



# Impact of rs174537 on Critically Ill Patients with Acute Lung Injury: A Secondary Analysis of the OMEGA Randomized Clinical Trial

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## ABSTRACT

**Background:** Nutrition in the intensive care unit is vital for patient care; however, immunomodulatory diets rich in PUFAs like  $\gamma$ -linolenic acid (GLA), EPA, and DHA remain controversial for patients with acute respiratory distress syndrome. We postulate that genetic variants impacting PUFA metabolism contribute to mixed responses to PUFA-rich diets.

**Objectives:** In this study, we aimed to test the effects of single nucleotide polymorphism (SNP) rs174537 on differential responses to PUFA-rich diets.

**Methods:** We performed a secondary analysis of the OMEGA trial (NCT00609180) where 129 subjects received placebo control diets and 143 received omega-oil. DNA was extracted from buffy coats and used to genotype rs174537; plasma was used to quantitate PUFAs. We tested for SNP–diet interactions on PUFA concentrations, inflammatory biomarkers, and patient outcomes.

**Results:** We observed that all individuals receiving omega-oil displayed significantly higher concentrations of GLA, EPA, and DHA (all  $P < 0.0001$ ), but they did not vary by genotype at rs174537. Statistically significant SNP–diet interactions were observed on circulating DHA concentrations in African Americans. Specifically, African American T-allele carriers on placebo illustrated elevated DHA concentrations. Additionally, all individuals receiving omega-oil had higher concentrations of EPA-derived urinary F3-isoprostane (Caucasians:  $P = 0.0011$ ; African Americans:  $P = 0.0002$ ). Despite these findings, we did not detect any significant SNP–diet interactions on pulmonary functional metrics, clinical outcomes, and mortality.

**Conclusions:** This study highlights the importance of genetic and racial contributions to PUFA metabolism and inflammation. In particular, rs174537 had a significant impact on circulating DHA and urinary isoprostane concentrations. Given our relatively small sample size, further investigations in larger multiethnic cohorts are needed to evaluate the impact of rs174537 on fatty acid metabolism and downstream inflammation. *Curr Dev Nutr* 2020;4:nzaa147.

**Keywords:** PUFA, FADS, rs174537, ARDS, omega-3, GLA, EPA, DHA, SNP–diet interactions, OMEGA trial

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Data described in this manuscript will be made available upon request.

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Supplemental Table 1 and Supplemental Figures 1 and 2 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/cdn/>.

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Abbreviations used: ARA, arachidonic acid; ARDS, acute respiratory distress syndrome; BioLINCC, Biological Specimen and Data Repository Information Coordinating Center; DGLA, dihomogamma-linolenic acid; DPA, docosapentaenoic acid; ELOVL, elongation of very-long-chain fatty acids; F2-IsoP, F2 series isoprostane; F3-IsoP, F3 series isoprostane; FADS, fatty acid desaturase; G, major allele at rs174537; GLA, gamma-linolenic acid; ICU, intensive care unit; LD, linkage disequilibrium; LTE4, leukotriene E4; SNP, single nucleotide polymorphism; T, minor allele at rs174537; VENT, ventilator; WFBMC, Wake Forest Baptist Medical Center; 5-HETE, 5-hydroxyeicosatetraenoic acid.

## Introduction

Acute respiratory distress syndrome (ARDS) is an acute, diffuse, inflammatory lung injury, associated with increased pulmonary vascular permeability, increased lung weight, loss of aerated lung tissue, and

significantly reduced lung compliance (1). It is also commonly referred to as acute lung injury. Globally, ARDS continues to plague hospitals and intensive care units (ICUs), ultimately contributing  $\leq 40\%$  of in-hospital mortalities (2). ARDS patients not only have poor respiratory function, but also tend to have higher concentrations of markers

for coagulation and epithelial/endothelial cell injury (3), as well as higher incidences of sepsis (4), demonstrating the inflammatory nature of this syndrome. Although mechanical ventilation remains the primary treatment of choice in ARDS patients, immunomodulatory diets have emerged as a secondary or adjuvant treatment for critically ill ARDS patients (5).

Immunomodulatory dietary blends were first proposed in 1985, with the key active ingredients being  $\omega$ -3 PUFAs such as EPA (6) and DHA (7). These PUFAs have been shown to exert anti-inflammatory effects by producing various specialized proresolving lipid mediators. In addition,  $\omega$ -6 PUFAs such as  $\gamma$ -linolenic acid (GLA) and arachidonic acid (ARA) serve as precursors to the production of various eicosanoids, leukotrienes, and isoprostanes (8, 9). As a result, companies like Abbott Nutrition and Nestlé created a suite of immunomodulatory diets for critically ill patients, such as: IMPACT (Nestlé), Vital (Abbott Nutrition), Pivot (Abbott Nutrition), and Oxepa (Abbott Nutrition), to name a few. The PUFA content of these commercially available diets is provided in **Supplemental Table 1**. Over the past 25 years, these diets have been used by hospitals and tested in several clinical trials (10–15).

For example, blunt force trauma patients receiving IMPACT experienced a decrease in major organ failure with no differences in ventilator-free days (VENT-free days), length of stay in the ICU, or length of stay in the hospital (10). In contrast, ARDS patients given Oxepa, a similar formulation enriched in  $\omega$ -3 fatty acids, experienced a significant reduction in the number of VENT-free and ICU-free days, suggesting a greater need for mechanical ventilation and time in the ICU (11, 12). Other clinical trials employing these commercially available dietary blends have reported no differences in survival in ARDS patients, but report a significant reduction in mortality in septic patients (13, 14). In the OMEGA trial, ARDS patients receiving a twice daily enteral supplementation of the Oxepa diet containing GLA, EPA, and DHA had 3 fewer VENT-free days and a 10% increase in 60-day mortality compared with those receiving the control diet (15). Consequently, the trial ended early, at its first interim analysis time point, and has contributed to the current controversy over the use of PUFA-enriched dietary blends in ARDS patients. Current nutritional guidelines and recommendations for critically ill ARDS patients remain heterogeneous and suboptimal (16–18). Therefore, there is a need to better understand which patients could benefit from these adjuvant dietary blends. Such an understanding will likely be borne from deeper understanding of the complex roles  $\omega$ -3 and  $\omega$ -6 fatty acids play during the acute phases of inflammation as well as appreciation of the genetic influences that govern PUFA metabolism.

The biosynthesis and metabolism of long-chain PUFAs occurs predominantly in the liver and is largely regulated by enzymes encoded by the fatty acid desaturase (*FADS*) and elongase (elongation of very-long-chain fatty acids; *ELOVL*) genes. Essential dietary PUFAs  $\alpha$ -linolenic and linoleic acids undergo multiple desaturation and elongation steps to form long-chain  $\omega$ -3 and  $\omega$ -6 PUFAs (i.e., DHA and ARA, respectively), with the desaturation steps often being the rate-limiting steps. Historically, it was thought that all individuals had similar metabolic capacities with respect to fatty acid metabolism and synthesis. However, in light of genome-wide association studies, several genetic variants near and within the *FADS* gene cluster have been identified and shown to be associated with differential metabolic capacities (19). Most notably, the

single nucleotide polymorphism (SNP) rs174537, which is located within an ~30-kb haplotype block near *FADS1*, accounts for nearly 20% of the variation in ARA concentrations and is strongly associated with *FADS1* enzymatic activity (20, 21). Further, the allele frequency of this SNP has been shown to exhibit large variations by ancestry and geographical location. Compared with Caucasian Americans, African Americans have higher frequencies of the major allele (G) at rs174537, which is associated with more efficient metabolic conversion of  $\omega$ -6 fatty acids than minor allele (T) carriers (22, 23).

