

Article

Lifelong *n*-3 PUFA Consumption Reduces HER2+ Mammary Tumour Growth and Alters Immune Markers Compared to Safflower- or Corn Oil-Based Sources of *n*-6 PUFA

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Abstract

Background: *n*-3 PUFA derived from marine sources, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exhibit potential for breast cancer prevention. In contrast, higher dietary intakes of *n*-6 PUFA, such as linoleic acid (LA), have been implicated in promoting mammary tumorigenesis. However, there is a need for further exploration into how *n*-3 PUFA influence breast cancer development in comparison to different amounts and sources of LA. **Objective:** The purpose of this study was to compare the effects of *n*-3 PUFA-enriched diets versus *n*-6 PUFA diets differing in LA content, including corn oil (50% LA) and safflower oil (70% LA), on mammary tumour development in a HER2+ breast cancer model. **Methods:** Using the HER2+ breast cancer MMTV-neu(ndl)YD5 transgenic mouse model, this study determined the effects of: (1) 10% *w/w* corn oil (CO, *n*-6 PUFA, *n* = 14), (2) 10% *w/w* safflower oil (SO, *n*-6 PUFA, *n* = 14), (3) 3% *w/w* menhaden oil + 7% *w/w* CO (3% FO 7% CO, *n*-3 PUFA, *n* = 12), and (4) 3% *w/w* menhaden oil + 7% *w/w* SO (3% FO 7% SO, *n*-3 PUFA, *n* = 14) on puberty onset, tumour incidence, tumour volume, and tumour number in utero until 20 weeks of age. **Results:** Mice fed the *n*-3 PUFA-enriched diets showed a lower trajectory of tumour development compared to the *n*-6 PUFA diets, although the differences for palpated tumour volume and number over time reached significance only between the 10% CO and 3% FO 7% CO groups. This suggests that high LA content in CO may represent a threshold for promoting tumour growth whereby further LA content marginally influences additional tumour development. Exposure to the CO *n*-6 PUFA diet further resulted in earlier onset of puberty compared to the *n*-3 PUFA-enriched diet containing CO. To investigate the underlying mechanisms, a qPCR analysis of mammary glands and tumour tissue revealed that the *n*-3 PUFA diets downregulated the expression of pro-tumourigenic immune markers, including CD206 and F4/80 in the mammary glands and the cannabinoid receptor CB2 in tumours, compared to the *n*-6 PUFA diets. **Conclusions:** These findings indicate that the presence of dietary *n*-3 PUFA plays a key role in modulating mammary tumour development, which may be further influenced by the underlying *n*-6 PUFA background. The associated changes in immune markers suggest that *n*-3 PUFA exert anticancer effects in part by shifting the tumour immune microenvironment toward an anti-tumour phenotype and modulating



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cannabinoid receptor signalling. Collectively, this work informs future human studies investigating the role of dietary fat composition in breast cancer risk.

Keywords: breast cancer; *n*-3 PUFA; *n*-6 PUFA; fish oil; fatty acid; MMTV-neu; eicosapentaenoic acid; docosahexaenoic acid

1. Introduction

Breast cancer is the most prevalent cancer among women worldwide, with approximately 2.3 million incident cases in 2022 [1], and it is estimated to increase to 3.19 million annual cases by 2040 [2]. This staggering statistic underscores the need to identify potential risk factors and strategies for the prevention of breast cancer. The risk of developing breast cancer is influenced by a myriad of factors, including lifestyle, physical activity, dietary patterns, genetic predisposition, sex, and age [3]. These multifaceted elements uniquely contribute to an individual's susceptibility to the disease.

Given that hereditary breast cancer comprises a small proportion (~5–10% of cases), there is a growing emphasis on identifying and addressing modifiable risk factors as a key strategy for reducing the future burden of breast cancer [4,5]. In particular, the role of dietary components in influencing the development of breast cancer continues to be a key area of interest as studies have identified associations between dietary habits and breast cancer risk [6]. Among these components, dietary fatty acids, particularly polyunsaturated fatty acids (PUFA), have been recognized for their potential role in modifying breast cancer risk. Specifically, *n*-3 and *n*-6 PUFA have been studied extensively in relation to breast cancer development. Epidemiological studies show that Asian populations that consume diets that are rich in marine-derived *n*-3 PUFA, such as eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), tend to exhibit a lower incidence of breast cancer [7,8]. In contrast, Western populations with elevated consumption of *n*-6 PUFA, including linoleic acid (LA, 18:2*n*-6) and arachidonic acid (AA, 20:4*n*-6), and comparatively lower *n*-3 PUFA intake than Asian diets, tend to have a higher risk of developing breast cancer [7,8]. The distinct fatty acid profiles of these two classes of PUFA, *n*-3 and *n*-6, are well-documented and known for exerting diverse effects on inflammation, immune responses, and cell signaling pathways [9–14]. These differences in biological effects suggest that the mechanisms through which *n*-3 and *n*-6 PUFA operate could be pivotal in their roles of either preventing or promoting breast cancer. Emerging human and animal studies show that *n*-3 PUFA, commonly abundant in fatty fish, flaxseed, and walnuts, are associated with an anti-inflammatory profile [9,15]. Conversely, *n*-6 PUFA, predominantly found in vegetable oils including corn, soybean, and safflower oils, have been associated with a pro-inflammatory profile [9,12,15]. This duality indicates that optimizing the balance between dietary *n*-3 and *n*-6 PUFA has potential implications for breast cancer risk and progression. However, the findings in human studies remain inconsistent, with some clinical trials reporting no significant effect of *n*-3 PUFA supplementation on breast cancer incidence or progression [16,17]. These discrepancies may be attributed to variations in dietary composition, baseline PUFA intake, and individual metabolic differences, highlighting the complexity of PUFA interactions in breast cancer. In contrast, a meta-analysis of prospective studies found that a higher intake ratio of *n*-3 to *n*-6 PUFA was associated with a lower risk of breast cancer among females, suggesting a potential protective effect of *n*-3 PUFA [8]. For example, the Shanghai Women's Health Study reported that, while individual PUFA intake was not directly associated with breast cancer risk, higher *n*-6 PUFA intake combined with low marine-derived *n*-3 PUFA intake was associated with a two-fold increase in breast cancer risk [18]. Despite these mixed results, preclinical studies

continue to support the potential anticancer properties of *n*-3 PUFA, warranting further mechanistic investigation [19].

The favourable and adverse effects of *n*-3 and *n*-6 PUFA, respectively, on mammary tumour outcomes are further supported by previous findings from our laboratory, which demonstrated that *n*-3 PUFA intake in mice (~7.8 mg/day of EPA and ~6.8 mg/day of DHA; corresponding to human intakes of ~3.5 g/day of EPA and DHA) reduced tumour growth and number and prolonged tumour latency compared to an *n*-6-PUFA-rich diet lacking EPA and DHA [12,20]. In support of these findings, studies also suggest that *n*-3 PUFA intake increases apoptosis and reduces cell proliferation and pro-inflammatory cytokine expression in breast cancer in humans and mouse models [13]. However, it remains unclear how different sources and levels of *n*-6 PUFA affect breast cancer development. Previous dietary interventions, including studies from our laboratory, have primarily used a single *n*-6 PUFA-rich diet, including corn oil- or safflower oil-based sources when assessing the effects of fatty acids on mammary tumourigenesis in vivo [11,20,21]. However, whether these *n*-6 PUFA-enriched oil sources result in differences in mammary tumourigenesis has yet to be evaluated extensively. While these oils belong to the same *n*-6 PUFA family, their fatty acid compositions and LA content differ. Safflower oil is higher in LA (70–80% of total fatty acids) compared to corn oil (50–60% of total fatty acids), with corn oil being more representative of the amounts consumed in the Western diet [22,23]. Thus, it can be hypothesized that diets rich in *n*-6 PUFA from safflower oil may predispose to less favourable tumour outcomes compared to diets containing comparatively lower *n*-6 PUFA content from corn oil. Given the variability in PUFA composition across dietary oils, further research is needed to determine whether the source of *n*-6 PUFA contributes to differential effects on breast cancer progression.

Beyond tumour growth, emerging evidence suggests that *n*-3 PUFA may exert anti-cancer effects through modulation of the tumour immune microenvironment. Tumour-associated macrophages (TAMs) are a key component of this microenvironment, with M1-like macrophages exerting anti-tumour effects and M2-like macrophages promoting tumour progression and immune suppression [24,25]. Increased M2-like macrophage infiltration has been associated with more aggressive breast cancer phenotypes [26–30]. Importantly, *n*-3 PUFA have been shown to influence macrophage polarization and reduce expression of TAMs in breast and prostate cancers [31–33]. In parallel, emerging research highlights a role for the endocannabinoid system (ECS) in cancer progression, particularly through cannabinoid receptors CB1 and CB2, which are expressed in immune cells and tumour tissues [34,35]. The CB2 receptor, in particular, is overexpressed in HER2-positive breast tumours and forms a heteromeric complex with HER2, driving oncogenic signaling and contributing to poor prognosis [31,36,37]. Notably, *n*-3 PUFA-derived lipid mediators such as docosahexaenoyl ethanolamide (DHEA) and eicosapentaenoyl ethanolamide (EPEA) can activate the CB2 receptor, potentially disrupting the HER2–CB2 receptor complex and attenuating downstream tumour-promoting pathways [31,36]. However, the influence of dietary PUFA on CB1 and CB2 receptor expression and macrophage polarization in vivo, particularly in HER2+ breast cancer, remains poorly understood.

This study aimed to assess the effect of *n*-3 PUFA on mammary tumourigenesis compared to different sources of *n*-6 PUFA diets that contain high or moderate levels of LA. Specifically, this study used safflower oil, a source with higher total *n*-6 PUFA, and corn oil, a source with comparatively lower *n*-6 PUFA content compared to safflower oil, to understand how the relative fatty acid compositions of these oils influence mammary tumour latency, growth, and weight in an aggressive mouse model of HER-2 breast cancer (mouse mammary tumour virus (MMTV)-neu(ndl)-YD5 model) [38]. By directly comparing two LA-rich *n*-6 PUFA sources with distinct fatty acid compositions within a controlled

dietary framework, this study extends beyond traditional single-oil *n*-6 PUFA models and provides a more nuanced assessment of how *n*-6 PUFA background influences mammary tumour development. Additionally, this study aimed to provide insight into the potential immunomodulatory and endocannabinoid-mediated mechanisms underlying the effects of varying dietary fatty acid compositions on tumour progression.

