fibrillation (VF), arising from myocardial infarctions is the most prominent cause of SCD. The isolated rat heart is widely used to model ischaemia-induced VF owing to its ability to produce high baseline incidences of VF and the ability to regulate several known arrhythmogenic mediators. A model that allows concurrent assessment of VF and contractile function would halve animal requirements. Unfortunately, in Langendorff perfused rat hearts, IVB inflation inhibits the ability of regional ischaemia to elicit VF.¹ However, perfusion with catecholamines facilitates ischaemia-induced VF (I-IVF) in IVB-free hearts when risk of VF is low due to a small ischaemic territory.² Therefore, it was sought to overcome the IVB-induced VF suppression by perfusion with exogenous catecholamines: rendering a model in which the antiarrhythmic properties and the effects on heart dynamics a potential drug has could be assessed simultaneously. Here we tested whether catecholamines can surmount the antiarrhythmic effect of IVB inflation.

Methods Male Wistar rat (285–400 g) hearts were Langendorff-perfused with an IVB positioned in the left ventricle and minimally inflated (0.01 ml) to give a detectable developed pressure (~30 mmHg; 'IVB' groups) or inflated substantially (0.12 ml) to give a developed pressure >100 mmHg ('IVB inflated'). Baseline assessment (–11 min) during perfusion with Krebs' (modified to contain 4 mM K⁺) was followed by randomized and blinded switch to test solution at –10 min, then left coronary artery ligation at time zero, with ischaemia maintained for 60 min. Test solution comprised Krebs' containing catecholamines (93.9 nM noradrenaline and 22.5 nM adrenaline in 15 mM ascorbate vehicle) or vehicle alone (n=9/group). Design (blinded and randomized) and data analysis (e.g. use of ANOVA for Gaussian-distributed data and Fisher's exact test for VF incidence) accorded with BPS guidelines.³

Results Heart rate and developed pressure were increased by catecholamines (Cat) from 1 min before ligation (–1 min) (294 ± 6, 310 ± 13 to 437 ± 10, 442 ± 13 Beats/min and 28 ± 2, 101 ± 3 to 94 ± 14, 177 ± 10 mmHg for IVB and IVB inflated groups respectively), and the effect on pressure was exacerbated by IVB inflation (IVB 30 ± 3 mmHg vs IVB inflation 101 ± 3 mmHg). Ischaemia reduced developed pressure, with effects sustained during ischaemia. Mean ischaemic zone size varied between groups from 53 ± 2 to 59% ± 1% (p > 0.05). Cats increased VF incidence (Vehicle 0% vs Cats 56%) (p < 0.05) and extended the susceptibility time window past 30 min of ischaemia, but IVB inflation was antiarrhythmic during ischaemia and surmounted the arrhythmia facilitating effect of catecholamines (IVB inflated +CATs 0% vs IVB +Cats 56%).

Conclusion Catecholamine perfusion was unable to surmount IVB-induced VF suppression. Unfortunately this rendered VF incidence too low to permit the assay to be suitable for evaluating drug effects on VF and contractile function contiguously.

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ZINC ATTENUATES UVA-DEPENDENT LABILE IRON INCREASE IN HUMAN DERMAL FIBROBLASTS: IMPLICATIONS FOR SKIN AGEING

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Introduction To protect the cell from damaging oxidative stress induced by Fenton reactions, the cytosolic labile iron pool (LIP) is normally kept low and tightly regulated. These reactions are initiated by interactions between iron and hydrogen peroxide and can be prolonged and increased with ascorbate. The source of cytosolic labile iron is unclear. It has been suggested that lysosomes are the main source of iron, since protease inhibitors reduce leakage of lysosomal contents including iron after UVA (ultraviolet light) exposure. However, the time resolution of the changes in enzyme activity required to assess lysosomal leakage is too long to establish whether lysosomal damage occurs due to secondary response to iron release from other sources, particularly ferritin. Other methods are therefore required to clarify the source of cytosolic labile iron. Zinc is a redox-inert metal that has anti-oxidant, anti-inflammatory and anti-proliferative properties which is notably reduced in patients with subclinical carotid atherosclerosis, acute coronary syndrome and heart failure. Zinc is known to block iron diffusion via ferritin threefold channels and can therefore be used as a tool to examine UV-induced iron release from ferritin.

Methods A fluorescence probe (FeRhoNox-1) specific for ferrous ion was used to determine the time dependent changes in human fibroblasts following exposure to UVA irradiation. Since it is known that low concentrations of zinc block iron diffusion via ferritin threefold channels, we reasoned that pre-incubating skin cells with low concentrations of ZnCl₂ would inhibit iron release from ferritin after photo-activation by UVA. Confluent monolayers of human dermal fibroblasts were incubated overnight with 10 μM FeSO₄ and 0, 10 and 40 μM ZnCl₂, before being loaded with FeRhoNox-1 and exposed to UVA (360 nm) for 1–10 min (8–80 J/m²), with fluorescent readings gathered at 1 min intervals.

Results The control cell iron dependent fluorescent signal increased by approximately 100% after 10 min UVA irradiation compared to pre-UV baseline (p < 0.001). This UVA-dependent increase in cell iron is prevented by both 10 and 40 μM ZnCl₂.

Conclusions These Results suggest that UVA causes a significant increase in skin fibroblast LIP and hence oxidative stress which may exacerbate the ageing phenotype of dermal cells. This LIP is likely to be sourced mainly from ferritin since this is completely blocked by relatively low concentrations of extracellular ZnSO₄.

