Pediatric RESEARCH

www.nature.com/pr

CLINICAL RESEARCH ARTICLE

Interplay of physical activity and genetic variants of the endothelial lipase on cardiovascular disease risk factors

Diego F. Salazar-Tortosa[1,2,3], José M. Pascual-Gamarra[1,4], Idoia Labayen[5], Azahara I. Rupérez[6,7,8], Laura Censi[9], Laurent Béghin[10], Nathalie Michels[11], Marcela González-Gross[12,13], Yannis Manios[14], Christina-Paulina Lambrinou[14], Luis A. Moreno[6,7,8,13,15], Aline Meirhaeghe[16], Manuel J. Castillo[4] and Jonatan R. Ruiz[1,17,18]

BACKGROUND: The aim of this study was to investigate the association of endothelial lipase gene (*LIPG*) polymorphisms with cardiovascular disease (CVD) risk factors in adolescents and their interaction with physical activity.
METHODS: Six polymorphisms of *LIPG* were genotyped in 1057 European adolescents (12–18 years old) enrolled in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study. CVD risk factors related to lipid profile, blood pressure, adiposity and glucose regulation were recorded. Physical activity was objectively measured by accelerometry.
RESULTS: The major C allele of rs2000813, the minor T allele of rs2276269 and the minor G allele of rs9951026 were associated with lower levels of several CVD risk factors related to lipid profile. We also found a significant association of the TTACA *LIPG* haplotype (rs2000812, rs2000813, rs8093249, rs2276269 and rs9951026) with higher concentrations of low-density cholesterol and apolipoprotein B. Finally, the interaction between physical activity and the polymorphisms rs2000813, rs2276269 and rs9951026 had a significant influence on several CVD risk factors.
CONCLUSIONS: *LIPG* polymorphisms were significantly associated with CVD risk factors in European adolescents. Interestingly, alleles of these polymorphisms were associated with a better cardiovascular profile in physically active adolescents only. High physical activity may reduce the development of CVD, modulating its genetic risk.

Pediatric Research (2022) 91:929–936; https://doi.org/10.1038/s41390-021-01519-1

IMPACT:

- Using gene-phenotype and gene × environment analyses, we detected associations between the endothelial lipase gene and cardiovascular risk factors, along with interactions with physical activity.
- This study shows that physical activity may modulate the influence of *LIPG* gene on cardiovascular risk in adolescents.
- These results bring insights into the mechanisms by which physical activity positively influences CVD in adolescents.

INTRODUCTION

Cardiovascular disease (CVD) is the result of a complex interplay between genetic and environmental factors. Several genes and polymorphisms have been associated with susceptibility to CVD by influencing risk factors such as blood pressure, and high-density lipoprotein and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) and triglyceride (TG) levels.[1] The influence of a number of genetic variants on stroke[2,3] and myocardial infarction[1,4–6] has also been reported. The development of CVD, therefore, appears to have a polygenic basis.[7]

Physical activity plays a key role in the prevention and treatment of CVD.[8,9] Regular physical activity provides a wide range of health benefits in the general population, including the improvement of blood pressure, sensitivity to insulin and tolerance to glucose (beneficial in diabetes), and increased HDL-C levels plus reduced TG, LDL-C and total cholesterol (TC) levels (improved lipid profile). Together these all reduce the overall risk of developing CVD.[8] Current evidence suggests that moderate-intensity physical activity reduces the incidence of all-cause mortality, and especially death associated with coronary artery

[1]PROFITH 'PROmoting FITness and Health through physical activity' research group, Sport and Health University Research Institute (iMUDS), University of Granada, Granada, Spain; [2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA; [3]Department of Ecology, Faculty of Sciences, University of Granada, Granada, Spain; [4]Department of Medical Physiology, Faculty of Medicine, University of Granada, Granada, Spain; [5]Department of Health Sciences, Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Navarra's Health Research Institute (IdiSNA), Public University of Navarra, Pamplona, Spain; [6]Growth, Exercise, Nutrition and Development (GENUD) Research Group, University of Zaragoza, Zaragoza, Spain; [7]Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain; [8]Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain; [9]Council for Agricultural Research and Economics (CREA), Research Centre for Food and Nutrition, Rome, Italy; [10]Univ. Lille, Inserm, CHU Lille, CIC 1403—Clinique Investigation Center and U1286—INFINITE—Institute for Translational Research in Inflammation, Lille, France; [11]Department of Public Health and Primary Care, Ghent University, Ghent, Belgium; [12]Department of Health and Human Performance, Universidad Politécnica de Madrid, Madrid, Spain; [13]Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, Madrid, Spain; [14]Department of Nutrition and Dietetics, Harokopio University of Athens, Athens, Greece; [15]Faculty of Health Sciences, University of Zaragoza, Zaragoza, Spain; [16]Inserm, Institut Pasteur de Lille, University Lille, UMR1167-RID-AGE-Risk factors and molecular determinants of aging-related diseases, Lille, France; [17]Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, Granada, Spain and [18]Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Huddinge, Sweden
Correspondence: Diego F. Salazar-Tortosa (dftortosa@email.arizona.edu)

Received: 14 September 2020 Revised: 19 March 2021 Accepted: 23 March 2021
Published online: 15 April 2021

disease (CAD).[8] The results of several cross-sectional and interventional studies also suggest that the genetic predisposition to CVD risk factors is lowered by moderate levels of physical activity in adults[10,11] and adolescents.[12–14]

The endothelial lipase (LIPG) is a member of the TG lipase family with only a minor role as triglyceride lipase, acting primarily as a phospholipase.[15] LIPG favours the internalization of HDL-C, LDL-C and very low-density cholesterol (VLDL-C), being especially relevant for HDL-C metabolism. Therefore, LIPG influences the levels of lipoprotein in plasma and facilitates the release of lipid precursors (fatty acids) inside the cell.[16] In addition, this lipase could favour atherogenesis by its pro-inflammatory effect via reduction of HDL-C (anti-inflammatory lipid) or the production of inflammatory markers like pro-inflammatory cytokines.[16,17] Accordingly, several genetic association studies have assessed the association between polymorphisms of *LIPG* and plasma lipids,[6,18,19] along with its interaction with physical activity.[10,20] Given the role of LIPG in the clearance of plasma lipids, polymorphisms of *LIPG* may influence the CVD risk factors affecting adolescents.

