Ratio of n6 to n-3 Fatty Acids in the Diet Affects Tumor Growth and Cachexia in Walker 256 Tumor-Bearing Rats

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Abstract: In this study we investigate the impact of the dietary ratio of n-6 to n-3 fatty acids (FAs) from postweaning until adult age upon tumor growth, lipid peroxidation in tumor tissue, and metabolic indicators of cancer cachexia in Walker 256 tumor-bearing rats. Weanling male Wistar rats received a normal low-fat (40 g/kg diet) chow diet or high-fat diets (300 g/kg) that included fish oil (FO) or sunflower oil or blends of FO and sunflower oil to yield n-6 to n-3 FA ratios of approximately 6:1, 30:1, and 60:1 ad libitum. After 8 wk, half of each group was inoculated with 1 ml of 2×10^7 Walker 256 cells. At the 14th day after tumor inoculation, the animals were killed, and tumors and blood were removed. The different diets did not modify the blood parameters in the absence of tumor bearing, except the high-FO diet, which decreased serum cholesterol and triacylglycerol concentrations. Tumor weight in chow-fed rats was 19 g, and these rats displayed cancer cachexia, characterized by hypoglycemia, hyperlacticidemia, hypertriacylglycerolemia, loss of body weight, and food intake reduction. Tumor weight in FO-fed rats was 7.7 g, and these animals gained body weight (14.6 g) and maintained blood metabolic parameters similar to non–tumor-bearing animals. Tumor weight in rats fed the diet with an n-6 to n-3 FA ratio of 6:1 was similar to tumor-bearing, chow-fed rats, but they gained 2 g in the body weight and blood metabolic parameters were similar to those in non–tumor-bearing rats. However, a further increase in the n-6 FA content of the diet did not change the cachectic state associated with tumor bearing. In this experimental model, a dietary n-6 to n-3 FA ratio of 6:1 was able to increase food intake and body weight, restore the biochemical blood parameters of cachexia, and prevent the development of cancer cachexia.

Introduction

Major changes in the type and amount of fat consumed by humans have occurred over the last 150 yr (1,2). Western societies have been characterized by an increase in the intake of omega-6 (n-6) fatty acids (FAs) and a decrease in the intake of omega-3 (n-3) FAs (2). It is proposed that the balance of n-6 to n-3 FAs in the diet is of importance to human health and disease (2). This may relate to the ability of certain n-6 and n-3 polyunsaturated FAs (PUFA) to be metabolized to form eicosanoids such as prostaglandins (PGs) and leukotrienes (3–6). Eicosanoids produced from the n-6 PUFA arachidonic acid enhance tumor cell proliferation and so are associated with the cancer development (3,7,8). Eicosanoids produced from the n-3 PUFA eicosapentaenoic acid are frequently much less potent (up to 100-fold) than the analogs produced from arachidonic acid (9). Hence, the relative amounts of n-6 and n-3 PUFA provided by the diet, and so present in blood and tissues, may be of importance to the development of some cancers (10–12). Indeed, chemically induced rat mammary carcinogenesis is promoted by dietary n-6 PUFA (13) but is inhibited by feeding n-3 PUFA-rich fish oil (FO) (14). Likewise, experimental rat colon carcinogenesis is inhibited by feeding a diet rich in n-3 PUFA (15–18). Furthermore, growth of human mammary and colon cancer cell lines as solid tumors in athymic ("nude") mice is inhibited by feeding a diet rich in n-3 PUFA (19,20). These and similar studies indicate the ability of n-3 PUFA to exert protective effects against some common cancers in animal models.

Cancer growth is accompanied by cachexia, which occurs in about one-half of untreated cancer patients (21). Cancer

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cachexia is characterized by anorexia, asthenia, anemia, weight loss, weakness, and intense peripheral catabolism with depletion of carbohydrate, lipid, and protein stores (21,22). As a result of a multifactorial etiology involving immune-metabolic pathways, the basic mechanisms that induce cancer cachexia are poorly known (22). A number of conditions have been postulated to play a key role in establishing cancer cachexia. These include excess production of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and interferon-γ (23), low plasma levels of insulin (24), high plasma levels of glucagon, cortisol, catecholamines, and vasopressin (25), and the presence of PGE_2 (26).

