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MATERIALS & METHODS

Measurement of LDL aggregation

Development of a method to quantify person-to-person variability in LDL aggregation-susceptibility

We began by testing inter-individual variability in LDL aggregation using agents that have been reported to cause LDL aggregation. LDL aggregation was induced at LDL concentration of 0.5 mg/ml by oxidation (incubation with 5 µM CuSO₄), by proteolysis (incubation with 0.1 mg/ml α-chymotrypsin from bovine pancreas (Sigma-Aldrich)), or by lipolysis (incubation with 50 mU/ml PLA₂ from bee venom (Sigma-Aldrich)), with 200 mU/ml SMase from Bacillus cereus (bcSMase, Sigma-Aldrich), or with 75 µg/ml hrSMase (a kind gift from Genzyme). The incubation buffer was 20 mM Tris-HCl, pH 7.4, containing 150 mM NaCl and 2 mM CaCl₂ and 2 mM MgCl₂, except in the case of hrSMase, where the incubation buffer was 20 mM MES, pH 5.5, containing 150 mM NaCl and 50 µM ZnCl₂. LDL size was determined at the beginning of the incubation and aggregate size was determined at the indicated time points by dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments, Malvern Works, UK).

Supplementary Figs. 1a-d confirms that oxidation, proteolysis, and lipolysis by PLA₂ and bcSMase each caused aggregation of LDL. The extent of aggregation of LDL from four representative fasting human donors was essentially identical, indicating that these assays are uninformative about possible variations in LDL quality. In contrast, Supplementary Fig. 1e shows large differences in aggregation of the same LDL samples when incubated in the presence of hrSMase at pH 5.5, which is a level of acidity often found in deeper regions of normal intima and within atherosclerotic plaques [1]. Moreover, the extent of LDL aggregation was far greater than with any of the other agents (compare y-axis scales). Therefore, we chose hrSMase-treatment at acidic pH for our systematic analysis of inter-individual differences in the susceptibility of LDL to aggregation.
We used several approaches to standardize the method. First, we found that hrSMase-induced aggregation of a single preparation of LDL was affected by the starting concentration of LDL. LDL aggregation was assayed at LDL concentrations of 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg/ml and aggregation was induced with hrSMase. The particle size was followed with DLS for up to 6 hours (Supplementary Fig. 2a) and concentration 0.2 mg/ml was chosen for standard assay. Second, we also standardized the concentration of hrSMase by its enzymatic activity towards LDL-SM and used a concentration of the enzyme (75 µg/ml) that was able to induce a nearly complete hydrolysis of LDL-SM within a 1-h incubation at pH 5.5. To define inter-assay variance we separately isolated LDL from one plasma sample that was divided into 26 aliquots and stored at -80 °C. The aggregation susceptibility of LDL was determined separately from these samples (Supplementary Fig. 2b). In preparation for analysis of biobanked plasma samples held at -80°C, we found that hrSMase-induced aggregation was unaffected by one freeze-thaw cycle and at most moderately affected by up to five freeze-thaw cycles (Supplementary Fig. 2c). LDL incubated under the same conditions in the absence of hrSMase did not aggregate (Initial LDL size 31.4±3.05 nm at 0h and 30.2±3.43 nm after incubation for 6h, n=94).

The standard assay is described in the article.

**SM-PC-, and LPC-enrichment of LDL ex vivo**

To exchange LDL surface phospholipids, LDL (2 mg/ml) was incubated with phospholipid vesicles (1 mM phosphatidylcholine 18:1/16:0 (1-palmitityl-2-oleyl-sn-glycero-3-phosphocholine), lysophosphatidylcholine 16:0 (1-Palmitoyl-2-Hydroxy-sn-Glycero-3-Phosphocoline) or sphingomyelin 16:0 (N-Palmitoyl-D-erythro-sphingosylphosphorylcholine) each from Avanti Polar Lipids) overnight at 37°C in buffer A (5 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.4). The vesicles had been prepared by sonicating 10 mM lipid solutions using a Branson Sonifier 250
sonicator (6 times for 5 minutes) in buffer A. Vesicles were removed with sequential ultracentrifugation (d<1.019 g/ml KBr) repeated twice.