Although the biochemical phenotypes of SNPs like rs174537 include differential capacities to metabolize PUFAs, this variant has a considerable impact on downstream inflammatory processes and diseases. Individuals homozygous for the major allele at rs174537 (i.e., GG) have been shown to possess an increased capacity to synthesize more proinflammatory eicosanoids like 5-hydroxyeicosatetraenoic acid (5-HETE), leukotriene B<sub>4</sub>, and 8-epi-PGF<sub>2</sub> $\alpha$  (24, 25). In addition, rs174537 has been associated with eczema (26), asthma (27), cardiovascular disease, and other inflammatory-related diseases (28). The next logical question is whether manipulation of dietary PUFA intake, in a genotype-dependent manner, could be leveraged to improve patient outcomes. This type of study has not yet been applied to patient populations. However, an SNP–diet interaction has been demonstrated in healthy subjects consuming daily omega-oil supplements in a double-blind, randomized, placebo-controlled crossover study (29).

Given the growing body of compelling data pointing to the importance of a genetic governance of fatty acid metabolism, we hypothesized that SNP rs174537 would contribute to the differential response to PUFA-rich diets and potentially influence patient outcomes. To test this hypothesis, we leveraged on the OMEGA randomized control trial, which evaluated the effect of twice-daily enteral supplementation of fatty acids GLA, EPA, DHA, and antioxidants on VENT-free days in patients with acute lung injury. The randomized control trial was terminated at the first interim analysis due to no evidence of improvement in the primary end point of VENT-free days or other clinical outcomes in patients with acute lung injury and there was some indication that the diet could be imposing harm (15). The primary objective of this study was to evaluate the impact of rs174537 on the response to PUFA-rich diets within the OMEGA trial and to assess SNP–diet interactions on downstream inflammatory pathways and patient outcomes.

## Methods

### Study population

The OMEGA study was a randomized, double-blind, placebo-controlled, multicenter trial conducted from January 2, 2008 through February 21, 2009 (NCT00609180). This study was funded by the National Heart, Lung, and Blood Institute and led by the Acute Respiratory Distress Syndrome Network. A total of 272 adults, aged between 18 and 89 y, who were within 48 h of developing ARDS and/or acute lung injury and required mechanical ventilation, were enrolled in the OMEGA trial. The complete study design for the trial is described in detail elsewhere (15). Briefly, patients were randomly allocated to receive either a twice-daily enteral supplementation of the omega-oil diet (Oxepa; Abbott Nutrition) containing GLA, EPA, DHA, plus antioxidants ( $n = 143$ ) or an

isocaloric-isovolemic carbohydrate-rich control/placebo diet ( $n = 129$ ). Plasma and urine samples were collected at 0, 3, 6, and 12 d postrandomization. All deidentified patient samples were frozen at  $-70^{\circ}\text{C}$  for further analysis and were provided by the Biological Specimen and Data Repository Information Coordinating Center (BioLINCC) through an approved material transfer agreement. Individual measurements for patient demographics, vital signs, pulmonary functional metrics, and clinical outcomes were also deidentified and compiled into a single database maintained by BioLINCC. This secondary analysis was approved both by BioLINCC and the Wake Forest Baptist Medical Center (WFBMC) Institutional Review Board (IRB#00035109). Biospecimens and other study data were received from BioLINCC for analyses at WFBMC for the purpose of this secondary analysis.

This secondary analysis of the OMEGA trial was performed on a total of 214 subjects, 116 randomly assigned to the omega-oil diet and 98 to the controlled placebo diet. We had to exclude 58 subjects from the original OMEGA trial due to missing DNA samples and/or missing race information. A flow chart illustrating the participants in each group of our secondary analysis is provided in **Supplemental Figure 1**.

### Supplement content and administration

The total energy contents of the omega-oil (480 kcal) and placebo control (474 kcal) diets were comparable, whereas the total protein (3.8 g compared with 20 g) and carbohydrate (4.2 g compared with 51.8 g) contents were much lower in the OMEGA diet. Additional amounts of EPA (6.84 g), DHA (3.40 g), and GLA (5.92 g) were supplemented in the omega-oil diet. Patients consumed (enterally) either the control diet or the omega-oil dietary supplement in the form of 4 oz (120 cc) servings every 12 h starting at the time of randomization, that is, 8 oz (240 cc) daily.

### Genotyping at rs174537

Banked buffy coats ( $n = 240$ ) from the OMEGA clinical trial were used as the source of DNA. Isolation of DNA was performed using a modified salting-out precipitation method commonly used for the purification of DNA (Gentra Puregene Blood Kit; Qiagen). Isolated DNA was subsequently used to identify genotype at rs174537. Sequencing was performed using an ABI 7500 real-time PCR machine. Hardy-Weinberg equilibrium was confirmed by calculating expected allele frequencies stratified by race. It should be noted that only self-reported race from the patient demographics was available.

### Fatty acid quantification

Fatty acid methyl esters were extracted from banked OMEGA trial plasma samples ( $n = 181$ ) collected at 0, 3, and 6 d postrandomization. GC with flame ionization detection was used to quantify the fatty acid methyl esters, as previously described (30). Briefly, total fatty acids were prepared in duplicate plasma samples of 100  $\mu\text{L}$  and then saponified from complex lipids and converted to methyl esters in the presence of 100  $\mu\text{g}$  triheptadecanoic internal standard (TAG of C17:0; Nu-Chek Prep). Fatty acids were analyzed on an Agilent J&W DB-23 column (30 m  $\times$  0.25 mm; film thickness = 0.25 mm) using an HP 7890 GC-FID (Agilent Technologies, Inc.). Accounting for  $\sim 99\%$  of the total fatty acid content, an average of 26 peaks were routinely identified in the plasma samples. Fatty acid data are presented as the percentage of total fatty acids in the samples.

### Urinary biomarkers

Urine was collected at days 0 and 6 postrandomization in 144 subjects from the original OMEGA trial. Urinary series 4 leukotrienes were previously quantified under the original study design, using ultraperformance LC followed by electrospray ionization-MS. F-series isoprostanes were quantified by using thin-layer chromatography followed by GC-MS. These urinary inflammatory biomarkers were normalized to creatinine concentrations (15).