In addition to effects on the production of eicosanoids involved in tumor cell proliferation and tumor growth, n-3 PUFA from FO have been demonstrated to decrease the production of cytokines such as TNF and IL-6 in healthy individuals (27–29) and in patients with pancreatic cancer (30). There are also reports of normalization of the metabolic response in pancreatic cancer–bearing patients supplemented with FO (30–32). These studies indicate that n-3 PUFA from FO might be efficacious in treating cachexia even in advanced stages of cancer.

Most previous studies carried out to investigate the effect of dietary FAs on tumor growth in laboratory animals have used young adult animals fed for a short period with a particular diet before or after induction of the tumor. Recently, we provided a dietary regimen of 1 g/kg body weight of FO for one generation that was able to reduce tumor growth and cancer cachexia in Walker 256 tumor-bearing rats (33). In this study, we investigate the impact of the dietary ratio of n-6 to n-3 PUFA from postweaning until adult age upon tumor growth, lipid peroxidation in tumor tissue, and metabolic indicators of cancer cachexia in Walker 256 tumor-bearing rats.

Material and Methods

Chemicals, Oils, Drugs, and Enzymes

Chemicals and enzymes used were obtained from Sigma Chemical Co. (St. Louis, MO). FO was kindly donated by the Herbarium Foundation (Curitiba, Brazil). The FO used was a mixed marine triacylglycerol (TAG) preparation containing 130 g eicosapentaenoic acid and 200 g docosahexaenoic acid per kilogram. Sunflower oil was from BUNGE Alimentos (Ponta Grossa, Brazil) and contained ~60% of linoleic acid,

and coconut oil was from Industria Brasileira de Gordura de Coco LTDA (Ponta Grossa, Brazil). The α-tocopherol contents of the oils used to prepare the diets were 11.9 (coconut oil), 19.5 (sunflower oil), and 12.4 (FO) μg/ml.

Study Design

Procedures involving animals were approved by the University Federal of Paraná Committee of Animal Welfare. Weanling male Wistar rats (aged 21 days) were maintained under controlled temperature (23°C), humidity, and 12 h/12 h light/dark cycle and were divided into six dietary groups. All diets contained the same amounts of protein (230 g/kg), fiber (60 g/kg), and vitamins and minerals (10 g/kg). One group (control) received a regular chow diet (fat content of 40 g/kg and carbohydrate content of 660 g/kg; Nuvital CR-1, Curitiba, Brazil). Five groups received a high-fat diet (fat content of 300 g/kg and carbohydrate content of 400 g/kg); in all cases 33% of the fat was as coconut oil. In two of these groups the other 66% of the fat was as FO or as sunflower oil; these diets are referred to as N-3 and N-6, respectively. In the other three groups 66% of the fat was as a blend of FO and sunflower oil to yield n-6 to n-3 PUFA ratios of approximately 6:1, 30:1, and 60:1; these diets are referred to as 6:1, 30:1, and 60:1, respectively. The oils were blended in such a way that the linoleic acid content was kept constant and so the n-6 to n-3 PUFA ratio was varied by changing the n-3 PUFA content. The FA compositions of the diets were analyzed by gas chromatography and are summarized in Table 1. Chemical analysis revealed that the n-6 to n-3 PUFA ratios of the diets were similar to those that were desired.

After 8 wk, half of each group was inoculated in the right flank with 1 ml of sterile suspension of 2×10^7 Walker 256 tumor cells obtained from an ascitic tumor-bearing rat. Thus, 12 groups of rats were set up; tumor bearing is indicated by the prefix W. Food intake and body weight were monitored every day. Food intake at Day 1 for each group was considered 100%, and the consumption was calculated relative to that. At the 14th day after tumor inoculation, the animals were killed by decapitation without anesthesia. Blood was collected via a funnel into 15-ml tubes and allowed to clot for 30 min at room temperature. Serum was prepared by centrifugation and used for the measurement of glucose, lactate, cholesterol, and TAG concentrations. Tumors were re-

Table 1. Summary of the Fatty Acid Compositions of the Diets Used[a]

