Published in final edited form as:

J Nucl Cardiol. 2014 April ; 21(2): 319–328. doi:10.1007/s12350-013-9843-7.

Lipid lowering and imaging protease activation in atherosclerosis Lipid therapy and MMP imaging in atherosclerosis

Mahmoud Razavian, PhD^{#1,2}, Lei Nie, PhD^{#1,2}, Azariyas Challa, MD, PhD^{1,2}, Jiasheng Zhang, MD^{1,2}, Reza Golestani, MD, PhD^{1,2}, Jae-Joon Jung, PhD^{1,2}, Simon Robinson, PhD³, and Mehran M. Sadeghi, MD^{1,2}

¹Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine and Yale Cardiovascular Research Center, Yale University School of Medicine, New Haven, CT

²VA Connecticut Healthcare System, West Haven, CT

³Lantheus Medical Imaging, North Billerica, MA

[#] These authors contributed equally to this work.

Abstract

Background—Lipid lowering is a mainstay of modern therapeutic approach to atherosclerosis. We sought to evaluate matrix metalloproteinase (MMP)-targeted microSPECT imaging for tracking of the effect of lipid-lowering interventions on plaque biology in atherosclerotic mice in vivo.

Methods and Results—ApoE^{−/−} mice fed on a high fat diet (HFD) for 2 months were randomly assigned to continuation of HFD, HFD plus simvastatin, HFD plus fenofibrate and high fat withdrawal (HFW). The animals underwent serial microSPECT/CT imaging using RP805, a ^{99m}Tc-labeled MMP-targeted tracer at 1 and 4 weeks after randomization. All three interventions reduced total blood cholesterol by 4 weeks. In animals on HFD, aortic arch RP805 uptake significantly increased from 1 week to 4 weeks. Tracer uptake in fenofibrate and HFW groups was significantly lower than uptake in the HFD group at 4 weeks. Similarly, CD 68 gene expression, reflecting plaque inflammation, was significantly lower in fenofibrate and HFW groups compared to HFD group. MMP tracer uptake significantly correlated with aortic CD68, but not VE-cadherin or smooth muscle α -actin expression.

Conclusions—MMP tracer uptake paralleled the effect of lipid-lowering interventions on plaque inflammation in atherosclerotic mice. MMP-targeted imaging may be used to track the effect of therapeutic interventions in atherosclerosis.

Keywords

Atherosclerosis; Molecular Imaging; Matrix metalloproteinases; Lipids; Inflammation

Address for correspondence: Mehran M. Sadeghi, M.D., VA Connecticut Healthcare System, 950 Campbell Avenue, 111B, West Haven, CT 06516, Fax: 203-937 3884, Phone: 203-932 5711 x3398, Mehran.sadeghi@yale.edu.

Disclosures: Simon Robinson is employee of Lantheus Medical Imaging. Mehran M. Sadeghi receives experimental tracers from Lantheus Medical Imaging.

Introduction

Lipid-lowering, in conjunction with anti-platelet and anti-hypertensive therapies are the mainstay of modern therapeutic approach to atherosclerosis (1). Part of the beneficial effects of lipid-lowering therapies, such as statins, may be linked to stabilizing effects on plaque biology rather than plaque regression (2). Inflammation is a key determinant of plaque vulnerability. Lipids, such as modified LDL, promote the recruitment and activation of inflammatory cells and trigger vessel wall inflammation in atherosclerosis (3). Macrophages (as well as other vascular cells) produce a number of proteases, including members of the matrix metalloproteinase (MMP) family (4). Activation of these proteases promotes further recruitment of inflammatory cells and directly contributes to plaque rupture through weakening of the fibrous cap (5). Some lipid lowering interventions, especially using statins, reduce vascular events linked to plaque rupture. However, there is little direct information on the effect of these interventions on vessel wall inflammation. More specifically, it is unclear whether lipid lowering, independent of the agent used, is associated with changes in MMP activation in atherosclerosis.

Molecular imaging provides an opportunity to evaluate regional and focal changes in vessel wall biology in vivo. As MMPs mediate vessel wall inflammation in atherosclerosis, MMP-targeted imaging may be a useful tool for assessing inflammation in atherosclerosis (6-8). Indeed, there is a correlation between macrophage marker expression and MMP activation detected by molecular imaging in atherosclerosis (9, 10). Whether this relation persists in the presence of various therapeutic interventions, and hence, MMP-imaging can be used for tracking the effect of specific therapeutic interventions remains to be determined. Here, we investigate whether lipid-lowering, regardless of the agent used, leads to a reduction in plaque inflammation and MMP activation in parallel with changes in blood cholesterol level; and whether the effect of various lipid lowering interventions on plaque inflammation in atherosclerotic mice can be tracked through non-invasive imaging of MMP activation.

