

# **Phenotyping ApoE knockout rats on a Western diet: endothelial dysfunction in small arteries as early signs of atherosclerosis**

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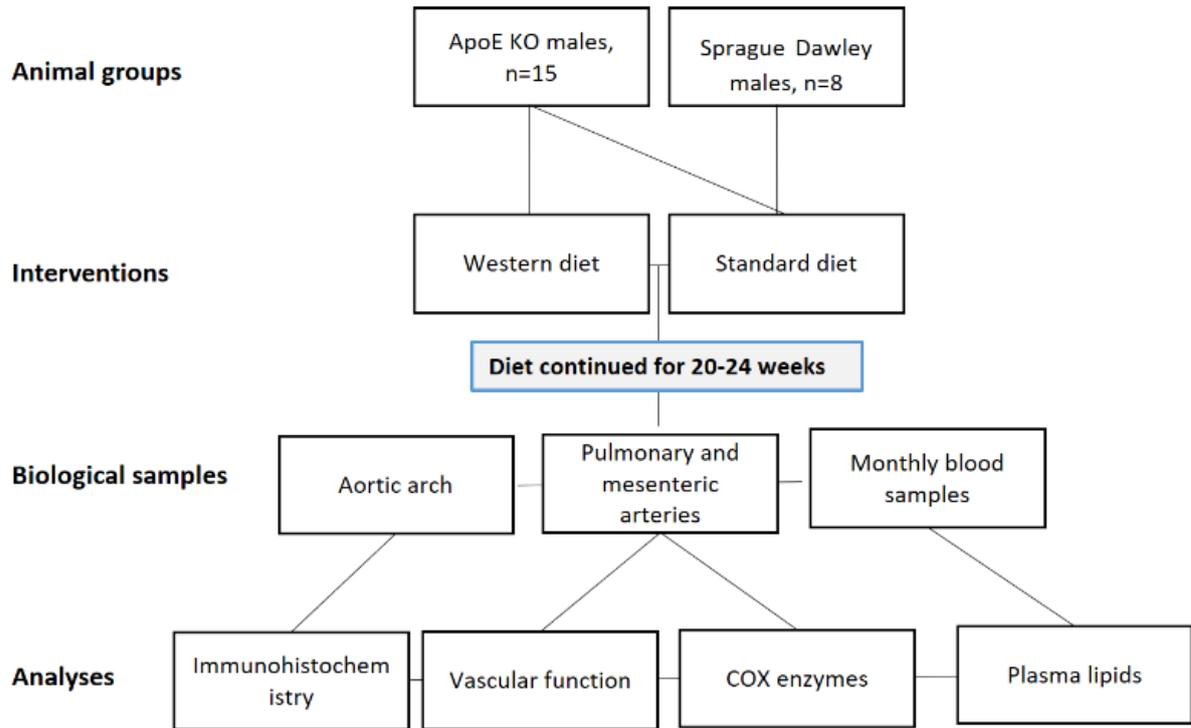
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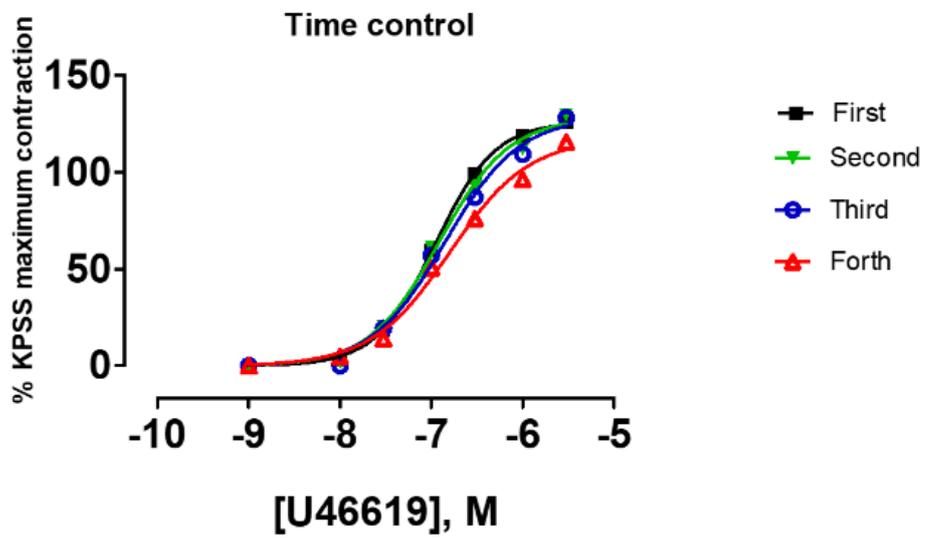
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DK8200 Aarhus N, Denmark

