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Effects of atorvastatin and diet interventions on atherosclerotic plaque inflammation and [ $^{18}\text{F}$ ]FDG uptake in *Ldlr*<sup>-/-</sup> *Apob*<sup>100/100</sup> mice

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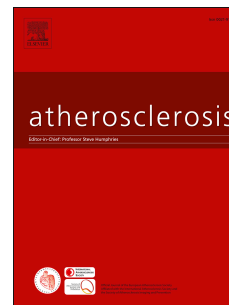
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**Effects of atorvastatin and diet interventions on atherosclerotic plaque inflammation and [<sup>18</sup>F]FDG uptake in *Ldlr*<sup>-/-</sup> *Apob*<sup>100/100</sup> mice**

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Abbreviations: [<sup>18</sup>F]FDG, 2-deoxy-2-[<sup>18</sup>F]-fluoro-*D*-glucose; ARG, autoradiography; HFD+A, high-fat diet with added atorvastatin; *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mouse model deficient in low-density lipoprotein receptor expressing only apolipoprotein B-100; LRP, low-density lipoprotein receptor-associated protein; MCP-1, Monocyte chemoattractant protein-1; PSL/mm<sup>2</sup>, photo-stimulated luminescence per square millimeter; SUV, standardized uptake value; TBR; target-to-background ratio

**Abstract**

**Background and aims:** Uptake of the positron emission tomography (PET) tracer 2-deoxy-2- $^{18}\text{F}$ -fluoro-D-glucose ( $^{18}\text{F}$ FDG) into macrophages is a sensitive marker of inflammation in atherosclerosis. To assess the anti-inflammatory effects of statins, we studied whether atorvastatin therapy reduces aortic  $^{18}\text{F}$ FDG uptake in hypercholesterolemic mice deficient in low-density lipoprotein receptor (Ldlr), and expressing only apolipoprotein B-100 (*Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup>).

**Methods:** Thirty-six *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mice were fed a high-fat diet (HFD) for 12 weeks and then allocated to receive a HFD (n=13), chow diet (Chow, n=12), or HFD with added atorvastatin (HFD+A, n=11), for another 12 weeks. In addition to aortic histopathology,  $^{18}\text{F}$ FDG uptake was studied *in vivo* using PET/computed tomography (CT), and *ex vivo* by gamma counting of excised aorta.

**Results:** Total cholesterol levels were lower in the Chow and HFD+A groups than in the HFD group (10±3.2, 23±4.9 and 34±9.2 mmol/l, respectively), with the Chow group also showing a lower plaque burden and lower numbers of macrophages in the lesions. Compared to the HFD group,  $^{18}\text{F}$ FDG uptake in the aorta (normalized for blood) was lower in the Chow group in both *in vivo* (2.1±0.21 vs. 1.7±0.25, *p*=0.018) and *ex vivo* (5.2±2.3 vs. 2.8±0.87, *p*=0.011) analyses, whereas atorvastatin had no effect on uptake (2.1±0.42 *in vivo* and 3.9±1.8 *ex vivo*).  $^{18}\text{F}$ FDG uptake correlated with plasma total cholesterol levels.

**Conclusions:** Atorvastatin therapy did not show cholesterol-independent effects on inflammation in atherosclerotic lesions in *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mice, as determined by histology and  $^{18}\text{F}$ FDG PET, whereas a cholesterol-lowering diet intervention was effective.

## Introduction

Inflammation plays a central role in the development of atherosclerosis and its complications, with macrophages being the most prominent inflammatory cells in atherosclerotic plaques [1]. Statin therapy can inhibit the progression of atherosclerotic plaques [2] and reduce the cardiovascular risk [3]. The main mechanism of action of statins is to reduce the blood cholesterol concentration by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, an enzyme that catalyzes endogenous cholesterol synthesis in the liver. However, statins have biological actions that are independent of cholesterol lowering, referred to as pleiotropic effects [4]. The reduction of cardiovascular risk is greater than expected on the basis of cholesterol reduction alone [5], and has been evident even before cholesterol lowering [6,7]. This is partly attributed to plaque-stabilizing effects related to the inhibition of the inflammatory response [8–10]. However, the contributions of pleiotropic effects of statins on cardiovascular risk reduction in individuals with elevated systemic inflammatory markers is very difficult to study in clinical trials, because cholesterol lowering itself is strongly atheroprotective [11,12].

Positron emission tomography/computed tomography (PET/CT) is a translational imaging method utilized in clinical studies and animal research. PET/CT imaging using 2-deoxy-2- $^{18}\text{F}$ -fluoro-*D*-glucose ( $^{18}\text{F}$ FDG) has been used as a tool to assess the effects of statins on atherosclerosis-associated vascular inflammation [13,14].  $^{18}\text{F}$ FDG is taken up by cells utilizing glucose, including active macrophages, which makes it a sensitive *in vivo* marker of atherosclerotic plaque inflammation [15]. A 4 week high-dose treatment with atorvastatin was shown to reduce  $^{18}\text{F}$ FDG uptake in the carotid arteries and aorta of patients at high risk of vascular inflammation, although the change in arterial  $^{18}\text{F}$ FDG uptake was not related to a change in blood low-density lipoprotein (LDL) level [13].

The responsiveness of LDL receptor-deficient (*Ldlr*<sup>-/-</sup>) mice to statin therapy is limited, as the cellular intake of cholesterol cannot be increased via the LDL receptor [16–18]. *Ldlr*<sup>-/-</sup> mice expressing only apolipoprotein B-100 (*Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup>) are expected to be even less responsive to statin-induced cholesterol lowering

than *Ldlr*<sup>-/-</sup> mice, since the lipoprotein clearance via LDL-related proteins (LRPs) is also impaired [19,20].

