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Incorporation of dietary *trans* monounsaturated fatty acids into tissues of Walker 256 tumor-bearing rats

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Abstract

The correlation between dietary *trans* fatty acids and neoplasia was examined in the present study. Walker 256 tumor-bearing and control rats were fed a *trans* monounsaturated fatty acid (MUFA)-rich diet for 8 weeks and the incorporation of *trans* fatty acids by tumor tissue was examined. Also, the effect of tumor growth on *trans* fatty acid composition of plasma and liver, and the content of thiobarbituric acid-reactive substances (TBARS) was determined. Walker 256 tumor cells presented both *trans* and *cis* MUFAs given in the diet. The equivalent diet proportions were 0.66 for *trans* and 1.14 for *cis*. Taking into consideration the proportion of *trans* MUFAs in plasma (11.47%), the tumor incorporated these fatty acids in a more efficient manner (18.27%) than the liver (9.34%). Therefore, the dietary *trans* fatty acids present in the diet are actively incorporated by the tumor. Tumor growth itself caused marked changes in the proportion of polyunsaturated fatty acids in the plasma and liver but provoked only slight modifications in both *trans* and *cis* MUFAs. Tumor growth also reduced the unsaturation index in both plasma and liver, from 97.79 to 86.83 and from 77.51 to 69.64, respectively. This effect was partially related to an increase in the occurrence of the lipid oxidation/peroxidation process of TBARS content which was increased in both plasma (from 0.428 to 0.505) and liver (from 9.425 to 127.792) due to tumor growth.

Key words
- *Trans* fatty acids
- Walker 256 tumor
- Fatty acid composition
- Lipid peroxidation

Introduction

The fatty acids present in tissues are obtained either from dietary fat or by biosynthesis. All the biologically important saturated and monounsaturated fatty acids (MUFAs) can be fully synthesized from acetyl CoA in mammals. In contrast, omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) cannot be completely synthesized (1). All of these PUFAs are ultimately derived from the diet (2). The dietary MUFAs, however, also modify the fatty acid composition of the tissues (3).

The same principles apply to malignant cells, which derive all of their PUFAs from the circulation of the tumor-bearing host. The PUFAs are supplied either preformed or as precursors that are elongated and further desaturated by the cancer cells. Therefore, the type and amount of PUFAs available to a tumor depend on what is present in the circu-

lation of the host, which, in turn, ultimately depends on the dietary fat intake. Because some of the phospholipid in tumor cells undergoes rapid turnover, certain membrane domains should respond quickly to changes in the fatty acid composition of the extracellular fluid. These observations suggest that it should be possible to change the fatty acid composition of a tumor by altering the dietary fat intake of the tumor-bearing host. Although we know that MUFAs can be synthesized by neoplastic tissue (4), it is not known if dietary MUFAs are actively incorporated by the tumor. However, if these changes occur they might alter the membrane functioning (5) and growth properties of the cancer cell or increase its sensitivity to cytotoxic agents (4).

The effects of *trans* fatty acids from partially hydrogenated vegetable oils and other sources have been extensively examined (6,7). Investigations of diverse animal species and multigenerational experiments have revealed some adverse effects on the occurrence of various diseases (8) including cancer (9). In fact, several studies have shown that *trans* fatty acids present carcinogenic properties. In 1981, Awad (10) indicated that ingestion of elaidic acid (9t-18:1) reduced the survival time of mice bearing the Ehrlich ascites solid tumor. Hogan and Shamsuddin (11) compared the effects of a 25% elaidic acid diet and a 25% oleic acid diet on the development of colon tumors induced in rats by azoxymethane. The animals were kept on the diets for at least 20 weeks after the beginning of carcinogen exposure and the incidence of colon carcinoma was higher in the elaidic acid group, i.e., 37% against 23%. This issue, however, still remains in debate. Ip and Marshall (6), in a review published in 1996, stated that there is no sufficient evidence to indicate that under properly controlled conditions the intake of *trans* fatty acids is a risk factor for cancer. The studies mentioned, however, did not determine many important aspects concerning the effects of *trans* fatty acids on neoplasias. For instance, the experiments lack the following determinations: 1) incorporation of *trans* fatty acids by the tumor tissue, 2) the effect of tumor growth on *trans* fatty acid composition of plasma and tissues, and 3) occurrence of lipid peroxidation in tumor-bearing rats fed a *trans* fatty acid-rich diet. To address these points, Walker 256 tumor-bearing and control rats were fed a *trans* fatty acid-rich diet for 7 weeks. After this period the following parameters were examined: 1) fatty acid composition of the tumor, plasma and liver, and 2) the content of thiobarbituric acid-reactive substances (TBARS) in the same tissues and plasma.

