SUPPLEMENTAL DATA

LDL subclass lipidomics in Atherogenic Dyslipidemia: Effect of Statin therapy on Bioactive lipids and Dense LDL

M. John Chapman, BSc (Hons), PhD, DSc[1]*, Alexina Orsoni, PhD[2], Ricardo Tan, BSc[3], Natalie A. Mellett, BSc[3], Anh Nguyen, BSc[3], Paul Robillard, BSc[1], Philippe Giral, MD[4], Patrice Thérond, PhD[2] and Peter J. Meikle, PhD[3]*

[1] Endocrinology Metabolism Division, Pitie–Salpetriere University Hospital, Sorbonne University and National Institute for Health and Medical Research (INSERM), Paris, France (Drs Chapman and Robillard); [2] Service de Biochimie, AP–HP, HUPS, Bicetre University Hospital, Le Kremlin Bicetre (Drs Orsoni and Therond), and EA 7357, Paris–Sud University and Paris–Saclay University, Chatenay–Malabry, France (Dr Therond); [3] Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria 3004, Australia (Drs Chapman, Tan, Nguyen, Mellett, and Meikle; and [4] INSERM UMR1166 and Cardiovascular Prevention Units, ICAN–Institute of CardioMetabolism and Nutrition, AP–HP, Pitie–Salpetriere University Hospital, Paris, France (Dr Giral)

*These authors contributed equally.

[1]Address correspondence to: Professor M. John Chapman, Endocrinology-Metabolism Division, Pitié-Salpêtrière University Hospital, 83, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France,

Email: john.chapman@upmc.fr; Tel: 331.4875632

Running title: Effect of Pitavastatin therapy on Lysolipids and Ceramides

METHODS:

***Characteristics of the Metabolic Syndrome subject cohort*:**

The patient cohort (biological, clinical and anthropometric characteristics), comprehensive inclusion and exclusion criteria, and the clinical protocol, were described earlier (1-4); essential features of this single-phase, unblinded study are briefly summarised here and in **Suppl Fig. S1**. The CAPITAIN Study (ClinicalTrials.gov, #NCT01595828) was monocentric and recruited obese, hypertriglyceridemic, hypercholesterolemic Caucasian male subjects (n=12; mean age 50 ± 3 years) with fasting plasma triglycerides > 150 mg/dL (≥1.7 mmol/l), LDL-C of 130–190 mg/dl (3.4–4.9 mmol/L), and a prediabetic phenotype of MetS determined according to strict International Diabetes Federation (IDF) criteria (5) (**Suppl Table S1);** data in the MetS group are compared to those in an age-matched group of non-obese, healthy normolipidemic, normoglycemic males (1-3,6); subjects exhibited moderate insulin resistance as evaluated by the HOMA index but did not display type 2 diabetes (4). Baseline levels of both HDL-C and apoAI were subnormal, and trended to minor increments on treatment (+ 4 and + 6% respectively)(2). Genotypically, subjects were predominantly apoE3 homozygotes (75%). Given strict inclusion and exclusion criteria (see below), and given the polygenic nature of mixed dyslipidemia, subjects acted as their own controls in order to limit potential confounding between separate placebo and treatment groups due to (i) variation in baseline phenotype, (response to statins is substantially phenotype-dependent) (7) and (ii) variation in genetic background and thus potential variability in pharmacogenomic response to statin therapy (8-10). This aspect is discussed in more detail under « Limitations » in the Discussion section of the main manuscript.

Inclusion criteria:

Inclusion criteria required participants to have central obesity (defined as a waist circumference ≥94 cm), plus any two of: (i) elevated (fasting) triglyceride level ≥1.7 mmol/l (>150 mg/dl), (ii) subnormal HDL-C <1.03 mmol/l (≤ 40 mg/dl) in males, (iii) controlled hypertension (systolic blood pressure (BP) ≥130 mmHg or diastolic BP ≥85 mmHg) or treatment for previously diagnosed hypertension with a calcium channel blocker which did not require treatment with a diuretic, beta-blocker, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker, or (iv) fasting plasma

glucose (FPG) ≥5.6 mmol/l (100 mg/dl). Key exclusion criteria were fasting triglyceride levels >400 mg/dl (4.5mmol/l), LDL-C >190 mg/dl (4.9 mmol/l) and excessive obesity defined as BMI > 35 kg/m^2. Recruitment was focused on subjects whose lifestyle and dietary profile was evaluated as consistent over time. Importantly, comprehensive lifestyle and dietary counseling throughout the study time course were reflected in a stable BMI (31.7±0.5 and 31.8±0.7 kg/m^2 at baseline and 180 days respectively) and by a stable homeostasis model of insulin resistance (2.7±0.5 and 2.2±0.3 at baseline and 180 days respectively; NS). Waist circumference was unchanged over the course of the study. All participants had been non-smokers for at least 12 months prior to inclusion and had previously smoked less than 25 cigarettes /day on a regular basis. Study participants had no history of cardiovascular disease or type 2 diabetes.

Exclusion criteria:

All subjects included in the study did not meet any of the following exclusion criteria:

- Women
- Non-Caucasian
- Excessive obesity defined as BMI above 35 kg/m2, rounded to the nearest whole number
- LDL-C >190 mg/dl at screening
- Fasting TGs >400 mg/dl at screening
- Diabetes mellitus, defined as a fasting glucose >7 mM, or taking diabetic therapy at screening
- History of symptomatic cardiovascular disease including angina pectoris, acute myocardial infarction, or peripheral arterial disease including intermittent claudication
- History of symptomatic cerebrovascular disease, including cerebrovascular hemorrhage, transient ischemic attack, or carotid endarterectomy
- A current smoker or have smoked in the preceding 12 months
- Consume >10 g of alcohol (equivalent to one 100 ml glass of table wine) per day
- Have received statins, fibric acid derivatives, bile acid sequestrants, cholesterol absorption inhibitors (including ezetimibe), or nicotinic acid >500 mg per day in the previous year

- Have uncontrolled hypertension (SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg). Patients may have their hypertension controlled with a calcium channel blocker but must not receive treatment with a diuretic, beta-blocker, angiotensin converting enzyme inhibitor, or angiotensin II Receptor blocker. If the patient has previously received treatment with these therapies, they must have been discontinued at least 2 months previously.

- Any conditions that cause secondary dyslipidemia or increase the risk of statin therapy including alcoholism, autoimmune disease, nephrotic syndrome, uremia, any viral hepatitis clinically active within 12 months before study entry, obstructive hepatic or biliary disease, dysglobulinemia or macroglobulinemia, multiple myeloma, glycogen storage disease, porphyria, and uncontrolled hypothyroidism or hyperthyroidism. Controlled thyroid disease [normal serum thyroid stimulating hormone and stable therapy for at least 3 months] is permitted.
- History of pancreatic injury or pancreatitis, or impaired pancreatic function/injury as indicated by abnormal lipase
- Liver injury as indicated by serum transaminase levels (alanine aminotransferase/serum glutamic pyruvic transaminase, aspartate aminotransaminase/serum glutamic oxaloacetic transaminase) >3× upper limit of the reference range (ULRR).
- Impaired renal function as indicated by serum creatinine levels >1.5× ULRR at screening or estimated glomerular filtration rate (eGFR) by Cockroft formula <60 ml/min.
- History of any muscle disease or unexplained elevation (>3× ULRR) of serum creatine kinase
- Any surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of the study drug, including the following: history of major gastrointestinal tract surgery (e.g., gastrectomy, gastroenterostomy or small bowel resection), gastritis or inflammatory bowel disease, current active ulcers, or gastrointestinal or rectal bleeding.
    - Current obstruction of the urinary tract or difficulty in voiding likely to require intervention during the course of the study

- Severe acute illness or severe trauma in the preceding 3 months
- Evidence of symptomatic heart failure (New York Heart Association class III or IV): significant heart block or cardiac arrhythmia
- History of uncontrolled complex ventricular arrhythmias, uncontrolled atrial fibrillation/flutter or uncontrolled supraventricular tachycardias with a ventricular response rate of >100 beats/min at rest. Patients whose electrophysiological instability is controlled with a pacemaker or implantable cardiac device are eligible.
- History of drug abuse
- History of allergy or intolerance to medication (including statins)
- Current or recent (within 1 week) use of supplements or medications known to alter lipid metabolism including soluble fiber (including >2 teaspoons Metamucil or psyllium-containing supplement per day) or other dietary fiber supplements, fish oils containing omega-3 oils, "fat blockers" (e.g., orlistat), or other products at the discretion of the investigator
- Any forbidden concomitant medication
- Within the exclusion period defined in the National Register for Healthy Volunteers of the French Ministry of Health
- Participation in any clinical trial with an investigational drug in the past 3 months preceding study entry
- Forfeit their freedom by administrative or legal award or who are under guardianship.

Clinical protocol, dietary counseling and compliance:

All subjects received high dose pitavastatin calcium (4mg/day) for a period of 180 days in an open label protocol **(Suppl. Fig. S1).** With the exception of study visits, the study drug was taken in the morning between 7:00 and 10:00 AM. At initiation of the protocol, each subject was admitted to the Clinical Unit at approximately 6:00 PM on day -2, and remained until baseline blood collection in the overnight fasting state on day 1. Thus, all subjects remained for 36 h in the Clinical Unit before blood sampling in order to ensure abstinence from alcohol, coffee, tea, or

sugared beverages; meals consumed on day -2 and on day -1, prior to collection of the baseline, pretreatment (D0) blood sample, were mixed meals consisting of 30–35% fat, 50–55% carbohydrate, and approximately 15% protein. Strenuous physical exercise was not allowed during the stay in the Clinical Unit. Subjects took the study medication at the end of the visit. All subjects were counseled by a dietician to abstain from alcohol, coffee, tea, or sugared beverages, or any beverages containing methylxanthines (theophylline, caffeine, or theobromine) during the 48h preceding the initial visit and subsequent visits. Furthermore, subjects were requested to limit, as much as possible, the consumption of all of the former beverages throughout the study duration (alcohol < 10 g/day). The consumption of starfruit, grapefruit, or grapefruit juice was not allowed starting from 1 week before dosing until discharge at the final visit at D180. Otherwise, overall dietary intake was not modified during the study. Subjects returned to the Clinical Unit on days 7, 30, 42, and 120 for compliance (drug intake was monitored with individual diary cards and by pill counts) and safety assessments; the investigator checked on the well-being of all subjects prior to discharge at each visit. At the end of each visit, the participant took the study medication. Furthermore, the dietary pattern and calorie consumption of each participant was controlled by the study dietician at each visit to the Clinical Unit; particular attention was accorded to alcohol consumption. Strenuous exercise was not allowed from the week preceding study initiation to the end of the clinical protocol. Finally, subjects returned to the Clinical Unit on day 180 (±7 days) in the morning, having fasted for at least 12 h, for blood sample collection and compliance and safety assessments. Tablet counts indicated that compliance with active treatment was 100%. The last meal taken before the study visit corresponded to a mixed meal, as described above at baseline and as counseled by the dietician. For further details, see Chapman et al (2) and Meikle et al (1).

Blood samples

Blood samples were collected after overnight fasting before initiation of statin treatment (baseline, D0) and at 180 days (D180) within 24 hours after the final intake of drug. Blood samples were withdrawn in the Clinical Unit by venipuncture from the antecubital vein into precooled (4°C) sterile, evacuated

tubes in the presence or absence (for serum isolation) of EDTA (final concentration 1 mg/ml) at pretreatment (D0) and post-treatment (D180) time points (1-3). Plasma or serum was separated from blood cells by low-speed centrifugation at 1,700 g for 20 min at 4°C; 0.6% sucrose was added to cryoprotect lipoproteins and plasma or serum aliquoted within 2 h of blood collection (11). After freezing in liquid nitrogen, samples were stored at -80°C until analysis; samples were thawed once and analysed directly. Earlier studies have documented the absence of lipid- or protein-derived oxidation products in the component lipoproteins of such samples (3, 12).

Analytical and Preparative Methods

Plasma lipid and apolipoprotein profiles

Plasma levels of triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C, and Lp(a) at baseline (D0) and at 180 days of statin treatment (D180) were quantified spectrophotometrically using Randox® (Randox Laboratories, Crumlin, N. Ireland) reagent kits (2). Non-HDL-C was calculated as TC minus HDL-C. Plasma concentrations of apoAI and apoB were determined by immunonephelometry on a Dade-Behring® autoanalyzer with Siemens® reagents. Intra- and inter-assay coefficients of variation were <10% for all assays.

Determination of plasma LCAT activity and plasma CETP mass and activity

The activity of LCAT in plasma was determined using an assay kit involving a fluorescent substrate (Roar Biomedical®) according to the instruction manual (2). CETP mass in plasma was measured using a sandwich immunoassay (ALPCO Diagnostics®), according to the instruction manual (2). Plasma CETP activity was determined using a fluorescent ex-vivo CETP activity assay kit (Roar Biomedical®) according to the manufacturer's instructions (2); this assay measures CETP activity against an exogenous substrate.

Determination of secretory phospholipase A2 mass in plasma

The plasma mass of secretory PLA2 (human type IIA) mass was determined using the colorimetric sPLA2 assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) based on 96 well plates

according to the manufacturer's instructions. The synthetic substrate is the 1,2-dithio analogue of diheptanoyl thiophosphorylcholine. Upon hydrolysis of the thioester bond at the *sn*-2 position by PLA_2, free thiols are detected using DTNB (5,5-dithio-*bis*-(2-nitrobenzoic acid) at 414 nm with a microwell plate reader (Dynex). Bee venom PLA2 was used as a positive control. Results are expressed as time-dependent absorbance increase using 5 points in time with 1-minute increments. Values are means of duplicate or triplicate measurements. Performance characteristics conformed to those described in the manufacturer's instruction manual.

Determination of oxidized LDL concentration in plasma

Circulating levels of oxidized LDL were quantitated with a solid phase two-site ELISA in a sandwich technique based on two different monoclonal antibodies and in accordance with the manufacturer's instruction manual (Mercodia, Sweden). This assay determines the content of aldehyde-conjugated epitopes in LDL-apoB100, and is therefore an indirect measure of the oxidative modification of LDL. Samples were assayed in duplicate or triplicate in multiwell plates; following reaction with peroxidase-conjugated anti-oxidised LDL, the absorbance at 450nm was read in each sample well using a Dynex multiwell plate reader (Perkin-Elmer). Intra- and inter-assay coefficients of variation were <10% for this assay.

