

antiplatelet therapy (DAPT) representing a risk factor for thrombosis. Coagulation pathways also play a central role and configuration of the fibrin network is a vital determinant of clot stability and outcome, with dense clots composed of compact thin fibres associated with thrombotic events. However, despite the interwoven nature of platelet activation and the coagulation system, studies relating both aspects of thrombosis in the same population are limited.

**Methods** Four groups of healthy volunteers ( $n = 8$ ) received single anti-platelet therapy aspirin (75 mg) or prasugrel (10 mg) or DAPT with aspirin (75 mg) plus prasugrel (10 mg) or aspirin (75mg) plus ticagrelor (90mg) for 7 days. In this study, we characterised the influences of these standard anti-platelet therapies on platelet function using standard tests of platelet activation and aggregation. We then associated platelet reactivity to fibrin clot microstructure which was determined using advanced rheological techniques to analyse the viscoelastic properties of incipient clots; fractal dimension ( $D_f$ ) and mean relative mass (RM).

**Results** Aspirin alone caused inhibition of platelet responses to arachidonic acid (AA), but did not reduce ATP release, P-selectin or PAC-1 binding. Aspirin also had no effect upon the measures of clot structure  $D_f$  ( $1.71 \pm 0.01$  to  $1.69 \pm 0.01$ ,  $p = 0.41$ ) or RM ( $-8 \pm 19\%$ ,  $p = 0.47$ ). Prasugrel significantly reduced  $D_f$  ( $1.72 \pm 0.02$  to  $1.67 \pm 0.01$ ,  $p = 0.03$ ) and RM ( $-40 \pm 11\%$ ,  $p = 0.03$ ), as well as caused a significant decrease in ATP release, PAC-1 and p-selectin expression ( $p < 0.05$ ). Both aspirin plus prasugrel and aspirin plus ticagrelor inhibited platelet responses to all agonists tested and decreased P-selectin expression. Significant platelet inhibition was associated with a reduction in  $D_f$ ;  $1.73 \pm 0.02$  to  $1.68 \pm 0.02$  ( $p = 0.03$ ) and  $1.72 \pm 0.03$  to  $1.62 \pm 0.02$  ( $p = 0.04$ ) by aspirin+prasugrel and aspirin+ticagrelor, respectively. This corresponded to reductions in RM both for aspirin plus prasugrel ( $-35 \pm 16\%$  change,  $p = 0.04$ ) and for aspirin plus ticagrelor ( $-45 \pm 14\%$  change,  $p = 0.04$ ).

**Conclusion** We demonstrate that platelets are important determinants of clot microstructure which is modifiable by anti-platelet therapies. These therapies may rely on their abilities to reduce thrombus density to exert their therapeutic effects with increased levels of platelet inhibition associated with the formation of more open, porous fibrin clots. We also suggest that it is activation of P2Y<sub>12</sub> receptors rather than TX (A<sub>2</sub>) receptors that are key to clot formation. We conclude that there lies both diagnostic and therapeutic potential in determining the functional relationship between platelet reactivity, eventual clot quality and clinical outcome and  $D_f$  could be a useful novel biomarker in risk stratification and tailored anti-platelet therapies.

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#### MITOCHONDRIAL DYSFUNCTION IN THE DIABETIC HEART A€ " IMBALANCE IN FUSION/FISSION AXIS?

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**Background** Cardiovascular disease is the leading cause of morbidity and mortality among diabetic patients. Diabetic cardiomyopathy is closely linked to mitochondrial dysfunction, however the pathophysiological mechanisms responsible are not known. Maintenance of mitochondrial function relies on

the balance between fusion and fission events. The fusion protein mitofusin-2 (Mfn2) has been implicated in the pathogenesis of diabetes. Alongside fusion, Mfn2 is widely believed to function as a molecular tether, binding mitochondria to the sarcoplasmic reticulum (SR) to form specialised Ca<sup>2+</sup> microdomains. Nonetheless, the role of Mfn2 in the heart is poorly characterised. Therefore, the aim of this study was to investigate changes to cardiac mitochondrial protein expression and function in diabetes with a particular focus upon the fusion/fission axis.

