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The role of circulating polyunsaturated fatty acids in mediating the effect of BMI on leukocyte telomere length: analysis using Mendelian randomization

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Abstract

Background polyunsaturated fatty acids (PUFAs) are a category of fatty acids that contain omega-3 and omega-6 fatty acids, which constitute a substantial portion of the Western diet and are vital for maintaining human wellness. The extent to which circulating PUFAs influence the effects of BMI on leukocyte telomere length (LTL) is unknown. Additionally, the impact of circulating PUFA on LTL remains controversial in observational studies.

Methods Using publicly accessible datasets, a genome-wide association study (GWAS) was carried out to determine genetic association estimates for BMI, circulating PUFAs, and LTL. The circulating PUFAs considered were omega-3 PUFAs (i.e., docosahexaenoic acid (DHA) and total omega-3 PUFAs) and omega-6 PUFAs (i.e., linoleic acid (LA) and total omega-6 PUFAs). Two-sample Mendelian randomization (MR) was used to investigate the causal relationships between BMI and PUFA with LTL. Additionally, we examined whether certain PUFA mediate the impact of BMI on LTL.

Results None of the evidence supported a causal effect of genetically predicted DHA and total omega-3 PUFA on LTL (DHA: $\beta = 0.001$, 95% CI: −0.023 to 0.026, $p = 0.926$; total omega-3 PUFA: $\beta = 0.008$, 95% CI: −0.013 to 0.029, $p = 0.466$). After conducting sensitivity analyses to account for various models of horizontal pleiotropy, the causal association between higher levels of LA and longer LTL persisted ($\beta = 0.034$, 95% CI 0.016 to 0.052, $p < 0.001$). Adjusting for LA in genetics reduced the effect of BMI on LTL from β = -0.039 (95% CI: -0.058 to -0.020, $p < 0.001$) to -0.034 (95% CI: -0.054 to -0.014, $p < 0.001$).

Conclusions This MR study indicates that an increase in genetically predicted circulating LA levels is associated with longer LTL. Additionally, it appears that circulating LA levels play a role in mediating some of the impact that BMI has on LTL.

Keywords Mendelian randomization, Genetic association, Circulating polyunsaturated fatty acids, BMI, Leukocyte telomere length

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Introduction

Leukocyte telomere length (LTL), a chromosomal end marker for biological ageing, is made up of repeating nucleotide sequences and related protein complexes [1]. As cells divide, the inability of their replicative machinery to fully copy chromosome ends leads to the loss of 50–100 base pairs, resulting in LTL attrition with increasing cellular age [2]. In cases where LTL becomes excessively shortened, it may induce replicative senescence, continued division, or cell death, mutation, and genetic abnormalities [3]. Furthermore, in the pursuit of understanding the complexities of aging, prior research has revealed a compelling association between shortened LTL and a range of age-related conditions, including, but not limited to, cancer and coronary artery disease [4].

Body mass index (BMI) is a commonly used indicator of obesity that has been linked to various health outcomes [5]. Observationally, empirical evidence indicates that a higher BMI tends to correspond to a shorter LTL [6, 7]. Several potential pathways have been proposed to clarify the connection between BMI and LTL, including inflammation, oxidative stress, and metabolic dysfunction [7]. However, some studies have come to completely opposite conclusions [8]. Moreover, most of the aforementioned studies have not explained in detail which specific biological processes link BMI and LTL together, making the relationship of causality between BMI and LTL ambiguous. Therefore, a better understanding of the interwoven causality between BMI and LTL and an uncovering of the deeper processes that mediate the effect of BMI on LTL are critical for the administration and avoidance of ageing and age-related diseases.

The potential mediating impact of circulating polyunsaturated fatty acids (PUFAs) on the relationship between BMI and LTL may be an important research topic. PUFAs are a category of fatty acids that contain omega-3 and omega-6 fatty acids, which constitute a substantial portion of the Western diet and are vital for maintaining human wellness [9]. They are involved in multiple physiological processes, including inflammation, oxidative stress, and cell signalling which are also interconnected with BMI and LTL [10, 11]. However, to the best of our knowledge, the causal link that exists between PUFAs and BMI or LTL has not been fully revealed. Several investigations have shown that the activities of various enzymes taking part in PUFA metabolic processes in individuals with high BMI, such as delta-6-desaturase and delta-5-desaturase, will change, thus affecting the level of PUFAs in vivo [12–14]. Recent evidence also suggests that the composition of gut microbiota could be an important factor linking BMI and PUFAs [15]. The Gut microbiota is essential to the regulation of the host's metabolism and energy balance, and thus may be involved in the metabolism of PUFAs [15]. Furthermore, significant compositional differences exist in gut microbiota between individuals with different BMIs, such as obese individuals and healthy individuals [16]. Several studies have reported that changes in gut microbiota composition, such as an increase in Lactobacillus, are associated with lower BMI and increased levels of certain PUFAs [15, 17, 18]. Meanwhile, PUFAs may have a direct impact on LTL in various ways, such as affecting inflammation, oxidative stress, and telomerase activity, among others [19, 20]. Finally, given the potential role of circulating PUFAs in both the BMI- PUFA and PUFA-LTL relationships, it is plausible that circulating PUFAs may mediate the effect of BMI on LTL. Further investigation into this potential mediating role could have important implications for understanding the underlying mechanisms linking obesity, PUFAs, and cellular ageing. Additionally, despite there being many previous studies reporting a potential causal relationship between PUFAs and LTL, the results are inconsistent [21–23]. This inconsistency highlights the necessity of integrating multiple lines of evidence to further explore their causal relationships.

Mendelian randomization (MR) is an innovative approach with the help of genetic variants as instrumental variables to provide more robust evidence for causal inference. It is possible to circumvent the biases associated with traditional research methods such as observational studies (e.g., reverse causality and residual confounding) by randomly allocating genotypes before conception, which simulates natural, randomized, controlled study circumstances [24]. Furthermore, MR has been increasingly used in epidemiological research to examine the causal connections between exposures and outcomes, including mediation effects [25].

With the help of MR, we aimed to determine to the causal relationships between BMI and PUFAs with LTL and whether the correlation between BMI and LTL is mediated by circulating PUFAs in the present study. MR provides a powerful tool for identifying causal associations based on genetic variants, which allows for the examination of relationships in human populations without the confounding effects of reverse causality or biases [24]. However, while MR analysis can suggest causal links, it is often limited by the availability of data and the complexity of underlying biological mechanisms. To further validate these findings and gain deeper mechanistic insights, we incorporated animal experiments.

Specifically, we examined whether circulating PUFAs, identified as potential mediators of the BMI-LTL relationship in MR studies, exhibit differential expression in an obesity mouse model, using C57BLKS/J db/db mice (DB mice), which have a homozygous mutation of the leptin receptor (LEPR) gene, leading to obesity, polydipsia, and polyphagia, and are commonly used to study

obesity [26], in comparison with control C57BL/6 mice (C57 mice). These animal experiments allow for more direct measurements of molecular and metabolic processes, providing experimental evidence that can help interpret the findings from MR studies. Additionally, we assessed differences in gut microbiota composition and predicted microbial functional profiles between the two groups, exploring potential associations between changes in relevant mediators, such as PUFAs, and gut microbiota differences. By integrating MR analysis with animal experiments, we aim to gain a more comprehensive understanding of the causal pathways and underlying biological mechanisms driving the relationship between BMI, PUFAs, and LTL.