**Mice**

*Assessment of atherosclerotic lesions*

Animals were euthanized ~24-h after the final i.p. injection of myriocin or vehicle, whole aortas were dissected, and fixed overnight in 4% paraformaldehyde in PBS. Cleaned aortas were stained with Sudan IV, stained lipid areas per section from 3 sections (aortic arch and thoracic and abdominal aorta) and from whole aortas was determined for each mouse.

**Lipid Mass Spectrometry analyses**

The lipid compositions of the SYSDIET-study LDL, the *in vitro*-manipulated human LDL and the myriocin mouse model LDL samples were determined by Agilent 6490 Triple Quad LC/MS with iFunnel technology (Agilent Technologies, California, USA) at the Helsinki University Lipidomics Unit. For the mass spectrometry, the total lipids were extracted from the LDL samples [2] and the extract was evaporated under nitrogen stream and immediately solved in choloform/methanol (1:2, v/v). The samples were spiked with a cocktail of several quantitative standards and 1% (v/v) of NH₃ was added into the samples just before the analysis to support ionization and prevent adduct formation. Lipids with choline head group were detected by using the specific scanning mode *i.e.* as precursors of the ion m/z 184. The mass spectra were recorded with MassHunter (Agilent Technologies) software and the quantitative analysis was performed by LIMSA [3] software, which employed the standards and a lipid library. The acyl chain assemblies in each lipid species were studied by recording negative ion mode product ion scans of the anion fragments for all common fatty acids [4]. For choline lipids, which do not ionize in negative mode as such, formate adducts served as mother ions, and yielded the anionic fragments of the acyl chains [5].
For Corogene and Health 2000 Health Examination Survey the LDL lipid composition was determined with 5500 QTRAP (SCIEX, Framingham, MA) mass spectrometer equipped with Eksigent 100-XL UHPLC system at Zora Biosciences (Espoo, Finland). Lipids were extracted with ethyl acetate:isopropanol (2:8, v/v) including protein precipitation solvent and internal standard solution. Just before the analysis 10 mM ammonium acetate in acetonitrile: 2-propanol (4:3, v/v) with 0.1% formic acid was added to the samples [6].

Circular dichroism (CD) analyses

Samples of control and SM-, PC- and LPC-enriched LDL particles (1mg/ml) were treated with SMase for 30-min and lipolysis was stopped by 10 mM EDTA. The LDL particles (50 μg/ml) were analyzed by CD as described previously [7, 8].

Cell culture

Macrophages

Human white blood cell fractions (buffy coats) were obtained from healthy volunteers (Finnish Red Cross Blood Service, Helsinki, Finland), monocytes were isolated and differentiated into macrophages as described before [9]. To study the effects of aggregated LDL on the macrophages, LDL was incubated with hrSMase for up to 24-h, followed by addition of EDTA to inhibit the enzymatic activity. Cells in 24-well plates or 8-well chamber well slides were incubated with 100 μg/ml of native LDL, hrSMase-aggregated LDL or acetylated LDL in serum-free RPMI 1640 media (Lonza) containing 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. After an overnight incubation, media in 24-well plate were collected and the MMP-1, -2, -3, -7, -8, -9, -10, -12, and -13 levels in culture media were measured with Human MMP Luminex Performanse magnetic panel (R&D systems) according to manufacturer’s instructions. The cells were washed and cellular lipids were extracted with isopropanol: hexane (3:2) and total cholesterol
was determined by cholesterol Amplex Red cholesterol kit (Invitrogen). Separately, foam cells in 8-well chamber well slides were stained with Oil Red as as described previously [10].

