Supplemental Material

Variation in coronary atherosclerosis severity related to a distinct LDL profile – findings from a familial hypercholesterolemia pig model

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Supplemental Methods

Follow-up time on atherogenic diet

After baseline imaging (T_1), the invasive imaging was repeated at 9 (T_2) and 10-13 months (T_3) after the start of the atherogenic diet. The timing of T_3 was determined based on the size of the coronary plaques at T_2. Because of the exponential growth pattern of the plaques and to prevent death due to a major cardiovascular event, pigs that displayed significant disease at T_2 (n=2) were sacrificed within 1 month (T_3 at 10 months). The other animals (n=8) were planned to be kept in experiment up to 12 months. Of these pigs, 1 pig died one day after the invasive imaging procedure at T_2 due to an unknown, but acute cause of death during feeding. Another pig had to be sacrificed between T_2 and T_3 due to suspected thrombosis in one leg.

Coronary histology

Upon sacrifice at the last time point, the heart was excised, the coronary arteries were dissected out, and sliced in 3mm blocks, which were embedded in Tissue Tek, slowly frozen on dry ice-cooled 2-propanol and stored at -80 °C. The 3mm blocks were sectioned in 5µm slides (Leica 3050s cryostat) and used for histological and immunohistochemical staining.
Haematoxylin and Eosin (HE), Resorcin-Fuchsin or Miller (collagen and elastin), Oil-red-O (ORO) (lipids) and Martius, Scarlet and Blue (MSB) (fibrin) stainings were performed. Immunohistochemical stains for CD68 (macrophages, primary antibody (Ab): Bio-Rad, MCA2317GA, mouse anti-pig, 1:1600; secondary antibody: ready-to-use EnVision™+ System/HRP, K4001, goat anti-mouse) and CD31 (endothelial cells, primary Ab: Bio-Rad, MCA1746GA, mouse anti-pig, 1:200; secondary Ab: DAKO, P0447, polyclonal goat anti-mouse, HRP labelled, 1:100) were used for further plaque characterization. A liquid DAB+ Substrate kit (DAKO, K3468) was used to detect the bound secondary antibodies.

To compare the plaque types found in the proximal versus the distal regions of the coronary arteries, the first half of the excised artery (up to 14 (LAD), 9 (LCX) and 16 (RCA) 3mm-blocks) were regarded as proximal and the other half distal. These numbers were determined based on a median split of the total number of blocks of the individual arteries. Furthermore, a 3mm region was marked as 'around a side branch' when a side branch was present in at least one of the slices derived from that respective block.

Positive staining for lipids and macrophages was quantified by using user-specified standard Hue-Saturation-Brightness values. Necrotic core was delineated manually as a lipid-rich area, scarce in nuclei and fibrous tissue with an overlaying fibrous cap[1]. Quantification of the average plaque area was based on all Oil-red-O, CD68 and Miller-stained sections and lipid, macrophage and necrotic core content were presented as an average percentage of the total plaque area. The presence of intraplaque haemorrhage was assessed on both HE and MSB stained sections as bright pink (HE) and red (MSB) stained areas. Neovascularisation in the plaque area could be detected using CD31 staining. The presence of calcifications was assessed on the Oil-red-O staining and calcifications were subdivided in micro (spotty appearance, <10µm/<5% of plaque area[2]) or macrocalcifications.

Imaging analysis

Before analysis, IVUS pullbacks were ECG-triggered by selecting the frame that was recorded 6 frames before the R-peak by in-house developed software. Hereby differences in lumen size were removed that were induced by movement of the catheter or because of cardiac contraction. The triggered IVUS pullbacks were analysed each frame by semi-automatic delineation (with manual correction) of the vessel wall and lumen contours. Based on these contours, total vessel area (VA), lumen area (LA) and plaque area (PA=VA-LA) (mm^2) were quantified. Plaque burden (PB) was calculated as PA/VA*100%. For final analysis, data were averaged over 3mm in longitudinal direction to reduce the influence of manual drawing errors and to reduce statistical dependence amongst the data points. The plaque size was also assessed by classifying the maximal intima-media thickness (IMT) per 3mm-segment into 4 grades (<0.5mm, 0.5-0.7mm, 0.7-1.0mm and >1.0mm) according to the method of Chatzizisis

et al.[3]. The percentage of the segments occupied by the respective grade was quantified per artery and averaged over all arteries.

For the OCT analysis, frames with a poor flush were excluded. Lumen contours were automatically segmented and plaque type and component angles were indicated manually. Fibrous plaques were defined as homogeneous, signal-rich intimal thickening with an IMT>0.5mm. Lipid-rich plaques were defined as displaying an inhomogeneous, fading signal combined with an absent 3-layered structure. Lipid-pools were identified when a signal-low region with a diffuse border was present with an overlying signal-rich layer: the fibrous cap. Minimal, maximal and average fibrous cap thickness were determined automatically by the QCU-CMS software. Plaque classification was based on the most severe classification in that respective frame: fibrous, lipid-rich or fibrous-cap atheroma (FCA). The latter classification was given when a lipid-pool was present in the plaque.

Plasma analysis pigs

At the start of every imaging procedure, blood samples were drawn from the carotid sheath into EDTA and clotting tubes. Blood tubes were spun at 1460 g for 10 minutes (Thermo scientific Heraeus centrifuge 3 S-R) to isolate the plasma and serum, which was stored at -80°C. Standard plasma analysis was performed on fresh plasma by the internal clinical chemistry department to determine leucocyte count and levels of total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

Patient plasma collection

Blood was collected in EDTA tubes from three homozygous FH (hoFH) patients who were under treatment at the Erasmus MC, The Netherlands. EDTA-plasma was isolated from blood by centrifugation (3000 rpm, 10 min, 4 °C) and stored at -80 °C until analysis. The diagnosis of hoFH was based on an LDL plasma level >13 mmol/L[4] and a genetic analysis.

Human pool plasma, used as a reference, was a mixture of plasma of approximately 100 non-FH patients that was residual material remaining after diagnostic analyses from the department of clinical chemistry. No informed consent was needed for the use of this residual material.

Lipoprotein profiling

Density-gradient ultracentrifugation

A detailed lipoprotein profile was obtained using 1 mL of EDTA plasma or serum by density-gradient ultracentrifugation (DGUC) as previously reported by Versmissen et al.[5–8]. In short, potassium bromide (KBr) (0.35 g/mL plasma) was added to plasma to obtain a density of 1.26 g/mL. 1 mL of this mix was placed in an ultracentrifuge tube (331372, Beckmann Coulter) and 1.9 mL of 1.21, 1.10, 1.063, 1.04, and 1.02 g/mL KBr in physiological salt was layered on top, followed by 1 mL of water. Samples were centrifuged at 207,000 g for 18 h at 4 °C using a SW41 rotor in an Optima XPN-80 Beckman ultracentrifuge (Beckman Instruments, Indianapolis, IN, USA). Thereafter, DGUC-fractions were collected starting from the bottom of the tube.