### Statistical analyses

Baseline characteristics are summarized using median and IQR for continuous variables like VENT-free and ICU-free days. Percentages and counts are used to summarize categorical variables like primary cause of lung injury. To estimate the effects of rs174537 genotype on patient responses to the omega-oil diet, we first ran a mixed linear model containing an SNP-diet interaction term. If the SNP-diet interaction was not statistically significant, we then performed a simple mixed linear model without the SNP-diet interaction term to assess the main effects of diet and genotype. Both models were stratified by race, adjusted for age and gender, and had an autoregressive correlation matrix to account for repeated measures. In this study, we selected an autoregressive correlation matrix because it is a commonly used correlation structure applied to time series and repeated measures modeling (31). A dominant genotype model (i.e., 0 = homozygous major allele "GG"; 1 = heterozygous and homozygous minor allele carriers "GT/TT") was also used in both models. Variables were either square root or log transformed to meet the linear regression assumptions of normality.

To estimate the effects of genotype and diet on patient outcomes such as VENT-free and ICU-free days, a Tobit regression model was used. Mortality at 30, 60, and 90 d was evaluated using a Cox proportional hazard regression model. All statistical analyses were stratified by race, adjusted for age and gender, and were considered statistically significant if  $P < 0.005$ . The reason for this lower  $P$  value is to account for multiple comparisons we made between the SNP and 5 PUFAs (i.e., GLA, EPA, DHA, ARA, and DPA) and 3 urinary markers of inflammation [F2-isoprostane (F2-IsoP), F3-isoprostane (F3-IsoP), and leukotriene E4 (LTE4)] included in our analysis. We employed a Bonferroni correction such that a  $P$  value of 0.005 was deemed statistically significant. All statistical analyses were performed in SAS (v9.4; SAS Institute, Inc.).

## Results

### Baseline characteristics of study participants

We successfully genotyped 240 subjects from the OMEGA trial; 174 were Caucasian, 40 were African Americans, and 26 were other races. Given our primary goal was to investigate SNP-diet interactions, we had to stratify our analyses by race and therefore were limited to the Caucasian and African American subsets. The observed allele frequency of the SNP was consistent with previous reports (22, 23) and met Hardy-Weinberg Equilibrium. Approximately 45% of Caucasians and 80% of African Americans were homozygous with the major allele G. The distribution of genotype by race is reported in **Table 1**.

Patient demographics and baseline study measurements are summarized by genotype and race in **Table 2**. Study participants were

**TABLE 1** Genotype at rs174537 from patients enrolled in the OMEGA trial<sup>1</sup>

Genotype at rs174537 (n = 214)	Caucasian Americans (n = 174)	African Americans (n = 40)
GG (52%)	45% (79)	80% (32)
GT (39%)	44% (77)	18% (7)
TT (9%)	11% (18)	2% (1)
p-value	0.157	0.158

<sup>1</sup>Distribution of homozygous major allele carriers (GG), heterozygotes (GT), and homozygous minor allele carriers (TT) are displayed as percentages and raw counts are provided in parentheses. Genotype counts are consistent with Hardy–Weinberg equilibrium. Fisher exact test was used to test for differences between allele frequencies in each race.

mostly Caucasian and accounted for 81% of patients included in the study. Pneumonia and sepsis were the two most common causes of lung injury. There were no statistically significant differences in patient demographics and baseline measurements based on genotype at rs174537 or diet groups. This is an important finding because the original trial reported significant differences in VENT-free days between diet groups, when you consider the entire study cohort. Indeed, we were able to reproduce this effect. However, when you repeat the analysis stratified by race, this effect is no longer observed suggesting that there are key racial differences in the response to omega-oil diet.

### Effects of diet and genotype on circulating fatty acid concentrations

We observed that all individuals who were randomly assigned to the omega-oil diet displayed significantly higher circulating concentrations of GLA, EPA, and DHA at days 3 and 6 (all  $P < 0.0001$ ) (Figure 1A–E). This was to be expected because the omega-oil diet was enriched with these 3 fatty acids. We then tested for SNP–diet interactions impacting GLA, EPA, and DHA and found that African Americans carrying the T allele (i.e., GT/TT) in the placebo group had high baseline DHA concentrations that were comparable to the OMEGA oil diet group. In fact, we detected a statistically significant SNP–diet interaction on DHA concentrations in African Americans (Figure 1F;  $P = 0.002$ ). This appears to be due to inherently higher DHA concentrations at day 0 prior to any dietary supplementation from the OMEGA trial.

Next, we focused on the metabolized long-chain fatty acids derived from GLA and EPA, respectively. GLA is metabolized first to dihomogamma-linolenic acid (DGLA), which can give rise to anti-inflammatory bioactive lipids, and then to ARA, the precursor of proinflammatory bioactive lipids. Because there was no evidence of statistically significant SNP–diet interactions on DGLA and ARA, we tested for the main effects of rs174537 and diet impacting circulating concentrations of these PUFAs. Using a mixed linear model stratified by race and adjusting for age and gender, we observed that Caucasian GGs had slightly higher concentrations of ARA compared with GT/TTs at all time points ( $P = 0.039$ ) (Supplemental Figure 2). However, after adjustment for multiple comparisons, this observation was no longer considered statistically significant. We subsequently calculated the ARA/DGLA ratio, which is often used as a surrogate marker for FADS1 enzymatic activity, and observed that Caucasian GGs had consistently higher ARA/DGLA ratios

at baseline ( $P = 0.0011$ ), day 3 ( $P = 0.0011$ ), and day 6 ( $P = 0.0042$ ) (Figure 2A–C). Though not statistically significant, this trend was similar in African American GGs, particularly if they received the omega-oil diet (Figure 2D–F). We believe that the smaller sample size of African Americans and the high variability in GGs on omega-oil on day 3 is contributing to the nonsignificant finding.

On the omega-3 side, EPA is metabolized into long-chain DPA and DHA. Individuals receiving the omega-oil diet displayed significantly increased concentrations of DPA on days 3 and 6 ( $P < 0.0001$ ), likely as a result of metabolized EPA from the diet (Figure 3). This pattern was consistent in both Caucasian and African American patients. Given that DHA was also supplemented in the diet, we were unable to tease out the contributions from exogenous DHA compared with endogenously produced DHA.

### Effects of diet and genotype on urinary inflammatory biomarkers

To test for both interactive and main effects of rs174537 and the omega-oil diet on urinary markers of inflammation, a mixed linear model stratified by race and adjusting for age and gender was used. Differences in concentrations of inflammation were considered statistically significant if  $P < 0.005$ , after adjusting for multiple comparisons.

Urinary F2-IsoP, an ARA-derived prostaglandin-like compound, serves as a marker of inflammation and oxidative stress. In Caucasians, F2-IsoP concentrations did not change significantly over time, between genotype or diet groups (Figure 4A). In contrast, African American GGs on omega-oil had significantly higher concentrations of F2-IsoP compared with GT/TTs ( $P = 0.0329$ ; Figure 4D). After our strict Bonferroni adjustment, however, this SNP–diet interaction was no longer considered statistically significant. Larger studies are needed to evaluate this potential interaction.

