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Abbreviations: AA, arachidonic acid; AdA, adrenic acid; ALA, α-Linolenic acid; COX, cyclooxygenase; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; GLA, γ-linolenic acid; GLMM, generalized linear mixed model; IsoPs, isoprostanes; LA, Linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acids; LOD, limit of detection; LOX, lipoxygenase; NeuroPs, neuroprostanes; PG, prostaglandin; PLS-DA, Partial Least Squares Discriminant Analysis; Rv, resolvins; STA, Stearidonic acid; TP, thromboxane receptor; TX, thromboxane.

ABSTRACT

Introduction: Cancer has been associated with increased oxidative stress and deregulation of bioactive oxylipins derived from long-chain polyunsaturated fatty acids (LC-PUFA) like arachidonic acid (AA). There is a debate whether ω-3 LC-PUFA could promote or prevent prostate tumor growth through immune modulation and reduction of oxidative stress. Our aim was to study the association between enzymatically or non-enzymatically produced oxidized-LC-PUFA metabolites and tumor growth in an immune-competent eugonadal and castrated C57BL/6 male mice injected with TRAMP-C2 prostate tumor cells, fed with ω-3 or ω-6 LC-PUFA-rich diets.

Materials and methods: Tumor fatty acids were profiled by gas chromatography and 26 metabolites derived from either AA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were assessed by liquid chromatography-mass spectrometry.

Results: The enriched ω-3 diet did not reduce oxidative stress overall in tumors but favored the formation of ω-3 rather than ω-6 derived isoprostanoids. We discovered that EPA and its oxidized-derivatives like F_3-isoprostanes and prostaglandin (PG)$F_{3\alpha}$, were inversely correlated with tumor volume (spearman correlations and T-test, $p<0.05$). In contrast, F_2-isoprostanes, adrenic acid, docosapentaenoic acid ($DPA_{\omega-6}$) and PGE_2 were positively correlated with tumor volume. Interestingly, F_4-neuroprostanes, PGD_2, $PGF_{2\alpha}$, and thromboxane were specifically increased in TRAMP-C2 tumors of castrated mice compared to those of eugonadal mice.

Discussion: Decreasing tumor growth under ω-3 diet could be attributed in part to increased levels of EPA and its oxidized-derivatives, a reduced level of pro-angiogenic PGE_2 and increased levels of F_4-neuroprostanes and resolvins content in tumors, suspected of having anti-proliferative and anti-inflammatory effects.

Keywords: Eugonadal mice, Castrated mice, Inflammation, Oxidative stress, Isoprostanoids, Oxylipins.

1. INTRODUCTION

Effects of long chain polyunsaturated fatty acids (LC-PUFA) from omega-3 (ω-3) on prostate cancer incidence and progression is controversial. A positive association between high plasma level of LC-ω-3PUFA and prostate cancer risk have been reported in some observational studies in humans (1, 2). In contrast, fats derived marine products were indirectly associated with a lower incidence of prostate cancer in Inuit populations (3, 4). A reduction of the ratio ω-3/ω-6 LC-PUFA in the prostate has been correlated with the increased incidence of prostate cancer in comparison to a benign hyperplasia control group (5). A decrease in proliferation index of prostate malignant cells in men undergoing prostatectomy following a fish-oil supplementation was documented (6). Interestingly, the eicosapentaenoic acid (EPA), a ω-3 LC-PUFA, was a specific marker of reduced risk of progression from low-grade to high-grade prostate cancer (7, 8). The heterogenous response to ω-3PUFA in prostate cancer has been associated with gene polymorphisms of inflammatory and oxidative stress pathways (reviewed in (9)). Polymorphism in the cyclooxygenase(COX)-2, a pro-inflammatory gene involved in the production of prostanoids has been associated with aggressiveness of prostate cancer in human (10).

LC-PUFA effects were also observed in the experimental immunocompetent TRAMP-C2 mouse model of prostate cancer (11). We observed a decrease in tumor growth under an ω-3 enrich diet compared to an ω-6 enriched diet or a standard diet. Moreover, the ω-3 diet was also shown to enhance Th1-mediated cytokine response in TRAMP-C2 tumors (11). However, the impact of these ω-3 and ω-6 enriched diets on the diversity and levels of oxylipins from LC-PUFA were not thoroughly investigated in TRAMP-C2 tumors so far.

LC-PUFA like arachidonic acid (AA), EPA and docosahexaenoic acid (DHA) contained in membrane phospholipids can be directly oxidized by oxidative process in tumor yielding up to several hundred isomers of F_2-isoprostanes (F_2-IsoPs), F_3-isoPs and F_4-neuroprostanes (F_4-NeuroPs) respectively (12) (Figure 1). The 15-F_{2t}-IsoP (also named 8-iso-$PGF_{2\alpha}$), the most studied F_2-IsoPs, has been shown to be increased in urine of prostate cancer patients compared to controls (13, 14). There are also COX and lipoxygenase (LOX) enzymatic pathways that produce LC-PUFA bioactive derivatives (Figure 1). Prostaglandin (PG)E_2 derived from the COX pathway was shown to promote angiogenesis and tumor growth in many cancers (15). Activation of thromboxane (TX) pathway was associated with a higher Gleason score and pathologic stage in human prostate cancer (16). Resolvins (RvD1-6) from DHA through the LOX pathway are believed to be involved in the reduction of tumor growth and inflammation (17).

We hypothesized that oxidized LC-PUFA metabolite from EPA and DHA reduce tumor growth in contrast to AA derivatives known to be pro-inflammatory and pro-angiogenic. The aim of this study was to measure and classify the relative importance of these metabolites from a large panel of oxidized metabolites from AA, EPA and DHA in TRAMP-C2 tumors. These tumors were implanted in immunocompetent eugonadal and castrated C57BL/6 male mice exposed to either, ω-6 or ω-3 enriched diets. The impact of androgen removal, through castration, and the diet were both investigated for all detectable metabolites.