Diet[b]	Linoleic Acid (% fatty acids)	Eicosapentaenoic + Docosahexaenoic Acids (% fatty acids)	n-6 to n-3 PUFA Ratio
Control	18.9	<0.5	>300
N-3	1.3	34.9	0.04
6:1	50.7	8.4	6.0
30:1	48.9	1.5	32.6
60:1	52.3	0.8	65.4
N-6	54.4	<0.1	>550

a: Abbreviation is as follows: PUFA, polyunsaturated fatty acid.

b: N-3, fish oil–rich diet; 6:1, 30:1, and 60:1, diets with differing ratios of n-6 to n-3 fatty acids; N-6, sunflower oil–rich diet.

moved and weighed, and samples were frozen in liquid nitrogen for the lipid peroxidation assay.

Determination of Blood Metabolite Concentrations

Serum glucose concentration was determined by using a glucose oxidase–based assay kit as described by Trinder (34) and quantified in a spectrophotometer (Ultrospec 2000; Pharmacia Biotech, São Paulo, Brazil) by measuring the absorbance at 505 nm. Serum total cholesterol and TAG concentrations were measured using the methods described by Jung et al. (35) and Young (36), respectively, and quantified by measuring the absorbance at 500 nm and 540 nm. For the lactate assay, serum (0.5 ml) was added to 0.1 ml of perchloric acid (25%) and left for 10 min at 4°C followed by centrifugation at 3,000 g for 5 min. The supernatant was collected and neutralized with Tris/KOH (2 M/0.5 M), and the concentration of lactate was determined as described by Engle and Jones (37) after measuring the absorbance at 340 nm.

Determination of the Products of Lipid Peroxidation

The products of lipid peroxidation were measured using the method described by Jiang et al. (38). Briefly, tumor tissue (0.1 mg) was homogenized in phosphate buffer solution (0.1 M), pH 7.4. The homogenate (0.1 ml) was added to 0.9 ml of reaction solution (100 µM xylenol orange, 250 µM Fe^{+2}, 25 mM H_2SO_4, and 4 mM butylated hydroxytoluene in 90% (vol/vol) methanol) and incubated for 30 min at room temperature prior to measurement at 560 nm. The concentration of hydroperoxides was calculated by using the extinction coefficient 4.3×10^{-4} M^{-1} cm^{-1}.

Statistical Analysis

The data are presented as mean ± SE. Statistical analysis was performed by two-way analysis of variance using diet and tumor bearing as factors. Post hoc tests were Bonferroni corrected for multiple comparisons. The value of $P < 0.05$ was taken to indicate statistical significance.

Results

Body Weight, Tumor Weight, and Food Intake

Rats supplemented with regular chow (C) increased their body weight by 28 g over 14 days (Table 2). In Walker 256 tumor-bearing rats (W) fed regular chow, tumor weight was 19 g, and carcass weight decreased by 18 g over 14 days. There was a significant effect of including n-3 PUFA in the diet on tumor growth and on body weight maintenance (Table 2). Tumor weight was 60% lower in the WN-3 group compared with the W group ($P < 0.001$), and carcass weight increased by 14.6 g ($P < 0.001$). Tumor weights in rats fed diets with different n-6 to n-3 PUFA ratios (W6:1, W30:1, and W60:1) and in the WN-6 group were not different from those in the W group ($P > 0.05$). Tumor-bearing rats fed the diet with n-6 to n-3 FA ratio of 6:1 (W6:1) showed weight gain of 2 g over 14 days. On the other hand, feeding the diet with the ratio of 30:1 (W30:1) or 60:1 (W60:1) or the high n-6 PUFA diet (WN-6) resulted in reductions in body weight (Table 2).

Food intake at Day 1 of tumor bearing was approximately 30 g per rat per day. Tumor growth in the W, W30:1, W60:1, and WN-6 groups was accompanied by a reduction in food intake ($P < 0.05$) when compared with non–tumor-bearing groups (Fig. 1). However, in the WN-3 and W6:1 groups, food intake was similar to that of non–tumor-bearing animals (C). Food intake and body weight in the non–tumor-bearing rats fed different diets were similar ($P > 0.05$; data not shown).