It was hypothesized that *n*-3 PUFA would delay tumour onset, reduce tumour number and tumour volume over time, and shift tumour immune and ECS profiles toward anti-tumorigenic states compared to mice fed an *n*-6 PUFA diet. In addition, it was hypothesized that mice fed a comparatively high-*n*-6 PUFA diet containing safflower oil would have more severe tumour outcomes compared to an *n*-6 PUFA diet containing corn oil.

2. Materials and Methods

2.1. Animals and Diet

Mice with the mammary tumour virus (MMTV)-neu(ndl)-YD5 genotype, bred on an FVB background, were sourced from an in-house breeding colony under the housing and breeding protocols described previously [12,39]. The MMTV-neu(ndl)-YD5 model is a useful model of HER-2 breast cancer to study prevention and treatment avenues, which contributes to 15–20% of all breast cancer cases [40–42]. Overexpression of HER-2 through the murine equivalent neu-oncogene leads to the rapid growth of mammary tumours, where 50% of MMTV transgenic mice develop tumours within 100 days of age [38]. Thus, this experimental model allows for the study of breast cancer development and response to dietary interventions.

Each harem was composed of a single male mouse carrying the heterozygous MMTV-neu(ndl)-YD5 genotype and three female FVB wild-type mice, yielding wild-type or heterozygous (MMTV) offspring. Harems were randomly assigned to one of four modified AIN93G diets (Research Diet Inc., New Brunswick, NJ, USA): (1) 10% *w/w* corn oil (CO, *n*-6 PUFA, *n* = 14), (2) 10% *w/w* safflower oil (SO, *n*-6 PUFA, *n* = 14), (3) 3% *w/w* menhaden oil + 7% *w/w* CO (3% FO 7% CO, *n*-3 PUFA, *n* = 12), or (4) 3% *w/w* menhaden oil + 7% *w/w* SO (3% FO 7% SO, *n*-3 PUFA, *n* = 14). Mice were bred until a minimum of 12 mice per diet group was achieved. This sample size was determined based on previous studies, which demonstrated that 12 mice per group were sufficient to detect significant differences in tumour size and multiplicity with 80% power at a significance level of 0.05 [12,20,43]. Variations in sample sizes across groups resulted from differences in litter sizes and exclusions due to health status. All diets were isocaloric. The 3% *w/w* menhaden oil diets approximate *n*-3 PUFA intake from high fish consumption, reflecting intake levels of traditional Japanese diets (1–2% of daily energy as marine *n*-3 PUFA) or levels that can be achieved through supplementation [44]. The 10% *w/w* SO and CO diets reflect Western dietary patterns, which are characterized by low *n*-3 PUFA intake and high LA consumption. While the *n*-6 PUFA diets represent the higher end of LA intake (with LA contributing approximately 12% and 16% of energy intake from 10% CO and 10% SO diets, respectively) and exceed the Institute of Medicine (IOM) recommendation of 5–10%, they remain within levels previously observed in human diets [45–47]. Gas chromatography was used to confirm the fatty acid composition of the diets (Table 1). The manufacturer supplied the macronutrient composition of the diets (Appendix A). Mice were provided food and double-distilled water *ad libitum*. Food intake was measured 3 times per week throughout the study period. Body weight (g) was measured weekly, starting at 4 weeks of age until study termination (i.e., 20 weeks). Female offspring were weaned and genotyped at three weeks of age as described previously [43]. Only female transgenic offspring were maintained on their parental diets, while male offspring were euthanized at weaning. Starting at 3 weeks of age, female mice were examined daily for the presence of vaginal opening, which serves as a

marker for the onset of puberty. Mice were co-housed in ventilated cages, with a maximum of four mice per cage. All research procedures were granted approval by the University of Guelph Animal Care Committee under Animal Utilization Protocol #4417 (approval date: 20 July 2020).

Table 1. Fatty acid composition (%) of experimental diets.

Fatty Acid	10% CO	10% SO	3% FO 7% CO	3% FO 7% SO
12:0	0.1	0.1	0.1	0.1
14:0	0.2	0.3	2.6	2.8
15:0	n.d.	0.1	0.3	0.3
16:0	12.1	7.2	14.7	10.2
16:1c9	0.2	0.1	3.6	3.9
17:1c10	n.d.	n.d.	n.d.	n.d.
18:0	1.8	3.5	2.2	2.8
18:1c9	27.9	12.5	22.0	13.9
18:1c11	0.7	0.7	1.3	1.4
18:2n6	54.7	70.5	40.3	50.0
18:3n6	n.d.	n.d.	0.2	0.1
19:0	n.d.	n.d.	0.1	0.2
19:1c7	n.d.	n.d.	n.d.	n.d.
18:3n3	1.0	0.1	1.2	0.6
18:4n3	n.d.	0.1	1.0	0.9
20:0	0.4	0.5	0.3	0.3
20:1c5&8	n.d.	n.d.	n.d.	n.d.
20:1c11	0.4	0.2	0.6	0.4
20:2n6	0.1	n.d.	0.1	0.1
20:3n6	n.d.	n.d.	0.2	0.1
20:4n6	n.d.	n.d.	0.5	0.7
20:3n3	n.d.	n.d.	0.1	0.1
20:5n3	0.1	n.d.	3.8	3.7
22:0	0.2	0.8	0.2	0.8
22:1n9	n.d.	0.2	0.1	0.2
22:2n6	n.d.	n.d.	0.1	n.d.
22:4n6	n.d.	n.d.	0.1	0.1
22:3n3	n.d.	n.d.	n.d.	n.d.
22:5n6	n.d.	0.1	0.2	0.2
22:5n3	n.d.	n.d.	0.7	0.9
24:0	0.2	0.6	0.2	0.5
22:6n3	n.d.	n.d.	2.7	2.9
24:1n9	n.d.	0.8	0.2	0.8
Total n-6	54.7	70.6	41.6	51.4
Total n-3	n.d.	0.2	9.6	9.3
Total SFA	15.0	13.0	20.9	18.0
Total MUFA	29.1	14.5	27.7	20.7
Total PUFA	55.8	70.8	51.1	60.6

Fatty acid composition (%) of 10% corn oil (10% CO), 10% safflower oil (10% SO), 7% corn oil plus 3% menhaden oil (3% FO 7% CO) and 7% safflower oil plus 3% menhaden oil (3% FO 7% SO) diets. Lipids were extracted and analyzed from two individual pellets per diet and analyzed by gas chromatography. Averages are shown. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n.d., not detected; c, cis configuration.

2.2. Measurement of Mammary Tumours and Collection of Tissue at Termination

Starting at 10 weeks of age, mice were palpated three times per week until termination (up to 20 weeks of age) for the presence of mammary tumours. When a new tumour was detected, a digital caliper was used to measure tumour dimensions along the sagittal (length) and transverse (width) planes, and tumour multiplicity was recorded. Tumour volume was calculated using the formula $[\text{length} \times (\text{width}^2)]/2$. When the cumulative dimension of tumours exceeded 17 mm in either length or width, mice were checked for estrous cycle and terminated by CO₂ overdose prior to the 20-week endpoint. The stage of

estrous cycle was assessed by vaginal flush with 30 μ L of phosphate buffer saline solution. The resulting solution was placed on a glass slide and observed under a microscope (Nikon Eclipse TS100, Neville Instruments, Melville, NY, USA). Mice were euthanized if in the proestrus, estrus, or metestrus stages of the estrous cycle. For mice in the diestrus stage, termination was delayed, and the estrous cycle was rechecked for a maximum of two days to control for hormone fluctuations. At termination, the mouse pelt with mammary glands and tumours still attached was removed to measure final tumour size using digital calipers. Subsequently, the tumours were excised, weighed, snap-frozen and stored at -80°C for fatty acid analysis. Final tumour weight, volume (sum of all tumour volumes) and multiplicity (total number of tumours) for each mouse were calculated.

2.3. Fatty Acid Analysis of Tumours

Lipids from mammary tumour tissue were extracted using the Folch method [48]. Briefly, lipids were extracted using a 2:1 chloroform:methanol mixture, separated by phospholipid class using thin-layer chromatography, and transmethylated with boron trifluoride prior to analysis, as described previously [43]. Fatty acid composition of tumours was analyzed by gas chromatography (7890A Agilent Technologies, SpectraLab, Markham, ON, Canada) and expressed as a percentage of total fatty acids.

2.4. RNA Isolation and qPCR Analysis of Tumours and Mammary Glands

Total RNA was isolated from mammary glands and tumour tissue ($n = 10$ per diet) using an RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) following the protocol by the manufacturer. RNA concentration and purity were assessed using a NanoDrop 2000 UV Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Further, 2 μ g of total extracted RNA per sample was used to make cDNA using a High-Capacity cDNA Reverse Transcription Kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Quantitative polymerase chain reaction (qPCR) analysis was then performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the following thermal cycling conditions: 2 min at 50°C , 10 min at 95°C , 15 s at 95°C , 60°C for 1 min, 15 s at 95°C and 15 s at 60°C for a total of 40 cycles. Primers were designed using the Primer-BLAST (National Center for Biotechnology Information, Bethesda, MD, USA; Primer3 version 2.5.0; Supplementary Table S1), and validated primer efficiencies ranged between 90 and 105%. Samples were run in duplicate in 96-well plates (Sarstedt, Nümbrecht, Germany; Cat #72.1980). Each 20 μ L reaction contained 0.4 μ L of 10 μ M primer solution, 4.6 μ L of dH_2O , and 10 μ L Power SYBRTM Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA; Cat #4367659) combined with 5 μ L cDNA. Gene expression levels were normalized to the mRNA expression of the housekeeping gene eukaryotic translation elongation factor 2 (*eEF2*), and the relative quantification of the *n*-3 PUFA treatment groups (3% FO 7% CO; 3% FO 7% SO) was calculated using the $\Delta\Delta\text{Ct}$ method in comparison to the respective *n*-6 PUFA control groups (10% CO; 10% SO). The fold change in gene expression ($2^{-\Delta\Delta\text{Ct}}$) was calculated, and averages were compared across the four diet groups for statistical analyses.