2. MATERIALS AND METHODS

2.1 Diets

Animal diets were purchased from Research Diets, Inc. (New Brunswick, NJ, USA). The AIN93G diet contained a main FA source from soybean oil (ω-3/ω-6 ratio of 0.15). The ω-6-enriched diet was the AIN93G diet modified by the addition of 10% safflower oil (w/w; final ω-3/ω-6 ratio of 0.002). The ω-3-enriched diet was the AIN93G diet modified by the addition of 1% safflower oil and 9% menhaden oil (w/w; ω-3/ω-6 ratio of 3.3). All diets were isocaloric with FA content representing 22% of daily Kcal intake which is representative of the normal North American diet as mentioned previously (11). The exact and detailed FA composition of diets were reported in supplemental data of a previous publication (11).

2.2 Mice Experiments

The immune-competent C57BL/6 mice were either fed with ω-3 or ω-6 supplemented diet. The same animals were used as previously described (11). Briefly, after 2 weeks of feeding, half of the mice were surgically castrated. After two additional weeks, 7 castrated and 7 eugonadal (non-castrated) mice were injected with 2 million TRAMP-C2 cells subcutaneously on both abdominal flanks; this was done for each of the 2 groups (ω-3 or ω-6 supplemented). After occurrence of the initial mass, tumor size was measured every other day. Mice were sacrificed when the tumor volume reached 2 cm^3 (around 40 to 42 days (11)). Tumors were collected from each mouse at the sacrifice and stored at -80˚C.

2.3 Measurement of fatty acid profiles in tumors.

FA profiles of TRAMP-C2 tumors were determined by gas chromatography coupled to flame ionization detection after extraction of total lipids as previously described (7, 11, 18).

2.4 Determination of LC-PUFA derived metabolite profiles

RvD1, 17(*R*)-RvD1, 17(*R*)-RvD1-d5, RvD2, RvD2-d5, RvD3, RvD5, RvE1, 17(*S*)-HDHA, 18-HEPE, 15-*epi*-15-F_{2t}-IsoP, 15-*epi*-15-F_{2t}-IsoP-d4, 15-F_{2t}-IsoP, 5-*trans*-$PGF_{2\alpha}$, $PGF_{2\alpha}$, $PGF_{2\alpha}$-d4, 8-F_{2t}-IsoP, 8-F_{2t}-IsoP-d4, 5-*epi*-5-F_{2t}-IsoP/5-F_{2t}-IsoP, 5-*epi*-5-F_{2t}-IsoP-d11/5-F_{2t}-IsoP-d11, 5(*RS*)-5-F_{2c}-IsoP, 5(*RS*)-5-F_{2c}-IsoP-d11, $PGF_{3\alpha}$, TXB_2, TXB_2-d4, PGE_2, PGD_2, 8-iso-PGE_2, PGE_2-d9, PGD_2-d4 were purchased from Cayman Chemical (Ann Arbor, MI, USA). The 4(*RS*)-4-F_{4t}-NeuroP, 10-F_{4t}-NeuroP-d4, 10-*epi*-10-F_{4t}-NeuroP-d4, 5-F_{3t}-IsoP, 8-F_{3t}-IsoP, 8-*epi*-8-F_{3t}-IsoP, 18-F_{3t}-IsoP, 18-*epi*-18-F_{3t}-IsoP were previously synthesized at IBMM (19-21). Methyl formate 97% was bought from Sigma-Aldrich (Oakville, ON, Canada). Sodium acetate trihydrate (ACS grade) was obtained from Laboratoire Mat (Québec, QC, Canada). N-hexane 95% was bought from Fisher Scientific (Ottawa, ON, Canada) and ethanol 99% was purchased from Commercial Alcohols (Toronto, ON, Canada). All other reagents and solvents were HPLC grades and were purchased from VWR International (Ville Mont-Royal, QC, Canada).

Mouse tumors (~35 mg) were homogenized manually using a small potter in 161 µL of water added with 10 µL of deuterated internal standard (25 ng/mL in ethanol), 7 µL of a solution containing 1% butylhydroxytoluene (BHT) and in presence of 625 µM indomethacin. Two procedures were used to either extract unbound or esterified oxylipins in homogenates. For free or unbound oxylipins (PGs or free F_x-IsoPs), potter homogenized tumors were diluted to 3 mL with 50 mM sodium acetate buffer (pH 3), centrifuged and then extracted by solid phase extraction (Strata-X, 60 mg/3cc, Phenomenex, Torrance, CA, USA) as described previously (22). For esterified F_x-isoPs, an alkaline hydrolysis was performed before solid phase extraction. Briefly, 340 µL of homogenate were incubated in 5.9% KOH (m/v) for 75 min at 37°C then, acidified with 81 µL of 5 N HCl, adjusted to 3 ml with 50 mM acetate buffer at pH 3 and centrifuged prior loading on the SPE cartridge as described previously (22). The nitrogen dried extracted samples were reconstituted in 60 µL of a solution containing 13.5% acetonitrile,

31.5% methanol and 0.01% acetic acid in water for HPLC-MS/MS determination. The reconstituted samples (40 µL) were injected to a Shimadzu Prominence HPLC (Columbia, MD, USA) coupled to a 3200 QTRAP® MS/MS from AB Sciex (Concord, ON, Canada) configured with a Turbo V^{TM} electrospray ionization probe operated in negative mode. The chromatography using a gradient of 3 solvents was carried out exactly as previously described (22) . The oxylipins were monitored in the multiple-reaction monitoring (MRM) mode using the transitions described in the Supplementary Table 1S. Acquisition was done with Analyst 1.6.2 and quantifications were performed using MultiQuant 3.0.2 software (AB Sciex).

2.5 Data analysis

Tumor growth was analyzed with a generalized linear mixed model (GLMM) using IBM SPSS statistics 26.0.0.2 for Mac OS (IBM Corp. Armonk, (NY) USA). The GENLINMIXED procedure was used with a repeated statement (days) and a covariance structure that minimize the Akaike criterion. The model was best fitted with a normal distribution using untransformed data. A fixed-intercept model for each subject appears to be the best fit. The fixed factors were castration (2 levels; Yes/No), diet (2 levels; ω-3/ω-6) and days (16 levels; 10/11/12/15/17/18/20/24 /27 /31/33/ 34/ 35/ 38/ 39 /40). Interactions between factors were also investigated.

