

# Enriched Marine Oil Supplement Increases Specific Plasma Specialized Pro-Resolving Mediators in Adults with Obesity

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## ABSTRACT

**Background:** Specialized pro-resolving mediators (SPMs), synthesized from PUFAs, resolve inflammation and return damaged tissue to homeostasis. Thus, increasing metabolites of the SPM biosynthetic pathway may have potential health benefits for select clinical populations, such as subjects with obesity who display dysregulation of SPM metabolism. However, the concentrations of SPMs and their metabolic intermediates in humans with obesity remains unclear.

**Objectives:** The primary objective of this study was to determine if a marine oil supplement increased specific metabolites of the SPM biosynthetic pathway in adults with obesity. The second objective was to determine if the supplement changed the relative abundance of key immune cell populations. Finally, given the critical role of antibodies in inflammation, we determined if ex vivo CD19 + B-cell antibody production was modified by marine oil intervention.

**Methods:** Twenty-three subjects [median age: 56 y; BMI (in kg/m<sup>2</sup>): 33.1] consumed 2 g/d of a marine oil supplement for 28–30 d. The supplement was particularly enriched with 18-hydroxyeicosapentaenoic (HEPE), 14-hydroxydocosahexaenoic acid (14-HDHA), and 17-HDHA. Blood was collected pre- and postsupplementation for plasma mass spectrometry oxylipin and fatty acid analyses, flow cytometry, and B-cell isolation. Paired *t*-tests and Wilcoxon tests were used for statistical analyses.

**Results:** Relative to preintervention, the supplement increased 6 different HEPEs and HDHAs accompanied by changes in plasma PUFAs. Resolvin E1 and docosapentaenoic acid–derived maresin 1 concentrations were increased 3.5- and 4.7-fold upon intervention, respectively. The supplement did not increase the concentration of D-series resolvins and had no effect on the abundance of immune cells. Ex vivo B-cell IgG but not IgM concentrations were lowered postsupplementation.

**Conclusions:** A marine oil supplement increased select SPMs and their metabolic intermediates in adults with obesity. Additional studies are needed to determine if increased concentrations of specific SPMs control the resolution of inflammation in humans with obesity. This trial was registered at clinicaltrials.gov as NCT04701138. *J Nutr* 2022;152:1783–1791.

**Keywords:** obesity, omega-3, polyunsaturated fatty acids, specialized pro-resolving mediators, inflammation, antibody

## Introduction

Specialized pro-resolving mediators (SPMs) are highly potent oxylipins synthesized from n-3 and n-6 PUFAs. SPMs have a critical role in resolving inflammation and returning damaged tissues to homeostasis (1, 2). SPMs are synthesized from the long chain n-3 PUFAs EPA, docosapentaenoic acid (DPA), DHA, and the n-6 PUFA arachidonic acid (AA) (3, 4). SPMs

are broadly categorized as resolvins, protectins, and maresins (Mars), as well as lipoxins derived from AA and EPA (3, 4). There is considerable interest in increasing the amounts of these metabolites in humans across a range of diseases that are associated with chronic inflammation, such as obesity. One approach toward increasing circulating concentrations of SPMs is to administer marine oils that are enriched in

metabolites of either the SPM biosynthetic pathways or their parent compounds.

There is debate on the bioavailability of SPMs in humans, particularly in response to marine oil intervention (5). This debate may stem from the analysis of differing human populations. Some laboratories have demonstrated that key circulating SPMs are not detectable in healthy humans but are present in response to inflammation. For instance, a study suggested that SPMs must be <0.1 nM in healthy individuals, as these metabolites were not detectable (6). Interestingly, SPMs were measurable in response to peritonitis or septic shock, suggesting that SPM concentrations are increased in the context of chronic inflammation (6, 7). Some studies have demonstrated that SPMs are robustly increased in healthy humans upon marine oil supplementation (8–10). Issues with SPM detection may also be related to methodological and/or analytical issues (11).

SPM bioavailability has not been effectively established in adults with obesity who are taking marine oil supplements. This population is of interest as obesity can drive deficiencies in SPMs and their metabolic intermediates (4, 12–17). Importantly, obesity and its comorbidities are associated with chronic inflammation that contributes to insulin resistance, hepatic steatosis, cardiovascular diseases, and poor responses to infections and vaccinations (4, 18–24). Notably, our research group and others have shown that SPMs and their metabolic intermediates can improve a range of obesity-related complications in mouse models (3, 12, 18, 20, 25–27). However, it remains unknown whether SPMs are sufficiently elevated in humans with obesity in response to a marine oil supplement that contains metabolites of the SPM biosynthetic pathway.

The primary objective of this study was to determine if a change from baseline in select PUFA-derived oxylipins occurs after 1 mo of supplementation in adults with obesity who are taking a marine oil supplement that contains several oxylipins but is particularly enriched in 14-hydroxydocosahexaenoic acid (14-HDHA), 17-HDHA, and 18-hydroxyeicosapentaenoic acid (HEPE). The secondary objective was to determine if 1 mo of supplementation modified the abundance of circulating peripheral blood mononuclear cells (PBMCs) from

**TABLE 1** Subject demographics at baseline<sup>1</sup>

| Characteristic            | Value             |
|---------------------------|-------------------|
| Age, y                    | 56 [52–61]        |
| Female sex, <i>n</i> (%)  | 13 (56.52)        |
| Race, <i>n</i> (%)        |                   |
| White or Caucasian        | 16 (69.57)        |
| Black or African American | 5 (21.74)         |
| Asian                     | 1 (4.35)          |
| Other                     | 1 (4.35)          |
| Weight, kg                | 96.4 [87.3–112]   |
| Height, cm                | 170.2 [160–177.8] |
| BMI                       | 33.1 [31–36]      |

<sup>1</sup>Values are medians [IQRs] or *n* (%). Total *n* data of 23 subjects are represented in the table.

baseline. The tertiary objective was to determine if 1 mo of supplementation changed antibody production compared with baseline. The rationale for focusing on antibodies was based on our previous work and the work of others, which have shown that SPM intermediates control antibody concentrations (12, 13, 24, 28). For instance, we previously demonstrated that oxylipins of the DHA-derived SPM pathway could lower circulating proinflammatory IgG2c in obese mice (13). To address these objectives, we conducted a clinical trial in which subjects with obesity consumed 2 g/d of an enriched marine oil supplement for 1 mo.