2.5. Statistical Analysis

Data for food intake, body weights, puberty onset, tumour latency, gene expression, and phospholipid species were analyzed by one-way ANOVA followed by Tukey's studentized range test if normally distributed or Kruskal–Wallis test followed by Wilcoxon rank-sum tests if normality assumption was not met. A two-way ANOVA with repeated measures was conducted for body weight, total palpated tumour volume, and multiplicity to determine differences between diet groups over the 20-week study period. Log-rank test was used to analyze the difference in proportion (%) of mice that remained tumour-free in

each diet group over the 20-week study period. Values are reported as mean $\pm$ standard error (SEM). All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA), with the statistical significance defined as $p \leq 0.05$.

3. Results

3.1. Body Weight and Food Intake

The initial and final body weights and average daily food intake per mouse are reported in Supplementary Table S2. There were no significant differences in initial body weight at 4 weeks of age across the four diet groups. The changes in body weight over 16 weeks of the diet intervention were significant across all the group comparisons except between the 10% CO and 3% FO 7% SO groups (Supplementary Figure S1). However, the final body weights at 20 weeks of age were only statistically lower for the 10% SO group compared to the 10% CO and 3% FO 7% CO groups ($p \leq 0.05$). The 10% SO group displayed lower body weight gain compared to the 3% FO 7% CO group only. Daily food intake was lower for the 10% SO group compared to the *n*-3 PUFA groups, 3% FO 7% CO and 3% FO 7% SO ($p \leq 0.05$). No differences in food intake were seen between the other diet groups.

3.2. Fatty Acid Composition of Tumour Phospholipids

A fatty acid analysis of phospholipid fractions from mammary tumours was conducted to determine the incorporation of *n*-6 and *n*-3 PUFA into target tissue. The percent fatty acid composition of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) fractions are shown in Table 2. The incorporation of fatty acids in sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS) fractions is reported in Supplementary Table S3. As expected, the mice fed the *n*-3 PUFA diets (3% FO 7% CO and 3% FO 7% SO) had higher levels of EPA (20:5*n*-3) and DHA (22:6*n*-3) in tumour PC, PE (Table 2), PI and PS (Supplementary Table S2) fractions compared to mice fed *n*-6 PUFA diets (10% CO and 10% SO). In contrast, the mice on the *n*-6 PUFA diets exhibited significantly higher incorporation of AA (20:4*n*-6) compared to their *n*-3 PUFA diet counterparts in the PC, PE, PI, and PS fractions. Interestingly, incorporation of LA (18:2*n*-6) across PC and PE tumour phospholipid fractions was higher in the 3% FO 7% CO group compared to the 10% CO group ($p \leq 0.05$), with no significant differences between the 7% SO 3% FO and 10% SO groups. Notably, the overall *n*-3 PUFA to *n*-6 PUFA ratios within individual tumour phospholipid fractions were higher in the *n*-3 PUFA groups. The phospholipid fractions of mammary tumours generally showed enrichment in different families of fatty acids according to the experimental diets.

Table 2. Fatty acid composition (%) of mammary tumour phospholipids.

Fatty Acids	PC				PE			
	10% CO	10% SO	3% FO 7% CO	3% FO 7% SO	10% CO	10% SO	3% FO 7% CO	3% FO 7% SO
12:0	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 $\pm$ 0.1	n.d.	n.d.
14:0	1.4 $\pm$ 0.1	1.5 $\pm$ 0.2	1.3 $\pm$ 0.1	1.6 $\pm$ 0.2	0.5 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
15:0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
16:0	31.8 $\pm$ 0.5	31.5 $\pm$ 0.5	33.0 $\pm$ 0.9	32.2 $\pm$ 0.3	6.8 $\pm$ 0.3	7.2 $\pm$ 0.2	7.2 $\pm$ 0.1	7.1 $\pm$ 0.4
18:0	8.3 $\pm$ 0.4	7.9 $\pm$ 0.5	7.3 $\pm$ 0.4	8.2 $\pm$ 0.5	14.4 $\pm$ 0.5	14.4 $\pm$ 0.6	14.1 $\pm$ 0.9	14.9 $\pm$ 1.1
19:0	n.d. ^a	0.1 $\pm$ 0.0 ^b	n.d. ^{ab}	0.1 $\pm$ 0.0 ^b	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0
20:0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	n.d.	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0
22:0	n.d. ^a	0.2 $\pm$ 0.0 ^b	0.2 $\pm$ 0.0 ^b	0.2 $\pm$ 0.0 ^b	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
24:0	0.1 $\pm$ 0.0	0.5 $\pm$ 0.2	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	1.1 $\pm$ 0.6	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
Total SFA	41.9 $\pm$ 0.7	42.1 $\pm$ 0.8	42.2 $\pm$ 1.0	42.8 $\pm$ 0.6	22.8 $\pm$ 0.6	23.4 $\pm$ 0.9	22.6 $\pm$ 0.9	23.3 $\pm$ 1.2
16:1c9	3.2 $\pm$ 0.1	3.4 $\pm$ 0.3	4.2 $\pm$ 0.6	4.2 $\pm$ 0.4	1.9 $\pm$ 0.1	2.0 $\pm$ 0.1	2.3 $\pm$ 0.3	2.3 $\pm$ 0.2

Table 2. Cont.

Fatty Acids	PC				PE			
	10% CO	10% SO	3% FO 7% CO	3% FO 7% SO	10% CO	10% SO	3% FO 7% CO	3% FO 7% SO
18:1c9	15.7 ± 0.3	13.1 ± 0.3	15.6 ± 1.0	14.2 ± 1.2	21.0 ± 0.6	19.3 ± 0.7	22.0 ± 1.8	19.9 ± 1.7
18:1c11	6.1 ± 0.2	5.3 ± 0.1	6.0 ± 0.5	5.7 ± 0.5	5.7 ± 0.2	5.2 ± 0.2	5.5 ± 0.6	5.3 ± 0.5
20:1c11	0.9 ± 0.1 ^a	0.7 ± 0.1 ^{ab}	0.8 ± 0.1 ^a	0.5 ± 0.0 ^b	1.0 ± 0.0 ^a	0.9 ± 0.1 ^{ab}	1.1 ± 0.2 ^a	0.7 ± 0.1 ^b
22:1n9	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
24:1n9	0.4 ± 0.1	0.8 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Total MUFA	26.7 ± 0.4	23.7 ± 0.6	27.3 ± 1.3	25.3 ± 1.4	30.6 ± 0.7	28.0 ± 0.8	31.9 ± 1.9	29.1 ± 1.8
18:2n6	10.3 ± 0.3 ^a	10.9 ± 0.7 ^a	13.8 ± 1.0 ^b	12.9 ± 0.6 ^{ab}	7.0 ± 0.2 ^a	7.4 ± 0.3 ^{ab}	9.4 ± 0.6 ^c	8.5 ± 0.4 ^{bc}
18:3n6	0.2 ± 0.0 ^a	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.1 ± 0.0 ^{ac}	0.2 ± 0.0 ^a	n.d. ^b	n.d. ^{bc}
18:3n3	0.1 ± 0.0	n.d.	0.1 ± 0.0	0.1 ± 0.0	n.d.	n.d.	n.d.	n.d.
20:2n6	1.4 ± 0.1 ^{ab}	1.7 ± 0.2 ^a	1.2 ± 0.2 ^{ab}	0.9 ± 0.0 ^b	1.2 ± 0.1 ^{ab}	1.4 ± 0.2 ^a	1.0 ± 0.1 ^{ab}	0.8 ± 0.1 ^b
20:3n6	2.9 ± 0.2	3.4 ± 0.4	3.1 ± 0.2	2.4 ± 0.3	3.8 ± 0.2	4.2 ± 0.4	4.0 ± 0.4	3.1 ± 0.4
20:4n6	13.6 ± 0.4 ^a	15.3 ± 0.6 ^a	8.6 ± 0.4 ^b	9.3 ± 0.6 ^b	22.9 ± 0.3 ^a	24.5 ± 0.7 ^a	17.0 ± 0.9 ^b	18.0 ± 1.3 ^b
20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20:5n3	n.d. ^a	n.d. ^a	1.0 ± 0.1 ^b	1.0 ± 0.1 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	2.1 ± 0.2 ^b	2.0 ± 0.2 ^b
22:2n6	0.3 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.2 ± 0.0 ^{ab}	0.2 ± 0.1 ^a	0.2 ± 0.0 ^{ab}	0.1 ± 0.0 ^b	0.1 ± 0.0 ^{ab}
22:4n6	0.6 ± 0.1 ^a	0.8 ± 0.1 ^a	0.2 ± 0.0 ^b	0.2 ± 0.0 ^b	2.4 ± 0.3 ^a	2.9 ± 0.3 ^a	0.5 ± 0.0 ^b	0.7 ± 0.1 ^b
22:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n6	0.4 ± 0.0 ^a	0.5 ± 0.1 ^a	0.0 ± 0.0 ^b	0.1 ± 0.0 ^b	1.7 ± 0.2 ^a	2.9 ± 0.5 ^a	n.d. ^b	0.2 ± 0.1 ^b
22:5n3	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.6 ± 0.1 ^b	1.0 ± 0.3 ^b	0.9 ± 0.1 ^a	0.6 ± 0.1 ^a	2.7 ± 0.3 ^b	3.0 ± 0.2 ^b
22:6n3	1.2 ± 0.1 ^a	0.8 ± 0.3 ^a	1.8 ± 0.2 ^b	3.6 ± 1.5 ^b	6.4 ± 0.4 ^{ac}	3.8 ± 1.3 ^a	8.6 ± 0.9 ^{bc}	11.2 ± 2.0 ^b
Total n-3	1.5 ± 0.1 ^a	1.0 ± 0.3 ^a	3.4 ± 0.3 ^b	5.7 ± 1.9 ^b	7.3 ± 0.4 ^a	4.5 ± 1.4 ^a	13.5 ± 1.3 ^b	16.2 ± 2.3 ^b
Total n-6	29.6 ± 0.7 ^a	33.2 ± 0.6 ^b	27.0 ± 0.9 ^{ac}	26.0 ± 1.0 ^c	39.3 ± 0.5 ^a	43.6 ± 0.8 ^b	32.0 ± 0.9 ^c	31.3 ± 1.7 ^c
n-6/n-3	20.3 ± 1.3 ^a	25.0 ± 4.3 ^a	8.1 ± 0.6 ^b	6.3 ± 1.2 ^b	5.5 ± 0.3 ^a	7.5 ± 1.8 ^a	2.5 ± 0.3 ^b	2.1 ± 0.3 ^b
Total PUFA	31.2 ± 0.6	34.2 ± 1.1	30.6 ± 1.1	31.8 ± 1.8	46.7 ± 0.7	48.2 ± 1.7	45.5 ± 1.5	47.5 ± 2.5