The individual lipid-derived metabolites were first analyzed with a generalized model. The GENLIN procedure of IBM SPSS was used with log-transformed data and a gamma distribution that minimized the Akaike criterion with fixed intercept. The values below the level of detection were replaced by a small value define as the limit of detection (LOD)/2. The fixed factors were castration (2 levels; Yes/No) and diet (2 levels; ω-3/ω-6) with the interaction (type III). Then, metabolites were analyzed altogether using multivariate analyses with bioinformatics tools offered by the Metaboanalyst web site

(https://www.metaboanalyst.ca). Volcano plot, Partial Least Squares Discriminant Analysis (PLS-DA) and spearman correlations through the pattern hunter module were performed according to instructions after normalization and range scaling centering (23). A *p*-value of less than 0.05 was considered significant for all statistics.

3. RESULTS

3.1 Enrichment of specific LC-PUFA in tumors by diet

TRAMP-C2 tumor contents in α-linolenic acid (ALA), stearidonic acid (STA), eicosatetraenoic acid (ETA) and EPA were barely detectable in the ω-6 diet-fed animals in contrast to mice fed with the ω-3 diet (Table 1). The levels of DHA and docosapentaenoic acid $(DPA)_{\omega-3}$ were 10 to 36-fold higher in the tumors of the ω-3 diet exposed animals compared to those of the mice fed with the ω-6 diet. In contrast, the ω-6 diet clearly enriched tumors in dihomo-γ-linolenic acid (DGLA), linoleic acid (LA), AA, $DPA_{\omega-6}$ and adrenic acid (AdA) from 2 to 15-fold respectively when compared to the ω-3 diet. Androgens removal by castration had mostly no significant impact on the fatty acid profiles of tumors, whatever the diet used with the exception of ALA and STA (Table 1).

A volcano plot and a multivariate analysis was used to classify the most important LC-PUFA affected by ω-3 and ω-6 diets. Figure 2 indicates in order of importance that EPA, ALA, ETA, AdA and $DPA_{\omega-3}$, were the five most important FA affected by diet change (see also Table 2S). This was also confirmed by a PLS-DA analysis showing that these same five LC-PUFA were major features (Figure 3). However, $DPA_{\omega-3}$ was considered slightly more important than AdA and ALA in the latter analysis (Figure 3B).

3.2 Tumoral oxidized LC-PUFA profile is related to the LC-PUFA dietary content

Various isomers of oxidative stress biomarkers, F_2- and F_3-IsoPs, were measured in tumors. F_2-IsoPs levels were lower in tumors of ω-3-fed mice compared to those of the ω-6-fed mice (Table 2). In contrast, ω-3-derived F_3-IsoPs and F_4-NeuroPs were more highly concentrated in tumors from the ω-3 fed than the ω-6 fed mice as expected (Table 2). However, the sum of all F_2- and F_3-IsoPs in tumors were not

different between diet groups (p=0.919). Only the ratios between F_2-IsoPs and the sum of F_3-IsoPs and F_4-NeuroPs differed between the ω-3 fed and the ω-6 fed mice (p<0.001). Some F_2- and F_3-IsoPs were also affected by androgen removal following castration, especially all F_4-NeuroPs, increased by an average of 38% following this procedure (p<0.003). Overall, F_2- and F_3-IsoPs only tend to be higher following castration (p=0.078).

Table 3 also showed that ω-3-enriched diet lowered ω-6-derived oxylipins in tumors and increase ω-3-derived metabolites from LOX and COX pathways. For example, levels of TXB_2, PGD_2 and PGE_2 were lower in ω-3-fed compared to the ω-6-fed group (Table 3). Levels of EPA-derived $PGF_{3\alpha}$ and DHA-derived RvD5 were higher in ω-3-fed group compared to ω-6 (Table 3). Also, we observed that androgen removal affected the RvE precursors 18-HEPE, $PGF_{2\alpha}$, PGD_2 and TXB_2. The castration procedure increased prostaglandin content from AA in tumors by roughly 2-fold under the ω-6 diet. Of note, most of the $PGF_{2\alpha}$, a prostaglandin and a potential isomer of F_2-IsoPs was under the free form (>80%) rather than bound to phospholipids in contrast to F_2-IsoPs, as shown in Table 3. Indeed, less than 50% of F_2-IsoPs were under the free form (Table 2).

The relative importance of all these oxidized metabolites in one diet compared to the other was revealed by the volcano plot and PLS-DA analyses (Figures 2 and 3). EPA derivatives like F_3-IsoPs and $PGF_{3\alpha}$ were the most important features compared to F_4-NeuroPs and F_2-IsoPs respectively.

3.3 Tumor ω-3 LC-PUFA and oxidized derivatives are related to tumor size and growth

In contrast to the fatty acid profiles of TRAMP-C2 reported earlier in Table 1, tumor growth was affected by both, the diet and castration as illustrated in Figure 4. The effect was more significant for diet than castration (p=0.006 vs p=0.031, respectively; Table 3S). The most significant contrasts between diets were observed at days 27, 31 and 34 (Table 4S).

The 31-day time point was selected to compare TRAMP-C2 tumor volume as a measure of growth with metabolites since this time point was overall the mostly significant for diet effect in castrated and eugonadal mice (Figure 4, Table 4S). As shown in Figure 5, tumor volume was positively correlated with ω-6 PUFA such as AdA and $DPA_{\omega-6}$ and also with 5-, 8- and 15-series F_2-IsoPs derived from AA. Of note, PGE_2 was the only enzymatic metabolite positively correlated with tumor volume (p<0.05). In contrast, tumor volume was inversely correlated with ω-3 LC-PUFA like ETA, STA, $DPA_{\omega-3}$, EPA and ALA respectively. Also, EPA derivatives from non-enzymatic oxidation such as F_3-IsoPs were all strongly inversely correlated with TRAMP-C2 tumor volume in mice. In addition, $PGF_{3\alpha}$ potentially produced by the COX pathway and derived from EPA also was inversely correlated with tumor size.