Biochemical Parameters of Cachexia

Tumor growth was accompanied by a reduction in blood glucose concentration by ~40% (Table 3) in the W, W30:1,

Table 2. Body Weight, Tumor Weight, and Carcass Weight of Non–Tumor-Bearing Rats (C), Walker 256 Tumor-Bearing Rats Fed a Control Diet (W), a Fish Oil–Rich Diet (WN-3), Diets With Differing Ratios of n-6 to n-3 Fatty Acids (W6:1, W30:1, and W60:1), or a Sunflower Oil–Rich Diet (WN-6)[a]

| Group	Body Weight Before Tumor Inoculation (g)	Body Weight 14 days Later (g)	Tumor Weight (g)	Carcass (g)	Weight Change (g)
C	267.2 ± 3.4	295.2 ± 4.2	–	295.2 ± 4.2	28.0 ± 2.7
W	257.2 ± 11.2	259.6 ± 9.7	19.4 ± 1.4	239.9 ± 9.7	−18.0 ± 3.9[b]
WN-3	256.7 ± 15.1	276.2 ± 14.3	7.7 ± 2.7[c]	271.3 ± 15.5	14.6 ± 0.7[b,c]
W6:1	260.5 ± 17.2	281.7 ± 12.6	20.6 ± 4.0	262.4 ± 11.5	2.0 ± 0.04[b,c,d]
W30:1	264.2 ± 12.7	302.7 ± 8.2	20.1 ± 2.7	263.0 ± 4.2	−1.06 ± 0.13[b,c,d,e]
W60:1	256.5 ± 14.4	268.5 ± 14.8	17.5 ± 2.9	251.7 ± 16.8	−4.8 ± 0.5[b,c,d,e]
WN-6	234.2 ± 15.4	248.7 ± 20.7	14.6 ± 2.7	232.5 ± 19.9	−2.3 ± 0.7[b,c,d,e]

a: Values are mean ± SE of 10 animals per group.

b: $P < 0.001$ compared with C.

c: $P < 0.001$ compared with W.

d: $P < 0.05$ compared with WN-3.

e: $P < 0.05$ compared with W6:1.
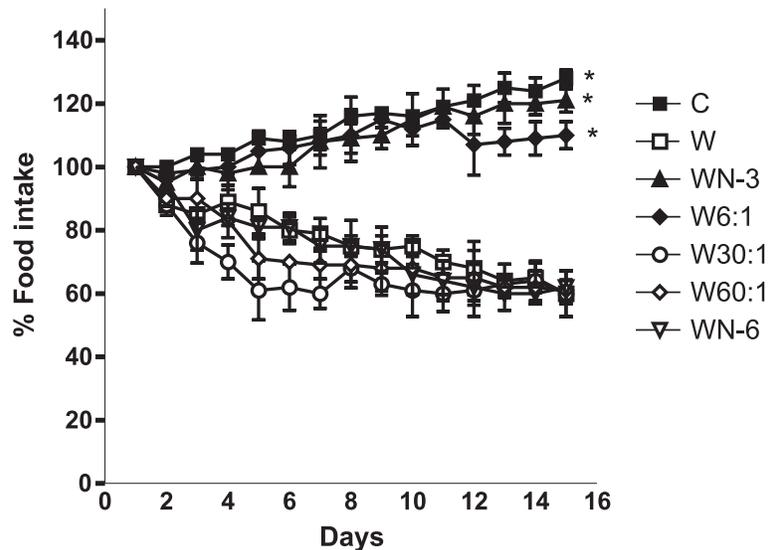
Figure 1. Food intake (% of Day 1) from Day 1 (tumor inoculation) to 14 days in rats fed normal chow (C) and tumor-bearing rats fed normal chow (W), fish oil (WN-3), sunflower oil (WN-6), or diets with different n-6 to n-3 polyunsaturated fatty acid ratios (W6:1, W30:1, and W60:1). Data are presented as mean ± SE of 10 animals per group. *$P < 0.05$ compared with W, W30:1, and W60:1.