F3-IsoP is an EPA-derived compound that serves as an inflammation-resolving metabolite that competes with and/or modulates the actions of F2-IsoP and other proinflammatory molecules derived from major  $\omega$ -6 fatty acids. All individuals on the omega-oil diet, regardless of race, had significantly higher concentrations of urinary F3-IsoP compared with those on placebo (Caucasians:  $P = 0.0011$ ; Figure 4B; African Americans:  $P = 0.0002$ ; Figure 4E). Genotype at rs174537 additionally had an effect on urinary F3-IsoP concentrations, where African American GT/TTs had lower concentrations (~50% lower) than GGs ( $P = 0.0221$ ; Figure 4E). After Bonferroni adjustment for multiple comparisons, this genotypic effect, however, was no longer considered statistically significant. Visually, the genotype effect appears to be additive, but larger studies are needed to verify this.

LTE4 is a potent ARA-derived eicosanoid involved in propagating inflammation. Caucasian GGs on the placebo diet displayed the largest reduction in urinary concentrations of LTE4 over 6 d, compared with GT/TTs on both placebo and omega-oil diets ( $P = 0.0355$ ; Figure 4C). African Americans, however, had more stable concentrations of LTE4 with less pronounced changes over time. This was especially true for minor allele carriers (GT/TT) ( $P = 0.0347$ ; Figure 4F). After adjustment for multiple comparisons, these main effects observed for diet in Caucasian Americans and rs174537 genotype in African Americans were no longer considered statistically significant.

**TABLE 2** Summary of patient demographics by genotype at rs174537 and race in the OMEGA trial. Caucasians ( $n = 174$ ) top panel, African Americans ( $n = 40$ ) bottom panel<sup>1</sup>

Caucasian Americans ( $n = 174$ ) Genotype at rs174537	Placebo ( $n = 83$ )		Omega oil ( $n = 91$ )		p-value diet effects	p-value SNP effects
	GG ( $n = 37$ )	GT/TT ( $n = 46$ )	GG ( $n = 42$ )	GT/TT ( $n = 49$ )		
Male	22% (18)	28% (23)	23% (21)	28% (25)	0.871	0.871
Age, y	53 (38, 65)	55 (41, 67)	52 (44, 66)	56 (46, 72)	0.240	0.418
Cause of lung injury					0.360	0.371
Pneumonia	21% (17)	30% (25)	21% (19)	30% (27)		
Sepsis	8% (7)	12% (10)	15% (14)	11% (10)		
Trauma	1% (1)	1% (1)	3% (3)	3% (3)		
Other	15% (12)	12% (10)	7% (6)	10% (9)		
Clinical outcomes						
Vent-free days	21 (7, 23)	23 (10, 26)	20 (0, 24)	21 (0, 24)	0.175	0.560
ICU-free days	19 (7, 23)	21 (12, 25)	20 (0, 24)	21 (1, 24)	0.423	0.246
Cumulative mortality						
At 14 days	5% (4)	6% (5)	8% (7)	10% (9)	0.369	0.904
At 30 days	6% (5)	8% (7)	10% (9)	12% (11)	0.352	0.956
At 90 days	8% (7)	11% (9)	12% (11)	13% (12)	0.591	0.661

Summary of patient demographics by genotype at rs174537 for African Americans in the OMEGA trial ( $n = 40$ )

African Americans ( $n = 40$ ) Genotype at rs174537	Placebo ( $n = 15$ )		Omega oil ( $n = 25$ )		p-value diet effects	p-value SNP effects
	GG ( $n = 12$ )	GG ( $n = 3$ )	GG ( $n = 20$ )	GT/TT ( $n = 5$ )		
Male	33% (5)	13% (2)	44% (11)	12% (3)	0.833	0.237
Age, y	50 (39, 58)	52 (51, 65)	46 (40, 62)	59 (53, 61)	0.864	0.122
Cause of lung injury					0.313	0.158
Pneumonia	73% (11)	13% (2)	52% (13)	8% (2)		
Sepsis	7% (1)	0% (0)	4% (1)	8% (2)		
Trauma	0% (0)	0% (0)	0% (0)	0% (0)		
Other	0% (0)	7% (1)	24% (6)	4% (1)		
Clinical outcomes						
Vent-free days	18 (0, 24)	14 (0, 27)	20 (11, 25)	10 (0, 18)	0.838	0.245
ICU-free days	17 (2, 22)	15 (12, 27)	19 (7, 24)	9 (0, 13)	0.283	0.521
Cumulative mortality						
At 14 days	13% (2)	0% (0)	12% (3)	8% (2)	0.195	0.600
At 30 days	13% (2)	0% (0)	16% (4)	12% (3)	0.053	0.419
At 90 days	13% (2)	0% (0)	16% (4)	12% (3)	0.053	0.419

<sup>1</sup>Baseline and study characteristics are reported as percentages and counts for categorical variables or as medians and IQRs for continuous variables. There were no statistically significant differences in patient demographics or outcomes by genotype or diet. In addition, SNP–diet interactions were not statistically significant. G, major allele at rs174537; ICU, intensive care unit; SNP, single nucleotide polymorphism; T, minor allele at rs174537.

### Effects of diet and genotype on pulmonary function

To determine the effects of SNP–diet interactions on pulmonary function, we evaluated the ratio of partial arterial oxygenation to fraction of inspired oxygen and positive end-expiratory pressure metrics. We did not observe any statistically significant SNP–diet interactions on any of these pulmonary metrics in either racial group. In addition, although we were able to detect some differences in the pulmonary function metrics by genotype, none of these met our adjusted Bonferroni  $P$  value of 0.005.

### Effects of diet and genotype on clinical outcomes

A Tobit model was used to estimate the effects of rs174537 genotype and diet on primary clinical outcomes (i.e., VENT-free and ICU-free days). We did not observe any significant differences in VENT-free or ICU-free days between genotype or the SNP–diet interaction when we stratified by race. Again, this is very important to note because the original OMEGA trial reported significant differences when the entire study population was evaluated as a whole. This secondary analysis reveals