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study (www.helenastudy.com) was designed to provide reliable data on nutrition and health-related phenotypes in a relatively large sample of European adolescents from nine countries. The data for this cohort includes information on 6 SNPs of the *LIPG* gene, as well as CVD risk factors. The aim of the present study was to assess the association between 6 *LIPG* polymorphisms and CVD risk factors in this population. The influence of the interaction between physical activity and *LIPG* polymorphisms on CVD risk factors was also examined.

METHODS

Participants

A total of 3865 subjects (12–18 years old) from ten cities in nine European countries (Austria, Belgium, France, Germany, Greece, Hungary, Italy, Spain and Sweden) were enrolled in the HELENA Study. All were randomly selected from public and private schools in each city between October 2006 and December 2007. Blood samples for genetic and clinical biochemistry analyses were obtained from one-third of the subjects ($N = 1155$), which were randomly selected. The data for 1057 (552 girls) of these subjects with information on *LIPG* polymorphisms and CVD risk factors were examined for the present study. The selected subjects did not differ with respect to the rest of the cohort in terms of age, sex or BMI ($P > 0.1$ in all cases; P values obtained with Kruskal–Wallis tests between groups). Within this sample, physical activity data were also available for 698 subjects. The subjects and their parents/guardians were fully informed regarding the aims and methods of the study, including the inclusion criteria,[21,22] and provided informed written consent for their data to be analysed. The study was conducted according to the ethical guidelines of the Declaration of Helsinki (Edinburgh 2000 revision), the Good Clinical Practice guidelines and adhering to the laws on clinical research in humans of all the participating countries. The human research committees of each involved centre approved the protocol followed.[23]

Assessment of cardiovascular risk factors

Thirty millilitres of venous blood was extracted between 8 and 10 a.m., 10 h after the last meal consumed (fasting conditions). The stability of samples had been tested before.[24] The CVD risk factors analysed included serum TC, HDL-C, LDL-C, apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), TG and glucose, all determined using the enzymatic methods of the Dimension RxL Clinical Chemistry System (Dade Behring, Schwalbach, Germany). Insulin was measured by solid-phase, two-site, chemiluminescent, immunometric assay using an Immulite 2000 analyser (DPC Biermann GmbH, Bad Nauheim, Germany). Leptin concentrations were measured in duplicate using the RayBio® Human Leptin ELISA Kit. The homeostatic model assessment (HOMA) index—an indicator of insulin resistance—was calculated as ([glycaemia × insulin]/22.5). The quantitative insulin sensitivity check index was also determined, as 1/[log(insulin) + log(glycaemia)]. Blood pressure was measured in the extended right arm using an OMRON M6 automatic oscillometric device. The subjects sat quietly for 5 min before taking two measurements 5 min apart. The mean (in mm Hg) was used in all analyses.

Anthropometric measurements were made in triplicate according to standardized techniques.[25] Weight was measured to the nearest 0.1 kg using a SECA 861 electronic scale (Seca Deutschland, Hamburg, Germany), height to the nearest 0.1 cm using a SECA 225 stadiometer and the skinfold thicknesses on the left side at the triceps and subscapularis to the nearest 0.2 mm using a Holtain Caliper (Holtain Ltd, Crymmych, Wales). A CVD risk score was computed from the mean of the standardized values [(value − mean)/standard deviation] of the following variables: TC/HDL, TG, HOMA, systolic blood pressure, and triceps and subscapular skinfolds, as done previously in other paediatric cohorts.[26,27]

Assessment of physical activity

Physical activity was assessed over 7 consecutive days using a GT1M uniaxial accelerometer (ActiGraph, Pensacola, FL) attached to the lower back.[28] Adolescents were instructed to wear the accelerometer during all time spent awake, removing it only during water-based activities. At least 3 days of recording for a minimum of 8 h/day were required for data to be used in analyses.[28] The time-sampling interval (epoch) was set at 15 s. The time engaged in at least moderate physical activity (≥3 metabolic equivalents) was calculated based on a standardized cut-off of 2000 counts/min or more. Moderate-to-vigorous physical activity (MVPA) was dichotomized into <60 min/day (inactive adolescents) and ≥60 min/day[28] (active adolescents).

Genotyping

Blood for DNA extraction was collected in EDTA K3 tubes, stored at the Analytical Laboratory at the University of Bonn and then sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille (Lille, France). DNA was extracted from white blood cells with the Puregene Kit (Qiagen, Courtaboeuf, France) and stored at −20 °C. In the context of the HELENA Study, polymorphism selection was performed using the previous literature and/or HapMap (tag SNPs with $r^2 > 0.8$) as previously described.[29,30] In the specific case of *LIPG* polymorphisms, the selection was based on previous literature on *LIPG* SNPs. Samples were genotyped by using an Illumina System (Illumina Inc., San Diego, CA) and the software used was GoldenGate Inc. (San Francisco, CA). A high rate of genotyping success was performed (≥99.8%) and each polymorphism respected the Hardy–Weinberg equilibrium (HWE) ($P \geq 0.05$ in all cases; Table 1). Several polymorphisms of the *LIPG* gene showed linkage disequilibrium between them (Fig. 1).

Statistical analysis

Deviations from the HWE were determined using an exact test with a significance set at $P < 0.01$.[31] Associations between genetic markers and CVD risk factors were assessed via linear regression models. Five genetic models (dominant, recessive, additive, codominant and overdominant) were contemplated in all analyses. The adjustment variables were body mass index (BMI; calculated as weight [in kg] divided by height in m^2), age, sex and centre. The influence of the interaction between physical activity and polymorphisms on CVD risk factors was also examined using the same models.

For each polymorphism, P values were computed using the likelihood ratio test (LRT) comparing a model with the polymorphism/the interaction term, and a null model without the term

Table 1. MAFs and results of an exact test to assess deviations from the HWE.

	Major allele	Minor allele	MAF	HWE
LIPG				
rs2000812	T	C	0.17	0.23
rs2000813	C	T	0.32	0.78
rs8093249	A	G	0.15	0.9
rs2276269	C	T	0.4	0.37
rs9951026	A	G	0.45	0.49
rs6507931	T	C	0.41	0.66

MAF minor allele frequencies, *HWE* Hardy–Weinberg equilibrium.

