SUPPLEMENTAL MATERIAL

Supplemental Methods

Animals

The experimental protocol was approved by the Harvard's Institutional Animal Care and Use Committee and conforms to the Guide for the Care and Use of Laboratory Animals. Five male Yorkshire swine, age 12-14 week, initially weighing 18.9±1.0 kg, were sedated with Telazol (10 mg/kg) and injected via ear vein with filter-sterilized b-cell cytotoxin streptozotocin (50 mg/kg in 0.1 mol/l Na-citrate, pH 4.5) daily for 3 days to induce diabetes.[1,2] Animals were subsequently given 25 g glucose twice daily at feeding for 2 days to offset insulin release from b-cells. Animals were maintained without exogenous insulin throughout the study protocol. Starting at the time of diabetes induction, the animals were fed a diet containing 1.5% cholesterol and 15% lard supplemented with sucrose, as previously described, in quantities titrated to maintain serum total cholesterol (TC) and blood glucose (BG) levels between 500-800 mg/dl and 150-350 mg/dl, respectively, while allowing steady weight gain.[1] If the serum cholesterol level exceeded 800 mg/dl the diet was adjusted by adding normal pig chow to the 1.5% cholesterol diet.

Animal weight was monitored every month, as well on the day of each catheterization procedure. TC and BG were monitored daily for 2 weeks, then weekly thereafter, following overnight (≥12 hours) fasting. Standard enzymatic assay kits were used for TC and BG measurement (Sigma, St. Louis, MO and Thermo Scientific, Waltham, MA, respectively). BG in particular was checked daily in cases in which the glucose level was >350 mg/dl. Blood (500 μl)

for all measurements was obtained without sedation by pricking an ear vein, cleaned with Nolvosan, with a lancet and collecting drops in a hematocrit tube.

Study protocol

All pigs underwent serial coronary angiography and IVUS, as described below, in all the major epicardial coronary arteries (left anterior descending, left circumflex, and right coronary artery) at five consecutive time points *in vivo*: 4, 11, 16, 23 and 36 weeks after the induction of diabetes and initiation of a high-fat diet (**Figure 1S**).

Serial cardiac catheterizations

Animals were fasted overnight before each catheterization procedure. Pre-procedural evaluation included physical examination and blood glucose measurement. Animals were at a surgical plane of anesthesia prior to each catheterization procedure, and remained under anesthesia for the entire procedure, as well as throughout euthanasia following the final procedure at week 36. The anesthesia protocol consisted of the combination of tiletamine with zolazepam (4.4 mg/kg, Telazol, Wyeth), Xylazine (2.2 mg/kg, Rompun, Bayer), and Atropine (0.05 mg/kg). After endotracheal intubation with 7-7.5mm sized tubes, the animals were ventilated with isoflurane (0.1% to 5.0%) and oxygen for maintenance of anesthesia throughout the procedure. Monitoring during each procedure included continuous electrocardiogram, arterial blood pressure and O_2 saturation (SaO_2) monitoring.

Access site for vascular access was the femoral groin area. The skin at the access site was aseptically prepared with povidone iodine (Betadine) solution. All incisions were made using a muscle-sparing technique and were kept as small as possible (~2.5 cm in length) in order to minimize injury, facilitate fast healing and minimize post-operative pain. After the incision and the arteriotomy, an appropriate sized introducer sheath was placed in the artery. The sheath was

removed immediately after completion of the procedure. All incisions were closed with a 3-layer closure of vicryl. Animals were monitored for post-operative pain and given analgesics (Buprenorphine, Buprenex, Reckitt & Colman, 0.05 mg/kg IM / BID for 24 hours, appropriately adjusted thereafter).