**Methods and results** Protein expression levels were measured in control and streptozotocin-treated (STZ) Wistar rat heart using Western Blot. Mitochondrial OXPHOS function was assessed using enzyme activity assays. Lastly, changes to the mitochondrial proteome were investigated using Mass Spectrometry (MS). Western Blot showed a significant increase in Mfn1 and Mfn2 expression levels in STZ compared to controls with no change to the fission protein Drp1. Enzymatic assays revealed that mitochondrial function was altered in the STZ rat heart compared to control. Lastly, MS identified 1437 proteins, of which there was an upregulation of proteins involved in beta oxidation in the STZ compared to controls. In contrast, there was a downregulation of proteins associated with OXPHOS in the STZ suggesting mitochondrial dysfunction that corroborates the functional data.

**Conclusion** These data suggest that mitochondrial dysfunction may be linked to an imbalance of the mitochondrial fusion/fission axis in the diabetic heart. Future work will focus on the 3-D reconstruction of the mitochondrial networks using electron microscopy to determine whether changes to mitochondrial function are linked to structural alterations. These studies will enhance our understanding of the pathogenesis of cardiac mitochondrial dysfunction in diabetes, with the hope to elucidate potential targets for therapeutic intervention.

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#### CHANGES IN PERIVASCULAR ADIPOSE TISSUE IN AGEING AND ATHEROSCLEROTIC MICE

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Perivascular adipose tissue (PVAT) encases the majority of blood vessels and is an important component of the vasculature. PVAT is an active endocrine organ but until recently was not considered important in the pathogenesis of atherosclerosis. In healthy vessels PVAT exerts an anti-contractile effect which is attenuated in states of metabolic disturbance. The following experiments were conducted to determine the influence of PVAT, age and progression of atherosclerotic disease on isolated arterial reactivity.

Upon weaning, male ApoE<sup>-/-</sup> or C57/BL6 (control) mice were fed a standard rodent chow diet for a period of eight or twenty-six weeks. Aortic atherosclerotic lesion area was analysed by en face quantification with Oil Red O. Aortic PVAT was collected and weighed. Contractile responses of the thoracic aortae to cumulative doses of phenylephrine ( $1 \times 10^{-10}$  –  $3 \times 10^{-5}$  M) were measured in PVAT intact or PVAT denuded vessels using myography. Adipokine expression from PVAT was examined using an adipokine proteome profiler.

Atherosclerotic lesions were present in the aortae of the ApoE<sup>-/-</sup> mice. A significant increase in total lesion area occupying the aorta was observed in the aged ApoE<sup>-/-</sup> mice compared to mice at the earlier time-point (0.11% vs. 2.78%,  $P = 0.006$ ,  $n = 6-7$  mice per group). A significant increase in the amount of PVAT surrounding the aortae of C57/BL6 mice was recorded in response to ageing ( $15.65 \pm 2.90$  mg versus  $26.87 \pm 2.84$  mg,  $P = 0.02$ ,  $n = 5-8$  mice per group). In contrast, no differences in the amount of aortic PVAT was observed between young and ageing ApoE<sup>-/-</sup> mice ( $14.08 \pm 1.64$  mg versus  $13.11 \pm 1.78$  mg,  $P=NS$ ,  $n = 3-5$ ). Aortic PVAT exerted anti-contractile effects in the young 8 week controls (PVAT versus no PVAT:  $P = 0.04$ ,  $n = 6$ ). However, the anti-contractile capacity of aortic PVAT was abolished in ageing control mice (PVAT versus no PVAT:  $P=NS$ ,  $n = 8$ ). The aortic PVAT from young ApoE<sup>-/-</sup> mice exhibited the characteristics of an aged phenotype, exerting no anti-contractile effect (PVAT versus no PVAT:  $P=NS$ ,  $n = 8$ ); this was sustained in ageing ApoE<sup>-/-</sup> mice (PVAT versus no PVAT:  $P=NS$ ,  $n = 8$ ). Modulations in adipokine expression were observed with ageing in both C57/BL6 and ApoE<sup>-/-</sup> mice.

Aortic PVAT from ApoE<sup>-/-</sup> mice displayed an aged phenotype even at 12 weeks of age. These findings may have important implications in the pathogenesis of atherosclerosis. Further investigation into the characteristics of aortic PVAT from ApoE<sup>-/-</sup> mice and the factors it releases in early and established atherosclerosis is ongoing.