These mice have a cholesterol profile resembling human familial hypercholesterolemia, and they develop extensive atherosclerosis in the aorta [19,21,22]. Therefore, to further study the possible cholesterol-independent effects of statin therapy on vascular inflammation in atherosclerosis, we used histology and [<sup>18</sup>F]FDG PET/CT imaging to compare the effects of diet and atorvastatin interventions on inflammation in atherosclerotic plaques in *Ldlr*<sup>-/-</sup> *Apob*<sup>100/100</sup> mice. In this study, after 12 weeks on a high-fat diet (HFD) to induce atherosclerosis, mice were allocated to either continue on the HFD, or switched to a chow diet (Chow) or a HFD combined with atorvastatin (HFD+A) for an additional 12 weeks. At the end of the intervention, [<sup>18</sup>F]FDG PET/CT was performed and the aortas were excised for gamma counting, histology of plaque burden and macrophage content, and evaluation of [<sup>18</sup>F]FDG uptake into lesions and lesion-free vessel walls by autoradiography (ARG). Total cholesterol, triglycerides, and biomarkers of inflammation and metabolism were measured from plasma.

### Animals and study design

The study protocol was approved by the National Animal Experiment Board in Finland and the Regional State Administrative Agency for Southern Finland. Studies were carried out according to the respective European Union directives on animal experimentation. The mice were bred and housed in standard conditions and a 12/12 hour light/dark cycle in the Laboratory Animal Unit of the University of Turku, with access to food and water *ad libitum*.

*Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mice (The Jackson Laboratory, Bar Harbor, ME, USA; n=41, both genders) were first fed a chow diet (9.1% of calories from fat, CRM [E], 801730, Special Diet Services, Essex, UK) for 8 weeks, and then a HFD (42% of calories from fat, 0.2% total cholesterol, TD 88137, Teklad, Harlan Laboratories, Madison, WI, USA) for 12 weeks. At the age of 20 weeks, a femoral vein blood sample was taken from each mouse. Thereafter, the mice were divided into the following interventional groups for an additional 12 weeks: 1) continuous HFD (HFD, n=18), 2) dietary intervention with chow diet (Chow, n=12), and 3) HFD combined with atorvastatin (HFD+A, n=12). The HFD+A diet contained 0.1 mg of atorvastatin per gram of food (Teklad Custom Research Diet, Harlan Laboratories, Madison, WI, USA). Food consumption and the weights of the mice were monitored weekly. For individual mice, the study was terminated if the mouse's well-being was compromised, judged on the basis of pre-defined criteria (weight reduction >15% or development of xanthomas affecting >10% of the skin area).

### [<sup>18</sup>F]FDG PET/CT

After the interventional period, all mice in the Chow and HFD+A groups, and eight mice in the HFD group, underwent *in vivo* [<sup>18</sup>F]FDG PET/CT imaging using an Inveon multimodality small animal PET/CT scanner (Siemens, Knoxville, TN, USA), as described in a previous study [23]. The mice were fasted for 4 hours, anesthetized with isoflurane (2–2.5%), and kept on a heating pad during the imaging. Glucose levels were measured with a glucometer (Contour, Bayer AG, Leverkusen, Germany). Approximately 10 MBq of [<sup>18</sup>F]FDG was injected via a tail vein catheter, and attenuation-corrected PET imaging was performed for a duration of 20 minutes, at 50 minutes post-injection. After the PET data were acquired, 100–150 µl of intravenous contrast agent (eXia 160XL, Binitio Biomedical, Ottawa, Canada) was injected, and high-

resolution CT was performed to visualize the blood vessels. The image reconstruction parameters were similar to those previously described [24]. The PET/CT image analysis was performed using Carimas 2.9 software (Turku PET Centre, Turku, Finland). Uptake was evaluated by defining regions of interest (ROIs) in the aorta, blood, myocardium, and muscle, using the contrast-enhanced CT images. The aortic ROI was placed in the arch, avoiding myocardial spillover. Blood radioactivity concentration was analyzed from the *vena cava*. Radioactivity concentrations were measured as mean standardized uptake values (SUV): (Tissue radioactivity/tissue volume)/(total injected radioactivity/mouse weight). Uptake in the aorta was normalized by calculating the target-to-background ratio (TBR) by dividing the aortic arch SUV by the blood SUV.

#### Ex vivo measurements and ARG

After imaging, the mice were sacrificed at 100-min post-injection by cardiac puncture and cervical dislocation. For the mice not imaged *in vivo*, [<sup>18</sup>F]FDG studies were performed *ex vivo* at the same time point. Selected tissues were excised, weighed, and measured for radioactivity with a gamma counter (Triathler 425-010, Hidex, Turku, Finland). The thoracic aorta was excised, cleaned, and rinsed with saline. Radioactivity concentrations in organs and blood were calculated as SUV: (tissue radioactivity/tissue weight)/(total injected radioactivity/mouse weight). The aortic TBR was defined as described above.

Digital ARG for longitudinal aortic cryosections was performed following methods described in a previous study [23]. The cryosections were placed under radiation-sensitive imaging plate for 4 hours and the plate was then scanned with a phosphoimager (Fuji BAS-5000 or FLA-5100, Fuji Photo Film Co., Ltd., Tokyo, Japan) to obtain digital ARG images. The ARG analysis was performed with Tina 2.1 software (Raytest isotopemessgeräte, GmbH, Straubenhardt, Germany). The ROIs in ARG images were defined in plaques (at



average 26 per mouse) and lesion-free vessel walls (at average 12 per mouse) on the basis of histology. Count densities (photo-stimulated luminescence per square millimeter, PSL/mm<sup>2</sup>) normalized for the injected radioactivity dose and the decay in radioactivity during the exposure, were then performed. The plaque-to-wall ratio was calculated for each animal by dividing the mean PSL/mm<sup>2</sup> values in the plaque by the mean PSL/mm<sup>2</sup> in the lesion-free vessel wall. The longitudinal 20 µm aortic sections utilized for the ARG were stored at -70°C and later stained with hematoxylin and eosin (H&E) for basic histology.