Methods

The STROBE-MR checklist of recommended items to address in reports of MR studies was followed in our study [27].

Overall design of the MR study

Using publicly accessible datasets, a genome-wide association study (GWAS) was carried out to determine genetic association estimates for BMI, circulating PUFAs, and LTL. The circulating PUFAs considered were omega-3 PUFAs (i.e., docosahexaenoic acid (DHA) and total omega-3 PUFAs) and omega-6 PUFAs (i.e., linoleic acid (LA) and total omega-6 PUFAs). Two-sample MR was applied to determine (1) the causal impacts of BMI on LTL, DHA, total omega-3 PUFA, LA, and total omega-6 PUFA and (2) the causal impacts of these circulating PUFA traits on LTL. For the circulating PUFA traits that showed causal relationships with BMI and LTL in steps 1 and 2, we further performed MR mediation analyses to evaluate to what extent they mediated the impact of BMI on LTL. Figure 1 illustrates the overall design of the study.

Furthermore, we align with previous studies on MR for mediation effects [28]. To meet the three core assumptions of MR, all of our two-sample univariable Mendelian randomization (UVMR) analyses, including the assessments of BMI on LTL, BMI on PUFAs, and the evaluation of PUFAs on LTL, as well as the subsequent Multivariable Mendelian randomization (MVMR) analysis evaluating the mediation effect of PUFAs in BMI on LTL, satisfy the three key assumptions: (1) Genetic variants must be strongly associated with the exposure in UVMR analyses and must be strongly associated with at least one of the multiple exposures in MVMR analyses (the relevance assumption); (2) Genetic variants must not be associated with confounders of the exposure-outcome relationship (the independence assumption); (3) The effects of genetic variants on the outcome variable must go only through each exposure and not through any alternative pathways (the exclusion restriction assumption).

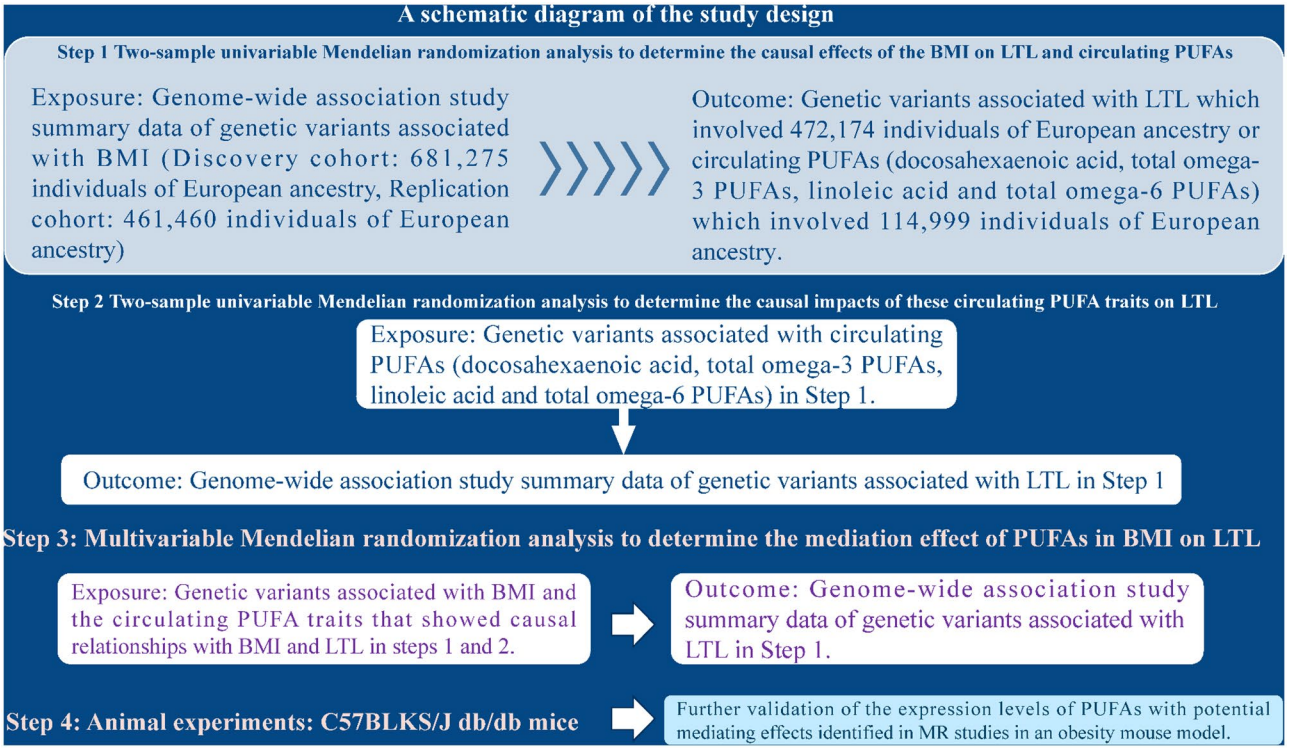

Fig. 1 The overall design of the study

The source of data

Genetic instrumental variable for BMI

A GWAS meta-analysis published in the Genetic Investigation of Anthropometric Traits provided the exposure variable data for the genetic variations connected to BMI (https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files, accessed on 2 January 2023) consortium (n=681,275 individuals of European ancestry) [29]. The IEU Open GWAS database, which contains the other BMI dataset (GWAS ID: ukb-b-19953, 461,460 people of European heritage), was extracted for sensitivity analysis. To enhance clarity and efficiency, we partition the aforementioned two databases into two distinct groups: BMI (GIANT_consortium) and BMI (ukb-b-19953). This delineation will persist throughout the subsequent text.

Potential mediators: fatty acids

The UK Biobank comprises a cohort based on a population size of approximately 500,000 individuals aged 40 to 69 who were selected over various facilities throughout the United Kingdom during the period of 2006 to 2010, representing approximately 5% of those invited [30, 31]. Previous publications have provided comprehensive information on the research project's design, individuals, and quality control procedures [31, 32]. The ethical permit for the UK Biobank has been obtained through the Research Ethics Committee, with reference number 11/NW/0382. Our study utilized data from the UK Biobank projects 30,418 and 15,825.

A high-throughput nuclear magnetic resonance (NMR) metabolomics platform was employed to measure circulating concentrations of omega-3 (including DHA and total omega-3) and omega-6 (including LA and total omega-6) fatty acids. Prerelease information concerning a subset of 126,846 nonfasting plasma samples obtained at baseline or first repeat evaluation was made accessible to initial detectors. A total of 121,577 samples were finally evaluated after duplicates and samples that failed quality control were eliminated. The NMR platform allowed for the simultaneous quantification of 249 metabolic constituents, including lipids, lipoprotein subclasses, fatty acid composition, and low-molecular weight metabolic products. Participants in the UK Biobank had mean DHA concentrations of 0.23 mmol/L (SD 0.08), total omega-3 concentrations of 0.53 mmol/L (SD 0.22), total omega-6 concentrations of 3.41 mmol/L (SD 0.69), and total omega-6 concentrations of 4.45 mmol/L (SD 0.68), which accounted for 0.02, 0.044, 0.29, and 0.38 of total fatty acids, respectively. This platform has been previously reviewed for technical details and epidemiological applications (for more information, see references [33–35]).