Determination of paraoxonase activity in serum

Paraoxonase activity was assayed as the organophosphatase activity of serum at baseline and after statin treatment (D180) using the EnzChek paraoxonase assay kit (Invitrogen, Thermo Fisher, Carlsbad, CA, USA). This assay is based on the hydrolysis of a fluorigenic organophosphate analogue; assays were performed in duplicate according to the manufacturer's instructions, and readout conducted on a microwell plate reader (TECAN) at 350nm.

Determination of serum amyloid A (SAA) in plasma

As an acute phase reactant, SAA was assayed in plasma samples at baseline and after statin treatment (D180). As 3 subjects displayed hsCRP levels at baseline superior to 5mg/L, they were excluded from

SAA assays. Plasma SAA levels were determined with a solid phase two-site ELISA based on a biotin-streptavidin-peroxidase system in 96 well plates according to the manufacturer's instructions, which included a standard curve based on human recombinant SAA (Invitrogen, Thermo Fisher, Carlsbad, CA, USA). Readout was performed at 450nm on a microwell plate reader (Dynex) following reaction with DTNB as substrate. Values are means of duplicate or triplicate assays.

Determination of plasma high sensitivity C-reactive protein, pro- and anti-inflammatory cytokines, soluble cytokine receptors, growth factors, and adhesion proteins

Determination of high sensitivity C-reactive protein (hsCRP) in plasma samples was performed using the MetS Array II, a multiplex (biochip) array, according to the manufacturer's instruction manual (Randox Laboratories, Crumlin, N. Ireland); quantitation was based on a chemiluminescent signal. After development, the multiplex arrays were read on a Randox Investigator BioChip Reader (Randox Laboratories, Crumlin, N. Ireland). The lower limit of the sensitivity of the hs-CRP assay was <0.67 µg/mL. Intra- and inter-assay CVs were in the range from 4 to 15%. It is noteworthy that 3 subjects displayed baseline hsCRP values in excess of 5mg/L, potentially due to mild infection; these subjects were excluded.

Pro- and anti-inflammatory cytokines, soluble cytokine receptors, growth factors and adhesion proteins were assayed as described earlier using multiplex biochip arrays (Randox Laboratories, Crumlin, N. Ireland) by the same technology as that indicated above for hsCRP, and in accordance with the manufacturers' instructions (13). Additional details of assay sensitivities are included in the Manufacturer's instruction manual.

Preparative isolation of plasma lipoprotein subfractions at baseline and following statin treatment

A single step, isopycnic non-denaturing density gradient procedure was employed to preparatively fractionate LDL subpopulations from plasma samples at each timepoint (D0 and D180) on the basis of their hydrated densities by ultracentrifugation in a Beckman SW41 Ti rotor at 40,000 rpm for 44 hours in a Beckman Optima XPN-80 ultracentrifuge at 15°C (14). Circulating LDL particles exhibit

hydrated densities ranging from 1.019 to 1.063 g/mL (14). Four LDL subpopulations are currently differentiated within this density range, notably large buoyant particles (d 1.019-1.023 g/ml), particles of medium or intermediate size and density (d 1.023-1.034 g/ml), small dense particles (sdLDL; d 1.034-1.063 g/ml); within the small dense LDL subclass, the smallest and densest subspecies is denoted very small dense LDL (d 1.050-1.063 g/ml)(15,16). We presently focused on apoB-containing lipoprotein fractions as follows: VLDL + IDL, d<1.019 g/mL, and 5 LDL subclasses: LDL1 (d=1.019-1.023 g/mL), LDL2 (d=1.023-1.029 g/mL), LDL3 (d=1.029-1.039 g/mL), LDL4 (d=1.039-1.050 g/mL) and LDL5 (d=1.050-1.063 g/mL). Light buoyant LDL were operationally defined as d 1.019-1.029 g/mL (ie. LDL1 + LDL2), intermediate or medium LDL as d 1.029-1.039 g/mL (ie. LDL3), small dense LDL as d 1.039 -1.050 g/mL (ie. LDL4), and very small dense LDL as d 1.050 -1.063 g/mL (ie. LDL5). It is especially relevant that MetS subjects with low plasma levels of Lp(a) (<10mg/dL) were preferentially recruited **(Suppl Table S1**), and in this way, co-isolation of dense LDL and Lp(a) was largely avoided (fig. 5A in ref. 2). Gradient fractions were desalted by dialysis at 4°C in SpectraPor tubing (Spectralabs.com) in the dark as described previously (6,14).

Chemical analysis of lipoprotein subfractions isolated by isopycnic density gradient ultracentrifugation:

Total cholesterol (TC), free cholesterol (COH), phospholipid (PL), triglyceride (TG) and total protein (TP) concentrations in the VLDL + IDL subfraction and in LDL1 to LDL5 at baseline (D0) and after treatment (D180) were determined by spectrophotometry with the following assay kits (for TC, FC, PL, Diasys Diagnostics Systems GmbH; for TG, Biomerieux and for protein, the BCA method of Thermo Scientific Pierce) as described previously (2,3,6,12) ; it is noteworthy that the phospholipid assay estimates choline enzymatically liberated from phosphatidylcholines and sphingomyelins, and thus non-choline-containing phospholipids, and notably those containing ethanolamine, inositol, or serine are not estimated. It is equally noteworthy that enzymatic assay of TG as performed here estimates glycerol content after lipolysis; this assay therefore includes glycerol derived from partial glycerides, such as DAG (see below). The concentration of cholesteryl esters was calculated from the formula (TC-FC) x1.67 (14). The total mass of each subfraction was calculated as the sum of FC, PL,

TG, CE and TP. ApoB concentration in individual apoB-containing subfractions was determined by immunoturbidimetry on the Konelab autoanalyser 20® instrument with Apolipoprotein B reagents, calibrators and "Lipotrol" as control (Thermo Scientific). Lp(a) concentration in each lipoprotein subfraction was determined on the Konelab® autoanalyser 20 instrument using a commercial assay kit (Randox®). Coefficients of intra- and inter-assay variation for the individual assays ranged from 2% to 9%. Concentrations were expressed as mg/dL plasma following correction for increase in plasma volume resulting from addition of solid KBr to raise the density to 1.21 g/mL for gradient fractionation (14).

Lipoprotein-associated phospholipase A2 mass and activity in plasma and lipoprotein subfractions

The PLAC® Test ELISA Kit, a solid phase sandwich enzyme immunoassay, was employed for determination of Lp-PLA2 mass in plasma and lipoprotein subfractions in microwell plates according to the manufacturer's instruction manual (Diadexus Inc, South San Francisco, CA, USA). This assay employed two distinct monoclonal antibodies to human Lp-PLA2 and was calibrated using human recombinant Lp-PLA2. The second antibody was conjugated to horseradish peroxidase; after reaction with the substrate, tetramethylbenzidine, absorbance was read at 450nm with a microwell plate reader (Dynex Technologies, VA, USA) and is directly proportional to the concentration of Lp-PLA2 present. Samples were analysed in duplicate or triplicate simultaneously with calibration standards. The performance characteristics of this assay corresponded to those quoted in the manufacturer's instruction manual.

The PLAC® test for Lp-PLA2 activity in plasma and lipoprotein subfractions was adapted to the Konelab® autoanalyser 20 instrument in collaboration with the manufacturer (Diadexus Inc, South San Francisco, CA, USA). Lp-PLA2 hydrolyses the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, to produce a colored reaction product, 4-nitrophenol. The rate of formation of 4-nitrophenol was measured spectrophotometrically at 410nm and Lp-PLA2 activity calculated from the rate of change in absorbance. A set of five Lp-PLA2 calibrators was used to generate a standard curve fit of change in absorbance versus Lp-PLA2 activity (nmol/min/mL), from which Lp-PLA2 activity in

each sample was derived. All samples were assayed in duplicate or triplicate; a set of calibration standards was measured in the same series. Performance characteristics were within the range of those quoted by the manufacturer.

RESULTS:

Plasma Lipid phenotype of the MetS subjects at baseline, effect of pitavastatin treatment, and comparison with normal healthy control subjects.

The plasma lipid phenotype of obese, mixed dyslipidemic, male MetS subjects at baseline, and following pitavastatin treatment (4mg/day; 180 days; D180), including lipid, lipoprotein and apolipoprotein profile, is summarized in **Suppl Table S1;** assays used in the clinical laboratory were employed, for which details are provided in the Methods section above. Comparison of the MetS phenotype at baseline and following statin treatment with corresponding values in normal, healthy control subjects has been reported earlier and is presented in **Suppl Table S1,** indicating that MetS subjects were both hypertriglyceridemic and hypercholesterolemic at baseline, and that this dyslipidemia was largely normalised by statin treatment to lipid levels within the respective normal ranges (1,2,6).

Baseline plasma mass concentrations and % weight chemical compositions of the VLDL+IDL fraction and LDL1-5 subclasses in MetS subjects and comparison with normal healthy controls : Effect of pitavastatin treatment

A wide overall range in plasma LDL mass concentrations in healthy control subjects has been reported from our laboratory using comparable analytical methodologies (225 – 303 mg/dL) (2,6). Consistent with this finding, we observed wide ranges in the mass concentrations of individual LDL subclasses in these earlier studies (Table S2)(2,6).

The effects of pitavastatin treatment (4mg/day; D180) on the % weight chemical compositions of the VLDL+IDL fraction and LDL1-5 subclasses in the MetS subjects at baseline (D0) are summarized in **Suppl Table S2** (taken from data reported earlier in ref. 2)**.** These findings are discussed in more detail in the Results section of the main manuscript.

Plasma concentrations of 23 lipid classes in the VLDL+IDL subfraction and the LDL1-5 subclasses: Effect of pitavastatin treatment

The absolute values for the plasma concentrations of the 23 lipid classes detected in the VLDL+IDL fraction and LDL1-LDL5 subclasses by mass spectrometry at baseline in obese, mixed dyslipidemic, male MetS subjects (expressed as pmol/mL plasma), and the effect of pitavastatin treatment (4mg/day; D180) on these concentrations (changes are expressed as %) are summarized in **Suppl Table S3**. These data are presented in schematic form as Figure 1 in the main manuscript.

Statistical comparisons between the Lipid class profiles normalized to apoB in the VLDL+IDL subfraction versus individual LDL1-5 subclasses at baseline: Effect of statin treatment.

In order to probe structural relationships between each lipid class and apoB in LDL particles, data were expressed as molar ratios of lipid classes to apoB. Individual apoB-normalized lipid profiles of VLDL+IDL were compared with each LDL subclass, and individual LDL subclasses with each other both at baseline D0 and at D180 in **Suppl Figure 2** (**panels A - AF)**.

Overall, the VLDL+IDL subfraction differed from each LDL subclass (LDL1 to LDL5) in 5 of the 22 lipid classes at baseline D0. Indeed, TAG (p<0.001; **fig.S2-C**), DAG (p<0.001; **fig.S2-E**), PE (p<0.001; **fig.S2-O**), and PI (0.001<p<0.01 vs LDL1, LDL2, LDL3 and LDL5 and p<0.001 vs LDL4; **fig.S2-R**) were more abundant per apoB in VLDL+IDL particles than in LDL1-5, whereas MHC (0.01<p<0.05 vs LDL1, 0.001<p<0.01 vs LDL4 and LDL5 and p<0.001 vs LDL2 and LDL3; **fig.S2-Y**) was deficient. In addition, SM/apoB ratio in VLDL+IDL (**figS.2-G**) was lower compared to light LDL (LDL1 and LDL2, 0.01<p<0.05 and 0.001<p<0.01, respectively) and to LDL3 (0.001<p<0.01). By contrast, PC per particle (ie. PC/apoB ratio; **fig.S2-I**) was enriched in VLDL+IDL compared to LDL2

($0.001<p<0.01$), LDL3 ($0.001<p<0.01$), LDL4 ($p<0.001$) and LDL5 ($0.001<p<0.01$); equally, VLDL+IDL were enriched in LPE comparatively to LDL3 and LDL4 ($0.01<p<0.05$; **fig. S2-P**). Interestingly, VLDL+IDL were enriched in Cer (**fig S.2-U**) compared to intermediate LDL3, ($0.01<p0.05$) and dense LDL (LDL4 and LDL5, $p<0.001$), whereas they were enriched in dhCer compared to dense LDL only ($0.01<p<0.05$; **fig. S2-W**). Finally, DHC (**fig.S2-AA**) and GM3 (**fig.S2-AE**) were deficient in VLDL+IDL compared to light LDL1 ($0.01<p<0.05$ and $0.001<p<0.01$, respectively) and LDL2 (both $p<0.001$), and equally to intermediate LDL3 ($0.001<p<0.01$ and $p<0.001$, respectively). Similarly, THC/apoB ratio (**fig.S2-AC**) was lower in VLDL+IDL as compared to light (LDL1 and LDL2; $0.001<p<0.01$ and $p<0.001$ respectively), to intermediate LDL ($p<0.001$), and to LDL5 ($0.01<p<0.05$). The following lipid classes did not differ in apoB molar ratio between VLDL+IDL and any LDL subfraction at baseline: COH, PC(P), PC(O) (**fig.S2-J**), and PE(P). With respect to lipid molar ratios, DAG/TAG was highest in the TG-rich subfraction, ie. VLDL+IDL (**fig. S2-AJ**) and was equally elevated as compared to light and intermediate LDL ($p<0.001$ vs LDL1, LDL2 and LDL3). In marked contrast, the SM/PC ratio showed the surface of VLDL+IDL to be deficient in SM relative to all LDL subfractions (**Suppl Fig S2-AK).** Furthermore, the CE/TAG ratio was up to some 10-fold lower in VLDL+IDL relative to LDL subfractions (**Suppl Fig S2-AG**).

Statistical comparisons between the Lipid class profiles normalized to apoB in the individual LDL1-5 subclasses at baseline: Effect of statin treatment.