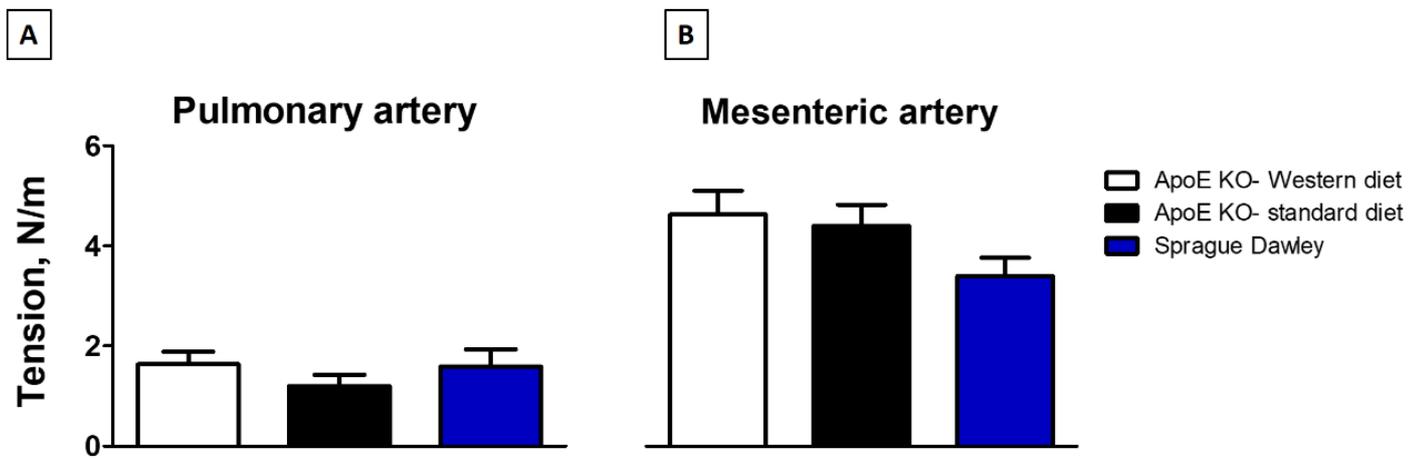
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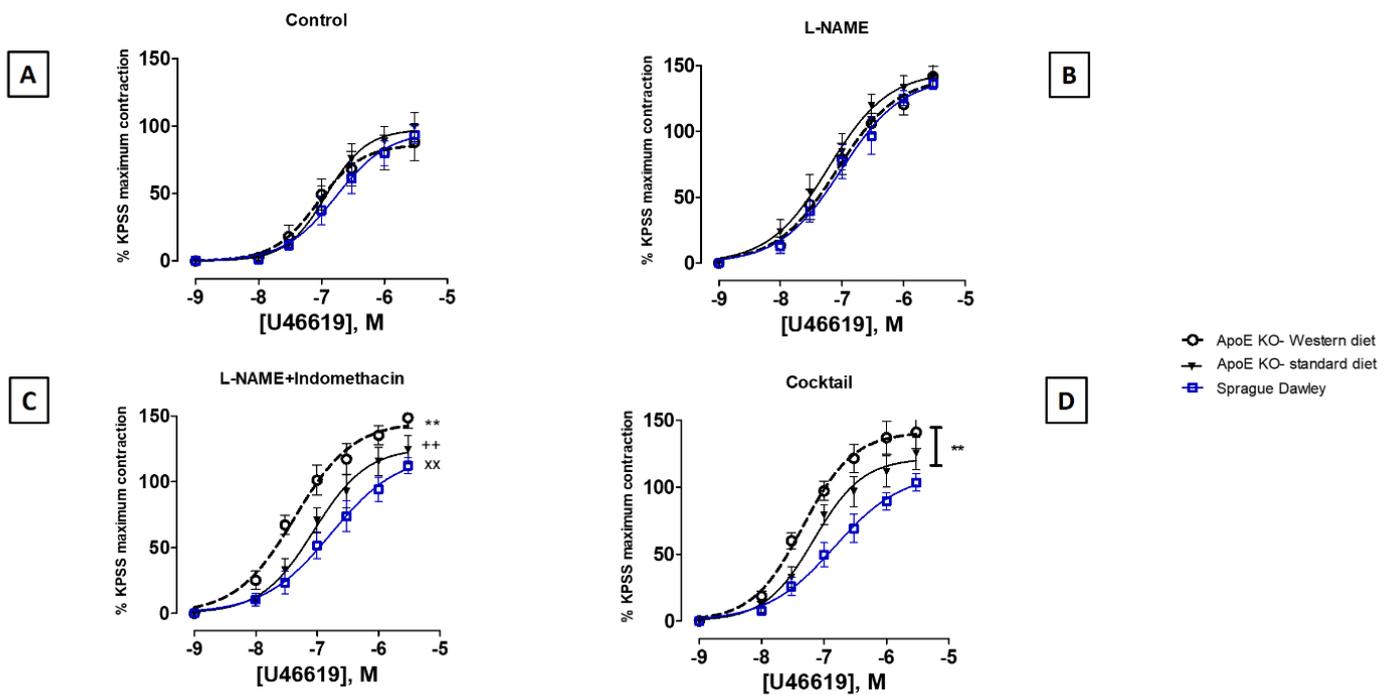
**Supplementary Figure S1. A summary chart of study design.** 15 male ApoE knockout (KO) rats and 8 Sprague Dawley rats were subjected to either a Western or standard diet for 20-24 weeks