Fatty acid composition (%) of mammary tumours from MMTV mice ($n = 6/\text{diet group}$) fed 10% CO, 10% SO, 3% FO 7% CO or 3% FO 7% SO at 20 weeks of age. For each fatty acid within each phospholipid class, values with different letters indicate significant difference ($p \leq 0.05$) between groups according to one-way ANOVA followed by Tukey's studentized range test. Values (expressed as % composition of total mammary tumour) are means $\pm$ SEM. 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil. PC, phosphatidylcholine; PE, phosphatidylethanolamine; n.d., not detected.

3.3. Puberty Onset

Puberty onset was delayed ($p \leq 0.05$) in mice fed the corn oil diet enriched with n -3 PUFA (3% FO 7% CO) (31.5 ± 1.0 days) compared to mice fed 10% CO (27.4 ± 0.9 days). There was no difference in puberty onset between the safflower diet enriched with n -3 PUFA (29.9 ± 0.9 days) and safflower n -6 PUFA group (30.9 ± 1.4 days) (Figure 1).

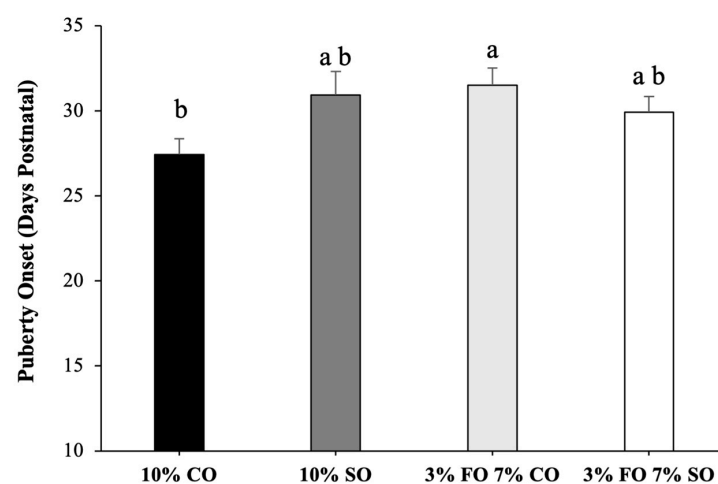


Figure 1. Average puberty onset (days postnatal) in mice fed either 10% corn oil ($n = 14$), 10% safflower oil ($n = 14$), 7% corn oil plus 3% menhaden oil ($n = 12$), or 7% safflower oil plus 3% menhaden oil ($n = 14$). Groups with a different letter are significantly different according to Tukey's studentized range test ($p \leq 0.05$). Bars represent mean values $\pm$ SEM. 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil.

3.4. Tumour-Free Status and Tumour Latency

The proportion of tumour-free mice over time is shown in Figure 2. The average median age at which mammary tumours were detected by palpation in 50% of mice (T₅₀ threshold) was delayed in both FO fed groups compared to CO fed mice, but the delay was not statistically significant (Figure 2). The average tumour latency was 94.1 ± 2.3 and 96.9 ± 3.2 days in mice fed the 10% CO and 10% SO diets, respectively, and 101.6 ± 2.6 and 99.1 ± 2.7 days in mice fed the 3% FO 7% CO and 3% FO 7% SO diets, respectively. There were no significant differences in tumour latency; however, the appearance of tumours occurred ~5 days earlier in mice fed *n*-6 (10% CO and 10% SO) versus *n*-3 (3% FO 7% CO and 3% FO 7% SO) (Figure 3).

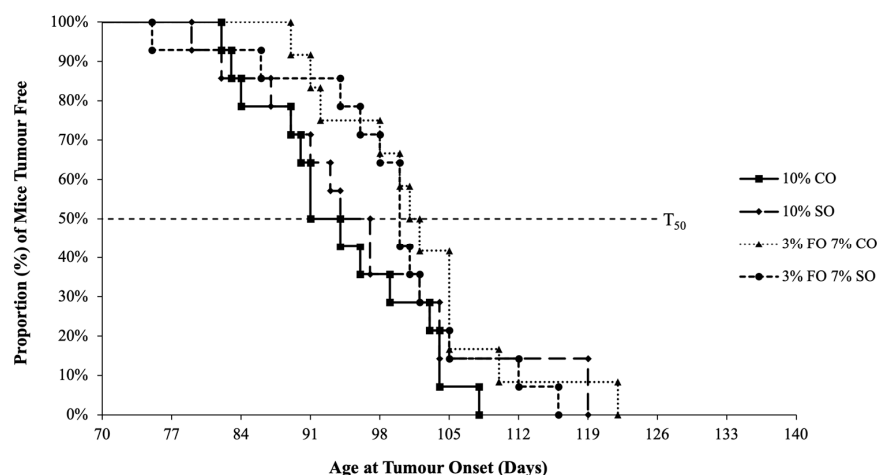


Figure 2. Proportion (%) of mice that remained tumour-free over the 20-week study period fed either 10% corn oil ($n = 14$), 10% safflower oil ($n = 14$), 7% corn oil plus 3% menhaden oil ($n = 12$), or 7% safflower oil plus 3% menhaden oil ($n = 14$). No significant differences across diet groups were found according to log-rank test. 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil.

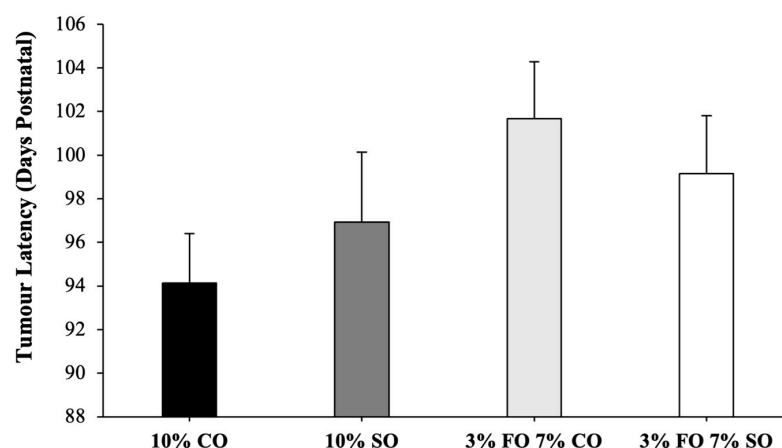


Figure 3. Average mammary tumour latency (days postnatal until the first detection of a palpable tumour). Bars represent mean values $\pm$ SEM ($n = 12$ – 14 /dietary group). Data was analyzed by one-way ANOVA. No significant differences were observed across diet groups. 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil.

3.5. Palpated Tumour Number and Tumour Volume

The palpated tumour number and tumour volume over time were lower in the *n*-3 PUFA-enriched corn oil group (3% FO 7% CO) compared to the *n*-6 PUFA (10% CO) diet

group ($p \leq 0.05$; Figure 4A,B). No differences in palpated tumour number and volume between the *n*-3 PUFA safflower oil (3% FO 7% SO) group and safflower oil (10% SO) group were detected over the 20-week period. The palpated tumour numbers over time were different between the two *n*-6 PUFA (10% CO vs. 10% SO) groups ($p \leq 0.05$) but not between the two *n*-3 PUFA groups (3% FO 7% CO vs. 3% FO 7% SO; Figure 4A). The palpated tumour volumes did not differ between the two *n*-6 PUFA (10% CO vs. 10% SO) groups but were significantly different among the two *n*-3 PUFA groups (3% FO 7% CO vs. 3% FO 7% SO; Figure 4B). At individual time points there were no differences in palpated tumour number or palpated tumour volume between the dietary groups.

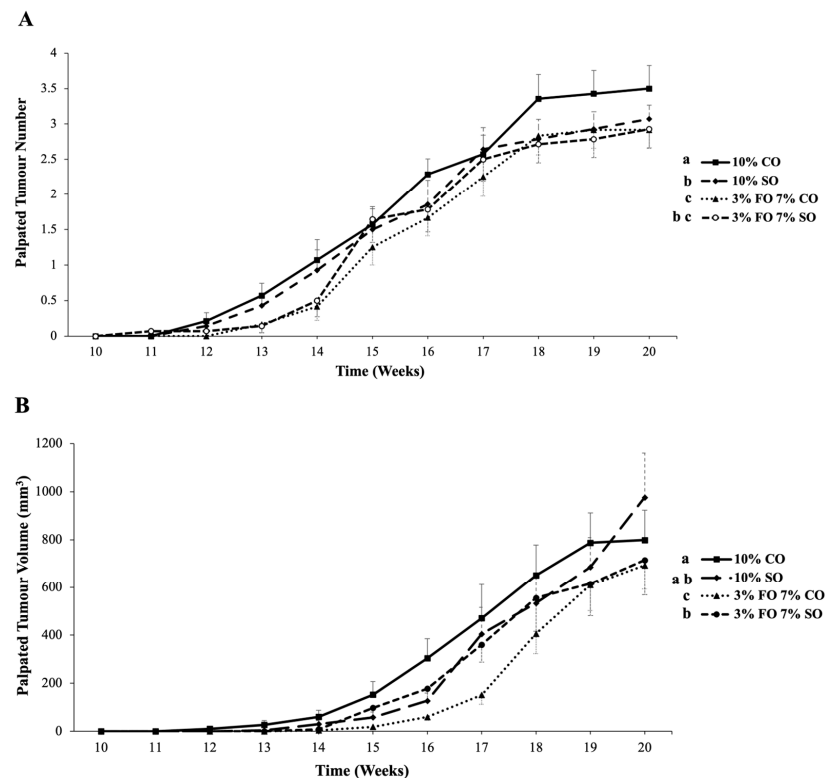


Figure 4. Average palpated (A) tumour number and (B) tumour volume per dietary intervention over the duration of the study in mice fed 10% corn oil ($n = 14$), 10% safflower oil ($n = 14$), 7% corn oil plus 3% menhaden oil ($n = 12$), and 7% safflower oil plus 3% menhaden oil ($n = 14$). Groups with a different letter indicate significantly different change in tumour volumes across time as determined by a repeated measures two-way ANOVA ($p \leq 0.05$). Values are mean $\pm$ SEM. Top error bars are displayed for the *n*-6 PUFA groups and bottom error bars are displayed for *n*-3 PUFA groups. 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil.

