1   **Title:** Circulating polyunsaturated fatty acids and COVID-19: a prospective cohort study and
2   Mendelian randomization analysis
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19   **ABSTRACT**

20   **Background:** Higher circulating polyunsaturated fatty acids (PUFAs), especially omega-3 ones,

21   have been linked to a better prognosis in patients of coronavirus disease 2019 (COVID-19).

22   However, the effects and causality of pre-infection PUFA levels remain unclear.

23   **Objective:** To investigate the observational and causal associations of circulating PUFAs with

24   COVID-19 susceptibility and severity.

25   **Design:** We first performed a prospective cohort study in UK Biobank, with 20,626 controls who

26   were tested negative and 4,101 COVID-19 patients, including 970 hospitalized ones. Plasma

27   PUFAs at baseline were measured by nuclear magnetic resonance, including total PUFAs, omega-

28   3 PUFAs, omega-6 PUFAs, docosahexaenoic acid (DHA), linoleic acid (LA), and the omega-

29   6/omega-3 ratio. Moreover, bidirectional two-sample Mendelian randomization (MR) analyses

30   were performed to examine the causal associations of eight individual PUFAs, measured in either

31   plasma or red blood cells, with COVID-19 susceptibility and severity using summary statistics

32   from existing genome-wide association studies.

33   **Results:** In the observational association analysis, total PUFAs, omega-3 PUFAs, omega-6 PUFAs,

34   DHA, and LA were associated with a lower risk of severe COVID-19. Omega-3 PUFAs and DHA

35   were also associated with a lower risk of testing positive for COVID-19. The omega-6/omega-3

36   ratio was positively associated with risks of both susceptibility and severity. The forward MR

37   analysis indicated that arachidonic acid (AA) and docosapentaenoic acid (DPA-n3) might be

38   causally associated with a lower risk of severe COVID-19, with OR (95% CI) per one SD increase

39   in the plasma level as 0.96 (0.94, 0.99) and 0.89 (0.81, 0.99), respectively. The reverse MR analysis

40   did not support any causal effect of COVID-19 on PUFAs.

41   **Conclusions:** Our observational analysis supported that higher circulating PUFAs, either omega-3 or omega-6, are protective against severe COVID-19, while omega-3 PUFAs, especially DHA, were also associated with reducing COVID-19 susceptibility. Our MR analysis further supported causal associations of AA and DPA-n3 with a lower risk of severe COVID-19.

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46   **Key Words:** COVID-19; polyunsaturated fatty acids; Mendelian randomization; prospective cohort; docosapentaenoic acid; arachidonic acid

48 **Introduction**

49 The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory

50 syndrome coronavirus 2 (SARS-CoV-2), has resulted in over five million deaths in less than two

51 years (1, 2). Understanding the role of nutrition in moderating susceptibility to and progression of

52 COVID-19 is critical for the development of evidence-based dietary recommendations to prevent

53 infection and to manage disease progression (3, 4). Omega-3 and omega-6 polyunsaturated fatty

54 acids (PUFAs) are of special interest because of their potent immunomodulatory effects, not only

55 in mounting immune responses against viral infection but also in promoting inflammation

56 resolution to avoid tissue damage (5-7). COVID-19 is an infectious disease characterized by

57 cytokine storm and hyperinflammation in severe cases (8), presenting multiple possible points of

58 action for PUFAs.

59

60 Recent observational studies have noted significant changes in the circulating levels of various

61 PUFAs when comparing COVID-19 patients to healthy controls and across severity subgroups of

62 patients. In general, total PUFAs, omega-6 PUFAs, linoleic acid (LA), and the omega-3 index

63 measured as the percentage of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in

64 red blood cell (RBC) fatty acids, are lower in COVID-19 patients and even lower in severe cases

65 (9-12). A higher omega-3 index in patients was further associated with lower risks of requiring

66 mechanical ventilation and death (9, 10). But conflicting patterns were also reported across cohorts

67 and studies (11, 12), such as elevated levels of LA and arachidonic acid (AA) in COVID-19

68 patients (12). Moreover, the circulating levels of PUFAs in patients are likely confounded by

69 immune responses to the viral infection and do not represent the effects of pre-infection circulating

70 levels. There is a prospective cohort study that compared hospitalized COVID-19 patients to non-

71   cases and found that almost all PUFA measurements, including total PUFAs, omega-6 PUFAs,

72   omega-3 PUFAs, LA, and DHA, are associated with a lower risk of severe COVID-19. The only

73   exception is the omega-6/omega-3 ratio, which exhibits a positive association (13). However, the

74   study did not distinguish the effects on susceptibility and severity, and the usage of non-cases

75   without COVID-19 status as the control did not correct for selection bias in those receiving tests.

76   Altogether, while these observational studies provide valuable insights, they are susceptible to

77   residual confounding and reverse causation. The causal effects of circulating PUFAs on COVID-

78   19 susceptibility and severity remain unclear.

79

80   Mendelian randomization (MR) is an analytic tool for inferring the causal effects of an exposure

81   on an outcome of interest (14). MR uses randomly allocated genetic variants related to the exposure

82   as instrumental variables, which are inborn and minimally affected by confounders and reverse

83   causation (15). This method has been widely utilized in recent studies to evaluate the causal roles

84   of specific risk factors in COVID-19, such as body mass index (BMI), white blood cells, some

85   circulating proteins, and smoking (16-19). On the other hand, MR studies have also provided

86   support for the causal clinical effects of circulating PUFAs (Supplemental Table 1). The

87   genetically predicted circulating levels of various PUFAs have been associated with clinical

88   biomarkers, such as blood lipids, white blood cell counts, and blood pressure (20-22). They were

89   also directly associated with risks of specific diseases, such as cardiovascular diseases, diabetes,

90   and cancers (23-27). Therefore, MR is a valuable and cost-effective tool to evaluate the causal

91   roles of circulating PUFAs in COVID-19 susceptibility and severity.

92

93   In this study, we first performed an observational analysis in a prospective cohort, UK Biobank,

94   with 4,101 COVID-19 patients, including 970 hospitalized ones, and 20,626 controls that were

95   tested negative. We performed multiple comparisons across different case and control groups to

96   evaluate the effects of six baseline plasma PUFA measures on COVID-19 susceptibility and

97   severity. Furthermore, we applied bidirectional two-sample MR analyses to examine the causal

98   associations between eight individual PUFAs and COVID-19. Genetic instruments for circulating

99   PUFAs were obtained from previous genome-wide association studies (GWAS) of corresponding

100   PUFAs measured in either plasma or RBC (28-30). Genetic associations with COVID-19

101   susceptibility and severity were obtained from GWAS meta-analyses conducted by the COVID-

102   19 Host Genetics Initiative (HGI) (31). Our study, integrating observational and genetics-

103   instrumented MR analyses, unraveled the effects of total and individual circulating PUFAs on the

104   risks of COVID-19 susceptibility and severity.