#### Assessment of plasma lipids, circulating biomarkers, plaque burden, and inflammation

Levels of total cholesterol and triglycerides in the circulation were measured in both baseline and end-of-study plasma samples [24]. Two Luminex kits (MILLIPLEX MAP Mouse Cytokine/Chemokine and Mouse Metabolic Hormone Magnetic Bead Panels, Merck Millipore, Billerica, MA, USA) were utilized to assess the inflammatory cytokine interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), as well as the metabolic markers insulin and leptin. The assays were performed on the end-of-study samples according to the manufacturer's instructions.

Paraffin-embedded aortic roots were cut to serial 5 µm sections at the level of the coronary ostia and stained with Movat's pentachrome, or with anti-Mac-3 antibody for macrophages [24]. The total area of intima and the Mac-3-positive area were measured using ImageJ software (NIH, Bethesda, MD, USA).

The levels of total cholesterol, plaque burden, and macrophages were compared with the measured aortic [<sup>18</sup>F]FDG uptake values.

#### Statistical analyses

The results are expressed as mean±SD. Analyses were performed with IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). One-way analysis of variance was used for the comparisons between interventional groups, followed by a Tukey or Tamhane *post-hoc* test in the respective cases of equal or unequal

variances, according to Levene's test. Pearson's correlation coefficient was calculated to assess correlations. Statistical significance was regarded as a  $p$ -value  $<0.05$ . The sample size was based on the assumption that average [ $^{18}\text{F}$ ]FDG uptake in atherosclerotic plaques (plaque-to-wall ratio in autoradiography) is  $1.95 \pm 0.33$  [25]. Then, 11 mice per group would be needed to detect 20 % difference in the uptake at 80 % probability with the alpha value of 0.05.

## Results

At the baseline age of 20 weeks, all the mouse groups showed similar characteristics (Table 1). The food consumption (g/mouse/day) was on average  $2.6\pm 0.39$  in the HFD group,  $3.1\pm 0.36$  in the Chow group, and  $2.3\pm 0.51$  in the HFD+A group, with the mean energy intake being  $12\pm 1.8$ ,  $11\pm 1.3$ , and  $10\pm 2.3$  kcal/mouse/day, respectively (the differences between groups were not significant). In the HFD+A group, the average intake of atorvastatin was  $8.2\pm 1.4$  mg/kg per day. Five mice in the HFD group and one mouse in the HFD+A group were terminated before the end of the study and excluded from the dataset, according to the pre-defined criteria described in the methods section. The final group sizes were 13, 12, and 11 for the HFD, Chow, and HFD+A groups, respectively. As the results contained no indications of gender differences, the results from male and female mice were pooled.

### Plasma lipids and biomarkers

Compared with baseline levels, plasma total cholesterol was lowered by 71% (from 35 to 10 mmol/l) in the Chow group and 39% (from 37 to 23 mmol/l) in the HFD+A group, whereas cholesterol levels in the HFD group were sustained (from 35 to 34 mmol/l; Table 1, Supplemental Fig. 1). At the end of the study, the differences in total cholesterol levels were significant between all groups ( $p<0.001$ ). Leptin levels were lower in the Chow group than in the HFD group. No significant differences between groups were observed in the levels of triglycerides, IL-6, MCP-1, or insulin.

### Plaque burden and inflammation

Histology was evaluated in the aortic roots (Fig. 1). The area of intima was 34% less ( $0.76\pm 0.27$  vs.  $1.1\pm 0.39$  mm<sup>2</sup>,  $p=0.031$ ) and the Mac-3-positive area was 61% less ( $0.049\pm 0.029$  vs.  $0.13\pm 0.039$  mm<sup>2</sup>,  $p<0.001$ ) in the Chow group compared with the HFD group. The HFD+A group did not significantly differ from the HFD group in either intimal area ( $0.96\pm 0.11$  mm<sup>2</sup>) or Mac-3-positive area ( $0.11\pm 0.039$  mm<sup>2</sup>).

*In vivo* PET/CT imaging showed visible [<sup>18</sup>F]FDG uptake in the aortic arch and brachiocephalic artery (Fig. 2). TBR in the aortas of the HFD group was higher than in the aortas of the Chow group ( $2.1 \pm 0.21$  vs.  $1.7 \pm 0.25$ ,  $p=0.018$ ; Fig. 2D, Supplemental Table 1), while TBR in the HFD+A group ( $2.1 \pm 0.42$ ) did not differ significantly from that in either the HFD or Chow group.

*Ex vivo* radioactivity measurements showed a 46% lower [<sup>18</sup>F]FDG uptake in the thoracic aorta in the Chow group compared with the HFD group (TBR:  $2.8 \pm 0.87$  vs.  $5.2 \pm 2.3$ ,  $p=0.011$ ; Fig. 2E, Supplemental Table 2). Uptake in the HFD+A group (TBR:  $3.9 \pm 1.8$ ) was between the other groups, but was not significantly different to either of them.

The ARG (Fig. 3) showed lower count densities in the Chow group than in the HFD group in both the plaque ( $100 \pm 70$  vs.  $210 \pm 93$  PSL/mm<sup>2</sup>,  $p=0.013$ ; Fig. 3D) and normal vessel wall ( $67 \pm 46$  vs.  $150 \pm 76$  PSL/mm<sup>2</sup>,  $p=0.027$ ). Count densities in the HFD+A group ( $160 \pm 88$  and  $120 \pm 81$  PSL/mm<sup>2</sup> in the plaque and wall, respectively) were not significantly different to those in the HFD or Chow groups. The plaque-to-wall ratio of [<sup>18</sup>F]FDG uptake showed no significant differences between the groups ( $1.6 \pm 0.28$ ,  $1.6 \pm 0.22$ , and  $1.4 \pm 0.31$  in the HFD, Chow, and HFD+A groups, respectively).