W60:1, and WN-6 groups ($P < 0.05$) when compared with non–tumor-bearing rats and also with the WN-3 and W6:1 groups. These two last groups, in turn, showed glycemia similar to non–tumor-bearing rats ($P > 0.05$). Tumor-bearing rats fed regular chow (W group) increased serum lactate concentration by 2.1-fold and those in the W30:1, W60:1, and WN-6 groups by 1.7-fold when compared with non–tumor-bearing rats ($P < 0.05$; Table 3). Tumor-bearing rats in the WN-3 and W6:1 groups had the same serum lactate concentrations as the non–tumor-bearing groups. Serum glucose and lactate concentrations were not different among non–tumor-bearing rats fed the different diets.

Non–tumor-bearing animals fed the high-FO diet showed lower ($P < 0.05$) serum TAG concentrations when compared with C (by 49%), 6:1 (by 37%), 30:1 (by 32%), 60:1 (by 39%), and N-6 (by 50%) (Table 3). Tumor-bearing rats in the W, W30:1, W60:1, and WN-6 groups had higher (~43%) serum TAG concentrations compared with non–tumor-bearing rats. However, in tumor-bearing rats in the WN-3 and W6:1 groups, the serum TAG concentrations were similar to those in non–tumor-bearing rats ($P > 0.05$).

Serum cholesterol concentration was significantly lower ($P < 0.05$; Table 3) in the non–tumor-bearing rats fed the FO diet (N-3 group) when compared with the other non–tumor-bearing groups. Serum cholesterol was also lower in the tumor-bearing rats fed regular chow (W) when compared with the other tumor-bearing rats ($P < 0.05$).

Lipid Peroxidation

Lipid peroxidation was 34% higher in the tumors obtained from tumor-bearing rats fed the FO diet ($P < 0.05$) when compared with the other tumor-bearing rats (Fig. 2). Lipid peroxidation was not different among the other groups ($P > 0.05$; Fig. 2).

Discussion

The Walker 256 tumor-bearing rat offers an opportunity to investigate cancer cachexia and tumor growth due to its characteristics of inducing a higher level of cachexia in 14 days accompanied by decreased food intake, depletion of liver and muscle glycogen stores, hypoglycemia, hypertriacylglycerolemia, and hyperlacticidemia (33). Thus, cachexia involves decreased food intake, body wasting, and altered profiles of blood metabolites. In the current study, bearing the Walker 256 tumor by rats fed a control diet was associated with reduced food intake (~40%), body weight loss (6.8%), hyperlacticidemia, hypoglycemia, and hypertriacylglycerolemia (Fig. 1, Tables 2 and 3).

Previous studies have demonstrated that dietary n-3 FAs, usually in the form of FO, can decrease tumor growth in various animals models compared with the enhancing effects of diets rich in saturated FAs or in n-6 FAs (14–17,19–20,33). However, most of these studies have used different approaches where feeding the modified diet started in early adulthood prior to tumor induction (either chemically or through inoculation of tumor cells) or immediately following tumor induction. In addition, in most of these studies the period of feeding the modified diet ranged from 2 to 8 wk. In this study, we chose to investigate the effect of chronic exposure (from weaning) to diets with a high content of fat and with an altered ratio n-6 to n-3 PUFA on tumor growth and cachexia in early adulthood. It was observed that chronic exposure (weaning to adulthood) to fat diet rich in FO (WN-3) decreased tumor growth by 60% and that this was accompanied by maintenance of normal serum glucose and lactate concentrations. Also, these animals increased body weight by 5.4% when compared with initial body weight (Table 2), and their food intake (Fig. 1) was identical to that of non–tumor-bearing rats.

Table 3. Serum Concentrations (mg/dl) of Glucose, Lactate, Triacylglycerol, and Cholesterol of Rats Fed the Different Diets[a]