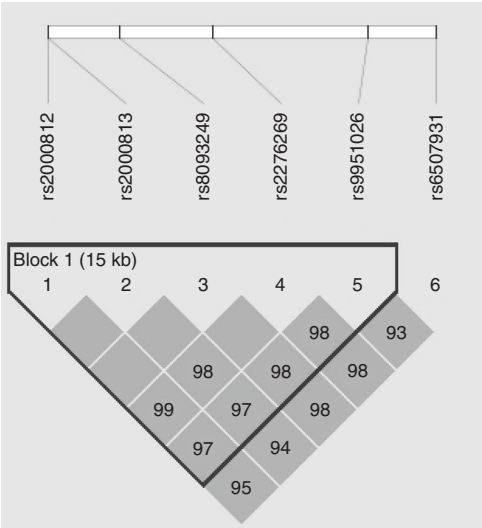

Fig. 1 Block 1 *LIPG* polymorphisms, which contains rs2000812, rs2000813, rs8093249, rs2276269 and rs9951026 according to the obtained genotyping data. Box numbers refer to linkage disequilibrium (D') between SNPs, while boxes with no number indicate 100% correlation ($D' = 1$). Logarithm of odds (LOD) score ≥2 and $D' = 1$ shown are as dark grey. See Haploview documentation about the standard colour scheme for further details (https://www.broadinstitute.org/haploview/ld-display).

of interest. Associations in which any combination of genotype and/or level of physical activity was represented by <10 subjects were discarded. All analyses were performed using the R software.[32] The associations between all SNPs and phenotypes under a given heritage model were contemplated as the family test, that is, the number of tests was equal to the number of SNPs analysed. Significant genotype–phenotype associations were selected for interaction and haplotype analysis. Given the exploratory nature of these analyses, and the reduced number of independent markers (these were in linkage disequilibrium; Fig. 1), the Bonferroni correction was deemed too stringent.[33] Instead, an exploratory selection of associations was undertaken using an approach that controls for the expected proportion of false positives (false discovery rate [FDR]).[34,35] Associations with an FDR <0.1 were used in the interaction and haplotype analyses.

Linkage disequilibrium between polymorphisms and haplotype block structures was examined using Haploview v.4.2 software (http://www.broad.mit.edu/mpg/haploview), haplo.stats software[36] and the "SNPassoc" R package.[31] First, haplotype blocks were generated by the four-gamete rule algorithm[37] (Fig. 1). For each block, it was determined whether the frequencies of haplotypes deviated from the expected under linkage equilibrium. Finally, the association between haplotypes and phenotypes was assessed using a permutation procedure. Only additive and dominant models were contemplated given the low frequency of some haplotypes. For significant associations, regressions between haplotypes and phenotypes were performed to search for significant differences between haplotype levels. Again, the FDR was calculated, using *P* values to spot differences between the reference (the most common) and other haplotypes. In the interaction analyses, a correction for multiple comparisons was made using the FDR (threshold 0.05). Note that no *LIPG* polymorphism was significantly associated with the time spent in MVPA ($P > 0.13$ in all cases; *P* value obtained by comparing linear models with and without each polymorphism as the predictor and including physical activity as a response variable, i.e. nested models).

RESULTS

Values of the CVD risk factors considered are shown across the whole cohort in Table 2, but also stratified according to CVD risk, using the median of CVD risk score as cut-off. Adolescents under lower CVD risk showed better lipid profile, lower adiposity, better glucose regulation and lower blood pressure, which is congruent with the calculation of CVD risk.

Association between *LIPG* polymorphisms and CVD risk factors

The exploratory analysis suggested that three of the six SNPs examined (rs2000813, rs2276269 and rs9951026) were associated with CVD risk factors (see Fig. 2 for significant associations under additive model; see Supplementary Appendix S1–2 for significant associations across all models). P values for all markers and heritage models are shown in the Supplementary Appendix S3–20.

The major C allele of rs2000813 was associated with lower TC ($P = 0.00168$; FDR = 0.0101), lower LDL-C ($P = 0.00031$; FDR = 0.0019), lower TC/HDL ratio ($P = 0.01209$; FDR = 0.0725), lower LDL-C/HDL-C ratio ($P = 0.00485$; FDR = 0.029), lower ApoB ($P = 0.00054$; FDR = 0.0033), and lower ApoB/ApoA1 ratio ($P = 0.00236$; FDR = 0.0141). Similarly, an association of the minor T allele of rs2276269 was observed with lower LDL-C/HDL-C and ApoB/ApoA1 ratios ($P = 0.04784$ and 0.03044; FDR = 0.0957 and 0.0609, respectively). Finally, an association was observed between the minor G allele of rs9951026 and lower LDL-C ($P = 0.03287$; FDR = 0.0986), along with lower LDL-C/HDL-C and lower ApoB/ApoA1 ratios ($P = 0.02961$ and 0.01989; FDR = 0.0888 and 0.0597, respectively).

Association between *LIPG* polymorphism haplotypes and CVD risk factors

LIPG block 1 contains rs2000812, rs2000813, rs8093249, rs2276269 and rs9951026 (Fig. 1). The TTACA haplotype of *LIPG* was significantly associated with higher LDL-C under the dominant model (global $P = 0.0116$) than were haplotypes TCATG (difference between groups 0.05; 95% confidence interval [CI] = 0.01-0.08; $P = 0.0112$; FDR = 0.02), TCACA (difference between groups 0.07; 95% CI = 0.02–0.11; $P = 0.0059$; FDR = 0.02) or CCATG (difference between groups 0.05; 95% CI = 0.01–0.09; $P = 0.0120$; FDR = 0.02). The same pattern was seen under the additive model: the TTACA haplotype was associated with higher LDL-C (global $P = 0.0181$) than was the TCATG (difference between groups 0.04; 95%CI = 0.01–0.07; $P = 0.0089$; FDR = 0.0154), TCACA (difference between groups 0.07; 95% CI = 0.02–0.11; $P = 0.0020$; FDR = 0.0101) or CCATG haplotype (difference between groups 0.05; 95% CI = 0.01–0.08; $P = 0.0092$; FDR = 0.0154).

Finally, the TTACA haplotype was significantly associated with higher ApoB values under the additive model (global $P = 0.043$) than was the TCATG (difference between groups 0.04; 95%

Table 2. Characteristics of the study population.