3.6. mRNA Expression of Mammary Glands and Tumours

Dietary fat composition differentially modulated immune-related gene expression in non-involved mammary gland and tumour tissues (Figures 5 and 6). In the mammary gland, expression of F4/80 (adhesion G protein-coupled receptor E1, Adgre1), a general macrophage marker, was highest in the *n*-6 PUFA groups, 10% SO and 10% CO, significantly decreased in the 3% FO 7% CO group, and intermediate in the 3% FO 7% SO group (Figure 5A, $p < 0.05$), suggesting that overall macrophage abundance may be influenced by dietary PUFA profiles. Similarly, expression of the M2-like macrophage marker CD206 (mannose receptor C-type 1, Mrc1) was markedly lower in the *n*-3 PUFA groups, 3% FO 7% CO and 3% FO 7% SO, compared to the *n*-6 PUFA groups, 10% CO and 10% SO (Figure 5B, $p < 0.05$), suggesting potential shifts in macrophage polarization. Expression of

CB1, (cannabinoid receptor 1, *Cnr1*) associated with immune regulation, was significantly reduced in the *n*-3 PUFA groups, 3% FO 7% CO and 3% FO 7% SO, compared to the *n*-6 PUFA groups, 10% CO and 10% SO (Figure 5C, $p < 0.05$). In contrast, no significant differences were observed in the expression of CB2 (cannabinoid receptor 2, *Cnr2*), M1-like marker CD86 (cluster of differentiation 86, *Cd86*), or M2-like marker ARG1 (arginase 1, *Arg1*) in mammary glands across the dietary groups (Supplementary Figure S2).

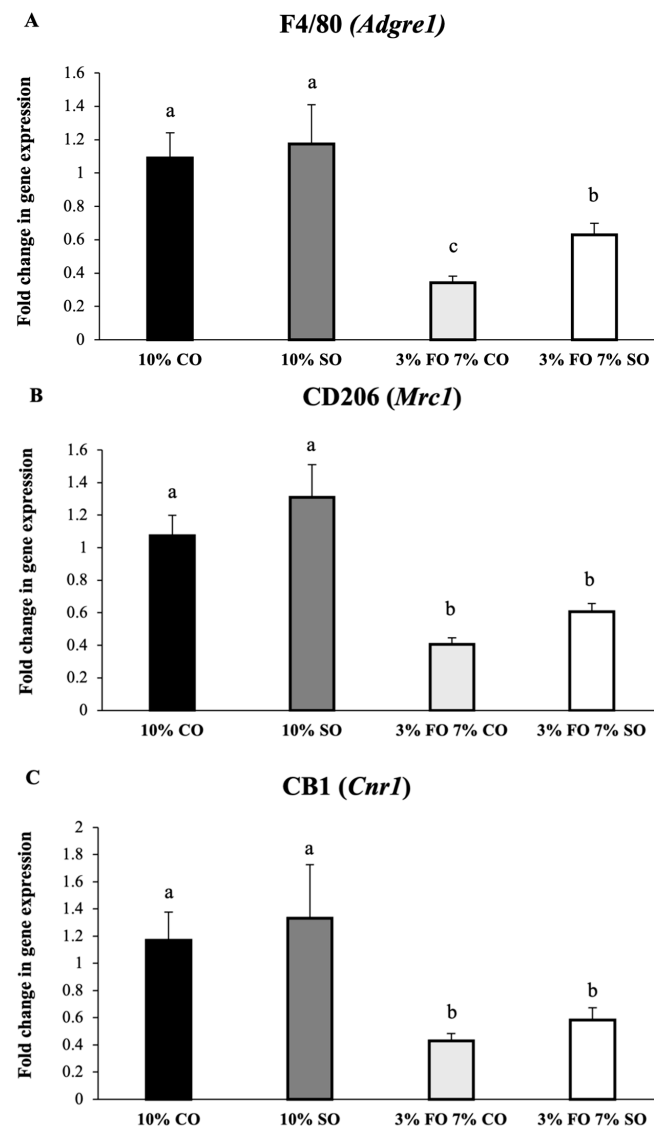


Figure 5. Gene expression in mammary glands of female MMTV mice at the time of termination (20 weeks of age). Bars represent mean values $\pm$ SEM ($n = 10$ /dietary group). Cycle threshold (Ct) values were normalized to the housekeeping gene *eEF2*, and $2^{-\Delta\Delta C_t}$ was used to calculate fold change in relative mRNA expression of the *n*-3 PUFA group (3% FO 7% CO; 3% FO 7% SO) compared to the respective *n*-6 PUFA (10% CO; 10% SO) group. Normalized fold-change expression levels of F4/80 (A), CD206 (B) and CB1 (C) are displayed as means $\pm$ SEM of two replicates. Data was analyzed by one-way ANOVA followed by Tukey's studentized range test or Kruskal–Wallis test followed by Wilcoxon rank-sum tests. Groups with a different letter are significantly different ($p \leq 0.05$). No significant differences in expression between CB2, CD86 (M1) and ARG1 (M2) genes between the groups (Supplementary Figure S2). 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil; *eEF2*, eukaryotic translation elongation factor 2; CB1 (*Cnr1*), cannabinoid receptor 1; CB2 (*Cnr2*), cannabinoid receptor 2; F4/80 (*Adgre1*), adhesion G protein-coupled receptor E1; CD206 (*Mrc1*), mannose receptor C-type 1; ARG1 (*Arg1*), arginase 1; CD86, (*Cd86*) cluster of differentiation 86.

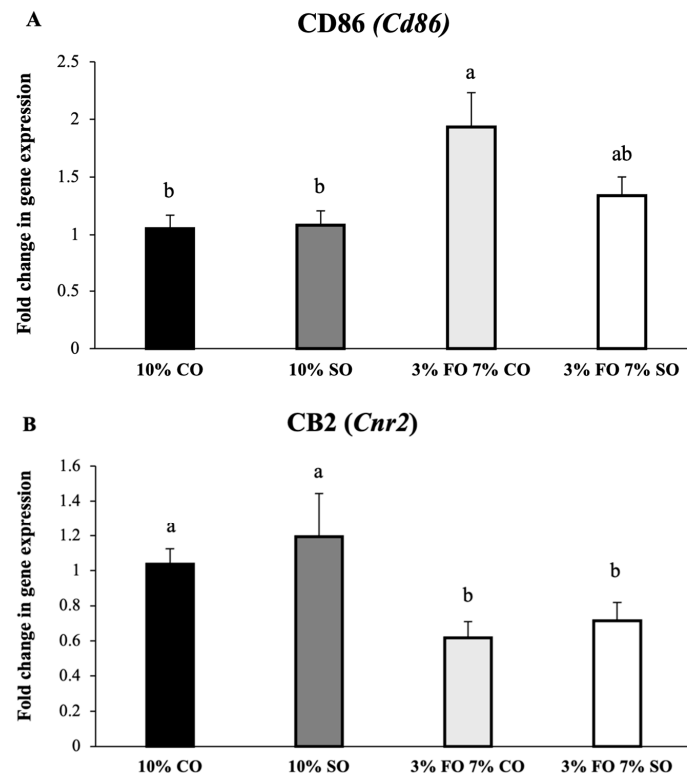


Figure 6. Gene expression in tumours of female MMTV mice at the time of termination (20 weeks of age). Bars represent mean values $\pm$ SEM ($n = 10$ /dietary group). Cycle threshold (C_t) values were normalized to the housekeeping gene *eEF2*, and $2^{-\Delta\Delta C_t}$ was used to calculate fold change in relative mRNA expression of the *n*-3 PUFA group (3% FO 7% CO; 3% FO 7% SO) compared to the respective *n*-6 PUFA (10% CO; 10% SO) group. Normalized fold-change expression levels of CD86 (**A**) and CB2 (**B**) are displayed as means $\pm$ SEM of at least two replicates. Data was analyzed by one-way ANOVA followed by Tukey's studentized range test or Kruskal–Wallis test followed by Wilcoxon rank-sum tests. Groups with a different letter are significantly different ($p \leq 0.05$). No significant differences in expression between CB1, F4/80, CD206 (M2) and ARG1 (M2) genes between the groups (Supplementary Figure S3). 10% CO, 10% corn oil; 10% SO, 10% safflower oil; 3% FO 7% CO, 7% corn oil plus 3% menhaden oil; 3% FO 7% SO, 7% safflower oil plus 3% menhaden oil; *eEF2*, eukaryotic translation elongation factor 2; CB1 (*Cnr1*), cannabinoid receptor 1; CB2 (*Cnr2*), cannabinoid receptor 2; F4/80 (*Adgre1*), adhesion G protein-coupled receptor E1; CD206 (*Mrc1*), mannose receptor C-type 1; ARG1 (*Arg1*), arginase 1; CD86, (*Cd86*) cluster of differentiation 86.