4. DISCUSSION AND CONCLUSIONS

Oxylipin levels are directly influenced by FA profiles of cell membranes, which in turn are regulated by diet. Oxidized LC-PUFA metabolites produced enzymatically by COX and LOX such as PGs and resolvins can modulate tumor cell proliferation, differentiation and apoptosis through multiple signaling pathways in tumors (24, 25). Our results showed that pro-inflammatory levels of PGs of series-2 (derived from AA) (15) were higher in faster-growing tumors of mice from ω6-fed than the ω3-fed group. The PGE_2 was positively correlated with tumor size in this study. PGE_2 is one of the most abundant PGs in cells and is known to inhibit tumor-cell apoptosis and induces tumor-cell proliferation by various mechanisms (26). It was also reported that TRAMP mice treated with the COX-2 inhibitor, celecoxib, showed limited tumor development and lower PGE_2 levels than controls (27). In contrast to PGE_2, less inflammatory or anti-inflammatory metabolites such as $PGF_{3\alpha}$ and RvD5 were higher in ω-3- compared to ω-6-fed group. As expected, DHA metabolites RvD5 were mostly detected in the ω3-fed group. Resolvins were recently identified as anti-inflammatory molecules that can orchestrate the timely resolution of inflammation in many inflammatory diseases including cancer (17, 28). Our experiment in mice identified for the first time RvD5 in a solid tissue sample; its presence was reported mostly in fluids like serum and milk (29, 30). RvD5 had already been reported to reduce blood levels of bacteria during infection and decrease pro-inflammatory cytokine such as IL-1β and TNF-α (31-33). Understanding the mechanisms of relatively unexplored resolvins such as RvD5 could provide many potential therapeutic targets to address diseases associated with chronic inflammation like prostate cancer.

The ω-3 FA like EPA and DHA could play an antioxidant role in the tumor microenvironment by potentially a) reducing inflammation, an indirect source of ROS; b) increasing antioxidant response through redox signaling or c) acting as a sacrificial antioxidant through unsaturated bonds (34, 35).

Interestingly, DHA was more readily oxidized into F_4-NeuroPs in faster-growing tumors of castrated mice. Indeed, F_4-NeuroPs appeared to be the most sensitive to castration as a group compared to other F_2- and F_3-IsoPs. Thus, the sum of F_4-NeuroPs could serve as sensitive markers of oxidation associated with growth under androgens. However, some specific isomers of F_2- (5 series) and F_3-isoPs were also affected by androgen removal, as F_4-NeuroPs, and were better correlated with tumor size. Increased levels of F_4-NeuroPs could help to reduce tumor growth since a report stated that F_4-NeuroPs exert anti-proliferative effects in the human breast cancer cell line MDA-MB-231 (36). These new findings are in accordance with the effect of androgen deprivation on increasing oxidative stress in human prostate tissues (37). The positive association between the levels of certain F_2-IsoP isomers derived from AA and tumor volume could therefore be attributed in part to increased oxidative metabolism associated with tumor growth, mainly stimulated by a ω-6 diet.

Levels of F_2-IsoPs, especially the widely measured 15-F_{2t}-IsoP represent one of the most accurate and recognized ways to assess oxidative stress (38-40). Our result showed that F_2-IsoP levels to be lower in ω3-fed than the ω-6-fed group. This strongly suggests that ω-3 LC-PUFA have anti-oxidative properties as reported (34, 41) and can actually reduce oxidative stress in our tumor model. Surprisingly, F_3-IsoPs and F_4-NeuroPs were significantly higher in tumors of mice fed with ω-3- than ω-6 rich diet. This led us to believe that oxidation of LC-PUFA is directly linked with the availability of the respective intact LC-PUFA. Overall, the oxidative stress remained the same in both groups following LC-PUFA substrate availability provided by the diet. Initially, we believed that the higher degree of unsaturation of EPA and DHA than AA could play a significant role in the neutralization of ROS, which is not the case in our system.

This work also emphasized that all isomers of IsoPs are not produced at the same rate/elimination in tumors. The 5- and 8- series of F_2-IsoPs have been significantly correlated with tumor size but not the classical 15-F_{2t}-IsoP. This specificity or signature was observed in other contexts and show the importance of the determination of a wide array of isomers to characterize the oxidative stress. Indeed, we have previously reported F_2-IsoPs profile specific and predictive to hypertension in pregnancy (5-*epi*-5-F_{2t}-IsoP/5-F_{2t}-IsoP) and gestational diabetes (15-*epi*-15-F_{2t}-IsoP) (42, 43).

Increase in F_2-IsoP during tumor growth could stimulate further growth and inflammation through eicosanoids receptors. The 15-F_{2t}-IsoP isomer like TXA_2 is able to stimulate the thromboxane receptor (TP) involved in vasoconstriction and platelet activation/aggregation. Signaling through TP is complex and has been associated with prostate cancer. Indeed, it was reported that high protein expression of enzyme and receptors of the TXA_2 pathways was associated with pathologic stage and Gleason score (16). The combined increase of TXB2, a stable metabolite of TXA_2, and F_2-IsoPs enhanced by the ω-6 diet could therefore in part stimulate tumor growth and progression.

In conclusion, we have shown under a diet favoring EPA in tumors that the decreased tumor growth could be attributed in part to increased levels of EPA and its oxidized metabolites, a reduced level of pro-angiogenic PGE_2 and an increased level of F_4-NeuroPs and resolvins (RvD5) in tumors, suspected of having antiproliferative and anti-inflammatory effects. The reasons for the importance of F_3-IsoPs remains to be further investigated since their biological properties like many other isoprostanoids produced are mostly unknown (12, 44). For example, it remains to be determined if the decreased tumor size alter oxylipins or if the oxylipins are changing the tumor growth. Yet, this work also underlines the importance of broad assessment of all LC-PUFA derived isoprostanoids for the determination of oxidative stress, especially if the relative proportion of LC-PUFA varies greatly between experimental

units.

5. AKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. Metabolites from ω-6/-3 LC-PUFA enzymatic and non-enzymatic oxidation investigated in TRAMP-C2 tumors. Arachidonic acid (AA) could be provided by the diet or from the desaturation and/or elongation of linoleic acid (LA), γ-linolenic acid (GLA) and dihomo-γ-linolenic acid (DGLA). AA can be further elongated and desaturated in adrenic acid (AdA) and docosapentaenoic acid ($DPA_{\omega-6}$) respectively. The α-linolenic acid (ALA), stearidonic acid (STA) and eicosatetraenoic acid (ETA) can be desatured/elongated in eicosatetraenoic acid (ETA) that can be desatured in eicosapentaenoic acid (EPA), elongated in docosapentaenoic acid ($DPA_{\omega-3}$) and further desatured in docosahexaenoic acid (DHA). The non-enzymatic oxidation of AA, EPA and DHA leads to several isomers of F_2-isoprostanes (F_2-IsoPs), F_3-IsoPs and F_4-neuroprostanes (F_4-NeuroPs), respectively. Enzymatic oxidation of AA through the cyclooxygenase (COX) pathway leads to several prostaglandins (PG) and the thromboxane (TX). EPA through the COX pathway yields to PGF_{3a} and other PGs. EPA through the lipoxygenase pathway yields to the resolvins (Rv)Es. DHA can be metabolized by the lipoxygenase pathway in RvDs.

Figure 2. Volcano plot of the important tumor features increased or decreased by ω-3 in comparison to ω-6 fed animals. Fold change (FC) and *t*-test for false discovery thresholds were set at 2 and 0.05 respectively (n=12-13/data point). The detailed list of metabolites can be found in Table 2S.

Figure 3. Targeted lipidomic analysis of TRAMP-C2 tumors of mice exposed to ω-3 or ω-6 diets. (A) Partial Least Squares Discriminant Analysis (PLS-DA), scores plot. (B) PLS-DA Variable Importance in the Projection (VIP) significant metabolites.

Figure 4. Effect of ω-3-enriched diet versus ω-6-enriched diet on TRAMP-C2 tumor growth in

eugonadal and castrated mice. Tumor growth curves were compared using a generalized linear mixed model (GLMM), see supplemental data Table 3S and 4S for details. Castration and diet, both impact tumor growth with $p < 0.031$. Errors bars are ± standard error (SE), n=10-15 per time point.

Figure 5. Top features in tumors positively or negatively correlated with tumor volume size at the 31-day time point. All spearman rank correlations are significant $p \leq 0.05$. Derived from the pattern hunter procedure of Metaboanalyst 4.0 (see materials and methods).

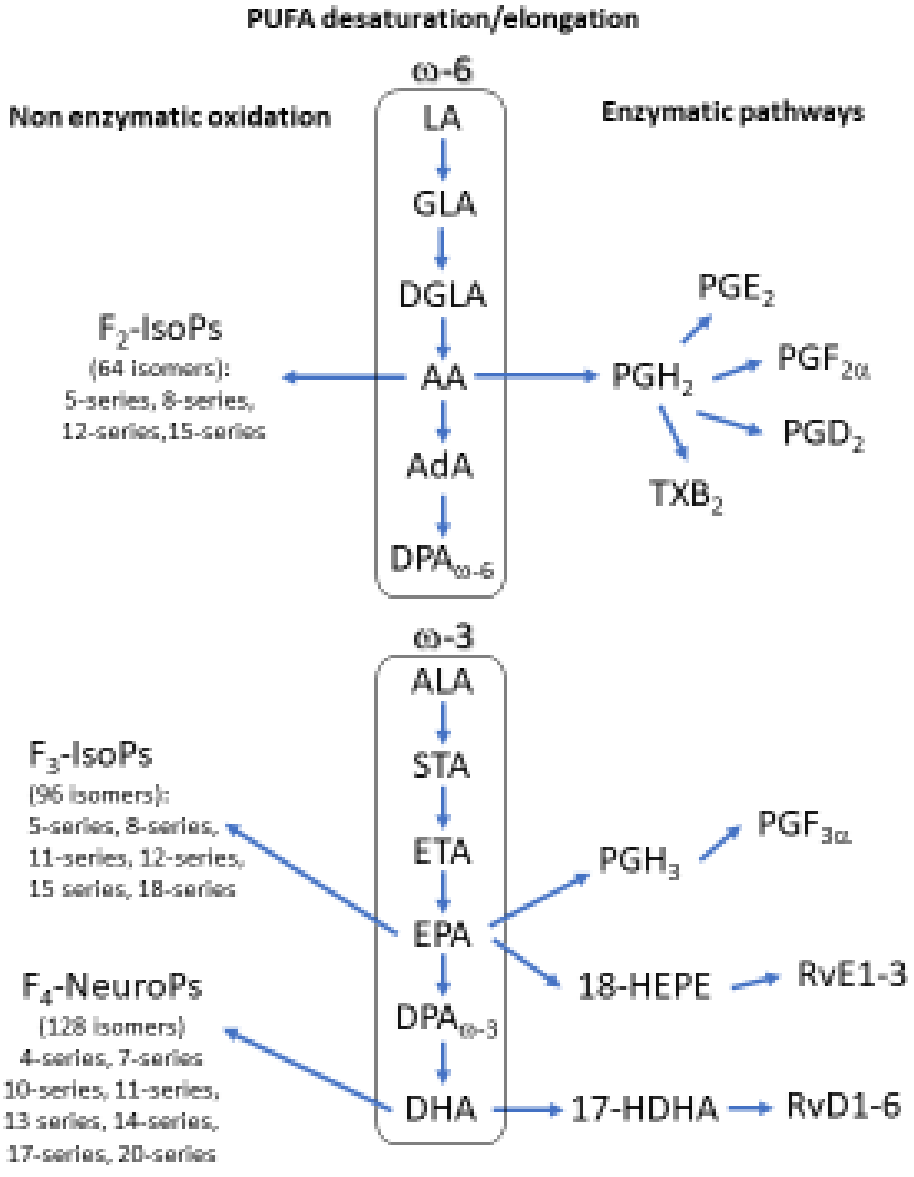
PUFA desaturation/elongation
ω-6
Non enzymatic oxidation
Enzymatic pathways
LA
GLA
DGLA
AA
AdA
DPAω-6
F2-IsoPs
(64 isomers):
5-series, 8-series,
12-series,15-series
PGE2
PGH2
PGF2α
PGD2
TXB2
ω-3
ALA
STA
ETA
EPA
DPAω-3
DHA
F3-IsoPs
(96 isomers):
5-series, 8-series,
11-series, 12-series,
15 series, 18-series
PGH3
PGF3α
18-HEPE
RvE1-3
F4-NeuroPs
(128 isomers)
4-series, 7-series
10-series, 11-series,
13 series, 14-series,
17-series, 20-series
17-HDHA
RvD1-6