## Subjects and Methods

### Study design and subject recruitment

We conducted a nonrandomized uncontrolled clinical trial (registered at clinicaltrials.gov as NCT04701138) in adults with obesity [BMI 30–40 (in kg/m<sup>2</sup>)]. All procedures and study protocols were approved according to the University of North Carolina's Institutional Review Board (IRB) guidelines and regulations. We recruited and enrolled 24 subjects (*n* = 13 females, 11 males), with 1 male subject as a loss to follow-up. Therefore, the study consisted of 23 participants with obesity (*n* = 13 females, 10 males) aged 50–65 y. We recruited only postmenopausal females to reduce confounding effects of estrogen on lipid metabolism during supplementation. Patients were also excluded if they had diagnosed type 1 or type 2 diabetes, had consumed n-3 PUFA supplements in the 3 mo prior to enrollment, regularly consumed >2 servings/wk of fatty fish (based on self-report), had an allergy to fish/shellfish, or took any of the following medications: anticoagulants, estrogen, testosterone, nonsteroidal anti-inflammatory drugs, asthma medications, or immunosuppressant therapy. Individuals with autoimmune disease, liver disease, coagulopathy, uncontrolled hypothyroidism, and/or active malignancy were also excluded. Eligible participants were recruited from the University of North Carolina's Family Medicine Center in Chapel Hill. Written informed consent was obtained from every subject. The baseline characteristics of the participants are shown in Table 1. The median age was 56 y, 56.5% of subjects were female, and approximately two-thirds identified as white or Caucasian (69.57%). At baseline, the median BMI was 33.1. The study from IRB approval through collecting blood samples from the last enrolled subject lasted 4 mo.

All participants received the marine oil supplement (SPM Active) provided by Metagenics. Participants were advised to take 4 capsules once daily of SPM Active for 4 wk (28–30 d). Two capsules contained 1 g active fractionated marine lipid concentrate that contains 14-HDHA, 17-HDHA, and 18-HEPE. The oxylipin and fatty acid composition of SPM Active has been previously described (8). We confirmed that these metabolites, in addition to several other oxylipins, were present in the capsules (Supplemental Table 1). Based on our analyses, the supplement provided 76.12 μg of 14-HDHA, 32.41 μg of 17-HDHA, and 100.96

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Supplemental Tables 1–2 and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/jn/>.

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Abbreviations used: AA, arachidonic acid; BH, Benjamini-Hochberg; DPA, docosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; LOX, lipoxygenase; MaR, maresin; PBMC, peripheral blood mononuclear cell; PG, prostaglandin; RvE1, resolvin E1; SPM, specialized pro-resolving mediator

$\mu\text{g}$  of 18-HEPE per capsule for a total daily dose of 837.96  $\mu\text{g}$  of 14-HDHA + 17-HDHA + 18-HEPE. We also measured the concentrations of the major fatty acids in the capsules (Supplemental Table 2).

The study required 2 face-to-face visits and 2 phone contacts. Subject compliance was assessed via phone by the study coordinator. Participants were required to fast for 8 h prior to each visit. Fasting blood samples were drawn by the laboratory staff at the clinic from which patients were recruited. The first visit consisted of a height and weight measurement, followed by a baseline blood draw (presupplementation), and the second face-to-face visit concluded the study with a second blood draw (postsupplementation). Each subject took their last dose of the supplement the morning of their second blood draw visit. We monitored subject health status via phone contact at the end of the second and third week of the 4-wk supplementation period. No subjects reported adverse events or changes in health status related to the supplement. During each phone contact, subjects were asked health questions related to COVID-19 to ensure they had not contracted the coronavirus. In addition, none of the subjects received the COVID-19 vaccine during supplementation. Any subjects who did receive the COVID-19 vaccine prior to supplementation were enrolled at least 4 wk after their completed vaccine doses.

### Plasma lipidomics

A blood sample from each subject was collected into a heparin-coated plasma tube and then centrifuged at  $900 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The upper plasma layer was then collected and frozen at  $-80^{\circ}\text{C}$  for lipidomic analysis by the Lipidomics Core Facility of Wayne State University following previously published protocols (10, 29–31). Metabolites that were under the detection limit for  $>80\%$  of the samples were not imputed and were excluded from the analysis. All other missing metabolite values were imputed with 0.0001 ng/mL. Quantitation limits were  $\sim 0.0005$  ng on the column. Variation in the internal standard quantitation method was typically 3–5% CV. All samples for lipidomics, including the fatty acid analyses described below, were processed and analyzed as one batch to avoid methodological issues.

### Plasma fatty acid analyses

Plasma fatty acids were extracted and analyzed using a Perkin Elmer 680/600S GC-MS as previously described (32).

### PBMC isolation and sample handling

PBMCs were separated using StemCell SepMate PBMC Isolation tubes (catalog no. 86450) with a gradient separation using the StemCell Lymphoprep solution (catalog no. 07851) followed by centrifugation (1200g for 10 min at  $23^{\circ}\text{C}$ ). The top layer of plasma was discarded without disturbing the buffy coat layer containing the PBMCs. The PBMC cell layer was collected and washed with sterile fluorescence-activated cell sorting buffer (2% fetal bovine serum in 1X PBS). Next, the PBMCs were treated with ammonium-chloride-potassium lysis buffer to remove any residual RBC contamination. The PBMCs were then transferred to cryogenic vials for storage. When thawing PBMCs, the protocol described in the Accucell package insert was utilized. After thawing, samples were placed in the incubator for a 16-h rest period prior to any experiments.

