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General Article

GENERAL ARTICLE

Fat metabolism is associated with telomere length in six population-based studies

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Abstract

Telomeres are repetitive DNA sequences located at the end of chromosomes, which are associated to biological aging, cardiovascular disease, cancer and mortality. Lipid and fatty acid metabolism have been associated with telomere

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shortening. We have conducted an in-depth study investigating the association of metabolic biomarkers with telomere length (LTL). We performed an association analysis of 226 metabolic biomarkers with LTL using data from 11 775 individuals from six independent population-based cohorts (BBMRI-NL consortium). Metabolic biomarkers include lipoprotein lipids and subclasses, fatty acids, amino acids, glycolysis measures and ketone bodies. LTL was measured by quantitative polymerase chain reaction or FlowFISH. Linear regression analysis was performed adjusting for age, sex, lipid-lowering medication and cohort-specific covariates (model 1) and additionally for body mass index (BMI) and smoking (model 2), followed by inverse variance-weighted meta-analyses (significance threshold $P_{meta} = 6.5 \times 10^{-4}$). We identified four metabolic biomarkers positively associated with LTL, including two cholesterol to lipid ratios in small VLDL (S-VLDL-C % and S-VLDL-CE %) and two omega-6 fatty acid ratios (FAw6/FA and LA/FA). After additionally adjusting for BMI and smoking, these metabolic biomarkers remained associated with LTL with similar effect estimates. In addition, cholesterol esters in very small VLDL (XS-VLDL-CE) became significantly associated with LTL ($P = 3.6 \times 10^{-4}$). We replicated the association of FAw6/FA with LTL in an independent dataset of 7845 individuals ($P = 1.9 \times 10^{-4}$). To conclude, we identified multiple metabolic biomarkers involved in lipid and fatty acid metabolism that may be involved in LTL biology. Longitudinal studies are needed to exclude reversed causation.

Introduction

Telomeres are repetitive DNA sequences located at the end of chromosomes that have an important role in the maintenance of genomic stability (1). Telomeres gradually shorten as a consequence of cell replication and damage accumulation with increasing age (2,3). Beyond a minimal critical telomere length, cells enter replicative senescence and this process of cellular senescence gradually affects multiple tissues during ageing and the viability of stem cells (4–6). Telomere length is therefore considered as a marker of biological aging. Although telomere shortening with age has a tissue-specific pace, telomere length in blood is considered a dynamic marker of physiological health and well-being in epidemiological and clinical studies (7). Both short and long leukocyte telomere length (LTL) have been associated with cancer (8–12), but only short LTL has been associated with several age-related diseases, including cardiovascular diseases (13–19), diabetes (20–22) and dementia (23,24). Multiple studies have also shown associations of shorter LTL with mortality (25–35), although findings have been inconsistent (13,36–39). Telomere length is highly heritable (h^2 between 44–86%) (40–42), and various genetic determinants have been identified (43–45). One of the most intriguing findings in studies investigating genetic determinants of LTL is that the identified genetic variants underscore the association between LTL and cardiovascular and metabolic diseases (18,43), Alzheimer's disease (46) and several cancers (47–49).

Lipid metabolism appears to play a key role in telomere length regulation. A relatively small study in 423 American Indians tested the association of 1364 distinct mass-to-charge ratio (m/z) features detected by untargeted liquid chromatography–mass spectrometry (LC/MS) with LTL (50). This study found nineteen metabolites significantly associated with LTL, independent of chronological age and other aging-related factors. These metabolites belong to the classes of glycerophosphoethanolamines, glycerophosphocholines, glycerolipids, bile acids, isoprenoids, fatty amides and carnitine esters (50). A second metabolomics study using an untargeted gas chromatography–mass spectrometry (GC/MS) and LC/MS platform showed associations of lysolipids and gamma-glutamyl amino acids with LTL in 3511 females from the TwinsUK cohort, suggesting the involvement of lipid metabolism, fatty acid metabolism and oxidative stress in telomere shortening (51). A third study identified phosphatidylcholines, amino acids and a carnitine associated with LTL using a targeted electrospray ionization tandem mass spectrometry (MS/MS) metabolomics platform in 7853 individuals, providing further support for the association of lipid metabolism, fatty acid metabolism and oxidative stress with LTL (52).

In this study, we investigated the association of LTL measured using quantitative polymerase chain reaction (qPCR) (44) or FlowFISH (53), a technique combining flow cytometry with fluorescent *in situ* hybridization, with metabolic biomarkers measured on a high-throughput proton nuclear magnetic resonance (NMR) platform (54) that targets lipoprotein subclasses and fatty acids specifically along with other low-weight molecules such as amino acids. We conducted the study in 11 775 participants from six Dutch cohorts, as part of the Biobanking for Medical Research Infrastructure of the Netherlands (BBMRI-NL) consortium.

