# Atheroprotective effects of semaglutide in the diet-induced obese Ldlr$^{-/-}$ mouse model of atherosclerosis


**Authors**

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## BACKGROUND & AIM

The glucagon like peptide 1 receptor (GLP-1R) agonist semaglutide are currently approved for diabetes and obesity treatment. Semaglutide has also demonstrated beneficial cardiovascular effects in these patient populations.

The present study aimed to explore the effect of semaglutide on obesity, dyslipidemia and aortic atherogenic plaque progression including leukocyte infiltration using quantitative 3D imaging in the diet-induced obese (DIO) low-density lipoprotein (LDL) receptor knock-out (DIO-LDLR-KO) mouse model of atherosclerosis.

## METHODS

Male LDLR$^{-/-}$ mice fed western diet (D12079B Research diets, 41 %-kcal fat, 0.21% cholesterol) for 17 weeks and administered (SC, QD) vehicle or semaglutide (30 nmol/kg) for 17 weeks as prophylactic intervention. Chow-fed LDLR$^{-/-}$ mice served as controls. Terminal endpoints included body weight, plasma markers of dyslipidemia (HDL/LDL-cholesterol, total cholesterol, triglycerides) and aortic atherogenic plaque load. Aortic branch whole-mounts were stained for CD45+ immune cells (leukocytes), cleared and imaged using 3D light sheet microscopy. Deep learning computational analyses was applied for rapid detection, anatomical mapping and quantification of atherogenic plaques (autofluorescence) and CD45+ infiltrates in the vascular wall.

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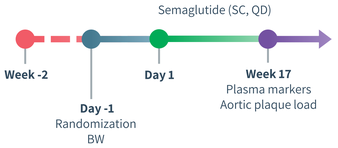
## 1 Study outline




| Group | Animal model | Treatment | Number of animals |
|---|---|---|---|
| 1 | CHOW-LDLR-KO | Vehicle | 12 |
| 2 | DIO-LDLR-KO | Vehicle | 16 |
| 3 | DIO-LDLR-KO | Semaglutide (30 nmol/kg) | 16 |

## 2 Metabolic and biochemical parameters

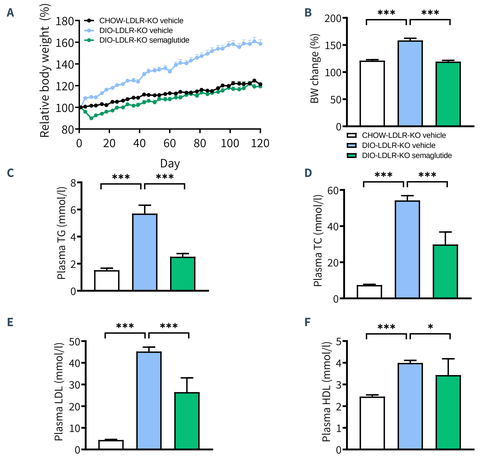



**Figure 1. Semaglutide improves metabolic and biochemical parameters in DIO-LDLR-KO mice.** **(A)** Body weight (BW) change relative to baseline (day 1) over study period. **(B)** Terminal body weight. **(C-F)** Terminal plasma markers of dyslipidimia. **(C)** Triglycerides (TG, nmol/l). **(D)** Total cholesterol (TC, nmol/l). **(E)** Low-density lipoprotein (LDL, nmol/l). **(F)** High-density lipoprotein (HDL, nmol/l). *p<0.05, ***p<0.001. Dunnett`s multiple comparisons test.

## 3 3D imaging pipeline for plaque detection

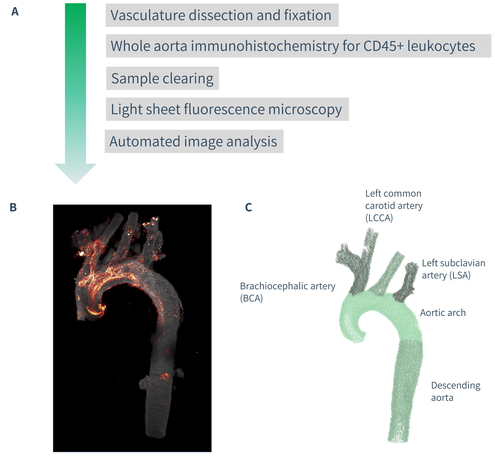



**Figure 2. 3D imaging pipeline for mapping and quantification of aortic atherogenic plaque load.** **(A)** Schematic overview of atherosclerosis analysis process. **(B)** Light sheet microscopy imaged aorta in DIO-LDLR-KO mouse. Tissue autofluorescence (grey), CD45-labelled cells (glow scale). **(C)** Light sheet microscopy data transferred to point cloud for segmentation of individual vascular branches.