Size exclusion chromatography (fast protein liquid chromatography)

A fast protein liquid chromatography (FPLC) profile was obtained from 200 µl plasma. Plasma was added to a Tricorn Superose200 10-300 GL column and a Tricorn Superdex 6 10-300 GL column with a flow rate of 0.5 ml/min. FPLC-fractions of 0.5 mL were collected.

Cholesterol and triglyceride content

Cholesterol and triglyceride content was determined in the fractions obtained by DGUC and FPLC using standard laboratory methods as previously described[7–9].

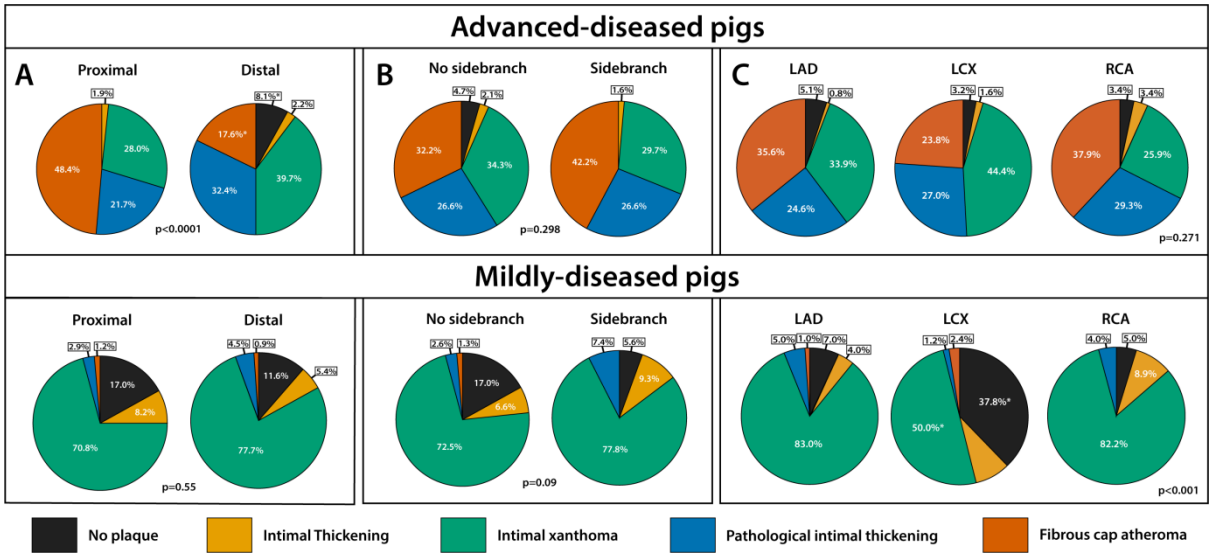
Sphingolipid content analysis by HPLC MS/MS

For the sphingolipid analysis, 10 µL plasma, or 50 µL FPLC-fraction was combined with 10 µl internal standard (2 µg/mL Cer(d18:1/17:0), 2 µg/mL Cer(d17:0/24:1), 0.2 µg/mL S1P-D7, and 10 µg/mL SM(d18:1/17:0) in methanol; Avanti Polar Lipids) and 10 µl 10 % TEA solution (triethylamine (10/90, v/v) in methanol/dichloromethane (DCM) (50/50, v/v)) and mixed thoroughly. Subsequently, 450 µl methanol/DCM (50/50, v/v) was added to this mixture. Samples were incubated under constant aggravation for 30 minutes at 4°C. After incubation, samples were centrifuged (14000 rpm, 20 min, 4°C) and supernatant was transferred to a glass vial, freeze dried and reconstituted in methanol before high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

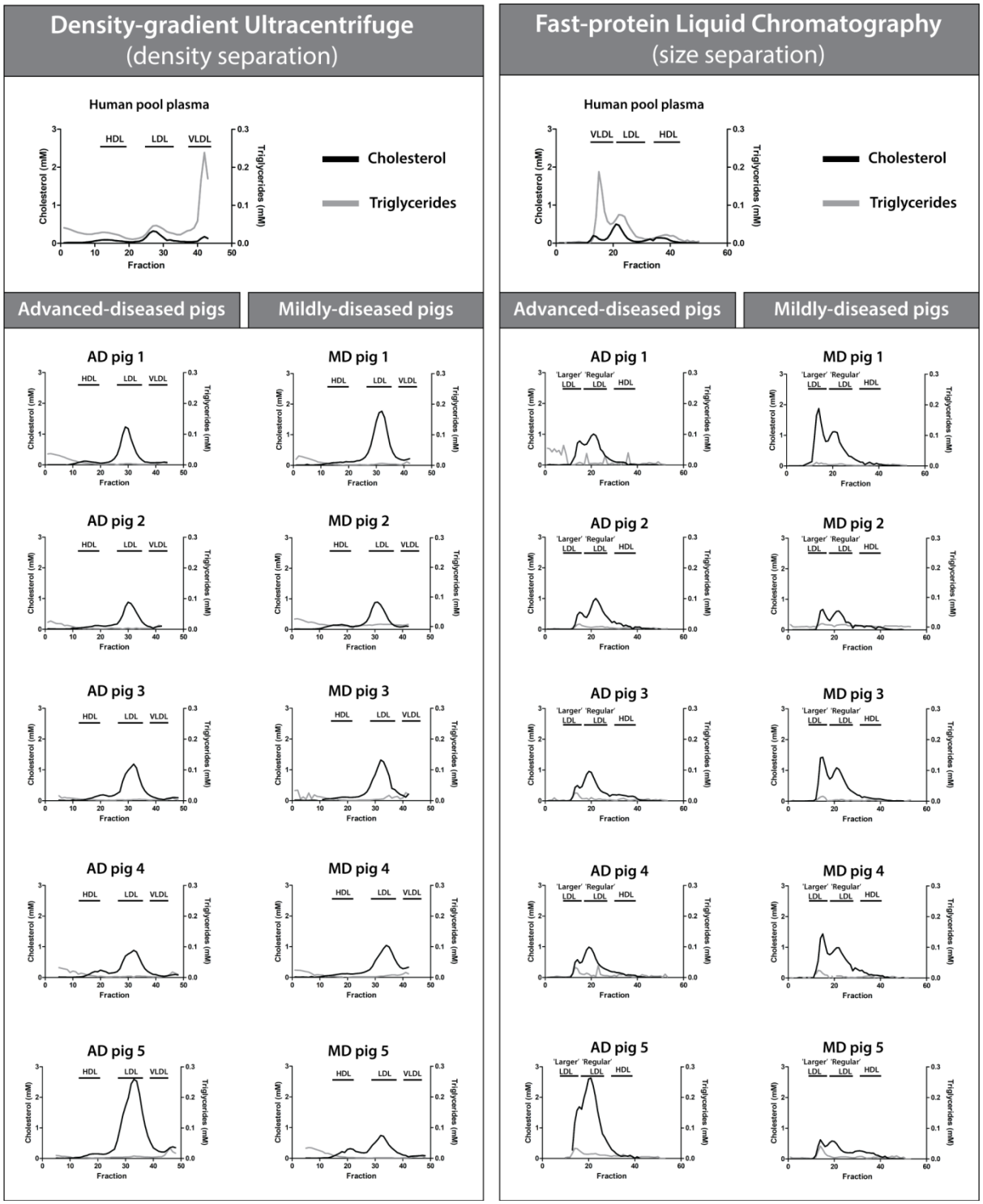
An autosampler (Shimadzu, Kyoto, Japan) injected 5 µL lipid extracts into a Shimadzu HPLC system (Shimadzu) equipped with a Kinetex C8 column (50 x 2.1 mm, 2.6 µm, Phenomenex, Maarssen, the Netherlands) at 30 °C using a gradient, starting from 95% mobile phase A (H_2O/MeOH (50/50, v/v) containing 1.5 mM ammonium formate and 0.1% formic acid) for 2 minutes and increased to 93% mobile phase B (100% MeOH containing 1 mM ammonium formate and 0.1% formic acid) at 5.5 minutes. After 10 minutes, the column was flushed with 99% mobile phase B for 2 minutes before a 2-minute re-equilibration. The flow rate was set at 0.25 ml/min and total run time was 14 minutes. The effluent was directed to a Sciex Qtrap 5500 quadruple mass spectrometer (AB Sciex Inc., Thornhill, Ontario, Canada) and analyzed in positive ion mode following electrospray ionization using multiple reaction monitoring (MRM). Detailed HPLC MS/MS settings are given in Supplemental table XII.