Phenotype	All ($n = 1057$)	Higher CVD risk ($n = 507$)	Lower CVD risk ($n = 506$)
Age (years)	14.7 ± 1.2	14.8 ± 1.2	14.6 ± 1.2
Weight (kg)	58.7 ± 12.7	63.5 ± 13.1	53.7 ± 9.5
Height (cm)	165.5 ± 9.3	165.6 ± 9.1	165.4 ± 9.7
Triceps skinfold (mm)	15.5 ± 6.9	18.6 ± 7.2	12.3 ± 4.9
Subescapular skinfold (mm)	12.8 ± 6.5	16 ± 7.2	9.6 ± 3.4
BMI (kg/m^2)	21.3 ± 3.7	23.1 ± 3.8	19.5 ± 2.3
TC (mg/dL)	160.74 ± 27.69	166.65 ± 28.68	154.42 ± 24.62
LDL-C (mg/dL)	94 ± 25	103 ± 25	86 ± 21
HDL-C (mg/dL)	55 ± 11	52 ± 10	59 ± 10
TC/HDL-C	2.99 ± 0.66	3.29 ± 0.67	2.66 ± 0.42
LDL-C/HDL-C	1.78 ± 0.63	2.05 ± 0.63	1.49 ± 0.42
TG (mg/dL)	69 ± 35	84 ± 41	53 ± 18
TG/HDL-C	1.33 ± 0.88	1.72 ± 1.04	0.93 ± 0.37
ApoA1 (mg/dL)	1.5 ± 0.22	1.46 ± 0.23	1.55 ± 0.2
ApoB (mg/dL)	0.65 ± 0.16	0.71 ± 0.16	0.59 ± 0.13
ApoB/ApoA1	0.44 ± 0.13	0.5 ± 0.13	0.39 ± 0.09
ApoB/LDL-C	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.03
Insulin (μIU/mL)	10 ± 8	13 ± 10	8 ± 3
Leptin (ng/mL)	20 ± 22	28 ± 26	12 ± 14
HOMA	2.35 ± 1.96	3.02 ± 2.51	1.69 ± 0.78
QUICKI	0.35 ± 0.03	0.33 ± 0.03	0.36 ± 0.03
SBP (mm Hg)	120 ± 13	125 ± 13	114 ± 11
DBP (mm Hg)	68 ± 9	71 ± 9	65 ± 8
CVD risk score	−0.01 ± 0.61	0.43 ± 0.56	−0.45 ± 0.23

Mean ± SD of CVD risk factors is shown across the whole cohort, but also stratified by CVD risk. The cohort is divided using the median CVD risk score as cut-off. Those individuals with a CVD risk score equal or higher than the cut-off were included in the category of "Higher CVD risk", while those below were included in "Lower CVD risk".
BMI body mass index, *TC* total cholesterol, *LDL-C* low-density lipoprotein, *HDL-C* high-density lipoprotein, *TG* triglycerides, *Apo* apolipoprotein, *HOMA* homeostatic model assessment, *QUICKI* quantitative insulin sensitivity check index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure.

CI = 0.01–0.07; $P = 0.0084$; FDR = 0.0211), TCACA (difference between groups 0.06; 95% CI = 0.02–0.09; $P = 0.0064$; FDR = 0.0211) or CCATG haplotype (difference between groups 0.04; 95% CI = 0.01–0.07; $P = 0.0171$; FDR = 0.0285).

Interaction between *LIPG* polymorphisms, physical activity and CVD risk factors

The alleles of rs2000813, rs2276269 and rs9951026 were associated with a lower cardiovascular risk only in the active adolescents (i.e. those who practiced ≥60 min/day of MVPA; see Fig. 3 for significant interactions under additive models; see Supplementary Appendix 21 for significant interactions across all models).

The major C allele of rs2000813 was associated with lower LDL-C levels only in those individuals performing moderate/high levels of physical activity ($P = 0.035417$; FDR = 0.035417). Similarly, active subjects carrying the minor T allele of rs2276269 showed lower LDL-C/HDL-C values ($P = 0.006504$; FDR = 0.022013), and lower ApoB/ApoA1 ratios ($P = 0.014327$; FDR = 0.035817). Finally, active subjects carrying the minor G allele of rs9951026 showed lower values of LDL-C ($P = 0.023001$; FDR = 0.035417), LDL-C/HDL-C ($P = 0.011235$; FDR = 0.022013) and ApoB/ApoA1 ($P = 0.007245$; FDR = 0.035817).

DISCUSSION

Our preliminary analyses showed associations between the C, T and G alleles of *LIPG* rs2000813, rs2276269 and rs9951026, respectively, and a better cardiovascular profile (lower TC, LDL-C, TC/HDL-C, LDL-C/HDL-C, ApoB/ApoA1 and ApoB values) in European adolescents. In addition, the *LIPG* haplotype block TTACA (rs2000812, rs2000813, rs8093249, rs2276269 and rs9951026) was significantly associated with higher levels of LDL-C and ApoB. Importantly, physical activity modulated the influence of *LIPG* polymorphisms on CVD risk factors; the alleles of rs2000813, rs2276269 and rs9951026 polymorphisms were associated with a better cardiovascular profile only in physically active adolescents.