Study outcome: telomere length

Genetic correlations with LTL were discovered in 472,174 European subjects from the largest GWAS on telomere length to date [36]. During the baseline evaluation, DNA was collected, and LTL measurements were taken using qPCR [37]. Measurements were presented using the T/S ratio and then log-transformed to roughly follow a normal distribution. Technical factors were managed and adjusted through various quality checks, as explained previously [37]. Z-standardized LTL values were used to compare LTL values with other datasets. There were no exclusions imposed, except for missing data. Similar tactics were used in a previous LTL study [37].

Instrument selection

Single-nucleotide polymorphisms (SNPs) with independent pairwise linkage disequilibrium (LD) $r^2<0.001$ and a genome-wide significant relationship with the exposure ($P<5\times10-8$) were chosen as the instruments for each exposure taken into account in univariable MR analysis. We gathered all SNPs related to BMI or the investigated mediators that were significant across the genome and clumped them with pairwise LD $r^2<0.001$, determined by the lowest P value for their association with any trait, when choosing instruments for MVMR analyses that investigated the mediators of the impact of genetically predicted BMI on LTL. The TwoSampleMR package in R was used for all clumping operations [38]. We did not use proxies and only took into account genetic variations for which association estimates were available for all characteristics in a given analysis. We calculated the minimum detectable sample size in MR analysis for each of the exposures independently, considering a type I error rate of 0.05, 0.8 power, corresponding beta-coefficients in univariable MR analysis, and the total amount of variance explained by the genetic instruments to assess the capacity to identify putative causal connections according to the accessible general statistics [39]. To assess the strength of each genetic instrument, we computed F statistics. Specifically, the cut-off F-statistic value in MR studies should be greater than 10, which indicates that bias in instrumental variable analysis is minimized, and the results are reliable [40].

Univariable mendelian randomization

The multiplicative random-effects inverse-variance weighted (IVW) MR approach was the main analysis applied to determine the influence of genetically predicted BMI and circulating PUFAs on LTL or BMI on circulating PUFAs [41]. The genetic association estimate for the impact of LTL was based on the beta coefficient of each genetic variant.

It is crucial to take into account any potential bias brought on by horizontal pleiotropy if numerous genetic

variations are to be used as instrumental factors in MR. This occurs under conditions where genetic variations have an impact on LTL via separate, unrelated mechanisms from the exposure under consideration. To mitigate this bias, we employed two sensitivity analyses, namely, MR–Egger and weighted median MR [42, 43]. MR–Egger entails weighting the estimates for the accuracy of the SNP-outcome association while regressing the genetic association estimates for SNP-outcome on SNP-exposure [42]. The MR estimate is given by the slope of the regression line, and directional pleiotropy can be assessed by determining whether the angle of the intercept is different from zero [42]. On the other hand, weighted median MR selects the median of the MR estimates from individual variants based on their precision-weighted magnitudes, and calculates the standard errors using bootstrapping [43]. This method can provide consistent estimates even when up to 50% of the instrumental variables are invalid and is shown to have better finite-sample Type 1 error rates than the IVW method, and is often used as a complement to the MR-Egger method [43]. We used the TwoSampleMR package of R to conduct all these univariable MR analyses. Finally, we compared the results of the main IVW MR analysis with the sensitivity analysis to identify any bias arising from pleiotropic variants. To determine the MR estimates, we calculated the impact of a one-unit increase in the exposure of interest, standardized by the corresponding standard deviation (SD). The SD values were obtained from the GWAS data previously described.

Multivariable mendelian randomization

For MVMR mediation analysis, the genetically predicted circulating PUFAs that demonstrated evidence of a causal effect on the LTL in univariable MR were advanced. Our goal was to determine the extent to which circulating PUFAs mediate the effect of BMI on LTL.

Each exposure's total effect on MVMR is divided into direct and indirect effects. This makes it possible to estimate any potential mediating effects as well as the percentage of the effect of the main exposure on the outcome that is mediated by additional exposures. Specifically, with the intercept set at zero and weighted for the precision (i.e., the inverse of their variance) of the variant-LTL genetic association estimates, the variant-LTL genetic association estimates were regressed on the variant-BMI and variant-circulating PUFA genetic association estimates. Both individually and collectively, the considered circulating PUFAs were taken into account in this model. The final beta coefficient of the impact of BMI on the LTL derived from MVMR was subsequently established. The following equation is used to calculate the percentage of the effect of genetically predicted BMI on LTL that was mediated by the taken into account circulating PUFAs [44]:

$$E\ (\%) = \frac{(\sum_{K=1}^{K} \beta 1 * \beta 2_{\mathrm{k}})}{(\sum_{K=1}^{K} \beta 3 + \beta 1 * \beta 2_{k})} \quad (1)$$

where the regression coefficients β1 represent the MR effects of BMI on mediators (such as LA), β2 represents the MR effect of mediator k with LTL adjusted for genetically determined BMI, and β3 represents the MR effect of BMI on LTL adjusted for genetically determined potential mediators. All regression coefficients were determined via MR instrumental analysis using IVW, assuming no correlation between the mediators.

Measuring the strength of evidence

The 0.05, 0.01 and 0.001 P values were utilized to determine statistical significance to varying degrees. We also consider the width of the confidence interval (CI) for the effect size measure (β) of interest as well as the consistency of the findings across the various sensitivity analyses employed when interpreting the evidence that the results provide.

Circulating PUFA levels in an obesity mouse model

We further investigated whether the expression levels of potential mediators involved in the BMI-LTL relationship, as identified in the MR study, differed between obese and nonobese mice in circulation. Detailed methods are provided in the supplementary materials.

Gut microbiota composition analysis and microbial functional profile prediction in an obesity mouse model

Detailed methodologies are available in the Supplementary methods.

Results

Research overview

Supplementary Tables 1–16 present all genetic association estimates and their F statistics that were used in the univariable and MVMR analyses. From 5.4% for BMI (GIANT_consortium) to 10.3% for total omega-6 PUFAs, the amount of variance explained by all of the variants we utilized as instrumental variables for the exposure and possible mediators ranged (Supplementary Table 17). The minimum detectable sample size in MR analysis for each exposure separately is given in Supplementary Table 17. All univariable MR results are provided in Supplementary Table 18. All genetic association estimates used in the univariable analyses are visualized by scatterplot in Supplementary Figs. 1–14. In Supplementary Table 19, MVMR results with estimated proportion mediated are presented. The STROBE-MR Checklist of Recommended