Results

In this study, we included 11 775 participants of predominantly European descent with data available on LTL as well as on metabolic biomarkers. The following six Dutch cohort studies were included: the Leiden Longevity Study (LLS), the Netherlands Study of Depression and Anxiety (NESDA), the Netherlands Twin Register (NTR), the Erasmus Rucphen Family (ERF) study, the Rotterdam Study (RS) and the LifeLines-DEEP (LLDeep) study. Descriptive statistics of the study participants are shown in Table 1. There was a higher proportion of female participants than male participants in all included cohort studies, with the highest percentage of females in NESDA and NTR (67%). The mean age of participants was between 39 and 75 years, covering a wide age range of 18 until 95 years across cohorts. The participants in the LLS and the RS were older ($\bar{x}_{age,\,LLS} = 59$ years and $\bar{x}_{age,\,RS} = 75$ years, respectively) than the participants of the other studies ($\bar{x}_{age,\,NESDA} = 42$ years, $\bar{x}_{age,\,NTR} = 39$ years, and $\bar{x}_{age,\,ERF} = 48$ years). Consequently, the mean LTL measured using qPCR was lower in the LLS ($\bar{x}_{LTL} = 1.5$) and RS ($\bar{x}_{LTL} = 0.9$) than in the younger cohorts ($\bar{x}_{LTL} = 1.8$ in ERF study and $\bar{x}_{LTL} = 2.8$ in NTR), with exception of NESDA ($\bar{x}_{LTL} = 1.1$). The mean LTL of the LLDeep study cannot be directly compared with the other studies as a different measurement technique for LTL was used (FlowFISH vs. qPCR). The proportion of participants that used lipid-lowering medication was on average between 4 and 11%, with the exception of the RS, where 20% of participants used lipid-lowering medication. This may be explained by the older age of the RS participants. BMI was comparable between studies with means of 24.5–27.4 kg/m^2, but the proportion of current

Table 1. Descriptive statistics of the study populations

		LLS	NESDA	NTR	ERF	RS	LLDEEP
Model 1	*N*	1858	2885	4170	1243	683	936
	N females (%)	1022 (55.0)	1920 (66.6)	2804 (67.2)	694 (55.8)	395 (58.0)	541 (57.8)
	Age range (years)	30–80	18–65	18–79	17–87	65–95	18–81
	Age mean (SD)	59.3 (6.6)	41.9 (13.0)	39.2 (13.0)	47.7 (14.0)	75.4 (6.2)	45.1 (13.6)
	LTL range (T/S ratio)	0.74–2.75	0.33–2.85	0.81–4.70	0.79–2.82	0.53–1.56	4.1–9.9*
	LTL mean (SD)	1.46 (0.27)	1.11 (0.31)	2.75 (0.48)	1.79 (0.36)	0.94 (0.16)	7.07 (0.96)*
	N lipid-lowering medication (%)	188 (10.1)	204 (7.1)	158 (3.8)	137 (11.0)	138 (20.2)	41 (4.4)
Model 2	*N*	1600	2883	4113	1235	650	927
	N females (%)	880 (55.0)	1918 (66.5)	2772 (67.4)	690 (55.9)	376 (57.8)	536 (57.8)
	Age range (years)	30–80	18–65	18–79	17–87	65–95	18–81
	Age mean (SD)	59.3 (6.5)	41.9 (13.0)	39.2 (13.0)	47.7 (14.0)	75.3 (6.2)	45.2 (13.5)
	LTL range (T/S ratio)	0.78–2.75	0.33–2.85	0.81–4.70	0.79–2.82	0.53–1.56	4.1–9.9*
	LTL mean (SD)	1.46 (0.27)	1.11 (0.31)	2.75 (0.48)	1.79 (0.36)	0.94 (0.16)	7.07 (0.95)*
	N lipid-lowering medication (%)	165 (10.3)	204 (7.1)	155 (3.8)	135 (10.9)	132 (20.3)	41 (4.4)
	BMI range (kg/m^2)	17.2–44.6	14.7–55.8	14.6–50.7	15.5–51.1	15.3–44.9	16.7–44.9
	BMI mean (SD)	25.5 (3.6)	25.6 (5.0)	24.5 (4.0)	26.9 (4.8)	27.4 (3.9)	25.1 (4.0)
	N current smokers (%)	210 (13.1)	1119 (38.8)	856 (20.8)	486 (39.5)	98 (15.1)	189 (20.4)

Abbreviations: LLS = Leiden Longevity Study; NESDA = Netherlands Study of Depression and Anxiety; NTR = Netherlands Twin Register; ERF = Erasmus Rucphen Family study; RS = Rotterdam Study; LLDEEP = LifeLines Deep study; *N* = number of participants; SD = standard deviation; T/S ratio = ratio of telomere repeat length (T) to copy number of a single copy gene *36B4* (S); BMI = Body Mass Index.
*LTL was measured in kilobases using FlowFISH in the LLDeep cohort.

smokers differed between studies, ranging from 13.1% in the LLS to 39.5% in the ERF study.