### PBMC flow cytometry analysis

All PBMCs were thawed and rested for 16 h prior to any flow cytometry analyses. Following the rest, each PBMC sample was split in half for staining with panel 1 and panel 2 fluorophores. Panel 1 samples were stained with the following fluorophore-tagged antibodies obtained from either BioLegend or BD: Zombie Fixable UV, CD19 (BUV737), CD38 (BV421), CD27 (APC), HLA-DR (FITC), CD23 (PE-Cy7), CD24 (PE), CD3 (BV570), CD56 (BV650), CD16 (PerCP-Cy5.5), and CD14 (APC-Cy7). Panel 2 samples were stained with the following fluorophore-tagged antibodies obtained from BioLegend: Zombie Fixable UV, CD3 (BV570), CD4 (APC-Cy7), CD8 (PerCP-Cy5.5), HLA-DR (FITC), and CD56 (BV711). The BD brilliant stain buffer (catalog no. 563794) was used in all staining steps to prevent interactions between the various brilliant violet dyes. Samples were fixed with 1% paraformaldehyde

for 12 h overnight at  $4^{\circ}\text{C}$ . The following PBMC subsets were adapted from the optimized multicolor immunofluorescence panels (OMIP-024 and OMIP-069) and analyzed using a BD LSRII flow cytometer: CD3-CD19+ (B cells), CD19 + CD38 + CD27 + HLA-DR+ (plasmablasts and plasma cells), CD19 + CD38-CD27-CD23+ (follicular B cells), CD19 + CD24hiCD38+ (B-regulatory cells), CD14 + CD16-HLA-DR+ (classical monocytes), CD14 + CD16 + HLA-DR+ (intermediate monocytes), CD14dimCD16 + HLA-DR+ (nonclassical monocytes), NK cell subsets (CD3-CD56hiCD16-, CD3-CD56dimCD16-, CD3-CD56hiCD16+, CD3-CD56dimCD16+, CD3-CD56-CD16+), CD3 + CD4+ (CD4 + T cells), CD3 + CD8+ (CD8 + T cells), CD3 + CD56+ (NKT cells), CD3 + CD4 + HLA-DR+ (activated CD4 + T cells), CD3 + CD8 + HLA-DR+ (activated CD8 + T cells), CD3 + CD4 + HLA-DR- (nonactivated CD4 + T cells), and CD3 + CD8 + HLA-DR- (nonactivated CD8 + T cells) (33, 34). All data were analyzed in FlowJo and R v.4.0.3. Gates were drawn from fluorescence minus one control.

### B-cell isolation/expansion

The Miltenyi Biotec's human B-Cell isolation kit II (catalog no. 130-091-151) was used for B cell isolation from PBMCs. Cell counts were performed following isolation and samples were plated in 12-well, flat-bottom plates (Genesee Scientific catalog no. 25-106) at a density of  $0.3 \times 10^6$  cells per well. For each subject, there were 4 wells: pre-supplement stimulated, pre-supplement unstimulated, post-supplement stimulated, and post-supplement unstimulated. Stimulated wells were treated with 16  $\mu\text{L}$  CD40L and 0.4  $\mu\text{L}$  IL4 (modeling T-dependent activation of B cells) as described in the Miltenyi Biotec human B-cell expansion kit (catalog no. 130-106-196) protocol. In addition, each subject's serum (100  $\mu\text{L}$ ) was combined with StemMACS media (1.9 mL), and then added to the stimulants for each sample well. Unstimulated wells were only plated with StemMACS expansion media and the subject's serum. Media exchanges were performed on day 7 and day 10. Cell supernatants were collected on day 14 and stored at  $-80^{\circ}\text{C}$  for ELISA analysis.

### ELISA analysis

Abcam's Human IgG ELISA kit (ab195215) and Human IgM ELISA kit (ab137982) were utilized to measure antibody concentrations from the supernatants of unstimulated and stimulated B cells. For the IgG ELISA, stimulated samples were diluted 1:100,000 and unstimulated samples were diluted 1:10. For the IgM ELISA, stimulated samples were diluted 1:100,000, and unstimulated samples were diluted 1:10,000. Plates were read at 450 nm using an automated plate reader (BMG Labtech FLUOstar Omega).