Materials and Methods

Reagents

Reagents were obtained from Sigma (St Louis, MI), unless otherwise specified. RP805, a ^{99m}Tc -labeled tracer with specificity for activated MMPs was provided by Lantheus Medical Imaging (North Billerica, MA) (11).

Animal model

Six to eight week old female apoE^{-/-} mice (Jackson Laboratory, Bar Harbor, ME) were fed a high fat diet (1.25% cholesterol, 15.8% fat, TD 90221, Harlan Teklad, Madison, WI) ad libitum for two months to induce atherosclerosis (n=58). After two months the animals were randomized to either continuation of high fat diet (HFD), high fat diet with simvastatin (50mg/kg/day, Sim), high fat diet with fenofibrate (30mg/kg/day, Fen) or return to normal chow (high fat withdrawal, HFW) for an additional 4 weeks (Supplemental Fig. 1). Experiments were performed according to regulations of VA Connecticut Healthcare System and Yale University Institutional Animal Care and Use Committees.

Measurement of blood cholesterol

Plasma total cholesterol concentration was determined by Yale Mouse Metabolic Phenotyping Center on blood samples collected after overnight fasting.

Imaging

MicroSPECT/CT imaging was performed as described previously with minor modifications (10, 12, 13). Briefly, one week after randomization 43.6 ($\pm$ 8.76) MBq RP805 (^{99m}Tc -labeled tracer targeting MMP activation epitope)(11) was administered through a jugular vein catheter placed under anesthesia (isoflurane 1-3%). Animals were imaged after 2 hours on a high-resolution small animal imaging system (X-SPECT, Gamma Medica-Ideas, Northridge, CA) equipped with 1-mm pinhole collimators. To verify the accuracy of image fusion 3 point sources with known activity (37 to 185 kBq) were placed in the field of view. The following acquisition parameters were used for microSPECT imaging: radius of rotation 27mm, 360°, 64 projections, 30 seconds/projection (~40 minute image acquisition), 140 keV photopeak $\pm$ 10% window, matrix:82x82. After completion of microSPECT imaging, animals were injected with a continuous infusion of iodinated CT contrast (iohexol 350 mg/mL, rate: 100 $\mu\text{L}/\text{min}$) over 2 minutes, and CT imaging was performed (energy 75 kVP/280 μA , matrix 512x512). After the first imaging at 2 months and 1 week the animals were allowed to recover and continued their corresponding diets and medication. Ibuprofen (0.1 mg/kg/day in drinking water) was used for postoperative analgesia. MicroSPECT/CT imaging was repeated after 3 weeks (2 months and 4 weeks after initiation of high fat diet). At the end of SPECT/CT imaging aorta was harvested for ex vivo planar imaging (distance: 28mm, acquisition time: 30 minutes). SPECT and CT image reconstruction and fusion was performed as described (12, 13). Multiplanar reformation of CTA and SPECT images of aorta was performed manually using Eviewbox software (<http://eviewbox.sourceforge.net>). For quantitative analysis of tracer uptake, a cylindrical (2x2x2 mm) region of interest (ROI) was drawn along the aortic arch starting at the level of right brachiocephalic artery. A ROI immediately anterior to thoracic descending aorta was used to calculate background activity. Data were expressed as background-corrected counts per voxel (cpv)/MBq injected. To quantify tracer uptake on ex vivo planar images a square (2x2 mm) ROI was drawn along the aortic arch. A ROI close to thoracic descending aorta was used to calculate background activity. Data were expressed as background- and decay-corrected counts per pixel (cpp)/MBq injected.

Oil Red O staining

Staining of explanted aortae with oil red O was performed after removal of adventitial fat as described (10). The aorta was photographed using a microscope equipped a digital camera (Leica, Heerbrugg, Switzerland). The red stained areas representing atherosclerotic lesions were measured over a 2 mm length of aorta, starting at the level of right brachiocephalic artery using NIH ImageJ software (National Institutes of Health, Bethesda, MD). The results were expressed as % total vessel area covered by atherosclerotic lesions.