Significant discontinuities between LDL subclasses were evident when the profiles of lipid/apoB ratios were considered at baseline **(Fig. 2A-V; Suppl Fig. S2-A-AF).** Thus, light LDL1 and LDL2 showed significant differences with intermediate LDL3, dense LDL4 and LDL5 for six lipid classes. Indeed, the CE /apoB ratio in LDL1 revealed CE enrichment (**Suppl Fig**.S2-**B**) as compared to both intermediate LDL ($0.001<p<0.01$) and dense LDL ($p<0.001$ vs LDL4 and $0.01<p<0.05$ vs LDL5). Moreover, both TAG (**Suppl Fig.S2-D**) and DAG (**Suppl Fig.S2-F**) /apoB ratios were elevated in LDL1 compared to LDL2

($0.001<p<0.001$ and $0.01<p<0.05$, respectively), intermediate LDL ($p<0.001$ for both) and dense LDL ($p<0.001$), LDL3, LDL4 and LDL5 ($p<0.001$). These findings were reflected in a lower CE/TAG ratio **(Suppl Fig.S2-AH**) in LDL1 relative to LDL2 ($0.01<p<0.05$), and to LDL3 to LDL5 ($p<0.001$). While the individual PC and SM ratios were not distinct in LDL1, nonetheless the SM / PC ratio (**Suppl fig.S2-AL**) was lower in LDL1 compared to LDL2 ($0.001<p<0.01$), and LDL3 ($0.001<p<0.01$).The Cer/apoB ratio of LDL1 (**Suppl fig.S2-V**) was also higher than that in LDL3, LDL4 and LDL5 ($p<0.001$ for all), while dhCer/apoB ratio (**Suppl fig.S2-X**) was higher in LDL1 than in dense LDL4 and 5 ($p<0.001$). The lipid/apoB profiles in LDL2 were distinct from those of LDL1, 3, 4 and 5 in five lipid classes, suggestive of a metabolic discontinuity concerning these specific lipids. Thus, LDL2 showed CE enrichment as compared to LDL4 only (**Suppl fig. 2-B;** $0.001<p<0.01$). The TAG/apoB ratio in LDL2 **(Suppl fig.2-D**) showed similar differences as seen above for LDL1, as LDL2 was enriched in TAG compared to LDL3 ($0.01<p<0.05$), LDL4 ($0.001<p<0.01$) and LDL5 ($0.01<p<0.05$). These observations are consistent with a lower CE to TAG ratio (**Suppl fig.S2-AH**) in LDL2 relative to LDL3 to LDL5 ($p<0.001$), and as seen for LDL1. The SM, DHC, THC and GM3 /apoB profiles in LDL2 were distinct from the remaining LDL subfractions. Indeed, molar SM (**Suppl fig.S2-H**) and GM3 (**Suppl fig.S2-AF**) /apoB ratios were increased in LDL2 compared to LDL4 ($0.01<p<0.05$ for both) and LDL5 ($0.01<p<0.05$, for both lipid classes); DHC (**Suppl fig.S2-AB**) and THC (**Suppl fig.S2-AD**) ratios were also higher in LDL2 compared to LDL4 ($0.01<p<0.05$ for both). Surprisingly, LDL2 showed a higher SM / PC ratio (**Suppl fig.S2-AL**) relative to LDL4 ($0.01<p<0.05$) and LDL5 ($p<0.001$), whereas this ratio in LDL1 was lower than that in both LDL2 and LDL3, giving a convex profile overall.

When the apoB-normalised profiles for the VLDL+IDL fraction and the LDL1-5 subclasses were considered following statin treatment, then an overall resemblance to the corresponding profiles at baseline was seen for each lipid class, with minor differences. The significant and preferential elevation in the lysolipid/apoB ratio for LPC, LPC(O) and LPI in LDL5 at both

baseline and following statin treatment is noteworthy (range of p values <0.05 – 0.001) (**Suppl fig.S2, K-N, T).**

Comparison of lipid class concentrations in the total LDL fraction in healthy, control subjects with those of MetS subjects at baseline: Normalisation by statin treatment

Important qualifications concerning the comparison of lipid class profiles in LDL (d1.019-1.063 g/mL) in control subjects with the corresponding LDL fraction in the MetS group at D0 and D180 are discussed in the main Results text. These profiles are presented in Suppl. Table S4; the salient findings are commented in the Results section.

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Supplementary Table S1: Baseline plasma lipid, lipoprotein and apolipoprotein concentrations in obese, mixed dyslipidemic, male MetS subjects, comparison with a healthy normal control group, and effect of pitavastatin calcium treatment (4mg/day; 180 days; D180).

	Healthy Controls	Baseline (D0)	Post-statin (D180)	% Change	p-value (D180 vs D0)
Lipid and Apolipoprotein Parameters (mg/dL)					
Total Cholesterol	171.4 ± 8.0*	232.2 ± 17.6	161.7 ± 5.7	-30%	<0.0005
Triglycerides	75.3 ±11.1 **	215.9 ± 16.0	127.7 ± 8.1	-41%	<0.0005
VLDL+IDL mass	56.0 ± 1.6**	135.9 ± 10.3	98.4 ± 8.1	-28%	0.0126
LDL-Cholesterol	100.4 ± 6.4*	153.0 ± 6.2	96.1 ± 5.8	-37%	<0.0001
ApoB	80.3 ± 12.6***	102.0 ± 4.2	72.8 ± 5.1	-29%	<0.0001
LDL mass	225.1 ±10.4*	320.6 ± 20.0	225.5 ± 12.6	-30%	<0.0001
HDL-Cholesterol	56.4 ±3.0**	46.3 ± 2.8	48.2 ± 3.6	+4%	ns
ApoAI	147.1 ±4.2**	100.3 ± 4.8	106.6 ± 5.7	+6%	ns
LDL-C/HDL-C ratio	1.8 ±0.1**	3.4 ± 0.2	2.1 ± 0.2	-38%	<0.0001
Lp(a)[a]	< 10.0	8.8 (0.5 – 24.9)	8.5 (0.9 – 32.2)	-3%	ns

Values in the MetS and control groups are expressed as means ± SEM (n=12 and n=10 respectively). [a]Due to its asymmetric distribution, Lp(a) levels are expressed as median (minimum-maximum). ns = non-significant. Values for the VLDL+IDL (density <1.019 g/mL) and total LDL (density 1.019-1.063g/mL) fractions are expressed as total mass (mg/dL). Data from Orsoni et al (3). VLDL+IDL mass and LDL mass represent the sum of the total concentrations of

free cholesterol, cholesteryl ester, triglyceride, phospholipid and protein as determined by absorptiometric assays in the respective subfractions (2,6,14). Values in the age-matched healthy male control group are taken from ref (2). *$p<0.0001$ for control group versus D0 value in the MetS group; **$p< 0.001$ for control group versus D0 and D180 values in the MetS group; ***$p< 0.05$ for the control group versus D0 values in the MetS group.

Supplementary Table S2: Effect of pitavastatin calcium treatment (4mg/day; 180 days; D180) on the weight % chemical composition and plasma concentrations of the VLDL+IDL fraction and LDL1-5 Subclasses from MetS Subjects at baseline (D0).

Component	Time point	VLDL+IDL	LDL1	LDL2	LDL3	LDL4	LDL5
TG	D0	50.0 ± 1.6	34.2 ± 2.1	18.7 ± 1.8	10.5 ± 1.3	9.0 ± 0.9	14.3 ± 1.6
	D180	**53.0 ± 1.6***	37.3 ± 1.8	19.9 ± 1.5	9.5 ± 1.0	7.2 ± 0.5	**10.7 ± 0.8***
CE	D0	19.3 ± 1.0	26.6 ± 1.1	34.9 ± 1.1	40.7 ± 1.6	40.4 ± 1.8	38.2 ± 1.6
	D180	**15.3 ± 0.8*****	**23.3 ± 1.0****	**32.3 ± 0.9***	40.3 ± 1.1	42.6 ± 1.0	38.9 ± 1.1
FC	D0	5.8 ± 0.2	7.3 ± 0.2	8.0 ± 0.3	8.0 ± 0.3	7.0 ± 0.3	7.2 ± 0.2
	D180	5.7 ± 0.2	7.4 ± 0.2	8.2 ± 0.2	7.9 ± 0.1	7.6 ± 0.2	8.6 ± 1.0
PL	D0	15.2 ± 0.5	18.6 ± 0.3	19.8 ± 0.7	19.6 ± 0.9	19.2 ± 0.9	19.8 ± 0.4
	D180	15.7 ± 0.5	19.0 ± 0.4	19.8 ± 0.4	19.8 ± 0.4	19.6 ± 0.2	**21.2 ± 0.4***
Total Protein	D0	9.8 ± 0.4	13.3 ± 1.2	18.6 ± 1.0	21.3 ± 0.8	24.5 ± 1.9	20.5 ± 1.9
	D180	**10.4 ± 0.5***	13.0 ± 1.0	19.8 ± 1.0	22.5 ± 0.6	23.0 ± 0.6	20.7 ± 1.1
CE/TG ratio	D0	0.40 ± 0.04	0.84 ± 0.10	2.10 ± 0.23	4.63 ± 0.71	5.12 ± 0.65	3.24 ± 0.52
	D180	**0.30 ± 0.02****	0.65 ± 0.06	1.75 ± 0.17	4.76 ± 0.46	6.26 ± 0.53	3.83 ± 0.28

Component	Time point	VLDL+IDL	LDL1	LDL2	LDL3	LDL4	LDL5
(PL+FC)/(CE+TG)	D0	0.30 ± 0.01	0.43 ± 0.01	0.53 ± 0.04	0.56 ± 0.05	0.54 ± 0.05	0.53 ± 0.04
ratio	D180	0.31 ± 0.01	0.44 ± 0.01	0.54 ± 0.02	0.56 ± 0.01	0.55 ± 0.01	0.61 ± 0.04
Total Plasma lipoprotein mass (mg/dL)	D0	135.8 ± 10.3	35.7 ± 5.2	46.2 ± 4.2	108.3 ± 7.9	109.7 ± 10.2	44.0 ± 6.9
	D180	**98.4 ± 8.1***	**23.9 ± 2.5***	**30.7 ± 3.1*****	**77.9 ± 7.5*****	**79.7 ± 7.5***	**29.4 ± 3.2***
Total Plasma Lipoprotein mass (mg/dL)	Control subjects	56 - 85	10.4 – 27.2	22.9 – 39.5	74.7 – 91.7	41.7 – 137.5	25.0- 57.7

Values are expressed either as means ± SEM (n=12) or as ranges (see below). Baseline corresponds to D0 and 180 days of statin treatment to D180. Mass determinations of lipid and protein components were performed with absorptiometric assays as outlined in the Methods section; the mass of individual components was expressed as % of total mass for each subfraction, with the exception of plasma concentrations and mass ratios. ***p<0.001, **0.001<p<0.01 and *0.01<p<0.05 vs D0. Data from Chapman et al. (2). Values for ranges of plasma concentrations of VLDL+IDL and LDL subclasses in groups of healthy normal control subjects were obtained using the same methodology as that described herein, and are taken from refs (2,6,14).

Supplementary Table S3: Lipid class composition of the plasma VLDL+IDL fraction and LDL1-5 subclasses at baseline in obese, mixed dyslipidemic, male MetS subjects (expressed as pmol/mL plasma), and the effect of pitavastatin calcium treatment (4mg/day; 180 days; D180).

Lipid	VLDL+IDL			LDL1			LDL2		
(pmol/mL plasma)	D0	% change[a]	p-value	D0	% change[a]	p-value	D0	% change[a]	p-value
Tot dhCer	123.6 ± 17.8	-10	0.6172	34.4 ± 6.6	-26	0.0994	63.9 ± 6.1	-14	0.3808
Tot Cer	917.8 ± 101.7	-2	0.9175	220.1 ± 37.9	-29	0.0660	381 ± 46	-19	0.2208
Tot MHC	579.3 ± 73.1	-25	0.2236	193.2 ± 32.6	**-47**	**0.0092**	446 ± 64	**-35**	**0.0342**
Tot DHC	656.1 ± 80.0	-26	0.1474	191.1 ± 30.1	**-46**	**0.0042**	386 ± 46	**-35**	**0.0342**
Tot THC	213.5 ± 21.8	-19	0.3035	71.8 ± 11.8	**-48**	**0.0069**	151 ± 20	**-38**	**0.0282**
Tot GM3	417.3 ± 41.8	-13	0.4925	142.4 ± 29.0	**-45**	**0.0234**	289 ± 47	**-32**	**0.0342**
Tot PG	52.67 ± 9.08	-12	0.5828	4.2 ± 1.8	-63	0.0840	4.0 ± 0.9	-31	0.1910
Tot CE	(1.9 ± 0.2)E+5	-28	0.0924	(0.5 ± 0.09)E+5	**-40**	**0.0216**	(0.8 ± 0.1)E+5	**-36**	**0.0115**
Tot COH	(1.4 ± 0.2)E+5	-23	0.2089	(0.3 ± 0.06)E+5	-37	0.0630	(0.6 ± 0.1)E+5	**-35**	**0.0342**
Tot SM	37016 ± 3970	-18	0.3380	11037 ± 2238	**-41**	**0.0267**	21455 ± 3009	-30	0.0640
Tot PC	(1.9 ± 0.2)E+5	-15	0.2675	(0.4 ± 0.07)E+5	**-41**	**0.0310**	(0.6 ± 0.07)E+5	**-33**	**0.0196**
Tot PC(O)	5111 ± 571	-16	0.3955	1042 ± 228	**-41**	**0.0093**	1773 ± 223	**-28**	**0.0210**
Tot PC(P)	2656 ± 256	-13	0.4561	536.0 ± 133	**-42**	**0.0161**	915 ± 130	**-29**	**0.0269**

Lipid (pmol/mL plasma)	VLDL+IDL			LDL1			LDL2		
	D0	% change[a]	p-value	D0	% change[a]	p-value	D0	% change[a]	p-value
Tot LPC	2302 ± 283	-26	0.1799	502 ± 104	**-52**	**0.0122**	933 ± 134	**-44**	**0.0186**
Tot LPC(O)	24.58 ± 3.38	-25	0.2362	5.9 ± 1.1	**-49**	**0.0122**	11.2 ± 1.8	**-37**	**0.0425**
Tot PE	5779 ± 691	+2	0.9105	528 ± 167	-50	0.0824	687 ± 136	**-44**	**0.0335**
Tot PE(O)	262.6 ± 23.9	-7	0.6662	50.0 ± 12.0	-35	0.1582	84 ± 16	-15	0.1099
Tot PE(P)	2774 ± 272	-9	0.6250	546 ± 140	-35	0.0923	929 ± 158	-23	0.0771
Tot LPE	521.8 ± 58.3	-22	0.2124	108 ± 23	-36	0.0522	169 ± 31	-31	0.1294
Tot PI	10988 ± 1148	-19	0.2159	1770 ± 344	**-50**	**0.0229**	3121 ± 497	**-45**	**0.0265**
Tot LPI	28.58 ± 4.81	-22	0.2832	5.96 ± 1.74	-42	0.1835	11.1 ± 2.6	-32	0.2224
Tot DAG	28267 ± 3450	-12	0.5313	2431 ± 679	-52	0.0566	2490 ± 344	**-45**	**0.0139**
Tot TAG	(1.1 ± 0.1)E+5	-16	0.1703	(0.1 ± 0.02)E+5	**-42**	**0.0441**	(0.14 ± 0.02)E+5	**-44**	**0.0128**
Tot SM/Tot PC	0.19 ± 0.01	-0.1	1.0000	0.30 ± 0.01	+1	0.8483	0.35 ± 0.01	+5	0.3087