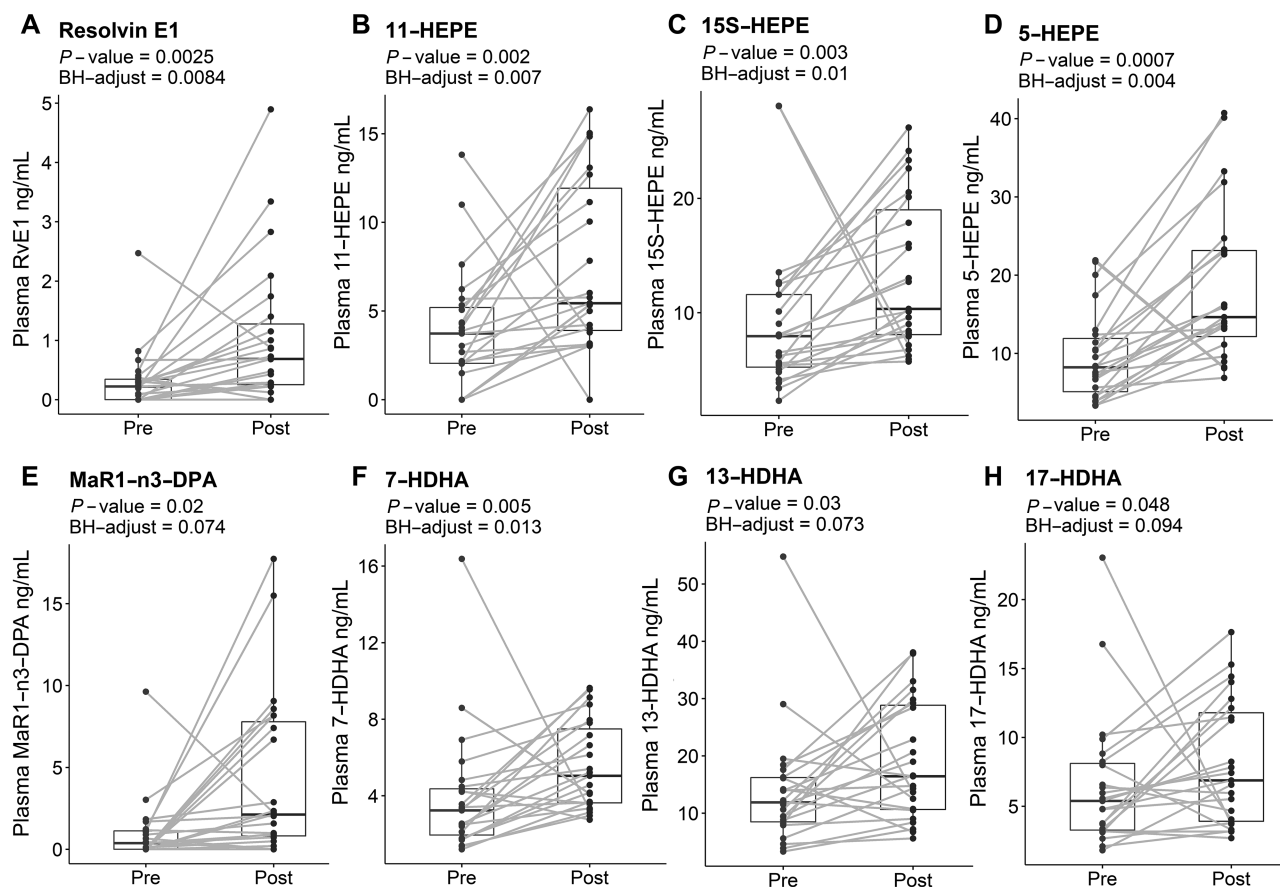
### Statistics

#### Statistical analyses.

All data were analyzed in R v.4.0.3. First, each dataset and supplementation time point (pre/post) was assessed for normality and heteroscedasticity via the Shapiro-Wilks test and Bartlett test, respectively. If the assumptions of normality and heteroscedasticity were met, a paired 2-sided Student *t*-test was performed between the pre- and postsupplementation participants. If the assumption of heteroscedasticity failed, a paired 2-sided Welch *t*-test was performed. Lastly, if the data failed both assumptions of normality and heteroscedasticity, a paired Wilcoxon test was performed. All outcomes in all the datasets underwent a Benjamini-Hochberg (BH) *P*-value adjustment with a false discovery rate of 10% to adjust for type 1 errors from multiple hypothesis testing. A BH-adjusted *P* value  $< 0.1$  was considered significant. Subject ID (SPM-16) was removed from all analyses due to loss to follow-up. Sample ID (SPM-21) was removed from all B-cell isolation and stimulation studies (ELISA data) due to PBMC cell death upon sample freezing. In addition, a Spearman correlation analysis was performed between baseline BMI and each fatty acid.

#### Sample size justification.

The primary aim of the study was powered to measure an increase in 18-HEPE, 17-HDHA, and 14-HDHA. Changes in remaining metabolites



**FIGURE 1** Marine oil supplement increases EPA-, DPA- and DHA-derived metabolites in adults with obesity. EPA-derived metabolites: RvE1 (A), 11-HEPE (B), 15S-HEPE (C), and 5-HEPE (D). DPA-derived: MaR1-n3-DPA (E). DHA-derived metabolites: 7-HDHA (F), 13-HDHA (G), and 17-HDHA (H);  $n = 23$  for all metabolites. *P* values and BH-adjusted *P* values are denoted underneath the header of each metabolite. Dots represent individual pre- and postsupplementation values, and the box plot represents median and IQR limits. DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; MaR1, maresin 1; SPM, specialized pro-resolving mediator.

were exploratory. At a family-wise error rate of 5% with a 2-sided alpha and an effect size of 1.4 controlling for testing of the 3 metabolites mentioned above,  $n = 9$  provided more than 80% power to detect a 50%-fold change increase in metabolite concentrations within each subject given a paired 2-sided *t*-test. We assumed a similar effect size for both males and females and doubled the sample size ( $n = 18$ , 9 per sex). To account for possible loss to follow-up, we increased the sample size from 18 to 24 ( $n = 12$  male + 12 female). Power analyses were conducted assuming a paired *t*-test on pre- and posttreatment metabolite concentrations for the 3 prespecified metabolites. Expected variability of within-subject differences in metabolite concentrations was assessed from a previous dataset in which the median and SD were calculated across all metabolites (35).