Quantitative RT-PCR

Aortic arch tissue freshly frozen in OCT was used for RNA isolation and quantitative RT-PCR as described (12). The following primer sets were used: MMP-2 (Mm00439506_m1), MMP-9 (Mm00442991_m1), MMP-12 (Mm00500554_m1), MMP-13 (Mm01168713_m1), CD68 (Mm00839636_g1), EMR1 (Mm00802529_m1); smooth muscle α -actin (Mm01546133_m1), VE-cadherin (Mm00486938_m1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Mm99999915_g1). The results were expressed as GAPDH-normalized values.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (La Jolla, CA). Data are presented as mean $\pm$ standard error (SE). Differences between two groups were tested using two-tailed paired Student's *t* test. Multiple groups were compared using one way ANOVA with Dunn's multiple comparison test or two way ANOVA followed by Bonferroni post-hoc analysis. Pearson correlation was used to test the association between 2 variables. Significance was set at the 0.05 level.

Results

To investigate the effect of lipid lowering on vessel wall inflammation and its imaging in atherosclerosis, apoE^{-/-} mice were fed a high fat diet for two months to induce atherosclerotic lesions along the aorta. Next, the animals were randomized to 4 groups: continuation of the high fat diet (HFD), high fat diet plus simvastatin (Sim), high fat diet plus fenofibrate (Fen) and normal chow [high fat withdrawal (HFW) group, Supplemental Fig. 1]. High fat withdrawal or fenofibrate treatment led to a significant reduction in total blood cholesterol level relative to HFD group as early as one week after randomization [1932 $\pm$ 132 mg/dl (HFD) vs 546 $\pm$ 39 mg/dl (HFW), $p < 0.001$ or 1460 $\pm$ 184 mg/dl (Fen), $p < 0.05$, $n = 5$ in each group, Fig. 1]. Consistent with previous reports on lipid lowering effects of statins in apoE^{-/-} mice (14), there was a trend toward a reduction in total blood cholesterol for the simvastatin-treated group relative to the HFD group at one week that became statistically significant at 4 weeks [2098 $\pm$ 167 (HFD) vs 1625 $\pm$ 72 mg/dl, $p < 0.05$ (Sim), $n = 5$ in each group, $p < 0.05$, Fig. 1].

MMP imaging of the effect of lipid-lowering interventions in atherosclerosis

To address the early and late effects of various lipid-lowering interventions on MMP activation in atherosclerosis, mice underwent serial RP805 (a ^{99m}Tc-labeled tracer that specificity targets MMP activation (11)) microSPECT-CT imaging at 1 and 4 weeks after randomization. There was considerable RP805 uptake in aortic arch on vivo images in the HFD group, which significantly increased from 1 to 4 weeks (0.09 $\pm$ 0.01 vs 0.13 $\pm$ 0.01 cpv/MBq, respectively for 1 and 4 weeks, $p < 0.01$, Fig. 2 and Supplemental Fig. 2). In addition, while there was no significant difference in RP805 uptake between different groups of animals at 1 week, tracer uptake was significantly lower in fenofibrate-treated and HFW groups relative to HFD animals at 4 weeks (0.11 $\pm$ 0.01, $p > 0.05$; 0.07 $\pm$ 0.02, $p < 0.05$; and 0.06 $\pm$ 0.01 cpv/MBq, $p < 0.01$; respectively for Sim, Fen and HFW groups, Fig. 2). Ex vivo planar imaging of aortas explanted after the second in vivo imaging at 4 weeks confirmed

MMP tracer uptake in aortic arch (Fig 3a). Consistent with the data obtained on in vivo images, aortic arch tracer uptake was significantly lower in Fen and HFW groups relative to HFD animals (Fig. 3b). Importantly, in vivo quantification of RP805 uptake correlated well with its ex vivo quantification ($r=0.66$, $p<0.001$, Fig. 3c), supporting the validity of in vivo quantification. Finally, RP805 uptake correlated well with total cholesterol level at 4 weeks ($r=0.95$, $p<0.05$, Supplemental Fig. 3).