In tumour tissues, CD86 expression, a M1-like macrophage marker, was significantly upregulated in the *n*-3 PUFA groups, 3% FO 7% CO and 3% FO 7% SO, relative to the *n*-6 PUFA groups, 10% CO and 10% SO (Figure 6A, $p < 0.05$), indicating enhanced immune activation within the tumour microenvironment. Additionally, CB2 expression was significantly decreased in the *n*-3 PUFA groups (3% FO 7% CO and 3% FO 7% SO) compared to *n*-6 PUFA groups (10% CO and 10% SO) (Figure 6B, $p < 0.05$), suggesting reduced immunosuppressive signaling. No significant differences were observed in CB1, F4/80, CD206, or ARG1 expression in tumours across diet groups (Supplementary Figure S3).

4. Discussion

The current study assessed the effects of *n*-3 PUFA compared to two different sources of *n*-6 PUFA (corn and safflower oil) on mammary tumour development in MMTV-neu(ntl)YD5 transgenic mice, a model of HER-2 positive breast cancer. Lifelong exposure to the *n*-3 PUFA-enriched corn oil diet delayed the onset of puberty and reduced palpated tumour volume and multiplicity compared to the *n*-6 PUFA corn oil diet. Overall, these re-

sults suggest that the presence or absence of *n*-3 PUFA plays a dominant role in influencing tumour development, with the specific *n*-6 PUFA background potentially modulating the extent of this effect.

4.1. Effects of Dietary PUFA on Mammary Tumour Development

The findings from this study are supported by evidence from previous preclinical studies by others and our group showing that diets high in *n*-3 PUFA improve tumour outcomes compared to diets high in *n*-6 PUFA [12,20,43,49]. Past studies from our laboratory using the same mouse model of HER-2 positive breast cancer revealed that *n*-3 PUFA (3% *w/w* menhaden FO plus 7% *w/w* safflower oil) attenuated mammary tumour development by delaying tumour onset, as well as lowering tumour volume and the occurrence of multiple tumours after 20 weeks compared to mice fed 10% *w/w* safflower oil [12,20]. In addition, preclinical studies from our laboratory have shown a dose-dependent anticancer effect of *n*-3 PUFA with experimental diets containing 3% or 9% (*w/w*) menhaden FO combined with 7% or 1% (*w/w*) safflower oil, respectively [39]. In this present study, we demonstrated a small dose–response effect by increasing the exposure level of *n*-6 PUFA relative to a fixed amount of *n*-3 PUFA. This provides a new perspective showing that higher levels of *n*-6 PUFA against a fixed amount of *n*-3 PUFA, while controlling for total fat, only attenuate the anticancer effects of *n*-3 PUFA to a small degree. Furthermore, the hypothesized dose–response due to higher LA (SO vs. CO) was not observed. Also, the tumour outcomes were not identical across *n*-6 PUFA sources. Mice fed the 10% SO diet, despite its higher LA content compared to 10% CO, had lower palpated tumour number. These findings appear to contradict the idea that higher *n*-6 PUFA LA content would consistently worsen tumour outcomes. One possible explanation is that compositional differences beyond LA, including minor fatty acids such as the saturated fatty acid palmitic acid (PA, 16:0), between safflower and corn oil may have influenced tumour development. PA (%) was higher in the corn oil-based diets (10% CO: 12.1% and 3% FO: 14.7%) compared to the safflower oil-based diets (10% SO: 7.2 and 3% FO 10.2%), although this did not translate to differences in PA in tumour phospholipid species across the diets. Some studies suggest that increased supplementation or circulating levels of PA may drive tumour initiation and metastasis in hormone receptor-negative breast cancer [50,51]; however, the findings are inconclusive across other studies, with some even suggesting a protective anti-tumour effect [52,53]. Alternatively, the tumour-suppressive effect of *n*-3 PUFA may be more dominant than the pro-tumourigenic effect of *n*-6 PUFA in this model. Although this discrepancy does not refute the broader evidence that excessive *n*-6 PUFA may promote tumourigenesis, it underscores the complexity of dietary fat interactions and highlights the need to consider both source and context in interpreting PUFA effects on cancer outcomes. This body of preclinical research is consistent with human observational studies showing that a greater dietary ratio of *n*-3/*n*-6 PUFA is associated with a lower risk of breast cancer [8]. Therefore, the totality of these findings highlights the opportunity to reduce breast cancer risk by both increasing dietary *n*-3 PUFA and decreasing *n*-6 PUFA.

Only a few studies have provided a direct comparison between the effects of diets with varying sources of *n*-6 PUFA LA on mammary tumour growth. An earlier study compared the effects of different sources of *n*-6 (corn, safflower and evening primrose oils) and *n*-3 PUFA (fish and linseed oils) on mammary tumour development in female C3H/Heston mice treated with DMBA [54]. It was found that the *n*-6 diets rich in LA, which included safflower oil and corn oil, had a notable tumour-promoting effect, while the evening primrose oil had an intermediate effect. In contrast, the *n*-3 PUFA-enriched diets, including linseed oil and fish oil, exerted a significant preventive effect. Further, no significant differences in tumour measures, such as tumour incidence, between the

safflower and corn oil diets were reported in the mice [54]. The results from our study support these earlier findings, which highlight the contrasting effects of *n*-6 and *n*-3 PUFA on tumour development and the comparable effects of the different *n*-6 diet sources on tumour incidence and volume.

Although inconclusive, there is some evidence that LA, the most abundant source of *n*-6 PUFA found in corn and safflower oils, exhibits tumour-promoting properties in excess [55]. The LA content in safflower oil (70+%) is higher compared to corn oil (50+%) [22,23]. However, this study found that both *n*-3 PUFA diets, regardless of *n*-6 PUFA oil background (corn or safflower) included in the diet composition, significantly reduced tumour multiplicity compared to the *n*-6 PUFA-alone diets. Repeated measures analysis revealed that tumour number was significantly higher in the 10% CO group relative to the 10% SO group. This finding suggests that safflower oil, despite being rich in LA, may attenuate tumour development over time compared to corn oil across certain measures. One possible explanation is that safflower oil may promote greater lipid oxidation compared to corn oil, leading to complex differential effects on tumour progression [45,56]. This finding challenges the assumption that higher dietary LA uniformly promotes tumour growth and highlights the complexity of oil composition beyond LA content alone. We also found that the *n*-3 PUFA diets (3% FO 7% CO and 3% FO 7% SO) greatly reduced the number and volume of palpated tumours over time when compared to the corn oil-based *n*-6 PUFA diet (10% CO), with no differences found in palpated tumour measures in comparison to the safflower oil diet (10% SO). However, differences in palpated tumour volume and number between the *n*-3 PUFA (3% FO 7% SO) and safflower oil (10% SO) *n*-6 PUFA diets have been observed previously [12,20,43]. Another experimental study compared the mammary gland gene expression profiles of Sprague-Dawley rats treated with DMBA and fed either corn oil (high in LA) or extra-virgin olive oil (low in LA) [57]. The study reported an upregulation of genes associated with cell proliferation and a downregulation of genes linked to apoptosis in rats fed corn oil compared to those on the extra-virgin olive oil diet. These findings suggest a potential mechanistic link that may explain the increased susceptibility of mammary tumourigenesis in rodents fed a corn oil diet [57].

4.2. Body Weight and Puberty Timing

While macronutrient composition and caloric density were controlled across the four diet groups, minor variations in final body weights, weight gain and average daily intake were observed. These findings suggest that the types of dietary fat may have metabolic impacts on body weight that extend beyond energy intake or palatability factors. Although food intake across the groups fell within a narrow range (2.1–2.3 g/day), the 10% SO group showed a slight but statistically significant reduction in daily intake compared to the *n*-3 PUFA-containing diets. However, we have previously shown no differences in food intake or body weight between mice fed *n*-3 PUFA and *n*-6 PUFA in safflower oil-based diets using the same mouse model [12,43]. The findings of this current study may indicate that, while palatability was likely not a driving factor, the fatty acid profile itself could play a role in modulating energy balance. Overall, these results underscore that, even within calorically matched and similarly palatable diets, specific fatty acid compositions may yield distinct physiological responses. In addition, previous studies have reported that tumour development may lead to weight loss in rodents [14,58,59]. Although not statistically significant, the comparatively higher tumour volumes observed in the 10% SO group ($1929.3 \pm 350.9 \text{ mm}^3$ for 10% SO vs. $1415.2 \pm 174.6 \text{ mm}^3$ and $1403.2 \pm 205.1 \text{ mm}^3$ for the *n*-3 PUFA-enriched groups) may have contributed to the reduced daily food intake and lower final body weight and weight gain measurements of these mice. These findings

highlight the potential significance of body weight in tumour development, warranting further investigation.

Vaginal opening is a marker for puberty onset, and pubertal delay has been correlated with a decreased risk of breast cancer. Conversely, early menarche has been associated with an increased risk for breast cancer [14,49,58,59]. Using the same experimental model, we have previously shown that *n*-3 PUFA can delay puberty onset when compared to mice fed an *n*-6 PUFA safflower oil diet [12,39]. As anticipated, the present study also observed a delay in puberty onset in the corn oil-based *n*-3 PUFA diet group compared to the *n*-6 PUFA corn oil diet. In contrast, this finding did not extend to the safflower oil-based diet groups. However, early puberty onset by safflower oil has been previously observed [11,12,20], and the discrepancy is likely due to the minimal sample size used to reduce animal use in accordance with animal care guidelines. While not statistically significant, the lower average initial body weight of 16.5 ± 0.3 g in the 10% SO, compared to 17.2 ± 0.2 g in the safflower oil-based *n*-3 PUFA group, may have also contributed to the delay in puberty onset in the 10% SO group. In comparison, similar average body weights were observed in the 10% CO (17.3 ± 0.3 g) and corn oil-based *n*-3 groups (17.2 ± 0.2 g). Nevertheless, these data suggest that *n*-6 PUFA results in early menarche or alternatively negates the potential delay of menarche by *n*-3 PUFA, with body weight as a potential mediator.