**T cells**

To investigate the effects of sphingomyelinase-modified LDL on apoB100apoB100-specific T-cell activation, we used the 48-5 T-cell hybridoma originally described by Hermansson et al [11]. Antigen presenting cells for the co-culture were prepared from irradiated splenocytes (25 Gy). Spleens were harvested from C57BL/6J mice (Jackson, Bar Harbor, Maine) and meshed through a 100 µm cell strainer followed by osmotic lysis of red blood cells (EL buffer, Qiagen, Hilden, Germany). Four-hundred thousand antigen presenting cells were incubated with 10⁵ 48-5 T-cell hybridoma and 10 µg/ml of LDL with different degrees of SMase modification. Native LDL and Concanavalin A were used as positive controls. The cells were incubated in 96-well plates with 200 µl serum-free RPMI 1640 medium containing ITS Premix (Corning, Bedford, Massachusetts), 0.1% bovine serum albumin, nonessential amino acids, L-glutamine, 1 mM Sodium pyruvate, and 50 µM β-ME for 24 hours at 37°C in a humid 5% CO2 atmosphere. Duplicates were used for all samples and the experiment was repeated three times. To assess T-cell activation, interleukin (IL)-2 in the supernatant was measured with ELISA (Mabtech, Nacka Strand, Sweden) according to the manufacturer’s protocol.

**Mathematical modeling and statistical analyses**

*Mixed-effects modeling of LDL aggregation curves to construct an aggregation susceptibility index*

For each individual LDL preparation, i, at each time point, t, we measured LDL aggregate size using DLS (at 0, 1, 2, 3, 4, 5, and 6 hours). To estimate the averaged response y_{i,t}, the data from all the human studies Health 2000 (n=100), Corogene (n=48), Sysdiet (n=57)) were modeled with the generalized logistic function (a slightly modified Richards’ curve) using a statistical modeling
framework developed and distributed as an open-source R package *hamlet* ([https://CRAN.R-project.org/package=hamlet](https://CRAN.R-project.org/package=hamlet)) as described in [12] together with the mixed-effects modeling package *lme4* ([https://CRAN.R-project.org/package=lme4](https://CRAN.R-project.org/package=lme4)). Of note, while the logistic function aims to bring the response variable within [0,1] and is commonly used to model binary outcomes, the generalized form allows much more flexible modeling with the aim of capturing e.g. a time-dependent growth phenomenon as described originally by Richards in [13]. While it retains its sigmoidal form, individual variation was allowed together with more precise inflation point and growth rate modeling, and the response variable has arbitrary constraints instead of the range [0,1]. Using non-linear mixed-effects modeling, the generalized equation of the logistic function presented with four population-based fixed parameters ($\alpha$, $\beta$, $\gamma$, and $\delta$) and was combined with two terms of individual specific variation ($u_\alpha$ and $u_\gamma$) and the error term $\varepsilon_{i,t}$ in equation (1). Thus, the modeled non-linear mixed-effects model was of form:

$$ y_{i,t} = \beta + \frac{\alpha + u_\alpha t - \beta}{1 + e^{\frac{\gamma - u_\gamma t}{\delta}}} + \varepsilon_{i,t} \tag{1} $$

The four population-based fixed effect parameters ($\alpha$, $\beta$, $\gamma$, and $\delta$) are interpreted as follows:

- $\alpha$: the right (top) asymptotical level that is obtained when $t \to \infty$ (i.e., theoretical maximal end-point size of the LDL aggregates).
- $\beta$: the left (bottom) asymptotical level that is obtained when $t \to -\infty$ (related to the original size of the LDL particles).
- $\gamma$: the point of inflection, i.e., the center point in time where the sigmoidal curve transitions from concave upwards to concave downwards. Thus, by definition, it is also the time point when the aggregation curve has the steepest upwards slope.
- $\delta$: the scale parameter that gives the rate of rise (slope) at the point of inflection.
The terms of individual specific variation in random effects are interpreted as follows:

- \( u_{\alpha,i} \sim N(0, \sigma_{\alpha}^2) \): Individual variation in the asymptotic higher levels specific for each individual \( i \), this model term captures variation in the higher horizontal levels that are achieved as \( t \to \infty \).
- \( u_{\gamma,i} \sim N(0, \sigma_{\gamma}^2) \): Individual variation in the point of inflection specific for each individual \( i \), this model term captures the variation for curves that start to raise earlier or later than population mean as a function of time.
- \( \epsilon_{i,t} \sim N(0, \sigma_{\epsilon}^2) \): The normally distributed i.i.d residual error term specific for each observation.