Supplementary Table S3: Continued

Lipid (pmol/mL plasma)	LDL3			LDL4			LDL5		
	D0	% change[a]	p-value	D0	% change[a]	p-value	D0	% change[a]	p-value
Tot dhCer	134 ± 15	-0.2	0.9895	89.3 ± 8.8	-7	0.4484	27.7 ± 3.2	-11	0.2790
Tot Cer	726 ± 69	-9	0.3349	493 ± 44	-13	0.2142	149 ± 20	-11	0.3745
Tot MHC	1402 ± 194	-28	0.0733	1268 ± 138	**-35**	**0.0041**	380 ± 50	**-33**	**0.0241**
Tot DHC	1079 ± 131	-27	0.0595	965 ± 98	**-32**	**0.0033**	310 ± 45	**-30**	**0.0312**
Tot THC	434 ± 47	**-31**	**0.0295**	377 ± 47	**-34**	**0.0063**	119 ± 14	**-29**	**0.0164**
Tot GM3	800 ± 79	-23	0.0910	700 ± 75	**-29**	**0.0088**	211 ± 22	**-25**	**0.0432**
Tot PG	11.0± 1.7	-35	0.1158	10.3 ± 1.5	**-50**	**0.0098**	3.7 ± 0.7	-42	0.1017
Tot CE	(1.9 ± 0.2)E+5	**-24**	**0.0061**	(1.9 ± 0.3)E+5	**-28**	**0.0426**	(0.7 ± 0.1)E+5	-30	0.0582
Tot COH	(1.6 ± 0.2)E+5	-21	0.0961	(1.6 ± 0.2)E+5	**-32**	**0.0056**	(0.47 ± 0.06)E+5	-27	0.0506
Tot SM	58256 ± 5786	-23	0.0637	51702 ± 5615	**-24**	**0.0149**	16070 ± 1867	**-24**	**0.0440**
Tot PC	(1.6 ± 0.1)E+5	**-27**	**0.0040**	(1.7 ± 0.2)E+5	**-30**	**0.0150**	(0.59 ± 0.08)E+5	-26	0.0744
Tot PC(O)	5080 ± 572	**-24**	**0.0498**	5252 ± 614	**-26**	**0.0391**	1810 ± 213	-22	0.0970
Tot PC(P)	2591 ± 306	-23	0.0508	2695 ± 339	-25	0.0607	938 ± 120	-23	0.0938
Tot LPC	2696 ± 381	**-38**	**0.0071**	2810 ± 337	**-38**	**0.0044**	1368 ± 161	**-35**	**0.0147**

Lipid	LDL3			LDL4			LDL5		
(pmol/mL plasma)	D0	% change[a]	p-value	D0	% change[a]	p-value	D0	% change[a]	p-value
Tot LPC(O)	32.2 ± 4.4	**-30**	**0.0281**	34.6 ± 3.9	**-33**	**0.0053**	20.6 ± 2.1	**-32**	**0.0101**
Tot PE	1608 ± 307	**-30**	**0.0493**	1730 ± 315	**-39**	**0.0255**	646 ± 107	-29	0.0958
Tot PE(O)	230 ± 30	-6	0.6589	249 ± 36	-16	0.4169	86.5 ± 10.3	-14	0.4262
Tot PE(P)	2605 ± 360	-15	0.2103	2919 ± 437	-22	0.1650	1010 ± 147	-19	0.2437
Tot LPE	416 ± 58	-18	0.1591	421 ± 57	-19	0.1509	162 ± 23	-19	0.1719
Tot PI	8605 ± 786	**-35**	**0.0032**	9799 ± 1328	**-43**	**0.0048**	3260 ± 435	**-37**	**0.0322**
Tot LPI	31.3 ± 4.4	-25	0.1040	42.2 ± 6.7	**-37**	**0.0311**	27.1 ± 3.3	**-37**	**0.0346**
Tot DAG	3750 ± 360	**-35**	**0.0038**	3523 ± 380	**-38**	**0.0019**	1628 ± 230	**-40**	**0.0169**
Tot TAG	(0.2 ± 0.02)E+5	**-30**	**0.0042**	(0.19 ± 0.03)E+5	**-36**	**0.0359**	(0.069 ± 0.008)E+5	-24	0.0825
Tot SM/Tot PC	0.35 ± 0.01	+5	0.2598	0.30 ± 0.01	+7	0.3074	0.28 ± 0.01	+3	0.5948

Values are expressed as means ± SEM in pmol/mL plasma (n=12). [a]Percent change from baseline (D0) subsequent to pitavastatin calcium (4mg/day) treatment for 180 days (D180). P values are from paired t-test analysis for parameters with a Gaussian distribution and from Wilcoxon non-parametric analysis for parameters that were non-Gaussian. All values highlighted in bold are statistically significant at the level of ≤0.05. Density ranges: VLDL+IDL <1.019 g/mL, LDL1 = 1.019-1.023 g/mL, LDL2 = 1.023-1.029 g/mL, LDL3 = 1.029-1.039 g/mL, LDL4 = 1.039-1.050 g/mL and LDL5 = 1.050-1.063 g/mL. dhCer, dihydroceramide; Cer, ceramide; MHC, monohexosylceramide; DHC, dihexosylceramide; THC, trihexosylceramide; GM3, monosialodihexosylganglioside;

PG, phosphatidylglycerol; CE, cholesteryl ester; COH, free cholesterol; SM, sphingomyelin; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), phosphatidylcholine plasmalogen; LPC, lysophosphatidylcholine; LPC(O), lysoalkylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), phosphatidylethanolamine plasmalogen; LPE, lysophosphatidylethanolamine; PI, phosphatidylinositol; LPI, lysophosphatidylinositol; DAG, diacylglycerol; TAG, triacylglycerol.

Supplementary Table S4. Comparison of lipid class concentrations in plasma-derived LDL fractions from heathy control subjects (n=12) with those of MetS subjects (n=12) at baseline (MetS D0) and following pitavastatin calcium treatment (MetS D180).

Lipid class	**Absolute levels (µM) - Mean (SEM)**			**MetS D0 vs. Healthy controls**		**MetS D180 vs. Healthy controls**	
	Healthy controls	MetS D0	MetS D180	% Difference	p-value	% Difference	p-value
Dihydroceramide (dhCer)	0.258 (0.019)	0.266 (0.016)	0.261 (0.024)	3.0	8.74E-01	1.1	9.65E-01
Ceramide (Cer)	1.57 (0.09)	1.43 (0.06)	1.33 (0.06)	-8.9	4.12E-01	-15.5	5.76E-02
Monohexosylceramide (MHC)	4.01 (0.31)	4.37 (0.36)	2.9 (0.2)	9.2	6.11E-01	-27.6	**1.51E-02**
Dihexosylceramide (DHC)	3.24 (0.21)	2.94 (0.13)	2.08 (0.09)	-9.2	4.12E-01	-35.8	**1.30E-03**
Trihexosylceramide (THC)	1.19 (0.10)	1.09 (0.06)	0.767 (0.045)	-8.9	5.25E-01	-35.8	**4.26E-03**
GM3 ganglioside (GM3)	2.24 (0.18)	2.03 (0.11)	1.53 (0.07)	-9.5	4.90E-01	-31.8	**6.55E-03**
Sphingomyelin (SM)	114 (8)	118 (4)	94.8 (4.7)	3.5	7.98E-01	-17.0	6.15E-02
Phosphatidylcholine (PC)	439 (24)	480 (16)	371 (15)	9.2	3.49E-01	-15.5	**4.62E-02**
Alkylphosphatidylcholine (PC(O))	15.3 (1)	15.1 (0.9)	12 (1)	-1.4	9.61E-01	-21.7	**1.99E-02**
Alkenylphosphatidylcholine (PC(P))	8.46 (0.65)	6.88 (0.38)	5.51 (0.29)	-18.7	2.22E-01	-34.9	**4.26E-03**
Lysophosphatidylcholine (LPC)	10.2 (0.7)	8.94 (0.53)	6.2 (0.4)	-12.7	3.49E-01	-39.5	**6.84E-04**
Lysoalkylphosphatidylcholine (LPC(O))	0.885 (0.015)	0.884 (0.012)	0.886 (0.010)	-0.1	9.65E-01	0.1	9.65E-01
Phosphatidylethanolamine (PE)	5.53 (0.90)	6.12 (0.66)	4.78 (0.50)	10.7	7.67E-01	-13.5	5.50E-01
Alkylphosphatidylethanolamine (PE(O)	0.775 (0.065)	0.618 (0.043)	0.562 (0.071)	-20.4	2.22E-01	-27.6	5.60E-02
Alkenylphosphatidylethanolamine (PE(P))	10.3 (0.7)	8.64 (0.66)	7.51 (0.59)	-16.3	3.08E-01	-27.3	**1.57E-02**
Lysophosphatidylethanolamine (LPE)	1.24 (0.08)	1.07 (0.08)	0.854 (0.063)	-13.2	3.49E-01	-30.9	**5.21E-03**
Phosphatidylinositol (PI)	10.4 (0.7)	12 (1)	7.91 (0.66)	15.9	2.90E-01	-23.6	**3.98E-02**
Lysophosphatidylinositol (LPI)	0.186 (0.02)	0.135 (0.009)	0.107 (0.011)	-27.4	2.02E-01	-42.5	**9.16E-03**
Phosphatidylglycerol (PG)	0.06 (0.01)	0.126 (0.015)	0.0754 (0.0167)	110.8	**1.08E-02**	25.7	5.28E-01
Cholesteryl ester (CE)	912 (68)	1030 (49)	867 (48)	13.1	3.49E-01	-4.9	6.49E-01
Free cholesterol (COH)	332 (21)	330 (10)	256 (10)	-0.7	9.62E-01	-23.0	**1.36E-02**
Diacylglycerol (DAG)	5.91 (0.68)	10.1 (0.8)	7.7 (0.4)	71.6	**7.28E-03**	30.3	5.60E-02
Triacylglycerol (TAG)	74.4 (4.8)	130 (9)	116 (6)	74.9	**1.57E-03**	55.4	**3.97E-04**

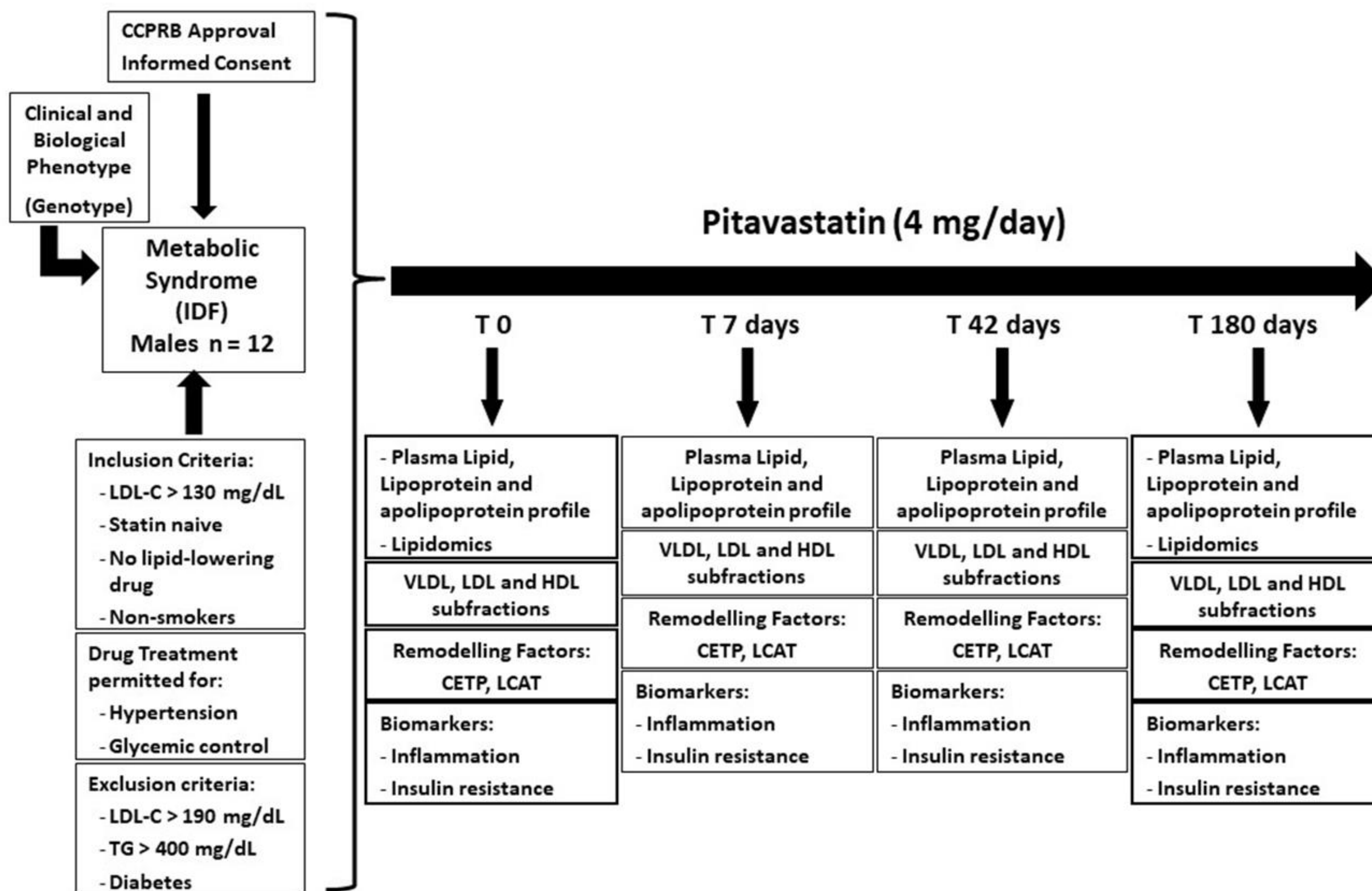
Data are expressed as mean µmolar plasma concentrations ±SEM (in parentheses) of individual lipid classes in total LDL fractions (d 1.019-1063 g/mL) isolated as indicated in the Methods section above, and determined by LC-MS. [1] Percentage difference was calculated based on the mean lipid levels of MetS subjects at baseline (MetS D0), or end of statin treatment (MetS D180), and compared to those of healthy control subjects. [2] Corrected p-values based on unpaired Student's t-test followed by Benjamini-Hochberg correction for multiple comparisons.