Nine-point calibration curves were constructed by plotting the analyte to the internal standard peak area ratios versus the corresponding analyte concentration for Cer(d18:1/14:0), Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0), Cer(d18:1/24:1), S1P(d18:1), SM(d18:1/16:0), SM(d18:1/18:0), SM(d18:1/18:1), SM(d18:1/20:0), SM(d18:1/22:0), SM(d18:1/24:0), and SM(d18:1/24:1) (Avanti polar lipids, Alabaster, AL, USA; SM(d18:1/20:0) and SM(d18:1/22:0) Matreya LLC, PA, USA). Obtained correlation coefficients (r^2) were >0.99. Sphingolipid concentrations were determined by fitting the identified sphingolipid species to these standard curves based on acyl-chain length. Instrument control and quantification of spectral data was performed using MultiQuant software (AB Sciex Inc.).

Supplemental Figures and Figure Legends



Supplemental figure I: Association between the anatomical location in the coronary artery and the histological plaque classification. Distribution of plaque in regions **A)** proximal versus the distal, **B)** Side branch (SB) versus no SB, **C)** LAD versus LCX versus RCA. The data are split on advanced diseased pigs (top figures) and mildly diseased pigs (bottom figures). P-values indicate overall significance. *z-score>1.96 for that respective plaque type compared to the other plaque types.



Supplemental figure II: Overview of the DGUC (density separation) and FPLC-derived (size separation) lipoprotein profiles from all pigs. The two top graphs demonstrate example profiles from human pool plasma for comparison. The other graphs indicate the DGUC and FPLC profiles for all individual advanced diseased pigs (ADs) and mildly diseased pigs (MDs). Both cholesterol levels (black line) and triglyceride levels (grey line) are indicated.

Supplemental Tables

Supplemental table I: General pig characteristics

	Advanced-diseased pigs			Mildly-diseased pigs			p-value
	T1	*T2*	*T3*	*T1*	*T2*	*T3*	*AD vs MD*
Weight (kg)	77 (58-105)	92 (68-106)	93 (68-106)	86 (69-90)	87 (79-99)	93 (78-106)	0.33
Leukocytes (x10^9/L)	6.8 (5.8-10.2)	6.7 (5.1-10.2)	6.2 (5.3-9.8)	7.6 (6.5-9.0)	6.3 (3.4-8.4)	6.2 (5.3-9.1)	0.93
Cholesterol (mmol/L)	11.0 (8.6-13.0)*	11.0 (10.0-23.5)	9.1 (8.5-21.0)	12.8 (9.6-17.1)	10.7 (9.2-12.4)	8.8 (7.8-10.9)	0.65
LDL-C (mmol/L)	9.1 (7.1-28.6)	8.9 (8.1-20.5)	7.6 (6.7-20.7)	10.7 (7.6-14.3)	8.4 (6.7-10.7)	6.5 (5.8-9.3)	0.14
HDL-C (mmol/L)	2.6 (1.9-4.5)	3.3 (2.3-4.5)	3.0 (2.9-3.2)	2.7 (2.5-4.6)	2.5 (2.4-5.0)	2.6 (2.2-5.1)	0.97
LDL-C/HDL/C ratio	4.2 (2.7-6.4)	3.3 (2.1-4.6)	2.6 (2.3-6.6)	3.3 (1.7-4.5)	3.5 (1.3-4.3)	2.8 (1.1-3.8)	0.08

Values expressed as median (range). * Value of 1 AD pig missing (with the highest LDL-C level).

Supplemental table II: Sphingolipid-ratios in 'regular' and 'larger' LDL at T1

	Advanced diseased pigs	Mildly diseased pigs	p-value
Cer(d18:1/16:0)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	0.3 (0.2 – 0.7)	0.3 (0.3 – 0.4)	0.84
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	1.1 (0.6 – 1.5)	1.0 (0.7 – 1.5)	0.84
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	1.1 (0.6 – 1.5)	0.8 (0.6 – 0.3)	0.84
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.0 (0.8 – 1.3)	1.2 (1.2 – 1.3)	0.10
Cer(d18:1/18:0)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	0.1 (0.1 – 0.2)	0.1 (0.1 – 0.1)	0.84
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	0.4 (0.2 – 0.8)	0.4 (0.3 – 1.1)	0.55
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	0.5 (0.2 – 0.7)	0.3 (0.2 – 0.4)	0.15
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	0.9 (0.8 – 1.3)	1.3 (1.2 – 2.5)	***0.032***
Cer(d18:1/24:1)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	1.0 (0.6 – 1.2)	1.0 (1.0 – 1.1)	0.55
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	1.0 (0.8 – 1.1)	1.0 (0.8 – 1.2)	0.69
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	0.9 (0.7 – 1.1)	0.9 (0.8 – 1.1)	0.69
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.2 (1.0 – 1.3)	1.1 (1.0 – 1.2)	0.69

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective peak. Data are presented as median (range). Significant values are indicated as italic and bold.

