which displays increased oxidative stress and triglyceride contents in the skeletal muscle. Experimental evidence suggests that ApoE KO mice have impaired regeneration after injury. Skeletal muscle stem cells are responsible for the regenerative capacity of skeletal muscle and undergo activation, proliferation and differentiation upon injury in order to support muscle repair and regeneration. The skeletal muscle stem cell function of ApoE KO mice remains largely unknown. We hypothesised that the attenuated regenerative capacity of the ApoE-deficient muscle derives from deficits in skeletal muscle stem cell function. Therefore, we investigated the impact of ApoE deficiency on the proliferation and differentiation profiles of muscle stem cells.

Methods Muscle stem cells from the extensor digitorum longus and biceps brachii were isolated from male ApoE KO and wild type mice for single fibre culture experiments *ex vivo* and stem cell cultures *in vitro*. Stem cell proliferation and differentiation, morphology, immunohistochemistry and gene expression analysis were performed. Cell culture experiments were conducted with $n=6-9$ technical replicates and $n=2-3$ independent experiments. Significant statistical differences were determined by the Mann-Whitney U test for $p<0.05$.

Results Single fibres of wild type and ApoE KO mice did not markedly differ in their myogenic proliferation over the first 48h in culture. After 72 hours however, impaired differentiation of ApoE KO mouse muscle stem cells was evident on single fibres *ex vivo* based on reduced expression of the muscle regulatory factor myogenin. Furthermore, treatment of cultures with 0.5mM palmitate for 24h resulted in loss of stemness and increased commitment to differentiation (i.e. Pax7-ve/MyoD+ve cells) as well as increased myogenic differentiation. Isolated muscle stem cells from ApoE KO mouse displayed impaired proliferation as well as differentiation, with morphological studies resulting in reduced myotube width and length, an indicator of reduced myotube fusion. These results were exacerbated by application of palmitate in both wild type and ApoE KO. These findings are strengthened by reduced gene expression data for key factors regulating skeletal muscle fusion of myogenic progenitors, cell fate and contractility (i.e. MyoD, Scrib1, myogenin, Bex1, tmem8c, srf, mhc1 and acta1).

Conclusion We report for the first time impaired skeletal muscle stem cell function in hyperlipidaemic ApoE KO mice, which may account for the impaired regenerative capacity of this mouse strain. These data establish a link between muscle stem biology and impaired myogenesis in an experimental model of atherosclerosis and systemic hyperlipidaemia with implications for the myopathies seen in cardiovascular disorders.

Conflict of interest None

BS56 REDOX-REGULATION OF ANGIO-INDUCED CEREBRAL MICROVASCULAR DAMAGE IN AGING

Li Geng*, Jian-Mei Li. *University of Reading*

10.1136/heartjnl-2019-BCS.217

Oxidative damage of cerebral vasculature has been found to play an important role in aging-related neurodegeneration. Endothelial oxidative stress attributable to the activation of a Nox2-containing NADPH oxidase is an early sign of age-related vascular abnormalities. However, the mechanism of

aging-related Nox2 activation and cerebral microvasculature rarefaction remains unclear. In this study we used littermates of wild-type (WT) and Nox2 knockout (Nox2KO) mice at young (3–4 m) and old age (20–22 m) to investigate the role of Nox2-derived ROS in oxidative damage of cerebral microvasculature in aging. Compared to WT young mice, WT (but not the Nox2KO) aging mice had higher blood pressure and elevated serum levels of AngII. WT aging brains had significantly higher levels of AngII, increased ROS production, elevated lipid peroxidation, reduced capillary density and increased expressions of cell apoptosis markers i.e. increased p53 expression and γ H2AX phosphorylation ($p<0.05$). However, these AngII-induced abnormalities were significantly reduced or absent in Nox2KO aging brains. The mechanism of AngII-induced Nox2 activation and cerebral endothelial oxidative damage was further investigated *in vitro* using primary cerebral microvascular endothelial cells isolated from mid-aged WT mice. AngII (100 μ mol/L) increased significantly the levels of endothelial ROS production, Nox2 expression and p47phox phosphorylation. These were accompanied by increased ERK1/2 and γ H2AX phosphorylation and p53 expression detected by Western Blot and damaged capillary formation on Matri-gels. However, all these AngII-induced oxidative responses were inhibited in the presence of a specific Nox2 inhibitor (Nox2-ds-tat). In conclusion, Nox2-derived ROS and redox signaling plays a critical role in regulation of AngII-induced cerebral microvascular rarefaction in aging.