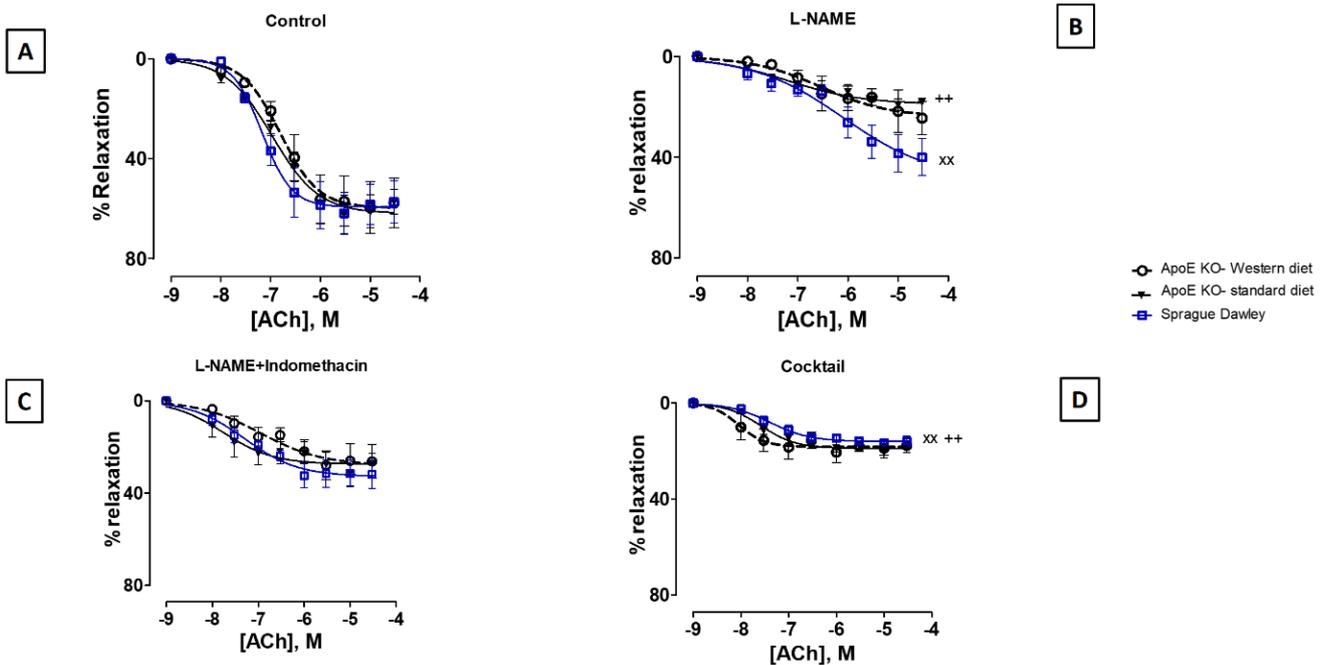
**Supplementary Figure S2. Time control in pulmonary arteries.** Percentage of contraction, expressed relative to the maximal contraction of KPSS, in response to U46619 in pulmonary arteries