Supplemental table III: Cholesterol content of 'regular' and 'larger' LDL at T2 and T3

		Advanced-diseased pigs	Mildly-diseased pigs	p-value
T2	Cholesterol 'regular' LDL	4.9 (4.7-13.3)	4.9 (3.8-5.9)	0.84
	Cholesterol 'larger' LDL	2.1 (0.8-3.6)	2.1 (0.7-3.1)	0.69
	Ratio cholesterol 'regular'/'larger' LDL	3.7 (2.0-6.0)	2.5 (1.9-5.7)	0.42
T3	Cholesterol 'regular' LDL	4.2 (4.0-11.7)	3.8 (2.3-5.3)	0.39
	Cholesterol 'larger' LDL	2.0 (0.5-5.0)	1.4 (1.0-2.7)	0.79
	Ratio cholesterol 'regular'/'larger' LDL	2.3 (2.1-8.6)	2.4 (1.9-2.7)	1.0

Data presented as median (range) of the area under the curve. Significant values are indicated as italic and bold.

Supplemental table IV: S1P and Ceramide content of 'regular' and 'larger' LDL at T2

	Advanced-diseased pigs	Mildly-diseased pigs	p-value
S1P(d18:1) total plasma ($x10^{-5}$)*	3.0 (1.6-4.0)	2.8 (2.3-3.8)	0.84
S1P(d18:1) 'regular' LDL ($x10^{-5}$)*	0.0 (0-0.2)	0.2 (0-0.9)	0.19
S1P(d18:1) 'larger' LDL ($x10^{-5}$)*	0.1 (0-0.2)	0.7 (0-2.2)	0.56
S1P(d18:1) 'regular'/'larger' LDL	0.1 (0-0.2)	0.9 (0.1-1.2)	0.40
Cer(d18:1/14:0) total plasma ($x10^{-5}$)	1.2 (1.0-1.8)	1.2 (1.1-1.6)	0.84
Cer(d18:1/14:0) 'regular' LDL ($x10^{-5}$)	0.9 (0.7-1.4)	1.3 (0.8-1.9)	0.31
Cer(d18:1/14:0) 'larger' LDL ($x10^{-5}$)	1.3 (1.0-1.5)	1.1 (0.6-2.1)	0.31
Cer(d18:1/14:0) 'regular'/'larger' LDL ratio	0.7 (0.6-1.2)	1.3 (0.7-1.7)	0.15
Cer(d18:1/16:0) total plasma ($x10^{-5}$)	8.2 (5.7-12.5)	6.1 (5.7-8.2)	0.55
Cer(d18:1/16:0) 'regular' LDL ($x10^{-5}$)	7.8 (6.3-12.3)	8.5 (5.2-11.1)	1.0
Cer(d18:1/16:0) 'larger' LDL ($x10^{-5}$)	8.7 (7.4-10.5)	8.6 (4.1-11.0)	0.84
Cer(d18:1/16:0) 'regular'/'larger' LDL ratio	0.9 (0.7-1.4)	1.1 (0.8-1.3)	0.55
Cer(d18:1/18:0) total plasma ($x10^{-5}$)	2.1 (1.3-2.9)	1.2 (0.8-1.3)	***0.016***
Cer(d18:1/18:0) 'regular' LDL ($x10^{-5}$)	3.2 (1.6-5.9)	2.7 (1.4-6.1)	1.0
Cer(d18:1/18:0) 'larger' LDL ($x10^{-5}$)	5.5 (2.0-6.4)	4.1 (1.2-10.5)	1.0
Cer(d18:1/18:0) 'regular'/'larger' LDL ratio	0.7 (0.6-0.9)	0.7 (0.6-1.2)	1.0
Cer(d18:1/20:0) total plasma ($x10^{-5}$)	8.6 (4.7-10.6)	6.4 (3.6-7.1)	0.15
Cer(d18:1/20:0) 'regular' LDL ($x10^{-5}$)	4.3 (2.6-5.4)	3.8 (2.6-4.1)	0.31
Cer(d18:1/20:0) 'larger' LDL ($x10^{-5}$)	3.6 (3.5-6.8)	3.5 (2.3-4.7)	0.42
Cer(d18:1/20:0) 'regular'/'larger' LDL ratio	0.9 (0.7-1.4)	1.0 (0.8-1.2)	0.84
Cer(d18:1/22:0) total plasma ($x10^{-5}$)	12.7 (7.2-15.4)	8.9 (5.9-10.2)	0.06
Cer(d18:1/22:0) 'regular' LDL ($x10^{-5}$)	6.2 (5.3-7.7)	5.8 (3.9-6.4)	0.55
Cer(d18:1/22:0) 'larger' LDL ($x10^{-5}$)	7.3 (5.3-10.0)	6.8 (3.4-11.1)	0.42
Cer(d18:1/22:0) 'regular'/'larger' LDL ratio	0.8 (0.7-1.1)	0.9 (0.5-1.1)	0.55
Cer(d18:1/24:0) total plasma ($x10^{-5}$)	18.7 (12.7-21.7)	11.9 (9.6-15.6)	***0.032***
Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	12.8 (7.8-17.6)	10.9 (6.7-12.0)	0.15
Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	12.9 (10.5-28.6)	12.7 (6.7-20.1)	0.42
Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	0.6 (0.5-1.2)	0.8 (0.6-1.1)	0.69
Cer(d18:1/24:1) total plasma ($x10^{-5}$)	16.9 (10.9-21.5)	13.0 (7.2-14.2)	0.10
Cer(d18:1/24:1) 'regular' LDL ($x10^{-5}$)	8.4 (6.5-10.5)	6.0 (5.5-9.0)	0.22
Cer(d18:1/24:1) 'larger' LDL ($x10^{-5}$)	7.7 (4.5-12.2)	6.7 (5.0-9.6)	0.55

Cer(d18:1/24:1) 'regular'/'larger' LDL ratio	0.9 (0.8-1.8)	1.0 (0.8-1.2)	1.0
Cer Total total plasma ($x10^{-5}$)	73.4 (44.1-82.3)	49.5 (34.1-57.4)	0.06
Cer Total 'regular' LDL ($x10^{-5}$)	44.0 (34.9-54.7)	40.4 (29.5-63.3)	0.31
Cer Total 'larger' LDL ($x10^{-5}$)	43.6 (40.2-74.9)	47.8 (23.4-63.3)	0.55
Cer Total 'regular'/'larger' LDL ratio	0.8 (0.7-1.3)	0.9 (0.6-1.3)	0.69

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective LDL peak. Data are presented as median (range) of the area under the curve. *Values around the minimum detection level, unreliable. Significant values are indicated as italic and bold.