Supplementary Fig. 3a shows the raw data of 100 LDL samples in the Health 2000 cohort (left panel), along with the corresponding model fits (right panel). The fixed effect parameters \( \beta \) (related to the original size of the LDL particles) and \( \delta \) (reflecting the maximal rate of LDL particle aggregation) did not vary significantly among the 100 samples. The aggregation-resistant samples had a lower \( \alpha \) (lower theoretical maximal end-point size), whereas the other samples could not be separated by this parameter. In contrast, the point of inflection \( \gamma \), was able to distinguish the 100 LDL samples according to their aggregation susceptibility. We further found that LDL aggregate size at 2 h \( (y_{i,t}=2\ h) \) correlated tightly and significantly with the individualized inflection point \( \gamma \) coupled with \( u_{\gamma,i} \) of the modeled data (Fig. 3b), but not with the individualized maximal end-point size \( \alpha \) coupled with \( u_{\alpha,i} \). Therefore, in further assays, aggregate size at this time point was used as a measure of LDL aggregation susceptibility.

**Statistical analyses**

The results are presented as average ± SD or if not normally distributed as medians and interquartile ranges. Statistical significance between groups was determined by Student’s t-test when comparing two groups, or by one-way ANOVA or Kruskal-Wallis test followed by post hoc tests. These tests
and two-tailed Spearman correlation were performed using IBM SPSS Software (version 22.0). P-values <0.05 were considered to be significant. No experiments, animals, or samples were excluded, except for technical failure in the lipidomic analyses of one sample in SYSDIET-study and five samples in Health 2000 Health Examination survey. One mouse developed an abdominal tumor in the myriocin experiment (myriocin group) and was therefore excluded.

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Supplementary Figure 1. Aggregation of LDL induced by oxidation, proteolysis, and phospholipolysis

LDL from four blood donors was incubated with (a) copper sulfate, (b) α-chymotrypsin, (c) PLA2, (d) bacterial SMase (bcSMase), or (e) hrSMase for the indicated times. Aggregation was followed by DLS. Notice the differences in y-axis and x-axis scales.
Supplementary Figure 2. Validation of LDL aggregation analysis

LDL was isolated from plasma, LDL aggregation was induced by hrSMase (75 µg/ml) and aggregation was followed by DLS. (a) To determine the effect of LDL concentration on LDL aggregation, the aggregation measurement was performed at the indicated LDL concentrations. (b) To determine the inter-assay variance in LDL aggregation, one plasma sample was divided into 26 aliquots, LDL was isolated from each aliquot separately, and LDL aggregation measurement was performed. The box plot diagram shows the variation in the sizes of LDL aggregates at the various time points. (c) One plasma sample was subjected to 0-5 freeze-thaw cycles as indicated, LDL was isolated, and LDL aggregation measurement was performed. (d) To determine the intra-individual day-to-day variation in LDL aggregation, plasma samples were collected from 4 healthy volunteers at day 0 and day 1, LDL was isolated, and LDL aggregation measurement was performed.
Supplementary Figure 3. Analysis of inter-individual variability in LDL aggregation

(a) LDL aggregation data and the corresponding logistic mixed-effects models in the Health 2000 dataset (n=100).

(b) Correlation of the point of inflection (vertical axis) and the 2h-aggregate size (horizontal axis) in the Health 2000 dataset.

