

Tumor Immunology

Effect of fish oil supplementation for 2 generations on changes in macrophage function induced by Walker 256 cancer cachexia in rats

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ABSTRACT

The effect of coconut fat (rich in medium saturated fatty acids) or fish oil (rich in ω -3 polyunsaturated fatty acids) supplementation for 2 generations on tumor growth, cancer cachexia, animal survival and macrophage function was investigated in Walker 256 tumor-bearing rats. Female Wistar rats were supplemented with coconut fat or fish oil prior to mating and then throughout pregnancy and gestation. Both supplementations were daily and orally given at 1 g per kg body weight as a single bolus. Same treatment was performed by the 2 following generations. At 90 days of age, male offspring (50%) from F2 generation were subcutaneously inoculated with 2×10^7 Walker 256 tumor cells. At 14 days after tumor implantation, rats not supplemented displayed cancer cachexia characterized by loss of body weight, hypoglycemia, hyperlactidemia, hypertriglyceridemia, decreased food intake and depletion of glycogen stores in the liver and skeletal muscles. Supplementation with coconut fat did not affect these parameters. However, supplementation with fish oil decreased tumor growth (59%), prevented body weight loss and food intake reduction and attenuated cancer cachexia. In addition, fish oil increased animal survival up to 20 days (from 25% in rats not supplemented to 67% in rats supplemented with fish oil) and improved macrophage function characterized by increased phagocytosis capacity and production of hydrogen peroxide and nitric oxide. These results suggest that fish oil supplementation for 2 generations improves macrophage function in association to reduced tumor growth and attenuated cancer cachexia, maintaining food intake and increasing animal survival. © 2006 Wiley-Liss, Inc.

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ARTICLE TEXT

Cancer cachexia syndrome is characterized by body weight loss, asthenia, weakness, anemia, edema, increased hepatic gluconeogenesis and resting energy expenditure, intense catabolism of carbohydrate, lipid and protein stores[1][2][3][4] and anorexia.[5] This debilitating condition leads to death of up to 70% of patients with cancer.[6][7] Moreover, deaths by secondary infections due to impairment of immune function are frequent in patients with cancer cachexia.[2] In fact, cachexia is considered the major complication to treat cancer patients.[8]

Current diet of the Western world has a high content of saturated fatty acids and ω -6 polyunsaturated fatty acids (ω -6 PUFA) and low content of ω -3 PUFA.[9] This fat dietary composition has been correlated with increased occurrence of certain neoplasias such as colon, colorectal, breast and prostate cancers.[10][11][12][13][14] Epidemiological and experimental studies have shown that ω -3 PUFA, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present anticancer effects[15][16][17][18][19][20][21] and improve action of other anticancer agents.[22][23][24] Greenland Eskimos and Alaskans that consume large quantity of ω -3 PUFA from fish oil present a lower colon cancer risk than North Americans that consume a low quantity of ω -3 PUFA and high of saturated fatty acids and ω -6 PUFA.[14] Diet enriched with ω -3 PUFA from fish oil inhibits mammary and colon carcinogenesis in rats,[25][26] growth of human breast cancer in nude mice[27][28] and of various tumor types in rats and athymic mice,[29][30] increases food intake in tumor-bearing rats[30] and pancreatic cancer patients[31] and raises survival of patients with malignant solid tumor.[32] Administration of large doses of fish oil in patients with advanced cancer attenuates cachexia, as demonstrated by stabilization or body weight gain.[3][31][33] In spite of the studies above, the mechanisms involved in the beneficial effects of ω -3 PUFA in cancer are poorly known.

Macrophages play an important role in the host defense against tumor.[34][35] They produce several cytokines and antitumor agents including hydrogen peroxide, superoxide anion and nitric oxide.[36][37] Dietary fish oil modulates the production of nitric oxide, hydrogen peroxide, and some cytokines such as IL-6 and TNF- α . [36][38] We recently investigated the effect of lifelong consumption of fish oil (1 generation) on tumor growth and cachexia in adult rats. The lifelong consumption of fish oil reduced tumor growth and cachexia, and increased food intake and survival in Walker 256 tumor-bearing rats.[39] In spite of this information, there is no study on the effect of lifelong fish oil supplementation on function of macrophages from tumor-bearing animals.