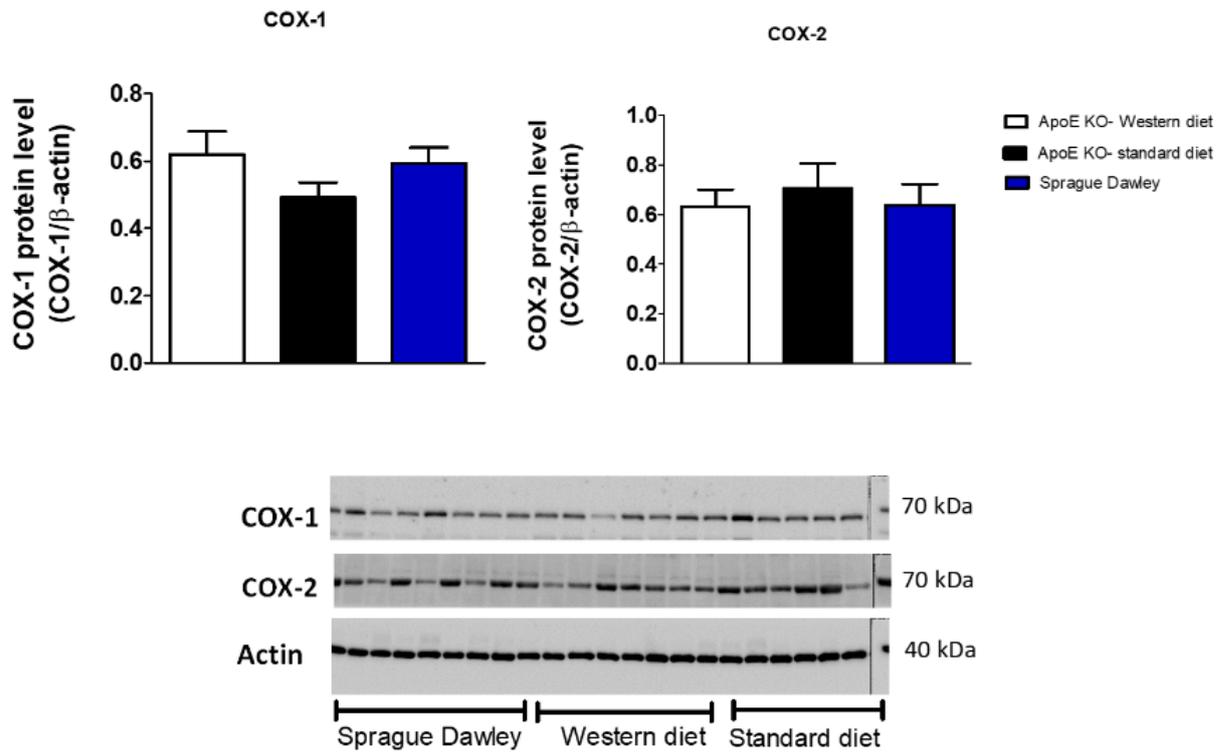
**Supplementary Figure S3. Maximal contraction in presence of KPSS.** (A) pulmonary (B) mesenteric arteries. KPSS, potassium physiological saline solution ANOVA, n=8, n=7.



**Supplementary Figure S4. U46619 concentration-response curves of pulmonary arteries from different experimental groups under different conditions;** (A), Control conditions; (B), In the presence L-NAME (100  $\mu$ M); (C), After 20 min incubation with L-NAME (100  $\mu$ M) and indomethacin (3  $\mu$ M), (\*\*),  $P < 0.0001$  for ApoE KO- Western diet vs ApoE- control diet, (++) ,  $P = 0.09$ , ApoE KO- control diet vs Sprague Dawley, (xx),  $P < 0.0001$ , Sprague Dawley vs ApoE KO- Western diet; (D), The effect of 20 min incubation with Cocktail: L-NAME, indomethacin, TRAM-34 and apamin, i.e. pharmacological inhibition of all major pathways for endothelium-dependent relaxation, (\*\*),  $p = 0.001$ , ApoE KO- western diet vs ApoE Ko- control diet, (++) ,  $p < 0.001$ , ApoE KO- standard diet vs Sprague Dawley, (xx),  $P < 0.0001$ , Sprague Dawley vs ApoE KO- Western diet, F test,  $n = 8, 7$ .



**Supplementary Figure S5. concentration-response curves of mesenteric arteries from different experimental groups under different conditions;** (A), Control conditions; (B), In the presence L-NAME (100  $\mu$ M), (++)  
 $P < 0.001$ , ApoE KO- control diet vs Sprague Dawley (xx),  $P = 0.003$ , Sprague Dawley vs ApoE KO Western diet;  
 (C), After 20 min incubation with L-NAME (100  $\mu$ M) and indomethacin (3  $\mu$ M); (D), The effect of 20 min  
 incubation with Cocktail: L-NAME, indomethacin, TRAM-34 and apamin, i.e. pharmacological inhibition of all  
 major pathways for endothelium-dependent relaxation, (++)  
 $p < 0.002$ , ApoE KO standard diet vs Sprague Dawley,  
 (xx),  $P < 0.002$ , Sprague Dawley vs ApoE KO Western diet,  $n = 8$ ,  $n = 7$ .



**Supplementary Figure S6. Relative expression of COX1 and COX 2.** Proteins quantified by Western blot and normalized to  $\beta$ -actin as loading control. ANOVA and t-test, n=8 ,7.