Top findings of the meta-analyses ($P_{\text{model 1}} < 6.5 \times 10^{-3}$) are depicted in Table 2 and Figure 1. The complete results of the meta-analyses for both models (models 1 and 2) are available in Supplementary Material, Table S2, and individual results per cohort are available in Supplementary Material, Table S3 (model 1) and Supplementary Material, Table S4 (model 2). After adjustment for age, sex, lipid-lowering medication and cohort-specific covariates (model 1), two ratios of very-low-density lipoprotein (VLDL) and two fatty acid ratios showed significant evidence of a positive association with LTL: total cholesterol to total lipids ratio in small VLDL (S-VLDL-C %, $P = 1.5 \times 10^{-4}$), cholesterol esters to total lipids ratio in small VLDL (S-VLDL-CE %, $P = 2.3 \times 10^{-4}$), ratio of omega-6 fatty acids to total fatty acids (FAw6/FA, $P = 4.2 \times 10^{-4}$) and ratio of 18:2 linoleic acid to total fatty acids (LA/FA, $P = 4.4 \times 10^{-4}$). However, these findings are not independent as S-VLDL-C % and S-VLDL-CE % were significantly correlated with each other ($r = 0.99$), as well as the omega-6 fatty acid measurements FAw6/FA and LA/FA ($r = 0.93$), as shown in Figure 2 (data of the ERF study). Additional adjustment for BMI and current smoking (model 2) had minimal effect on all four metabolic biomarkers as effect sizes remained similar (Table 2). Although the metabolic biomarker cholesterol esters in very small VLDL (XS-VLDL-CE) was not significantly associated with LTL after adjusting for multiple testing in model 1, this metabolic biomarker was significantly associated with LTL in model 2 ($P = 3.6 \times 10^{-4}$).

We next performed a sensitivity analysis to determine whether the analyses were influenced by the data of the LLDeep cohort, which used a different method to measure LTL (FlowFISH vs. qPCR). After excluding the LLDeep cohort from the meta-analysis, all metabolites remained significantly associated with LTL in model 1 and XS-VLDL-CE was also significantly associated with LTL (Supplementary Material, Table S5). In model 2, the results also remained similar and S-VLDL-C % and S-VLDL-CE % became significantly associated with LTL (Supplementary Material, Table S5).

In the replication analyses, where a lookup of the significant metabolic biomarkers was performed in the results of the study performed by Couto Alves et al. (see Materials and Methods and Supplementary Material), we were able to replicate the association FAw6/FA ($N = 7845$, $P_{model1} = 1.9 \times 10^{-4}$, $P_{model2} = 1.4 \times 10^{-2}$) with LTL only (Table 3). Unfortunately, the other four metabolic biomarkers were not available in the replication data and their association with LTL could not be confirmed. In the sex-stratified analysis, the top findings were consistent in males and females and showed a similar effect size as in the total cohort (Supplementary Material, Fig. S1), but were not statistically significant, most likely due to a reduction in sample size. Results of the sex-stratified meta-analyses are available in Supplementary Material, Tables S6 and 7 for models 1 and 2, respectively.

Discussion

We performed an association analysis of NMR-based metabolic biomarkers with LTL using data of 11 775 participants from six Dutch cohorts. We found higher levels of five metabolic biomarkers, three lipid subtypes (S-VLDL-C %, S-VLDL-CE % and XS-VLDL-CE) and two fatty acid ratios (FAw6/FA and LA/FA), associated with higher LTL values. We were able to replicate the association of FAw6/FA in a large sample of 7845 individuals.

Both fatty acid ratios that were significantly associated with LTL are omega-6 fatty acids, where linoleic acid (LA) is the major dietary omega-6 fatty acid in most Western diets (55). The association of omega-6 fatty acids with health remains unclear because of the pro-inflammatory as well as anti-inflammatory properties of omega-6 fatty acids (56–58). LA intake has been shown to be inversely associated with the risk of cardiovascular heart disease (59–61), death of cardiovascular disease and mortality (62). This is in agreement with the positive

Table 2. Overview results meta-analysis (*P* in model $1 < 6.5 \times 10^{-3}$)