The aim of the present study was to investigate (i) if the anticachetic effects previously observed in rats supplemented with fish oil for 1 generation are maintained or pronounced when this supplementation is given for 2 generations and (ii) the effect of this supplementation protocol on macrophage function from rats bearing Walker 256 tumor for 14 days.

Material and methods

Materials

All enzymes and reagents for buffers were obtained from Sigma Chemical (St. Louis, MO). Fish oil from cold sea was purchased from Herbarium (Paraná, Brazil) and coconut fat from Refino de Óleos (Bahia, Brazil). Regular chow diet containing 4% fat was obtained from Nuvital Nutrients (Curitiba, Brazil). Total fatty acid composition of the oils and chow (Table I) was determined by high-performance liquid chromatographer as described previously (Shimadzu model LC-10A, Shimadzu, Kyoto, Japan).[40][41]

Table I. Fatty Acid Composition of Fish Oil, Coconut Fat and Regular Chow Used in the Present Study

Fatty acids	Fish oil	Coconut fat	Regular chow
Lauric (12:0)	0.50	61.06	0.10
Myristic (14:0)	11.66	18.93	1.36
Palmitic (16:0)	14.44	5.57	22.23
Stearic (18:0)	3.23	2.62	8.61
Oleic (18:1)	8.85	7.04	35.91
Linoleic (18:2)	0.20	2.86	31.69
γ -Linolenic (18:3)	1.76	0.50	0.10
Arachidonic (20:4)	12.33	-	-
Eicosapentaenoic (20:5)	26.48	1.42	-
Docosahexaenoic (22:6)	20.55	-	-

The results are shown as % of the total fatty acids.

Animals

Wistar rats were obtained from the Department of Physiology, Biological Sciences Building, Federal University of Paraná, Curitiba, Brazil. The animals were housed at 12:12 hr light-dark cycle, (23 ± 1)°C, and received the regular chow diet and water *ad libitum*. The Brazilian Animal Ethics Committee (CEEA) approved this study.

Study design

After the nursing period (21 days), female rats were divided into 3 groups: (i) control group (not supplemented) (CN), (ii) group supplemented with fish oil (FO), and (iii) group supplemented with coconut fat (CO). Both oils were daily and orally given at 1 g per kg body weight as a single bolus. After 3 months of supplementation, female rats were mated with male Wistar rats fed on a normal chow diet (not supplemented). The female rats continued to receive supplementation throughout gestation and lactation. Same treatment was performed to the 2 following generations. Coconut fat presented mainly medium chain saturated fatty acids (80%) and it was used as control in this study because there is no clear correlation between this group of fatty acids and cancer risk. At 90 days of age, male offspring (50%) from F2 generation were injected in the right flank with 1 mL of a sterile suspension of 2×10^7 Walker 256 tumor cells, obtained from an ascitic tumor-bearing rat.[42] Thus, the following groups were studied: nontumor-bearing rats subdivided in the CN, FO and CO groups and tumor-bearing rats subdivided in not supplemented (WKNS), and supplemented with coconut fat (WKCO) or fish oil (WKFO). Body weight and food intake were determined during the experimental period. At day 14 after tumor implantation, the rats were killed and the following parameters were determined: tumor weight, liver and muscle glycogen content, blood levels of glucose and lactate and macrophage function (phagocytosis, lysosomal volume and production of H₂O₂, anion superoxide and nitric oxide). Some tumor-bearing rats were randomly used for determination of survival up to 20 days.

Determination of blood metabolites

Serum glucose concentration was determined by the glucose oxidase method.[43] Serum triglycerides concentration was evaluated by the method described by Young.[44] For lactate measurement, 0.1 mL of 25% perchloric acid was added to 0.5 mL of serum for deproteinization. After incubation for 10 min, at 4°C, the samples were centrifuged for 5 min at 3,000g. The supernatant was neutralized with 1 N NaOH solution and the lactate content determined by the method described by Engel and Jones.[45]

Determination of glycogen content in the liver and skeletal muscle

Liver, soleus muscle and white portion of gastrocnemius muscle (70 mg) were digested in 0.5 mL of 1 M KOH solution for 20 min, at 70°C. After digestion, 0.1 mL was added to 0.5 mL assay buffer I (75 mM acetic acid, 120 mM acetate sodium and 6.5 U/mL amyloglucosidase, at pH 4.8) and incubated for 2 hr at room temperature. The samples were then centrifuged for 5 min at 800g and added to 1.0 mL of assay buffer II (375 mM triethanolamine, 3 mM MgSO₄, 10 mM ATP, 0.65 mM NADP, 4.5 U/mL hexokinase, and 1.5 U/mL glucose-6-phosphate dehydrogenase, at pH 7.5). Glycogen content was quantified by measurement at 340 nm as previously described by Foot *et al.*[46]