Previous studies have shown significant associations between lipid profile and some of the polymorphisms analysed in the present study in adults. Halverstadt et al.[20] found higher levels of some HDL-C fractions for carriers of the CC genotype of rs2000813. Moreover, Liu et al.[38] reported an association between this polymorphism and higher HDL-C in two ethnic Chinese groups, but for the genotype TT. They also showed an association between rs2000813 genotype and ApoB levels in one of the groups. A similar pattern was found by Hutter et al.[19] in Japanese Americans, an haplotype constituted by rs2000813 T and rs3813082 C alleles was associated with higher HDL-C and ApoA1, along with lower LDL-C and ApoB. Paradis et al.[39] reported the same pattern, with higher HDL-C values related to the rs2000813 T allele in white women. Similarly, Elnaggar et al.[6] showed that T carriers had higher values of HDL-C and lower risk of CAD in an Egyptian cohort, with T allele being a protective factor for CAD independently of plasma lipids. Mank-Seymour et al.[18] also detected a relationship between HDL-C and one of the polymorphisms considered in this study, as white carriers of the TT allele of rs2276269 had lower HDL-C levels. Finally, other studies have failed to detect any association between lipid profile and *LIPG* polymorphisms analysed in the present study. For example, Smith et al.[10] found no association between either rs2000813 or rs6507931 polymorphisms with HDL-C or LDL-C when interaction with lifestyle was not considered. Other studies have found no differences between genotypes of rs2000813 and LDL-C levels.[6,20,39] Similarly, DeLemos et al.[40] found no differences in the genotype frequencies of rs2000813 between white individuals with high HDL-C levels and normal level controls. The same lack of significance was reported by Yamakawa-Kobayashi et al.[41] for rs2000813 in a cohort of Japanese schoolchildren. To our knowledge, there is no previous evidence relating the rs9951026 polymorphism to plasma lipoprotein levels in European adolescents or any other population. There is an important inconsistency between studies about the influence of *LIPG* on lipid profile, including the present study. Although we found associations between *LIPG* polymorphisms and ratios including HDL-C or ApoA1, no significant association was found for these variables in isolation. In addition, we found a strong link between *LIPG*, LDL-C and ApoB as previous studies, but in the opposite sense. In our study, T allele of rs2000813 was associated with higher LDL-C. Variation between study results can be caused by several factors. For example, the measurement of variables differed between studies, with some of them estimating LDL-C through the Friedewald's formula[42] instead of using direct measurements like in the present study. In addition, the existence of false positives/negatives in these studies cannot be disregarded, although the present study showed strong significant associations for LDL-C and ApoB even after applying stringent corrections for multiple comparisons (see "Methods" section). Other relevant factors for explaining this variability could be differences in population-dependent penetrance and allele frequencies, subject age, ethnicity and sample size.

Exercise can affect the lipid profile,[43] but its effect varies among individuals,[44] suggesting the existence of heritable

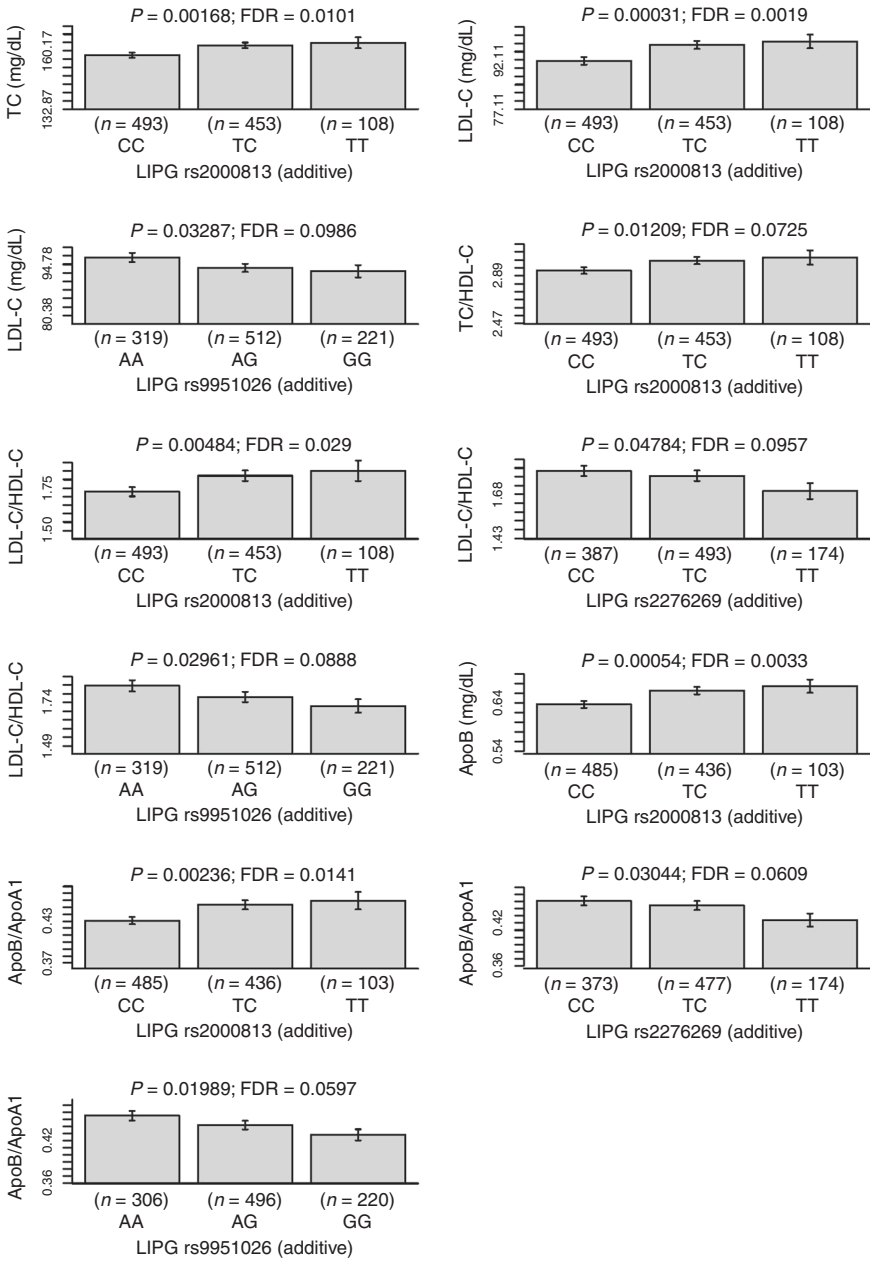

Fig. 2 Significant associations (FDR < 0.1) between *LIPG* polymorphisms and CVD risk factors under an additive model. The *P* value and false-positive discovery rate (FDR) are shown for each association. Values are adjusted for body mass index, centre, sex and age.