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DIETARY SULFORAPHANE REDUCES PERI-INFARCT CONSTRICTION FREQUENCY IN MOUSE BRAIN FOLLOWING FOCAL CEREBRAL ISCHAEMIA VIA A NRF2-INDEPENDENT MECHANISM

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Introduction The dietary isothiocyanate sulforaphane (SFN) obtained from precursors in cruciferous vegetables such as broccoli is a potent inducer of endogenous antioxidant defences. As a strong electrophile, it oxidises cysteine residues in the redox sensor Keap1 inducing activity of the transcription factor Nrf2 and upregulation of target antioxidant and detoxifying genes. We have previously demonstrated that oral administration of doses achievable through diet for three days

prior to surgically induced ischaemic stroke results in a reduced infarct volume 70 hour later in wild type but not Nrf2 deficient mice, possibly as a consequence of enhanced Nrf2-mediated redox protection in the context of ischaemic-reperfusion injury.

Materials and methods To further investigate mechanisms by which dietary sulforaphane affords neuroprotection, we measured acute blood flow changes during and after focal cerebral ischaemia in mice fed vehicle (corn oil) or sulforaphane at 5 mg/kg for 3 days. Ischaemic stroke was induced via the Longa procedure, employing a surgically inserted filament to transiently occlude the middle cerebral artery under stereotaxic anaesthesia. Ischaemia followed by 60 min of reperfusion resulted in a reproducible temporal lobe stroke. Transcranial laser speckle imaging (Moor FLIPI 2) was used to assess blood flow with high temporal (20 s) and spatial (20 μ m) resolution, enabling discrimination of three functional regions during stroke in the unaffected contralateral hemisphere, partially responsive peri-infarct region and the ischaemic infarct core.

Results While sulforaphane did not alter initial baseline blood flow or affect the depth of ischaemia at the start of the procedure, a clear effect was resolved on the number of evoked peri infarct constriction (PIC) waves. While in both vehicle and treatment groups, an initial PIC was observed in all animals within 10 min of the onset of ischaemia, sulforaphane pre-treatment in wild type mice significantly reduced the number of subsequent PICs. In Nrf2 deficient mice, an equivalent and statistically significant reduction in PIC number was seen associated with sulforaphane pre-treatment, implying that the effects of sulforaphane on PIC frequency are unlikely to be mediated by Nrf2 but rather associated with associated redox gene induction. As sulforaphane significantly improved post-reperfusion core blood flow in wild type not Nrf2 deficient mice.

Conclusions Our findings suggest that peri-infarct depolarisation waves or baseline Nrf2 genetic status are not major contributors to post-reperfusion, no-reflow in ischaemic stroke. However, activation of Nrf2 target genes by dietary sulforaphane protects against no-reflow pathology with persistent benefits post stroke.

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DEFINING THE NORMAL SPECTRUM OF ELECTROCARDIOGRAPHIC AND STRUCTURAL ADAPTATIONS OF WHITE, BLACK AND MIXED-RACE ADOLESCENT ATHLETES' HEART

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Introduction This study aimed to describe the normal spectrum of EKG changes and left ventricular dimensions in a large cohort of healthy white, black and mixed-race, male and female adolescent soccer players. There are a paucity of data describing the adolescent athlete's heart with the vast majority of studies based on adult white athletes. Not only are adolescent athletes (aged 14–18 years old) significantly understudied compared with adults but are more vulnerable to sudden cardiac death than adults. There is a four to six-fold higher incidence of sudden cardiac death (SCD) during exercise among young black athletes (aged 14–35 years) compared with young white athletes. Such a significant disparity has never before

been systematically explored, due to the limitations in identifying individuals at risk and a consequent lack of a well-defined population. The population of black individuals in the UK is 8 million, making up 13% of the overall population. In professional sport however, black athletes constitute up to 30% of all sportsmen and sportswomen. Moreover, the mixed-race athlete's EKG and echocardiogram has not before been described, though this is the fastest growing ethnic group participating in sport in the UK and US and for whom no such previous data exist.

Methods Between 1996 and 2016, 11 435 consecutive adolescent soccer players underwent pre-participation screening. 11 168 soccer players (mean age 16.4 ± 1.3 years) were evaluated with an EKG and echocardiogram measuring maximal left ventricular wall thickness (MLVWT) and left ventricular end diastolic diameter (LVEDD). Ethnicity was self-reported in accordance with UK census classification.

Results The cohort included 10 581 (95%) males and 587 (5%) females. There were 9664 (87%) white, 1004 (9%) black and 500 (4%) mixed-race athletes.

Black male athletes exhibited a greater MLVWT compared with both white (10.9 vs. 10.6 mm; $p=0.0323$). (Figure 1). White male athletes demonstrated the largest LV cavity sizes compared with black athletes (52.4 vs. 50.6 mm; $p=0.0477$).

Black female athletes revealed a greater MLVWT compared with white female athletes (8.6 vs. 8.2 mm; $p=0.0352$). As with males, white female athletes demonstrated larger LV cavity sizes than black athletes (46.3 vs. 45.2 mm; $p=0.0088$) and mixed-race athletes (46.3 vs. 45.4 mm; $p=0.0316$).

For white males, a proposed upper limit for MLVWT is 13 mm and for black and mixed-race males <14 mm. For females of all three ethnic groups this cut off is <11 mm.

Abstract 8 Figure 1 Bar chart demonstrating the distribution of maximal left ventricular wall thickness in adolescent (A, top) male and (B, bottom) female athletes of white, black and mixed-race ethnicity. Upper limits according to 2 standard deviations (SD) are also shown