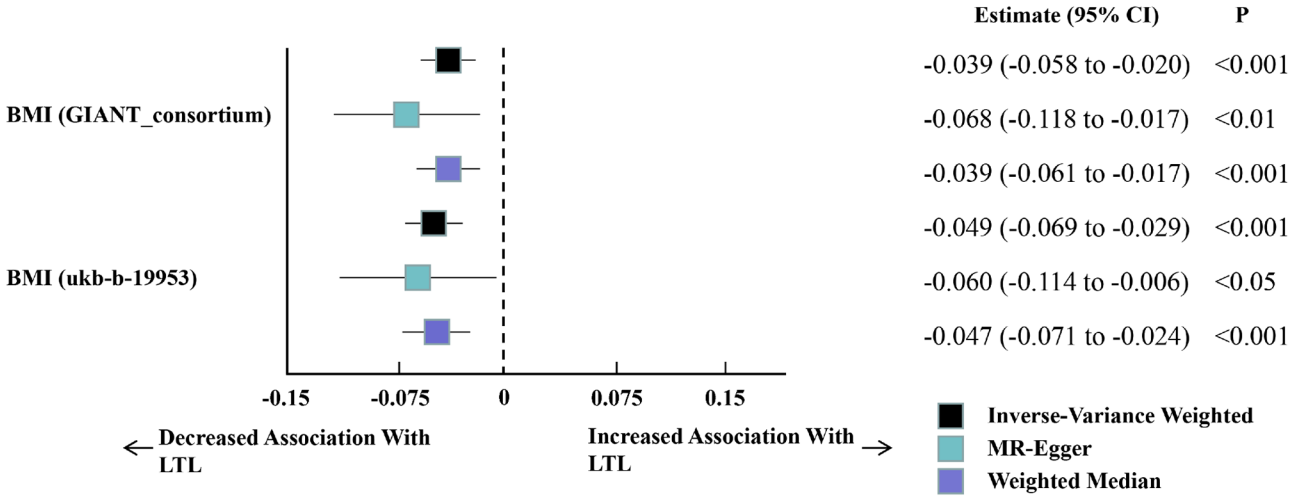

Fig. 2 Effects of genetically predicted BMI on LTL in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

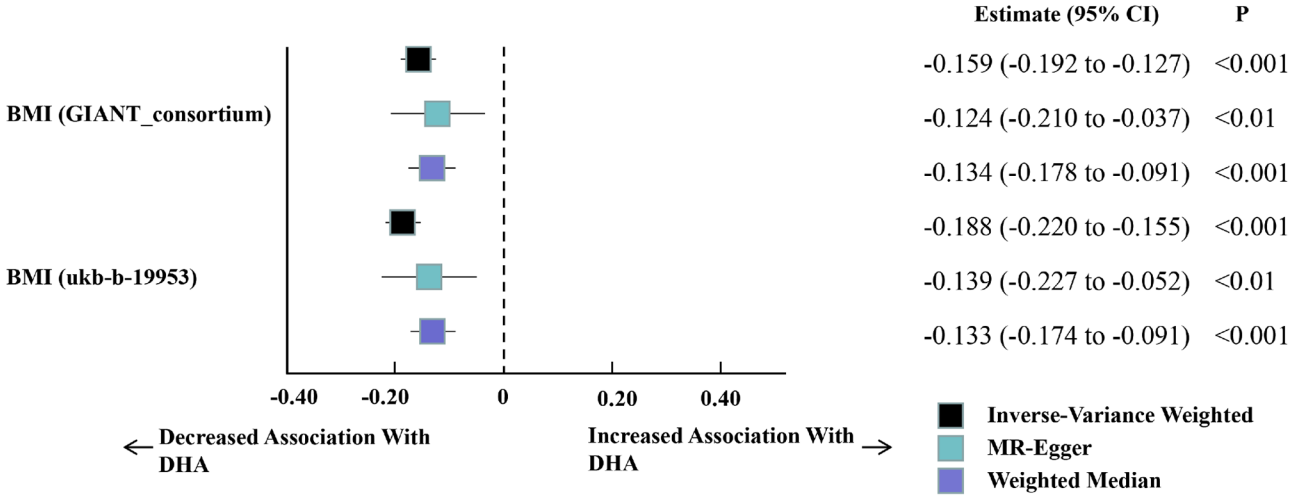

Fig. 3 Effects of genetically predicted BMI on DHA in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

Items to Address in Reports of MR Studies is shown in Supplementary Table 20.

Effects of BMI on LTL

In the univariable MR, there was an unfavourable effect of genetically predicted BMI (GIANT_consortium) on LTL in the main IVW analyses (β = -0.039, 95% confidence interval (CI): -0.058 to -0.020, $p<0.001$), with consistent findings in sensitivity analyses including two other MR methods and other summary statistics of BMI (ukb-b-19953) (Fig. 2).

Effects of BMI on circulating PUFAs

In the univariable MR, there was a causal effect of higher BMI on lower levels of DHA, LA and total omega-6 PUFA in the main IVW analyses (DHA: β = –0.159, 95% CI: –0.192 to –0.127, $p<0.001$; LA: β = –0.120, 95% CI: –0.160 to –0.081, $p<0.001$; total omega-6 PUFA: β = –0.093, 95% CI: –0.134 to –0.052, $p<0.001$), with consistent findings in sensitivity analyses (Figs. 3, 4 and 5). In addition, the main IVW analyses did not support a causal effect of BMI on total omega-3 PUFAs (β = -0.036, 95% CI: –0.075 to 0.003, $p=0.069$), with consistent findings in sensitivity analyses (Fig. 6).

Effects of circulating PUFAs on LTL

As shown in Fig. 7, in the univariate MR analysis, we only found a positive causal effect of LA on LTL in the main IVW analysis (β=0.034, 95% CI 0.016 to 0.052, $p<0.001$), with consistent findings in sensitivity analyses. Additionally, due to the presence of directional horizontal pleiotropy detected by the MR-Egger intercept ($p=0.02$) in

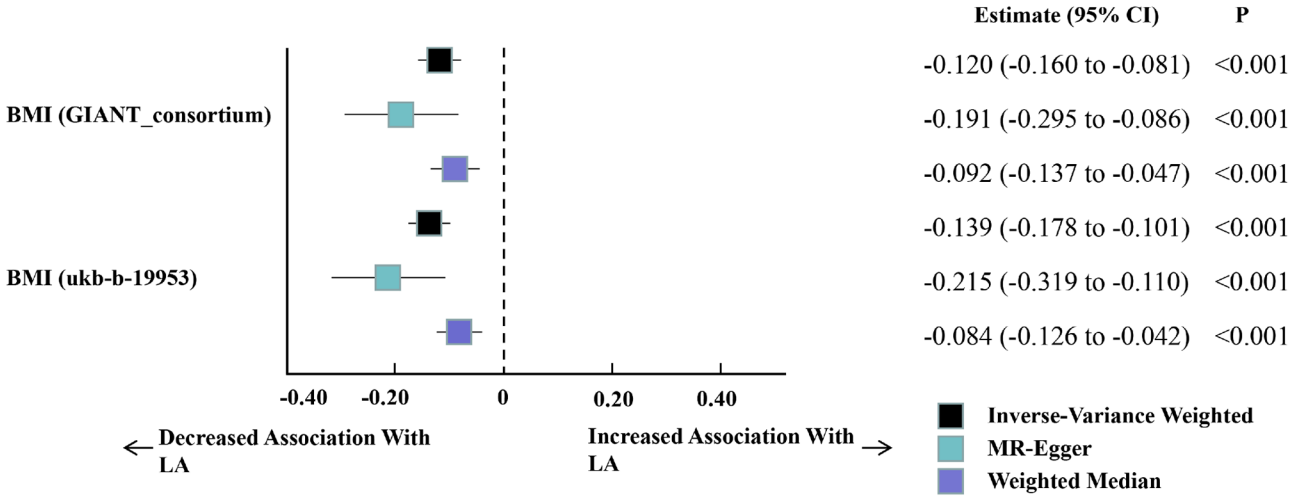

Fig. 4 Effects of genetically predicted BMI on LA in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