Methods

Ethical considerations

The usage of individual-level data for this study was approved by the University of Georgia Institutional Review Board and UK Biobank (application no. 48818). All participants of UK Biobank and the Framingham Heart Study (FHS) provided written informed consent before joining these studies. Informed consent was not required for publicly available summary statistics. Our study follows the guidelines for strengthening the reporting of observational studies in epidemiology (STROBE, Supplemental Table 2) and strengthening the reporting of Mendelian randomization studies (STROBE-MR, Supplemental Table 3) (32).

Participants and study design

We performed an observational cohort study based on UK Biobank and then a bidirectional two-sample MR study with summary statistics from GWAS of PUFAs and COVID-19. UK Biobank is a population-based prospective study, including >500,000 participants aged 37–73 years at recruitment from 2006 to 2010 in the United Kingdom (33). The observational analysis was performed to examine the associations between six plasma PUFA measures and COVID-19 status in UK Biobank. The six plasma PUFA measures include total PUFAs, omega-3 PUFAs, omega-6 PUFAs, DHA, LA, and the calculated omega-6/omega-3 ratio. The MR study investigated the causal effects of eight individual PUFAs on COVID-19 susceptibility and severity. Genetic instruments for plasma PUFAs were obtained directly from published GWAS (28, 29). Genetic instruments for RBC PUFAs were determined based on a published GWAS, but their summary statistics, not reported in the original study, were calculated by ourselves with the same statistical model and individual-level data from 2,462 FHS participants (30). Six PUFAs have genetic

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128   instruments for their circulating levels in both plasma and RBC, including α-Linolenic acid (ALA),

129   docosapentaenoic acid (DPA-n3), LA, γ-Linoleic acid (GLA), dihomo-γ-linoleic acid (DGLA),

130   and AA. Docosatetraenoic acid (DTA) only has genetic instruments for its RBC level, while DHA

131   only for its plasma level.

132

Observational analysis

134   Figure 1 displays the flow of participants throughout the observational study. To minimize the

135   possibility of bias due to population stratification, the analysis was restricted to individuals of

136   European descent. In addition, we removed participants who had mismatched self-reported sex

137   and genetic sex, sex chromosome aneuploidy, ten or more third-degree or closer relatives, or had

138   withdrawn from UK Biobank. Our exposure variables were six PUFAs, as measured by nuclear

139   magnetic resonance (NMR) in plasma samples collected between 2007 and 2010 (13, 33, 34). We

140   used the COVID-19 testing result and inpatient status as our outcome (data accessed on June 21,

141   2021). The specimen collection dates were March 16, 2020 to June 14, 2021 for those in England;

142   February 11, 2020 to March 18, 2021 in Scotland; and January 13, 2020 to June 7, 2021 in Wales.

143   Hospitalized COVID-19 patients were identified as those with positive PCR-based diagnosis and

144   explicit evidence of being inpatients. Of note, being an inpatient does not necessarily indicate

145   hospitalization for COVID-19 because patients in hospitals for any reason may be prioritized for

146   COVID-19 testing (35). Inpatient status was not available for assessment centers in Scotland and

147   Wales. To test the association with COVID-19 severity, we performed two separate analyses with

148   different controls: 1) non-hospitalized COVID-19 patients, and 2) individuals who tested negative.

149   To examine the association with COVID-19 susceptibility, we focused on all COVID-19 cases

150   which were tested positive for SARS-CoV-2. Individuals with negative tests were used as the

8

151   control. This analysis of susceptibility was performed in two datasets: 1) participants from England,

152   and 2) participants from England, Scotland, and Wales. For the 24,727 participants with both

153   plasma PUFA measures and COVID-19 status, we applied logistic regression models on various

154   case and control groups to estimate the associations of PUFAs with COVID-19 susceptibility and

155   severity. Covariates included continuous variables, age, BMI, and Townsend deprivation index,

156   and categorical variables, sex, ethnicity, and assessment center. Individuals with missing

157   information in PUFA measures, COVID-19 status, or covariates were excluded. The comparable

158   effect sizes were expressed per one standard deviation (SD) increase in the plasma PUFAs. All

159   analyses in the observational study were conducted using R version 4.0.0, and nominal

160   significance was set at p-value < 0.05. Bonferroni correction for multiple testing [corrected P

161   significance cutoff: 0.05/2 (outcomes)/6 (exposures) = 0.0042] was used to avoid the type I error

162   (36).

163

164   **Genetic associations with PUFAs**

165   Two types of circulating PUFAs were evaluated in our MR analyses, plasma and RBC PUFAs.

166   For plasma PUFAs, single nucleotide polymorphisms (SNPs) were obtained from published

167   GWAS of omega-3 PUFAs (n = 8,866) and omega-6 PUFAs (n = 8,631) in participants of

168   European ancestry (28, 29). We selected SNPs for each plasma omega-3 and omega-6 PUFA,

169   which reached genome-wide significance level ($P < 5 \times 10^{-8}$) and were restricted by linkage

170   disequilibrium (LD) clumping to ensure independence ($R^2 < 0.001$ within a 10 Mb window). To

171   ensure robustness and reduce false positives, we also used less stringent LD cutoffs ($R^2 < 0.01$,

172   0.1, and 0.3) to select SNPs associated with plasma omega-3 PUFAs. The same LD-related

173   sensitivity analysis was not possible for plasma omega-6 PUFAs because their genome-wide

174   summary statistics were not available. To examine the effects of RBC PUFAs, we obtained genetic

175   associations at a genome-wide significance level ($P < 5 \times 10^{-8}$) identified by Tintle *et al.* (30). We

176   used the individual-level data from the FHS to confirm the significance of these SNPs and calculate

177   their effect sizes and standard errors. In the same linear mixed model, covariates included age, sex,

178   and matrix of kinship coefficients in the FHS. We respectively selected independent ($R^2 < 0.001$,

179   0.01, 0.1, and 0.3 within a 10 Mb window) SNPs predicting RBC PUFAs at genome-wide

180   significance ($P < 5 \times 10^{-8}$). We calculated F-statistics to test instrument strength (F-statistics >10

181   for all plasma and RBC PUFAs) (37). Summary statistics for the genetic instruments for plasma

182   and RBC PUFAs are openly available for public access (Supplemental Tables 4 and 5).

183

184   **Genetic associations with COVID-19**

185   To assess genetic associations with COVID-19 severity, we used three GWAS meta-analyses

186   conducted by the HGI (release 5, released on January 18, 2021) (31). First, we used the GWAS of

187   severe COVID-19, labeled as study A2, that compared patients confirmed with very severe

188   respiratory symptoms (n = 5,101) to the control group of general population samples (n =

189   1,383,241). Second, another HGI GWAS, labeled as study B2, compared hospitalized COVID-19

190   patients (n = 9,986) to general population samples (n = 1,877,672). The third severe COVID-19

191   GWAS utilized in our study, labeled as B1, compared hospitalized COVID-19 patients (n = 4,829)

192   to non-hospitalized COVID-19 patients (n = 11,816). To assess genetic associations with COVID-

193   19 susceptibility, we used one GWAS by HGI, labeled as study C2, that compared any COVID-

194   19 case (n = 38,984) to population controls (n = 1,644,784). In addition to these four COVID-19

195   GWAS used in our primary analysis, we repeated MR analyses using the study A2, B1, B2, and

196   C2 from HGI release 4 (released on October 20, 2020), to examine the consistency of our findings

197   across different data releases. Detailed information about these GWAS is available at the COVID-

198   19 HGI website (https://www.covid19hg.org/results/).