Supplemental table V: S1P and Ceramide content of 'regular' and 'larger' LDL at T3

	Advanced-diseased pigs	Mildly-diseased pigs	p-value
S1P(d18:1) total plasma ($x10^{-5}$)#	2.6 (1.7-3.5)*	3.1 (2.9-5.0)	0.39
S1P(d18:1) 'regular' LDL ($x10^{-5}$)#	0.0 (0.0-0.1)*	0.1 (0.0-0.6)	0.39
S1P(d18:1) 'larger' LDL ($x10^{-5}$)#	0.0 (0.0-0.1)*	0.0 (0.0-2.1)	0.79
S1P(d18:1) 'regular'/'larger' LDL	1.1 (1.1-1.1)*	0.5 (0.1-0.8)	0.67
Cer(d18:1/14:0) total plasma ($x10^{-5}$)	0.9 (0.8-1.6)*	1.2 (0.9-1.2)	0.79
Cer(d18:1/14:0) 'regular' LDL ($x10^{-5}$)	1.3 (0.5-1.6)*	1.2 (0.8-2.2)	0.79
Cer(d18:1/14:0) 'larger' LDL ($x10^{-5}$)	0.9 (0.8-1.3)*	1.2 (0.8-2.3)	0.57
Cer(d18:1/14:0) 'regular'/'larger' LDL ratio	1.2 (0.6-1.7)*	1.0 (0.8-1.5)	1.0
Cer(d18:1/16:0) total plasma ($x10^{-5}$)	4.6 (4.5-11.0)*	6.3 (5.1-9.1)	0.57
Cer(d18:1/16:0) 'regular' LDL ($x10^{-5}$)	5.5 (5.5-14.1)*	6.9 (5.8-8.5)	0.57
Cer(d18:1/16:0) 'larger' LDL ($x10^{-5}$)	8.3 (4.1-12.5)*	8.7 (6.7-9.9)	1.0
Cer(d18:1/16:0) 'regular'/'larger' LDL ratio	1.1 (0.7-1.3)*	0.9 (0.8-1.0)	0.57
Cer(d18:1/18:0) total plasma ($x10^{-5}$)	1.3 (1.3-2.5)*	1.4 (1.1-1.7)	0.79
Cer(d18:1/18:0) 'regular' LDL ($x10^{-5}$)	2.9 (1.3-5.9)*	2.3 (2.0-4.9)	1.0
Cer(d18:1/18:0) 'larger' LDL ($x10^{-5}$)	2.8 (2.2-9.1)*	4.0 (2.7-8.9)	0.77
Cer(d18:1/18:0) 'regular'/'larger' LDL ratio	0.6 (0.6-1.0)*	0.6 (0.5-0.8)	0.39
Cer(d18:1/20:0) total plasma ($x10^{-5}$)	6.0 (4.1-6.0)*	6.1 (4.8-8.4)	0.39
Cer(d18:1/20:0) 'regular' LDL ($x10^{-5}$)	3.4 (2.5-4.8)*	3.5 (3.4-4.4)	1.0
Cer(d18:1/20:0) 'larger' LDL ($x10^{-5}$)	4.5 (2.2-5.1)*	4.5 (3.3-5.0)	1.0
Cer(d18:1/20:0) 'regular'/'larger' LDL ratio	1.1 (0.7-1.1)*	0.8 (0.8-1.0)	0.57
Cer(d18:1/22:0) total plasma ($x10^{-5}$)	8.1 (6.8-10.3)*	7.2 (6.2-8.1)	0.39
Cer(d18:1/22:0) 'regular' LDL ($x10^{-5}$)	5.0 (3.8-8.1)*	6.0 (4.5-6.1)	1.0
Cer(d18:1/22:0) 'larger' LDL ($x10^{-5}$)	5.9 (4.8-12.9)*	6.7 (5.5-8.9)	0.79
Cer(d18:1/22:0) 'regular'/'larger' LDL ratio	0.7 (0.6-1.0)*	0.8 (0.7-0.9)	0.57
Cer(d18:1/24:0) total plasma ($x10^{-5}$)	11.1 (10.8-15.9)*	11.8 (9.6-13.4)	0.79
Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	9.8 (8.0-12.9)*	8.9 (6.9-12.4)	0.79
Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	12.7 (12.5-18.4)*	13.7 (8.6-24.6)	0.79
Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	0.7 (0.6-0.8)*	0.7 (0.5-0.8)	1.0
Cer(d18:1/24:1) total plasma ($x10^{-5}$)	11.2 (9.9-11.4)*	11.3 (8.2-16.7)	1.0
Cer(d18:1/24:1) 'regular' LDL ($x10^{-5}$)	6.7 (5.9-8.1)*	6.7 (5.1-7.5)	0.57
Cer(d18:1/24:1) 'larger' LDL ($x10^{-5}$)	6.7 (5.4-9.3)*	7.0 (6.7-8.8)	0.79

Cer(d18:1/24:1) 'regular'/'larger' LDL ratio	1.1 (0.7-1.2)*	0.9 (0.8-1.0)	0.57
Cer Total total plasma ($x10^{-5}$)	48.2 (38.3-53.7)*	45.1 (36.0-58.5)	0.79
Cer Total 'regular' LDL ($x10^{-5}$)	35.1 (31.4-55.4)*	36.8 (35.9-42.4)	0.57
Cer Total 'larger' LDL ($x10^{-5}$)	44.5 (32.7-65.3)*	48.4 (34.3-63.3)	0.79
Cer Total 'regular'/'larger' LDL ratio	0.8 (0.7-1.1)*	0.8 (0.7-1.1)	0.57

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective LDL peak. Data are presented as median (range) of the area under the curve.* n=3 pigs. #Values around the minimum detection level, unreliable. Significant values are indicated as italic and bold.

Supplemental table VI: Sphingolipid-ratios in 'regular' and 'larger' LDL at T2

	Advanced diseased pigs	**Mildly diseased pigs**	**p-value**
Cer(d18:1/16:0)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	0.5 (0.3-0.7)	0.5 (0.4-0.6)	0.42
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	0.6 (0.5-1.0)	0.8 (0.5-1.1)	0.55
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	0.6 (0.3-0.8)	0.6 (0.5-0.8)	0.69
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.1 (1.1-1.9)	1.3 (1.0-1.5)	1.0
Cer(d18:1/18:0)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0.69
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	0.2 (0.1-0.5)	0.2 (0.2-0.6)	0.55
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	0.2 (0.2-0.6)	0.3 (0.2-0.8)	0.31
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	0.9 (0.8-1.4)	0.9 (0.7-1.2)	0.69
Cer(d18:1/24:1)/Cer(d18:1/24:0) total plasma ($x10^{-5}$)	0.9 (0.7-1.0)	0.9 (0.7-1.1)	0.69
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular' LDL ($x10^{-5}$)	0.7 (0.5-0.8)	0.6 (0.5-0.8)	1.0
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'larger' LDL ($x10^{-5}$)	0.4 (0.3-0.7)	0.7 (0.3-0.8)	0.42
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.4 (1.2-2.0)	1.2 (1.0-1.6)	0.22

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective peak. Data are presented as median (range). Significant values are indicated as italic and bold.