## Results

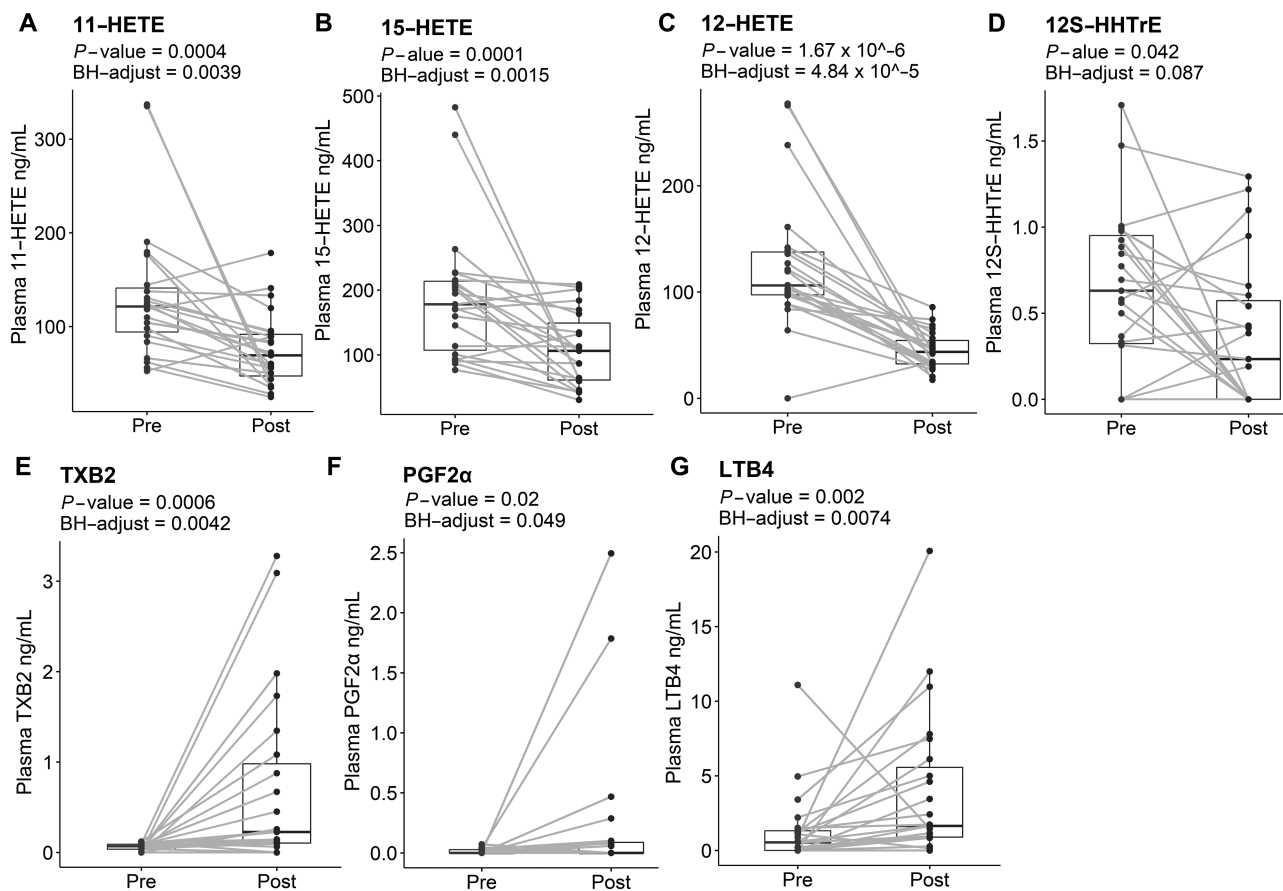
### Marine oil supplementation increases plasma EPA-, DPA-, and DHA-derived metabolites

MS-based plasma lipidomic analyses (Figure 1, Supplemental Figure 1) revealed a significant increase in EPA-derived metabolites such as resolvin E1 (RvE1; BH  $P = 0.008$ ) with a 3.5-fold increase (Figure 1A), 11-hydroxy-EPA (11-HEPE, BH  $P = 0.007$ ) with a 2-fold increase (Figure 1B), 15S-hydroxy-EPA (15S-HEPE, BH  $P = 0.01$ ) with a 1.5-fold increase (Figure 1C), and 5-hydroxy-EPA (5-HEPE, BH  $P = 0.004$ ) with a 2-fold increase (Figure 1D) in response to supplementation relative

to baseline. In addition, DPA- and DHA-derived mediators were also significantly elevated postsupplementation relative to baseline, such as DPA-derived MaR1 (MaR1-n3-DPA; BH  $P = 0.074$ ) with a 4.7-fold increase (Figure 1E), 7-hydroxy-DHA (7-HDHA, BH  $P = 0.013$ ) with a 1.5-fold increase (Figure 1F), 13-hydroxy-DHA (13-HDHA, BH  $P = 0.073$ ) with a 1.4-fold increase (Figure 1G), and 17-hydroxy-DHA (17-HDHA, BH  $P = 0.094$ ) with a 1.2-fold increase (Figure 1H). EPA-, DPA-, and DHA-derived metabolites that were measured in the samples but were not statistically significant between pre/postsupplementation included 12-hydroxy-EPA (12-HEPE), 18-hydroxy-EPA (18-HEPE), 5S,6R-dihydroxy eicosatetraenoic acid [5(S),6(R)-DiHETE], 5S,15S-dihydroxy EPA [5(S),15(S)-DiHEPE], 14-hydroxy DHA (14-HDHA), 4-hydroxy DHA (4-HDHA), DPA-derived resolvin D5 (RvD5-n3-DPA), resolvin D1 (RvD1), and resolvin D2 (RvD2) (Supplemental Figure 2).

### Marine oil supplementation exerts differential effects on plasma AA-derived metabolites

Subjects had some decreased plasma AA-derived oxylipins postsupplementation relative to baseline. Those included hydroxyeicosatetraenoic acid (HETE) 11-HETE (BH  $P = 0.0039$ ) with a 1.7-fold decrease (Figure 2A), 15-HETE (BH  $P = 0.0015$ ) with a 1.7-fold decrease (Figure 2B), 12-HETE (BH  $P = 4.84 \times 10^{-5}$ ) with a 3-fold decrease (Figure 2C), and 12S-hydroxy



**FIGURE 2** Marine oil supplement differentially influences AA-derived metabolites in adults with obesity. AA-derived metabolites: 11-HETE (A), 15-HETE (B), 12-HETE (C), 12S-HHTrE (D), TXB2 (E), PGF2 $\alpha$  (F), and LTB4 (G);  $n = 23$  for all metabolites.  $P$  values and BH-adjusted  $P$  values are denoted underneath the header of each metabolite. Dots represent individual pre- and postsupplementation values, and the box plot represents median and IQR limits. AA, arachidonic acid; HETE, hydroxyeicosatetraenoic acid; LTB4, leukotriene B4; PGF2 $\alpha$ , prostaglandin F2 $\alpha$ ; SPM, specialized pro-resolving mediator; TXB2, thromboxane B2; 12S-HHTrE, 12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid.