Metabolic biomarkers	Model 1					Model 2					Description
	Beta	**SE**	***P*-value**	**Direction***	**N**	**Beta**	**SE**	***P*-value**	**Direction***	**N**	
S-VLDL-C %	0.015	0.004	**1.51 × 10–4**	+++−+−	11766	0.014	0.004	8.04 × 10–4	+++−+−	11399	Total cholesterol to total lipids ratio in small VLDL
S-VLDL-CE %	0.014	0.004	**2.30 × 10–4**	+++−+−	11766	0.013	0.004	8.27 × 10–4	+++−+−	11399	Cholesterol esters to total lipids ratio in small VLDL
FAw6/FA	0.013	0.004	**4.15 × 10–4**	++++++	11514	0.012	0.004	1.32 × 10–3	+++++−	11157	Ratio of omega-6 fatty acids to total fatty acids
LA/FA	0.013	0.004	**4.45 × 10–4**	++++++	11515	0.013	0.004	8.48 × 10–4	+++++−	11158	Ratio of 18:2 linoleic acid to total fatty acids
M-VLDL-CE %	0.013	0.004	8.52 × 10–4	+++−+−	11772	0.011	0.004	4.78 × 10–3	+++−+−	11405	Cholesterol esters to total lipids ratio in medium VLDL
S-VLDL-TG %	−0.013	0.004	8.70 × 10–4	−−−+−−	11766	−0.011	0.004	6.78 × 10–3	−−−+−+	11399	Triglycerides to total lipids ratio in small VLDL
XS-VLDL-CE	0.013	0.004	1.06 × 10–3	+++++−	11774	0.014	0.004	**3.65 × 10–4**	+++++−	11407	Cholesterol esters in very small VLDL
PUFA/FA	0.012	0.004	1.19 × 10–3	++++++	11515	0.011	0.004	4.07 × 10–3	+++++−	11158	Ratio of polyunsaturated fatty acids to total fatty acids
XS-VLDL-C %	0.011	0.004	1.92 × 10–3	+++−+−	11773	0.010	0.004	7.70 × 10–3	+++−+−	11406	Total cholesterol to total lipids ratio in very small VLDL
Phe	−0.011	0.004	2.01 × 10–3	−−−+−+	11687	−0.010	0.004	5.55 × 10–3	−−−+−+	11322	Phenylalanine
XS-VLDL-TG %	−0.011	0.004	2.06 × 10–3	−−−+−−	11773	−0.010	0.004	1.11 × 10–2	−−−+−+	11406	Triglycerides to total lipids ratio in very small VLDL
XS-VLDL-C	0.012	0.004	2.16 × 10–3	+++++−	11774	0.014	0.004	7.17 × 10–4	+++++−	11407	Total cholesterol in very small VLDL
S-HDL-PL	−0.011	0.004	2.39 × 10–3	−−−−++	11775	−0.011	0.004	2.92 × 10–3	−−−−++	11408	Phospholipids in small HDL
M-VLDL-C %	0.011	0.004	3.06 × 10–3	+++−+−	11772	0.010	0.004	1.09 × 10–2	+++−+−	11405	Total cholesterol to total lipids ratio in medium VLDL
XS-VLDL-CE %	0.010	0.004	3.26 × 10–3	+++−+−	11773	0.009	0.004	1.54 × 10–2	+++−+−	11406	Cholesterol esters to total lipids ratio in very small VLDL
VLDL-D	−0.011	0.004	3.56 × 10–3	−−+−−−	11775	−0.009	0.004	2.49 × 10–2	−−+−−−	11408	Mean diameter for VLDL particles
Alb	0.010	0.004	3.60 × 10–3	++++++	11775	0.008	0.004	2.66 × 10–2	−+++++	11408	Albumin
S-VLDL-FC %	0.012	0.004	4.30 × 10–3	++++++	11766	0.009	0.004	4.25 × 10–2	++++++	11399	Free cholesterol to total lipids ratio in small VLDL
S-LDL-TG %	−0.010	0.004	4.76 × 10–3	−−+−−−	11735	−0.009	0.004	1.93 × 10–2	−−+−−−	11369	Triglycerides to total lipids ratio in small LDL
XL-HDL-C	0.011	0.004	5.45 × 10–3	+++−++	11775	0.009	0.004	2.20 × 10–2	+−+−+−	11408	Total cholesterol in very large HDL
IDL-FC	0.011	0.004	5.53 × 10–3	+++++−	11773	0.011	0.004	5.85 × 10–3	++++++	11406	Free cholesterol in IDL
HDL-D	0.011	0.004	6.31 × 10–3	++++++	11775	0.008	0.005	7.21 × 10–2	+++++−	11408	Mean diameter for HDL particles

Abbreviations: SE = standard error, *N* = sample size. Model 1: linear regression analysis with LTL as dependent variable adjusted for metabolic biomarker, age, sex, lipid-lowering medication (yes/no), and if necessary, for batch effects, case–control status or familial relationships. Model 2: model 1 + additional adjustment for BMI and smoking.
*Order of cohorts in direction column: LLS, NESDA, NTR, ERF, RS, LLDEEP; direction of effect represented by - (negative association) + (positive association) or? (not available). *P*-values in bold surpassed the significance threshold.