As shown in Fig. 4, aortic [<sup>18</sup>F]FDG uptake measured by *in vivo* PET/CT correlated with total cholesterol level. Aortic [<sup>18</sup>F]FDG uptake measured *ex vivo*, and plaque uptake measured by ARG, correlated with both plaque burden and the quantity of macrophages, in addition to the plasma total cholesterol level. The correlations between the different methods of measuring [<sup>18</sup>F]FDG uptake are shown in the on-line supplement.

The main finding of the study was that oral atorvastatin intervention had no significant effect on inflammation in the atherosclerotic *Ldlr*<sup>-/-</sup>*ApoB*<sup>100/100</sup> mouse aorta, as determined by macrophage content or [<sup>18</sup>F]FDG uptake. By contrast, the chow diet intervention was associated with a markedly lower cholesterol level and reduced plaque burden and inflammation. Furthermore, the degree of arterial inflammation correlated with the level of cholesterol. Thus, in this mouse model of atherosclerosis, [<sup>18</sup>F]FDG did not demonstrate cholesterol-independent effects of atorvastatin on metabolic activity in atherosclerotic lesions.

In addition to the differences in vascular inflammation, there was also a difference in the degree of cholesterol lowering between the dietary and atorvastatin intervention. Marked reductions in cholesterol levels and reduced vascular inflammation on switching to a chow diet are consistent with a previous study in *Ldlr*<sup>-/-</sup>*ApoB*<sup>100/100</sup> mice [26]. Although there are no previous reports on the effects of statins in *Ldlr*<sup>-/-</sup>*ApoB*<sup>100/100</sup> mice, the relatively small effect on cholesterol level was expected, since *Ldlr*<sup>-/-</sup> mice have limited responsiveness to statins [16–18], and lipoprotein clearance in *Ldlr*<sup>-/-</sup>*ApoB*<sup>100/100</sup> mice is even further impaired. The effect of statin on inhibition of cholesterol biosynthesis is, however, still functional. The statin-induced cholesterol reductions seen in *Ldlr*<sup>-/-</sup> mice may be explained by clearance mediated by Ldlr-related proteins (LRPs), other receptors and receptor-independent uptake into hepatocytes, following the inhibition of cholesterol biosynthesis [19,20,27]. Definition of the exact mechanisms underlying cholesterol lowering is beyond the scope of this study.

Statin treatment in *Ldlr*<sup>-/-</sup> mice has generally led to reduced atherosclerotic lesion size, regardless of the effects on plasma cholesterol levels [16,17,28,29]. Reduction of the inflammatory process occurring in neointimal thickening in a vascular injury model has been observed with simvastatin treatment [17], but the effects of statins on atherosclerotic inflammation induced by HFD feeding in *Ldlr*<sup>-/-</sup> mice are not well known. Our results are somewhat contradictory to the previous studies, as atorvastatin treatment showed no effect on plaque burden or macrophage content. This might be related to the duration and timing of the interventions, as large inflamed atherosclerotic lesions were already present in the *Ldlr*<sup>-/-</sup>*ApoB*<sup>100/100</sup> mice at baseline [23]. The dose of atorvastatin was selected to roughly correspond to the high-dose therapy in

humans, while taking into account the high baseline cholesterol level in the mice [16]. The choice of statin

may also have a role, as the pharmacokinetic properties of different statins, such as lipophilicity, vary [4].

The present study extends earlier findings, in that aortic [ $^{18}\text{F}$ ]FDG uptake in *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mice was not significantly lowered by high-dose atorvastatin indicating that orally administered atorvastatin has limited direct effects on metabolic activity in atherosclerotic lesions in the absence of cholesterol lowering. [ $^{18}\text{F}$ ]FDG PET is a sensitive marker of metabolic activity in atherosclerotic lesions; this is primarily attributed to inflammation [13,15]. Statins have been shown to exert many anti-inflammatory cellular effects [4,9] and to reduce [ $^{18}\text{F}$ ]FDG uptake in humans [13]. Although it is likely that there are pro-inflammatory pathways in macrophages that are not directly reflected by [ $^{18}\text{F}$ ]FDG uptake [9], it did correlate with plasma cholesterol levels and with the quantity of macrophages in plaques in our study. In line with our results, prolonged atorvastatin therapy in apolipoprotein E-deficient mice with a mutation in the fibrillin-1 gene had no effect on plaque macrophage content despite reduced systemic inflammatory markers [10]. However, in that model statin reduced other features of plaque vulnerability, such as fibrous cap thickness, metalloproteinase activity and pro-inflammatory cytokine expression in the absence of cholesterol-lowering [10]. Thus, more studies are needed to understand the significance of various potential cholesterol-independent anti-inflammatory and plaque-stabilizing effects of statins.

The small size of atherosclerotic lesions in mice makes their PET imaging challenging. Compared with *ex vivo* quantification, *in vivo* measurements underestimated [ $^{18}\text{F}$ ]FDG uptake; this was probably due to partial volume effects. The lack of baseline [ $^{18}\text{F}$ ]FDG imaging was a limitation, as it would have enabled the assessment of the treatment effect on vascular inflammation in each individual animal. The high-resolution

CT protocol was, however, considered too risky for the animals due to large amount of contrast agent and

high radiation exposure. Due to prolonged duration of HFD, there was extensive atherosclerosis throughout the aorta, and finding lesion-free vessel wall areas was challenging. Thus, both total [ $^{18}\text{F}$ ]FDG uptake in plaques and relative uptake (plaque-to-wall ratio) were measured. Limited sample size precludes detection of small differences between treatments that might still be significant in long-term disease progression. Due to limited responsiveness of *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mice to statins, similar studies in other animal models are warranted to obtain further information on the effects of statins on vascular inflammation.