Vascular profiling for ESS calculation

Intracoronary vascular profiling methodology has been previously described and validated in-vivo.[3-6] In brief, the 3D anatomy of the coronary artery was reconstructed from IVUS images and biplane coronary angiography. IVUS (ClearView, Boston Scientific, Natick, MA) was performed with automated pullback at 0.5 mm/sec. The arterial lumen and external elastic membrane (EEM) were segmented from digitized end-diastolic IVUS images.[7] The physical 3D path of the IVUS transducer during pullback was reconstructed using the corresponding biplane angiographic projections, and the segmented IVUS images were located along this path and oriented appropriately. Lumen and EEM boundary points were connected by spline curves to rebuild the lumen and EEM geometry in 3D space, respectively. A structured grid was employed to represent the lumen volume. Coronary blood flow for the reconstructed arterial segment was calculated directly from the time required for opacified blood to fill a known volume of coronary artery during a contrast injection.[3,4] Blood was considered as Newtonian fluid and its viscosity was estimated using the hematocrit and serum TC.[4] Detailed intravascular flow characteristics were obtained by computational fluid dynamics solving the transport equations governing the conservation of mass and momentum (PHOENICS, Cham Ltd, London, UK).[4] The governing equations of blood flow were determined assuming that the arterial wall is stiff, blood is incompressible, and coronary blood flow is steady with uniform inlet flow velocity.[3] ESS at the

lumen surface of the geometrically correct 3D reconstructed artery was calculated at all time points as the product of viscosity and the gradient of blood velocity at the wall.[4]

Each 3D reconstructed artery was divided at week 36 into 3mm-long segments along its entire length. To locate the segments in IVUS investigations at multiple time points, baseline and follow-up reconstructed arteries were matched using IVUS-derived anatomical landmarks (i.e. side branches, veins, calcified areas) for accurate comparison of the same segments over time.[3,4,8] Mean ESS was calculated in each 3D segment at all time points.

Assessment of vascular remodeling

The nature of the remodeling response to plaque growth was assessed over time in each arterial segment that contained significant plaque at final week 36, defined as maxIMT≥0.5mm by IVUS. Remodeling was assessed in these segments at each time point by comparing the local remodeling behavior of each individual segment with the global remodeling response of the entire artery, as previously described.[2,9] Briefly, the EEM areas of all the IVUS cross-sections along each reconstructed artery were measured at each time point and plotted against the corresponding intima-media areas. The global reference of the entire reconstructed artery at each time point was determined by the linear regression line and its 90% prediction band in the EEM area vs. intima-media area plot. The EEM and intima-media area of each individual segment were then identified within the corresponding plot, and three local remodeling patterns were defined: (a) excessive expansive remodeling if the EEM area of the segment was above the upper limit of the 90% prediction band of the entire artery remodeling behavior, (b) compensatory expansive remodeling if the EEM area of the segment was within the 90% prediction band, and (c) constrictive remodeling if the EEM area of the segment was below the lower limit of the 90% prediction band.

Supplemental Results

To assess the lifetime exposure of pigs to hyperlipidemia and hyperglycemia, the time-average of TC and BG was calculated for all measurements once the pigs were rendered diabetic and started on the high-fat diet. The time-averaged TC and BG during the 36-week study duration were 728±161 mg/dl and 265±53 mg/dl, respectively.

The mean body weight at study entry was 18.9±1.0 kg (range: 17.9-20.2kg). At the end of the study the body weight increased to 49.9±2.9 kg (range: 46.1-53.3 kg), representing a 164±16.9% increase (**Table 1S**). Animal body weight and the rate of body weight gain did not exhibit significant differences among the animals throughout the study protocol. The most marked body weight gain, quantified as absolute increase of body weight (21.0±2.6 kg), percent rate of weight gain (73.0±9.9%), and weight gain rate (1.6±0.2 kg/week), was observed during the last of the four intervals of the study period, i.e. between weeks 23 and 36.

Study Limitations

We acknowledge some assumptions we made concerning blood flow. [4] Instead of using intracoronary Doppler flow-wire, the coronary flow was measured by applying a previously published methodology based on the fundamental definition of flow rate, i.e. the time required for opacified blood to fill a known volume of coronary artery.[2-4] We determined the true volume of the arterial segment under study and measured the time required to fill that volume by tracking the wave front of contrast medium through it. Utilizing our technique for flow measurement we were able to minimize the use of additional catheters, thereby minimizing the risk of complications, such as vascular injury that could affect the progression of native atherosclerotic disease. The assumption of steady-state coronary blood flow ignored phasic phenomena, but, as we previously demonstrated, using the average flow in steady flow calculations yields essentially the same values of ESS as calculating the average ESS from the phasic solution.[10,11] The errors produced by the assumptions of Newtonian viscosity and rigid arterial walls were insignificant in the flow ranges observed in the current study.[10,11] At the Reynolds numbers observed in this study, the distortions introduced by the assumption of uniform inlet velocity were also insignificant for inlet diameters above 1 mm^3.