The Walker 256 carcinosarcoma was discovered in 1928 as a spontaneous mammary tumor (12). This tumor cell line has been used in many studies because it is easily transplanted and is species specific for rats (13). This tumor grows rapidly, is quite invasive, and causes important metabolic and ionic changes in the host animal (14,15). The growth of this tumor leads to marked hormonal changes such as reduced insulinemia (16), which has been assumed to result in the establishment of a cachexia state in two weeks (17). Thus, this tumor model was chosen to investigate the above questions concerning *trans* fatty acids.

Material and Methods

Animals

Male Wistar rats (28 days old) weighing 45-50 g were obtained from the animal breeding unit, Faculty of Pharmaceutical Sciences, USP, São Paulo. Animals were maintained in a temperature-controlled room at 23°C on a 12-h light-12-h dark cycle. During the 7-week experimental period, the rats were divided into two groups of 8 animals each, i.e., Walker 256 tumor-bearing rats and controls. The rats were fed a diet whose composition

is shown in Table 1, with casein contributing 23.63% of the total calories as protein. The diets were prepared fresh each week and stored at 0°-4°C until the time for use.

Chemicals

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) or Merck (São Paulo, SP, Brazil).

Tumor implantation

A Walker 256 cell suspension (approximately 3 x 10^4 cells in 1.0 ml) was subcutaneously injected into the left flank of the rats, and the controls were sham injected with 1.0 ml 0.9% NaCl solution (w/v) without anesthesia. The amount implanted ensured that the tumor mass was 8-10% of the carcass weight at the time of the experiments. All experiments were started between 8:00 and 10:00 h, 14-15 days after implantation.

Analysis of fatty acid composition

The total lipid fractions of the diets were analyzed for their fatty acid components using gas-liquid chromatography. Lipids were extracted from the diet and the tissues using the chloroform:methanol (2:1, v/v) mixture described by Folch et al. (18). The samples were methylated by the method of Hartman and Lago (19). After removing the solvent under a stream of N_2, fatty acid methyl esters were resuspended in chloroform and analyzed with a CG-500 gas chromatograph equipped with a flame ionization detector linked to a CG-30021 integrator-processor (CG Instrumentos Científicos Ltda., São Paulo, SP, Brazil). The gas liquid chromatography column was a SP-2560, 100 m x 0.25 mm ID, 20-µm film thickness (Supelco Park, Bellefonte, PA, USA). The column temperature was programmed from 125° to 175°C at a rate of 1°C/min and held at 175°C for 25 min. The detector and the injector temperatures were 235° and 225°C, respectively. The retention times of the fatty acid methyl ester peaks were compared with those of known standards, and the relative levels of individual fatty acids were calculated as a percentage of the total fatty acids. The fatty acids containing more than 18 carbons and 3 double bonds were less than 10% and were not considered for the calculations. In fact, this study was focused on incorporation of the fatty acids given in the diet, mainly the *trans* fatty acids.

Unsaturation index

The unsaturation index of the fatty acids present in the tissues and plasma was calculated. The percentage of each fatty acid was multiplied by the number of double bonds present in the molecule (5).

Table 1 - Basic composition of the diet.

Data are reported as mean ± SD for 5 samples. Unsaturation index = (proportion of fatty acids) x (number of unsaturation of each fatty acid).

Component (g/100 g diet)	
Protein (casein)	23.63 ± 0.40
Fat	15.48 ± 2.47
Moisture	14.54 ± 0.05
Ash	6.47 ± 0.18
Nifext	39.88
Fatty acid composition (g/100 g)	
Saturated	28.18
C16:0	13.66 ± 0.91
C18:0	14.13 ± 0.26
C20:0	0.39 ± 0.05
trans monounsaturated	27.50
C18:1 6-8t	7.88 ± 0.43
C18:1 9t	6.00 ± 0.82
C18:1 10t	8.39 ± 0.55
C18:1 11t	5.23 ± 0.20
cis monounsaturated	36.86
C18:1 9c + 13t + 14t	29.72 ± 6.70
C18:1 11c	3.59 ± 0.17
C18:1 12c + 15t	2.78 ± 0.22
C18:1 13c	0.77 ± 0.07
cis polyunsaturated	7.47
C18:2 9c, 12c	7.47 ± 1.86
Unsaturation index	79.30

TBARS determination

The extraction medium used for the measurement of TBARS content in tissues and plasma contained 0.10 M sodium phosphate, pH 7.0 (20). TBARS were measured as previously described by Winterbourn et al. (21). The spectrophotometric measurements were carried out at 25°C and the results expressed as µmol per mg tissue protein.