Groups	C	N-3	6:1	30:1	60:1	N-6	W	WN-3	W6:1	W30:1	W60:1	WN-6
Glucose	146.2 ± 5.5	148.0 ± 0.1	149.8 ± 5.2	146 ± 6.3	141.3 ± 8.2	144.9 ± 5.6	89.0 ± 6.4[b]	142.7 ± 2.5[c]	130.2 ± 3.1[c]	88.5 ± 7.8[b]	103.8 ± 5.1[b]	102.9 ± 6.8[b]
Lactate	1.12 ± 0.41	1.36 ± 0.1	1.22 ± 0.08	1.31 ± 0.12	1.15 ± 0.13	1.30 ± 0.07	2.55 ± 0.12[b]	1.17 ± 0.7[c]	1.35 ± 0.1[c]	1.98 ± 0.17[b]	1.97 ± 0.21[b]	1.99 ± 0.19[b]
Triacylglycerol	123.4 ± 10.7	63.6 ± 10.[d]	99.9 ± 12.1	94.1 ± 7.7	104.1 ± 12.7	105.1 ± 9.8	169.1 ± 10.4[b]	96.2 ± 5.8[c]	138.5 ± 17.3	183.7 ± 17.3[b]	189.7 ± 15.7[b]	187.4 ± 15.4[b]
Cholesterol	84.6 ± 6.7	53.8 ± 7[d]	79.9 ± 11.5	89.2 ± 8.1	89.6 ± 17.2	79.3 ± 7.5	48.5 ± 2.5[e]	79.3 ± 7.3[f]	84.8 ± 9.1	100.3 ± 9.9	96.5 ± 11.3	82.6 ± 8.6

a: Diets are as follows: C, control; N-3, fish oil–rich diet; 6:1, 30:1, and 60:1, diets with differing ratios of n-6 to n-3 fatty acids; N-6, sunflower oil–rich diet. W, Walker 256 tumor-bearing rats fed a control diet; WN-3, a fish oil–rich diet; W6:1, W30:1, and W60:1, diets with differing ratios of n-6 to n-3 fatty acids; WN-6, sunflower oil–rich diet.
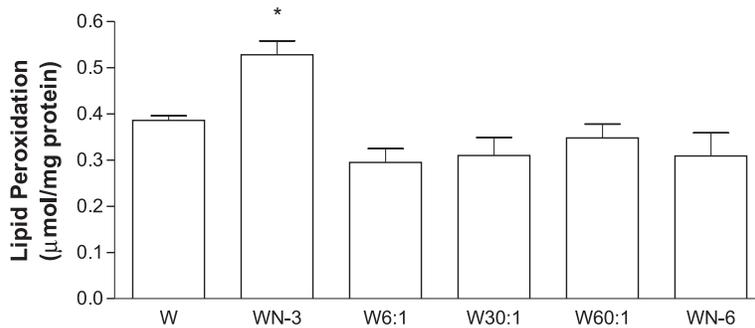
b: $P < 0.05$ compared with non–tumor-bearing rats fed the same diet.

c: $P < 0.05$ compared with W, W30:1, W60:1, and WN-6.

d: $P < 0.05$ compared with C.

e: $P < 0.05$ compared with C, 6:1, 30:1, 60:1, and N-6.

f: $P < 0.05$ compared with N-3.

Figure 2. Lipid peroxidation (μmol/mg protein) in tumor tissue of rats fed regular chow (W) or diets rich in fish oil (WN-3) or sunflower oil (WN-6) or with different ratios of n-6 to n-3 fatty acids (W6:1, W30:1, and W60:1). Data are presented as mean ± SE of 10 animals per group. $^*P < 0.05$ compared with W, W6:1, W30:1, W60:1, and WN-6.

In contrast, tumor-bearing rats fed a diet very rich in n-6 PUFA (WN-6 group) had pronounced tumor growth (representing 5.8% of body weight) and decreased food intake (reduced by 35% when compared with non–tumor-bearing rats), which were similar to the W group, as well as similar glucose, lactate, and TAG concentrations. Despite that, interestingly, these animals lost only 0.72% of body weight. Thus, nutritional intervention with FO led to a remarkable effect on tumor growth and metabolic response in tumor-bearing rats (WN-3 group), which was not observed with the n-6 PUFA-rich diet except concerning body weight. The mechanisms by which FO inhibits tumor growth and the development of cachexia are still unknown (12). This may occur through reducing cellular proliferation (18,39), increasing lipid peroxidation (40), decreasing eicosanoid production within the tumor (41), an altered host immune response (42,43), and/or reduced intracellular signaling (18,44–46). n-3 PUFA are more prone to peroxidation than are saturated or monosaturated FAs or n-6 PUFA, and it is known that peroxidation products may alter cellular membrane structure leading to cell death (40). Togni et al. (33) showed that tumors from Walker 256 tumor-bearing rats supplemented with FO contained more n-3 PUFA than those from rats fed a control diet or a saturated fat–rich diet. Thus, there would be more substrate available for peroxidation. In the current study, tumor lipid peroxidation products increased significantly in rats in the WN-3 group (34%) when compared with the other tumor-bearing groups. This may explain the reduced tumor growth rate. However, increased peroxidation may not be solely responsible for the changes observed in tumor growth, and the other factors mentioned previously could also play a role. Lipid peroxidation in nontumor tissues such as spleen, thymus, liver, kidney, cerebral cortex, and hippocampus was not different (data not shown).