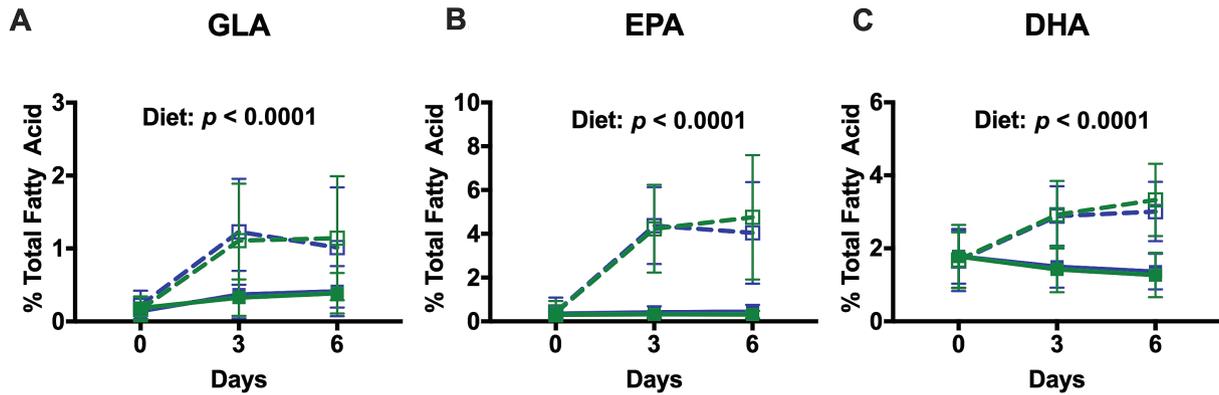
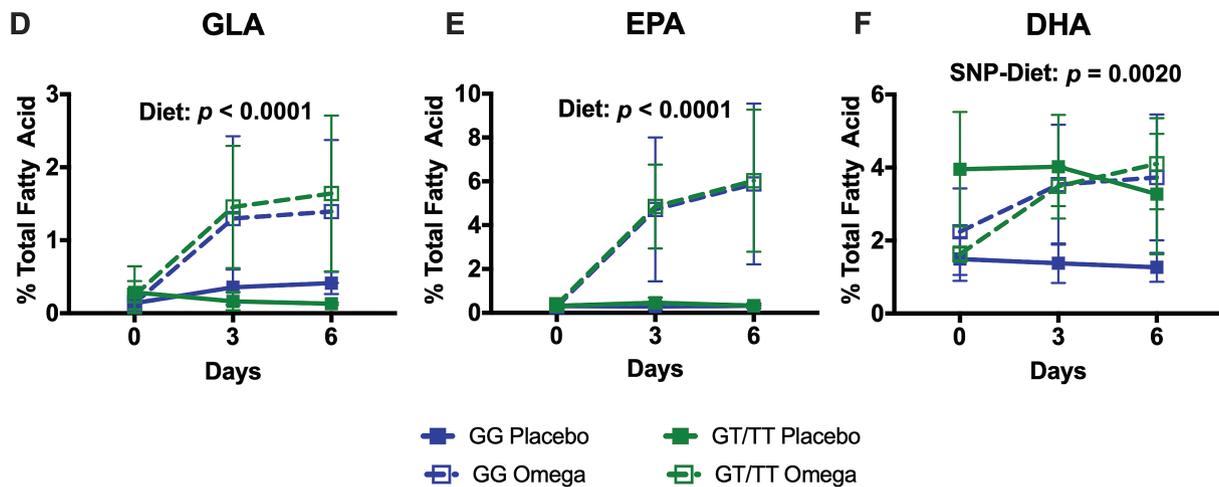
potential racial differences in ARDS patients that should be considered for all future studies.

### Effects of diet and genotype on mortality

Overall, there were 44 deaths in Caucasian Americans and 10 deaths in African Americans 90 d postrandomization. To determine the impact of genotype, diet, and SNP–diet interactions on mortality we used a Cox proportional hazard model that adjusted for age and gender. No statistically significant differences in mortality were observed between genotype or diet in the overall study population (Figure 5A). After stratifying by race, this effect remained nonsignificant in both racial cohorts (Caucasian Americans: HR: 1.25; 95% CI: 0.659, 2.372;  $P = 0.49$ , Figure 5B; African Americans: HR: 2.47; 95% CI: 0.507, 12.017;  $P = 0.26$ , Figure 5C).

### Discussion

It is well known that  $\omega$ -3 and  $\omega$ -6 fatty acids are key players of innate immunity and can modulate the acute phases of inflammation.

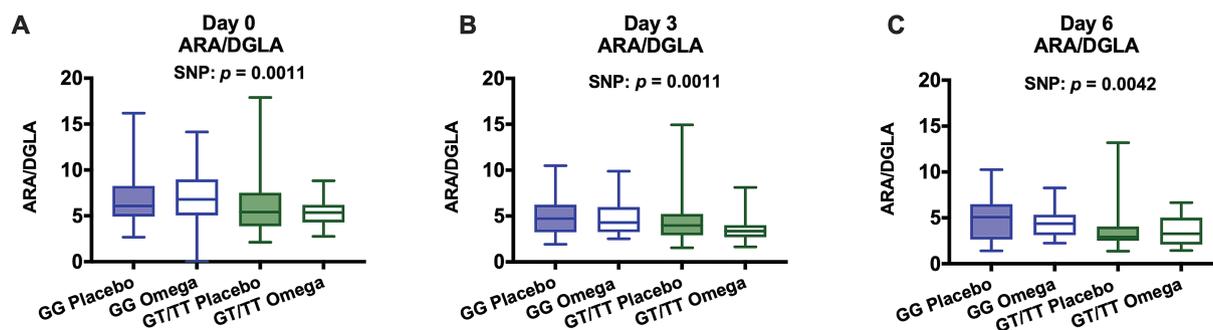
Caucasian Americans ( $n = 117$ )African Americans ( $n = 27$ )

**FIGURE 1** Circulating concentrations of GLA, EPA, and DHA by genotype in the OMEGA trial. Means (squares) and standard deviations (error bars) are illustrated for each study time point (i.e. days 0, 3 and 6). A mixed linear model with an autoregressive covariance structure was stratified by race and adjusted for age and gender to estimate the interactive and additive effects of diet and genotype at rs174537 on concentrations of GLA, EPA, and DHA. GLA and EPA variables were log transformed to meet the linear regression assumptions of normality. Differences in circulating concentrations of these fatty acids between diet and genotype were assessed using a dominant genotype model (i.e., GG = 0 vs. GT/TT = 1) and considered statistically significant if  $P < 0.005$ , based on our Bonferroni correction for multiple comparisons. We observed significant effects of diet on circulating concentrations of GLA, EPA, and DHA in both races (A–F;  $P < 0.0001$ ), regardless of genotype. Interestingly, we observed a statistically significant SNP–diet interaction on circulating concentrations of DHA in African Americans (F;  $P = 0.0020$ ), where T-allele carriers on placebo illustrated elevated concentrations of DHA, comparable to those on the omega-oil diet. G, major allele at rs174537; GLA,  $\gamma$ -linolenic acid; T, minor allele at rs174537.

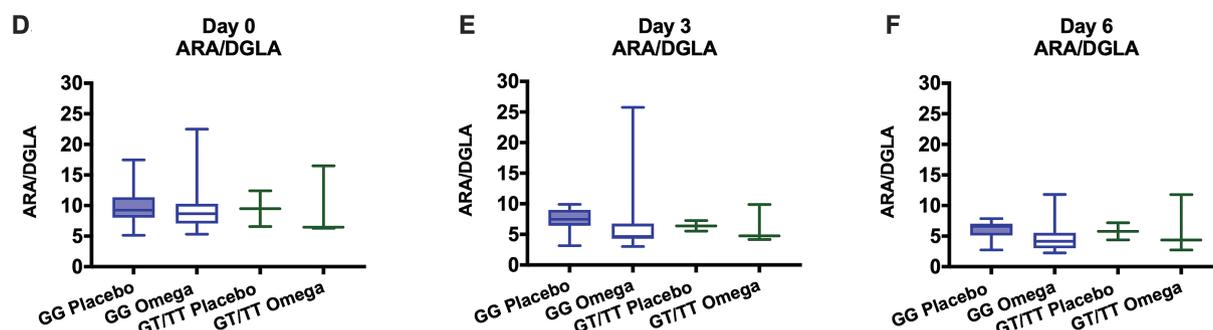
However, the utility of  $\omega$ -3 PUFA-enriched diets for modulation of inflammation continues to be debated due to conflicting results from several clinical trials in critically ill patients, including those with ARDS. Genetic variants spanning the *FADS* gene cluster and those in strong linkage disequilibrium (LD) with the *FADS* region (e.g., rs174537) can directly impact concentrations of tissue and circulating  $\omega$ -3 and  $\omega$ -6 fatty acids, which we postulated can also contribute to the variability in response to such diets. This study describes for the first time SNP–diet interactions impacting patient responses to PUFA-enriched diets in an ARDS cohort. We show that 1) the omega-oil diet significantly impacts