Effect of lipid lowering on plaque burden and composition

As expected, oil red O staining of explanted aortas showed considerable development of atherosclerotic lesions along aortic arch in apoE^{-/-} mice fed on the high fat diet for three months (relative plaque burden $61.4\pm3.8\%$, Fig. 4). Switch to normal chow at two month (HFW group) led to a significant reduction in aortic arch plaque burden at 3 months ($44.2\pm4.4\%$, $n=5$ in each group, $p<0.05$), while treatment with simvastatin or fenofibrate led to a non-statistically significant reduction in aortic arch plaque burden relative to HFD group [relative plaque burden $55.9\pm4.8\%$, (simvastatin) and $51.3\pm3.5\%$ (fenofibrate), $n=5$ in each group, Fig. 4].

Aortic arch gene expression analysis at 4 weeks after randomization showed a significant reduction in CD68 and EMR1 (macrophages), but not VE-cadherin (endothelial cells) and smooth muscle α -actin (vascular smooth muscle cells) mRNA expression in Fen and HFW groups relative to HFD group of animals (Fig. 5a). Similarly, in simvastatin-treated animals there was a non-statistically significant trend toward reduction in CD68 and EMR1 expression. CD68 expression correlated well with total blood cholesterol at 4 weeks ($r=0.92$, $p<0.05$, Supplemental Fig. 4). Assessment of MMP gene expression showed that MMP-2 and -9 were significantly reduced in all lipid lowering groups, whereas MMP-12 was lower in Fen and HFW groups and MMP-13 was reduced only on HFW group relative to HFD group of animals (Fig. 5b).

Correlates of MMP activation in vivo

MMP activation detected by molecular imaging has been linked to vessel wall inflammation in atherosclerosis (10). To ascertain the validity of this observation in the presence various lipid lowering interventions we investigated the correlation between RP805 uptake and cellular composition of aortic arch atherosclerotic lesions. While there was no significant correlation between RP805 uptake and VE-cadherin (endothelial cell marker) or smooth muscle α -actin (vascular smooth muscle cell marker) expression, MMP tracer uptake significantly correlated with macrophage marker, CD68 expression ($r=0.92$, $p<0.05$, Fig. 6 and Supplemental Fig. 5), establishing the effectiveness of RP805 imaging for tracking the effect of lipid-lowering interventions on vessel wall macrophage content in atherosclerosis.

Discussion

Our data demonstrate the feasibility and effectiveness of serial in vivo MMP-targeted molecular imaging for monitoring changes in atherosclerotic plaque inflammation in response to therapeutic interventions, and define the correlates of tracer uptake in the vessel wall. Lipid accumulation and inflammation are hallmarks of atherosclerosis and play a

central role in its pathogenesis. Sub-endothelial accumulation of LDL and its oxidative modification promote vessel wall inflammation through upregulation of adhesion molecules and chemokine production, and ultimately lead to immune cell recruitment (1, 3). Macrophages and possibly smooth muscle cells engulf retained lipid particles and transform into foam cells. In advanced atherosclerotic lesions foam cells and apoptotic debris compose a necrotic core. In humans, the presence of a large necrotic core and abundance of inflammatory cells in conjunction with a thin fibrous cap are key characteristics of vulnerable plaque prone to rupture. In the absence of plaque rupture in our apoE^{-/-} mouse model of atherosclerosis, discussion of plaque vulnerability in this model may be criticized. However, mouse models provide useful experimental tools for plaque characterization. Lipid lowering interventions in our study led to a reduction in vessel wall inflammation in atherosclerotic mice. This was associated with a reduction in MMP expression and activation, although considerable differences in the magnitude of the response to therapy were noted for specific members of the MMP family. Interestingly, there was a good correlation between vessel wall macrophage content (and MMP activation) in atherosclerosis and plasma cholesterol level in spite of the variety of lipid-lowering interventions employed.

Lipid-lowering is the mainstay of primary and secondary prevention in atherosclerosis. 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitors, e.g., simvastatin, reduce cardiovascular events; an effect which is believed to be to some extent related to their cholesterol-independent anti-inflammatory effects mediated in part through regulation of protein prenylation (15). Unlike their effects in humans, the effects of statins on serum lipid levels and plaque burden and composition in the mouse are inconsistent and appear to depend on experimental conditions (14, 16, 17). This may explain the differences between our findings and those of previous investigations in rabbits (9, 18). Fibrates, such as fenofibrate, regulate blood lipid levels through their agonistic effect on peroxisome proliferator receptor (PPAR) α signaling (19). Although fibrates reduce the extent of atherosclerosis (20, 21), there is considerable uncertainty about their effects on cardiovascular events (22). The effects of other lipid-lowering interventions, such as dietary intervention on cardiovascular outcome are even less clear. Little is known about the effect of any of these or emerging (1) lipid lowering interventions on vessel wall inflammation in human atherosclerosis. This is in major part due to lack of appropriate imaging tools for assessing vessel wall inflammation in vivo. Indirect anatomic measures (e.g., carotid intima media thickness (23)) have been used extensively as surrogates for predicting cardiovascular outcome in response to therapeutic interventions, and not surprisingly, their effectiveness has been found to be questionable (24, 25).