Macrophage function

Isolation of macrophages

Resident macrophages were obtained by intraperitoneal lavage with 10 mL of sterile phosphate-buffered saline (PBS). The peritoneal cells were collected by centrifugation (290g, 4°C, for 5 min), washed and then resuspended in PBS medium after counting in a Neubauer chamber by optical microscopy, using a Trypan blue solution (1%). The number of macrophages in tumor-bearing animals was higher compared to the nontumor-bearing rats. Macrophages were further purified by incubating peritoneal cells in tissue culture plates for 2 hr and then washed 3 times with PBS to remove the nonadherent cells.[47] The final preparation had at least 98% macrophages.

Phagocytosis

Aliquots (0.1 mL) of macrophage suspension were added to a 96-well flat bottomed tissue culture plate and left to adhere for 1 hr. Then, 50 μ L of neutral-red stained zymosan (1×10^8 particles per mL) were added to each well. After incubation for 30 min, the cells were fixed with Baker's formal-calcium (4% formaldehyde, 2% sodium chloride, 1% calcium acetate) for 30 min. The cells were then washed 2 times by centrifugation (453g for 5 min). The neutral-red stain was solubilized by adding 0.1 mL of acidified alcohol (10% acetic acid, 40% ethanol in distilled water) to each well. After 30 min, the absorbance at 550 nm of each well was read on a plate reader (Spectra MaxPlus, Molecular Devices, CA, USA).

Phagocytosis capacity was calculated from a standard curve using known amounts of stained zymosan and the results expressed per mg protein.[48]

Lysosomal volume

The uptake of the cationic dye neutral red, which concentrates in cell lysosomes, was used to assess the volume of the macrophage lysosomal system. 20 μ L of 3% neutral-red in PBS were added to 0.1 mL of macrophage suspension per well for 30 min. The cells were then washed twice with PBS by centrifugation (453g for 5 min). Neutral red was solubilized by a 30 min incubation adding 0.1 mL of 10% acetic acid plus 40% ethanol solution. The absorbance was read at 550 nm and neutral red uptake calculated per mg protein.[48]

H₂O₂ production

Hydrogen peroxide production by peritoneal macrophages was measured as described by Pick and Mizel.[49] This assay is based on the horseradish peroxidase (HRPO)-dependent conversion of phenol red into a colored compound by H₂O₂. Macrophages (final volume 0.1 mL) were incubated in the presence of glucose (5 mM), phenol red solution (0.56 mM) and HRPO (8.5 U/mL) in the dark for 1 hr at 20°C. After this period, the absorbance was measured at 620 nm on a plate reader. The concentration of H₂O₂ was determined from a standard curve prepared in parallel. H₂O₂ production is expressed as μ mol per mg protein.

Production of superoxide anion

Superoxide anion production was estimated by the nitroblue tetrazolium (NBT) reduction assay. Macrophages (0.45 mL) suspended in PBS were incubated for 1 hr at 37°C in the presence of 0.03 mL of phorbol myristate acetate (PMA, 5 μ M final concentration) and 0.1% NBT. The reaction was stopped by adding 0.45 mL of acetic acid. The mixture was then centrifuged for 30 s at 2,500g. Formation of blue formazan from NBT reduction was spectrophotometrically measured (560 nm). The results are expressed as absorbance per mg protein.[50]

Nitric oxide production

Nitric oxide was measured as nitrite (NO₂⁻). Macrophages (2×10^5 in a final volume of 0.2 mL) were incubated for 24 hr in the presence of LPS (10 μ g/mL final concentration). Nitrite concentration was measured by the Greiss reaction. Equal volumes of cell culture supernatant and Greiss reagent were incubated for 10 min at room temperature and the absorbance measured at 550 nm. NO₂⁻ concentration was determined from a standard curve generated by using NaNO₂. [51] Nitrite production is expressed as μ mol per L.

Protein determination

Protein concentrations of macrophage preparations were measured by the method of Bradford, using bovine serum albumin as standard.[52]

Statistical analysis

The results are presented as mean \pm S.E.M. and analysed by two-way ANOVA followed by the post hoc Bonferroni test. $p < 0.05$ was taken to indicate statistical significance.