## 4 AI-based detection of aortic plaques

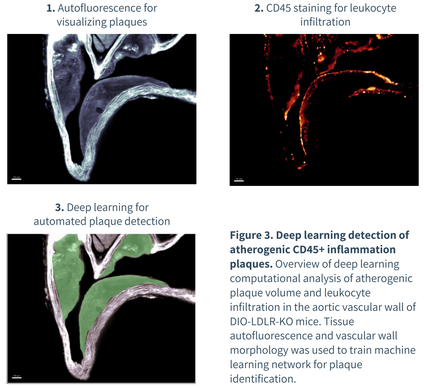



**Figure 3. Deep learning detection of atherogenic CD45+ inflammation plaques.** Overview of deep learning computational analysis of atherogenic plaque volume and leukocyte infiltration in the aortic vascular wall of DIO-LDLR-KO mice. Tissue autofluorescence and vascular wall morphology was used to train machine learning network for plaque identification.

## 5 Aortic plaque volume

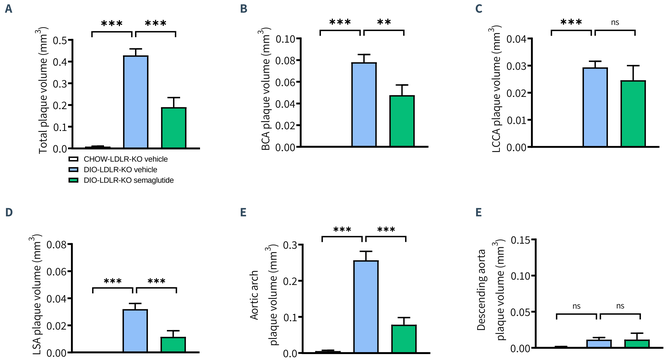



**Figure 4. Semaglutide reduces aortic plaque load in DIO-LDLR-KO mice.** **(A)** Total plaque volume in the aorta. Plaque volume in the **(B)** brachiocephalic artery (BCA), **(C)** left common carotid artery (LCCA), **(D)** left subclavian artery (LSA), **(E)** aortic arch, **(F)** descending aorta. **p<0.01, ***p<0.001; ns, not significant. Dunnett`s multiple comparisons test.

## 6 Aortic inflammation volume

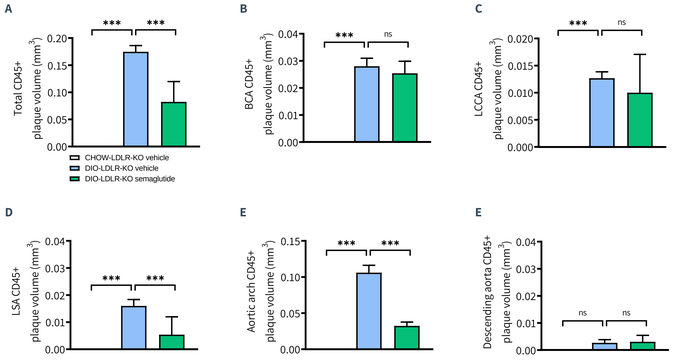



**Figure 5. Semaglutide reduces aortic inflammation load in DIO-LDLR-KO mice.** **(A)** Total plaque CD45+ immunofluorescence volume in the aorta. Plaque CD45+ immunofluorescence volume in the **(B)** brachiocephalic artery (BCA), **(C)** left common carotid artery (LCCA), **(D)** left subclavian artery (LSA), **(E)** aortic arch, **(F)** descending aorta. ***p<0.001; ns, not significant. Dunnett`s multiple comparisons test.

## CONCLUSION

- High-throughput, automated 3D light sheet imaging enables accurate volumetric assessment of aortic atherogenic plaque and inflammation load in DIO-LDLR-KO mice.
- Semaglutide induces weight loss and improves dyslipidemia/hypercholesterolemia in DIO-LDLR-KO mice
- Semaglutide markedly reduces total atherogenic plaque volume due to reduced plaque lesions in almost all aortic compartments in DIO-LDLR-KO mice
- Semaglutide reduces total aortic plaque leukocyte volume, driven by reductions of CD45+ leukocyte infiltrates in the LSA and aortic arch in DIO-LDLR-KO mice

**The DIO-LDLR-KO mouse represents a translational model for evaluating drug effects on clinical endpoints focusing on progression of dyslipidemia and atherosclerosis**