circulating fatty acid concentrations, urinary F3-IsoP concentrations, and clinical outcomes, some more disproportionately in African Americans; 2) genotype at rs174537 is significantly associated with ARA/DGLA ratios, particularly in Caucasians; and 3) there is some indication of SNP–diet interactions on DHA and concentrations of both F2-IsoPs and F3-IsoPs, although only the impact on DHA in African Americans remained statistically significant after we made an adjustment for multiple comparisons. Larger studies are needed to evaluate the impact of rs174537 and other relevant genetic variants influencing fatty acid metabolism. To the best of our knowledge, this is

## Caucasian Americans (n = 117)



## African Americans (n = 27)



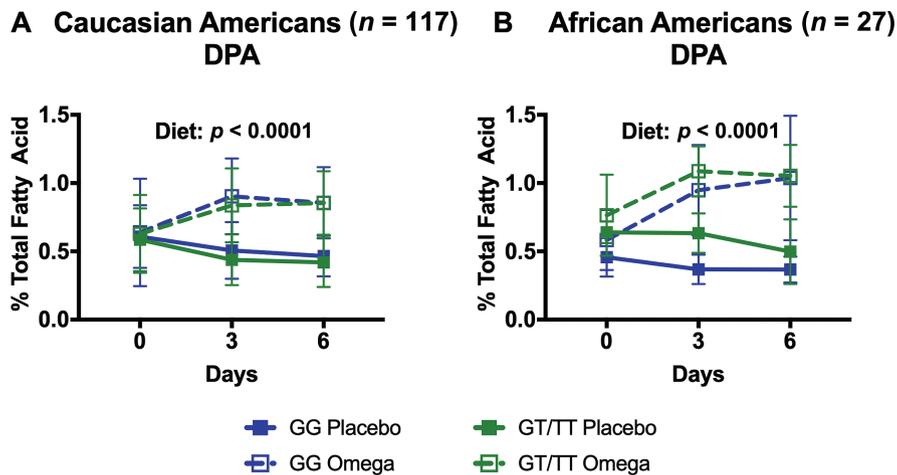
**FIGURE 2** ARA/DGLA ratios, representing fatty acid desaturase 1 enzymatic efficiency (also known as delta 5 desaturase), is illustrated over time between ethnicities and genotype at rs174537. Median and interquartile range (IQR) values are reported in the form of box-whisker plots. Caucasian GGs at SNP rs174537 have higher ARA/DGLA ratios. ARA and DGLA variables were transformed to meet the linear regression assumptions of normality. A mixed linear model with an autoregressive covariance structure was stratified by race and adjusted for age and gender to estimate the interactive and additive effects of diet and genotype at rs174537 on ARA/DGLA ratios over time. Differences in ARA/DGLA ratios between diet and genotype were assessed using a dominant genotype model (i.e., GG = 0 vs. GT/TT = 1) and considered statistically significant if  $P < 0.005$ , based on our Bonferroni correction for multiple comparisons. There was no evidence of SNP–diet interactions observed in either racial cohort. After Bonferroni adjustments for multiple comparisons, we observed a significant genotype effect impacting concentrations of ARA/DLGA ratios in Caucasian Americans at baseline ( $P = 0.0011$ ; A), day 3 ( $P = 0.0011$ ; B), and day 6 ( $P = 0.0042$ ; C). No significant genotype effects were observed in African Americans (D–F). ARA, arachidonic acid; DGLA, dihomo- $\gamma$ -linolenic acid; G, major allele at rs174537; SNP, single nucleotide polymorphism; T, minor allele at rs174537.

the first study assessing potential SNP–diet interactions impacting the patient response to PUFA-enriched diets in critically ill patients with ARDS.

In this study, major allele carriers (i.e., GG) at rs174537 displayed the highest ARA/DGLA ratios at all time points, particularly in Caucasians, implying fast and efficient FADS1 enzymatic activity (also known as  $\Delta^5$ -desaturase activity). However, this did not translate to significantly different concentrations of ARA by genotype. One explanation for this finding is the high amount of GLA given to participants randomly assigned to the omega-oil diet group. When GLA is given in the diet, it is metabolized into DGLA, which can cause a bottleneck effect at the FADS1 step in T-allele carriers because they are unable to efficiently metabolize excess DGLA into ARA. Further, the high dose of both GLA and EPA could impact the FADS1 activity, ultimately causing circulating ARA concentrations to remain low. In a study of healthy

adults given 3 g/d fish oil supplements for 6 wk, minor allele carriers at rs174546, which is in high LD with rs174537 ( $r^2 = 0.978$ ), had the smallest change in  $\Delta^5$ -desaturase activity after 6 wk of fish oil supplementation (32). Though it is clear that this SNP impacts FADS1 activity, more information is needed to better understand how diet modulates its activity.

In our smaller African American subset, we observed a significant SNP–diet interaction effect on DHA concentrations even after correcting for multiple comparisons. African American T-allele carriers (i.e., GT/TT) on placebo displayed elevated concentrations of circulating DHA, comparable to participants receiving the omega-oil diet. This observation reveals a potential confounding factor from the original OMEGA trial. Not only were baseline DHA concentrations higher in this subset, but our secondary analysis reveals dramatic differences between races.



**FIGURE 3** Circulating DPA concentrations are significantly higher in patients receiving the omega-oil diet. Means (squares) and standard deviations (error bars) are reported. DPA variables were log transformed to meet the linear regression assumptions of normality. A mixed linear model with an autoregressive covariance structure was stratified by race and adjusted for age and gender to estimate the interactive and additive effects of diet and genotype at rs174537 on concentrations of DPA. Differences in circulating DPA concentrations between diet randomization and genotype groups were assessed using a dominant genotype model (i.e., GG = 0 vs. GT/TT = 1) and were considered statistically significant if  $P < 0.005$ , based on our Bonferroni correction for multiple comparisons. There was no evidence for a SNP–diet interaction in either racial cohort. However, all individuals on the omega-oil diet exhibited significantly higher concentrations of DPA compared with those on placebo (as indicated by diet  $p$ -value in figure), in both races (A, B;  $P < 0.0001$ ). DPA, docosapentaenoic acid; G, major allele at rs174537; SNP, single nucleotide polymorphism; T, minor allele at rs174537.