Protein determination

Protein content was assessed by the method of Lowry et al. (22), using BSA (Sigma) as standard.

Statistical analysis

Results are reported as means ± SD for 8 rats. The paired Student *t*-test was employed to compare the effect of tumor growth, and the level of significance was set at $P<0.05$. The GraphPad Instat software, version 2.01, was used for statistical analysis.

Table 2 - Fatty acids as a percentage of the total lipid fraction and unsaturation index of the tumor tissue from two-week-Walker 256 tumor-bearing rats.

Data are reported as mean ± SD for 8 rats. Unsaturation index = (proportion of fatty acids) x (number of unsaturation of each fatty acid).

Fatty acids	Tumor
Saturated	28.03
C16:0	14.34 ± 3.01
C18:0	13.69 ± 4.91
trans monounsaturated	18.27
C18:1 6-8t	3.17 ± 0.69
C18:1 9t	4.91 ± 1.22
C18:1 10t	3.80 ± 0.89
C18:1 11t	4.54 ± 1.11
C18:1 12t	1.85 ± 0.56
cis monounsaturated	42.35
C18:1 9c + 13t + 14t	32.34 ± 2.41
C18:1 10c	1.55 ± 0.28
C18:1 11c	4.98 ± 1.36
C18:1 12c + 15t	2.12 ± 0.32
C18:1 13c	0.55 ± 0.07
C20:1	0.81 ± 0.45
cis polyunsaturated	10.56
C18:2 9c, 12c	10.56 ± 2.81
Unsaturation index	81.74

Results

The proportion of saturated fatty acids in tumor tissue was similar to that present in the diet, i.e., 28% (Tables 1 and 2). The proportion of *trans* MUFAs was 9.23% lower, whereas that of *cis* MUFAs and *cis* PUFAs was 5.49 and 3.09% higher, respectively, as compared to the diet. However, the unsaturation index was quite similar.

The Walker 256 tumor growth did not markedly alter the proportion of saturated and *trans* MUFAs in plasma (Table 3). However, the presence of the tumor raised the percentage of *cis* MUFAs (mainly C18:1 9c + 13t + 14t) by 6.05% and the percentage of *cis* PUFAs by 3.69%. The *trans* PUFA (C18:2 9c, 12t) was not detected in plasma of tumor-bearing rats. The unsaturation index of the plasma fatty acids was decreased due to the presence of the tumor (10.96%).

Tumor growth decreased the proportion of C18:0 in the liver, whereas C14:0 was found in the tumor group only, leading to a 6.83% increase in the proportion of total saturated fatty acids in the latter group (Table 4). The proportion of total *trans* MUFAs in the liver was not modified by the presence of the tumor. However, C18:1 6-8t decreased, C18:1 9t and C18:1 12t appeared, and C18:1 11t decreased due to neoplasia. The proportion of all *cis* MUFAs was reduced by tumor growth in the liver. *Trans* PUFAs were enhanced in the liver of tumor-bearing rats due to the detection of C18:2 tc, C18:2 9t, 12t and C18:2 9t, 12c. C18:2 9c, 12c was decreased but C18:3 9c, 12c, 15t and C18:3 9c, 12c, 15c appeared in the hepatic tissue of the tumor group, so that the total proportion of *cis* PUFAs was unchanged.

TBARS content in the tumor was 5-fold

higher than in the liver of the control group. Tumor growth raised the TBARS content in plasma by 18% and caused a remarkable 14-fold increment of these compounds in the liver (Table 5).

Discussion

Several studies have shown that the fatty acid composition of tissues is changed by altering the dietary fat (5), particularly PUFAs. Tumor cells can synthesize MUFAs and so the incorporation of these fatty acids given in the diet by the tumor was an important aspect to be examined. The first question addressed in this study was: are the *tran*s and *cis* MUFAs similarly incorporated by the tumor?