199

200   To assess reverse causality, we obtained strong ($P < 5 \times 10^{-8}$) and independent ($R^2 < 0.001$ within

201   a 10 Mb clumping window) SNPs associated with COVID-19 phenotypes as genetic instruments.

202   We also used a less stringent selection criterion ($P < 5 \times 10^{-6}$) to determine the robustness of our

203   results.

204

205   **MR analyses**

206   MR was used to infer causality between PUFAs and COVID-19 by leveraging genetic data as

207   instrumental variables. We scaled the odds ratio (OR) estimates per SD increment of plasma and

208   RBC PUFAs (% of total fatty acids). We obtained the SNP-specific Wald estimate (ratio of the

209   SNP-outcome effect divided by the SNP-exposure effect) when only one SNP was available. The

210   inverse variance-weighted (IVW) method with a multiplicative random-effects model ($\geq$2 SNPs)

211   was used as the primary analysis (38-40). We used the MR-Egger intercept test to evaluate the

212   extent of unbalanced horizontal pleiotropy, which can lead to a biased causal effect estimate (39).

213   In sensitivity analyses, we applied the MR-Egger and weighted median (WM) methods to account

214   for pleiotropy (39-41). The MR-Egger method provides an unbiased causal estimate even when all

215   SNPs are invalid instruments as long as that the horizontal pleiotropic effects are balanced across

216   SNPs (39). However, MR-Egger can be imprecise and suffer from low statistical power,

217   particularly when based on a small number of SNPs (e.g., < 10) (39). The WM method gives robust

218   causal estimates even when up to 50% of SNPs are invalid genetic instruments (41). To test the

219   presence of heterogeneity among genetic instruments, we calculated Cochran's Q statistic for the

11

220   IVW method and an extended version of Cochran's Q statistic (Rücker's Q′) for the MR-Egger

221   method (42, 43). We utilized Bonferroni correction [corrected P significance cutoff: 0.05/2

222   (outcomes)/7 (exposures) = 0.0036] for multiple testing. Additionally, we required a relationship

223   to be nominally significant ($P < 0.05$) with both measures of the same PUFA (plasma and RBC)

224   and in the case of COVID-19 severity, with different outcome GWAS (study A2, B2, and B1). All

225   MR analyses were performed in R version 4.0.0 with the TwoSampleMR package version 3.6.9

226   (44).

227   **Results**

228   **Baseline characteristics**

229   The flow of UK Biobank participants throughout the observational study is described in Figure 1,

230   while their baseline characteristics are summarized in Table 1. Across all assessment centers in

231   England, Scotland, and Wales, there were 104,112 participants with COVID-19 status. Among

232   them, 17,395 were tested positive for COVID-19. Inpatient status was only reported by assessment

233   centers in England. Of the 92,756 participants with COVID-19 status in England, 16,449 were

234   tested positive, and 4,209 had confirmed inpatient status. Across England, Scotland, and Wales,

235   COVID-19 patients were more likely to be male (t-test, $P = 0.008$), with higher BMI ($P = 9.34 \times$

236   10^{-14}), but younger than participants with negative testing results ($P < 2.2 \times 10^{-16}$). Across

237   assessment centers in England, hospitalized COVID-19 patients were older ($P < 2.2 \times 10^{-16}$), were

238   more likely to be male ($P = 2.44 \times 10^{-5}$), and had higher BMI ($P = 1.13 \times 10^{-14}$), when compared

239   to non-hospitalized COVID-19 patients.

240

241   **Observational association analysis**

242   Table 2 shows the observational associations between baseline plasma PUFAs and COVID-19

243   susceptibility and severity. Among participants from England who also had plasma data, there

244   were 18,293 with negative testing results and 3,873 with positive tests. Among the COVID-19

245   patients, 970 were hospitalized and the other 2,903 were non-hospitalized. Comparing hospitalized

246   patients to those tested negative, we observed a lower risk of COVID-19 severity per SD increase

247   in total PUFAs (OR: 0.88; 95% confidence interval (CI): 0.82, 0.95; $P = 0.0005$), omega-3 PUFAs

248   (OR: 0.82; 95% CI: 0.76, 0.89; $P = 8.1 \times 10^{-7}$), omega-6 PUFAs (OR: 0.91; 95% CI: 0.85, 0.98;

249    $P = 0.0121$), DHA (OR: 0.78; 95% CI: 0.72, 0.85; $P = 4.6 \times 10^{-9}$), and LA (OR: 0.92; 95% CI:

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250   0.86, 0.99; P = 0.0228). Using 2,903 non-hospitalized COVID-19 patients as the control group,

251   there were consistently inverse associations of COVID-19 severity with total PUFAs (P = 0.0012),

252   omega-3 PUFAs (P = 0.0013), omega-6 PUFAs (P = 0.0047), DHA (P = 8.9×10^{-5}), and LA (P

253   = 0.0079).

254

255   We further evaluated the effects of baseline plasma PUFAs on COVID-19 susceptibility by

256   comparing COVID-19 patients to those tested negative. Among 24,727 participants in England,

257   Scotland, and Wales, we found a lower risk of getting COVID-19 per SD increase in omega-3

258   PUFAs (OR: 0.92; 95% CI: 0.89, 0.96; P = 2.3×10^{-5}) and DHA (OR: 0.91; 95% CI: 0.87, 0.94;

259   P = 1.4×10^{-6}). Among 22,166 individuals in England only, we also observed consistently

260   significant associations for omega-3 PUFAs (OR: 0.92; 95% CI: 0.88, 0.96; P = 4.3×10^{-5}) and

261   DHA (OR: 0.91; 95% CI: 0.87, 0.94; P = 3.0×10^{-6}).

262

263   The omega-6/omega-3 ratio was significantly associated with an increased risk of severe COVID-

264   19, either by comparing hospitalized patients to participants who tested negative (OR: 1.13; 95%

265   CI: 1.07, 1.20; P = 1.5×10^{-5}) or to non-hospitalized patients (OR: 1.12; 95% CI: 1.03, 1.22; P =

266   0.0061). The ratio was also positively associated with COVID-19 susceptibility when comparing

267   COVID-19 patients to those tested negative in England, Scotland, and Wales (OR: 1.06; 95% CI:

268   1.03, 1.10; P = 0.0005) or in England only (OR: 1.05; 95% CI: 1.02, 1.09; P = 0.0030). Overall,

269   our observational analysis showed that individuals with lower baseline levels of all five examined

270   PUFAs were associated with a higher risk of hospitalized COVID-19, and those with lower levels

271   of omega-3 PUFAs and DHA were also at a higher risk of COVID-19 susceptibility. On the other

272   hand, the omega-6/omega-3 ratio was positively associated with the risks of both COVID-19

273   susceptibility and severity.