Figure 1

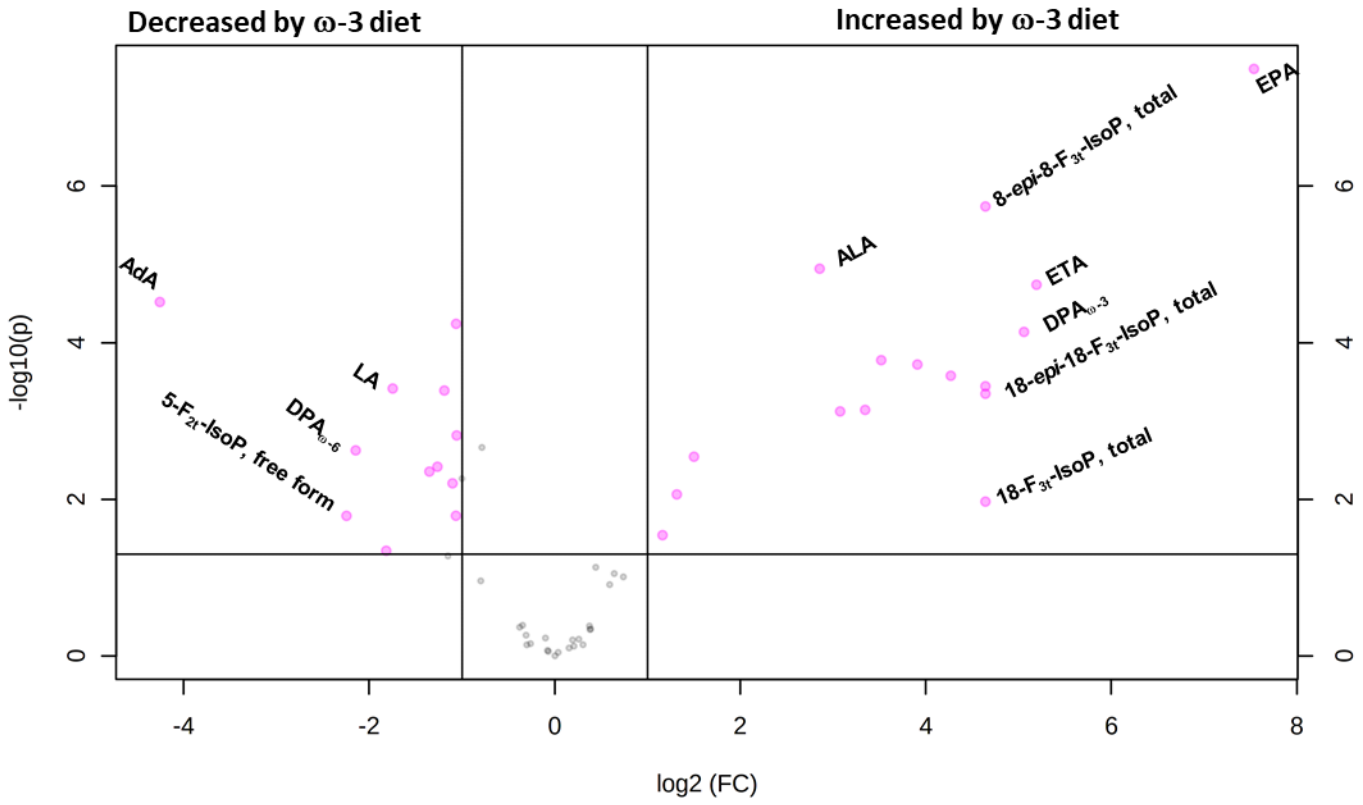
Decreased by ω-3 diet
Increased by ω-3 diet
AdA
LA
DPA ω-6
5-F2t-IsoP, free form
ALA
8-epi-8-F3t-IsoP, total
EPA
ETA
DPA ω-3
18-epi-18-F3t-IsoP, total
18-F3t-IsoP, total
-log10(p)
log2 (FC)
0
2
4
6
-4
-2
0
2
4
6
8