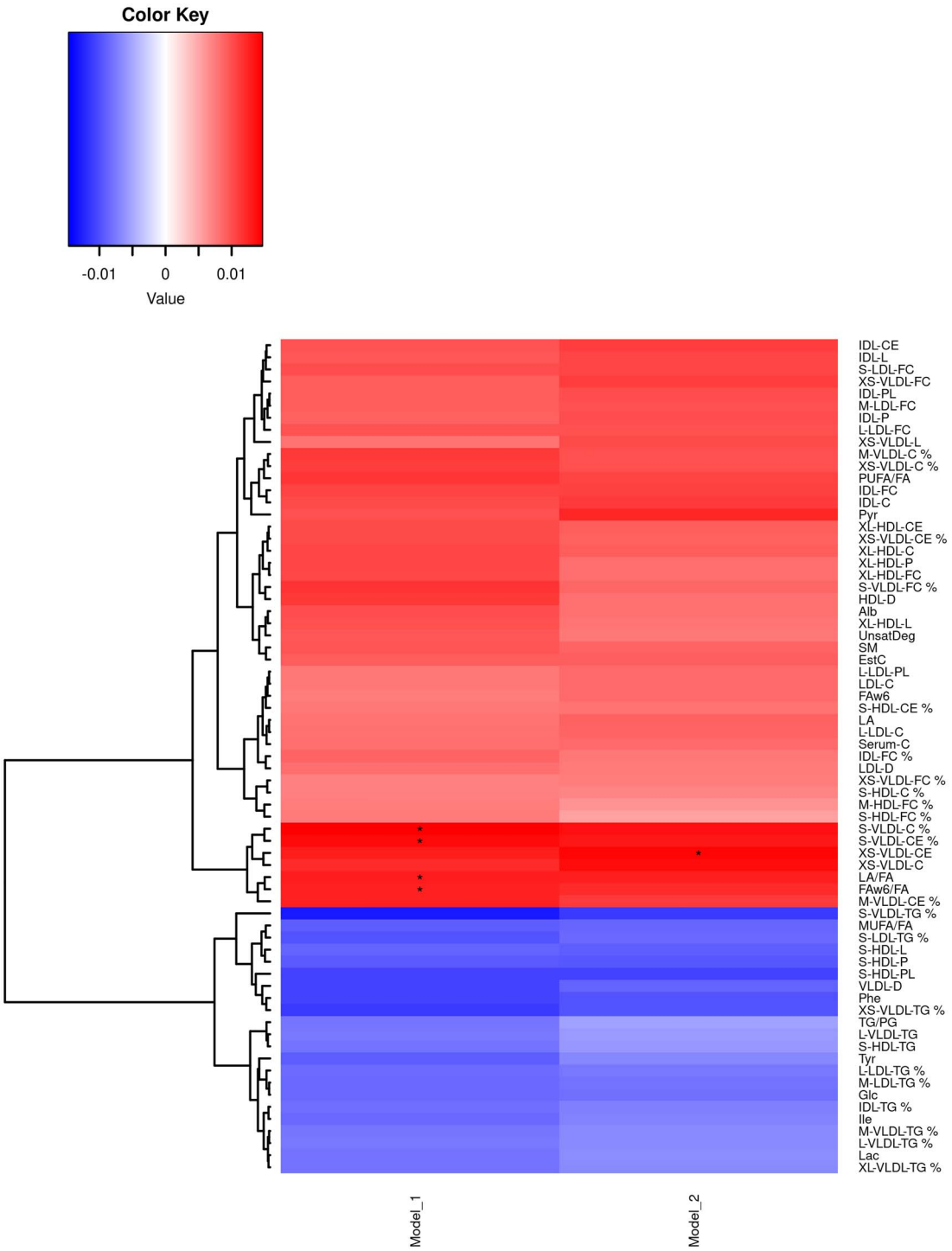

Figure 1. Heat map showing cluster analysis of the metabolic biomarker correlations with LTL. Metabolic biomarkers with $P < 1.0 \times 10^{-2}$ in model 1 were included in this Figure. The metabolic biomarkers are displayed vertically (*y*-axis) and the two models used in this study on the horizontal axis (*x*-axis). The association analysis was adjusted for age, sex and lipid-lowering medication in model 1 and additionally for BMI and smoking in model 2. A blue colour represents a negative correlation of the metabolite with LTL, and a red colour represents a positive correlation. Metabolic biomarkers that were significantly associated with LTL ($P < 6.5 \times 10^{-4}$) are labelled with a star.

association of the ratio of omega-6 fatty acids to total fatty acids (FAw6/FA) and the ratio of LA to total fatty acids (LA/FA) with LTL. Our findings are in contrast with the findings in the Nurses' Health Study ($N = 2284$ females) (63), where LA intake was negatively associated with LTL (P for trend 0.05). Of note, the findings of these studies are difficult to compare as we have used quantitative measures of metabolic biomarkers instead of dietary data derived from food frequency questionnaires. More importantly, our data agree with the recent large meta-analysis of the Fatty Acids and Outcomes Research Consortium (FORCE)

Table 3. Replication of the association between LTL and FAw6/FA

	Metabolic biomarkers	Discovery					Replication			
		Beta	SE	*P*-value	Direction*	N	Beta	*P*-value	Direction**	N
Model 1	FAw6/FA	0.013	0.004	$4.15 \times 10–4$	++++++	11 514	0.042	$1.87 \times 10–4$	++++−	7845
Model 2	FAw6/FA	0.012	0.004	$1.32 \times 10–3$	+++++−	11 157	0.029	$1.43 \times 10–2$	+−+−−	7670

*Order of cohorts in direction column: LLS, NESDA, NTR, ERF, RS, LLDEEP; direction of effect represented by–(negative association) or + (positive association).
**Order of cohorts in direction column: NFBC1966, KORA, HBCS, TWINFAT, TWINACTIVE; direction of effect represented by–(negative association) or + (positive association).