274

275   **Bidirectional MR analyses**

276   We performed bidirectional MR analyses to examine the causal relationships between individual

277   PUFAs and COVID-19. First, we performed a forward MR analysis to investigate the effects of

278   PUFAs on COVID-19 susceptibility and severity. Second, we conducted a reverse MR analysis to

279   evaluate the causal effects of genetically instrumented COVID-19 on PUFAs. All genetic

280   instruments for PUFAs (F-statistics >31.43) and COVID-19 (F-statistics >30.81) were strong

281   instruments. Six individual PUFAs have existing GWAS for their levels in plasma and RBC, and

282   there are three GWAS on severe COVID-19 (i.e., HGI study A2, B2, B1). Only results that were

283   consistent across these different GWAS were reported here.

284

285   In the forward MR study of plasma PUFAs, genetically instrumented one-SD increase in AA (OR:

286   0.96; 95% CI: 0.94, 0.99; $P = 0.007$) and DPA-n3 (OR: 0.89; 95% CI: 0.81, 0.99; $P = 0.026$) were

287   associated with a lower risk of very severe respiratory symptoms of COVID-19 based on HGI

288   study A2 (Figure 2A). Consistently, genetically instrumented AA (OR: 0.96; 95% CI: 0.96, 0.97;

289    $P = 3.23 \times 10^{-20}$) and DPA-n3 (OR: 0.93; 95% CI: 0.92, 0.95; $P = 4.73 \times 10^{-20}$) were associated

290   with a lower risk of hospitalized COVID-19 based on HGI study B2, which used general

291   population samples as the control (Figure 2B). Similar results were observed with HGI study B1,

292   which used non-hospitalized COVID-19 patients as the control (Figure 2C). Besides plasma

293   PUFAs, MR analyses with RBC PUFAs consistently support the protective effects of AA against

294   severe COVID-19 based on HGI A2 (OR: 0.97; 95% CI: 0.94, 1.00; $P = 0.048$), B2 (OR: 0.95;

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295   95% CI: 0.93, 0.97; $P = 1.32 \times 10^{-5}$), and B1 (OR: 0.84; 95% CI: 0.83, 0.85; $P = 8.57 \times 10^{-130}$)

296   studies (Figures 2). For DPA-n3, its genetically instrumented RBC level was consistently

297   associated with a lower risk of COVID-19 severity in our forward MR analysis with study A2 (OR:

298   0.79; 95% CI: 0.63, 0.99; $P = 0.041$), B2 (OR: 0.88; 95% CI: 0.82, 0.94; $P = 9.30 \times 10^{-5}$), and B1

299   (OR: 0.76; 95% CI: 0.59, 0.98; $P = 0.036$) (Figures 2). To ensure the robustness of findings, we

300   selected genetic instruments based on various LD categories ($R^2 < 0.001$, 0.01, 0.1, and 0.3). The

301   causal estimates of AA and DPA-n3 were consistent and at least nominally significant throughout

302   all MR analyses (Supplemental Tables 6–8). Causal estimates for AA and DPA-n3 maintained the

303   same effect directions in MR-Egger and WM methods, and sensitivity tests identified no evidence

304   of horizontal pleiotropy or heterogeneity of effects (Supplemental Tables 6–8). Of note, while

305   there were nominally significant associations between plasma DHA and very severe COVID-19

306   with HGI A2 and between RBC DTA and hospitalized COVID-19 with HGI B1, these two

307   relationships were not replicated in analyses with the other two GWAS of severe COVID-19

308   (Figure 2).

309

310   In terms of COVID-19 susceptibility, we found that genetically instrumented one-SD increase of

311   plasma DGLA (OR: 1.01; 95% CI: 1.00, 1.02; $P = 0.031$) was associated with an increased risk of

312   any SARS-CoV-2 infection (Figure 3). MR analysis with RBC DGLA showed a similar pattern

313   (OR: 1.01; 95% CI: 1.00, 1.02; $P = 0.007$). However, the association of genetically instrumented

314   DGLA with the risk of testing positive for COVID-19 was not statistically significant using any

315   other LD criteria for genetic instruments (Supplemental Table 9). Notably, our forward MR

316   findings were confirmed using additional COVID-19 GWAS from HGI release 4 (Supplemental

16

317   Tables 10–13). In summary, our forward MR analyses suggest that higher circulating levels of AA

318   and DPA-n3 are associated with a lower risk of developing severe forms of COVID-19.

319

320   We further applied reverse MR analyses to investigate the causal effects of COVID-19 on each

321   PUFA. Although several reverse MR analyses showed that genetically instrumented COVID-19

322   susceptibility or severity was associated with ALA, DHA, GLA, or DGLA, there was no consistent

323   evidence for an effect of COVID-19 on these PUFAs using the conventional genome-wide

324   significance threshold ($P < 5 \times 10^{-8}$) and the more lenient threshold ($P < 5 \times 10^{-6}$) for COVID-19

325   SNPs from HGI release 5 (Supplemental Tables 14–21). In addition, we used SNPs associated

326   with COVID-19 from HGI release 4, and we did not observe any causal effect of COVID-19 on

327   PUFAs (Supplemental Tables 22–29). Importantly, the reverse MR results showed no significant

328   association of genetically predicted COVID-19 severity with AA and DPA-n3, suggesting that the

329   significant forward MR results are unlikely to be confounded by reverse causation.

Discussion

330

331   Our observational analysis in a prospective cohort showed that total PUFAs, omega-3 PUFAs,

332   omega-6 PUFAs, DHA, and LA in baseline plasma samples were inversely associated with the

333   risk of severe COVID-19. There were also inverse associations of omega-3 PUFAs and DHA with

334   COVID-19 susceptibility. In contrast, the omega-6/omega-3 ratio was positively associated with

335   both COVID-19 susceptibility and severity. In our bidirectional two-sample MR analyses, we

336   provided evidence for the potential causal roles of higher circulating AA and DPA-n3 in a lower

337   risk of COVID-19 severity.