Supplemental table VII: Sphingolipid-ratios in 'regular' and 'larger' LDL at T3

	Advanced diseased pigs	Mildly diseased pigs	p-value
Cer(d18:1/16:0)/Cer(d18:1/24:0) total plasma (x10^{-5})	0.4 (0.3-1.0)*	0.5 (0.4-0.7)	0.57
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular' LDL (x10^{-5})	0.7 (0.6-1.1)*	0.7 (0.7-1.0)	0.79
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'larger' LDL (x10^{-5})	0.7 (0.3-0.7)*	0.6 (0.4-0.8)	0.79
Cer(d18:1/16:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.6 (1.0-1.7)*	1.3 (1.0-1.8)	0.79
Cer(d18:1/18:0)/Cer(d18:1/24:0) total plasma (x10^{-5})	0.1 (0.1-0.2)*	0.1 (0.1-0.2)	1.0
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular' LDL (x10^{-5})	0.3 (0.2-0.5)*	0.3 (0.2-0.4)	1.0
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'larger' LDL (x10^{-5})	0.2 (0.2-0.5)*	0.3 (0.2-0.7)	0.39
Cer(d18:1/18:0)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	0.9 (0.9-1.3)*	1.0 (0.6-1.3)	1.0
Cer(d18:1/24:1)/Cer(d18:1/24:0) total plasma (x10^{-5})	0.9 (0.7-1.0)*	0.9 (0.9-1.2)	0.79
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular' LDL (x10^{-5})	0.6 (0.6-0.8)*	0.8 (0.4-1.0)	1.0
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'larger' LDL (x10^{-5})	0.4 (0.4-0.7)*	0.6 (0.3-0.8)	0.79
Cer(d18:1/24:1)/Cer(d18:1/24:0) 'regular'/'larger' LDL ratio	1.4 (1.2-1.7)*	1.3 (1.1-1.5)	0.57

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective peak. Data are presented as median (range). *n=3 pigs. Significant values are indicated as italic and bold.

Supplemental table VIII: Sphingomyelin content of 'regular' and 'larger' LDL at T2

	Advanced-diseased pigs	Mildly-diseased pigs	p-value
SM(d18:1/16:0) total plasma ($x10^{-3}$)	29.4 (18.0-29.7)	30.7 (27.5-31.8)	0.10
SM(d18:1/16:0) 'regular' LDL ($x10^{-3}$)	26.8 (16.5-29.4)	25.6 (21.6-31.2)	1.0
SM(d18:1/16:0) 'larger' LDL ($x10^{-3}$)	24.5 (20.4-33.1)	26.2 (16.6-30.4)	1.0
SM(d18:1/16:0) 'regular'/'larger' LDL	1.0 (0.8-1.4)	1.0 (0.8-1.3)	0.84
SM(d18:1/18:0) total plasma ($x10^{-3}$)	4.8 (2.9-5.5)	4.1 (4.0-5.2)	0.84
SM(d18:1/18:0) 'regular' LDL ($x10^{-3}$)	4.4 (2.2-5.9)	4.2 (3.0-4.5)	0.69
SM(d18:1/18:0) 'larger' LDL ($x10^{-3}$)	3.5 (2.9-6.4)	4.7 (2.4-5.9)	0.84
SM(d18:1/18:0) 'regular'/'larger' LDL ratio	1.0 (0.7-1.5)	1.0 (0.7-1.2)	1.0
SM(d18:1/18:1) total plasma ($x10^{-3}$)	1.1 (0.7-1.1)	1.0 (0.9-1.1)	0.31
SM(d18:1/18:1) 'regular' LDL ($x10^{-3}$)	0.7 (0.5-0.9)	6.3 (5.0-7.5)	0.55
SM(d18:1/18:1) 'larger' LDL ($x10^{-3}$)	0.5 (0.5-0.9)	5.2 (3.8-7.4)	0.69
SM(d18:1/18:1) 'regular'/'larger' LDL ratio	1.2 (0.8-1.6)	1.1 (0.8-1.4)	1.0
SM(d18:1/20:0) total plasma ($x10^{-3}$)	15.8 (10.8-22.4)	20.2 (16.0-24.8)	0.22
SM(d18:1/20:0) 'regular' LDL ($x10^{-3}$)	18.5 (7.2-22.4)	19.1 (13.9-24.6)	0.55
SM(d18:1/20:0) 'larger' LDL ($x10^{-3}$)	16.8 (11.6-28.4)	23.2 (11.6-28.6)	0.69
SM(d18:1/20:0) 'regular'/'larger' LDL ratio	1.0 (0.6-1.2)	0.9 (0.7-1.2)	0.84
SM(d18:1/22:0) total plasma ($x10^{-3}$)	31.1 (25.8-39.4)	35.3 (30.6-38.2)	0.42
SM(d18:1/22:0) 'regular' LDL ($x10^{-3}$)	15.2 (6.7-17.1)	13.8 (9.6-19.5)	1.0
SM(d18:1/22:0) 'larger' LDL ($x10^{-3}$)	12.6 (7.1-18.7)	16.4 (7.6-20.3)	0.31
SM(d18:1/22:0) 'regular'/'larger' LDL ratio	1.1 (0.8-1.9)	1.0 (0.7-1.3)	0.69
SM(d18:1/24:0) total plasma ($x10^{-3}$)	6.2 (4.6-7.6)	6.3 (5.4-6.6)	1.0
SM(d18:1/24:0) 'regular' LDL ($x10^{-3}$)	2.5 (1.0-3.3)	2.8 (1.5-3.2)	1.0
SM(d18:1/24:0) 'larger' LDL ($x10^{-3}$)	2.0 (1.3-3.2)	3.0 (1.2-3.5)	0.69
SM(d18:1/24:0) 'regular'/'larger' LDL ratio	1.0 (0.8-1.7)	1.0 (0.8-1.2)	1.0
SM(d18:1/24:1) total plasma ($x10^{-3}$)	12.3 (10.9-14.8)	13.5 (11.8-15.0)	0.22
SM(d18:1/24:1) 'regular' LDL ($x10^{-3}$)	5.4 (3.2-7.2)	6.6 (4.4-7.1)	0.84
SM(d18:1/24:1) 'larger' LDL ($x10^{-3}$)	4.9 (3.5-6.5)	6.5 (3.5-7.7)	0.31
SM(d18:1/24:1) 'regular'/'larger' LDL ratio	1.2 (0.8-1.5)	1.1 (0.8-1.2)	0.55
SM Total total plasma ($x10^{-3}$)	102.6 (75.9-115.8)	112.2 (99.7-119.8)	0.31
SM Total 'regular' LDL ($x10^{-3}$)	75.3 (39.0-84.1)	71.3 (54.4-88.8)	1.0
SM Total 'larger' LDL ($x10^{-3}$)	66.0 (49.2-97.1)	78.1 (43.3-94.2)	0.94
SM Total 'regular'/'larger' LDL ratio	1.1 (0.8-1.4)	0.9 (0.8-1.3)	1.0

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective LDL peak. Data are presented as median (range) of the area under the curve. Significant values are indicated as italic and bold.