Results

Body weight and tumor growth

Body weight was not altered by supplementation with fish oil or coconut fat before and after 14 days of Walker 256 tumor implantation (Table II). Coconut oil supplementation did not affect tumor weight, whereas fish oil supplementation reduced tumor weight by 59% as compared to the WKNS group ($p < 0.001$). Carcass weight, calculated by subtracting tumor weight from body weight, was reduced in the WKNS and WKCO groups (Table II). Supplementation with fish oil however partially prevented the loss of body weight induced by tumor growth (Table II).

Table II. Effect of Fish Oil and Coconut Fat Supplementation for 2 Generations on Body Weight and Tumor Growth in Walker 256 Tumor-Bearing Rats Fed Normal

Chow (WKNS), Supplemented with Coconut Fat (WKCO) or Fish Oil (WKFO)

Groups	WKNS	WKCO	WKFO
Body weight (g)			
Before tumor implantation	259.3 ± 10.7	272.2 ± 10.3	281.4 ± 9.7
After 14 days of tumor implantation	259.9 ± 8.9	280.2 ± 6.8	276.8 ± 11.3
Tumor weight (g)	18.7 ± 1.7	18.79 ± 1.2	7.7 ± 1.2 ¹
Carcass weight (g)	237.0 ± 9.2	253.7 ± 7.3	268.0 ± 11.6
Body Weight change (%)	-6.9 ± 1.2	-6.4 ± 2.7	-1.4 ± 1.0 ¹

The results are shown as media ± S.E.M. of 15 animals per group.

¹ *p* < 0.001 versus WKNS and WKCO.

Food intake

Food intake was not modified by the supplementation with coconut fat (CO) or fish oil (FO). In the WKNS rats, daily food intake was reduced from 22.8 ± 1.7 g at the beginning of the study to 13.9 ± 3.0 g 14 days after tumor implantation. WKCO rats also had a significant reduction in daily food intake; from 25.1 ± 1.1 g to 14.8 ± 1.5 g. Tumor-induced reduction of food intake was prevented in the WKFO group; 26.0 ± 1.3 g and 22.8 ± 1.5 g per day at the beginning and fourteen days afterwards, respectively (Table III).

Table III. Food Intake and Blood Levels of Glucose, Lactate and Triglycerides (TG) in Nontumor-Bearing Rats Fed Normal Chow (CN), Supplemented with Coconut Fat (CO) or Fish Oil (FO), and Walker 256 TUMOR-Bearing Rats Fed Normal Chow (WKNS), Supplemented with Coconut Fat (WKCO) or Fish Oil (WKFO)

Groups	CN	CO	FO	WKNS	WKCO	WKFO
Food intake (g)	22.8 ± 1.7	25.1 ± 1.1	26.0 ± 1.3	13.9 ± 3.0 ²	14.80 ± 1.5 ²	22.8 ± 1.5 ³
Blood lactate levels (μmol/mL)	1.32 ± 0.10	1.28 ± 0.12	1.11 ± 0.07	2.63 ± 0.13 ²	2.51 ± 0.19 ²	1.39 ± 0.12 ⁴
Blood glucose levels (mg/100 mL)	125.2 ± 3.4	123.6 ± 4.6	121.1 ± 2.7	100.2 ± 3.8 ¹	97.7 ± 6.2 ¹	124.3 ± 3.0 ⁴
Blood TG levels (mg/100 mL)	79.6 ± 6.5	99.2 ± 9.9	80.1 ± 5.9	136.7 ± 10 ¹	140.0 ± 10 ¹	92.6 ± 5.8 ³

Data are as mean ± S.E.M. of 15 rats per group.

¹ *p* < 0.05 and

² *p* < 0.001 versus CN.

³ *p* < 0.05 and

⁴ *p* < 0.01 versus WKNS and WKCO.

Blood metabolite concentrations

Blood concentration of glucose, lactate, and triglyceride was not altered in the CO and FO groups. After 14 days of tumor implantation, however, serum glucose concentration was reduced and serum lactate and triglyceride concentration was increased in the WKNS and WKCO groups. These effects were partially prevented by fish oil supplementation (WKFO group) (Table III).

Liver and muscle glycogen content

Glycogen content in the liver and soleus and white gastrocnemius muscles was reduced in WKNS and WKCO rats. Fish oil supplementation (WKFO group) partially prevented this reduction (Table IV).