Figure 2

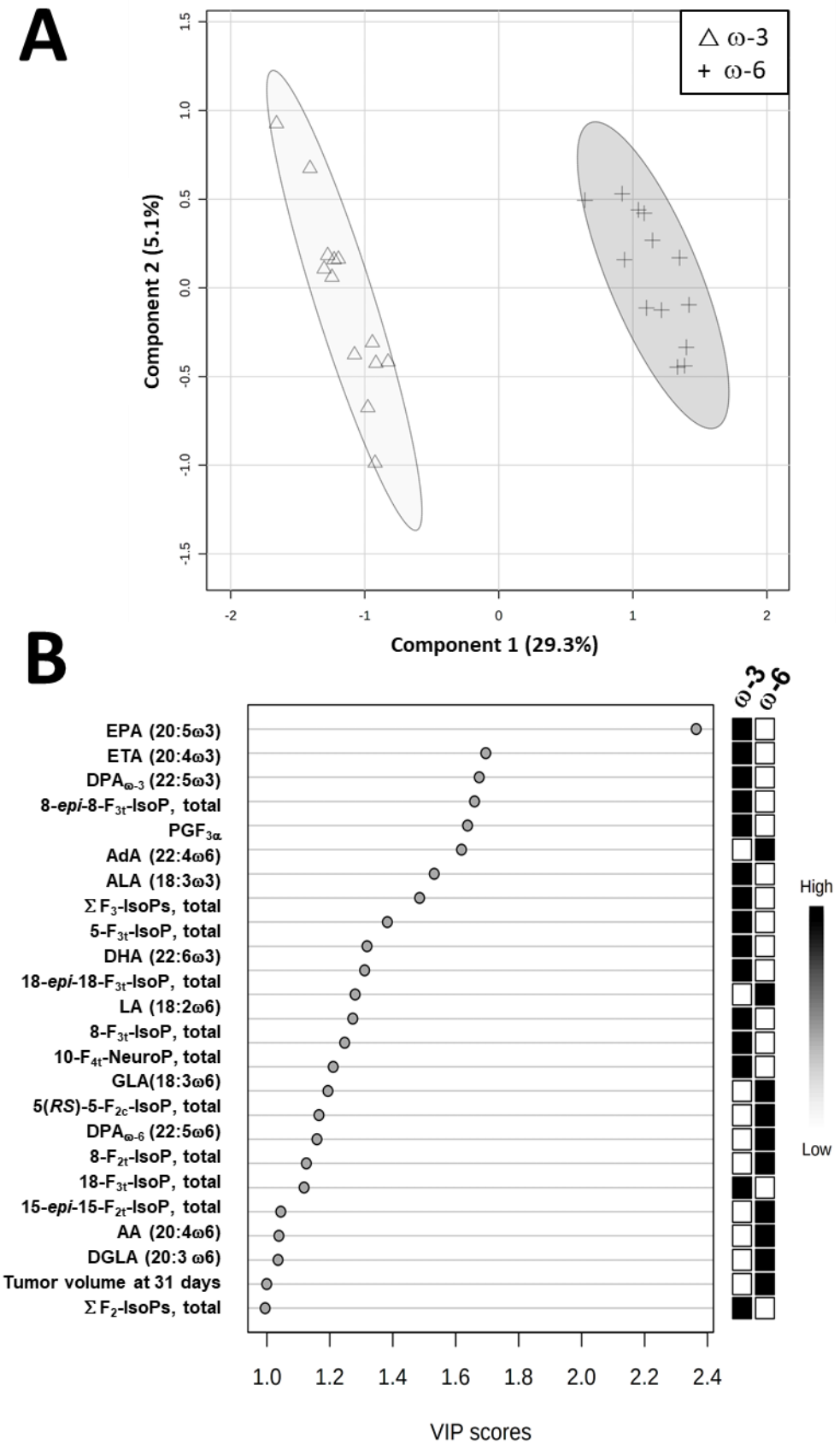
A
△ ω-3
+ ω-6
Component 2 (5.1%)
Component 1 (29.3%)
B
ω-3
ω-6
EPA (20:5ω3)
ETA (20:4ω3)
DPA$_{ω-3}$ (22:5ω3)
8-*epi*-8-F$_{3t}$-IsoP, total
PGF$_{3α}$
AdA (22:4ω6)
ALA (18:3ω3)
ΣF$_3$-IsoPs, total
5-F$_{3t}$-IsoP, total
DHA (22:6ω3)
18-*epi*-18-F$_{3t}$-IsoP, total
LA (18:2ω6)
8-F$_{3t}$-IsoP, total
10-F$_{4t}$-NeuroP, total
GLA(18:3ω6)
5(*RS*)-5-F$_{2c}$-IsoP, total
DPA$_{ω-6}$ (22:5ω6)
8-F$_{2t}$-IsoP, total
18-F$_{3t}$-IsoP, total
15-*epi*-15-F$_{2t}$-IsoP, total
AA (20:4ω6)
DGLA (20:3 ω6)
Tumor volume at 31 days
ΣF$_2$-IsoPs, total
High
Low
VIP scores

Figure 3

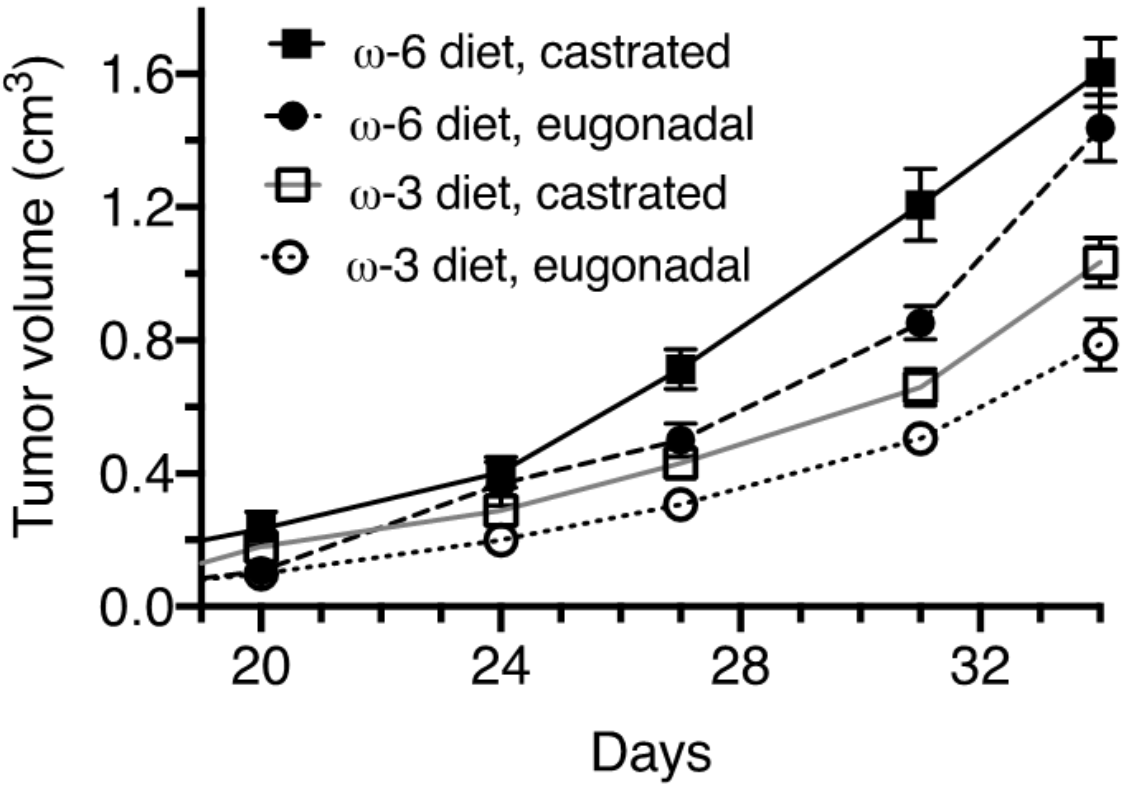
ω-6 diet, castrated
ω-6 diet, eugonadal
ω-3 diet, castrated
ω-3 diet, eugonadal
Tumor volume (cm3)
1.6
1.2
0.8
0.4
0.0
20
24
28
32
Days