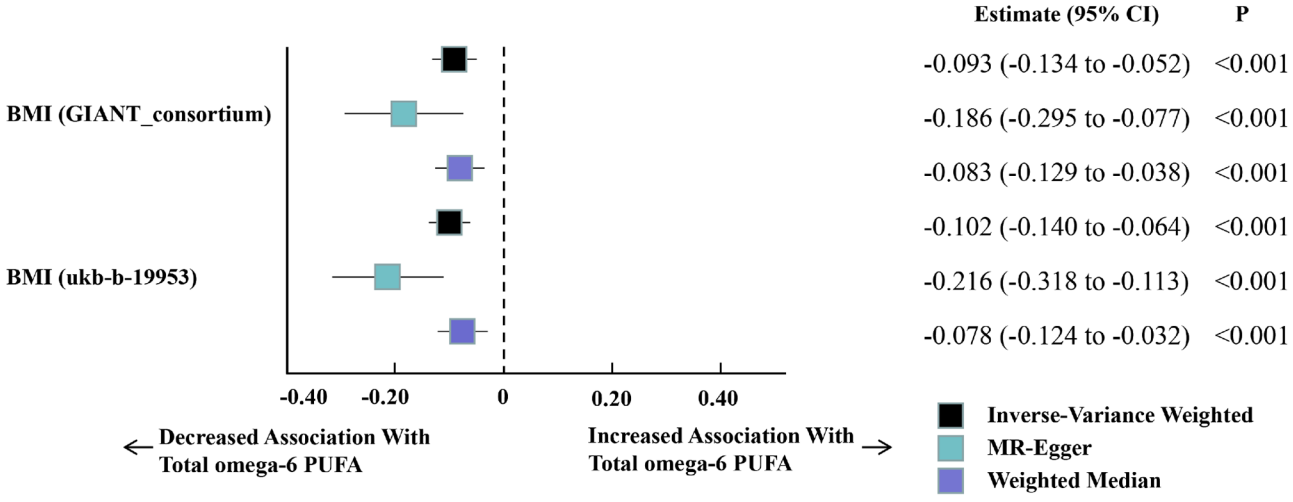

Fig. 5 Effects of genetically predicted BMI on total omega-6 PUFAs in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

the causal effect of LA on LTL (Supplementary Table 18), we further compared the estimate of effect (slope) from MR−Egger with horizontal pleiotropy removed to the IVW results. We found a stronger causal effect of LA on LTL (β=0.068, 95% CI 0.034 to 0.103, $p<0.001$), which further confirms the positive causal effect of LA on LTL. However, although the main IVW analysis supported a positive causal effect of total omega-6 on LTL (β=0.030, 95% CI: 0.012 to 0.048, $p<0.001$), the weighted median method in sensitivity analyses demonstrated a negative result (β=0.016, 95% CI: -0.004 to 0.037, $p=0.115$), which suggests an unstable conclusion. Moreover, none of the evidence supported a causal effect of genetically predicted DHA and total omega-3 PUFA on LTL in the main IVW MR analyses (DHA: β=0.001, 95% CI: –0.023 to 0.026, $p=0.926$; total omega-3 PUFA: β=0.008, 95% CI: –0.013 to 0.029, $p=0.466$), with consistent outcomes acquired in sensitivity analyses.

Mediating effects of circulating PUFAs on BMI–LTL effects

As shown in Fig. 8 and Supplementary Table 19, in this study, we investigated the potential mediator LA, which had causal support from MR for both the effect of BMI on LA (step 1) and its effect on LTL (step 2). The effect of BMI on LTL decreased from β = -0.039 (95% CI: -0.058 to -0.020, $p<0.001$) in univariable IVW analysis to β = -0.034 (95% CI: -0.054 to -0.014, $p<0.001$) after adjusting for genetically predicted LA in MVMR analysis. Sensitivity analyses using other summary statistics of BMI (ukb-b-19953) still showed the existence of the effect (IVW univariable analysis: β = -0.049, 95% CI: -0.069 to -0.029, $p<0.001$; Adjusting for LA in MVMR: β = -0.046, 95%

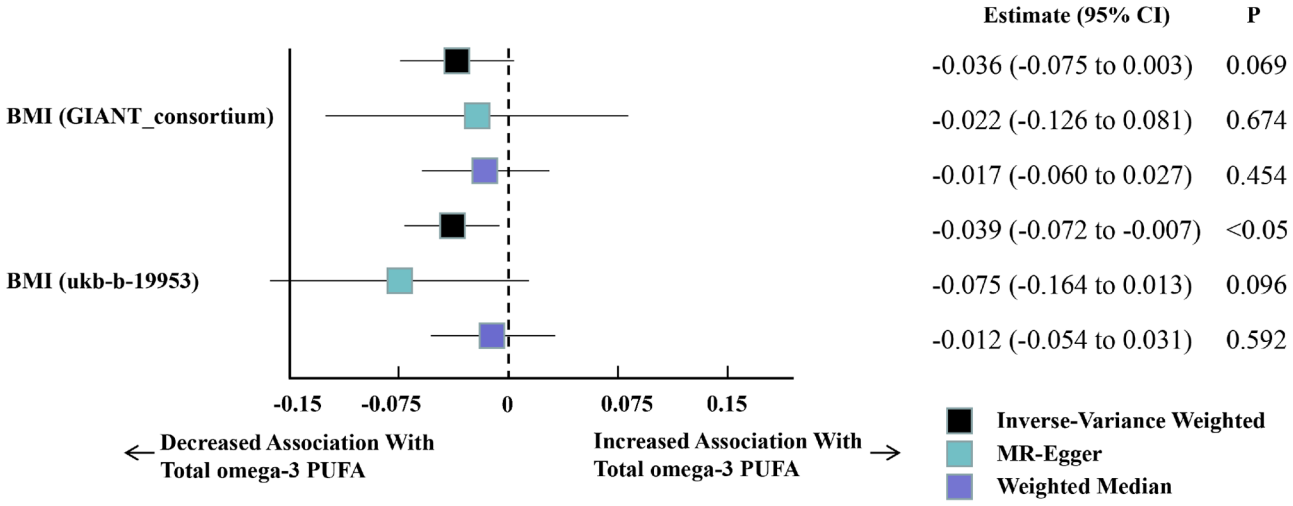

Fig. 6 Effects of genetically predicted BMI on total omega-3 PUFAs in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