The Walker 256 tumor cells incorporated both *trans* and *cis* MUFAs given in the diet. The equivalent diet proportions were 0.66 for *trans* and 1.14 for *cis*. Clearly, the proportion of *trans* MUFAs was much lower in the tumor compared to the proportion in the diet. In fact, the *cis* MUFAs/*trans* MUFAs ratio was 1.34 in the diet and 2.31 in the tumor. These findings permit us to assume that *trans* MUFAs may not be actively incorporated by Walker 256 tumor tissue. However, the dietary fatty acids have to be digested and absorbed as chylomicrons before being incorporated by the tissues, showing that the cells can only incorporate the lipids in the surroundings. As compared with the proportion of *trans* MUFAs in the diet (27.5%), the percentage of these fatty acids was low in plasma (11.47%) and still lower in the liver (9.34%), equivalent to dietary proportions of 0.42 and 0.33, respectively. Thus, the proportion of *trans* MUFAs in the tumor (18.27%) was about 9% higher than the values found in plasma and liver. Therefore, taking into consideration the proportion of these fatty acids in plasma, the tumor incorporated *trans* MUFAs in a more efficient manner than the liver. On the other hand, the equivalent plasma proportion was 1.32 and 1.12 for *trans* and *cis* MUFAs, respectively, in the tumor-bearing rats. These findings support the proposition that *trans* MUFAs may be more actively incorporated by the tumor than *cis* MUFAs. Experiments in cultured cells are now required to further examine this matter (23). Our observations, however, suggest that it should be possible to change the MUFA composition of a tumor by altering the type of dietary fat ingested by the tumor-bearing host. Membrane functioning and production of vital lipid mediators may be altered, possibly leading to changes in the growth properties of the cancer tissue (24). As a result, this may also raise the sensitivity of the neoplastic cells to cytotoxic agents.

Table 3 - Fatty acids as a percentage of the total lipid fraction and unsaturation index of plasma from the Walker 256 tumor-bearing and control groups.

Data are reported as mean ± SD for 8 rats. *P<0.05 compared with the control group (Tukey-Kramer test). Unsaturation index = (proportion of fatty acids) x (number of unsaturation of each fatty acid). ND = Not detected.

Fatty acids	Groups	
	Control	Tumor-bearing
Saturated	29.56	31.33
C16:0	16.64 ± 2.21	16.64 ± 4.90
C18:0	11.47 ± 3.31	13.39 ± 3.54
C20:0	1.14 ± 0.61	1.30 ± 0.43
trans monounsaturated	11.47	13.89
C18:1 6-8t	1.78 ± 0.90	2.16 ± 0.81
C18:1 9t	2.64 ± 1.82	3.44 ± 1.52
C18:1 10t	2.78 ± 1.04	3.35 ± 1.02
C18:1 11t	1.34 ± 1.16	2.91 ± 1.13
C18:1 12t	1.92 ± 0.88	2.03 ± 0.80
cis monounsaturated	31.63	37.68
C18:1 9c + 13t + 14t	26.38 ± 2.41	30.95 ± 6.64*
C18:1 11c	2.85 ± 0.36	3.58 ± 0.99
C18:1 12c + 15t	1.47 ± 0.37	1.82 ± 0.72
C18:1 13c	0.94 ± 0.14	1.33 ± 0.70
trans polyunsaturated	14.82	1.13
C18:2 tt	ND	1.13 ± 0.95
C18:2 9c, 12t	14.82 ± 4.85	ND
cis polyunsaturated	12.52	16.21
C18:2 9c, 12c	12.52 ± 6.07	15.63 ± 6.10
C18:3 9c, 12c, 15c	ND	0.58 ± 0.57
Unsaturation index	97.79	86.83

Table 4 - Fatty acids as a percentage of the total lipid fraction and unsaturation index of the liver from the Walker 256 tumor-bearing and control groups.

Data are reported as mean ± SD for 8 rats. *P<0.05 compared with the control group (Tukey-Kramer test). Unsaturation index = (proportion of fatty acids) x (number of unsaturation of each fatty acid). ND = Not detected.