LEGENDS TO FIGURES

Supplementary Figure S1: Overall clinical protocol for the CAPITAIN study (**ClinicalTrials.gov, #NCT01595828)** in male subjects exhibiting the criteria of Metabolic syndrome as defined by IDF criteria (1-5). Key inclusion and exclusion criteria, medications permitted during the period of the clinical study and which do not significantly impact lipid metabolism or lipid profile, and the time course of plasma lipid, lipoprotein and biomarker analyses over the 180 day duration of pitavastatin treatment (4mg/day; open label) are indicated.

Supplemental Figure S2: Statistical comparison at baseline (D0) levels of 23 individual lipid classes in plasma VLDL+IDL, LDL1, LDL2, LDL3, LDL4 and LDL5, and the effect of pitavastatin treatment (4mg/day) for 180 days on individual lipid classes. Values are expressed as means ± SEM (n=12) in pmol of lipid class / pmol apoB. ***p<0.001; **0.001<p<0.01 and *0.01<p<0.05 vs each subfraction; p values were calculated by Bonferroni test as a post-test to a two-way repeated measure ANOVA test. Density ranges: VLDL+IDL <1.019 g/mL, LDL1 = 1.019-1.023 g/mL, LDL2 = 1.023-1.029 g/mL, LDL3 = 1.029-1.039 g/mL, LDL4 = 1.039-1.050 g/mL and LDL5 = 1.050-1.063 g/mL. ***<u>Panels A to AL</u>*: A and B** - CE: cholesteryl ester; **C and D** - TAG: triacylglycerol; **E and F** - DAG: diacylglycerol; **G and H** - SM: sphingomyelin; **I** - PC: phosphatidylcholine; **J** - PC(O): alkylphosphatidylcholine; **K & L** - LPC: lysophosphatidylcholine; **M & N** - LPC(O): lysoalkylphosphatidylcholine; **O** - PE: phosphatidylethanolamine; **P & Q** - LPE: lysophosphatidylethanolamine; **R** - PI: phosphatidylinositol; **S & T** - LPI: lysophosphatidylinositol; **U & V** - Cer: ceramide; **W & X** - dhCer: dihydroceramide; **Y & Z** - MHC: monohexosylceramide; **AA & AB** -. DHC: dihexosylceramide; **AC & AD** – THC: trihexosylceramide; **AE & AF** – GM3: monosialodihexosylganglioside; **AG & AH** – CE/TAG ratio; **AI & AJ** – DAG/TAG ratio; **AK & AL** – SM/PC ratio.

Supplementary Figure S1:



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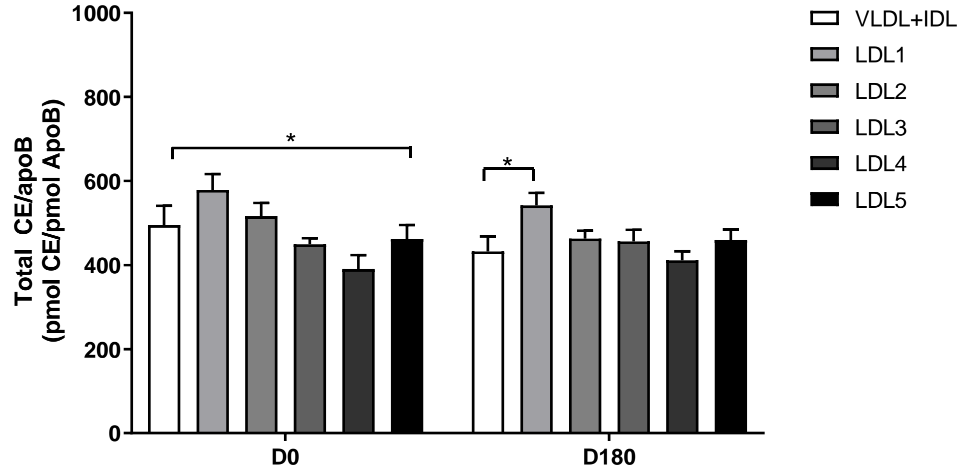

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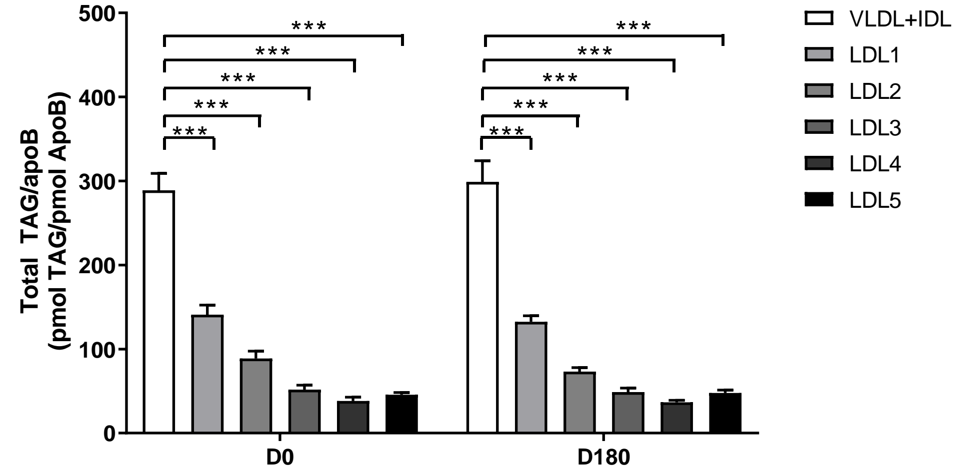

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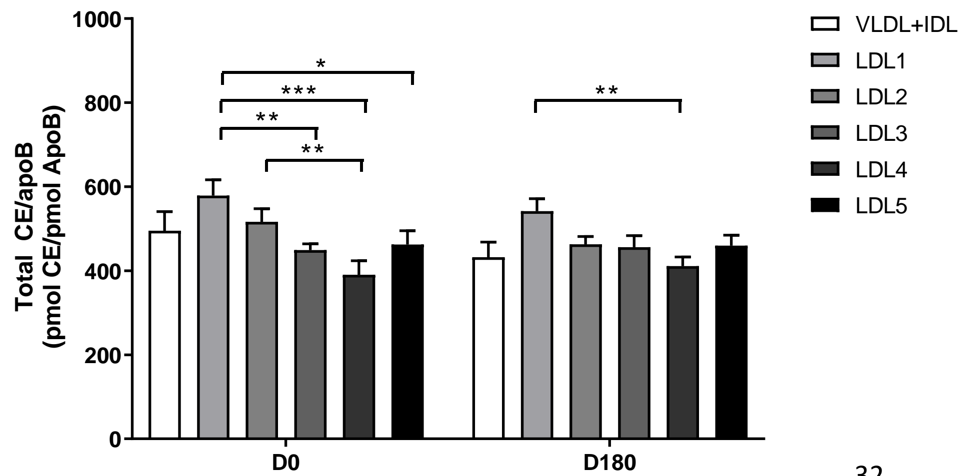

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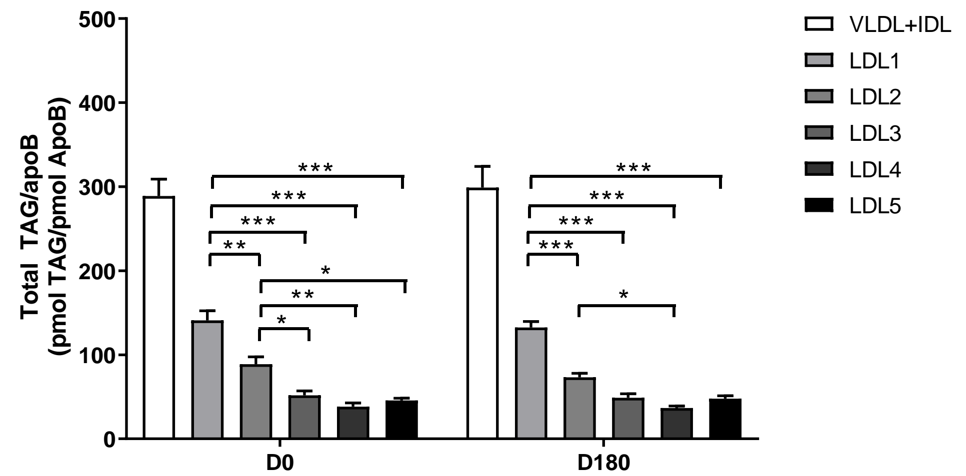

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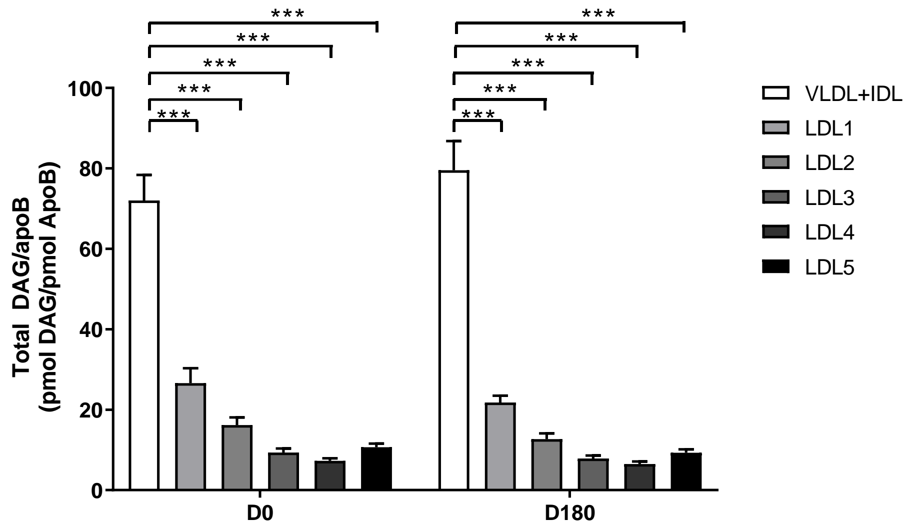

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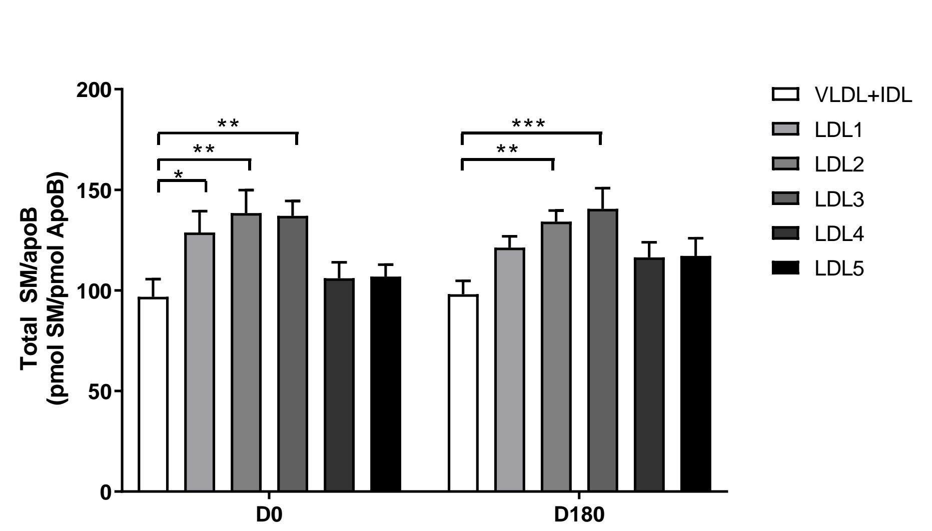

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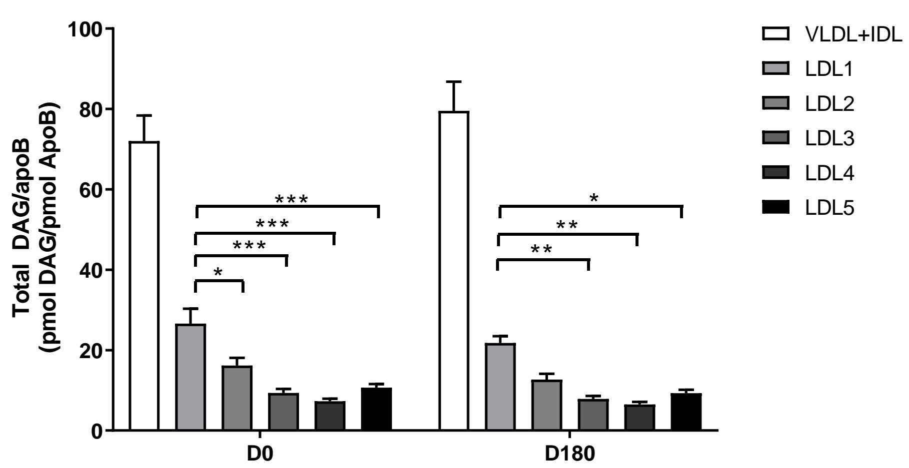

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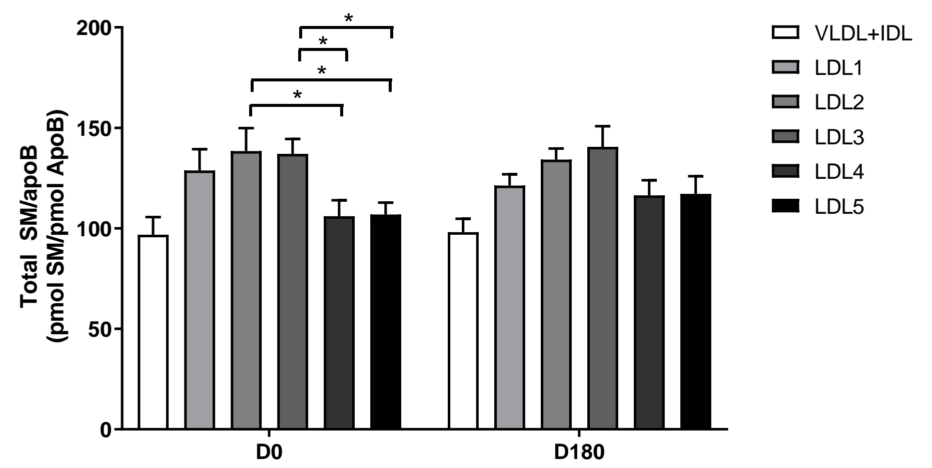