n-6 FAs are associated with higher synthesis of inflammatory metabolites such as the eicosanoid PGE$_2$. Walker 256 tumor-bearing rats have high circulating levels of PGE$_2$ compared with normal rats, with the tumor being the main source of PGE$_2$ (26). High rates of PGE$_2$ production by tumors are associated with enhanced tumor development (3,7,8). We and others have shown that anti-inflammatory agents that inhibit cyclooxygenase-2 (COX-2), and so block PGE$_2$ production, prevent the growth of some tumors (47–49). COX-2 expression has been shown to down-regulate the apoptotic pathway, probably via Bcl-2 (50), which might explain the promotion of tumor growth by PGE$_2$. In addition, the growth of solid tumors depends on angiogenesis, which is promoted by many factors, including PGs (51,52) and 12-hydroxyperoxyeicosatetraenoic acid, also derivative of arachidonic acid (53). Increased dietary supply of n-3 PUFA has been shown to decrease arachidonic acid levels in host tissues and within tumors (20,33), and this would result in decreased formation of PGE$_2$ and related eicosanoids (41,54). In this study, we did not measure the arachidonic acid:eicosapentaenoic acid ratio in the tumors. However, dietary regimens used previously have shown that alterations in the ratio of these FAs occur (20,33), and so a suppressive effect of FO on PGE$_2$ production, accounting for some of the observed effects, cannot be ruled out.

Genetic and environmental factors may contribute to the development of many types of cancer. It is estimated that 30% of cancers are affected by food intake, and high-fat diets have a positive relationship with the incidence of some cancers (55). Experimental and epidemiological studies have shown that amount of fat consumed as well as type of fat are important to the initiation and development of cancer (12,55). Nowadays, in Western diets, the ratio of n-6 to n-3 PUFA is about 10 to 20:1, and this ratio may be associated with the incidence of different chronic diseases. Some authors (2) have suggested a healthy n-6 to n-3 PUFA ratio of about 4 to 6:1, which could reduce the development of diseases such as coronary heart disease, type 2 diabetes, inflammatory and autoimmune disorders, and cancer.

In the current study, the n-6 to n-3 PUFA ratio of 6:1, advocated as a healthy one, did not modify tumor growth rate that was similar to that seen in W rats. However, it was able to increase body weight gain, and food intake was only slightly lower than control and WN-3 groups. In addition, the blood metabolic profile, used here to indicate cachexia, was similar to that of non–tumor-bearing animals. Thus, the regimen with the ratio of 6:1 has a remarkable effect upon cachexia, although it did not reduce the tumor growth rate. As indicated previously, decreased cachexia in the WN-3 group may be due to a lower tumor burden resulting from the mechanisms

described previously. However, our finding in the W6:1 group suggests that this is not the case because, despite the larger tumors in the latter group, cachexia was reduced significantly. This is an interesting finding because usually a lower tumor growth rate is related to reduced cachexia, but here we show otherwise. In addition, n-3 PUFA from FO might exert a more active effect upon cachexia because they have the ability to decrease production of cytokines involved in cancer cachexia, including TNF, IL-1, and IL-6 (27–30).

In summary, the ratio of n-6 and n-3 FAs in the diet affects the progression of the Walker 256 tumor and influences the metabolic effects of tumor bearing. In this experimental model, a dietary n-6 to n-3 PUFA ratio of 6:1 was able to increase food intake and body weight, restore the biochemical blood parameters of cachexia, and prevent the development of cancer cachexia.

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