Table IV. Effect of Fish Oil and Coconut Fat Supplementations for 2 Generations on Glycogen Content in the Liver and Skeletal Muscles in Nontumor-Bearing Rats Fed Normal Chow (CN), Supplemented with Coconut Fat (CO) or Fish Oil (FO), and

Walker 256 Tumor-Bearing Rats Fed Normal Chow (WKNS), Supplemented with Coconut Fat (WKCO) or Fish Oil (WKFO)

Glycogen content ($\mu\text{mol/g}$ wet weight)	Nontumor-bearing rats			Tumor-bearing rats		
	CN	CO	FO	WKNS	WKCO	WKFO
Liver	131.1 \pm 6.4	131.6 \pm 6.3	135.0 \pm 6.9	75.6 \pm 7.7 ¹	77.8 \pm 7.3 ¹	106.9 \pm 5.9 ¹²
Soleus muscle	47.8 \pm 2.8	45.8 \pm 3.2	46.8 \pm 2.6	33.5 \pm 1.9 ¹	32.8 \pm 1.7 ¹	38.9 \pm 1.9
Gastrocnemius muscle	54.3 \pm 2.3	54.8 \pm 3.2	55.3 \pm 2.9	38.6 \pm 1.2 ¹	33.2 \pm 2.1 ¹	49.7 \pm 1.8 ¹²

The results are shown as media \pm SEM of 15 animals per group.

¹ $p < 0.05$ versus CN.

² $p < 0.05$ versus WKNS and WKCO.

Survival

The survival of tumor-bearing rats was increased by fish oil supplementation (WKFO rats). After 20 days of tumor implantation, the percentage of tumor-bearing rats that survived was of 25, 8 and 67% in the WKNS, WKCO, and WKFO groups, respectively (Fig. 1).

Figure 1. Survival of Walker 256 tumor-bearing rats fed normal chow (WKCEN), supplemented with coconut fat (WKCO) or fish oil (WKFO). Data are mean \pm SEM of 8 rats per treatment group. ^a $p < 0.001$ versus WKNS and WKCO.

[Normal View 6K | Magnified View 11K]

Macrophage function

Peritoneal macrophages from nontumor-bearing rats supplemented with fish oil (FO group) exhibited increased phagocytosis capacity, which was about 30% higher than in the control and CO groups (Fig. 2a). Tumor-bearing state (WKNS group) markedly increased macrophage phagocytosis. There was no difference in macrophage phagocytosis between WKNS and WKCO groups. However, macrophage phagocytosis was increased in the WKFO group when compared to WKNS and WKCO groups ($p < 0.05$). Macrophages from WKNS, WKCO and WKFO groups showed a 50% increase in the phagocytosis capacity when compared to nontumor-bearing rats (CN, CO and FO groups).

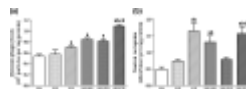


Figure 2. Zymosan phagocytosis (a) and neutral red uptake (b) by peritoneal macrophages from nontumor-bearing rats fed normal chow (CN), supplemented with coconut fat (CO) or fish oil (FO), Walker 256 tumor-bearing rats fed normal chow (WKNS), supplemented with coconut fat (WKCO) or fish oil (WKFO). Data are mean \pm SEM of 15 rats per treatment group. ^a $p < 0.05$ versus CN. ^b $p < 0.05$ versus CO. ^c $p < 0.05$ versus WKNS. ^d $p < 0.05$ versus WKCO.

[Normal View 39K | Magnified View 127K]

Uptake of neutral red was higher by macrophages from the FO group as compared to the control and CO groups (Fig. 2b). Tumor-bearing state increased neutral red uptake by macrophages from the WKNS group but it had no effect on cells from the WKCO and WKFO groups. There was no difference in neutral red uptake by macrophages between CN and CO rats and between WK and WKCO rats.

Hydrogen peroxide production by peritoneal macrophages from the CO group was 46% higher when compared to control rats (Fig. 3a). Supplementation with fish oil (FO group) raised H_2O_2 production by 80 and 23% when compared to the control and CO groups, respectively ($p < 0.05$). Tumor-bearing state increased H_2O_2 production by macrophages in all groups (Fig. 3a). In WKNS rats, there was an increase of 62% in H_2O_2 production when compared to the control group. H_2O_2 production by macrophages from the WKCO and WKFO groups was increased by 21 and 16%, compared to WKNS rats, respectively. Macrophages from the WKCO group presented an increase of 34% in H_2O_2 production when compared to CO rats, whereas there was no difference between the WKFO and FO groups (Fig. 3a).