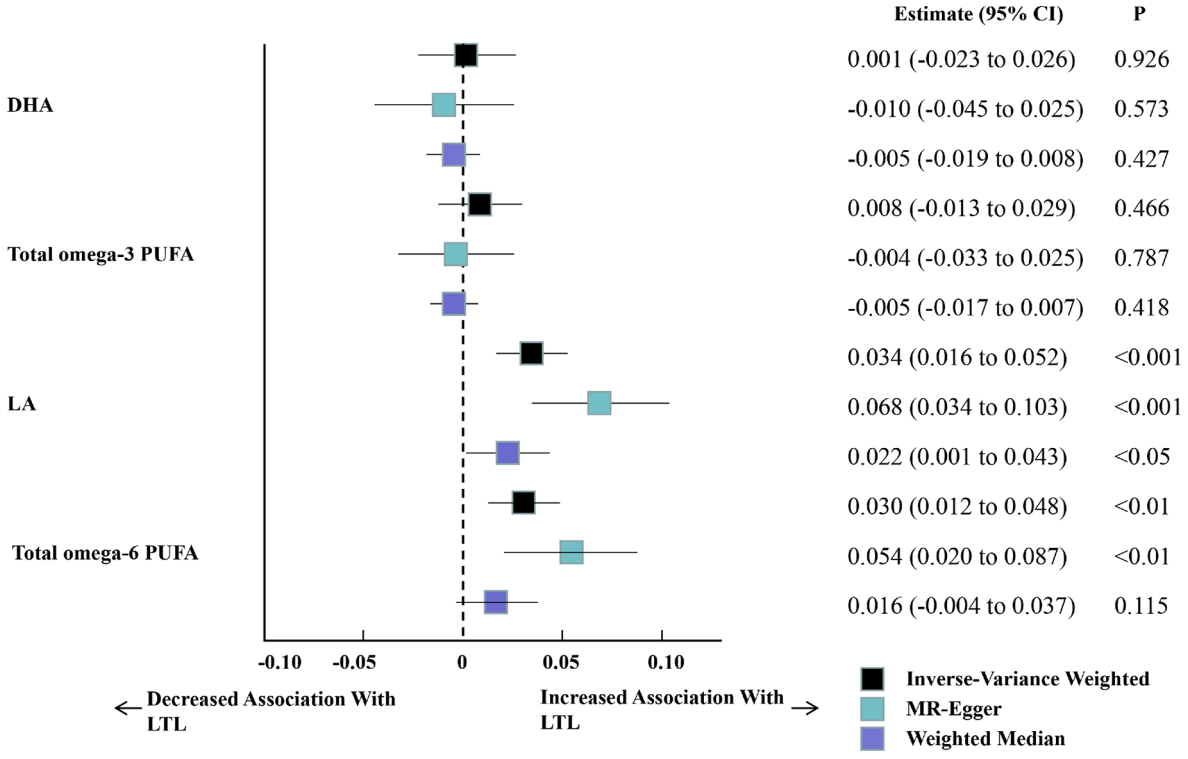

Fig. 7 Effects of genetically predicted circulating PUFAs on LTL in univariable MR analyses. Inverse-variance weighted (IVW), MR–Egger and weighted median represent different Mendelian randomization models. CI = confidence interval

CI: -0.067 to -0.025, $p<0.001$). We estimated the proportion of the effect of genetically predicted BMI mediated through genetically predicted LA as 8.1% and 7.3% in the main and sensitivity analyses, respectively.

Analysis of circulating LA levels, gut microbiota composition, and microbial functional profiles in an obesity mouse model

DB mice and C57 mice were fed a normal diet and monitored for weight, food intake, and water consumption every week for 4 weeks. The results showed that the obesity mouse model was successfully established, with DB mice having significantly higher body weight, food intake,

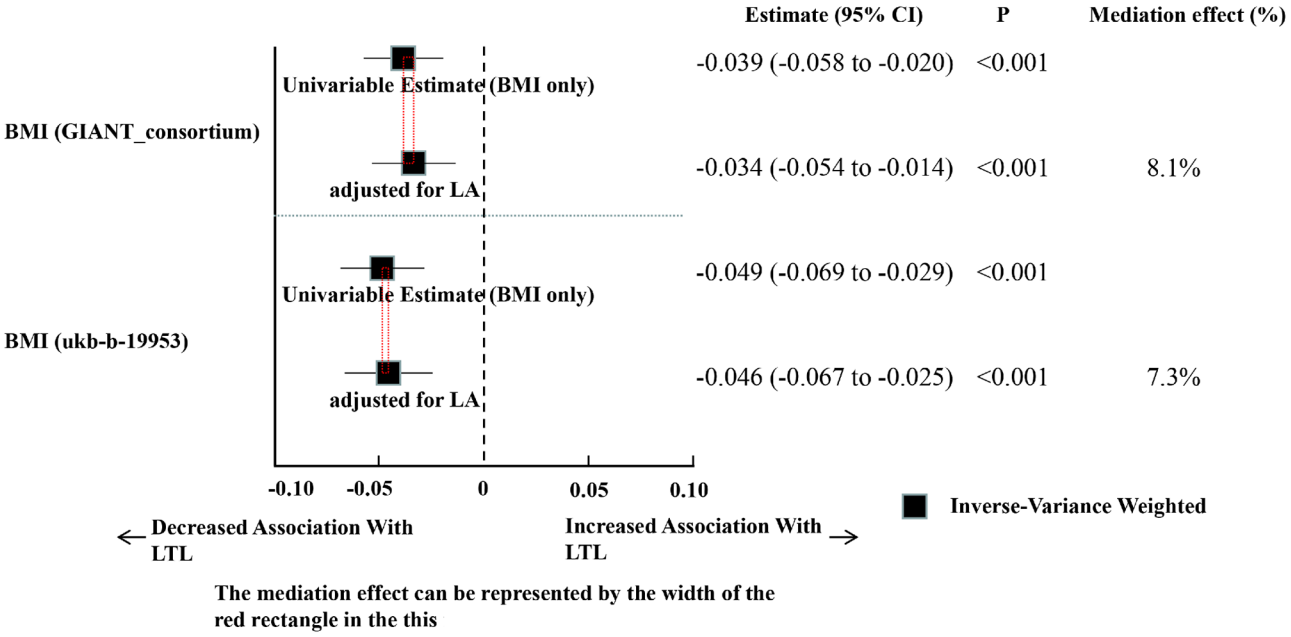

Fig. 8 Multivariable and univariable MR analyses were conducted to assess the effect of genetically predicted BMI on LTL. The multivariable MR analyses were adjusted for genetically predicted LA using the inverse-variance weighted (IVW) MR model. The results of the multivariable MR analyses showed the indirect effect of genetically predicted BMI on LTL via LA. The effects of genetically predicted BMI on LTL in univariable MR analyses were also presented to compare the mediation effect. CI = confidence interval

water consumption, and mean adipocyte diameter than C57 mice (Supplementary Fig. 15). We further investigated and found that the expression level of the potential mediator LA, which has been identified in MR studies as mediating the relationship between BMI and LTL, was lower in the circulation of DB mice (LA: 204.91 μmol/L) than in that of C57 mice (LA: 226.56 μmol/L), although there was no significant difference ($p > 0.05$) (Supplementary Fig. 16a).