differences in the response to physical activity. In the present work, carriers of certain genotypes of the rs2000813, rs2276269 and rs9951026 polymorphisms showed a better lipid profile under moderate/high levels of physical activity. Halverstadt et al.[20] showed the interaction between physical activity and rs2000813 to significantly influence HDL-C levels, reporting higher levels of HDL-C in CC compared to CT-TT genotypes under the training intervention. Interestingly, CT-TT carriers showed a better lipid profile than the CC carriers before the training intervention, suggesting that these genotypes may be protective under sedentary conditions. This might an additional factor to explain the discrepancy among studies assessing the association between *LIPG* polymorphism and HDL-C levels without contemplating physical activity, as a given genotype could be protective or not depending on the level of physical activity. Smith et al.[10] also found a significant interaction between *LIPG* and physical activity. Women spending ≥2.6 h/day of on-screen time and who carried the TT genotype of the rs6507931 polymorphism had lower total HDL-C and higher small LDL-C, suggesting a worse lipid profile compared to the CT-CC genotypes. In contrast with rs6507931, no association was found for the rs2000813 polymorphism in that study. Our results follow the inconsistency of previous studies, as we found an interaction between *LIPG* rs2000813 polymorphism and physical activity on LDL-C and related ratios, while no significant interaction was found for rs6507931 or HDL-C. Discrepancies between these studies could be due to the method used for monitoring study variables. Halverstadt et al.[20] estimated LDL-C instead of using direct measurements, which was the approach followed in the present study and by Smith et al.[10] In addition, the monitoring of physical activity differs between studies. In contrast with Halverstadt et al.[20] and the present study, Smith et al.[10] did not objectively measure physical activity; rather, they used the time spent in front of screens as a proxy of activity

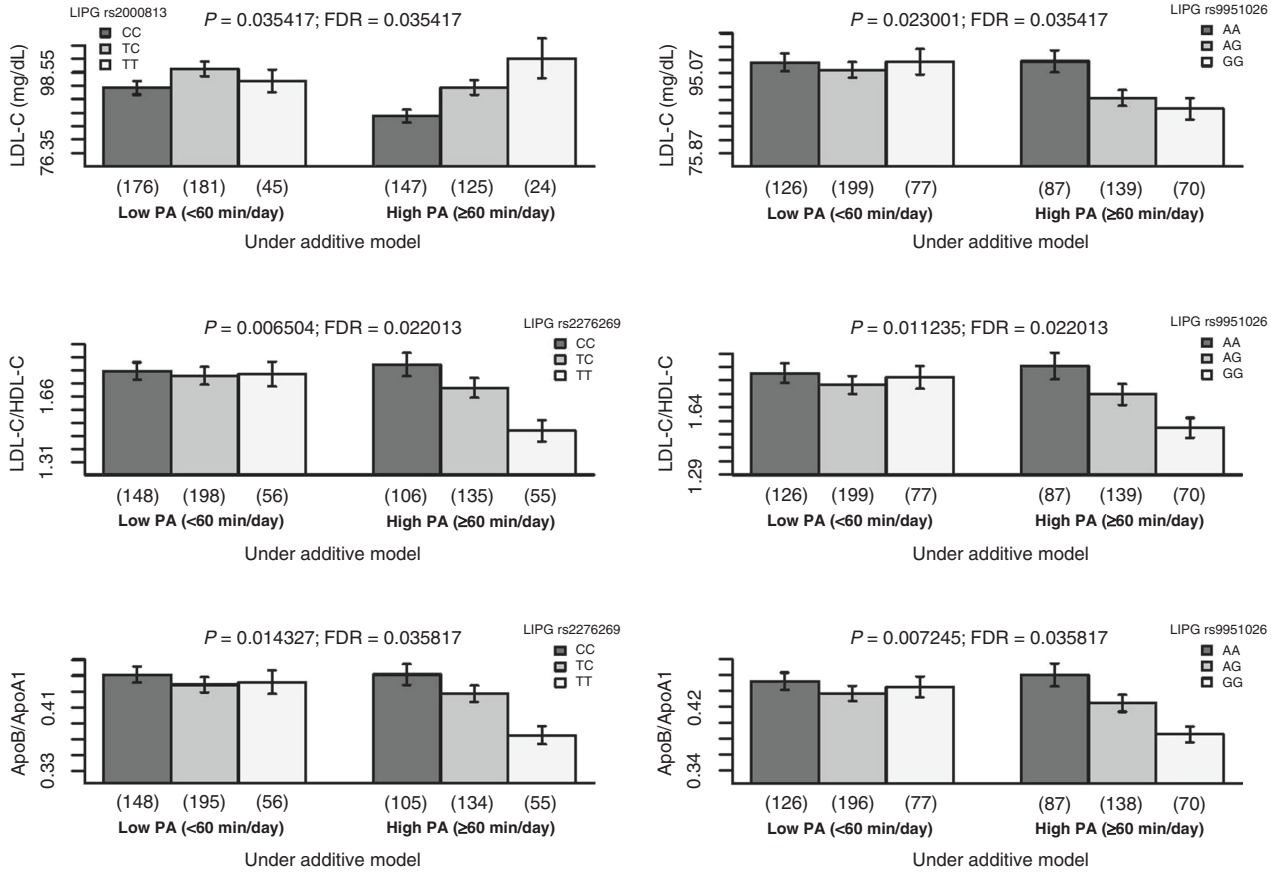

Fig. 3 Significant interactions (FDR < 0.05) between the studied SNPs and level of physical activity under an additive model. The *P* value and false-positive discovery rate (FDR) are shown for each association. Values are adjusted for body mass index, centre, sex and age. PA physical activity.

level. These factors, along with differences in the sample characteristics could explain the observed discrepancies.

Endothelial lipase is involved in the metabolism of plasma lipoproteins such as LDL-C, VLDL-C, and HDL-C.[16] This is congruent with the association detected between *LIPG* polymorphism and plasma lipoproteins. LIPG is also involved in the uptake of ApoA1 and ApoB (constituents of HDL-C and LDL-C particles, respectively) in mice, via heparin sulfate proteoglycans that bind to LIPG in the cell membrane.[45,46] Accordingly, in the present work, significant associations were found between *LIPG* polymorphisms and ApoB levels, and the ApoB/ApoA1 ratio. In contrast with other lipases of its family, LIPG has only minor triglyceride lipase activity,[16] which agrees with the lack of significance of any association with TG reported here or in previous studies.[20,38,47]

The interaction between physical activity and *LIPG* could be explained by blood pressure. Regular physical activity is known to reduce the blood pressure,[8,48] which in turn could affect LIPG production via endothelial shear stress. Support for this hypothesis comes from evidence showing that LIPG messenger RNA increases under fluid shear stress in cultured endothelial cells and in hypertensive animals.[49,50] Another cause could be inflammation, as long-term inflammation impairs the effects of exercise (e.g. increased anabolic resistance under low-grade chronic inflammation in elderly[51]). LIPG has been positively associated with inflammation,[52,53] thus polymorphisms that reduce the functionality of this lipase might generate a more favourable environment for the development of beneficial effects of exercise. Lower LIPG activity might reduce inflammation via a reduction in HDL hydrolysis (HDL-C has anti-inflammatory activity),[16] but this would not fully explain our results given the lack of associations between *LIPG* and HDL-C. However, LIPG can also influence inflammation through facilitation of monocyte–endothelial adhesion and reduction of pro-inflammatory cytokines production.[16,17,54] Given the lack of data in plasma for endothelial lipase in the present study, possible mechanisms of action can only be hypothesized. Further research is needed to elucidate the enzymatic mechanism of action and impact on plasma lipids.