Our model did not enable us to study pathophysiologic mechanisms implicated in abrupt plaque progression, including healing of subclinical plaque rupture.[12] However, we have previously shown no histological evidence of plaque rupture using the same experimental model in a similar time frame of investigation.[2] We may therefore conclude that plaque progression assessed by IVUS represents increase of plaque volume itself.

Supplemental Tables

Table 1S. Progression of individual animal body weight over time. No significant differences were observed among animals at any time point.

Animal	Total Body Weight (kg)				
	Week 4	Week 11	Week 16	Week 23	Week 36
#1	17.9	22.2	21.4	26.7	49
#2	18.6	25.5	23.0	29.3	46.1
#3	19.9	23.9	24.6	28.7	49
#4	20.2	27.1	26.7	30.5	53.3
#5	18.3	23.5	23.9	29.3	52.3

Supplemental Figure Legends

Figure 1S. Schematic presentation of the study protocol.

Figure 2S. Evolution of plaque burden in segments that culminated in compensatory (Comp.), excessive expansive (Excess), and constrictive remodeling (Constrict.) at week 36. Plaques that culminated in constrictive remodeling had on average plaque burden >50% at week 36, as opposed to plaques that culminated in compensatory, or excessive expansive remodeling. Values are presented as mean ± SEM.

Supplemental Figures

Figure 1S.

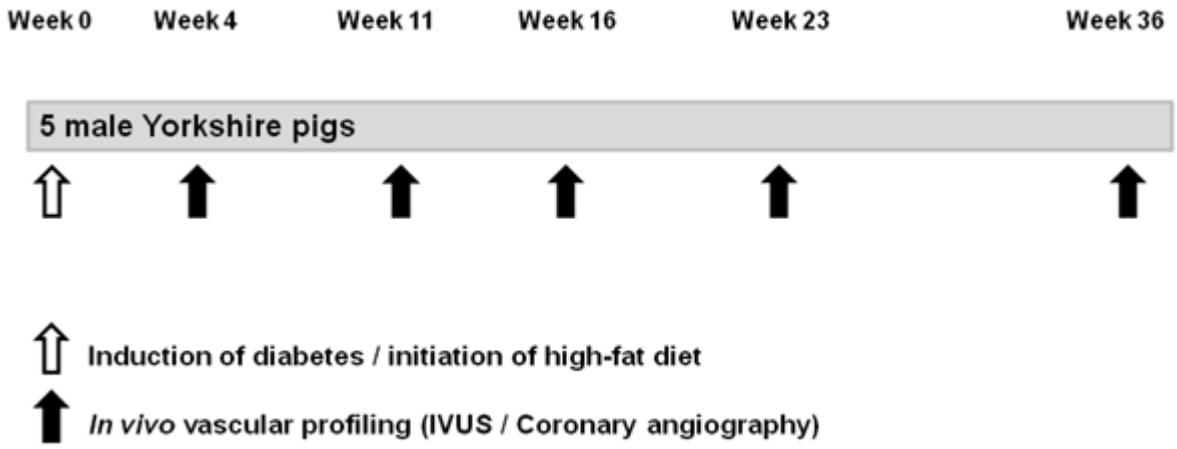
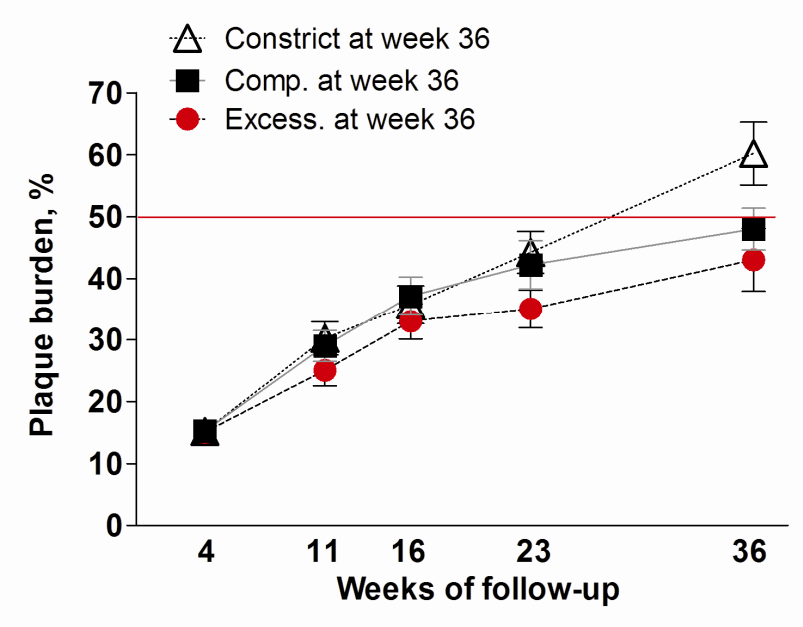