Fatty acids	Groups	
	Control	Tumor-bearing
Saturated	42.81	49.64
C14:0	ND	15.11 ± 2.62
C16:0	15.53 ± 1.71	16.26 ± 5.58
C18:0	27.28 ± 3.83	17.98 ± 1.88*
C20:0	ND	0.28 ± 0.09
trans monounsaturated	9.34	9.08
C18:1 6-8t	3.14 ± 2.27	0.50 ± 0.348*
C18:1 9t	ND	3.13 ± 0.95
C18:1 10t	2.85 ± 0.39	2.00 ± 1.03*
C18:1 11t	3.35 ± 0.28	1.92 ± 0.68*
C18:1 12t	ND	1.52 ± 0.27
cis monounsaturated	27.55	22.68
C18:1 9c + 13t + 14t	20.89 ± 2.14	17.98 ± 3.02*
C18:1 11c	3.16 ± 0.28	2.33 ± 0.21
C18:1 12c + 5t	2.27 ± 0.25	1.52 ± 0.27*
C18:1 13c	0.73 ± 0.12	0.53 ± 0.17*
C18:1 14c	0.49 ± 0.19	0.32 ± 0.09*
trans polyunsaturated	0.55	1.34
C18:2 tt	0.55 ± 0.31	0.59 ± 0.43
C18:2 tc	ND	0.31 ± 0.11
C18:2 9t, 12t	ND	0.22 ± 0.07
C18:2 9t, 12c	ND	0.22 ± 0.16
cis polyunsaturated	19.76	17.27
C18:2 9c, 12c	19.76 ± 2.95	16.61 ± 2.02*
C18:3 9c, 12c, 15t	ND	0.24 ± 0.10
C18:3 9c, 12c, 15c	ND	0.42 ± 0.25
Unsaturation index	77.51	69.64

Table 5 - Content of thiobarbituric acid-reactive substances (TBARS) in plasma, liver and tumor of the tumor-bearing and control groups.

Data are reported as mean ± SD for 8 rats. *P<0.05 compared with the control group (Tukey-Kramer test).

Groups	TBARS (µmol per mg protein)		
	Plasma	Liver	Tumor
Control	0.428 ± 0.101	9.425 ± 0.855*	-
Tumor-bearing	0.505 ± 0.038*	127.792 ± 39.177*	47.40 ± 9.62

The second question addressed was: what is the effect of tumor growth on *trans* MUFA composition in tissues and plasma? Walker 256 tumor grows very rapidly, stimulates catabolic pathways and leads to cachexia in about two weeks (25,26). Among the metabolic pathways, lipolysis is markedly activated, possibly causing important changes in the proportion of fatty acids in tissues. Tumor growth caused only slight changes in the proportion of *trans* and *cis* MUFAs in plasma, a *cis*/*trans* ratio of 2.76 in the control and 2.71 in the tumor-bearing groups. Nevertheless, the proportion of *trans* PUFAs was markedly reduced. In fact, the *cis* PUFA/*trans* PUFA ratio was 0.84 in the control and 14.35 in the tumor-bearing groups. In the liver, a similar observation was made for MUFAs. The *cis* MUFAs/*trans* MUFAs ratio was 2.9 in controls and 2.5 in tumor-bearing rats. However, the proportion of *cis* PUFAs to *trans* PUFAs was markedly decreased due to tumor growth, i.e., 12.39 compared to 35.93. These findings support the proposition that tumor growth causes important changes in the proportion of PUFAs but provokes only slight modifications in both *trans* and *cis* MUFAs. The preferential effect of tumor growth on PUFAs is an interesting finding which remains to be elucidated. One possible explanation for this observation may be related to the acylation/deacylation process (27), which might be activated under these conditions.

It is noteworthy that tumor growth reduced the unsaturation index in both plasma and liver. This decrease in the abundance of double bonds could be the result of an increased occurrence of lipid oxidation/peroxidation. The oxidation/peroxidation process of double bonds facilitates the removal of unsaturated fatty acids from the phospholipid moieties (28), thus reducing the proportion of these types of fatty acids in the cells. Therefore, the third point addressed was the measurement of TBARS content in plasma and tissues. It has been postulated

that the lipid peroxidation/oxidation process plays a key role in tumor growth and invasiveness (29). This may explain the high content of TBARS in the tumor as compared to liver and plasma of the control group. In addition, however, tumor growth slightly increased TBARS content in plasma but greatly increased this compound in the liver. Therefore, tumor growth, by leading to hormonal, metabolic and ionic changes in the host, may favor the occurrence of lipid oxidation/peroxidation in non-tumor tissues and plasma. However, the tumor itself may release products of lipid oxidation/peroxidation that are exported to the liver.

The results presented here led us to postulate that *trans* fatty acids present in the diet are actively incorporated by the tumor. This fact can be used as a dietary strategy during cancer treatment if *trans* fatty acids somehow affect the rate of tumor growth. This is an important issue to be investigated. The tumor growth itself also alters the proportion of fatty acids in tissues and plasma and this may partially result from an increased occurrence of lipid oxidation/peroxidation.

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