Imaging vessel wall biology is complicated by the small size of the target and its proximity to blood. Much of the work in this area has focused on fluorescent imaging. Although a powerful tool for basic investigation, optical imaging in its current form is not applicable to non-invasive imaging of the vasculature in humans. Nuclear imaging is used extensively for diagnosis and risk stratification in cardiovascular medicine. Hybrid imaging combining high sensitivity nuclear imaging in combination with high resolution anatomic imaging by CT or MRI appears to be especially suitable for imaging inflammation in atherosclerosis. ¹⁸F-fluoro deoxyglucose (FDG), an FDA-approved tracer, which is used for imaging enhanced

metabolism in cancer and inflammation has been introduced recently as a tool for detecting vessel wall inflammation in atherosclerosis (26-29). While there are uncertainties about the target(s) and regulators of ^{18}F -FDG uptake in the vessel wall (30), a number of ongoing clinical trials have incorporated ^{18}F -FDG PET imaging in their assessment of the effect of therapeutic interventions in atherosclerosis (31-33).

MMPs are produced by inflammatory (and other) cells in atherosclerotic lesions and contribute to thinning and eventual rupture of the fibrous cap, which in turn may lead to in situ thrombus and acute coronary syndrome (34-36). As such, imaging MMP activation can potentially provide information on atherosclerotic plaques' inflammation and propensity to rupture. RP805 binds with high affinity to an activation epitope of MMPs and has favorable pharmacokinetics for vascular imaging (11). Previous work by our group and others has established $^{99\text{m}}\text{Tc}$ -labeled RP805 (or its homologue ^{111}In -labeled RP782) SPECT as a promising approach for detection of protease activation and inflammation in vascular remodeling and atherosclerosis (9, 10, 12, 13, 37, 38). Signal specificity for this family of tracers was demonstrated in blocking experiments (9, 13). Here we showed that despite considerable differences in response to therapy for individual members of MMP family, the correlation between MMP tracer uptake (reflecting MMP activation in general) and vessel wall macrophage content persists following lipid-lowering interventions in atherosclerosis, further supporting the effectiveness of this approach for tracking the effect of therapeutic interventions on vessel wall biology. The relative potency of various lipid-lowering interventions on vessel wall inflammation (and MMP activation) observed in our murine model of atherosclerosis does not reflect their relative effect on outcome in humans, and may be explained by well-recognized differences in vessel wall biology and response to therapy between the two species. Nevertheless, murine models serve as valuable experimental tools for proof of principle, e.g., in this case demonstrating the potential value of molecular imaging for tracking the effect of therapeutic interventions on vessel wall biology in vivo. The observed heterogeneity of the response to treatments in drug-therapy groups highlights the value of serial molecular imaging in follow up and optimization of therapy. The underlying causes of this heterogeneity in response to therapy can be established in future studies.

The small size of mouse arteries creates a number of technical challenges for imaging and quantification of tracer uptake. The diameter of murine aorta is close to spatial resolution of microSPECT systems and partial volume effect and scatter from perivascular structures could interfere with quantification of aortic uptake. Therefore, to validate our in vivo quantification methodology, we relied on ex vivo data as "gold standard", establishing a good correlation between in vivo and ex vivo quantification of tracer uptake in this model. The larger size of arteries in humans should facilitate clinical translation of molecular imaging. While imaging of aorta and carotid arteries in humans is relatively straightforward with modern technology, cardiac motion remains a challenge for molecular imaging of coronary arteries. As such ^{18}F -FDG PET imaging of the vessel wall biology has remained limited to aorta, carotid arteries and left main and proximal coronaries (39). This limitation could be overcome with the use of gating or a global score which would reflect the composite effect of the treatment on coronary vasculature (40). While the application of the latter to ^{18}F -FDG PET images may be hindered by uptake of the tracer in normal

myocardium, the limited myocardial uptake of RP805 makes this agent particularly suitable for this analytical methodology.