4.3. Early-Life Exposure to Dietary PUFA and Implications for Breast Cancer Risk

While the ratio of *n*-6/*n*-3 PUFA and dietary *n*-3 PUFA intake alone has previously been linked to reduced breast cancer risk, the limited evidence for the absolute role of *n*-6 PUFA on breast cancer outcomes has been inconclusive. Findings from observational studies indicate no significant association, and, in some cases, an inverse association between *n*-6 PUFA intake and risk of breast cancer [18,60]. However, one study in rats using a chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumour model found that maternal exposure to a corn oil diet high in *n*-6 PUFA resulted in shorter tumour latency in the female offspring compared to offspring from mothers fed the low-fat corn oil diet [49]. This finding was later supported by a similar study using the same chemical carcinogenesis model that found a transgenerational increase in mammary tumour risk, whereby tumour latency was shorter in mice whose mothers were exposed to a high-*n*-6 PUFA diet compared to a control diet. Further, the offspring from the high-*n*-6 PUFA group exhibited altered mammary gland gene expression profiles consistent with increased cancer susceptibility (e.g., upregulation of *ID4*, *TBX2*, and *JAM3*) and impaired anticancer immune response (e.g., upregulation of *EGR3* and downregulation of *ZBP1*) [61]. These findings suggest that maternal intake of a high-*n*-6 PUFA diet during pregnancy can lead to a transgenerational increase in mammary cancer risk in mice. Interestingly, in the non-involved mammary gland, we found that *n*-3 PUFA diets significantly reduced expression of F4/80, a pan-macrophage marker, as well as CB1 and CD206, which are involved in immune signaling and M2 macrophage polarization, respectively [62,63]. These findings raise the possibility that *n*-3 PUFA may lower basal immune cell recruitment or immune activation in normal mammary tissue, aligning with their known anti-inflammatory effects and providing further support for their cancer-preventive role during early-life exposures.

4.4. Tumour Phospholipid Composition and Fatty Acid Metabolism

While high LA in corn and safflower oil diets is described as tumour-promoting, research suggests that LA may exhibit a dual effect on tumour cell growth in different cancer models [64]. Consistent with past studies [12,20,43], the LA content on a relative basis in tumour phospholipids was found to be higher in tumours from the *n*-3 PUFA diets compared to *n*-6 PUFA diets. For example, in the PC fraction, the relative LA content

was $13.8 \pm 1.0\%$ and $12.9 \pm 0.6\%$ for the corn- and safflower oil-based *n*-3 PUFA groups, respectively, compared with 10.4 ± 0.3 and 10.9 ± 0.7 for the *n*-6 PUFA 10% CO and 10% SO groups. However, the difference in LA content was statistically significant only between the corn oil-based groups.

Although the overall differences in tumour phospholipid LA content across the *n*-3 PUFA and *n*-6 PUFA groups were relatively small, the higher LA content observed in the corn oil-based *n*-3 PUFA diet within the PE and PC fractions may be partially attributed to competitive fatty acid metabolism. LA and α -linolenic acid (ALA, 18:3*n*-3) compete for the same elongation and desaturation enzymes. When dietary *n*-3 PUFA are high, less LA is converted into AA because ALA competes with LA for Δ 6-desaturase, reducing AA synthesis. Consequently, it may be possible that more LA remains unconverted and available for incorporation into phospholipids. In contrast, in the *n*-6 PUFA-fed mice, a greater proportion of LA is converted to AA, resulting in less available LA for direct incorporation into PC and PE. In the *n*-3 PUFA-fed mice, EPA and DHA inhibit AA synthesis, potentially leading to higher residual LA incorporation into phospholipids.

Interestingly, one prior study on tumour lipid metabolism in a rat sarcoma model found that tumour phospholipids tend to retain lower LA levels despite dietary abundance, likely due to rapid turnover [65]. This study showed significantly lower LA content in tumour phospholipids compared to normal muscle, suggesting either inefficient incorporation or accelerated depletion of LA in tumours. The study further demonstrated that, while dietary LA supplementation increased LA incorporation into tumour phospholipids, this effect was transient as LA was rapidly depleted when dietary LA intake was reduced [65]. Furthermore, oxidized LA metabolites (OXLAMs) are known to contribute to lipid peroxidation and cellular stress, which may influence LA retention in tumour phospholipids [45]. LA is particularly susceptible to oxidation when embedded within membrane lipids, and excessive dietary intake of *n*-6 PUFA has been linked to increased formation of these bioactive lipid peroxidation products [45]. Given the oxidative environment of tumours, it is plausible that dietary LA in high-*n*-6 PUFA groups is more readily converted to OXLAMs, reducing its presence in tumour phospholipids. This mechanism may contribute to the observed discrepancy in LA content between the *n*-3 and *n*-6 PUFA diet groups. These findings may align with our observation that tumour phospholipid LA levels were lower in high-LA (10% *n*-6 PUFA) diets compared to *n*-3 PUFA-supplemented diets despite the latter containing less LA, further highlighting the dynamic nature of tumour fatty acid metabolism. Nonetheless, given that the *n*-3 PUFA menhaden oil diets contained 7% safflower oil or corn oil, both of which are rich in LA, some level of LA incorporation was expected. However, importantly, the presence and preferential incorporation of EPA, docosapentaenoic acid (DPA, 22:5*n*-3) and DHA in tumour phospholipids suggest that *n*-3 PUFA are prioritized as a substrate, further supporting the competitive metabolic shifts induced by dietary PUFA composition. Overall, these findings highlight the potential differences between dietary and tissue LA. This pattern suggests that LA incorporation into tumour phospholipids alone does not directly reflect tumour-promoting activity but rather that tumour-promoting effects appear to be strongly influenced by metabolic context and downstream conversion pathways. These findings raise the possibility of a functional threshold beyond which additional LA incorporation into phospholipid species exerts only marginal effects on tumour development. Additionally, while only trace amounts of LA product, AA, were present in the *n*-6 PUFA diets, higher levels of AA were observed in the tumour PC and PE fractions of the *n*-6 PUFA groups compared to the *n*-3 PUFA groups, suggesting endogenous production of AA. Similar increases in AA incorporation were also observed in the PI and PS fractions across the *n*-6 PUFA diets, with a comparable but non-significant directional trend in the SM fraction.

4.5. Immune and Endocannabinoid Signaling Mechanisms

The observed tumour-suppressive effect of *n*-3 PUFA in this study may be partly attributed to immune modulation. Cannabinoid receptors CB1 and CB2 are G protein-coupled receptors involved in diverse physiological processes, including inflammation and immune signaling [66]. CB2 is predominantly expressed on immune cells, including macrophages, and has been implicated in tumour immune evasion and immunosuppressive signaling [34–36,66]. In contrast, CB1 is primarily expressed in the central nervous system but is also present in peripheral tissues such as mammary gland [66]. Previous studies have shown that CB2 overexpression in breast cancer models is associated with reduced T-cell infiltration, increased immune suppression and poor prognosis [67,68]. In contrast, CB2 knockout or inhibition has been shown to restore anti-tumour immunity and reduce tumour burden in different cancers [69]. Our findings of reduced CB2 expression in tumours from *n*-3 PUFA-fed mice align with these observations and suggest that *n*-3 PUFA may reduce CB2-mediated immune suppression. Although CB1 expression was lower in the mammary glands of the *n*-3 PUFA-fed groups, no significant changes were seen in tumours, suggesting tissue-specific regulation or potentially lower CB1 expression in tumour-resident immune cells. In this study, the *n*-3 PUFA diets were also shown to significantly increase the expression of CD86, a marker overexpressed in M1 macrophages with anti-tumour effects [70], compared to the *n*-6 PUFA diets. These overall molecular shifts suggest that *n*-3 PUFA intake may enhance anti-tumour immune activity within the tumour microenvironment, contributing to reduced tumour multiplicity and volume.

Despite some similarities in tumour outcomes between the background corn and safflower oil diets, the gene expression patterns revealed subtle differences in the immune microenvironment, particularly in the tumour tissue. Both *n*-3 PUFA-enriched diets, regardless of background source (corn or safflower oil), led to reduced CB2 expression compared to *n*-6 PUFA. However, CD86 expression was significantly higher only in the 3% FO 7% CO group compared to both *n*-6 PUFA diets (10% CO and 10% SO), suggesting that the corn oil background may allow for a more pronounced immunostimulatory response when partially substituted by *n*-3 PUFA. Although the CD86 expression was also modestly elevated in the 3% FO 7% SO group, this increase did not reach significance compared to the *n*-6 PUFA diets. This nuance underscores how not only the presence of *n*-3 PUFA but also the specific *n*-6 PUFA background may influence specific markers involved in immune-related mechanisms in tumour suppression.

CD86 was evaluated as a marker of M1-like macrophage activation due to its role as a co-stimulatory molecule that facilitates antigen presentation and T-cell activation and its strong association with classical macrophage activation [63,70]. Although CD86 is not a macrophage-specific or definitive indicator of classical activation, it is widely used to infer M1-like polarization and immune-stimulatory capacity [63,71,72]. Recent work demonstrated that physiological states associated with immune remodeling, such as lactation, promote the accumulation and activation of CD8⁺ T cells in mammary tissue, conferring long-term protection against breast cancer. These findings underscore the importance of antigen presentation and T-cell activation in shaping mammary immune surveillance [73]. Accordingly, the observed upregulation of CD86 in response to *n*-3 PUFA may reflect enhanced macrophage activation and antigen-presenting potential within the tumour microenvironment. In contrast, no significant differences were observed in the expression of M2-associated (CD206 and ARG1) markers in tumours across the diet groups. This divergence may reflect complex macrophage plasticity within the tumour microenvironment, where cells exhibit overlapping features rather than a strict binary M1/M2 phenotype.

Emerging research supports the role of *n*-3 PUFA in macrophage modulation. In vitro and in vivo studies have shown that EPA and DHA may suppress M2-associated markers, such as CD206 and ARG1, while promoting M1 polarization and enhancing anti-tumour activity in different cancer models [31–33]. Together, our findings are consistent with these reports and demonstrate tissue-specific modulation, with increased CD86 expression in tumours and reduced CD206 and F4/80 in normal mammary glands, highlighting the nuanced role of *n*-3 PUFA in shaping immune cell phenotypes in both healthy and tumour contexts.