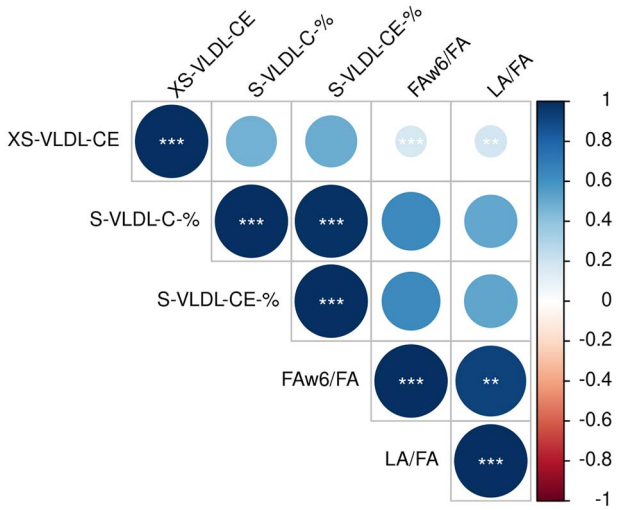

Figure 2. Correlation plot of the metabolic biomarkers associated with LTL after correction for multiple testing using ERF data. Positive correlations are displayed in blue and negative correlations in red. Colour intensity and the size of the circle are proportional to the correlation coefficients, with larger and darker circles indicating higher correlation point estimates. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

consortium (64). This study used data of 68 659 participants from 30 prospective studies and observed that higher circulating LA levels were associated with a lower risk of cardiovascular disease, cardiovascular mortality and ischemic stroke (64). The observations with regard to the other type of polyunsaturated fatty acids (PUFA), the omega-3 fatty acids (55), are also of interest. Higher levels of omega-3 fatty acids have been associated with lower levels of pro-inflammatory cytokines, higher levels of anti-inflammatory cytokines and reduced oxidative stress (56). Higher omega-3 fatty acids levels have also been associated with a reduced rate of telomere length shortening (65). Although the PUFA/FA ratio (i.e. the ratio of polyunsaturated fatty acids to total fatty acids) showed a nominally significant association with longer LTL ($P = 1.2 \times 10^{-3}$), this is most likely driven by the omega-6 fatty acid ratios as we observed no association of omega-3 with LTL.

The observed significant association of PUFA ratios (FAw6/FA and LA/FA) with LTL, a predictor of mortality (25–35), is in line with one of our previous studies in which we found that PUFA/FA was one of the 14 independent circulating biomarkers that were significantly associated with all-cause mortality (HR = 0.78, 95% confidence interval (CI) = 0.75–0.80, $P = 1.1 \times 10^{-47}$) (66). PUFAs are hydrocarbon chains containing two or more double bonds, which are further classified as either an omega-3 PUFA (FAw3) or omega-6 PUFA (FAw6), which is based on the position of the first double bond relative to the methyl end of the fatty acid (67). Although we found the strongest association of FAw6/FA with LTL, the PUFA/FA ratio also showed a nominally significant positive association with LTL ($\beta = 0.012$, SE = 0.004, $P = 1.2 \times 10^{-3}$). PUFA/FA and FAw6/FA appear to denote the same entity when studying LTL, as their correlation is very high ($r = 0.96$), and, as a result, when including both PUFA/FA and FAw6/FA in the regression analysis in the Rotterdam Study we found a high level of multicollinearity (variance inflation factor (VIF)$_{PUFA/FA} = 13.3$, $VIF_{FAw6/FA} = 13.4$). When re-examining the association of the FAw6/FA ratio with all-cause mortality (66), we found that FAw6/FA was more strongly associated with mortality (HR = 0.85, 95% CI = 0.83–0.87, $P = 3.0 \times 10^{-39}$) than FAw3/FA (HR = 0.91, 95% CI = 0.89–0.94, $P = 2.8 \times 10^{-11}$) (66).