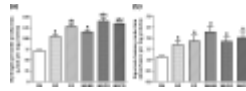


Figure 3. Production of hydrogen peroxide (a) and superoxide anion (b) by peritoneal macrophages from nontumor-bearing rats fed normal chow (CN), supplemented with coconut fat (CO) or fish oil (FO), Walker 256 tumor-bearing rats fed normal chow (WKNS), supplemented with coconut fat (WKCO) or fish oil (WKFO). Data are mean \pm SEM of 15 rats per treatment group. ^a $p < 0.05$ versus CN. ^b $p < 0.05$ versus CO. ^c $p < 0.05$ versus WKNS.

[Normal View 43K | Magnified View 142K]

Superoxide production by peritoneal macrophages (Fig. 3b) from the CO and FO groups was higher ($p < 0.05$) as compared with control rats. Tumor-bearing state (WKNS group) increased (by 60%) superoxide production by macrophages as compared to cells from control rats. In contrast, superoxide production by macrophages from the WKFO group was not altered as compared to FO rats (Fig. 3b).

Under unstimulated conditions, peritoneal macrophages from the FO group showed higher production of NO as compared to the control and CO groups (Fig. 4). NO production by macrophages from the WKFO group was greater than that of the WKNS and WKCO groups (Fig. 4). LPS stimulation increased (by 2.0- to 2.8-fold) NO production by macrophages from CN, CO, WKNS and WKCO rats (Fig. 4). In contrast, LPS stimulation did not increase NO production by cells from rats that had been supplemented with FO, regardless the presence of the tumor (Fig. 4). The highest production of NO was observed in macrophages from tumor-bearing rats supplemented with fish oil (Fig. 4).

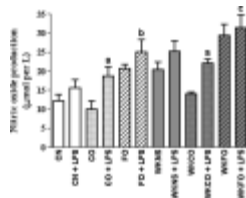


Figure 4. Production of nitric oxide by unstimulated and LPS-stimulated peritoneal macrophages from nontumor-bearing rats fed normal chow (CN), supplemented with coconut fat (CO) or fish oil (FO), Walker 256 tumor-bearing rats fed normal chow (WKNS), supplemented with coconut fat (WKCO) or fish oil (WKFO). Data are mean \pm SEM. of 8 rats per treatment group. ^a $p < 0.05$ versus same diet without LPS. ^b $p < 0.05$ versus CN, CO, WKNS and WKCO groups without LPS. ^c $p < 0.05$ versus all other groups with LPS.

[Normal View 31K | Magnified View 85K]

Discussion

Depletions of glycogen stores in the liver and skeletal muscle, of proteins in skeletal muscle and of triacylglycerol in adipose tissue occur as a result of the intense catabolism in tumor-bearing rats.[3][4] In association to decreased food intake, there is weight loss, asthenia, weakness and anemia in cancer cachexia. The predominant anaerobic metabolism in the tumor results in reduced glycemia and increased blood lactate levels.[2][3][4][53] In the present study, Walker 256 tumor growth caused body weight loss, depletion of skeletal muscle and liver glycogen content, hypoglycemia, increased serum lactate and triglyceride levels and reduced food intake and survival. These changes are indicative of the establishment of the cancer cachexia state. Fish oil supplementation (high levels - up to 70% fat intake) for a short period of time has been shown to attenuate cancer cachexia and to reduce tumor growth.[6][31][33] In this study, fish oil supplementation (at low levels - 0.1% of body weight) for 2 generations partially prevented the development of cancer cachexia in Walker 256 tumor-bearing rats by reducing tumor growth and body weight loss and increasing daily food intake and survival. Thus, a nutritional supplementation of fish oil at low dose leads to remarkable effects on tumor growth and cancer cachexia with no apparent side effects.

In a previous study, we demonstrated beneficial effects of fish oil supplementation for 1 generation in Walker 256 tumor bearing rats.[39] The results obtained herein were very similar. Thus, the beneficial effects of fish oil supplementation for 1 generation (reducing tumor growth, partially preventing cachexia and increasing food intake and survival) are maintained when the study is carried out for 2 generations in Walker 256 tumor-bearing rats.