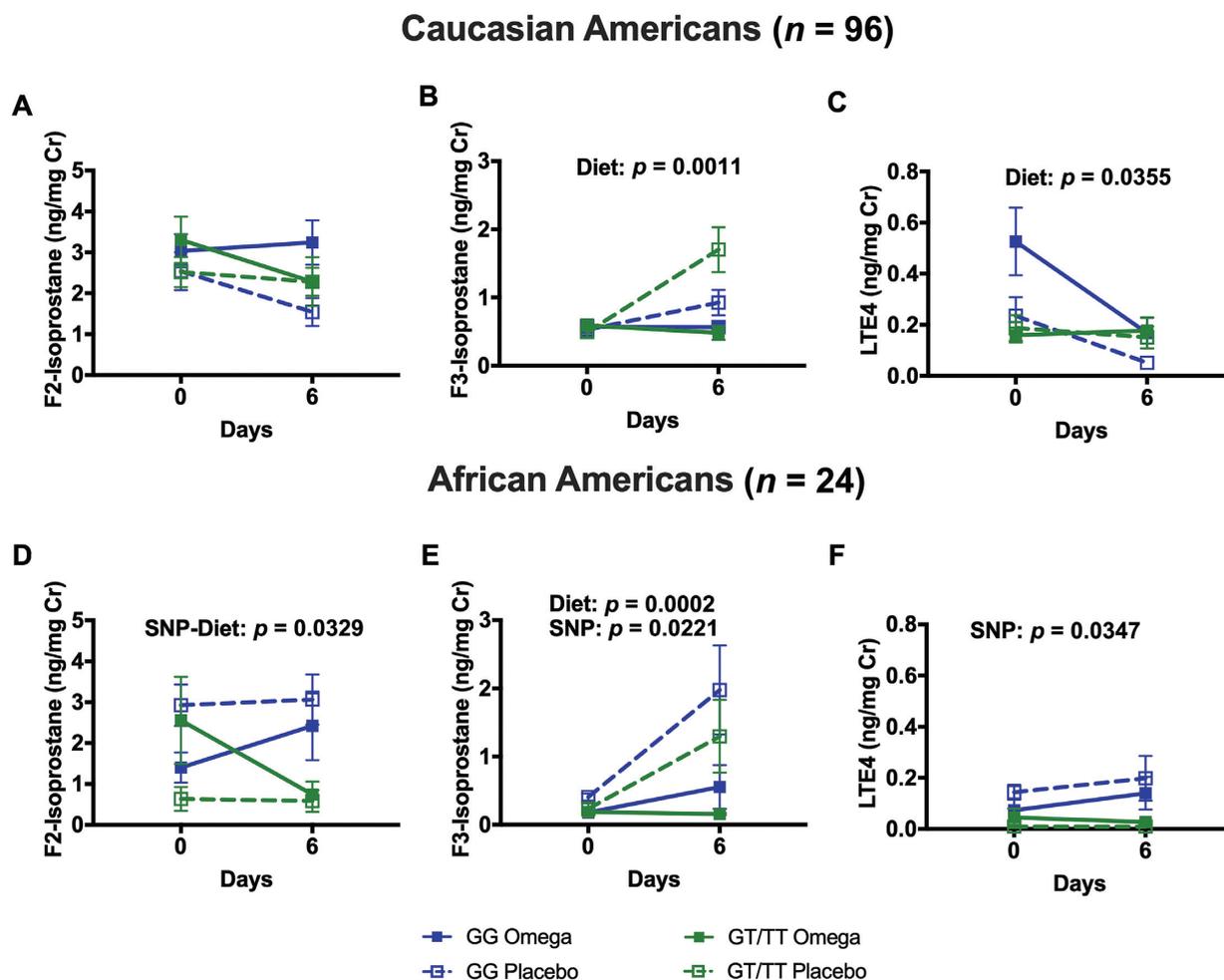
To date, reports addressing the relative benefit of  $\omega$ -3 fatty acid-enriched diets in ARDS patients have focused primarily on clinical outcomes like VENT-free and ICU-free days. However, the sole reliance on clinical outcomes fails to highlight PUFA-related biochemical pathways, mechanisms by which  $\omega$ -3 and  $\omega$ -6 fatty acids modulate inflammation, and biological targets worth investigating. In this study, we observed that individuals on the omega-oil diet had significantly elevated concentrations of the EPA-derived urinary metabolite F3-IsoP, with the T-allele carriers generally displaying the highest concentrations. This effect was consistently observed in both Caucasian and African American subjects. The excretion of EPA-derived metabolites raises a question about the dosing of EPA within the diet and how it might potentially provide immunomodulatory benefit. Additionally, we observed that individuals receiving the omega-oil diet generally had lower concentrations of ARA-derived inflammatory markers (based on unadjusted  $P$  values for multiple comparison). However, larger studies are needed to evaluate this effect more fully.

Although the current study did not measure PGs, thromboxanes, or leukotrienes other than LTE<sub>4</sub>, other studies have observed associations between rs174537 and the generation of several eicosanoids including leukotriene B<sub>4</sub> and 5-HETE, 2 proinflammatory eicosanoids that were elevated in Caucasian major allele carriers (24). Similarly, other studies have demonstrated strong associations between major allele carriers of rs174537 and elevated 8-epi-PGF<sub>2 $\alpha$</sub>  concentrations (25, 33). Considering the strong allele-specific associations that have been uncovered between major allele carriers of rs174537 and elevated ARA concentrations, it is plausible that the increase in ARA-derived eicosanoids is a result of an increased metabolic capacity to metabolize  $\omega$ -6 fatty acids toward proinflammatory mediators of inflammation. To date,

few studies have highlighted potential associations between rs174537 and  $\omega$ -3 fatty acids (34). It would be interesting to see if rs174537, and other related SNPs, impact the production of  $\omega$ -3-derived lipid mediators and consequently modulate inflammatory processes (35, 36).

Despite observing significant diet, genotype, and SNP–diet interactions on the circulating PUFAs and inflammatory biomarkers, we did not detect any significant differences in patient outcomes, including pulmonary functional metrics, VENT-free days, ICU-free days, or mortality. The worse mortality rates were observed in the African American T-allele carriers (i.e., GT/TT) receiving the omega-oil diet. Although we were unable to detect statistically significant differences between genotype and diet groups, larger studies are necessary to confirm these trends.