It is of interest that we identified three VLDL metabolic biomarkers associated with LTL, which were all lipid measures to lipid ratios in VLDL (i.e. S-VLDL-C %, S-VLDL-CE % and XS-VLDL-CE). We did not find associations of absolute lipid measures of the VLDL sub-fractions, which may imply that type of lipids (i.e. small VLDL) and their composition drive the association with LTL and not the total lipids. Although we were unable to replicate our findings because of the unavailability of these metabolites in other studies, lipid metabolism has been associated before with LTL (51,52). The mechanisms through which lipids relate to LTL have been discussed before but are far from understood. One mechanism through which lipid as well as fatty acid metabolism may influence telomere length is oxidative stress, which is proposed as a cause of aging (68–70) and is known to attenuate telomere length attrition (71–73). Fat accumulation has been associated with oxidative stress (74), and previous studies have shown that oxidative stress is involved in the development of age-related diseases, including the metabolic syndrome (74,75). The metabolic syndrome is defined by clinical and biochemical alterations characterized by multiple components such as obesity, dyslipidaemia, arterial hypertension, hyperglycaemia and insulin resistance (75,76). These components have been associated with oxidative damage at DNA and lipid level and also with shorter telomere length (75). Interestingly, statins have been shown to prevent telomere length shortening through decreasing oxidative stress (77). Interventions promoting healthy lifestyle may delay telomere shortening and development of the metabolic syndrome. Because of the multifactorial process, development of interventions remains a challenge and these associations should receive attention in future studies. However, alternative mechanisms are plausible including the effect that lipid metabolism has on inflammation, a major driver of telomere length in blood (78–80). Also, inflammation and oxidative stress are intertwined (70,78). As most of the mechanisms are based on epidemiological cross-sectional studies and functional studies are lacking, further functional studies are therefore needed to explore these mechanisms.

The strength of our study is that metabolic biomarkers were measured in a standardized way with the same platform in all cohorts. Using a targeted metabolomics platform is both a strength as well as a limitation for this study. This platform contains a detailed catalogue of lipid subfractions, cholesterol and triglyceride measures, fatty acids and various low-molecular metabolites, which enabled us to find supportive evidence for the association of fatty acid metabolism with LTL and more in-depth information on the association of lipid metabolism with LTL. However, using this platform limits us to study other metabolic biomarkers and pathways that might also be related to LTL such as phosphatidylcholines and the methionine-homocysteine pathway, which were previously found to be associated with LTL (52). Another limitation of this study is that participants included in this study were predominantly from European descent, which makes it more difficult to generalize the findings to other populations. Lastly, we did not have longitudinal data to investigate the changes in telomere length over time. A previous study has found an inverse relationship between omega-3 fatty acids intake at baseline and telomere length shortening rate over 5 years, while there was no association between omega-3 fatty acid levels and telomere length at baseline (65). Longitudinal data might therefore provide further insights into telomere length biology and the opportunity to investigate reverse causality.

To conclude, we found subclasses of VLDL and ratios of omega-6 fatty acids to total fatty acids significantly associated with LTL. We were able to replicate the association of higher FAw6/FA levels with longer LTL in an independent dataset. These findings further support the association of lipid and fatty acid metabolism with LTL and provide more detailed information on the association of specific lipoprotein subclasses and fatty acids with telomere length. In the future, these findings might help to create prevention and therapeutic strategies to increase healthy aging.

Materials and Methods

Discovery populations

LLS. Long-lived siblings of Dutch descent were recruited together with their offspring and the partners of the offspring. Families were included if at least two long-lived siblings were alive and fulfilled the age criterion of 89 years or older for males and 91 years or older for females. In total, 944 long-lived proband siblings from 421 families with a mean age of 94 years (range, 89–104), 1671 offspring (61 years, 39–81) and 744 offspring's partners (60 years, 36–79) were included in the study (81). DNA was extracted from samples (non-fasted) at baseline using conventional methods (82). For the current analysis, only the offspring and their partners were used of whom LTL and Nightingale metabolomics data were available ($N = 1858$).

NESDA. A multi-centre study consisting of 2981 participants aged 18 to 65 years with depression or anxiety disorders (current and in the past) and healthy controls (83). Baseline data, collected between 2004 and 2007, of 2885 participants were included in this analysis. DNA and plasma levels were collected after overnight fast in a standardized manner.

NTR. Twins and their siblings and parents were recruited to study the causes of individual differences in health, behaviour and lifestyle. Participants are followed longitudinally and details about the cohort have been published previously (84,85). A subsample of unselected twins and their family members has taken part in the NTR-Biobank (86) in which biological samples, including DNA and RNA, were collected in a standardized manner after overnight fasting. In total, 4170 NTR participants with LTL measures were included in this study.

ERF study. A family-based study consisting of ~3000 inhabitants from an isolated population in the southwest of the Netherlands (87,88). ERF participants are descendants of 22 founder couples that had at least six children baptized in the community church. At baseline, participants were screened for many quantitative traits related to common diseases of interest. Samples were collected after overnight fasting in a standardized manner. Baseline data were collected between 2002 and 2005, and data of 1243 participants were included in this study.