Compared to C57 mice, DB mice exhibited a significant decrease in gut microbiota α-diversity (as indicated by a significant decrease in the Shannon index) ($p < 0.05$) (Supplementary Fig. 16b). The Firmicutes/Bacteroidetes (F/B) ratio was higher in the DB mice than in C57 mice, although there was no significant difference ($p > 0.05$) (Supplementary Fig. 16c). Supplementary Fig. 16d displays the relative abundances (%) of the top ten most abundant genera in db mice and C57 mice. In db mice, the most abundant genus was Muribaculaceae (26.0%, 38.8%, 34.7% in each sample, respectively), followed by Lactobacillus (24.1%, 26.3%, 25.6% in each sample, respectively). However, in C57 mice, the most abundant genera were Muribaculaceae (40.2%, 39.1%, 50.6% in each sample, respectively) and Lachnospiraceae (12.9%, 12.9%, 8.7% in each sample, respectively).

The microbiota of the gut interacts with metabolites to affect the host metabolism [45]. We predicted alterations in metabolic functions within the microbiome by KEGG analysis of gene expression differences among DB mouse and C57 mouse groups. The results showed that the pathways of "lipid metabolism" ($p < 0.01$) and "nucleotide metabolism" ($p < 0.01$) were upregulated in DB mice (Supplementary Fig. 16e).

Discussion

The key findings of the MR study

This is the first MR study to investigate the extent to which circulating PUFAs contribute to the causal connection between BMI and LTL. Consistent with previous cross-sectional studies, which reported a shorter LTL in individuals with high BMI, such as those with obesity, suggesting a negative correlation between BMI and LTL [6, 46], we further demonstrate using genetic variants that a higher BMI results in shorter LTL. Our findings also imply that LA plays significant roles in modulating the causal relationship between BMI and LTL. In contrast, our findings do not indicate any causal relationships for the connection between DHA, total omega-3 PUFAs, and total omega-6 PUFAs with LTL. Due to the enormous number of individuals who are overweight or obese worldwide as well as the lack of scalable, efficient treatments for obesity, secondary prevention, which seeks to lower the incidence of obesity-related disorders by focusing on causal mediators, is crucial [47, 48]. Treating causal mediators of the impact of BMI on LTL could attenuate its effect, but limitations in traditional epidemiological approaches to investigating mediation have hampered our comprehension of which variables mediate the

effects of BMI [49, 50]. Overall, our results offer compelling evidence that RCTs involving obese adults should be conducted to investigate the impact of LA on LTL, which has significant therapeutic ramifications for the administration of treatment for and avoidance of ageing and age-related illnesses.

Inflammation and oxidative stress: explaining the negative BMI-LTL causality in MR studies

Cellular senescence is a process that occurs when there is a decline in LTL [36]. Several factors can influence the LTL, including smoking status, socioeconomic status, stress level, lifestyle, reactive oxygen species and inflammation [51, 52]. Our findings support previous research that found a causal association between BMI and LTL, which is in line with earlier research that has shown a correlation between higher BMI and shorter telomeres in obese individuals compared to healthy individuals. This may be due to increased inflammation and oxidative stress in obese individuals, which can contribute to telomere dysfunction [53]. Excessive adiposity leads to the production of adipokines, such as cytokines, hormones, and immunologic factors, that have proinflammatory effects, thus contributing to telomere dysfunction [54]. Previous research has demonstrated that high levels of inflammation, including amyloid A, C-reactive protein, and interleukin (IL)-6, can increase the generation of oxygen free radicals by neutrophils [55]. This, in turn, can damage telomeric DNA and contribute to telomere shortening, as telomeres are highly sensitive to oxidative stress [56].

Potential explanation for the causality between circulating omega-6 PUFA levels and LTL in MR studies

In addition to our study demonstrating a positive correlation between the level of LA in circulating PUFAs and LTL, which is consistent with some previous observational studies [21], it also highlights inconsistencies with others [57]. Over the past decade, there has been significant controversy surrounding the potential causal relationship between circulating PUFAs and LTL, which requires further investigation to determine whether a causal relationship exists [19, 20].

Circulating PUFAs refer to two separate categories of fatty acids: omega-6 and omega-3. Omega-3 and omega-6 PUFAs are necessary lipids that govern many vital body functions; nevertheless, they are metabolically and functionally unique [58]. For instance, the generation of essential components or mediators that control biological processes such as inflammation and oxidative stress is modulated differently by omega-3 and omega-6 PUFAs [59]. These two biological pathways are strongly associated with LTL [60, 61]. Moreover, the impact of omega-3 and omega-6 PUFAs in maintaining LTL might be multifaceted and intertwined, as oxidative stress and inflammation are two closely related and interdependent pathophysiological processes that can both be activated or inhibited by these PUFAs [62]. However, the interaction between omega-3 and omega-6 PUFAs in the backdrop of inflammation and oxidative stress is intricate, and currently, it is poorly understood [63].

Previously, it was thought that consuming more LA (the common omega-6 PUFA) would cause inflammation [64]. However, recent research in healthy human adults has indicated that consuming more LA does not always correlate with higher amounts of several inflammatory markers and may even be associated with lower inflammation [65–67]. In fact, numerous in vitro studies have confirmed the crucial role of LA in anti-inflammatory and antioxidant stress responses, challenging our traditional understanding of LA [68, 69]. Considering that LA may have anti-inflammatory and antioxidant attributes, it is reasonable to suggest that it may increase LTL, as discussed earlier. Several observational studies have supported this viewpoint. For instance, a randomized controlled trial found that LTL was positively correlated with LA levels but was not related to other omega-3 or omega-6 PUFAs [21]. Furthermore, some studies have indirectly suggested the potential benefits of LA by showing its association with reduced mortality in many diseases [70]. Interestingly, we did not discover a direct relationship between total omega-6 PUFAs and LTL, as sensitivity analysis showed inconsistency. This further demonstrates the specificity of LA and suggests that it may be of greater importance for antiaging than other metabolites.

Potential explanation for the causality between circulating omega-3 PUFA levels and LTL in MR studies

Regarding the results of our study, we found that the effect of DHA (the main omega-3 PUFA) and total omega-3 PUFA on LTL is insignificant. This conclusion is inconsistent with many previous studies, which suggest that DHA and other omega-3 PUFAs (such as EPA) are linked to reduced blood levels of F2-isoprostanes and increased levels of the antioxidant enzymes catalase and superoxide dismutase which are crucial in reducing inflammation and preventing LTL shortening [22]. However, these are only potential assumptions and are not fully supported by the current observational study. For instance, a meta-analysis that included six studies found no statistically significant effect of omega-3 PUFA supplementation on LTL in half of the studies [23].

Furthermore, the instability of current research may be due to the potential impact of circulating PUFAs on the direct effect of telomerase on LTL [71]. LTL maintenance is canonically carried out by telomerase, a ribonucleoprotein that expands telomere ends with tandem arrays

of TTAGGG repeats [72]. According to some research, regardless of telomere length, omega-3 and omega-6 PUFAs both dramatically lower telomerase activity [73]. Another study discovered that PUFAs, particularly DHA, downregulate the transcription of telomerase in addition to directly inhibiting its enzymatic activity [74]. Therefore, considering the negative impact of telomerase activity inhibition on telomere length, the inconsistency of current research findings can be easily explained, as the effect of circulating PUFAs on the production of LTL by affecting telomerase is exactly opposite to its effect on LTL through anti-inflammatory and antioxidant pathways. At the same time, some limitations of observational studies, such as reverse causality, control of confounding factors, and the use of self-report data in designing experiments, may make the research conclusions unstable.