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#### DIFFERENTIAL EFFECTS OF RESVERATROL ON ACETYLCHOLINE-INDUCED AND FLOW-MEDIATED DILATION OF THE MOUSE FEMORAL ARTERY

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Despite the widespread use of nutritional supplements, insight into their potential beneficial or harmful biological effects is frequently lacking. Resveratrol (RV) is a polyphenol found in berries and grape skin and is often marketed as beneficial for vascular health. Here, we tested this assumption and hypothesised that RV enhances endothelial function and flow-mediated dilation (FMD) in mouse femoral arteries.

C57BL/6 male mice (12 weeks of age) were humanely euthanised in accordance with the *Animals (Scientific Procedures) Act 1986* and Institutional guidelines. Sections of the femoral artery were dissected and mounted between two glass cannulae on a modified pressure myograph chamber. Arteries were pressurised to an intravascular pressure of 60 mmHg. Endothelial-dependent dilator responses to acetylcholine (ACh) and intraluminal flow in pre-constricted vessels (Phe  $10^{-5}$  M) were assessed in femoral arteries incubated for 1 h in either 30  $\mu$ M RV or physiological saline solution (PSS). The contribution of different vasodilator pathways was investigated by treating sections of the femoral artery with L-NG-nitro-L-arginine (L-NNA; 100 $\mu$ M) or indomethacin (10  $\mu$ M). The drugs were applied intraluminally for 30 min using a 1-mL syringe inserted into one end of a  $\frac{3}{8}$  way luer

connexion on the side of the pressure myograph chamber while incubated in PSS or RV. After 30 min incubation, dilator responses to ACh ( $10^{-9}$ – $10^{-3}$  M) and intraluminal flow (5–10  $\mu$ L $\cdot$ min<sup>-1</sup>) in the presence or absence of these drugs were determined.

Maximal degrees of dilation were reached upon perfusing the arteries with of  $10^{-5}$  to  $10^{-3}$  M ACh. RV treatment significantly enhanced dilation in response to ACh ( $p < 0.05$ ). Whereas dilation of the arteries in response to ACh was (mean  $\pm$  SEM)  $68.1 \pm 13.7\%$  ( $n = 9$ ) of the passive diameter when incubated with PSS, treatment with RV resulted in dilation of  $92.2 \pm 13.4\%$  ( $n = 6$ ). However, RV significantly reduced FMD ( $p < 0.05$ ); dilation in response to intraluminal flow of 8  $\mu$ L $\cdot$ min<sup>-1</sup> was  $22.5 \pm 7.1\%$  ( $n = 9$ ) and  $0.67 \pm 1.06\%$  ( $n = 6$ ) of the passive when the sections were incubated with PSS and RV, respectively. Incubation with L-NNA reduced dilation to ACh in the presence of RV. However, the effects of RV on ACh dilation were maintained in the presence of indomethacin. The effects of RV on FMD were not significantly modulated by L-NNA or indomethacin. The responses to intraluminal flow were still compromised and no significant dilation was present. Our data suggest that RV may have differential effects on ACh-dependent and FMD responses in the mouse femoral artery. The use of RV as a nutritional supplement still warrants caution.

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#### INVESTIGATING PLATELET FUNCTIONAL HETEROGENEITY USING DROPLET MICROFLUIDICS

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Platelet activation is an important step in arterial thrombosis, the acute complication of atherosclerosis. However, current diagnostic techniques for platelet function have been shown to be inadequate to predict thrombosis. In spite of this, many patients are prescribed aspirin to prevent (further) occurrence of arterial thrombosis, introducing bleeding risk. Platelets have been shown to be heterogeneous in a number of features such as size, volume and density, and this variety may underpin overall system behaviour. Functional heterogeneity has been suggested in several studies but current methods are not suitable to reliably study single platelet function. Such a method should provide a high throughput means to profile large platelet populations for the identification of, potentially rare, hyperactive platelets. In addition, the technique must prevent paracrine signalling (platelet-mediated activation of neighbouring platelets), necessitating the isolation of single platelets. This study adapts a droplet microfluidics approach to investigate single platelet functionality.

Here, single platelet sensitivity is studied by adding the agonist (convulxin, specific ligand of the GPVI receptor for collagen) during encapsulation in droplets. After an incubation period the platelets are retrieved from the droplets into fixative, followed by flow cytometry analysis of markers for activation. The PAC-1 antibody is used to identify the active conformation of the  $\alpha$ IIb $\beta$ 3 receptor, important for aggregation and adhesion, and anti-CD62P (p-selectin) to identify degranulation of the platelets. Platelets are identified with CD42b, which is a platelet specific receptor.