The present study has some limitations. First, it is observational in design, so no cause–effect relationships can be established. The results should be tested in future experimental studies looking for causal correlations between *LIPG* polymorphisms, CVD risk factors and physical activity, along with their impact on hard endpoints like myocardial infraction to quantify their clinical relevance. In addition, these associations could be modified by gene–gene and other gene–environment interactions. Finally, no information is available regarding patterns of relatedness between subjects, and the ethnic origins of the sample members are unknown. Our results should be considered carefully; studies with a larger sample size could help to further confirm the role of *LIPG* gene on the development of CVD and its interaction with physical activity.

In summary, an association was found between *LIPG* polymorphisms and CVD risk in European adolescents. The results also suggest that the influence of the rs2000813, rs2276269 and rs9951026 polymorphisms on the lipid profile can be modulated by physical activity. *LIPG* polymorphisms were only associated with a better cardiovascular profile in those individuals meeting the recommendations of daily physical activity. Therefore, increased physical activity may modulate the genetic risk of CVD development.

DISCLAIMER

The content of this article reflects only the authors' views, and the European Community is not liable for any use that may be made of the information contained therein.

ACKNOWLEDGEMENTS

The HELENA Study received funding from via contract FOOD-CT-2005-007034 within the European Community Sixth RTD Framework Programme. The study was also supported by the Spanish Ministry of Education (FPU13/03410; FPU16/02127), via *Redes Temáticas de Investigación Cooperativa RETIC* grant (Red SAMID RD16/0022), by the University of Granada *Plan Propio de Investigación 2016* (Excellence actions: Unit of Excellence on Exercise and Health [UCEES]), by the European Regional Development Fund (ERDF), by the *Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades* (ref. SOMM17/6107/UGR) and by grants from the Public University of Navarra, "Ayudas a Grupos de Investigación (2019)". We wish to thank Rosa Maria Torres, Petra Pickert and Anke Berchtold for their contribution to laboratory work.

AUTHOR CONTRIBUTIONS

J.M.P.-G., I.L., A.I.R., L.C., L.B., N.M., M.G.-G., Y.M., C.-P.L., L.A.M., A.M., M.J.C., D.F.S.-T. and J.R.R. designed the study; D.F.S.-T. performed all analyses; D.F.S.-T. and J.R.R. wrote the initial draft and all co-authors significantly contributed to the final version.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41390-021-01519-1.

Competing interests: The authors declare no competing interests.