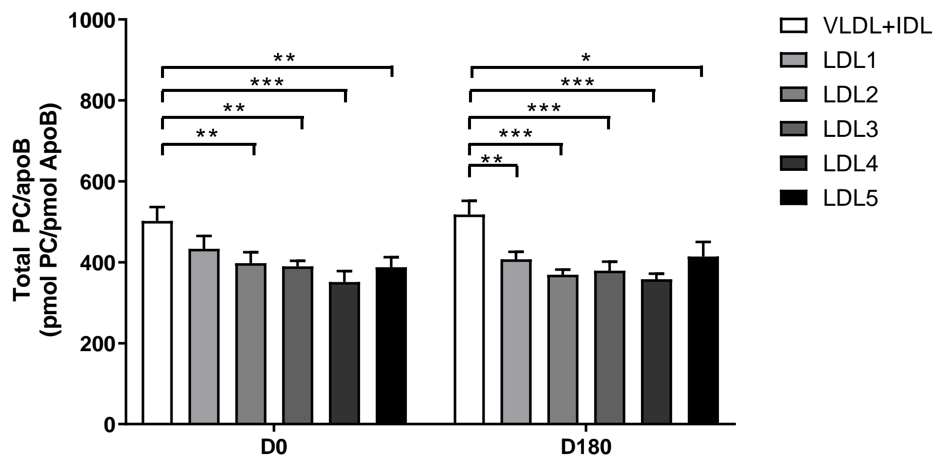


Fig.S2-I
1000
800
600
400
200
0
Total PC/apoB
(pmol PC/pmol ApoB)
**
***
**
**
*
***
***
***
**
D0
D180
VLDL+IDL
LDL1
LDL2
LDL3
LDL4
LDL5

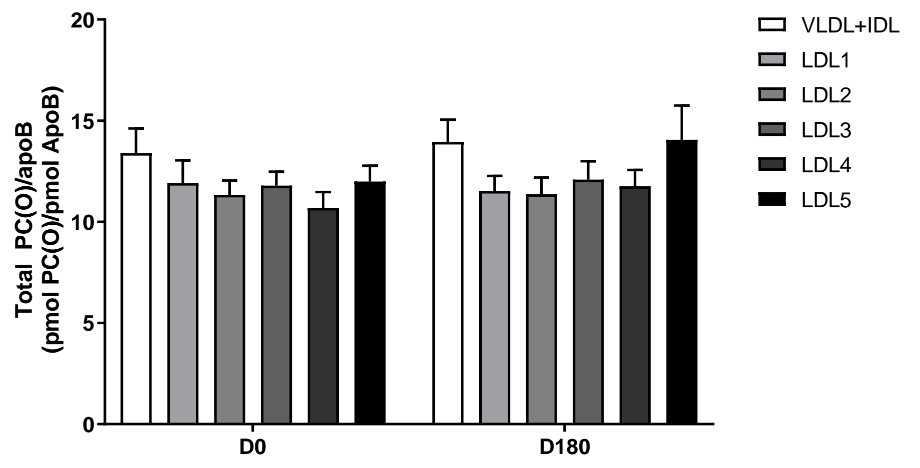
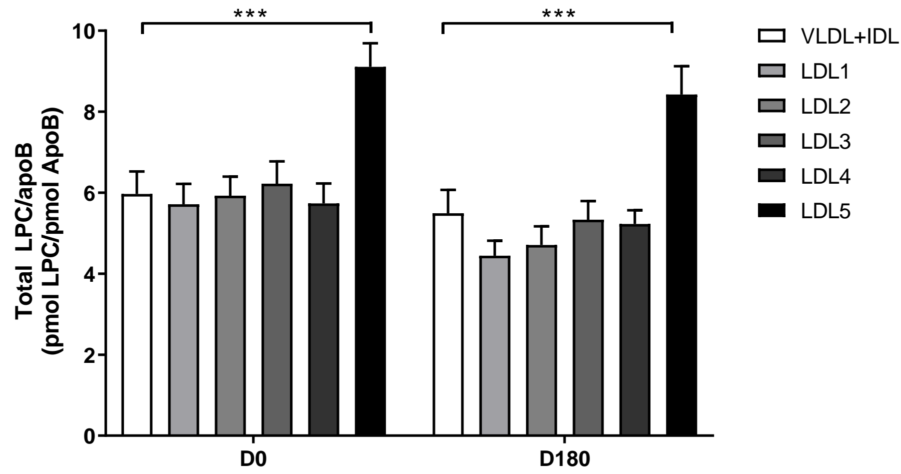


Fig.S2-J
20
15
10
5
0
Total PC(O)/apoB
(pmol PC(O)/pmol ApoB)
D0
D180
VLDL+IDL
LDL1
LDL2
LDL3
LDL4
LDL5

Fig.S2-K



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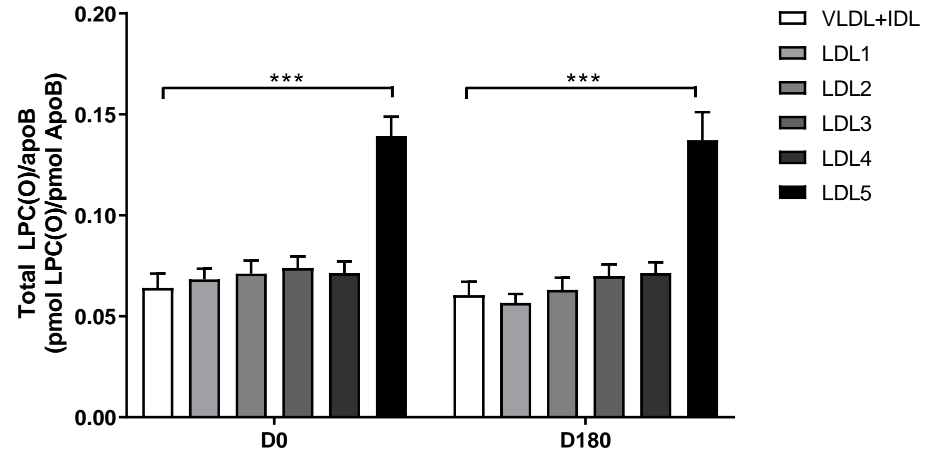

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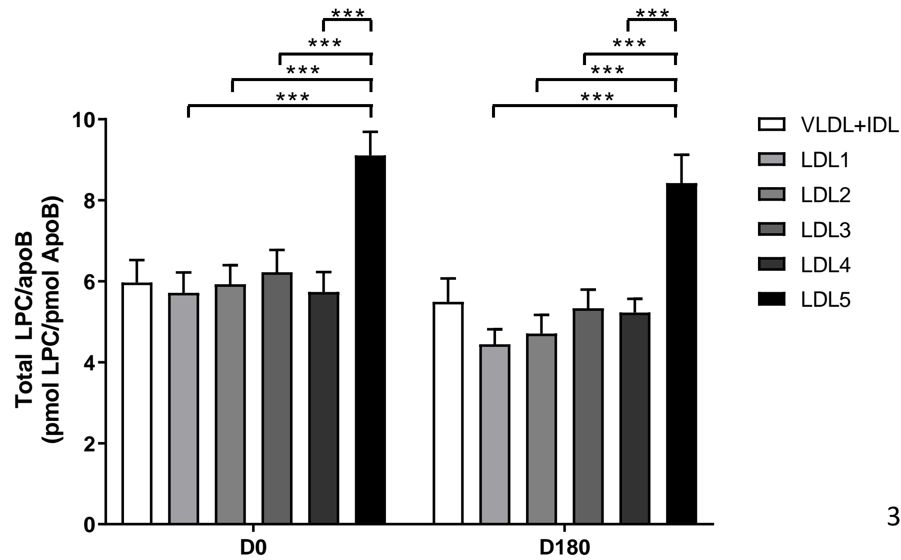

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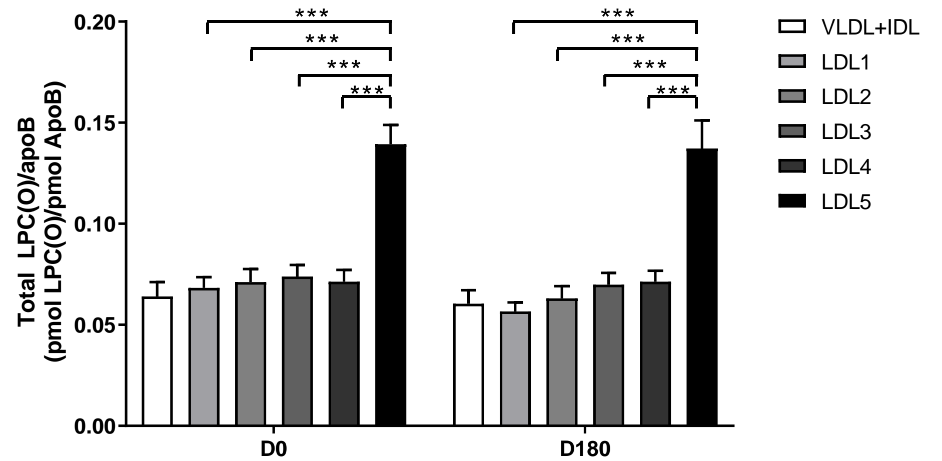

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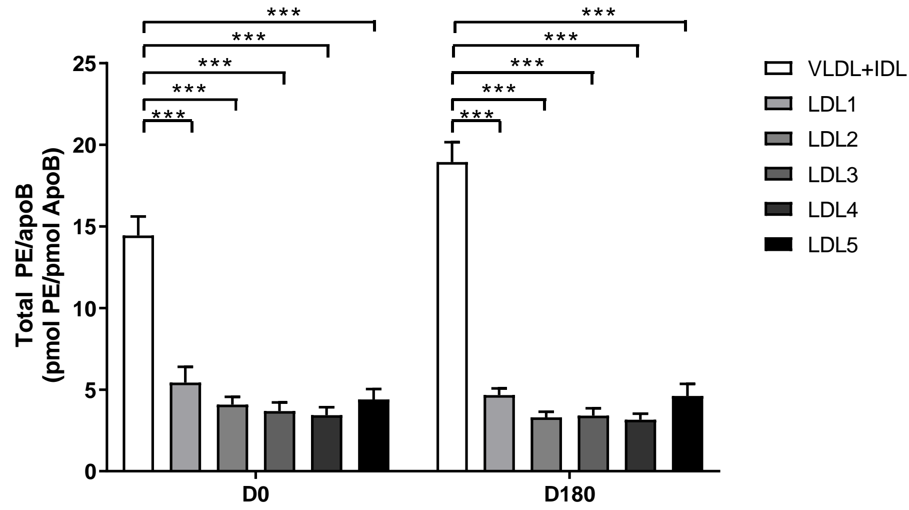

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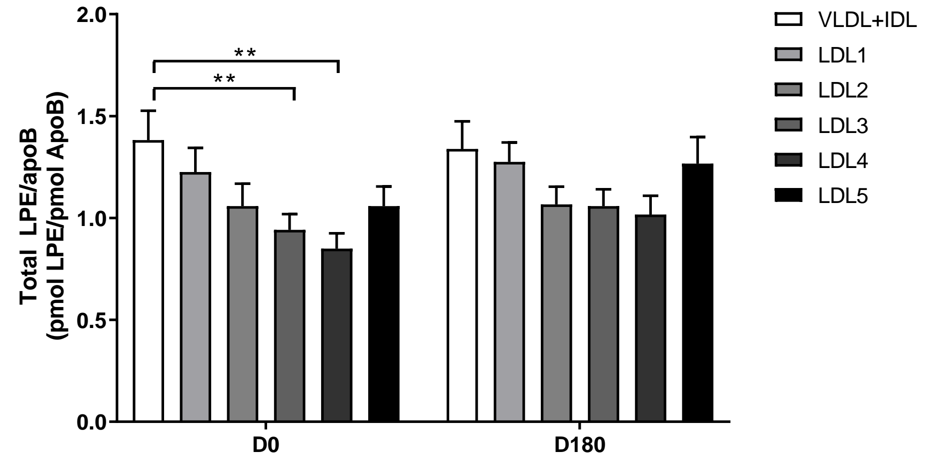

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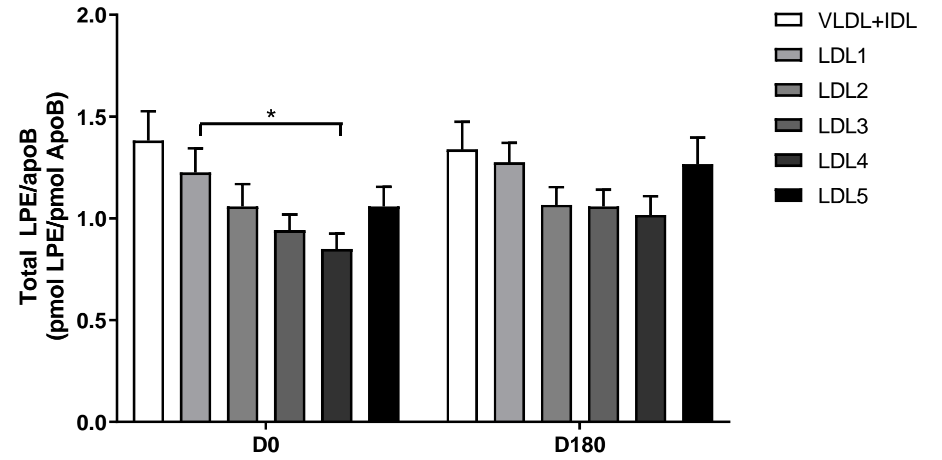