Conflict of interest No

BS57 ABSTRACT WITHDRAWN

BS58 DOES CHANGE IN RENAL FUNCTION AFFECT WARFARIN'S EFFICACY IN PATIENTS WITH ATRIAL FIBRILLATION?

¹Ahsan Khan*, ²Eduard Shantsila, ²Y.C. Lau, ³Lewis Hardy, ³Helen Philippou, ¹Gregory Lip.
¹Liverpool Heart & Chest Hospital NHS Foundation Trust; ²University of Birmingham;
³University of Leeds

10.1136/heartjnl-2019-BCS.218

Introduction Anticoagulation use in atrial fibrillation (AF) is common, but concurrent chronic kidney disease (CKD) reduces its efficacy and potentiates the risk of bleeding. Patients with AF have a higher incidence of renal impairment and the latter predisposes to incident AF. The co-existence of both conditions leads to a higher risk for thromboembolic-related adverse events but also a paradoxical increased haemorrhagic risk with devastating consequences for the individual. Assessing the effect of renal function on plasma clot properties in anticoagulated AF patients may help explain reasons behind this.

Aim To observe how progressive renal dysfunction affects plasma clot properties in AF patients on warfarin.

Methods We studied 192 patients with AF on warfarin and divided them in 4 groups based on estimated glomerular filtration rate (eGFR), eGFR>90 ($n=30$), eGFR 60–89 ($n=89$), eGFR 30–59 ($n=59$) and eGFR<30 ($n=14$). Plasma samples were obtained from patients using venepuncture and centrifuged to prepare platelet poor plasma. Assays were performed

Abstract BS58 Table 1 Effect of renal function on plasma clot properties in AF patients on warfarin

eGFR group mL/min/1.73 m2	≥90 n=30	60-89 n=89	30-59 n=59	<30 n=14	p	
Demographic and clinical characteristics						
Age, years	61 [50-76] ^{abc}	74 [66-79] ^{bc}	78 [74-83]	81 [75-86]	<0.001	
Males, n (%)	22 (73%)	43 (48%)	29 (57%)	5 (36%)	0.08	
Systolic BP, mmHg	132 [124-144]	130 [118-145]	125 [114-141]	133 [110-152]	0.47	
Diastolic BP, mmHg	79 [68-90] ^{bc}	74 [67-83] ^b	70 [61-76]	70 [65-74]	0.01	
Body mass index, kg/m ²	28 [24-32]	29 [26-33]	28 [25-33]	27 [25-32]	0.61	
Smoking, n (%)	3 (10%)	2 (2%)	2 (4%)	0	0.23	
Creatinine, n (%)	71 [66-75]	85 [75-94]	130 [112-160]	195 [175-238]	<0.001	
eGFR, n (%)	102 [96-110]	73 [65-78]	41 [36-52]	25 [23-28]	<0.001	
Creatinine clearance, n (%)	115 [87-130]	74 [62-84]	44 [32-52]	24 [17-28]	<0.001	
Coronary artery disease, n (%)	8 (27%)	36 (40%)	23 (45%)	8 (57%)	0.22	
Diabetes, n (%)	11 (37%)	30 (34%)	26 (51%)	9 (64%)	0.06	
Hypertension, n (%)	20 (67%)	77 (87%)	42 (82%)	13 (93%)	0.06	
Peripheral artery disease, n (%)	3 (10%)	8 (9%)	5 (10%)	1 (7%)	0.99	
Parameters of clot turbidity and lysis (non-normalised values)						
Maximum OD	PPP	0.53 [0.47-0.62]	0.55 [0.44-0.65]	0.55 [0.49-0.74]	0.64 [0.52-0.80]	0.13
	APPT	0.48 [0.42-0.57]	0.50 [0.41-0.61]	0.50 [0.43-0.66]	0.58 [0.45-0.77]	0.16
	Thrombin	0.57 [0.52-0.65]	0.57 [0.47-0.68]	0.59 [0.54-0.77]	0.66 [0.51-0.86]	0.07
Time to 50% maximum OD	PPP	440 [396-496]	476 [400-556]	456 [392-492]	448 [412-496]	0.25
	APPT	350 [308-404]	356 [316-400]	348 [328-380]	352 [324-428]	0.98
	Thrombin	242 [220-292]	272 [220-348]	268 [228-308]	250 [196-336]	0.25
Time between 50% clotting and 50% lysis	PPP	472 [412-576] ^{ab}	544 [460-692]	576 [464-752]	546 [508-600]	0.03
	APPT	532 [484-692] ^{abc}	632 [528-812]	648 [532-880]	640 [584-688]	0.02
	Thrombin	500 [448-584] ^{abc}	552 [488-692]	580 [500-776]	586 [536-636]	0.03
Time to 50% lysis from maximum OD	PPP	300 [248-376] ^{ab}	360 [284-508]	400 [292-560]	388 [340-424]	0.04
	APPT	418 [364-528]	500 [396-680]	520 [404-744]	520 [480-544]	0.07
	Thrombin	310 [272-360] ^{abc}	368 [308-476]	376 [316-544]	376 [328-460]	0.02