Oral atorvastatin intervention showed no significant effect on inflammation in the atherosclerotic *Ldlr*<sup>-/-</sup>*Apob*<sup>100/100</sup> mouse aorta as determined by lesion size, macrophage content, or [ $^{18}\text{F}$ ]FDG uptake.

#### **Conflict of interest**

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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**Author contributions**

S.H. contributed to the study design, acquired, analyzed and interpreted data, and drafted the manuscript. S.S., H.L., J.V., J.M.U.S., M.S., N.S. and J.M. acquired, analyzed and interpreted data, and participated in manuscript drafting. M.J., P.S., S.Y-H., P.N., J.K., A.R. and A.S. contributed to the study conception and design, interpreted data, and contributed to the manuscript writing enhancing its intellectual content. All authors read and approved the final manuscript.

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**Fig. legends****Fig. 1.** Histology of aortic roots.

(A) Representative Movat-stained plaques in the aortic root. Lipids are stained green, muscle red, collagen yellow, and cell nuclei black. Scale bar: 100  $\mu\text{m}$ . (B) Boxplots showing mean and quartiles, and whiskers representing the minimum and maximum. The total intimal area is smaller in the Chow than HFD group. NS, not significant. (C) Corresponding Mac-3-staining (brown color) of macrophages in plaques. (D) The Mac-3-positive area in the intima is significantly lower in the Chow than HFD group.

**Fig. 2.** *In vivo* [ $^{18}\text{F}$ ]FDG PET/CT imaging and *ex vivo* measurements.

(A) Left panel: contrast-enhanced computed tomography (CT) of mouse thorax shows the heart (LV, left ventricle), aortic arch (AA), and brachiocephalic trunk (Br). Scale bar: 1 cm. Middle panel: [ $^{18}\text{F}$ ]FDG positron emission tomography (PET) and CT superimposed. Right panel: PET images show high [ $^{18}\text{F}$ ]FDG uptake in the myocardium. Uptake is also seen in the aortic arch (arrow) and brachiocephalic trunk (arrowhead) in high-fat diet (HFD) and high-fat diet plus atorvastatin (HFD+A) groups, but in Chow group the uptake is lower. (B) Quantitative results of *in vivo* [ $^{18}\text{F}$ ]FDG PET/CT analyses show lower uptake in the Chow group than in the HFD group. TBR, target-to-background ratio (tracer uptake in aorta/uptake in blood). Boxplots show the mean and quartiles with whiskers from minimum to maximum. (C) Quantitative results of [ $^{18}\text{F}$ ]FDG *ex vivo* radioactivity measurements are in line with the *in vivo* data.

**Fig. 3.** Autoradiography of aortic tissue sections.

(A) Left panel: hematoxylin-eosin-stained sections of the aortic arch showing vessels with large atherosclerotic plaques (arrows). HFD, high-fat diet; HFD+A, high-fat diet plus atorvastatin; L, lumen; B, brachiocephalic artery; LC, left common carotid artery; LS, left subclavian artery. Scale bar: 1 mm. Middle panel: autoradiography shows high [ $^{18}\text{F}$ ]FDG uptake in some of the plaques. Right panel: Mac-3-stained

adjacent sections show the presence of macrophages (brown color) in the plaques. (B) Quantitative results

of autoradiography. Boxplots show the mean and quartiles with whiskers from minimum to maximum.

**Fig. 4.** Correlation of aortic [ $^{18}\text{F}$ ]FDG uptake with plasma cholesterol, plaque burden, and intimal macrophages.

Red markers, HFD group; green, Chow group; and blue, HFD+A group. Aortic [ $^{18}\text{F}$ ]FDG uptake *in vivo* was measured from PET/CT images as target-to-background ratio (TBR, uptake in aortic arch/uptake in venous blood) and *ex vivo* samples by tissue gamma counting (TBR, uptake in thoracic aorta/uptake in blood). [ $^{18}\text{F}$ ]FDG uptake in aortic plaques was measured by autoradiography as count density (photo-stimulated luminescence per square millimeter, PSL/mm<sup>2</sup>). Plaque burden (total intimal area) and quantity of macrophages (intimal Mac-3-positive staining) were determined from aortic root sections.

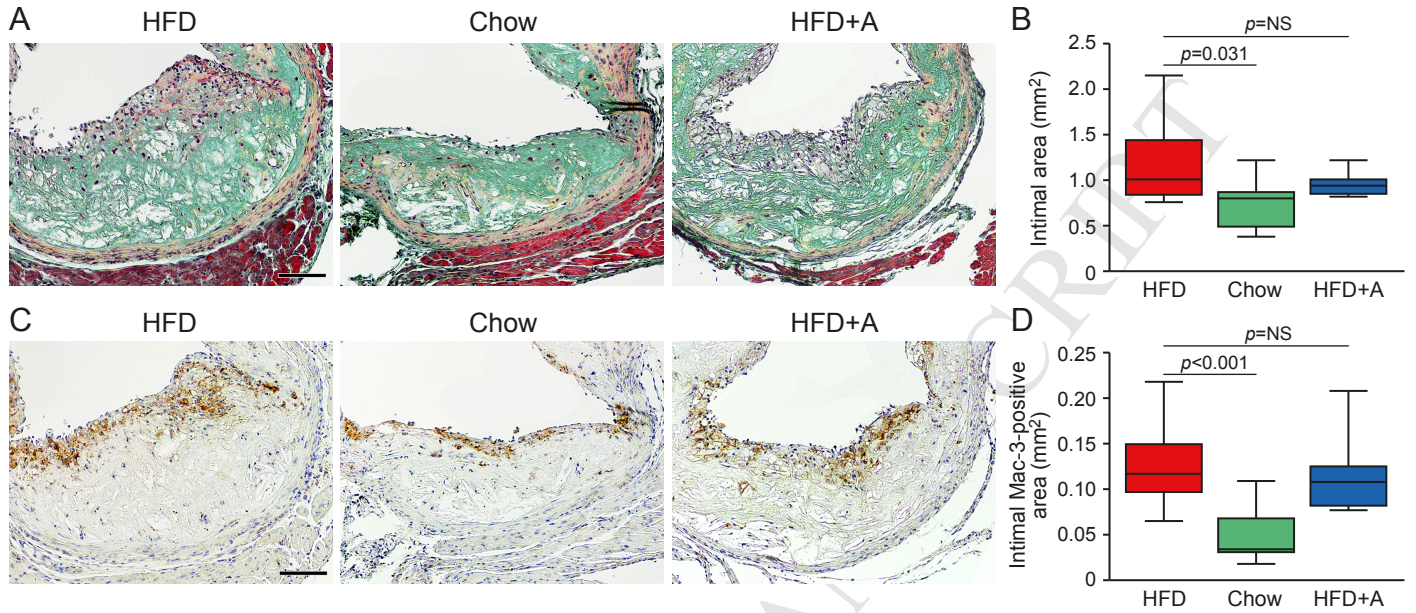
**Table****Table 1.** Characteristics of study animals.