338

339   Our observational findings are broadly consistent with previous observational studies and a pilot

340   clinical trial. Julkunen *et al.* also examined the UK Biobank cohort, although with smaller sample

341   sizes and different controls. They showed that for total PUFAs, omega-3 PUFAs, omega-6 PUFAs,

342   DHA, and LA, their absolute levels and relative percentages in total fatty acids were both inversely

343   associated with the risk of severe COVID-19 when comparing patients to non-cases with unknown

344   COVID-19 status (13). Our study corrected for potential selection bias by restricting the analysis

345   to individuals with COVID-19 testing status and used those with negative tests or non-hospitalized

346   patients as the controls. We confirmed the same inverse association patterns for severe COVID-

347   19. We further showed that omega-3 PUFAs and DHA were inversely associated with COVID-19

348   susceptibility. Another study investigated the metabolic fingerprint of COVID-19 severity in 581

349   samples from three cohorts, revealing inverse associations with severity for total PUFAs, omega-

350   6 PUFAs, and LA. But inconsistent associations of omega-3 PUFAs, DHA, and the omega-

351   6/omega-3 ratio were also observed across cohorts (11). Comparing the lipid profile of 42 severe

352   COVID-19 patients to 22 healthy subjects, a study by Perez-Torres *et al.* found that plasma GLA,

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353   DGLA, and EPA were decreased in COVID-19 patients, but LA and AA were elevated (12). Two

354   small studies found that the omega-3 index was significantly lower in COVID-19 patients and was

355   inversely associated with risks of requiring mechanical ventilation and death (9, 10). The

356   differences in these observational studies are likely results of uncontrolled confounding factors or

357   the usage of patients at different disease stages. In support of the associated protective effect of

358   omega-3 fatty acids, the first randomized clinical trial of supplementing 1000 mg omega-3 fatty

359   acids in 128 critically ill COVID-19 patients showed that the intervention group has a significantly

360   higher one-month survival rate and improved respiratory and renal function (45). Altogether with

361   the existing literature, our study supports the protective effects of omega-3 fatty acids against the

362   development of severe COVID-19 and likely also against viral infection. There are probably

363   protective benefits of omega-6 fatty acids against severe COVID-19, but a high omega-6/omega-

364   3 ratio may increase the risks of both COVID-19 susceptibility and severity.

365

366   In our MR study, we examined whether specific individual PUFAs play causal roles in COVID-

367   19 susceptibility and severity. We found that genetically instrumented circulating levels of AA

368   and DPA-n3 are associated with a lower risk of severe COVID-19. AA is an omega-6 fatty acid,

369   while DPA-n3 is an omega-3 fatty acid. Although these two specific PUFAs were not available in

370   our observational analysis, their potentially causal protective effects are consistent with the inverse

371   associations of both omega-6 PUFAs and omega-3 PUFAs with severe COVID-19. The potential

372   protective roles of AA and DPA-n3 in severe COVID-19 have mechanistic support. Both of them

373   are well-known to serve as precursors of specialized pro-resolving mediators, such as lipoxins

374   derived from AA, resolvins, protectins and maresins derived from DPA-n3, playing essential roles

375   in promoting the resolution of inflammatory responses and tissue repair (5, 7, 46). Notably, it has

376   been highlighted that the roles of AA in initiating timely inflammatory responses through its

377   derived prostaglandins (PGs), such as PGE_2, may be as important as its roles in inflammatory

378   resolution through lipoxins (6, 47). Another possible mechanistic route for AA could be drawn

379   from a human cell line study (48). Huh-7 cells, a hepatocyte-derived carcinoma cell line, when

380   infected with human coronavirus 229E (HCoV-229E), exhibit significantly elevated levels of LA

381   and AA, a pattern also observed in a study of severe COVID-19 patients (12). Interestingly,

382   exogenous supplementation of LA and AA in HCoV-229E-infected cells significantly suppressed

383   HCoV-229E virus replication. Similar suppressive effects were observed for the highly pathogenic

384   Middle East respiratory syndrome coronavirus (MERS-CoV) (48), suggesting a possible general

385   mechanism of LA and AA on coronavirus. Additionally, LA has been shown to directly and tightly

386   bind the SARS-CoV-2 spike glycoprotein, reducing its interaction with the human ACE2 receptor

387   (49). Similar inhibitory effects were observed for ALA, EPA, and DHA in a ligand screening study

388   (50), which did not include AA and DPA-n3. Our MR findings call for future studies into the

389   mechanistic roles of AA and DPA-n3 in the development of severe COVID-19.

390

391   Our study has a number of strengths and novel features. To our knowledge, this is the first MR

392   study examining the causal effects of PUFAs on COVID-19. It is also the first MR study of PUFAs

393   that used genetic variants for RBC PUFAs, in addition to plasma PUFAs. We applied bidirectional

394   two-sample MR analyses to evaluate the direction of the causality and to rule out the impacts of

395   reverse causation. To obtain robust evidence, we required the reported patterns to be observed with

396   both plasma and RBC PUFAs. Similarly, to ensure reproducibility across data releases, we

397   confirmed the results with analyses based on four COVID-19 GWAS (A2, B2, B1, and C2) from

398   HGI releases 5 and 4. Bonferroni correction was used to overcome the issue of multiple testing.

399   Another strength is the sensitivity analysis with various LD cutoffs. Additionally, comparing our

400   MR results between severe COVID-19 and any SARS-CoV-2 infection, we found that AA and

401   DPA-n3 might mainly impact the severity of disease progression but not susceptibility to viral

402   infection.

403

404   Our study has several limitations. First, we could not completely rule out the possibility that some

405   genetic variants might be pleiotropic, although we applied multiple sensitivity analyses, including

406   the heterogeneity test, MR-Egger, and WM method. Second, a limitation of this MR study is that

407   the effect of endogenous PUFAs may be different from the effect of dietary PUFAs, and our study

408   did not directly examine dietary PUFAs. However, leveraging genetic instruments yields novel

409   insights and minimizes the measurement error from self-reported dietary consumption in nutrition

410   studies. Third, another limitation is that the population controls were utilized with no information

411   on COVID-19 status in three COVID-19 GWAS used in our primary analysis, including the HGI

412   A2, B2, and C2 studies. To mitigate this issue, we also utilized the HGI B1 study, which is another

413   GWAS of COVID-19 using non-hospitalized patients as the control group. Fourth, in the

414   observational study, UK Biobank recruited healthier individuals and thus may not be

415   representative of the general population. Fifth, the NMR-based measurements of plasma PUFAs

416   were collected over ten years before the COVID-19 pandemic, and the time lag likely attenuates

417   the magnitude of association. Sixth, our observational study could be affected by ascertainment

418   bias in differential healthcare seeking and testing. Seventh, our findings might not be extrapolated

419   to other ethnicities because the study only focused on participants of European descent. Eighth,

420   our study can not thoroughly explain the mechanisms. Further mechanistic research is necessary

421   to investigate the biological pathways underpinning the roles of PUFAs in severe COVID-19.

422

423   In conclusion, our observational analysis in a prospective cohort shows that total PUFAs, omega-

424   3 PUFAs, omega-6 PUFAs, DHA, and LA are inversely associated with the risk of severe COVID-

425   19. Omega-3 and DHA may also be protective against SARS-CoV-2. A higher omega-6/omega-3

426   ratio has adverse effects on both COVID-19 susceptibility and severity. Our MR study further

427   suggests a possible causal role of AA and DPA-n3 in reducing the risk of severe COVID-19. Our

428   findings call for further studies into the mechanistic roles of PUFAs in COVID-19. They also

429   support the possible usage of circulating PUFA levels as biomarkers for identifying high-risk

430   individuals and as therapeutic targets for managing COVID-19 patients.

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588

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592

593 The authors' responsibilities were as follows—YS and KY: designed the study, provided statistical

594 advice, and interpreted the data; YS: performed data analysis and prepared visualizations; YS, RC,

595 and AR: provided material support during the study; YS: wrote the paper; KY: critically revised

596 the paper; and all authors: read and approved the final manuscript and took responsibility for the

597 integrity of the work as a whole. The authors report no conflicts of interest.