Figure 4

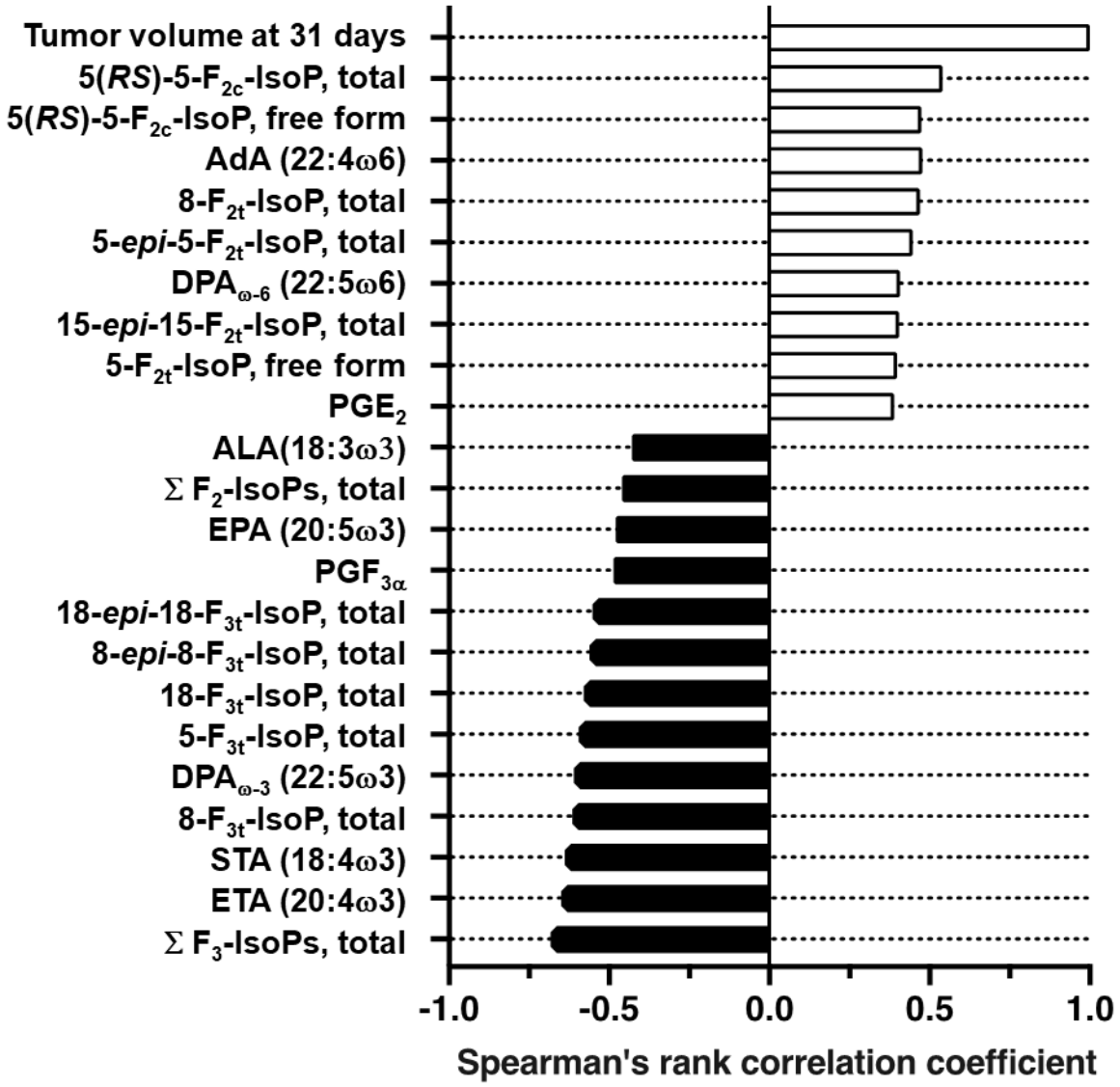
Tumor volume at 31 days
5(RS)-5-F2c-IsoP, total
5(RS)-5-F2c-IsoP, free form
AdA (22:4ω6)
8-F2t-IsoP, total
5-epi-5-F2t-IsoP, total
DPAω-6 (22:5ω6)
15-epi-15-F2t-IsoP, total
5-F2t-IsoP, free form
PGE2
ALA(18:3ω3)
Σ F2-IsoPs, total
EPA (20:5ω3)
PGF3α
18-epi-18-F3t-IsoP, total
8-epi-8-F3t-IsoP, total
18-F3t-IsoP, total
5-F3t-IsoP, total
DPAω-3 (22:5ω3)
8-F3t-IsoP, total
STA (18:4ω3)
ETA (20:4ω3)
Σ F3-IsoPs, total
-1.0
-0.5
0.0
0.5
1.0
Spearman's rank correlation coefficient

Figure 5