The reports on macrophage function in cancer are controversial mainly due to differences in experimental design, animal species or strain used, type of tumor used and macrophage subpopulation studied.[54] Several antitumor agents produced by macrophages have been shown to be altered by dietary fish oil.[38] To our knowledge, this is the first study carried out to compare the effects of coconut fat and fish oil supplementation in pregnant and lactating rats and subsequently in the offspring for 2 generations on peritoneal macrophage function in tumor-bearing rats. Walker 256 tumor growth increased phagocytosis capacity, lysosomal volume and production of hydrogen peroxide and superoxide anion and did not alter

NO production by macrophages.

Dietary fats had different effects on nontumor and tumor-bearing animals in this study. Fish oil supplementation was able to induce an increase in zymosan phagocytosis, uptake of neutral red and production of superoxide anion, hydrogen peroxide and nitric oxide by macrophages from nontumor-bearing rats. These responses are important steps of the host defense[36][37] and lifelong fish oil supplementation appears to improve innate immune response. Increased phagocytosis by macrophages and monocytes has been shown in other studies with ω -3 PUFA[36][38] and seems to result from altered membrane fluidity.[55] The increase in the cationic dye neutral-red uptake, which concentrates in cell lysosomes, indicates lysosomal swelling. This means that the cells are able to phagocytose this particle and then process it. This is consistent with improved phagocytosis and cell killing capacity, and partially explains the attenuating effect of fish oil supplementation on tumor growth and cancer cachexia.

Reactive oxygen metabolites and nitric oxide are toxic to pathogens and to tumor cells. The enzymes responsible for the synthesis of reactive oxygen species and NO are regulated by eicosanoids, cytokines and protein kinase C (PKC). ω -3 PUFA affect the production of eicosanoids and cytokine[29][56] and modulate PKC activity.[57] Walker 256 tumor-bearing rats have high circulating levels of PGE₂ compared to controls being the tumor tissue the main source of this eicosanoid.[39] Increased dietary supply of ω -3 PUFAs has been shown to decrease arachidonic acid levels in host tissues and in the tumor,[29] and this would be expected to result in decreased formation of PGE₂ and related eicosanoids. Thus, the mechanism by which dietary fish oil increases production of these cytotoxic mediators by macrophages from control rats might involve altered eicosanoid and cytokine levels and PKC activation.[38][56] Superoxide, H₂O₂ and NO are macrophage-derived cytotoxic agents and so fish oil could enhance the macrophage killing capacity of foreign cells by leading to an increase in the production of these metabolites. However, some studies have shown that high amounts of fish oil present inhibitory effects on macrophage function.[56] Lysosomal volume and production of superoxide anion were not affected by the diet in tumor-bearing rats, whereas NO production, phagocytosis capacity and H₂O₂ release were increased in tumor-bearing rats supplemented with fish oil. Thus, the mechanism by which fish oil is able to cause tumor growth reduction may involve enhanced macrophage activity. In addition, however, fish oil may affect the metabolism of Walker 256 tumor-bearing rats by changing eicosanoid production, cyclooxygenase-2 expression, lipid peroxidation and angiogenesis.[16][56] In a recent study, Sauer *et al.*[58] found that EPA inhibits cell proliferation in MCF-7 human breast cancer xenografts in nude rats associated with a reduction in intracellular cAMP content, uptake of others fatty acids (saturated, monounsaturated and ω -6 PUFA) and production of 13-hydroxyoctadecadienoic acid by the tumoral cells. Therefore, the effects of fish oil supplementation on macrophage function could be a consequence of a lower tumor growth. So far, this issue still remains to be fully clarified.

In summary, fish oil supplementation at low dose (1 g per kg body weight per day) for a long period of time (1 or 2 generations) has anticancer and anticachetic effects in Walker 256 tumor-bearing rats, as demonstrated by reduced tumor growth and body weight loss, and increased food intake and survival. Fish oil supplementation for 2 generations enhanced macrophage function what partially explains its attenuating effect on Walker 256 cancer cachexia (e.g. increased phagocytosis capacity and production of NO and H₂O₂). Thus, a low intake of fish oil for generations is an efficient strategy to prevent tumor growth and cancer cachexia and to improve the innate immune function. Whether these positive effects of fish oil supplementation on cancer also occur in humans remains to be investigated.

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