Supplemental table IX: Sphingomyelin content of 'regular' and 'larger' LDL at T3

	Advanced-diseased pigs	Mildly-diseased pigs	p-value
SM(d18:1/16:0) total plasma (x10^{-3})	31.1 (18.7-31.1)	31.7 (29.3-33.2)	0.39
SM(d18:1/16:0) 'regular' LDL (x10^{-3})	28.8 (22.7-35.2)	27.3 (23.6-32.6)	1.0
SM(d18:1/16:0) 'larger' LDL (x10^{-3})	31.4 (21.9-36.6)	31.7 (22.3-37.1)	0.79
SM(d18:1/16:0) 'regular'/'larger' LDL	1.0 (0.7-1.3)	0.9 (0.8-1.1)	1.0
SM(d18:1/18:0) total plasma (x10^{-3})	4.8 (3.2-5.6)	4.7 (4.6-5.4)	1.0
SM(d18:1/18:0) 'regular' LDL (x10^{-3})	5.5 (3.5-6.2)	4.5 (3.4-6.0)	0.57
SM(d18:1/18:0) 'larger' LDL (x10^{-3})	4.8 (4.7-5.2)	5.8 (3.2-7.8)	0.57
SM(d18:1/18:0) 'regular'/'larger' LDL ratio	1.2 (0.7-1.2)	0.8 (0.7-1.1)	0.39
SM(d18:1/18:1) total plasma (x10^{-3})	1.1 (0.8-1.1)	1.0 (1.0-1.4)	1.0
SM(d18:1/18:1) 'regular' LDL (x10^{-3})	0.8 (0.7-0.9)	0.7 (0.6-1.0)	0.25
SM(d18:1/18:1) 'larger' LDL (x10^{-3})	0.7 (0.5-1.0)	0.7 (0.5-1.1)	1.0
SM(d18:1/18:1) 'regular'/'larger' LDL ratio	1.3 (0.8-1.4)	1.0 (0.9-1.3)	0.79
SM(d18:1/20:0) total plasma (x10^{-3})	14.5 (12.0-18.7)	21.9 (19.1-25.0)	***0.04***
SM(d18:1/20:0) 'regular' LDL (x10^{-3})	22.1 (14.5-22.7)	24.5 (17.8-25.4)	0.39
SM(d18:1/20:0) 'larger' LDL (x10^{-3})	20.2 (20.1-20.4)	25.6 (17.1-34.7)	0.25
SM(d18:1/20:0) 'regular'/'larger' LDL ratio	1.1 (0.6-1.1)	0.9 (0.7-1.0)	0.57
SM(d18:1/22:0) total plasma (x10^{-3})	30.9 (27.4-37.8)	39.9 (29.1-44.0)	0.25
SM(d18:1/22:0) 'regular' LDL (x10^{-3})	13.5 (11.4-15.7)	15.5 (9.9-18.3)	0.57
SM(d18:1/22:0) 'larger' LDL (x10^{-3})	47.8 (14.3-15.7)	18.3 (9.1-22.4)	0.25
SM(d18:1/22:0) 'regular'/'larger' LDL ratio	0.9 (0.7-1.1)	0.9 (0.7-1.1)	1.0
SM(d18:1/24:0) total plasma (x10^{-3})	6.6 (4.2-7.5)	6.1 (5.3-8.2)	1.0
SM(d18:1/24:0) 'regular' LDL (x10^{-3})	2.6 (2.2-3.1)	2.6 (1.8-3.4)	1.0
SM(d18:1/24:0) 'larger' LDL (x10^{-3})	2.7 (2.1-2.8)	3.0 (1.8-4.5)	0.25
SM(d18:1/24:0) 'regular'/'larger' LDL ratio	1.1 (0.8-1.2)	0.8 (0.7-1.0)	0.25
SM(d18:1/24:1) total plasma (x10^{-3})	11.1 (11.0-15.0)	14.7 (13.0-17.4)	0.25
SM(d18:1/24:1) 'regular' LDL (x10^{-3})	6.4 (5.8-7.5)	6.1 (5.8-8.5)	1.0
SM(d18:1/24:1) 'larger' LDL (x10^{-3})	5.4 (5.2-7.8)	7.5 (4.8-9.2)	0.57
SM(d18:1/24:1) 'regular'/'larger' LDL ratio	1.2 (0.7-1.4)	0.9 (0.8-1.3)	0.79
SM Total total plasma (x10^{-3})	97.6 (79.8-116.6)	122.8 (104.6-131.6)	0.14
SM Total 'regular' LDL (x10^{-3})	82.9 (60.8-88.0)	81.0 (62.9-90.2)	0.79
SM Total 'larger' LDL (x10^{-3})	84.5 (70.0-87.5)	88.6 (59.5-115.7)	0.39

SM Total ‘regular’/’larger’ LDL ratio	1.0 (0.7-1.2)	0.9 (0.8-1.1)	0.79

All sphingolipid data were expressed relative to the cholesterol concentration in total plasma or the respective LDL peak. Data are presented as median (range) of the area under the curve. Significant values are indicated as italic and bold.

Supplemental table X: Homozygous FH patient characteristics

	Patient 1	Patient 2	Patient 3
Age (years)	23	31	28
Sex (m/f)	m	m	m
BMI	27.2	23.0	18.7
Hypertension	no	no	no
Diabetes	no	no	no
Smoker	no	former	no
Statines	yes	yes	yes
Ezetimibe	yes	yes	yes
Other lipid lowering medication	Lomitapide	Lomitapide	Lomitapide
FH mutation	LDLR Null/null G352D, exon8/ 2417insG, exon 17	LDLR Null/null 1685delACT, exon 11/ 1685delACT, exon 11	LDLR Null/null 4.4Kb dupl, exon 12/ 2.5Kb del exon 7 and 8
Highest cholesterol (mmol/L)	23.6	17.2	20.6
Cholesterol (mmol/L)	16.0	2.8	15.1
LDL (mmol/L)	14.5	1.7	14.6
HDL (mmol/L)	0.9	1.1	1.2
Triglycerides (mmol/L)	1.6	0.3	0.4

Supplemental table XI: Overview porcine studies of natural coronary atherosclerosis development with histological analysis