Spearman rho = -0.961
p<0.01
Supplementary Figure 4. SM-, PC-, and LPC-vesicle-induced phospholipid changes in LDL particles in vitro

(a) The amounts of SM, PC, and LPC in LDL before and after treatment with SM-, PC-, and LPC-vesicles was determined by mass spectrometry (n=4). T-test was used to compare the groups.

(b) LDL particles from four healthy volunteer blood donors were treated without vesicles (Control) or with SM-, PC- or LPC-vesicles after which the LDL particles were re-isolated. Control LDL and the SM-, PC- and LPC-enriched LDL particles were treated with hrSMase and particle aggregation was determined by DLS.

(c) The amount of α-helix in control LDL and in SM-, PC- and LPC-enriched LDL from the same donor before and after SMase-treatment was determined by circular dichroism. The average±SD of individual LDL preparations are shown, (n=6, except for LPC-enriched n=4). T-test was used to compare groups.

(d) Representative CD spectra of the LDL samples described above. * indicates p-value <0.05.
Supplementary Figure 5. Changes in the reported consumption of different micro- and macronutrients and their correlation with LDL composition and aggregation in the SYSDIET-study

(a) Changes in intake of the various micro- and macronutrients were calculated by dividing the 18- or 24-week value by the corresponding 0-week value, except for alcohol consumption, which was calculated by subtraction, because some study subjects consumed no alcohol. The columns show averages±SDs. Healthy Nordic diet, n=33, Control diet, n=24. * p<0.05, ** p<0.01, *** p<0.001 by Student's t-test. The p-values were corrected for false discovery rate using the Benjamini-Hochberg method.

(b) Associations of changes in dietary Vitamin E, dietary PUFA, LDL-PC, LDL-SM, and LDL aggregation.

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(a) Changes in intake of the various micro- and macronutrients were calculated by dividing the 18- or 24-week value by the corresponding 0-week value, except for alcohol consumption, which was calculated by subtraction, because some study subjects consumed no alcohol. The columns show averages±SDs. Healthy Nordic diet, n=33, Control diet, n=24. * p<0.05, ** p<0.01, *** p<0.001 by Student's t-test. The p-values were corrected for false discovery rate using the Benjamini-Hochberg method.

(b) Associations of changes in dietary Vitamin E, dietary PUFA, LDL-PC, LDL-SM, and LDL aggregation.
Supplementary Figure 6. Correlation of the changes in the susceptibility of LDL to aggregate with changes in LDL surface lipid composition.

Volcano plots showing Spearman correlation coefficients changes in LDL aggregate size at 2 h vs. changes in LDL surface lipids (a) in the control samples of the SYSDIET study and (b) in placebo samples of the EQUATOR study. Red circles indicate positive correlations, and blue circles indicate negative correlations. The identities of only lipids with significant correlation values (p<0.05) are indicated.
Supplementary Figure 7. LEV-induced lipid changes in LDL particles in mice

(a) The ratio of SM to the sum of PC and LPC, and (b) the ratio of free cholesterol (FC) to cholesteryl esters (CE) in LDL isolated from mice treated with PBS (Control) or with LEVs. T-test was used to compare the groups. ** indicates p-values < 0.01.
Supplementary Figure 8. Plasma lipid levels in myriocin-treated and control mice

(a) Plasma levels of cholesterol (b) and triglycerides in LDLR-/-apoB100/100-mice and LDLR-/-apoB100/100-mice treated with myriocin for various periods of time.
(c) The ratio of SM to the sum of PC and LPC in LDL isolated from the pooled plasma of control mice and mice treated with myriocin. T-test was used to compare the groups.
Supplementary Table 1. Spearman correlation coefficients of aggregate size at 2h with clinical parameters measured in the Health 2000 study.