HFD	n (male/female)	Chow	HFD+A	<i>p</i> -value	<i>p</i> -value	
				HFD vs. Chow	HFD vs. HFD+A	
	13 (6/7)	12 (5/7)	11 (4/7)			
Baseline	Weight (g)	31 ± 7.7	27 ± 6.1	26 ± 5.7	NS	NS
	Fasting glucose (mmol/L)	9.5 ± 1.7	8.8 ± 1.2	9.2 ± 1.4	NS	NS
	Total cholesterol (mmol/L)	35 ± 4.3	35 ± 14	37 ± 9.5	NS	NS
	Triglycerides (mmol/L)	3.0 ± 0.49	2.6 ± 1.3	2.3 ± 1.0	NS	NS
End of study	Weight (g)	33 ± 8.8	28 ± 5.3	28 ± 5.7	NS	NS
	Fasting glucose (mmol/L)	9.4 ± 1.9	11 ± 2.5	10 ± 2.4	NS	NS
	Total cholesterol (mmol/L)	34 ± 9.2	10 ± 3.2	23 ± 4.9 <sup>a</sup>	<0.001	<0.001
	Triglycerides (mmol/L)	3.6 ± 1.4	2.8 ± 0.33	3.0 ± 0.59	NS	NS
	IL-6 (pg/ml)	60 ± 110	34 ± 40	81 ± 140	NS	NS
	MCP-1 (pg/ml)	100 ± 100	35 ± 26	56 ± 58	NS	NS
	Insulin (ng/ml)	2.1 ± 1.6	2.6 ± 2.4	2.8 ± 2.1	NS	NS
	Leptin (ng/ml)	7.3 ± 5.9	2.1 ± 1.9	4.0 ± 3.4	0.037	NS