Consent statement: The subjects and their parents/guardians were fully informed regarding the aims and methods of the study, including the inclusion criteria, and provided informed written consent for their data to be analysed.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Schunkert, H. et al. Genetics of coronary artery disease in the light of genome-wide association studies. *Clin. Res. Cardiol.* **107**, 2–9 (2018).
2. Akhter, M. S. et al. Screening of the NOS3 gene identifies the variants 894G/T, 1998C/G and 2479G/A to be associated with acute onset ischemic stroke in young Asian Indians. *J. Neurol. Sci.* **344**, 69–75 (2014).
3. Man, B. L. et al. Genetic polymorphisms of Chinese patients with ischemic stroke and concurrent stenoses of extracranial and intracranial vessels. *J. Clin. Neurosci.* **17**, 1244–1247 (2010).
4. Nasibullin, T. R. et al. Genotype/allelic combinations as potential predictors of myocardial infarction. *Mol. Biol. Rep.* **43**, 11–16 (2016).
5. Buysschaert, I. et al. A variant at chromosome 9p21 is associated with recurrent myocardial infarction and cardiac death after acute coronary syndrome: the GRACE genetics study. *Eur. Heart J.* **31**, 1132–1141 (2010).
6. Elnaggar, I. Z., Hussein, S., Amin, M. I. & Abdelaziz, E. A. Association of 584C/T polymorphism in endothelial lipase gene with risk of coronary artery disease. *J. Cell. Biochem.* **120**, 14414–14420 (2019).
7. Khera, A. V. et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat. Genet.* **50**, 1219–1224 (2018).
8. Smith, J. K. Exercise and atherogenesis. *Exerc. Sport Sci. Rev.* **29**, 49–53 (2001).
9. NIH Consensus Development Panel on Physical Activity and Cardiovascular Health. Physical activity and cardiovascular health. *JAMA* **276**, 241–246 (1996).
10. Smith, C. E. et al. Physical inactivity interacts with an endothelial lipase polymorphism to modulate high density lipoprotein cholesterol in the GOLDN study. *Atherosclerosis* **206**, 500–504 (2009).
11. Franks, P. W. et al. Assessing gene-treatment interactions at the FTO and INSIG2 loci on obesity-related traits in the Diabetes Prevention Program. *Diabetologia* **51**, 2214–2223 (2008).
12. Ruiz, J. R. et al. Attenuation of the effect of the FTO rs9939609 polymorphism on total and central body fat by physical activity in adolescents: The HELENA study. *Arch. Pediatr. Adolesc. Med.* **164**, 328–333 (2010).
13. Pascual-Gamarra, J. M. et al. Association between *UCP1*, *UCP2*, and *UCP3* gene polymorphisms with markers of adiposity in European adolescents: The HELENA study. *Pediatr. Obes.* **14**, e12504 (2019).
14. Salazar-Tortosa, D. F. et al. Association between lipoprotein lipase gene polymorphisms and cardiovascular disease risk factors in European adolescents: The Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Pediatr. Diabetes* **21**, 747–757 (2020).
15. Maugeais, C. et al. Dose-dependent acceleration of high-density lipoprotein catabolism by endothelial lipase. *Circulation* **108**, 2121–2126 (2003).
16. Yu, J. E., Han, S. Y., Wolfson, B. & Zhou, Q. The role of endothelial lipase in lipid metabolism, inflammation and cancer. *Histol. Histopathol.* **33**, 1–10 (2018).
17. Badellino, K. O., Wolfe, M. L., Reilly, M. P. & Rader, D. J. Endothelial lipase is increased in vivo by inflammation in humans. *Circulation* **117**, 678–685 (2008).
18. Mank-Seymour, A. R. et al. Association between single-nucleotide polymorphisms in the endothelial lipase (LIPG) gene and high-density lipoprotein cholesterol levels. *Biochim. Biophys. Acta* **1636**, 40–46 (2004).
19. Hutter, C. M. et al. Association of endothelial lipase gene (LIPG) haplotypes with high-density lipoprotein cholesterol subfractions and apolipoprotein AI plasma levels in Japanese Americans. *Atherosclerosis* **185**, 78–86 (2006).
20. Halverstadt, A. et al. High-density lipoprotein-cholesterol, its subfractions, and responses to exercise training are dependent on endothelial lipase genotype. *Metabolism* **52**, 1505–1511 (2003).
21. Moreno, L. A. et al. Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr.* **11**, 288–299 (2007).
22. Moreno, L. A. et al. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *Int. J. Obes.* **32**, S4–S11 (2008).
23. Béghin, L. et al. Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. *Int. J. Obes.* **32** (Suppl. 5), S12–S18 (2008).
24. González-Gross, M. et al. Sampling and processing of fresh blood samples within a European multicenter nutritional study: Evaluation of biomarker stability during transport and storage. *Int. J. Obes.* **32**, S66–S75 (2008).
25. Nagy, E. et al. Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. *Int. J. Obes.* **32**, S58–S65 (2008).
26. Lobelo, F. et al. Validity of cardiorespiratory fitness criterion-referenced standards for adolescents. *Med. Sci. Sports Exerc.* **41**, 1222–1229 (2009).
27. Ruiz, J. R. et al. High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. *Pediatr. Res.* **61**, 350–355 (2007).
28. Ruiz, J. R. et al. Objectively measured physical activity and sedentary time in European adolescents. *Am. J. Epidemiol.* **174**, 173–184 (2011).
29. Bokor, S. et al. Single-nucleotide polymorphism of cd36 locus and obesity in European adolescents. *Obesity* **18**, 1398–1403 (2010).
30. Bokor, S. et al. Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. *J. Lipid Res.* **51**, 2325–2333 (2010).
31. González, J. R. et al. SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* **23**, 644–645 (2007).
32. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2017).
33. Sham, P. C. & Purcell, S. M. Statistical power and significance testing in large-scale genetic studies. *Nat. Rev. Genet.* **15**, 335–346 (2014).
34. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc.* **57**, 289–300 (1995).
35. Qu, H-Q., Tien, M. & Polychronakos, C. Statistical significance in genetic association studies. *Clin. Investig. Med.* **33**, E266–E270 (2010).
36. Sinnwell, J. P. & Schaid, D. J. haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous. https://cran.r-project.org/package=haplo.stats (2016).
37. Wang, N. et al. Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. *Am. J. Hum. Genet.* **71**, 1227–1234 (2002).
38. Liu, W. Y. et al. Association of the LIPG 584C > T polymorphism and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. *Lipids Health Dis.* **9**, 110 (2010).
39. Paradis, M. E. et al. The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women. *J. Lipid Res.* **44**, 1902–1908 (2003).
40. DeLemos, A. S. et al. Identification of genetic variants in endothelial lipase in persons with elevated high-density lipoprotein cholesterol. *Circulation* **106**, 1321–1326 (2002).

41. Yamakawa-Kobayashi, K. et al. Relationship between serum HDL-C levels and common genetic variants of the endothelial lipase gene in Japanese school-aged children. *Hum. Genet.* **113**, 311–315 (2003).
42. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18**, 499–502 (1972).
43. Huffman, K. M. et al. Exercise effects on lipids in persons with varying dietary patterns - Does diet matter if they exercise? Responses in STRRIDE I. *Am. Heart J.* **164**, 117–124 (2012).
44. Leon, A. S. et al. Variability in the response of HDL cholesterol to exercise training in the HERITAGE Family Study. *Int. J. Sports Med.* **23**, 1–9 (2002).
45. Fuki, I. V. et al. Endogenously produced endothelial lipase enhances binding and cellular processing of plasma lipoproteins via heparan sulfate proteoglycan-mediated pathway. *J. Biol. Chem.* **278**, 34331–34338 (2003).
46. Strauss, J. G. et al. Endothelial cell-derived lipase mediates uptake and binding of high-density lipoprotein (HDL) particles and the selective uptake of HDL-associated cholesterol esters independent of its enzymic activity. *Biochem. J.* **368**, 69–79 (2002).
47. Edmondson, A. C. et al. Loss-of-function variants in endothelial lipase are a cause of elevated HDL cholesterol in humans. *J. Clin. Invest.* **119**, 1042–1050 (2009).
48. Ishikawa-Takata, K., Ohta, T. & Tanaka, H. How much exercise is required to reduce blood pressure in essential hypertensives: a dose-response study. *Am. J. Hypertens.* **16**, 629–633 (2003).
49. Shimokawa, Y. et al. Increased expression of endothelial lipase in rat models of hypertension. *Cardiovasc. Res.* **66**, 594–600 (2005).
50. Hirata, K. I. et al. Regulated expression of endothelial cell-derived lipase. *Biochem. Biophys. Res. Commun.* **272**, 90–93 (2000).
51. Degens, H. The role of systemic inflammation in age-related muscle weakness and wasting. *Scand. J. Med. Sci. Sports* **20**, 28–38 (2010).
52. Badellino, K. O., Wolfe, M. L., Reilly, M. P. & Rader, D. J. Endothelial lipase concentrations are increased in metabolic syndrome and associated with coronary atherosclerosis. *PLoS Med.* **3**, 0245–0252 (2006).
53. Paradis, M. E. et al. Endothelial lipase is associated with inflammation in humans. *J. Lipid Res.* **47**, 2808–2813 (2006).
54. Kojma, Y. et al. Endothelial lipase modulates monocyte adhesion to the vessel wall. A potential role in inflammation. *J. Biol. Chem.* **279**, 54032–54038 (2004).