Reference	Breed	Sex	Age at start study (weeks)	Max. FU time (weeks)	Induction of atherosclerosis	Plaque size (PA unless otherwise indicated)	IMR	Plaque classification (% coverage of artery)	Used imaging technique
Prescott 1991 [10]	Rapacz	Unknown	Mature	234	None	Occlusive	-	FCA	-
Neeb 2010 [11]	Ossabaw (O) Yucatan (Y)	Male	Unknown	40	Atherogenic diet	-	-	-	IVUS
Thim 2010 [12]	FBM minipig	Castrated male	39	18	Atherogenic diet	NP/IT/IX: 0.28mm^2* PIT: 0.93 mm^2* FCA: 2.53 mm^2*	NP/IT/IX: 0.19* PIT: 0.42* FCA: 1.26*	-	VH-IVUS‖
Al-Mashhadi 2013 [13]	Yucatan PCSK9 mutation	Male(m) and female(f)	Unknown	46	Atherogenic diet	Male: 0.29 mm^2*† Female: 0.25 mm^2*†	-	NP: 0%* IX: 0%(m) / 25%(f)* PIT: 100%(m) / 25%(f)* FCA: 0%(m) / 50%(f)*	-
Davis et al. 2014[14]	Yucatan LDLR$^{-/-}$	Female and castrated male	22	6	Atherogenic diet	0.2 mm^2	-	-	-
Pedrigi et al. 2015 [15]	Yucatan PCSK9 mutation	Female	Unknown	34	Atherogenic diet	0.2 mm^2	-	NP:41% IT: 30% IX: 5% PIT: 24%	OCT‖

Poulsen et al. 2016[16]	FBM minipig	Castrated female	29	38	Atherogenic diet	Average maximal PA*: 1.8 mm^2	-	NP: 11% IX: 60% PIT: 18% FCA:11%	-
Shim et al. 2017[17]	Yucatan $ApoE^{-/-}$	Unknown	8	44	Atherogenic diet	Average maximal PA*†: 0.15 mm^2	-	IX: 75%*† PIT: 25%*†	-
Badin 2018	Ossabaw	Female	130 (young) or 458 (old)	48	Atherogenic diet	-	Average: 0.3 (young)†; 0.8 (old)†	-	IVUS
Tharp 2019	Rapacz	Castrated male	60	26	Atherogenic diet	-	-	-	IVUS
Hoogendoorn 2019	**FBM minipig**	**Castrated male**	**147**	**52**	**Atherogenic diet**	**IT: 0.9 mm^2†§** **IX: 0.9 mm^2†§** **PIT: 2.1 mm^2†§** **FCA: 4.2 mm^2†§** **Average: 2.0 mm^2**	**IT: 0.6†§** **IX: 0.7†§** **PIT: 1.8†§** **FCA: 2.6†§** **Average: 1.4**	**NP: 4%†§** **IT: 2%†§** **IX: 33%†§** **PIT: 27%†§** **FCA: 34%†§**	**IVUS and OCT**
Gerrity 2001 [18]	Yorkshire	Male	8-12	48	Atherogenic diet or Atherogenic diet + diabetes	Stenosis degree: 86%†	-	-	-
Chatzizisis 2008 [19]	Yorkshire	Male	Unknown	30	Atherogenic diet + diabetes	-	Average: 0.55 – 1.3*†	IT: 18.3%* IX/PIT: 39.4%* FCA:42.3%*	IVUS

Koskinas 2010/2013 [20,21]	Yorkshire	Male	12-14	36	Atherogenic diet + diabetes	Average: 1.6 – 2.7 mm^2*†	-	IT: 6%* IX/PIT: 25%* FCA: 69%*	IVUS
Patel 2013 [22]	Yorkshire	Male	Unknown	39	Atherogenic diet + diabetes	-	-	No plaque: 13% IT/IX: 22% PIT: 22% FCA: 43%	IVUS
Ludvigsen 2015 [23]	Göttingen	Castrated male	11	43	Atherogenic diet + diabetes	0.27 mm^2†	0.23†	IX: 33% PIT: 33% FCA: 33%	-
Ditzhuijzen 2016 [24]	Yorkshire/ Landrace	Male	11	65	Atherogenic diet or Atherogenic diet + diabetes	-	-	IT - FCA	OCT

Plaque size and classification are based on histological data. Sizes based on imaging are mentioned in the text if the manuscript. Majority of the numbers are estimated from graphs. NP=no plaque, IT=intimal thickening, IX=intimal xanthoma, PIT=pathological intimal thickening, FCA=fibrous cap atheroma, IMR=intima-media ratio. *Only of the largest lesions, †Tissue not pressure fixed, ‡ Of all lesions, not of the whole artery, [§]Only of the advanced-diseased pigs. [||]No imaging results reported on spontaneous plaque development.

Supplemental table XII: HPLC MS/MS specifications

Component	MRM transition	CE (Volt)	Internal standard
Cer(d18:1/14:0)	510.6 → 264.2	30	Cer(d18:1/17:0)
Cer(d18:1/16:0)	538.6→ 264.2	30	Cer(d18:1/17:0)
Cer(d18:1/18:0)	566.6→ 264.2	30	Cer(d18:1/17:0)
Cer(d18:1/20:0)	594.6→264.2	30	Cer(d18:1/17:0)
Cer(d18:1/22:0)	622.6→264.2	35	Cer(d18:1/17:0)
Cer(d18:1/24:0)	650.6→264.2	40	Cer(d17:0/24:1)
Cer(d18:1/24:1)	648.6→264.2	40	Cer(d17:0/24:1)
S1P(d18:1)	380.4→264.2	20	S1P(d18:1)-D7
SM(d18:1/16:0)	706.6→186.2	40	SM(d18:1/17:0)
SM(d18:1/18:0)	734.6→186.2	40	SM(d18:1/17:0)
SM(d18:1/20:0)	762.6→ 186.2	40	SM(d18:1/17:0)
SM(d18:1/22:0)	790.6→186.2	40	SM(d18:1/17:0)
SM(d18:1/24:0)	818.6→ 186.2	47	SM(d18:1/17:0)
SM(d18:1/24:1)	816.6→186.2	47	SM(d18:1/17:0)
S1P(d18:1)-D7	387.4→ 271.2	20	-
Cer(d18:1/17:0)	552.6→ 264.2	30	-
Cer(d17:0/24:1)	634.6→ 250.2	35	-
SM(d18:1/17:0)	720.6→ 186.2	40	-

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Familial hypercholesterolemia Bretonchelles Meishan Pigs (homozygous R84C *LDLR* mutation)	Department of Angiohematology, Hospital Lariboisiere, France	Rapacz x Chinese Meishan x Bretonchelles minipig	Castrated male

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
CD68	Bio-Rad	MCA2317GA	0.6 µg/mL
CD31	Bio-Rad	MCA1746GA	5 µg/mL
Secondary Ab for CD68 (EnVision™+ System/HRP)	Agilent	K4001	N/A (Ready-to-use)
Secondary Ab for CD31	DAKO	P0447	10 µg/mL

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