598

Data availability

599

600 The COVID-19 data (GWAS summary statistics) used in this study are freely accessible in the

601 COVID-19 Host Genetics Initiative (https://www.covid19hg.org/). The code for the analyses is

602 available at https://github.com/yitangsun/COVID19_PUFA_MR.

603

Notes

604

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610

611 Supplemental Tables 1–29 are available from the "Supplementary data" link in the online posting

612 of the article and from the same link in the online table of contents at

613 https://academic.oup.com/ajcn/.

614

615 Abbreviations used: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory

616 syndrome coronavirus 2; PUFAs, polyunsaturated fatty acids; LA: linoleic acid; EPA,

617 eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cell; AA, arachidonic acid;

618 MR, Mendelian randomization; BMI, body mass index; GWAS, genome-wide association studies;

619 HGI, COVID-19 Host Genetics Initiative; FHS, Framingham Heart Study; STROBE,

620 strengthening the reporting of observational studies in epidemiology; STROBE-MR, strengthening

621 the reporting of Mendelian randomization studies; ALA, α-Linolenic acid; DPA-n3,

622 docosapentaenoic acid; GLA, γ-Linoleic acid; DGLA, dihomo-γ-linoleic acid; DTA,

623 docosatetraenoic acid; NMR, nuclear magnetic resonance; SD, standard deviation; SNPs, single

624 nucleotide polymorphisms; LD, linkage disequilibrium; OR, odds ratio; IVW, inverse variance-

625 weighted; WM, weighted median; CI, confidence interval; PGs, prostaglandins; HCoV-229E,

626 human coronavirus 229E; MERS-CoV, Middle East respiratory syndrome coronavirus.

Tables

TABLE 1 Characteristics of the UK Biobank participants at baseline[1]

Characteristics	England				England, Scotland, and Wales	
	Hospitalized COVID-19	Non-hospitalized COVID-19	Test positive	Test negative	Test positive	Test negative
Participants, n	4,209	12,240	16,449	76,307	17,395	86,717
Participants with plasma PUFA measures, n	970	2,903	3,873	18,293	4,101	20,626
Age, y	59 [40-70]	51 [40-70]	52 [40-70]	59 [40-70]	52 [40-70]	59 [40-70]
Females, n (%)	445 (46)	1,559 (54)	2,004 (52)	9,771 (53)	2,123 (52)	11,145 (54)
Body mass index, kg/m2 (SD)	29.55 (5.61)	27.96 (4.94)	28.36 (5.16)	27.69 (4.88)	28.36 (5.14)	27.71 (4.89)
PUFAs, mmol/l (SD)	4.82 (0.81)	4.92 (0.78)	4.89 (0.79)	4.97 (0.804)	4.89 (0.78)	4.96 (0.801)
Omega-3 PUFAs, mmol/l (SD)	0.48 (0.203)	0.49 (0.205)	0.49 (0.204)	0.53 (0.22)	0.49 (0.203)	0.53 (0.22)
DHA, mmol/l (SD)	0.21 (0.07)	0.22 (0.08)	0.22 (0.08)	0.24 (0.08)	0.22 (0.08)	0.23 (0.08)
Omega-6 PUFAs, mmol/l (SD)	4.34 (0.699)	4.42 (0.66)	4.401 (0.67)	4.44 (0.68)	4.402 (0.67)	4.44 (0.68)
LA, mmol/l (SD)	3.29 (0.698)	3.39 (0.65)	3.37 (0.67)	3.39 (0.69)	3.37 (0.66)	3.39 (0.68)

1   Values are numbers (%) for categorical variables, mean (SD) or medians [range] for continuous variables. PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; LA linoleic acid.

TABLE 2 Associations of PUFAs concentrations with COVID-19 susceptibility and severity[1]

Plasma PUFA measures	COVID-19 severity						COVID-19 susceptibility					
	Hospitalized *vs.* Non-hospitalized (*n* = 3,873)			Hospitalized *vs.* Test negative (*n* = 19,263)			Test positive *vs.* Test negative (*n* = 22,166)[2]			Test positive vs. Test negative (*n* = 24,727)[3]		
	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
PUFAs	-0.139	0.043	0.0012	-0.127	0.037	0.0005	-0.029	0.019	0.1285	-0.027	0.018	0.1337
Omega-3 PUFAs	-0.140	0.044	0.0013	-0.197	0.040	8.1×10^{-7}	-0.083	0.020	4.3×10^{-5}	-0.082	0.019	2.3×10^{-5}
DHA	-0.176	0.045	8.9×10^{-5}	-0.247	0.042	4.6×10^{-9}	-0.098	0.021	3.0×10^{-6}	-0.097	0.020	1.4×10^{-6}
Omega-6 PUFAs	-0.121	0.043	0.0047	-0.090	0.036	0.0121	-0.010	0.019	0.6183	-0.008	0.018	0.6656
LA	-0.113	0.043	0.0079	-0.082	0.036	0.0228	-0.007	0.019	0.7289	-0.006	0.018	0.7299
Omega-6/omega-3	0.114	0.042	0.0061	0.124	0.029	1.5×10^{-5}	0.053	0.018	0.0030	0.058	0.017	0.0005

1   Effect sizes (β) per SD increase in the exposure, SEs, and *P* values were obtained from the logistic regression analysis of COVID-19 susceptibility and severity. All models were adjusted for age, sex, ethnicity, BMI, Townsend deprivation index, and assessment center. PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; LA linoleic acid.

2   Data from England only.

3   Data from England, Scotland, and Wales.

Figure Legends

Figure 1. Flowchart of the UK Biobank participants from recruitment to inclusion in the observational analysis.