heptadecatrienoic acid (12S-HHTrE) (BH  $P = 0.087$ ) with a 1.9-fold decrease (Figure 2D) compared with presupplementation. We also found significantly upregulated AA-derived mediators postsupplementation, such as thromboxane B2 (TXB2, BH  $P = 0.0042$ ) with a 10.7-fold increase (Figure 2E), prostaglandin F2 $\alpha$  (PGF2 $\alpha$ , BH  $P = 0.049$ ) with a 17.7 increase (Figure 2F), and leukotriene B4 (LTB4, BH  $P = 0.0074$ ) with a 2.8-fold increase (Figure 2G). AA-derived metabolites that were measured in the samples but were not statistically significant between pre- and postsupplementation included 5S,12S-dihydroxy eicosatetraenoic acid [5(S),12(S)-DiHETE], prostaglandin E2 (PGE2), PGD2, lipoxin A4 (LXA4), and 5-HETE (Supplemental Figure 3).

### Plasma fatty acid analyses

Plasma fatty acid analyses revealed changes in response to the supplement when comparing postsupplementation to baseline (Figure 3). Myristic (14:0) (Figure 3A) and palmitic (16:0) (Figure 3A) acid concentrations were slightly increased by 1.2- and 1.1-fold, respectively, upon supplementation. AA concentrations were decreased by 0.9-fold (Figure 3C) upon supplementation relative to baseline. EPA (20:5) concentrations were increased upon supplementation by 2.2-fold compared with preintervention (Figure 3D). DTA (22:4n-6) concentrations (Figure 3E) were decreased by 0.7-fold upon supplementation relative to baseline. The concentration of DHA (Figure 3F) was increased by 1.6-fold compared with baseline. DPA (22:5n-3)

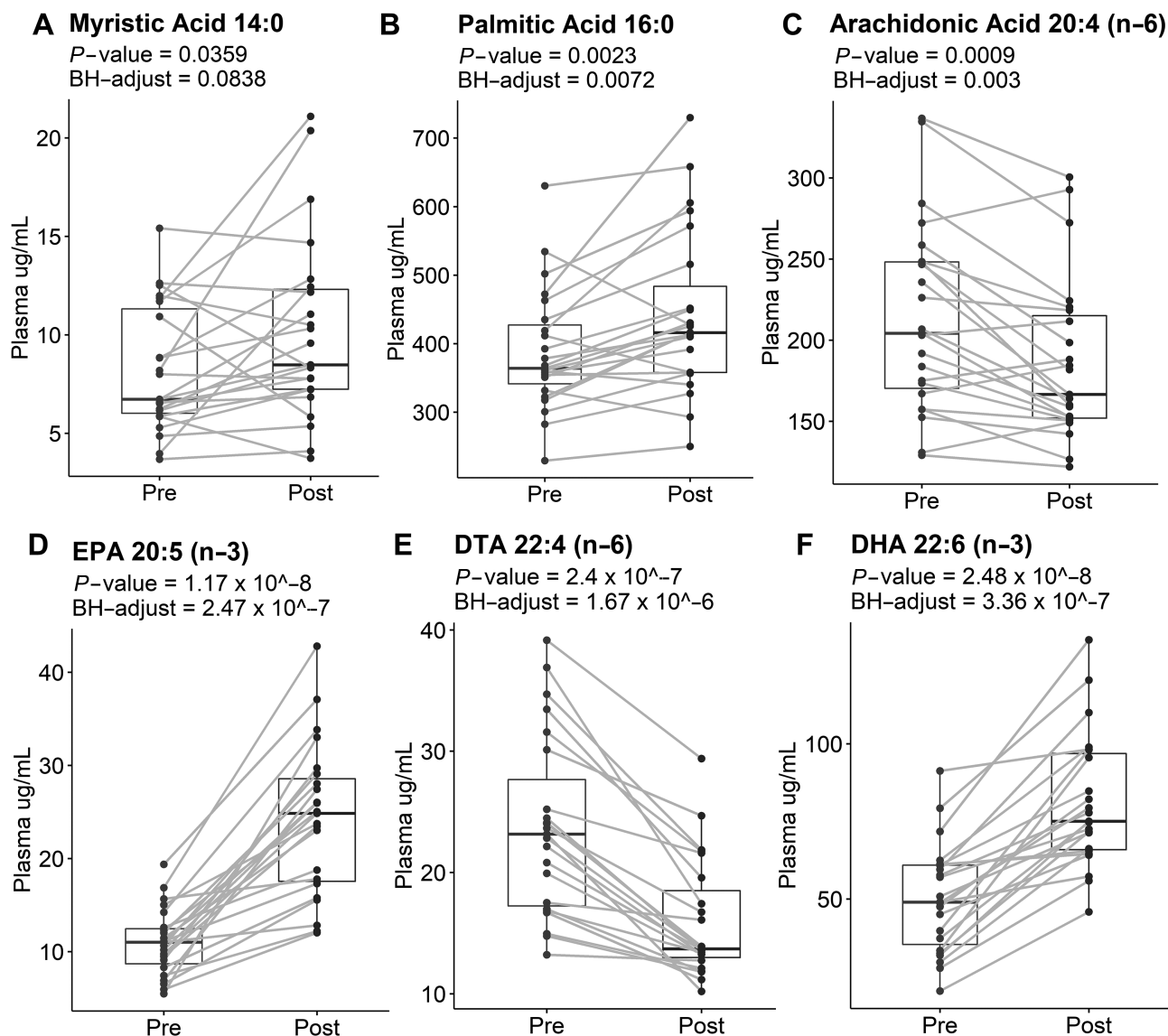
concentrations were not modified in response to the supplement (data not shown). In addition, we found no correlation with the concentration of these fatty acids with baseline BMI (data not shown).