Moreover, while the tumour phospholipid profiles reflected competitive fatty acid incorporation between *n*-3 and *n*-6 PUFA, the gene expression results suggest parallel shifts in immune cell signaling. In particular, diets with higher *n*-3 PUFA content led to preferential incorporation of EPA and DHA in phospholipids alongside downregulation of CB2 in tumours, possibly reflecting a suppression of immunoregulatory endocannabinoid signaling derived from arachidonic acid metabolism. EPA and DHA are also precursors to endocannabinoid-like molecules (e.g., DHEA and EPEA), which can act on CB1 and CB2 with anti-inflammatory effects [35,74,75]. It is therefore plausible that dietary *n*-3 PUFA alter the ligand landscape of these receptors, further contributing to immune activation and tumour suppression.

An increasing body of research has been dedicated to elucidating the mechanisms through which *n*-3 and *n*-6 PUFA exert their contrasting effects on tumour development, with *n*-3 PUFA often demonstrating anticancer properties, while *n*-6 PUFA have been implicated in promoting tumourigenesis [9,13,76–78]. While the present study did not directly examine downstream signaling or cellular localization, our findings suggest that dietary PUFA composition modulates the tumour microenvironment through both metabolic and immunological pathways. The upregulation of CD86 and downregulation of CB2 in tumours from *n*-3 PUFA-fed mice point toward enhanced immune activation and reduced immune suppression, respectively. In contrast, the downregulation of CD206 and F4/80 in the mammary gland suggests a dampening of resident macrophage populations and M2-like polarization in non-tumour tissue [63]. Together, these tissue-specific changes highlight a novel role for dietary PUFA in shaping the immune landscape of both normal and cancerous mammary tissue.

Although the present study only explored mechanisms of action relating to the expression of immune-related genes, several concurrent pathways contribute to these effects. The anticancer effects of *n*-3 PUFA, including EPA and DHA, are in part attributed to their incorporation into cellular membranes, replacing *n*-6 PUFA [79,80]. This membrane remodeling alters lipid composition, disrupting the organization of lipid rafts and modulating key tumourigenic signaling pathways, including PI3K/Akt and HER2, thereby suppressing tumour cell proliferation [12,13]. This was evident in the present study as significant increases in *n*-3 PUFA were detected within individual phospholipid fractions of mammary tumours from mice exposed to the *n*-3 PUFA diets. Furthermore, *n*-3 PUFA promote pro-apoptotic signaling in tumour cells by activating factors such as Bax, caspase-3, 8, and 9 and increasing reactive oxygen species production, ultimately leading to apoptosis [10,81–83]. The effects of *n*-3 and *n*-6 PUFA on lipid mediators and inflammation are also distinct, whereby oxylipins derived from *n*-6 PUFA (e.g., prostaglandins, leukotrienes and thromboxanes) tend to exhibit more potent pro-inflammatory and proliferative properties compared to oxylipins produced from *n*-3 PUFA [84]. The tumour-promoting effect of *n*-6 PUFA, dietary LA, and its product, AA, can also be attributed to their ability to influence inflammatory signalling pathways, increase cell proliferation and angiogenesis, and inhibit caspase-dependent apoptosis [85]. Given the complexity of PUFA metabolism, it is likely that multiple concurrent putative mechanisms contribute to the opposing effects of *n*-3 and

n-6 PUFA on tumour development, including membrane composition changes, oxylipin signaling, inflammation, oxidative stress, and apoptosis regulation. However, the goal of the present study was specifically aimed to examine how different dietary sources of *n*-6 PUFA influence tumour growth outcomes. Future research should continue to dissect these pathways to provide deeper insights into the molecular and cellular mechanisms that mediate the effects of dietary PUFA composition on tumour progression.

4.6. Strengths, Limitations, and Future Directions

This study employed a well-established MMTV-neu mouse model that mimics HER-2 overexpression, which is a significant area of clinical interest for the aggressive HER-2-driven breast cancer subtype [38]. This experimental model, along with the use of lifelong exposure to *n*-3 PUFA, offers a robust investigation of the preventative effects of dietary fatty acids on mammary tumour development, controlling for pregnancy, lactation and puberty. Further, the inclusion of corn oil in this study as a positive control diet that promotes tumour development enhances translatability to the human diet. Compared to safflower oil, corn oil more closely reflects the saturated, monounsaturated and *n*-6 PUFA profile of edible oils consumed in the Western diet [86]. Overall, this research contributes valuable insights into the distinct impacts of different sources of fatty acids on breast cancer development. However, there are limitations that should be considered when interpreting the results and designing future research. The findings from this study are based on a specific mouse model of HER-2 positive breast cancer and may not be directly generalizable to all subtypes of breast cancer or clinical populations. Since breast cancer is a heterogeneous disease, the effects of dietary factors may differ among the various subtypes. Variability also exists in the human diet; therefore, the diets used in this study, while valuable for isolating the effects of specific dietary components like PUFA, may not fully replicate the complexity of real-world dietary patterns. In addition, although the diets were calorically matched, minor differences in body weight and energy intake across the diet groups may have partially contributed to the observed differences in tumour outcomes. Also, the immune-related interpretations in this study are based on a focused panel of mRNA markers to provide directional, rather than definitive, insight into immune cell infiltration or macrophage polarization within the tumour microenvironment. Future studies should build on these findings by incorporating complementary immune phenotyping approaches, such as protein-level analyses or flow cytometry. While the study was designed to compare dietary PUFA sources and contexts, future studies could benefit from applying further interaction-focused statistical analyses, including the role of pubertal timing, to more fully delineate how *n*-3 PUFA effects are modified by the underlying *n*-6 PUFA background. Extension of this work to additional breast cancer models and to more complex dietary patterns will further clarify the translational relevance of dietary PUFA composition in breast cancer prevention.

5. Conclusions

This study demonstrates that lifelong dietary exposure to *n*-3 PUFA attenuates mammary tumour development in a HER2+ mouse model by reducing tumour volume and multiplicity over time in a diet-dependent manner. While the tumour outcomes were not uniformly different across all the dietary contexts, the *n*-3 PUFA-enriched diets resulted in lower tumour burden relative to the corn oil-based *n*-6 PUFA diet. In contrast, comparable tumour-suppressive effects were not consistently observed when safflower oil was used as the *n*-6 PUFA source, suggesting that the source of *n*-6 PUFA can modulate the magnitude of benefit associated with *n*-3 PUFA intake. Collectively, these findings suggest that, while the presence of *n*-3 PUFA plays a key role in modulating tumour develop-

ment, the underlying *n*-6 PUFA background may further shape these effects. Notably, the lack of additional tumour promotion with higher LA content in safflower oil compared with corn oil raises the possibility that high LA exposure may reach a threshold beyond which further increases exert only marginal effects on tumour development. These tumour outcomes were accompanied by alterations in macrophage-associated immune- and endocannabinoid-related gene expression, suggesting that immune modulation contributes to the observed diet-dependent effects. Together, these findings highlight the importance of dietary PUFA composition and context in shaping mammary tumour development and refine the current understanding of how interactions between *n*-3 and *n*-6 PUFA may influence breast cancer risk.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu18040606/s1>, Table S1: Primer sequences; Table S2: Body weight and food intake; Figure S1: Changes in body weight; Table S3: Fatty acid composition (%) of mammary tumour phospholipids in SM, PI and PS fractions; Figure S2: Gene expression in mammary glands; Figure S3: Gene expression in tumours.

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Abbreviations

The following abbreviations are used in this manuscript:

10% CO	10% corn oil
10% SO	10% safflower oil
3% FO 7% CO	7% corn oil plus 3% menhaden oil
3% FO 7% SO	7% safflower oil plus 3% menhaden oil
PUFA	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid

LA	Linoleic acid
AA	Arachidonic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
SM	Sphingomyelin
eEF2	Eukaryotic translation elongation factor 2
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
F4/80	Adhesion G protein-coupled receptor E1
CD206	Mannose receptor C-type 1
ARG1	Arginase 1
CD86	Cluster of differentiation 86
EGR3	Early growth response 3
ID4	DNA-binding protein inhibitor ID-4
JAM3	Junctional adhesion molecule 3
TBX2	T-box 2
ZBP1	Z-DNA binding protein 1

Appendix A

Table A1. Composition of modified AIN-93G rodent diets (Research Diets).

	10% CO (D04092704R)		10% SO (D04092701)		3% FO 7% CO (D21100508)		3% FO 7% SO (D04092703)	
Macronutrient	g%	kcal%	g%	kcal%	g%	kcal%	g%	kcal%
Protein	21	20	21	20	21	20	21	20
Carbohydrate	60	58	60	58	60	58	60	58
Fat	10	22	10	22	10	22	10	22
Total		100		100		100		100
kcal/g	4		4		4		4	
Ingredient	g	kcal	g	kcal	g	kcal	g	kcal
Casein	200	800	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12	3	12
Corn starch	337	1347	337	1347	337	1347	337	1347
Maltodextrin 10	132	528	132	528	132	528	132	528
Sucrose	100	400	100	400	100	400	100	400
Cellulose, BW200	50	0	50	0	50	0	50	0
Corn oil	97	873	0	0	68	611	0	0
Safflower oil	0	0	97	873	0	0	68	611
Menhaden oil	0	0	0	0	29	262	29	262
t-Butylhydroquinone	0.02	0	0.02	0	0.02	0	0.02	0
Mineral mix S10022	35	0	35	0	35	0	35	0
Vitamin mix V10037	10	40	10	40	10	40	10	40
Choline bitartrate	2.5	0	2.5	0	2.5	0	2.5	0
Total	966	4000	966	4000	966	4000	966	4000

Composition of modified AIN-93G diets with 10% corn oil (10% CO), 10% safflower oil (10% SO), 7% corn oil plus 3% menhaden oil (3% FO 7% CO), and 7% safflower oil plus 3% menhaden oil (3% FO 7% SO), as provided by manufacturer, Research Diets (New Brunswick, NJ, USA).

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