RS. A prospective population-based cohort consisting of 14 926 participants from the Ommoord district in Rotterdam, the Netherlands, aiming at investigating the occurrence and causes of diseases that are frequent in the elderly (89). The RS cohort was initialized in 1990 including participants of 55 years and older (RS-I, $N = 7983$). At baseline, all participants were interviewed at home and had an extensive set of examinations, which were repeated every 3–4 years. The cohort was further extended in 2000 (RS-II) and 2005 (RS-III). For this study, the fourth follow-up visit from the first RS cohort was used (RSI-4, $N = 683$). Samples were collected after overnight fasting in a standardized manner.

LLDeep study. A population-based cohort study in the northern Netherlands including 1539 participants of 18 years and older (90). We included 936 participants in this analysis, for whom both telomere length and NMR data were available. Samples were collected after overnight fasting.

All participants provided written informed consent, and all studies were approved by the relevant institutional boards.

Leukocyte telomere length measurements

In all cohorts, except in the LLDeep study, mean LTL was measured with a quantitative PCR-based technique (qPCR) as previously described (44,52,91,92). Telomere length was expressed as the ratio (T/S) of telomere repeat length (T) to copy number of the single copy gene *36B4* (S) in each sample. In the LLDeep cohort, mean LTL was measured by FlowFISH using the previously published protocols (53). Telomere length measurements in lymphocytes were used for this analysis.

Metabolic measurements

The metabolic biomarkers were quantified from EDTA plasma and serum samples using a high-throughput proton NMR metabolomics platform (Nightingale Health, Helsinki, Finland), as described earlier (54,66,93). The NMR platform enables quantification of 14 lipoprotein subclasses, their lipid concentrations and composition, apolipoproteins, various cholesterol and triglyceride measures, albumin, fatty acids and other small metabolites including amino acids, glycolysis-related measures and ketone bodies. In this study, we included all 226 available metabolic biomarkers, of which a full list is shown in Supplementary Material, Table S1. Quality control of metabolic biomarkers was done in a standardized manner. First, metabolic biomarkers that failed quality control as indicated by

Nightingale Health were excluded from the analysis. Second, metabolic biomarkers with more than 10% missing values were removed. Third, a value of one was added to all metabolic biomarkers included in the analysis to take into account metabolic biomarkers with values below the detection limit, followed by a natural logarithm (LN) transformation to adjust for deviation from a normal distribution. Finally, all metabolic biomarkers were scaled to standard deviation units in order to standardize measurements across cohorts.

Statistical analysis

Linear regression analysis was performed over all metabolic traits per cohort using LTL as outcome variable and each metabolite as independent variable, adjusting for age, sex, lipid-lowering medication (yes/no), and if necessary, for batch effects, case–control status or familial relationships (model 1). In the second model (model 2), we additionally adjusted for body mass index (BMI) and smoking (current smoking: yes/no) as these factors might have an effect on both LTL as well as the human metabolome (94–99). BMI (kg/m^2) was calculated using the standard formula: weight (kg) divided by height in meters squared (m^2). Both models were analyzed in the total sample and in males and females separately. Inverse variance-weighted fixed effects meta-analyses were performed using METAL software (100). Heterogeneity was assessed using Cochran's Q-test as implemented by METAL software. We additionally performed a sensitivity analysis to test the robustness of the results and repeated the meta-analysis excluding the LLDeep cohort as telomere length was measured using a different method (FlowFISH vs. qPCR). To correct for multiple testing, we calculated the number of independent metabolic biomarkers in the Rotterdam Study (101). There were 77 independent metabolic biomarkers, resulting in a Bonferroni corrected *P-value* of 6.5×10^{-4} (= 0.05/77).

Replication analysis

We performed a lookup of the metabolites that were significantly associated with LTL in the results of Couto Alves et al. (Supplementary Material). In this study, data on up to 20 155 individuals of European ancestry from 11 cohorts were used to replicate our findings (102). Metabolite data were generated on one or two NMR platforms (54,103). Included cohorts were: Northern Finland Birth Cohort 1966, Northern Finland Birth Cohort 1986, LLS, NTR, ERF study, Cooperative Health Research in the Augsburg Region study (KORA), Estonian Genome Center of University of Tartu Cohort (EGCUT), Helsinki Birth Cohort Study (HBCS), TWINFAT, TWINACTIVE and HRT Twins. Although three of these cohorts (LLS, NTR and ERF study) overlap with the cohorts included in the discovery population of the current study, there is no overlap in individuals between the discovery and replication populations for the metabolites examined for replication. Couto Alves et al. used two statistical models in their analyses, where in the first model, LTL was regressed on metabolite levels adjusting for age and sex and for family structure if needed, and the second model was additionally adjusted for BMI.

Supplementary Material

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. The authors declare the following competing interests: A.S. is an employee and option holder of the company SkylineDx. G.A. is a part-time employee of Repeat Dx, a company that specializes in clinical telomere length measurements. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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