New knowledge gained

MMP-targeted molecular imaging can track the effect of lipid-lowering interventions on plaque inflammation in atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding: This work was supported by National Institutes of Health R01 HL112992, R01 HL114703, R01-HL085093, and Department of Veterans Affairs Merit Award IO-BX001750.

Abbreviations

MMP	matrix metalloproteinase
HFD	high fat diet
HFW	high fat withdrawal
Fen	fenofibrate
Sim	simvastatin
ROI	region of interest

References

- (1). Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med.* 2011; 17:1410–22. [PubMed: 22064431]
- (2). Yla-Herttuala S, Bentzon JF, Daemen M, Falk E, Garcia-Garcia HM, Herrmann J, et al. Stabilisation of atherosclerotic plaques. Position paper of the European Society of Cardiology (ESC) Working Group on atherosclerosis and vascular biology. *Thromb Haemost.* 2011; 106:1–19. [PubMed: 21670845]
- (3). Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell.* 2011; 145:341–55. [PubMed: 21529710]
- (4). Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. *Febs J.* 2011; 278:28–45. [PubMed: 21087458]
- (5). Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. *Arterioscler Thromb Vasc Biol.* 2010; 30:1282–92. [PubMed: 20554950]
- (6). Nahrendorf M, Sosnovik DE, French BA, Swirski FK, Bengel F, Sadeghi MM, et al. Multimodality cardiovascular molecular imaging, Part II. *Circ Cardiovasc Imaging.* 2009; 2:56–70. [PubMed: 19808565]
- (7). Sadeghi MM, Glover DK, Lanza GM, Fayad ZA, Johnson LL. Imaging atherosclerosis and vulnerable plaque. *J Nucl Med.* 2010; 51(Suppl 1):51S–65S. [PubMed: 20395341]
- (8). Quillard T, Libby P. Molecular imaging of atherosclerosis for improving diagnostic and therapeutic development. *Circ Res.* 2012; 111:231–44. [PubMed: 22773426]

- (9). Fujimoto S, Hartung D, Ohshima S, Edwards DS, Zhou J, Yalamanchili P, et al. Molecular imaging of matrix metalloproteinase in atherosclerotic lesions: resolution with dietary modification and statin therapy. *J Am Coll Cardiol*. 2008; 52:1847–57. [PubMed: 19038682]
- (10). Razavian M, Tavakoli S, Zhang J, Nie L, Dobrucki LW, Sinusas AJ, et al. Atherosclerosis plaque heterogeneity and response to therapy detected by in vivo molecular imaging of matrix metalloproteinase activation. *J Nucl Med*. 2011; 52:1795–802. [PubMed: 21969358]
- (11). Su H, Spinale FG, Dobrucki LW, Song J, Hua J, Sweterlitsch S, et al. Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. *Circulation*. 2005; 112:3157–67. [PubMed: 16275862]
- (12). Tavakoli S, Razavian M, Zhang J, Nie L, Marfatia R, Dobrucki LW, et al. Matrix metalloproteinase activation predicts amelioration of remodeling after dietary modification in injured arteries. *Arterioscler Thromb Vasc Biol*. 2011; 31:102–9. [PubMed: 20947820]
- (13). Razavian M, Zhang J, Nie L, Tavakoli S, Razavian N, Dobrucki LW, et al. Molecular imaging of matrix metalloproteinase activation to predict murine aneurysm expansion in vivo. *J Nucl Med*. 2010; 51:1107–15. [PubMed: 20554725]
- (14). Zedelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM, et al. Mouse models for atherosclerosis and pharmaceutical modifiers. *Arterioscler Thromb Vasc Biol*. 2007; 27:1706–21. [PubMed: 17541027]
- (15). Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends in Molecular Medicine*. 2008; 14:37–44. [PubMed: 18068482]
- (16). Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, et al. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. *Arterioscler Thromb Vasc Biol*. 2001; 21:115–21. [PubMed: 11145942]
- (17). Bea F, Blessing E, Bennett B, Levitz M, Wallace EP, Rosenfeld ME. Simvastatin promotes atherosclerotic plaque stability in apoE-deficient mice independently of lipid lowering. *Arterioscler Thromb Vasc Biol*. 2002; 22:1832–7. [PubMed: 12426212]
- (18). Ohshima S, Fujimoto S, Petrov A, Nakagami H, Haider N, Zhou J, et al. Effect of an antimicrobial agent on atherosclerotic plaques: assessment of metalloproteinase activity by molecular imaging. *J Am Coll Cardiol*. 2010; 55:1240–9. [PubMed: 20298932]
- (19). Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation*. 1998; 98:2088, 93. [PubMed: 9808609]
- (20). Ericsson CG, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U. Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet*. 1996; 347:849–53. [PubMed: 8622389]
- (21). Frick MH, Syvanne M, Nieminen MS, Kauma H, Majahalme S, Virtanen V, et al. Lipid Coronary Angiography Trial (LOCAT) Study Group. Prevention of the angiographic progression of coronary and vein-graft atherosclerosis by gemfibrozil after coronary bypass surgery in men with low levels of HDL cholesterol. *Circulation*. 1997; 96:2137–43. [PubMed: 9337181]
- (22). Jun M, Foote C, Lv J, Neal B, Patel A, Nicholls SJ, et al. Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. *Lancet*. 2010; 375:1875–84. [PubMed: 20462635]
- (23). Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation*. 2007; 115:459–67. [PubMed: 17242284]
- (24). Stein EA. Additional lipid lowering trials using surrogate measurements of atherosclerosis by carotid intima-media thickness: more clarity or confusion? *J Am Coll Cardiol*. 2008; 52:2206–9. [PubMed: 19095140]
- (25). Colatsky TJ. Reassessing the validity of surrogate markers of drug efficacy in the treatment of coronary artery disease. *Curr Opin Investig Drugs*. 2009; 10:239–44.
- (26). Yun M, Yeh D, Araujo LI, Jang S, Newberg A, Alavi A. F-18 FDG uptake in the large arteries: a new observation. *Clin Nucl Med*. 2001; 26:314–9. [PubMed: 11290891]