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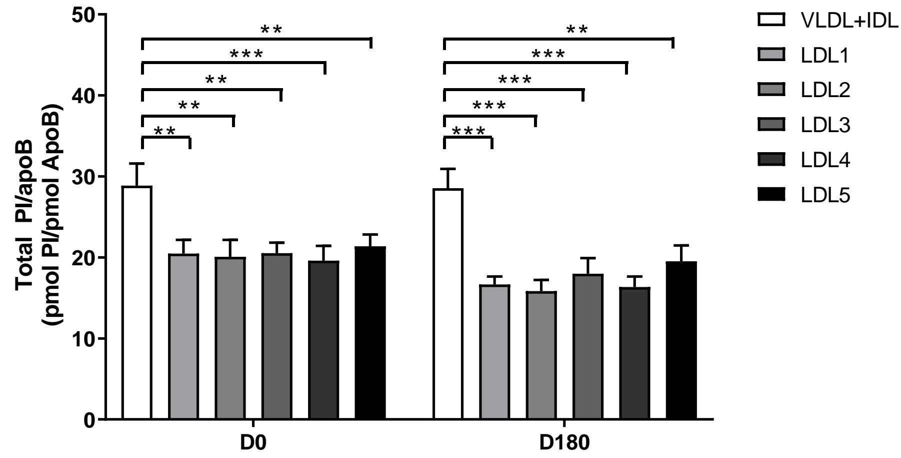

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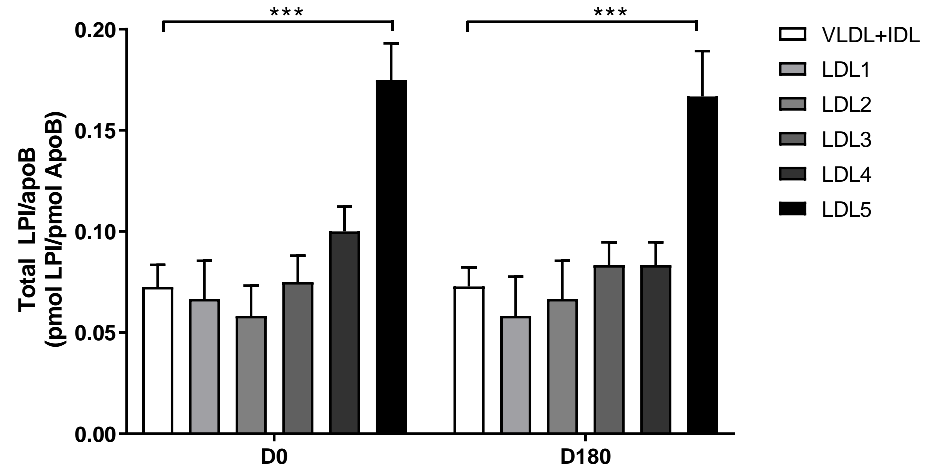

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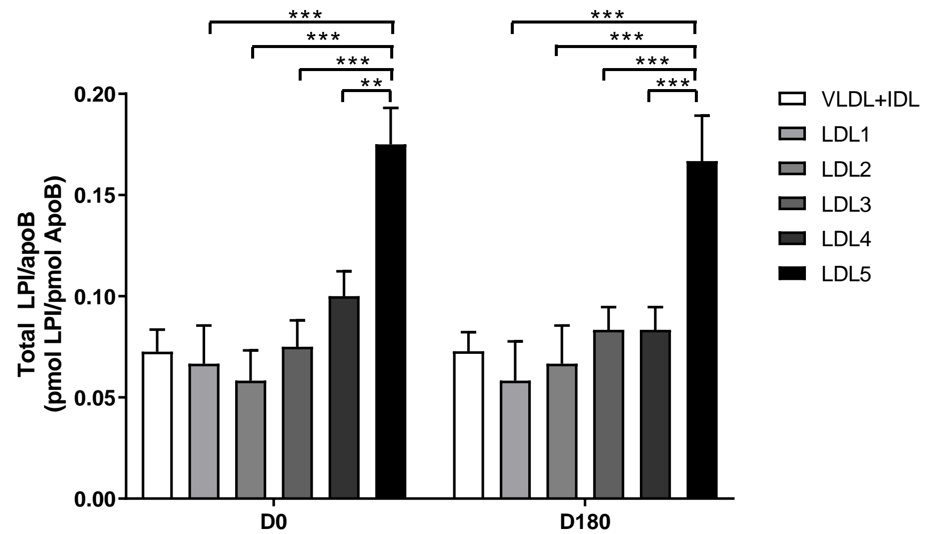

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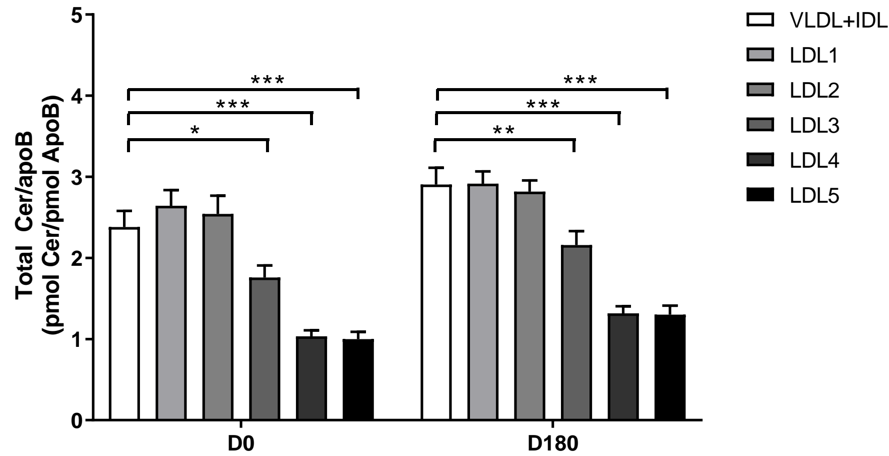

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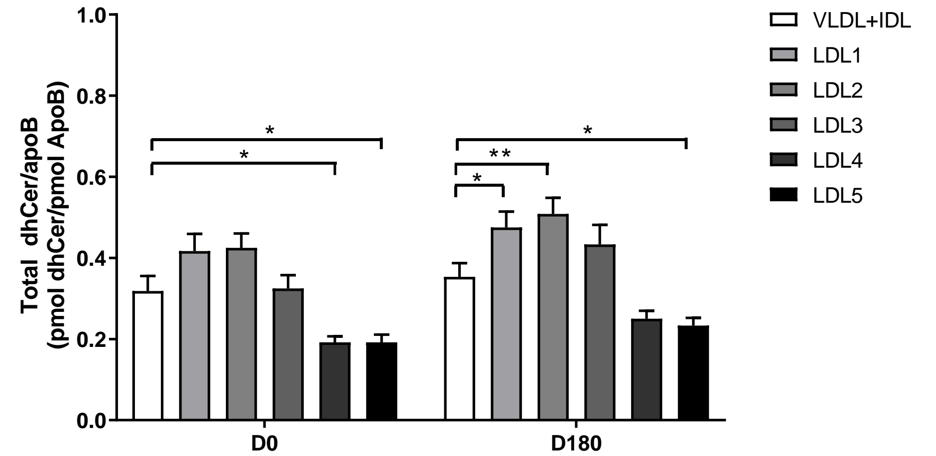

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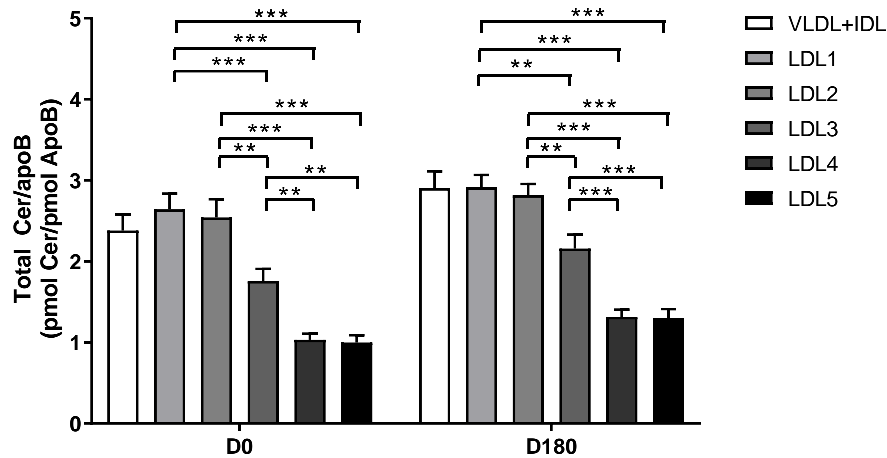

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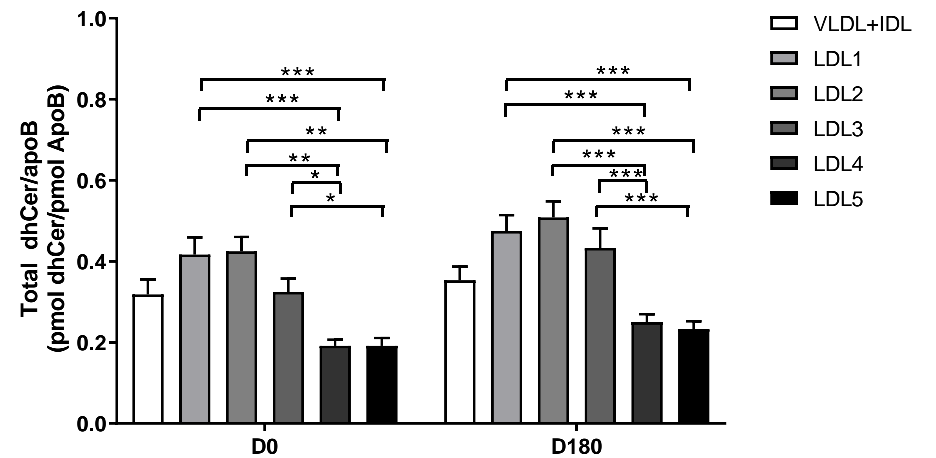

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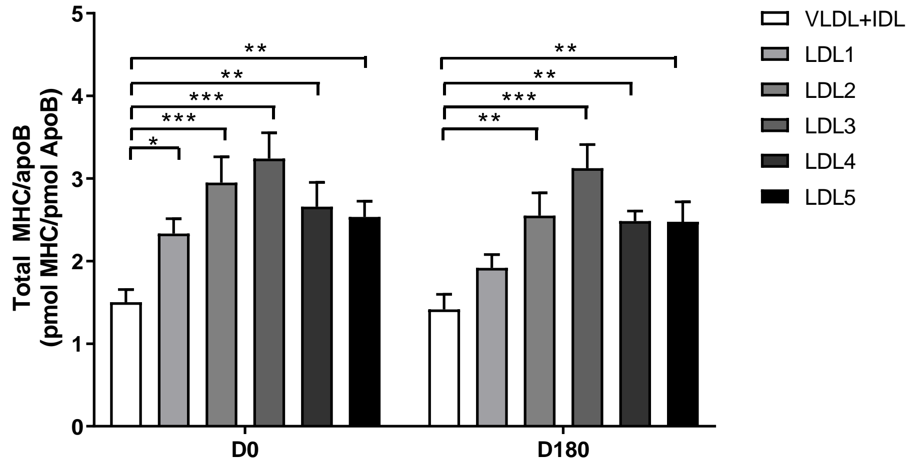

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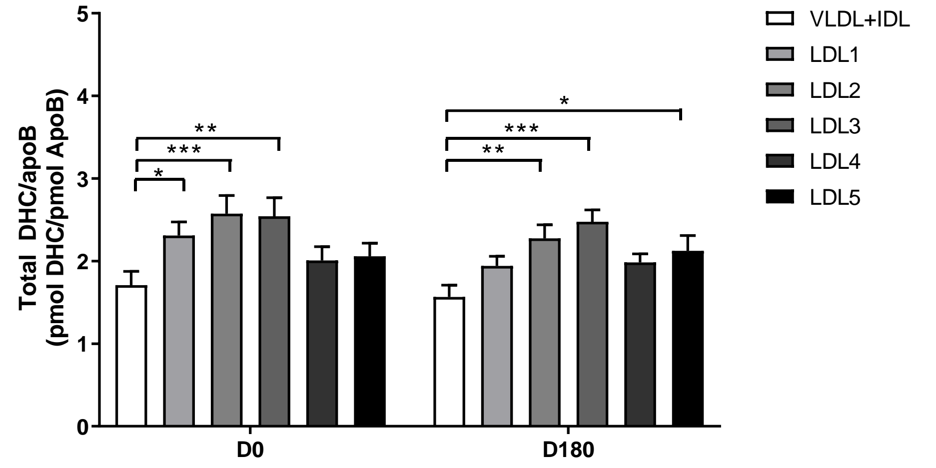

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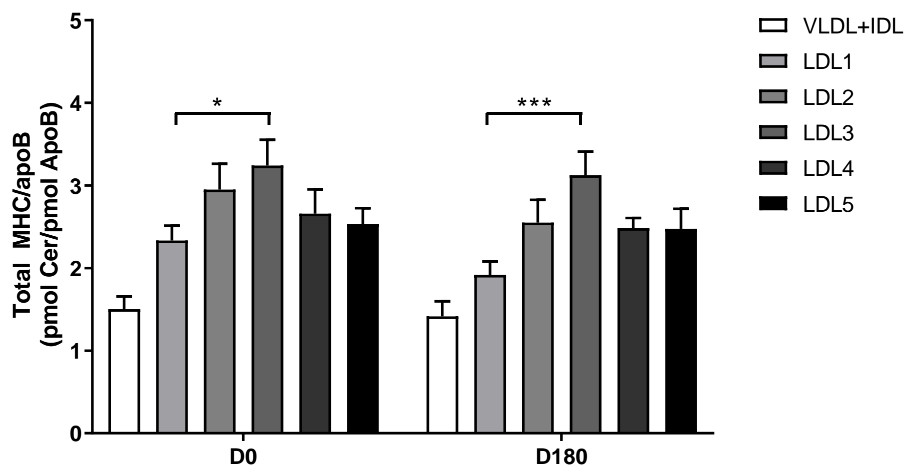