in 96-well polystyrene microtiter plates. Reagents were diluted in standard buffer (10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid [HEPES], pH 7.4, 150 mM NaCl). Patient plasma samples (25 %) were incubated with tissue plasminogen activator (500 ng.mL⁻¹) for 10 minutes at 37°C before the addition of CaCl₂ (7.5 mM). Either PPP reagent (2.5 %), aPPT reagent (2.5 %), or thrombin (0.5 U.mL⁻¹) were then added to initiate coagulation. Polymerisation of fibrin in plasma was monitored (ΔOD340 nm) using a Synergy H1 hybrid multi-mode plate reader, readings were taken in 12 second intervals for up to 60 minutes.

Results Comparison between the 4 groups was performed using Kruskal-Wallis test with Dunn's post-hoc analysis and Holm-Sidak adjustment. There was no statistical difference in optical density reflecting clot turbidity or rate of clot formation between the eGFR groups (table 1). Plasma clot lysis time was noted to be significantly prolonged in patients with worsening renal function with all 3 reagents used (PPP (p=0.03), APPT (p=0.02) and thrombin (p=0.03) respectively). Time for 50% of the clot to lyse was also significantly slower with worsening renal function with PPP (p=0.04) and thrombin (p=0.02) reagents suggesting modulation of warfarin action.

Conclusion Worsening renal function in AF patients on warfarin leads to increased clot lysis time, hence leading to a potentially increased risk of thrombosis.

Conflict of interest None

BS59

LOSS OF SIRT1 IN DIABETES ENHANCES DNA DAMAGE AND REDUCES MRN COMPLEX ACTIVATION RESULTING IN INCREASED VASCULAR CALCIFICATION

Francesca Bartoli-Leonard*, Yvonne Alexander, Ria Weston. *Manchester Metropolitan University*

10.1136/heartjnl-2019-BCS.219

Introduction Patients with type II diabetes have considerably higher risk of developing cardiovascular disease and often have an elevated prevalence of vascular calcification combined with a loss of SIRT1, which induces osteogenic differentiation within the smooth muscle. This study aims to determine whether the loss of SIRT1 contributes to the enhanced DNA damage associated with vascular calcification and diabetes.

Methods Popliteal arteries, harvested from diabetic patients undergoing limb amputation, and non-atherosclerotic internal thoracic arteries, were stained for a range of DNA damage markers. Human coronary artery smooth muscle cells (vSMCs) were used for an in vitro calcification model (5mM βGP and 2.6mM CaCl₂) in the presence or absence of a diabetic environment (high glucose (25mM), or control (5mM) DMEM respectively. DNA damage was induced using 200μM H₂O₂ and SIRT1 was activated or inhibited pharmacologically using SRT1720 or Sirtinol respectively. DNA damage was assessed via a comet assay, γH2A.X and p21 immunocytochemistry, whilst DNA damage-induced SIRT1 activation was assessed via