### Flow cytometry analyses reveal no differences in abundance of resting PBMCs pre- and postsupplementation

We conducted a flow cytometry analysis of the resting PBMCs pre- and postsupplementation to determine if marine oil supplementation could impact the abundance of key immune cell populations in circulation, as in the gating strategies shown in panel 1 (Supplemental Figure 4A) and panel 2 (Supplemental Figure 4B). When analyzing both the absolute counts and frequencies of these cell populations, we found no significant differences between pre- and postsupplementation (Supplemental Figures 4C and D).

### IgG but not IgM antibody concentrations decrease upon activation

Finally, we assessed whether marine oil supplementation affects antibody concentrations. The rationale was driven by previous work from our laboratory and others to show that SPMs and their metabolic intermediates control antibody concentrations (12, 13, 24, 36). Unstimulated (Figure 4A) and stimulated (Figure 4B) B cells did not produce significantly differing amounts of IgM pre- compared with postsupplementation.



**FIGURE 3** Marine oil supplement increases the concentration of EPA and DHA while lowering AA in adults with obesity. Plasma concentration of 14:0 (A), 16:0 (B), 20:4(n=6) (C), 20:5(n=3) (D), 22:4(n=6) (E), and 22:6(n=3) (F);  $n = 23$  for all fatty acids.  $P$  values and BH-adjusted  $P$  values are denoted for each fatty acid. Dots represent individual pre- and postsupplementation values, and the box plot represents median and IQR limits. AA, arachidonic acid; DHA, docosahexaenoic acid; SPM, specialized pro-resolving mediator.

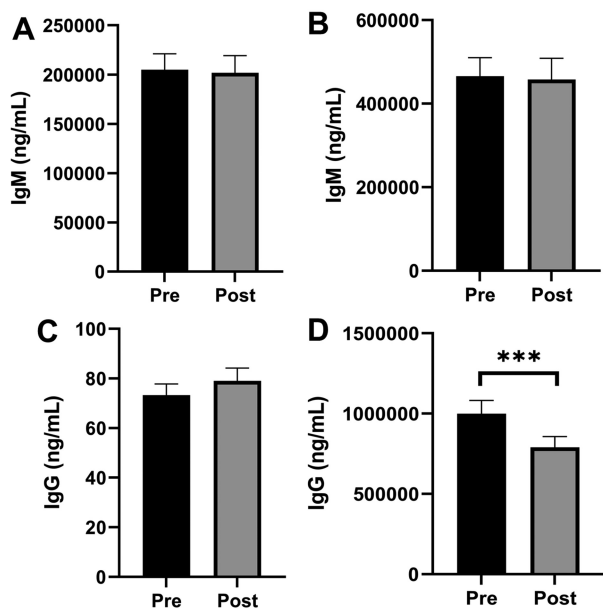
Similarly, unstimulated B cells did not produce significantly different amounts of IgG (Figure 4C). However, stimulated B cells produced significantly lower concentrations of IgG postsupplementation (Figure 4D), with  $P = 0.003$  and BH-adjusted  $P = 0.01$ .

## Discussion

Adults with obesity present with a dysregulated SPM signature that may contribute to chronic low-grade inflammation and thereby a wide range of health complications (25). The impaired SPM signature in adults with obesity is mirrored in preclinical studies. Several laboratories have reported that mice that were obese in response to a high-fat diet or genetically obese show significantly lower concentrations of SPMs or their metabolic intermediates across differing tissues than lean mice (4, 12–17). Therefore, increasing the concentration of circulating SPMs

and their metabolic intermediates with specific dietary or pharmacological supplements may have a therapeutic role in preventing or treating conditions associated with obesity. In this study related to obesity, we investigated the plasma lipidomic signature in response to a marine oil supplement, which was particularly enriched in 14-HDHA, 17-HDHA, and 18-HEPE.

We found that the marine oil supplement significantly increased some oxylipins of the SPM biosynthetic pathway. Notably, RvE1 concentrations were increased 3.5-fold. RvE1 is of interest as it has an emerging role in controlling glucose homeostasis in mice (17, 37, 38). In addition, high RvE1 concentrations are associated with plaque regression in coronary artery disease patients and exerts protective effects in murine models of atherosclerosis (7, 17, 39–41). Interestingly, we did not find an increase in the concentration of D-series resolvins upon intervention, although several DHA-derived metabolites were increased. This could be due to an inability of adults with obesity to generate D-series resolvins. Previous work



**FIGURE 4** Ex vivo IgG but not IgM concentrations are decreased postsupplementation with a marine oil supplement in adults with obesity. Unstimulated (A) and stimulated B-cell IgM production (B). Unstimulated (C) and stimulated (D) B-cell IgG production. *P* value is denoted by \*\*\* < 0.005. Bar plots are presented as mean ± SEM; *n* = 22.

demonstrated that n-3 PUFA supplementation with aspirin led an increase in EPA-derived SPMs but not DHA-derived SPMs in subjects with the metabolic syndrome (42). There is also evidence that adults with morbid obesity have impaired 15-lipoxygenase (LOX) activity, a reduction in 5-LOX expression, and decreased 5-LOX serine-271 phosphorylation (25). There may be a possibility that a higher dose of the marine oil supplement or a longer intervention time could lead to increased concentrations of D-series resolvins and other oxylipins of the SPM family.