Figure 2S.


Constrict at week 36
Comp. at week 36
Excess. at week 36
Plaque burden, %
70
60
50
40
30
20
10
0
4
11
16
23
36
Weeks of follow-up

Supplemental References

1. Gerrity RG, Natarajan R, Nadler JL, Kimsey T. Diabetes-induced accelerated atherosclerosis in swine. *Diabetes* 2001;50:1654-1665.

2. Chatzizisis YS, Jonas M, Coskun AU, et al. Prediction of the localization of high risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: an intravascular ultrasound and histopathology natural history study. Circulation 2008;117:993–1002.

3. Stone PH, Coskun AU, Kinlay S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J, Kuntz RE, Feldman CL. Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans: in vivo 6-month follow-up study. *Circulation* 2003;108:438-444.

4. Coskun AU, Yeghiazarians Y, Kinlay S, Clark ME, Ilegbusi OJ, Wahle A, Sonka M, Popma JJ, Kuntz RE, Feldman CL, Stone PH. Reproducibility of coronary lumen, plaque, and vessel wall reconstruction and of endothelial shear stress measurements in vivo in humans. *Catheter Cardiovasc Interv* 2003;60:67-78.

5. Giannoglou GD, Chatzizisis YS, Sianos G, Tsikaderis D, Matakos A, Koutkias V, Diamantopoulos P, Maglaveras N, Parcharidis GE, Louridas GE. In-vivo validation of spatially correct three-dimensional reconstruction of human coronary arteries by integrating intravascular ultrasound and biplane angiography. *Coron Artery Dis* 2006;17:533-543.

6. Slager CJ, Wentzel JJ, Schuurbiers JC, Oomen JA, Kloet J, Krams R, von Birgelen C, van der Giessen WJ, Serruys PW, de Feyter PJ. True 3-dimensional reconstruction of coronary arteries in patients by fusion of angiography and IVUS (ANGUS) and its quantitative validation. *Circulation* 2000;102:511-516.

7. Mintz GS, Nissen SE, Anderson WD, Bailey SR, Erbel R, Fitzgerald PJ, Pinto FJ, Rosenfield K, Siegel RJ, Tuzcu EM, Yock PG. American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 2001;37:1478-1492.

8. Stone PH, Coskun AU, Kinlay S, Popma JJ, Sonka M, Wahle A, Yeghiazarians Y, Kuntz RE, Feldman CL. Regions of low endothelial shear stress are sites where coronary plaque progress and vascular remodeling occurs in humans: An in-vivo serial study. *Eur Heart J* 2007;28:705-710.

9. Feldman CL, Coskun AU, Yeghiazarians Y, Kinlay S, Wahle A, Olszewski ME, Rossen JD, Sonka M, Popma JJ, Orav J, Kuntz RE, Stone PH. Remodeling characteristics of minimally diseased coronary arteries are consistent along the length of the artery. *Am J Cardiol.* 2006;97:13-16.

10. Feldman CL, Ilegbusi OJ, Hu Z, Nesto R, Waxman S, Stone PH. Determination of in vivo velocity and endothelial shear stress patterns with phasic flow in human coronary arteries: a methodology to predict progression of coronary atherosclerosis. *Am Heart J* 2002;143:931-939.

11. Soulis JV, Giannoglou GD, Chatzizisis YS, Farmakis TM, Giannakoulas GA, Parcharidis GE, Louridas GE. Spatial and phasic oscillation of non-Newtonian wall shear stress in human left coronary artery bifurcation: an insight to atherogenesis. *Coron Artery Dis* 2006;17:351-358.

12. Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. *Circulation*. 2001;103:934–940.