- (27). Lederman RJ, Raylman RR, Fisher SJ, Kison PV, San H, Nabel EG, et al. Detection of atherosclerosis using a novel positron-sensitive probe and 18-fluorodeoxyglucose (FDG). *Nucl Med Commun.* 2001; 22:747–53. [PubMed: 11453046]
- (28). Tawakol A, Migrino RQ, Hoffmann U, Abbata S, Houser S, Gewirtz H, et al. Noninvasive in vivo measurement of vascular inflammation with F-18 fluorodeoxyglucose positron emission tomography. *J Nucl Cardiol.* 2005; 12:294–301. [PubMed: 15944534]
- (29). Rudd JH, Narula J, Strauss HW, Virmani R, Machac J, Klimas M, et al. Imaging atherosclerotic plaque inflammation by fluorodeoxyglucose with positron emission tomography: ready for prime time? *J Am Coll Cardiol.* 2010; 55:2527–35. [PubMed: 20513592]
- (30). Folco EJ, Sheikine Y, Rocha VZ, Christen T, Shvartz E, Sukhova GK, et al. Hypoxia but not inflammation augments glucose uptake in human macrophages implications for imaging atherosclerosis with (18)fluorine-labeled 2-deoxy-d-glucose positron emission tomography. *J Am Coll Cardiol.* 2011; 58:603–14. [PubMed: 21798423]
- (31). Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. *Lancet.* 2011; 378:1547–59. [PubMed: 21908036]
- (32). Mizoguchi M, Tahara N, Tahara A, Nitta Y, Kodama N, Oba T, et al. Pioglitazone attenuates atherosclerotic plaque inflammation in patients with impaired glucose tolerance or diabetes a prospective, randomized, comparator-controlled study using serial FDG PET/CT imaging study of carotid artery and ascending aorta. *JACC Cardiovasc Imaging.* 2011; 4:1110–8. [PubMed: 21999871]
- (33). Elkhawad M, Rudd JH, Sarov-Blat L, Cai G, Wells R, Davies LC, et al. Effects of p38 mitogen-activated protein kinase inhibition on vascular and systemic inflammation in patients with atherosclerosis. *JACC Cardiovasc Imaging.* 2012; 5:911–22. [PubMed: 22974804]
- (34). Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res.* 2002; 90:251–62. [PubMed: 11861412]
- (35). Dollery CM, Libby P. Atherosclerosis and proteinase activation. *Cardiovasc Res.* 2006; 69:625–35. [PubMed: 16376322]
- (36). Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques. *Trends Cardiovasc Med.* 2007; 17:253–8. [PubMed: 18021934]
- (37). Zhang J, Nie L, Razavian M, Ahmed M, Dobrucki LW, Asadi A, et al. Molecular imaging of activated matrix metalloproteinases in vascular remodeling. *Circulation.* 2008; 118:1953–60. [PubMed: 18936327]
- (38). Ohshima S, Petrov A, Fujimoto S, Zhou J, Azure M, Edwards DS, et al. Molecular imaging of matrix metalloproteinase expression in atherosclerotic plaques of mice deficient in apolipoprotein e or low-density-lipoprotein receptor. *J Nucl Med.* 2009; 50:612–7. [PubMed: 19289429]
- (39). Rogers IS, Nasir K, Figueroa AL, Cury RC, Hoffmann U, Vermylen DA, et al. Feasibility of FDG imaging of the coronary arteries: comparison between acute coronary syndrome and stable angina. *JACC Cardiovasc Imaging.* 2010; 3:388–97. [PubMed: 20394901]
- (40). Beheshti M, Saboury B, Mehta NN, Torigian DA, Werner T, Mohler E, et al. Detection and global quantification of cardiovascular molecular calcification by fluoro18-fluoride positron emission tomography/computed tomography--a novel concept. *Hell J Nucl Med.* 2011; 14:114–20. [PubMed: 21761011]