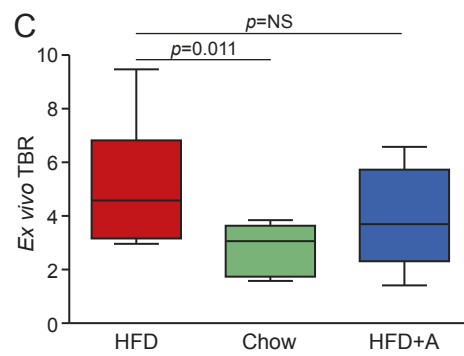
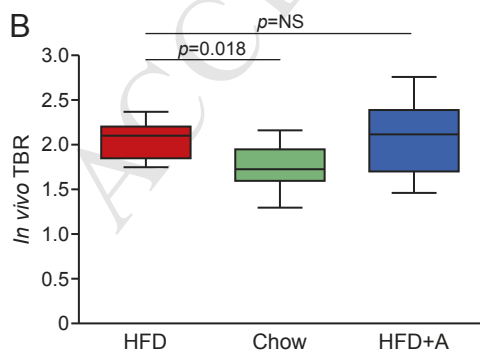
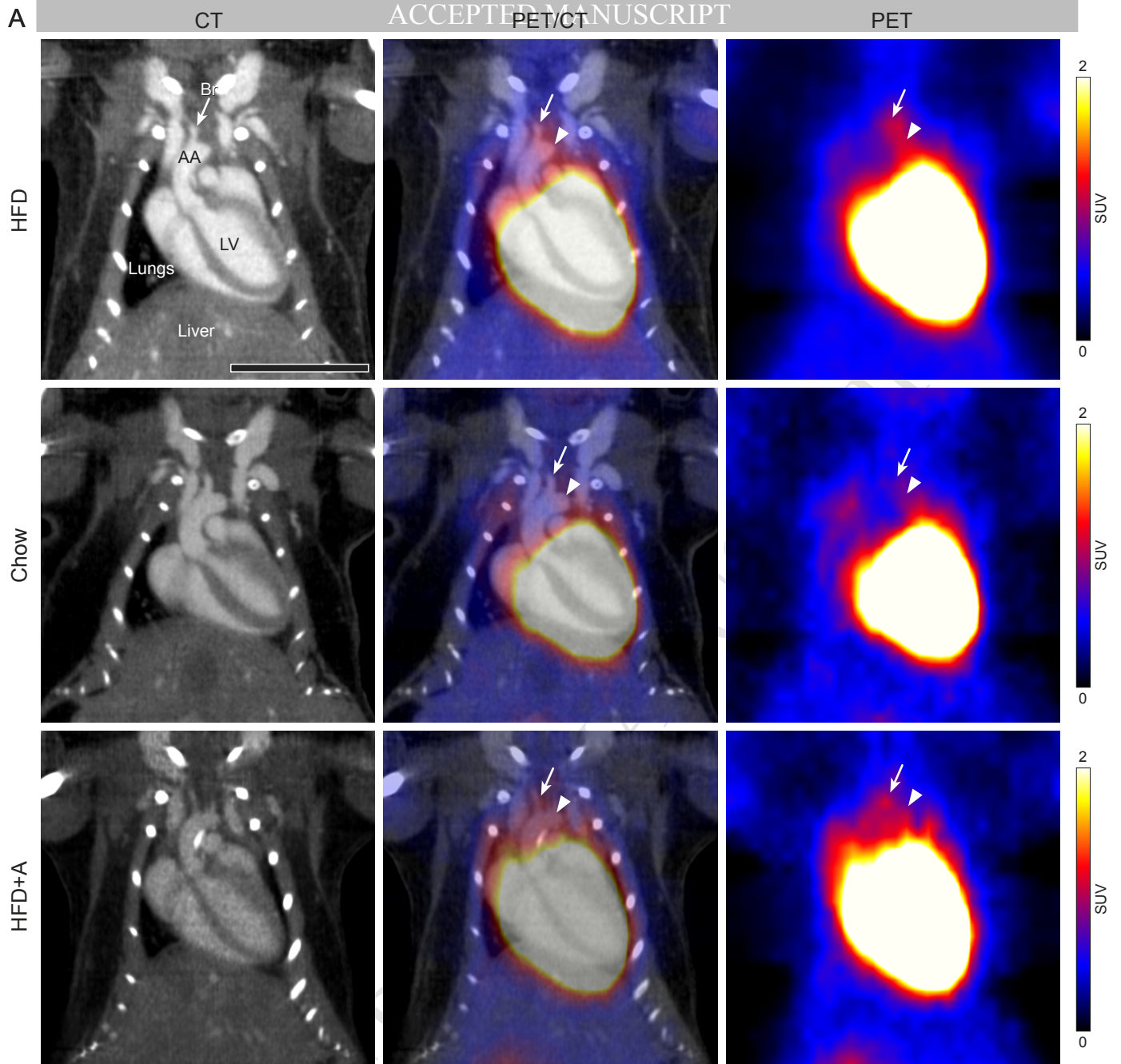
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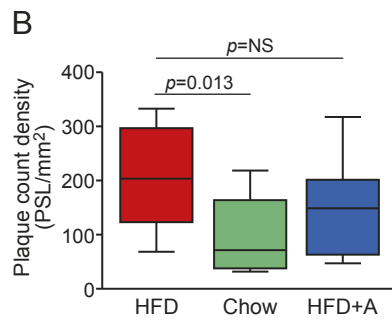
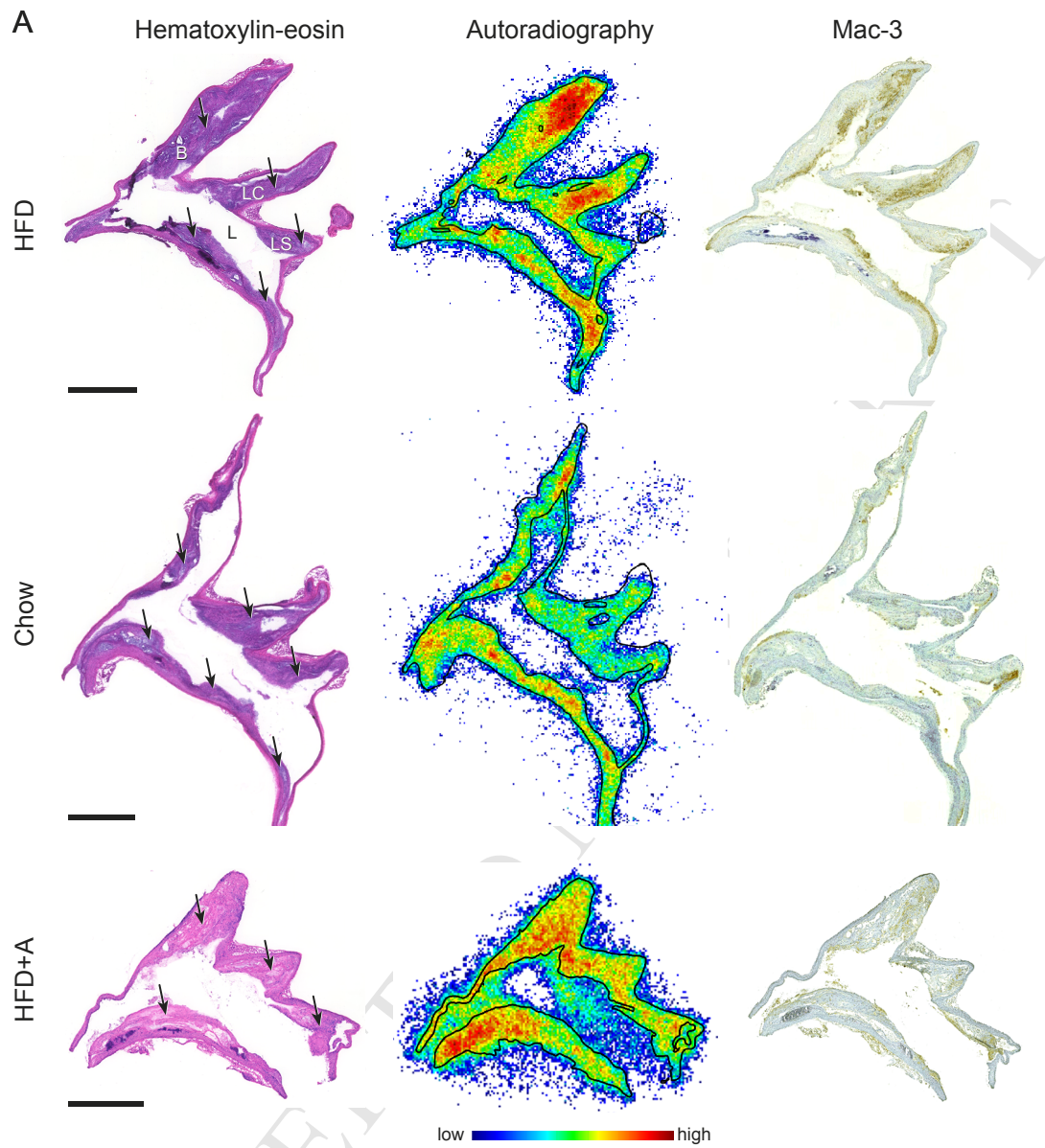
NS, not significant; HFD, high-fat diet group; HFD+A, HFD and atorvastatin group.

