	16·3^{d}	0·6
	12	23·9^{e}	0·9	23·5^{c}	1·2	26·4^{e}	3·0	18·8^{e}	0·7	17·0^{d}	1·0	17·7^{d}	1·1
	16	22·5de	4·8	32·9^{d}	1·1	25·0^{e}	2·1	—	—	—	—	—	—
22:4*n*6	0	2·3^{abc}	0·2	3·1^{ac}	0·5	1·4^{bc}	0·5	1·5	0·2	1·6	0·1	1·8^{c}	0·1
	4	2·2^{c}	0·4	2·8^{c}	0·3	1·8^{c}	0·8	—	—	—	—	—	—
	8	5·0^{d}	0·3	6·6^{d}	0·5	6·6^{d}	0·8	1·7	0·1	2·3	0·3	2·4^{c}	0·2
	12	5·8^{ad}	0·4	7·8^{bd}	0·6	9·3^{bd}	0·9	1·7^{a}	0·1	1·5^{a}	0·5	3·1^{d}	0·2
	16	5·3^{ad}	1·4	14·0^{be}	0·1	8·0^{ad}	1·3	—	—	—	—	—	—
22:6*n*3	0	19·3^{c}	4·3	17·4^{c}	3·8	20·5^{c}	0·8	25·6	0·4	25·2	0·2	25·0^{c}	0·7
	4	18·9^{c}	2·3	18·9^{c}	0·6	19·1^{c}	3·0	—	—	—	—	—	—
	8	5·7^{d}	0·8	5·5^{d}	0·5	3·6^{d}	0·8	26·7	0·3	26·1	1·8	24·2^{c}	0·7
	12	4·6^{d}	0·2	4·4^{d}	0·7	4·1^{d}	1·1	24·7	1·7	23·8	0·6	21·4^{d}	0·9
	16	8·6^{d}	4·3	2·0^{d}	0·1	6·4^{d}	2·6	—	—	—	—	—	—

^{a,b} Means with different superscript letters were significantly different between diets ($P < 0·05$).
^{c,d,e} Means with different superscript letters were significantly different for period (d) on diet ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 284–286.

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Table 6. *Fatty acids (mol/100 mol) of choline phosphoglyceride and ethanolamine phosphoglyceride extracted from regressing or growing tumours in experimental rats fed on different levels of maize oil (MO) from 7 d before tumour transplant for 23 d**

		Choline phosphoglyceride				Ethanolamine phosphoglyceride			
Tumour status...		Regressed		Grew		Regressed		Grew	
Fatty acid	Dietary fat (g MO/kg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
18:1*n*9	20	19·2^{c}	1·2	24·7^{c}	1·9	8·7^{a}	2·0	15·0^{bc}	0·5
	100	10·8^{d}	0·4	14·6^{d}	1·0	9·1	1·2	10·5^{d}	0·5
	200	11·1^{d}	0·7	11·2^{d}	0·5	9·7	1·2	9·2^{d}	0·4
18:2*n*6	20	12·0	3·3	20·9	1·5	3·3^{a}	0·5	11·6^{bc}	0·5
	100	12·0^{a}	1·5	21·6^{b}	1·3	6·2^{a}	2·9	11·7^{bc}	0·9
	200	20·6	2·1	25·4	1·5	9·0^{a}	1·6	15·2^{bd}	0·6
20:4*n*6	20	17·4^{a}	2·0	7·2^{b}	1·0	22·8	6·8	23·2	0·9
	100	23·4^{a}	0·0	10·3^{b}	1·0	31·2^{a}	1·8	23·3^{b}	1·3
	200	16·5	2·1	8·6	0·3	27·3	2·3	21·6	0·5
22:4*n*6	20	2·1^{a}	0·3	0·8^{bc}	0·2	5·7^{c}	1·9	5·8^{c}	0·5
	100	3·3^{a}	0·0	2·0^{bd}	0·3	11·6^{d}	2·4	8·2^{d}	0·7
	200	2·0	0·3	1·8^{d}	0·1	9·0^{cd}	1·0	7·9^{d}	0·4
22:6*n*3	20	0·6	0·3	1·3	0·3	10·5	5·6	4·3	0·3
	100	0·8^{a}	0·0	1·8^{b}	0·2	2·3	0·3	4·4	0·9
	200	0·9	0·3	1·7	0·2	5·3	1·8	4·8	0·6

^{a,b} Means with different superscript letter were significantly different for tumour growth ($P < 0·05$).
^{c,d} Means with different superscript letters were significantly different between diets ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 284–286.

LA and AA in PC of normal muscle changed with dietary CO content (Table 4). Thus, muscle LA decreased with time on 20 g MO/kg, to a lesser extent on 100 g MO/kg whereas it increased with time in the muscle from animals consuming 200 g MO/kg (Table 4). AA tended to increase over time on all three diets.

The fatty acid composition of tumour PC was influenced by duration of diet regardless of level of dietary MO (Table 4). LA decreased while AA increased slightly with time (Table 4). Tumour docosa-7,10,13,16-tetraenoic (DTA) levels increased with all diets over time whereas it did not change in muscle. These changes indicated that, in contrast to muscle, the tumour possessed an active elongase enzyme which readily converted AA to DTA.

LA content was higher in the PL of growing than in regressing tumours (Table 6). Growing tumours had lower AA levels than did regressing tumours in animals on the different diets. The lower AA content in growing tumours was reflected in lower DTA as well.

Prostaglandin E_2

Serum PGE_2 concentrations were similar among animals on the different dietary levels of MO (Table 7) while PGE_2 concentrations in tumours varied with diet and time but showed no particular pattern. The levels of PGE_2 were lower in serum from animals with regressing tumours (Table 8). The PGE_2 was very variable in both regressing and growing tumours. The mean concentration of PGE_2 in normal contralateral muscle tissue was lower (6·0 (SE 2·7) ng PGE_2/mg tissue) than that observed for most of the tumour tissue (Tables 7 and 8).