Figure 2. Mendelian randomization estimates of the effects of polyunsaturated fatty acids on COVID-19 severity risk. (A) Mendelian randomization analysis based on the release 5 HGI A2. (B) Mendelian randomization analysis based on the release 5 HGI B2. (C) Mendelian randomization analysis based on the release 5 HGI B1. Odds ratios are scaled to a genetically predicted SD increase in polyunsaturated fatty acids. Associations with p-value < 0.05 were indicated with diamonds, while others with squares. Detailed summary statistics are available in Supplemental Tables 6–8. PUFA, polyunsaturated fatty acid; ALA, α-Linolenic acid; LA: linoleic acid; GLA, γ-Linoleic acid; DGLA, dihomo-γ-linoleic acid; AA, arachidonic acid; DPA-n3, docosapentaenoic acid; DTA, docosatetraenoic acid; DHA, docosahexaenoic acid; OR, odds ratio.
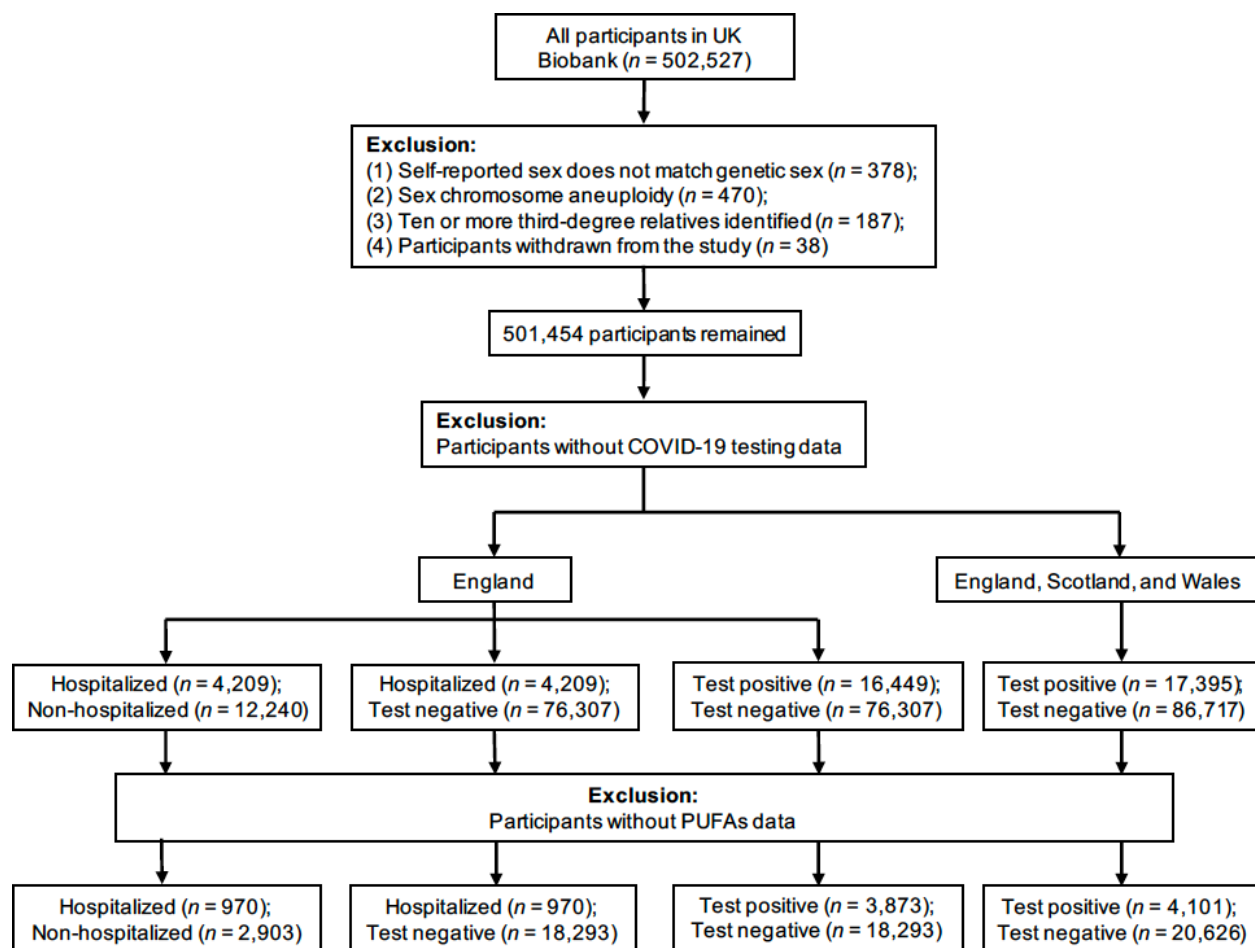
Figure 3. Mendelian randomization estimates of the effects of polyunsaturated fatty acids on COVID-19 susceptibility risk based on the release 5 HGI C2. Odds ratios are scaled to a genetically predicted SD increase in polyunsaturated fatty acids. Associations with p-value < 0.05 were indicated with diamonds, while others with squares. Detailed summary statistics are available in Supplemental Table 9. PUFA, polyunsaturated fatty acid; ALA, α-Linolenic acid; LA: linoleic acid; GLA, γ-Linoleic acid; DGLA, dihomo-γ-linoleic acid; AA, arachidonic acid; DPA-n3, docosapentaenoic acid; DTA, docosatetraenoic acid; DHA, docosahexaenoic acid; OR, odds ratio.
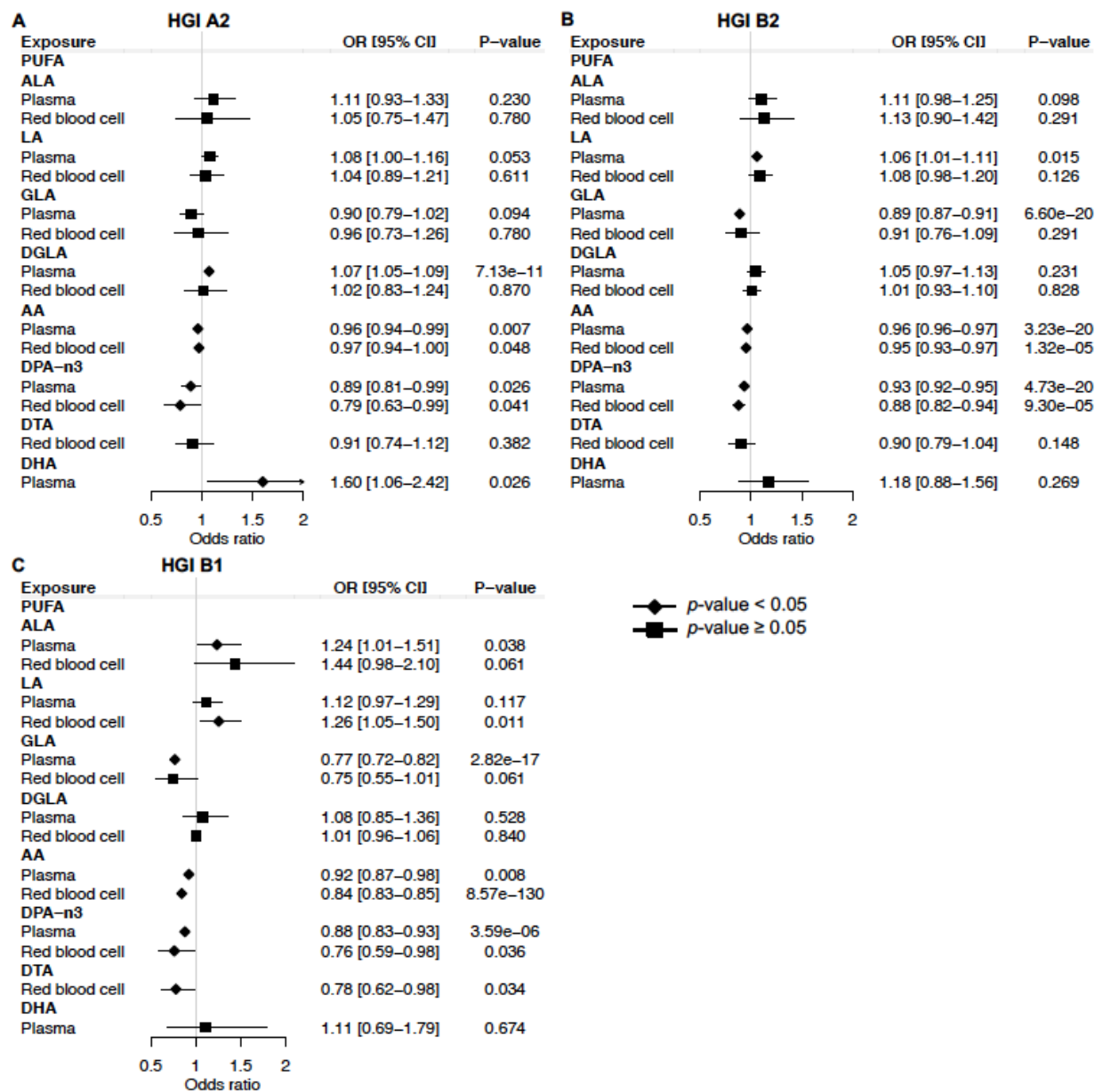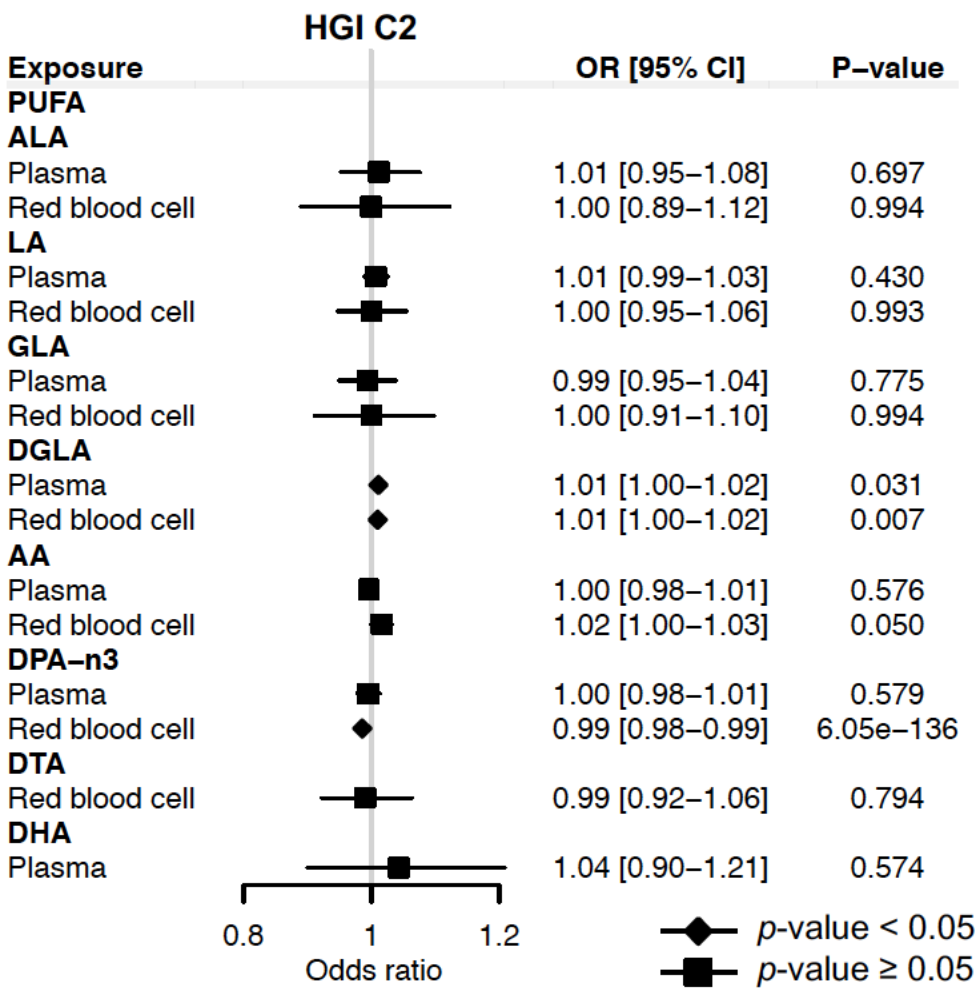
Figures