|                | Age   | Gender | BMI   | TC    | TG    | LDL-C  | HDL-C  | CRP   |
|----------------|-------|--------|-------|-------|-------|--------|--------|-------|
| Aggregate size at 2h | 0.13  | 0.15   | -0.09 | -0.17 | -0.28* | -0.17  | 0.25*  | -0.05 |
| Age            | -0.16 | 0.09   | 0.29* | -0.03 | 0.25*  | 0.19   | -0.11  |       |
| Gender         | -0.23*| 0.21   | -0.22 | -0.35*| 0.40** | 0.07   |        |       |
| BMI            | 0.32**| 0.41** | 0.29* | -0.31**| 0.28*  |        |        |       |
| TC             | 0.34**| 0.90** | -0.04 | -0.04 |       |        |        |       |
| TG             | 0.24  | -0.48**| 0.26* |       |        |        |        |       |
| LDL-C          | -0.29*| -0.10  |       |        |        |        |        |       |
| HDL-C          |       | -0.11  |       |        |        |        |        |       |

* indicates p-values <0.05 and ** <0.01. The p-values were corrected for false discovery rate using Benjamini-Hochberg method. BMI=body-mass-index; TC=total cholesterol; TG=triglycerides; LDL-C=LDL-cholesterol; HDL-C=HDL-cholesterol; CRP=C-reactive protein.
Supplementary Table 2. Spearman correlation coefficients of aggregate size at 2h with clinical parameters measured in the Corogene study.

| Age   | BMI   | TC    | TG    | LDL-C | HDL-C | ApoB  | ApoA-I | ApoA-II | CRP   | Lp(a) | Lp-PLA2 | LDL size | Statin | Smoking |
|-------|-------|-------|-------|-------|-------|-------|--------|---------|-------|-------|---------|----------|--------|---------|
| 0.38  | -0.19 | -0.17 | 0.17  | 0.22  | -0.05 | -0.13 | -0.04  | -0.01   | -0.04 | 0.00  | 0.00    | 0.08     |        |         |
| Age   | 0.36  | -0.10 | 0.20  | 0.18  | 0.133 | -0.08 | 0.07   | -0.06   | -0.07 | 0.17  | -0.00   | 0.08     |        |         |
| BMI   | -0.08 | 0.22  | -0.22 | -0.09 | -0.12 | 0.092 | 0.06   | 0.23    | -0.10 | 0.028 | -0.04   |          |        |         |
| TC    | 0.33  | 0.93  | 0.43  | 0.91  | 0.33  | 0.46  | 0.08   | 0.10    | 0.63  | 0.46  | -0.29   | -0.05    |        |         |
| TG    | 0.14  | -0.18 | 0.42  | 0.03  | 0.20  | 0.14  | -0.10  | 0.18    | -0.15 | 0.04  | -0.02   |          |        |         |
| LDL-C | 0.27  | 0.90  | 0.12  | 0.26  | 0.20  | 0.16  | 0.69   | 0.57    | -0.39 | -0.05 |          |          |        |         |
| HDL-C | 0.15  | 0.90  | 0.65  | 0.38  | -0.062 | -0.09 | 0.21  | 0.16    |        |      | 0.01    |          |        |         |
| ApoB  | 0.07  | 0.24  | 0.23  | 0.13  | 0.65  | 0.38  | -0.30  | -0.01   |      |      |         |          |        |         |
| ApoA-I| 0.78  | -0.48 | -0.20 | -0.14 | -0.03 | 0.26  | 0.05   |         |      |      |         |          |        |         |
| ApoA-II| 0.37  | -0.07 | 0.05  | -0.01 | 0.16  | -0.01 |        |         |      |      |         |          |        |         |
| CRP   | 0.154 | 0.263 | 0.038 | 0.219 | -0.085 |      |        |         |      |      |         |          |        |         |
| Lp(a) | 0.079 | 0.163 | 0.113 | 0.129 |        |      |        |         |      |      |         |          |        |         |
| Lp-PLA2 | 0.51  | -0.47 | 0.042 | 0.042 |        |      |        |         |      |      |         |          |        |         |
| LDL size | -0.62 | -0.21 |        |      |      |      |        |         |      |      |         |          |        |         |
| Statin | 0.30  |      |      |      |      |      |        |         |      |      |         |          |        |         |