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Table 7. *Prostaglandin E_2 (PGE_2) concentration of serum and tumour from experimental rats fed on different levels of maize oil (MO) from 7 d before tumour transplant for up to 23 d**

(Values are means with their standard errors for six rats per group)

Dietary fat (g MO/kg)...		20		100		200	
Tissue	Period post-transplant (d)	Mean	SE	Mean	SE	Mean	SE
Serum (ng PGE_2/0·1 ml)	0	3·4[ab]	0·3	4·1	0·3	4·2	0·5
	4	3·0[a]	0·2	3·8	0·3	3·6	0·4
	8	3·6[ab]	0·3	3·8	0·4	4·4	0·7
	12	4·5[b]	0·9	4·0	0·5	3·4	0·8
	16	3·8[ab]	0·2	4·3	0·5	4·0	0·4
Tumour (ng PGE_2/mg protein)	0	16·8	6·2	4·4[a]	1·9	4·5	1·7
	4	15·4	3·3	8·9[a]	0·9	9·1	1·5
	8	15·7	5·9	8·2[a]	1·7	13·8	1·5
	12	8·6	2·8	29·5[b]	9·7	23·9	15·5
	16	22·3	12·7	18·4[ab]	7·0	12·6	3·5

[a,b] Means with different superscript letters were significantly different for period (d) on diet ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 284–286.

Table 8. *Serum and tumour prostaglandin E_2 (PGE_2) in regressing or growing tumours from experimental rats fed on different levels of maize oil (MO) from 7 d before tumour transplant for 23 d**

(Values are means with their standard errors for eight rats per group)

Tumour status...		Regressing		Growing	
Tissue	Dietary fat (g MO/kg)	Mean	SE	Mean	SE
Serum (ng PGE_2/0·1 ml)	20	3·4	0·5	4·8	0·8
	100	3·2[a]	0·5	4·6[b]	0·3
	200	3·2	0·6	4·6	0·6
Tumour (ng PGE_2/mg protein)	20	26·0	21·0	10·2[c]	2·5
	100	12·7	2·8	29·6[d]	7·4
	200	18·2	7·5	13·8[c]	1·5

[a,b] Means with different superscript letters were significantly different with tumour growth ($P < 0·05$).
[c,d] Means with different superscript letters were significantly different between diets ($P < 0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 284–286.

DISCUSSION

Although tumour growth rate during the first 8 d post-transplant was apparently not affected, the amount of MO in the diet affected the regression of Walker 256 carcinosarcoma. With the highest amounts of MO in the diet there was a greater number of regressing tumours, a lower number of growing tumours after 8 d, and fewer animals died on the 200 g MO/kg diet. Dietary MO did not alter significantly the fatty acid composition of either tumour or muscle tissue. The LA levels in phospholipids of regressing tumours were lower than in growing tumours while AA levels were somewhat higher, reflecting desaturase and elongase activities involved in the conversion of LA to AA (Kinsella *et al.* 1990). Higher LA and lower AA in both PC and PE indicated a decreased desaturase–elongase system which reduced the conversion of LA to AA. Several researchers

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have shown that inhibition of liver Δ6-desaturase results in increased LA and lower AA in rats (Bailey & Dunbar, 1971; Shimp *et al.* 1982). Bailey & Dunbar (1971) proposed that deletion of Δ6-desaturase activity signified the initiation of undifferentiated tumour cell proliferation; however, Δ6-desaturase has been reported for other continuous tumour cell lines in culture (Maeda *et al.* 1978).

The lower AA levels in growing Walker 256 tumours were not associated with lower PGE_2 levels, although variability was high. The PGE_2 levels in the tumour tissues were higher than those in the contralateral muscle. Serum PGE_2 was lower in animals with regressing tumours and this was, perhaps, more significant because PGE_2 may be a general immunosuppressant (Kinsella & Lokesh, 1990). Conceivably, the lower PGE_2 levels in circulation allowed tumour-killing cells to be more effective.

The lower levels of *n*-3 PUFA, e.g. DHA, in tumour tissue were noteworthy. The *n*-3 PUFA can suppress PGE_2 synthesis and reduce tumour growth in animals (Kinsella & Lokesh, 1989; O'Connor *et al.* 1989; Kinsella *et al.* 1990). It is possible that there was less inhibition of the conversion of AA to PGE_2 by *n*-3 PUFA in animals with tumours. Kwong *et al.* (1984) reported that Walker 256 carcinosarcoma grew unhindered in rats fed on a diet high in MO fatty acids but regressed in rats fed on the same diet with the addition of the non-saponifiable matter of MO. However, we controlled the vitamin E content, a major component of non-saponifiable matter in the MO, by equalizing the tocopherol level in all diets. There was greater tumour regression and fewer deaths from tumour growth with increasing MO. Other researchers have reported that vitamin E level alone did not inhibit tumour formation but vitamin E exerted a synergistic effect with Se in inhibiting mammary tumour growth (Horvath & Ip, 1983).

In conclusion, dietary MO affected the regression of Walker 256 carcinosarcoma in rats. As dietary MO increased, fewer animals died and more tumours regressed. The present studies are consistent with the previous observation of Kwong *et al.* (1984) and suggest that agents other than tocopherol in the non-saponifiable matter of MO, e.g. tocotrienols, be involved in the antitumour effect. This will be examined in future studies.

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Printed in Great Britain

https://doi.org/10.1079/BJN19940134 Published online by Cambridge University Press