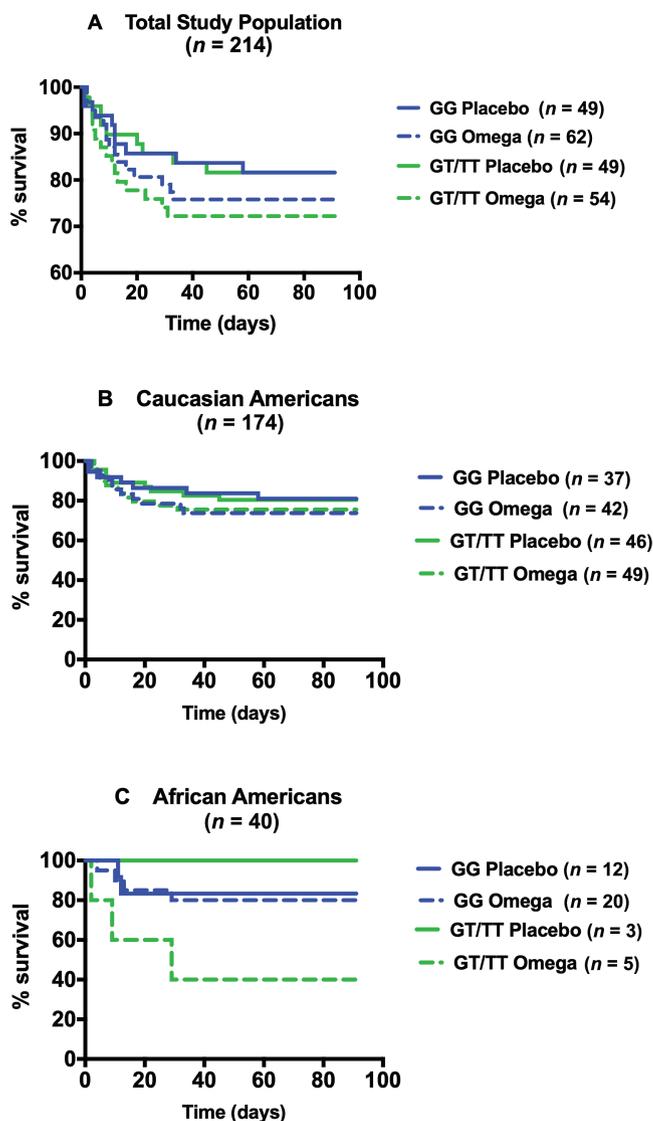
Although this secondary analysis of the OMEGA trial has revealed potentially important targets involving SNP–diet interactions on fatty acid metabolism and inflammation, there are a few limitations worth noting. First, we acknowledge that sample size was relatively small, particularly in the African American subset because we had to stratify our analyses by both race and genotype. Additional studies are needed to investigate the impact of this SNP and others within the *FADS* region in larger ethnic populations and to confirm the SNP and racial effects we observed in this study. Further, the OMEGA trial only provided self-reported race. Larger studies, potentially with ancestral markers, are needed to further delineate the effects of rs174537 and other genetic variants on PUFA-rich diets. Second, we acknowledge that the carbohydrate and protein compositions of the randomized diets were also different and might have influenced patient outcomes. However, disentangling the interdependencies among the nutritional



**FIGURE 4** The effect of genotype at rs174537 and diet on urinary inflammatory biomarkers. Means (squares) and standard deviations (error bars) are reported. F-series isoprostane variables were log transformed and series 4 leukotriene variables were square root transformed to meet the linear regression assumptions of normality. Urinary biomarkers were normalized to creatinine (Cr) levels. A mixed linear model with an autoregressive covariance structure was stratified by race and adjusted for age and gender to estimate the interactive and additive effects of diet and genotype at rs174537 on urinary biomarkers of inflammation. Differences between diet randomization and genotype groups were assessed using a dominant genotype model (i.e., GG = 0 vs. GT/TT = 1) and considered significant if  $P < 0.005$ , based on our Bonferroni correction for multiple comparisons. Unadjusted  $P$  values were observed for the following variables: F2-isoprostane: for the SNP–diet interaction  $P = 0.0329$  in African Americans (D); F3-isoprostane: diet  $P = 0.0011$  in Caucasians (B), and diet  $P = 0.0002$  and genotype  $P = 0.0221$  in African Americans (E); leukotriene E4: diet  $P = 0.0355$  in Caucasians (C) and genotype  $P = 0.0347$  in African Americans (F). After Bonferroni adjustments for multiple comparisons, only the effect of omega-oil diet on F3-isoprostane concentrations remained statistically significant in both races. This means that the increase in urinary F3-isoprostane is mainly driven by the high concentrations of EPA in the omega-oil diet. Cr, creatinine; G, major allele at rs174537; LTE4, leukotriene E4; SNP, single nucleotide polymorphism; T, minor allele at rs174537.

components of the diet is beyond the scope of our current study. Third, we initially focused on SNP rs174537, but there is a need to perform a comprehensive scan of all relevant *FADS* and *ELOVL* SNPs to identify which genetic variants most strongly impact PUFA metabolism and inflammation in the context of ARDS. Fourth, this study only focused on 3 major urinary inflammatory biomarkers, which were measured in the original OMEGA trial. Other key inflammatory markers that could be evaluated for SNP–diet interactions in the future include plasma cytokines, PUFA-derived eicosanoids, and specialized

proresolving lipid mediators, which can be altered by fatty acids obtained from the diet. Lastly, we could not assess epigenetic changes to *FADS* and *ELOVL* genes because DNA was only collected at baseline (i.e., day 0). Future studies could benefit from investigating genetic and epigenetic regulation of these genes in response to dietary fish oil supplements. Despite these limitations, this study is the first to report potential SNP–diet interactions impacting fatty acid metabolism and inflammation in ARDS patients. We show some promising evidence that rs174537 and potentially other SNPs in high LD could play an



**FIGURE 5** Comparison of survival rates in Caucasian and African Americans by genotype and randomized diet group. A Cox proportional hazard regression model stratified by race and adjusting for age gender was used to assess differences in 30-d, 60-d, and 90-d mortality. Differences in mortality between diet and genotype were assessed using a dominant genotype model (i.e., GG = 0 vs. GT/TT = 1) and was considered statistically significant if  $P < 0.005$ , based on our Bonferroni correction for multiple comparisons. Using this model, we did not observe any statistically significant genotype, diet, or SNP–diet effects impacting 30-d, 60-d, or 90-d mortality in either racial groups. However, African American T-allele carriers on the omega-oil diet displayed the worst survival rates. G, major allele at rs174537; T, minor allele at rs174537.

important role on the subsequent inflammation following lung injury in ARDS patients.

To conclude, in this study we evaluated the impact of rs174537 on the response to diets enriched in omega-oil by performing a secondary data analysis of the OMEGA trial. We observed significant SNP–diet interactions on circulating DHA concentrations, within African Americans.

Caucasian GGs consistently had higher ARA/DGLA ratios at all time points. Both races receiving the omega-oil diet demonstrated elevated concentrations of EPA-derived urinary F3-IsoP compared with placebo, but this did not translate to improved patient outcomes. Our secondary data analysis reveals the importance of FADS-related genetic variants and race on circulating PUFA concentrations, particularly DHA. In addition, rs174537 was significantly associated with higher concentrations of urinary inflammatory markers, particularly in African Americans receiving the omega-oil diet. A “one size fits all” approach to PUFA supplementation might not be appropriate for human populations (34). Future studies should investigate other *FADS* and *ELOVL* genetic variants to identify more precisely which patient populations and racial groups might benefit from adjuvant  $\omega$ -3-enriched diets to reduce inflammation and improve outcomes following acute lung injury.

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