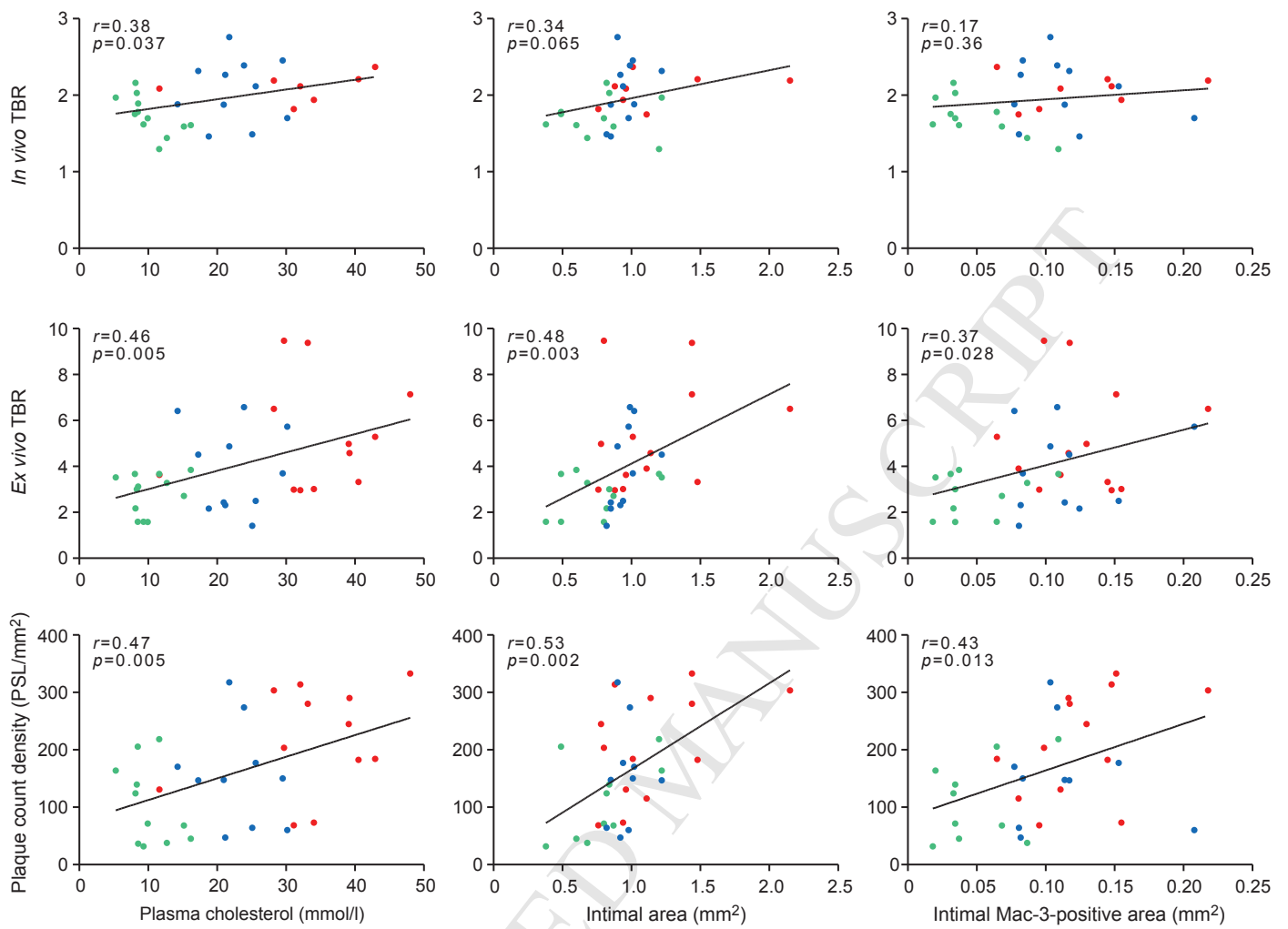
<sup>a</sup>Also significantly higher than in the Chow group ( $p < 0.001$ ).











Effects of atorvastatin and diet interventions on atherosclerotic plaque inflammation and [<sup>18</sup>F]FDG uptake in *Ldlr*<sup>-/-</sup> *Apob*<sup>100/100</sup> mice

Highlights

- PET tracer [<sup>18</sup>F]FDG is a sensitive marker of atherosclerotic plaque inflammation.
- Potential anti-inflammatory effects of interventions were studied by [<sup>18</sup>F]FDG.
- Atorvastatin had no effect on plaque inflammation in *Ldlr*<sup>-/-</sup> *Apob*<sup>100/100</sup> mice.
- Chow diet reduced plasma cholesterol, plaque size and inflammation.
- We found no evidence of cholesterol-independent effects on vascular inflammation.