The supplement, despite being enriched in 14-HDHA, 17-HDHA, and 18-HEPE, only led to an increase in 17-HDHA concentrations upon supplementation, relative to baseline, but had no effect on the concentration of 14-HDHA or 18-HEPE. The oxylipin 18-HEPE may have converted to RvE1, which was significantly elevated with supplementation as described above. Thus, it is possible that a higher dose of 14-HDHA, 17-HDHA, and 18-HEPE may be needed to elevate these oxylipins in adults with obesity. Interestingly, the supplement contained some HETEs, which were lowered in response to the marine oil supplement. Furthermore, since several other n-3 PUFA-derived oxylipins besides 14-HDHA, 17-HDHA, and 18-HEPE were increased upon supplementation relative to baseline, it is possible that the elevation in oxylipins may be a direct consequence of the other oxylipins that were also in the supplement (8). In addition, EPA, DHA, and other fatty acids in the supplement may also contribute to changes in oxylipins.

The supplement decreased some HETEs, which are synthesized from AA. There is a need to further investigate how oxylipins of the SPM family and their parent compounds decrease HETE concentrations, as these molecules may have an important role in the pathogenesis of obesity. For instance, 20-HETE interferes with insulin signaling in mouse models

of obesity (43). In humans, increasing concentrations of 5-, 11-, and 15-HETE are positively associated with BMI and serum leptin (44). We also observed a significant increase in several AA-derived metabolites. The results with AA-derived metabolites were generally consistent with a previous clinical trial testing the same supplement on PUFA-derived metabolites in healthy subjects (8). In this previous study, the same marine oil supplement increased LTB<sub>4</sub> concentrations (8). It is interesting that the supplement lowered plasma concentrations of arachidonic acid, even though the supplement contained a small amount of AA. We acknowledge that other fatty acids and oxylipins in the marine oil supplement could also impact the concentration of SPMs and their metabolic intermediates.

We observed an increase in TXB<sub>2</sub> and PGF<sub>2</sub>α in response to the marine oil supplement. The underlying mechanism by which a marine oil supplement would increase the concentration of these eicosanoids is unclear. The changes in these eicosanoids could be linked to the effects of select drugs (45). There is considerable variability in the literature on how marine oil supplements may influence differing AA-derived oxylipins, which warrants further investigation. For instance, fish oil supplementation can increase TXB<sub>2</sub> and PGF<sub>2</sub>α, whereas other studies show fish oil can lower some of these eicosanoids in either circulation or upon LPS stimulation of PBMCs, whereas other studies suggest no effects of fish oil on some of these metabolites (46–48).

A notable outcome, albeit highly exploratory, was that ex vivo B-cell stimulation led to a decrease in IgG concentrations in culture upon intervention. This may mimic the resolution phase of the stimulation response, by which antibody production is lowered to the homeostatic state. The reduction in antibody concentrations was consistent with the literature reporting that some SPM pathway markers can lower the concentration of antibodies, whereas others increase antibody concentrations (49). We previously reported that a cocktail of metabolites including 17-HDHA, which was modestly elevated with the subjects in this study, could lower the concentration of circulating IgG<sub>2</sub>c concentrations in obese mice (13). IgG<sub>2</sub>c is of interest since it is proinflammatory and contributes toward insulin resistance (50). We have also previously reported that a fish oil concentrate could lower IgM but not IgG concentrations upon ex vivo activation for 3 d with a mixture of agonists to stimulate the Toll-like receptor 9 agonist and B-cell receptor (35). Therefore, future studies will need to discriminate how differing PUFA-derived metabolites, which are either increased or decreased with marine oil supplementation, may manipulate antibody concentrations using a wide range of stimulation protocols that target different signaling pathways.

There are several limitations to this study. We did not use a placebo control and thereby did not conduct a randomized clinical trial. Thus, there is potential for selection bias as this was not a randomized study (51). Also, we were not powered to discriminate between females and males. There are likely sex differences in oxylipins within the population with obesity. Previous studies show strong sex differences in the concentration of PUFA-derived metabolites, including SPMs in mice and humans (13, 52–54). The study did not test for the concentration of metabolites of the SPM biosynthetic pathway in subjects that are younger with obesity, which may be a good population to intervene with to prevent some of the later complications of obesity. Furthermore, the study design did not allow us to entirely blind the samples; therefore,

future studies should be designed as double-blind randomized placebo-controlled clinical trials. Another limitation is that we were not powered to study inflammatory biomarkers in circulation, which would be an area for future investigation. Subsequent studies incorporating adults who are not obese will be an important comparison to establish whether any increases in these metabolites in adults with obesity with intervention rescues metabolite concentrations to amounts similar to those adults without obesity.

This study did not account for heterogeneity in the population with obesity, which may explain why not every individual had the same set of detectable oxylipins and the same response to the intervention (55). Heterogeneity may be driven by differences in microbiome profiles, food intake, baseline metabolic status, and ethnic differences (55). These measures should be considered in future trials. As an example, food recalls or FFQs can be used to account for differences in food intake between subjects and for each subject during the course of the intervention. Moreover, we did not account for host genetics as there are well-identified polymorphisms in the biosynthetic pathways for differing oxylipins, including SPMs (4). A recent study, which underscores this point, demonstrated that the *APOE4* genotype controls the concentration of specific oxylipins (56).

In summary, the findings from this study show that 17-HDHA, but not 14-HDHA or 18-HEPE, are increased in response to supplementation relative to baseline. In addition, some SPMs such as RvE1 and other oxylipins, notably HEPEs, are significantly increased in adults with obesity upon intervention with a marine oil supplement that is particularly enriched with 14-HDHA, 17-HDHA, and 18-HEPE. We also observed that many SPMs were not increased, particularly D-series resolvins, whereas other select DHA-derived metabolites were increased upon intervention. In addition, AA-derived metabolites were both increased and decreased with the marine oil supplement. Collectively, these results provide a framework for future studies on the use of a marine oil supplement to examine the effects of how SPMs and their metabolic intermediates control varying aspects of inflammation and immunity, including antibody concentrations, in subjects with obesity.

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## Data Availability

Data described in the manuscript and analytic code will be made publicly and freely available without restriction at <https://github.com/abrar-alshaer>

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