BMI-mediated LA level reduction and its effect on LTL: insights from MR study and animal experiments

Our MR results also demonstrated a significant correlation between higher BMI and lower levels of LA, and MVMR analysis suggests that LA may mediate the effect of BMI on LTL. To further construct experimental evidence validating the MR study and explore whether circulating PUFAs, identified as potential mediators of the BMI-LTL relationship in MR studies, have changed, while also examining potential biological mechanisms beyond causal pathways, we conducted animal experiments. These experiments allowed for more direct measurement of molecular and metabolic processes, potentially providing evidence to explain the underlying biological mechanisms. Specifically, we evaluated differences in circulating LA levels and gut microbiota composition between obese (DB mice) and normal mice (C57 mice), and predicted microbial functional features. The study showed that, compared to C57 mice, DB mice had lower LA levels. This trend is consistent with our MR results, which indicate a significant correlation between higher BMI and lower LA levels.

Previous studies have highlighted the crucial role of the gut microbiota in regulating host metabolism and energy balance, suggesting its potential involvement in PUFA metabolism [15, 75]. A comparison of gut microbiota between DB and C57 mice revealed significantly lower α-diversity and circulating LA levels in DB mice. These findings are consistent with previous observations of a strong positive correlation between gut microbiota α-diversity and circulating LA levels [76]. In addition, we found that Lactobacillus were significantly more abundant in DB mice than in the C57 mice. Research has shown that lactobacillus can convert LA into other fatty acid metabolites by influencing the activity of enzymes involved in LA biotransformation, such as linoleate isomerase and dehydrogenase [77]. This also suggests that the lower circulating LA levels in individuals with higher BMI or obesity may be caused by the higher abundance of lactobacillus in the gut microbiota.

Furthermore, we performed KEGG enrichment analysis and found that the characteristic gut microbiota in DB mice showed functional enrichment in lipid metabolism. This further suggests that the characteristic changes in the gut microbiota of individuals with higher BMI or obesity may contribute to alterations in LA metabolism levels in obese individuals.

This conclusion that higher BMI is associated with lower levels of LA is also supported by other studies, which generally suggest that the increase in delta-6-desaturase activity (an essential enzyme in the metabolism of linoleic acid) in obesity leads to an increase in the conversion rate of LA, thus keeping LA levels lower in obesity [12, 13]. Furthermore, recent compelling evidence suggests that there is a sixth taste modality designed to detect oral-gustatory PUFAs [78]. This modality is mediated by the genetic polymorphism of CD36 [79]. Studies have found a significant difference in the threshold of sensitivity to LA between individuals with low BMI and high BMI. Specifically, those with low BMI are more sensitive to LA [80]. Although there is no evidence indicating that this difference in sensory perception directly leads to the consumption of more LA among those with low BMI, we have previously discussed that LA may be a potential anti-inflammatory and antioxidative stress substance. Therefore, the reverse causal relationship between BMI and LA identified in our research may also be related to the fact that the taste system of individuals with low BMI is more sensitive to LA. As a result, they may be more likely to consume a diet that is naturally higher in LA.

Finally, based on the three conclusions from our study: (1) a negative correlation between BMI and LTL; (2) a negative correlation between BMI and LA; and (3) a positive correlation between LA and LTL, we conducted a mediation MR analysis and confirmed that LA may have a mediating effect on the LTL shortening caused by high BMI, although we cannot speculate on which specific metabolic pathways involving LA are involved in this mediation effect based on current research. However, based on the above discussion, individuals with a high BMI may reduce their LA levels in circulation in various potential ways, such as characteristic changes in gut microbiota composition that influence LA metabolism, increasing delta-6-desaturase activity, and consuming relatively low levels of LA. Given that LA may play a critical role in anti-inflammatory and antioxidant stress responses, the BMI-related decrease in LA levels may subsequently lead to shortened LTL. This may partially explain the observed mediation effects in this study.

Study limitations and areas for further investigation

Our MR study has limitations. First, we were constrained by the limited information available from the GWAS data, which prevented us from adjusting for variables related to exposure, mediator, and outcome, such as age, sex, disease status, environmental factors, stress factors, and other environmental factors, and conducting subgroup analyses. This may have some impact on the stability of our conclusions. Second, based on the first assumption of the MR principle of relevance, we selected instrumental variables that were strongly correlated with their features to replace exposure and mediator [81]. However, many SNPs that do not have statistically significant differences could still have some impact on complex traits such as BMI, which further weakens the statistical power of this study [82]. Third, because the GWAS data used in this study are derived from Europeans, the generalizability of our conclusions to other races may be limited. Fourth, some MR results, such as the MR-Egger intercept (P=0.02) in the study of the causal effect of LA on LTL, are consistent with a previous MR study, where the MR-Egger intercept P-value was <0.05, suggesting the possibility of horizontal pleiotropy [48]. Nonetheless, compared with common observational and randomized controlled studies with low compliance, small sample sizes, and interfering factors such as reverse causality and confounding, this study still has many advantages. These include using a large sample-size GWAS and Mendelian randomization (using genetic association data for fatty acids from more than 114,000 UK Biobank participants, which is the largest known fatty acid GWAS database), and overcoming the influence of reverse causality that is typical with the previous observational studies and the common confounding factors on the results (using SNPs as instrumental variables following the Mendelian second genetic law). Additionally, sensitivity tests were performed (using two BMI databases as exposure and two supplemental MR methods), and they showed consistent results.

Conclusion

In summary, this study utilized genetic data analysis via MR to expand upon the understanding of the causal networks among BMI-LTL, BMI-PUFAs, and PUFAs-LTL. Specifically, it demonstrated the protective effect of higher levels of LA and the detrimental impact of higher BMI on LTL. Furthermore, it indicated that the effect of BMI on LTL is partially mediated by LA. These findings emphasize the potential shared mechanisms of obesity and LA in influencing LTL and suggest that LA could serve as a suitable target for secondary prevention of aging and age-related diseases caused by obesity.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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NA.

Author contributions

L.T. conducted research and was in charge of task management and research conception. M.-M.Z., Y.-Q.Z, Y.F., Q.Y., J.H., Z.-Y.O.-Y. were in charge of choosing the research topics and gathering the data. N.-X.C., X.-N.S., Q.Z., Q.L., H.Y.and M.-Y.W. conducted the statistical analyses. Y.-Z. F and Y.G. supervised the project. Each author contributed to the analysis and writing of the publication.

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Data availability

Anyone can find the data created or used in this study at the data repositories mentioned in this published paper.

Declarations

Ethical approval

The MR studies from which the data were gathered previously got the necessary ethical approval and participant agreement, and all the data used in this research are openly accessible. The animal experiments were conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of the Second Xiangya Hospital of Central South University (protocol code KQ2019FY01, approved on March 20, 2019) and the Animal Ethics Committee of Central South University (2018sydw0179).

Disclosure summary

All the authors declare that they have no financial or non-financial interests that could create a conflict of interest with the subject matter of this disclosure.

Competing interests

The authors declare no competing interests.

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