Fig. 1.

Total blood cholesterol measured in high fat fed atherosclerotic mice at 1 and 4 weeks after randomization to different intervention groups. HFD: high fat diet, Sim: high fat diet + simvastatin, Fen: high fat diet + fenofibrate, HFW: high fat withdrawal, n=5 in each group.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (two way ANOVA).

Fig. 2.

In vivo RP805 microSPECT/CT imaging of aortic MMP activation in atherosclerotic mice.

a) Examples of in vivo CT angiography, RP805 microSPECT and fused images of atherosclerotic mice at 4 weeks after randomization. Arrows point to aorta. b) Examples of multiplanar reformation of aortic tracer uptake (right image for each group) and CT angiography (left image for each group) at 4 weeks after randomization to different

intervention groups. c) Quantification of aortic arch tracer uptake on in vivo images at 1 and 4 weeks after randomization, *: $p < 0.05$, **: $p < 0.01$ (two way ANOVA). $n = 5-6$ in each group. HFD: high fat diet, Sim: high fat diet + simvastatin, Fen: high fat diet + fenofibrate, HFW: high fat withdrawal, cpv: counts per voxel.

Fig. 3.

Ex vivo RP805 planar imaging of aortic MMP activation in atherosclerotic mice. Examples (a) and quantification (b) of aortic arch tracer uptake on planar images of explanted aortas at 4 weeks after randomization to different intervention groups. c) Correlation between in vivo and ex vivo quantification of aortic arch tracer uptake. *: $p < 0.05$, **: $p < 0.01$ (one way ANOVA). $n=5-6$ in each group. HFD: high fat diet, Sim: high fat diet + simvastatin, Fen: high fat diet + fenofibrate, HFW: high fat withdrawal, cpp: counts per pixel, cpv: counts per voxel.

Fig. 4.

Aortic arch atherosclerotic plaque burden in high fat fed atherosclerotic mice 4 weeks after randomization to different intervention groups (3 months). a) Examples of Oil Red O staining of atherosclerotic lesions in explanted aortas. b) Quantification of the area covered by atherosclerosis in aortic arch. *: $p < 0.05$ (one way ANOVA). $n = 5$ in each group. HFD: high fat diet, Sim: high fat diet + simvastatin, Fen: high fat diet + fenofibrate, HFW: high fat withdrawal.

Fig. 5.

Real time PCR analysis of aortic arch composition. GAPDH-normalized cell marker (a) and MMP (b) expression at 4 weeks after randomization to different intervention groups. *: $p < 0.05$, **: $p < 0.01$ (one way ANOVA). $n = 5$ in each group. HFD: high fat diet, Sim: high fat diet + simvastatin, Fen: high fat diet + fenofibrate, HFW: high fat withdrawal.

Fig. 6. Correlation between MMP tracer uptake on in vivo microSPECT/CT images and GAPDH-normalized macrophage marker (CD68) expression in aortic arch at 4 weeks after randomization to different intervention groups. cpv: counts per voxel.