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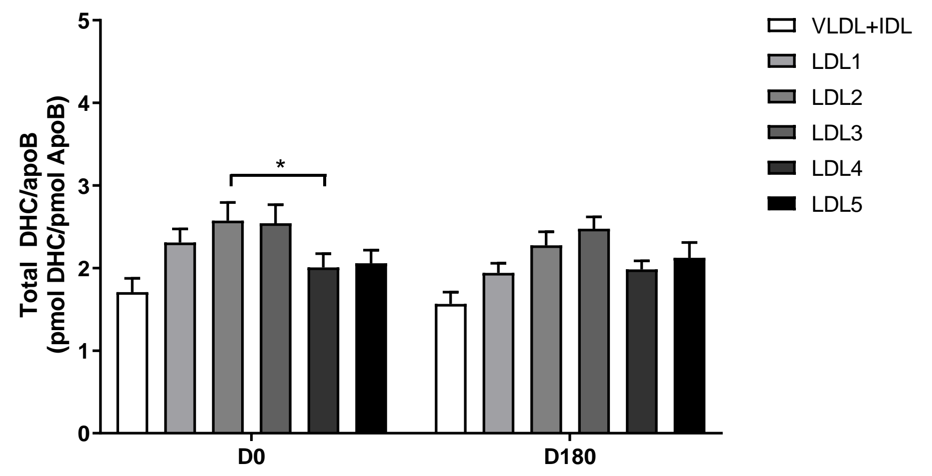

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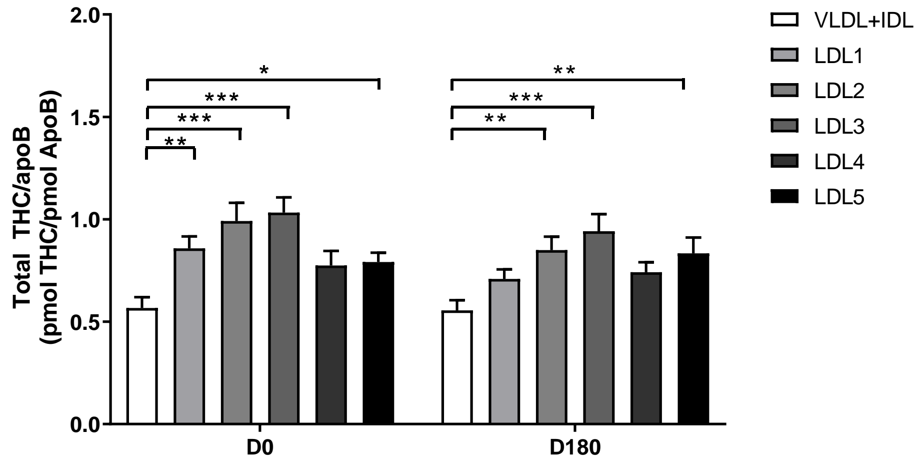

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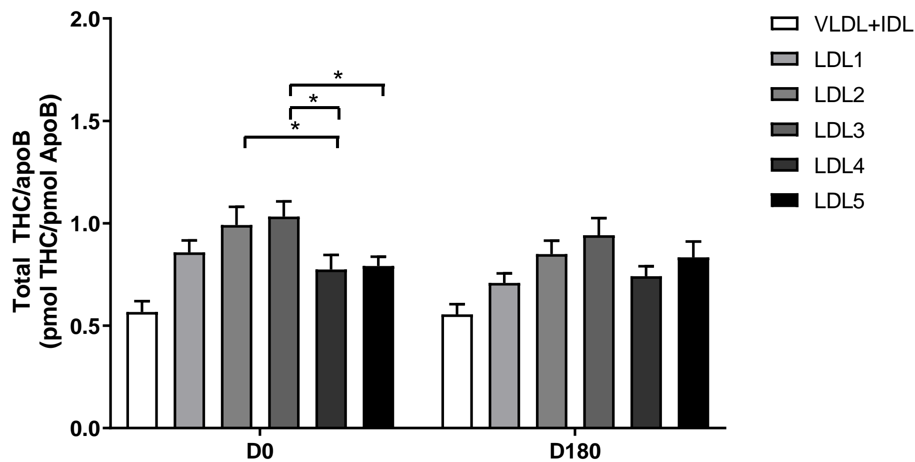

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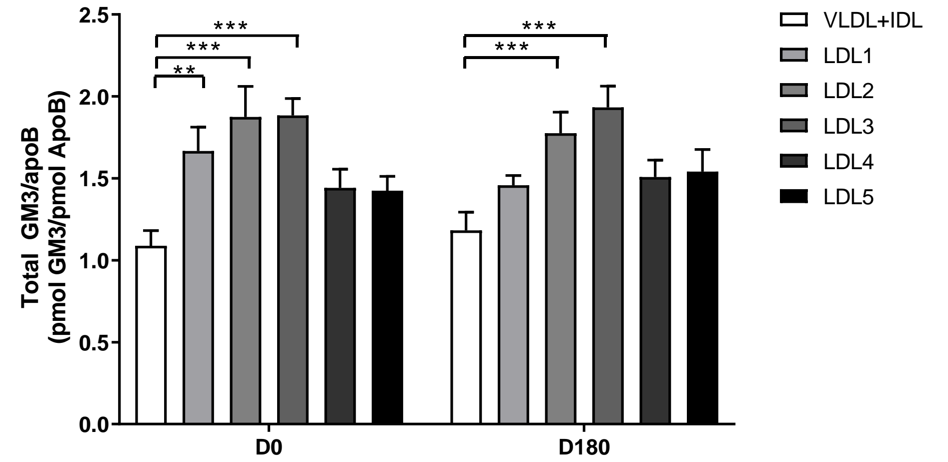

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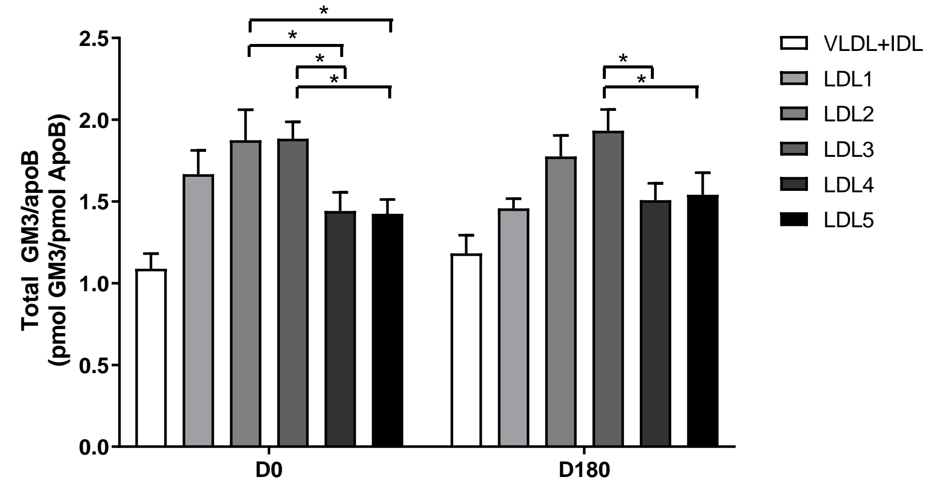

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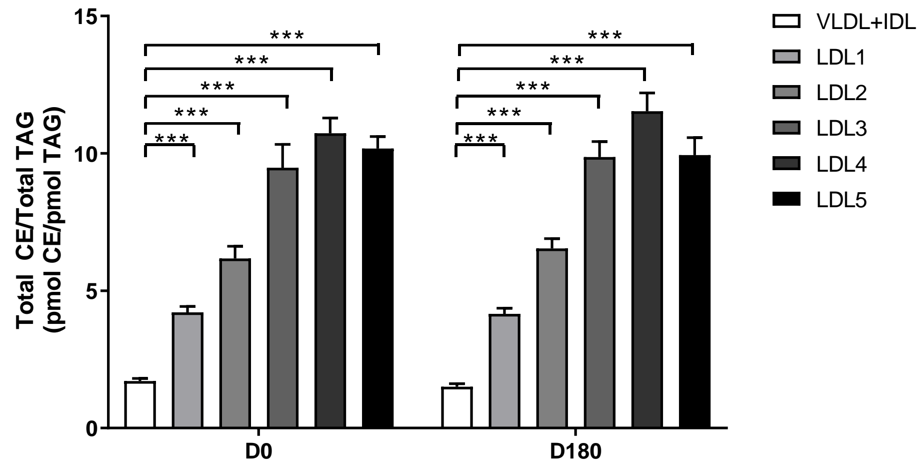

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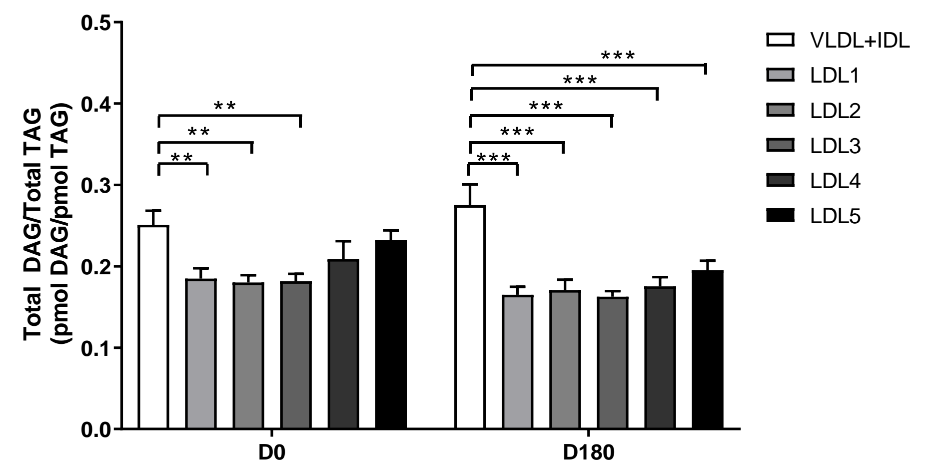

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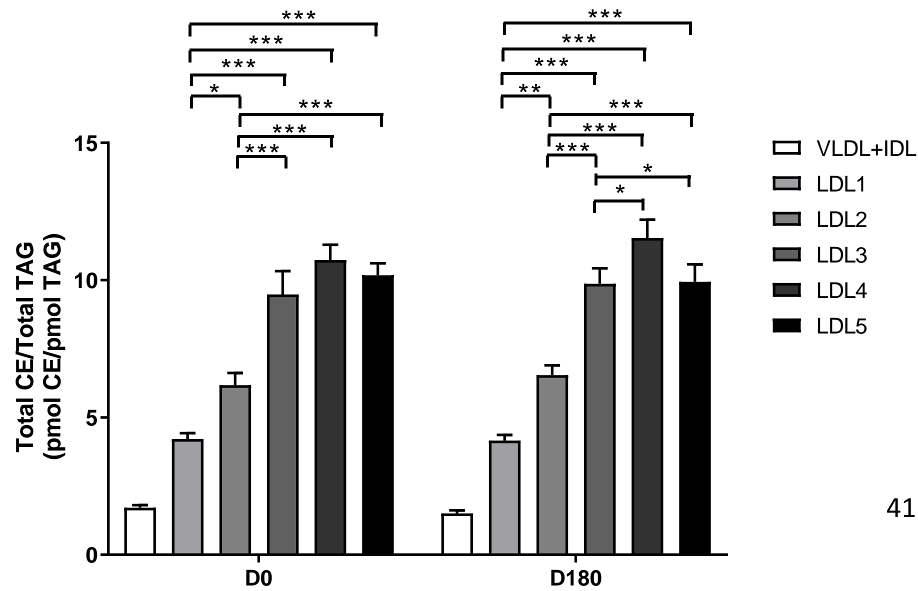

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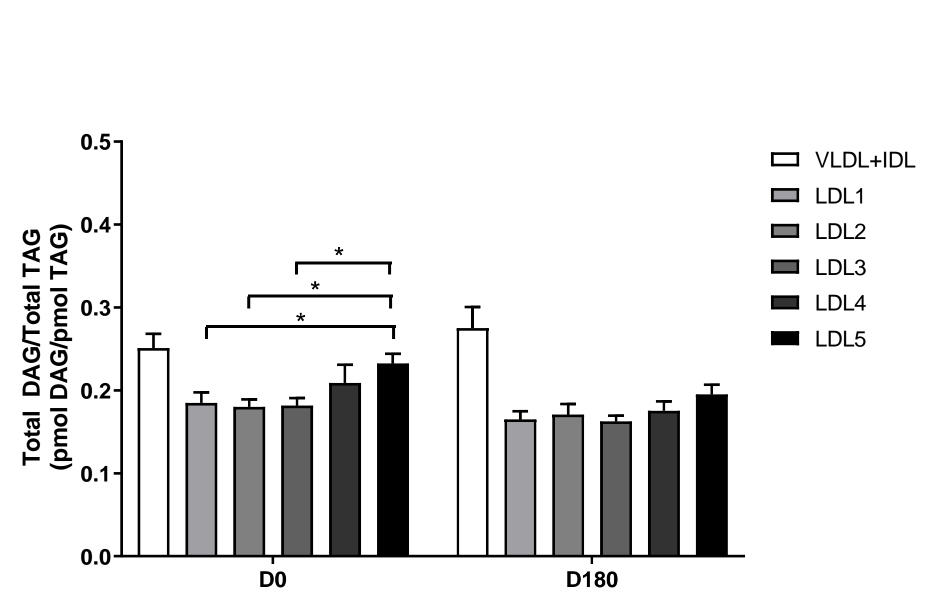

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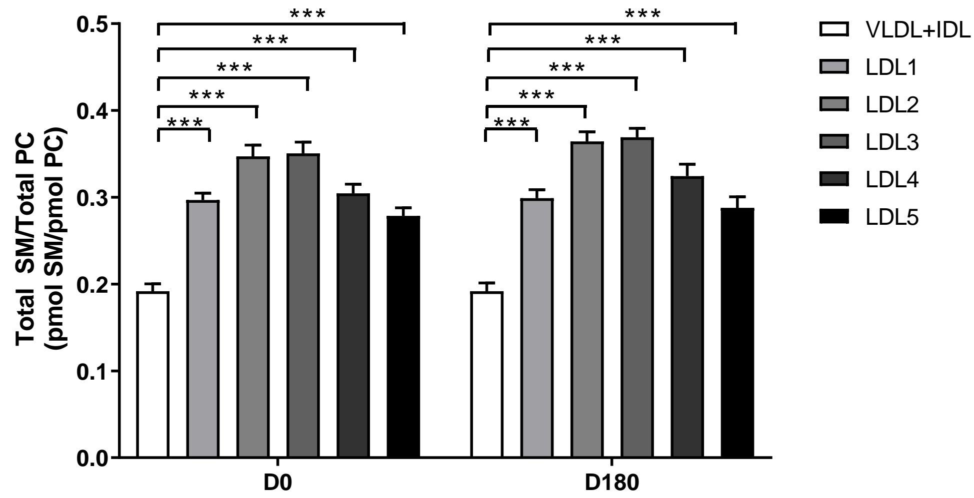

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