FIGURE 1

FIGURE 2

FIGURE 3

Supplemental Tables

Supplemental Table 1. Notable Mendelian randomization studies of polyunsaturated fatty acids.

Supplemental Table 2. STROBE Statement—checklist of items that should be included in reports of cohort studies.

Supplemental Table 3. STROBE-MR checklist.

Supplemental Table 4. Genetic instruments for plasma polyunsaturated fatty acids.

Supplemental Table 5. Significant SNPs for red blood cell polyunsaturated fatty acids.

Supplemental Table 6. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 5 HGI A2.

Supplemental Table 7. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 5 HGI B2.

Supplemental Table 8. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 5 HGI B1.

Supplemental Table 9. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 susceptibility based on the release 5 HGI C2.

Supplemental Table 10. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 4 HGI A2.

Supplemental Table 11. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 4 HGI B2.

Supplemental Table 12. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 severity based on the release 4 HGI B1.

Supplemental Table 13. Forward Mendelian randomization estimates of associations of genetically predicted polyunsaturated fatty acids with COVID-19 susceptibility based on the release 4 HGI C2.

Supplemental Table 14. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI A2 (COVID-19 SNP P < 5e-8).

Supplemental Table 15. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI B2 (COVID-19 SNP P < 5e-8).

Supplemental Table 16. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI B1 (COVID-19 SNP P < 5e-8).

Supplemental Table 17. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 susceptibility with polyunsaturated fatty acids based on the release 5 HGI C2 (COVID-19 SNP P < 5e-8).

Supplemental Table 18. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI A2 (COVID-19 SNP P < 5e-6).

Supplemental Table 19. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI B2 (COVID-19 SNP P < 5e-6).

Supplemental Table 20. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 5 HGI B1 (COVID-19 SNP P < 5e-6).

Supplemental Table 21. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 susceptibility with polyunsaturated fatty acids based on the release 5 HGI C2 (COVID-19 SNP P < 5e-6).

Supplemental Table 22. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI A2 (COVID-19 SNP P < 5e-8).

Supplemental Table 23. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI B2 (COVID-19 SNP P < 5e-8).

Supplemental Table 24. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI B1 (COVID-19 SNP P < 5e-8).

Supplemental Table 25. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 susceptibility with polyunsaturated fatty acids based on the release 4 HGI C2 (COVID-19 SNP P < 5e-8).

Supplemental Table 26. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI A2 (COVID-19 SNP P < 5e-6).

Supplemental Table 27. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI B2 (COVID-19 SNP P < 5e-6).

Supplemental Table 28. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 severity with polyunsaturated fatty acids based on the release 4 HGI B1 (COVID-19 SNP P < 5e-6).

Supplemental Table 29. Reverse Mendelian randomization estimates of associations of genetically predicted COVID-19 susceptibility with polyunsaturated fatty acids based on the release 4 HGI C2 (COVID-19 SNP P < 5e-6).