* indicates p-values <0.05 and ** <0.01. The p-values were corrected for false discovery rate using Benjamini-Hochberg method. BMI=body mass index; TC=total cholesterol; TG=triglycerides; LDL-C=LDL-cholesterol; HDL-C=HDL-cholesterol; Apo=Apolipoprotein; CRP=C-reactive protein; Lp=lipoprotein
Supplementary Table 3. Clinical characteristics of the SYSDIET-study participants assessed in this study.

| Characteristics                       | Control Diet | Healthy Nordic diet |
|---------------------------------------|--------------|---------------------|
| Number of subjects                   | 24           | 33                  |
| Male/female                          | 8/16 (33%/66%)| 13/20 (39%/61%)    |
| Age (years)                           | 57 (50-61)   | 54 (45-59)          |
| Current smoker                        | 0 (0%)       | 0 (0%)              |
| Diabetes                              | 0 (0%)       | 0 (0%)              |
| Study weeks                           | 0            | 18/24               |
| Blood pressure: syst/diast (mm Hg)²   | 130/86 (124-143/74-93) | 128/84 (121-136/78-92) | 133/88 (124-138/81-95) | 129/86 (123-140/78-91) |
| BMI (kg/m²)²                          | 31.6 (29.6-34.0) | 32.0 (30.5-34.5) | 29.7 (28.5-33.1) | 29.8 (28.1-33.6) |
| Glucose (mmol/L)²                     | 5.6 (5.4-6.2) | 5.6 (5.3-6.1) | 5.9 (5.4-6.2) | 5.6 (5.3-6.1) |
| Statin                                | 7 (29%)      | 7 (29%)            | 7 (21%)       | 7 (21%)       |
| Total cholesterol (mmol/L)²           | 5.4 (4.7-6.3) | 5.5 (4.6-6.0) | 5.2 (4.9-6.1) | 5.1 (4.6-5.8) |
| LDL-C (mmol/L)²                       | 3.4 (3.0-4.1) | 3.7 (2.5-4.0) | 3.2 (2.6-4.1) | 3.0 (2.5-3.6) |
| HDL-C (mmol/L)²                       | 1.2 (1.1-1.5) | 1.3 (1.1-1.7) | 1.5 (1.2-1.7) | 1.6 (1.3-1.8) |
| TG (mmol/L)²                          | 1.2 (1.0-1.9) | 1.4 (0.9-1.8) | 1.4 (0.8-1.9) | 1.3 (1-1.7) |
| C-reactive protein (mg/L)²            | 1.7 (0.9-4.2) | 1.8 (1.2-2.7) | 1.5 (0.8-2.8) | 1.6 (0.8-4.1) |

¹ Number of cases (%)
² Median (interquartile range)
Supplementary Table 4. Clinical characteristics of the EQUATOR-study participants assessed in this study.

| Characteristics                          | RG7652       | Placebo      |
|-----------------------------------------|--------------|--------------|
| Number of subjects                      | 25           | 15           |
| Age (years)                             | 64 (56-70)   | 67 (62-70)   |
| BMI (kg/m²)                             | 29.1 (24.8-31.2) | 29.1 (26.9-31.1) |
| Male/Female                             | 15/10 (60/40%) | 8/7 (53/47%) |
| Statin¹                                 | 15 (60%)     | 11 (73%)     |
| Total cholesterol (mmol/L)²             | 5.5 (4.9-6.2) | 5.4 (4.6-5.6) |
| LDL-C (mmol/L)²                         | 3.3 (2.8-4.1) | 3.4 (2.9-4.0) |
| HDL-C (mmol/L)²                         | 1.2 (0.9-1.5) | 1.4 (1.2-1.6) |
| TG (mmol/L)²                            | 1.8 (1.3-2.1) | 1.3 (1.5-1.9) |

¹ Number of cases (%)
² Median (interquartile range)