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Changes to both cardiac metabolism and performance accompany acute reductions in functional capillary supply

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ABSTRACT

Background: The relative importance of arteriole supply or ability to switch between substrates to preserve cardiac performance is currently unclear, but may be critically important in conditions such as diabetes.
Methods: Metabolism of substrates was measured before and after infusion of polystyrene microspheres in the perfused working heart to mimic random capillary loss due to microvascular disease. The effect of acute loss of functional capillary supply on palmitate and glucose metabolism together with function was quantified, and theoretical tissue oxygen distribution calculated from histological samples and ventricular VO_2 estimated.
Results: Microsphere infusion led to a dose-dependent decrease in rate-pressure product (RPP) and oxygen consumption ($P < 0.001$). Microsphere infusion also increased work/unit oxygen consumption of hearts ('efficiency') by 25% ($P < 0.01$). When corrected for cardiac work palmitate oxidation remained tightly coupled to very low workloads (RPP < 2500 mm Hg/min), illustrating a high degree of metabolic control. Arteriole occlusion by microspheres decreased the density of patent capillaries ($P < 0.001$) and correspondingly increased the average capillary supply area by 40% ($P < 0.01$). Calculated rates of oxygen consumption declined from 16.6 ± 7.2 ml/100 ml/min to 12.4 ± 9 ml/100 ml/min following arteriole occlusion, coupled with increases in size of regions of myocardial hypoxia (Control = 22.0% vs. Microspheres = 42.2%).
Conclusions: Cardiac mechanical performance is very sensitive to arteriolar blockade, but metabolite switching from fatty acid to glucose utilisation may also support cardiac function in regions of declining PO_2.
General significance: Preserving functional capillary supply may be critical for maintenance of cardiac function when metabolic flexibility is lost, as in diabetes.

1. Introduction

The heart has a high and unremitting demand for energy derived largely from oxidation of lipids, with a lesser contribution from glucose [1]. Lipid oxidation requires an adequate supply of oxygenated blood to facilitate maximum ATP production from the fewest molecules of substrate. However, complete oxidation of glucose yields more ATP per molecule of oxygen consumed, and is hence more efficient where oxygen availability may be limiting. Indeed, inhibition of fatty acid β-oxidation with trimetazidine led to increased glucose oxidation, coupled with increases in cardiac mechanical work despite no changes in oxygen consumption for both rat [2] and human hearts [3], suggesting more efficient ATP production. Given the relationship between oxygen consumption and coronary flow [4], substrate selection by the working heart may be dictated by the availability of oxygen and/or coronary flow. Hence, conditions such as compensated cardiac hypertrophy that are characterised by regions of tissue ischaemia within the myocardium [5] exhibit increased glucose oxidation and decreased fatty acid β-oxidation [6]. Further compromise to oxygen supply, e.g. during decompensated cardiac failure and ischaemic injury, leads to an increased reliance on glycolysis, enabling production of ATP without requiring oxygen [7]. This is inefficient in terms of substrate use, characterised by the heart transitioning from being a net importer of lactate to the synthesis and export of excess lactate [8]. Therefore,

Abbreviations: CD, capillary density; HR, heart rate; KH, Krebs Henseleit buffer; LAD, left anterior descending coronary artery; NN, nearest neighbour; PO_2, partial pressure of oxygen; RPP, rate-pressure product; SAN, sino-atrial node.

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capillary supply of oxygenated blood is critically important to the optimisation of myocardial ATP production. This may be of particular consequence following rarefaction of capillary supply to the heart, as occurs in conditions such as diabetes [9] and hypertension [10].

Recent experiments document the heterogeneous nature of blood flow distribution within the adult heart *in vivo*, suggesting that not all capillaries are continuously perfused. Indeed, periods of reduced myocardial flow may be a normal cardiac phenomenon, largely controlled by arteriolar autoregulation [11]. Other factors may influence oxygen delivery beyond arteriolar dilatation, and hence perfusion. For example, diabetes led to a thickening of myocyte capillary basement membranes in both rats [12] and humans [13] with resulting increases in capillary diffusion distance presenting physical barriers to oxygen exchange [14]. Furthermore, increases in cardiac muscle fibre diameter (as typified by cardiac hypertrophy) lead to increased intercapillary distance [15] and reduced cardiac performance [16]. In addition, heterogeneity of capillary supply may be an independent variable in limiting oxygen delivery [17], with increases in heterogeneity associated with greater severity of cardiac impairment [18].

Decreased perfusion of cardiac muscle and impaired coronary flow reserve may predict mortality in humans [10] with microvascular diseases such as diabetes [11], as a close correlation between oxygen demand and capillary distribution normally matches delivery with utilisation [19]. We therefore postulated that if microvascular units (an individual arteriole and its associated capillaries supplied downstream) are responsible for local provision of oxygen to the myocardium, loss of functional capillaries by occlusion of individual arterioles would decrease work performed by the heart, and hence decreased total metabolism, without altering the balance between lipid and glucose metabolism for the remaining muscle fibres as no 'spillover' of oxygen into adjacent capillary domains may occur. Conversely, if diffusion of oxygen over wider distances supports metabolism in neighbouring fibres/capillary domains then metabolism will be altered to improve the efficiency of ATP production through 'metabolic flexibility'. This response involves switching of substrate use to efficiently exploit the prevailing oxygen supply, exemplified by a decrease in fatty acid oxidation and increased reliance on glucose metabolism.

The isolated crystalloid-perfused heart offers the best method to investigate the effects of altered functional capillary supply in the heart. This overcomes autoregulation of coronary arterioles by perfusing with a high oxygen partial pressure yet low content medium [20,21] enabling the contribution of individual, maximally-dilated arterioles to contractile performance and regional metabolism in the myocardium to be investigated. Exploiting microspheres, we selectively occluded arterioles in the perfused heart from naive rats. Histological analysis was used to discriminate between patent capillaries and those with microsphere-occluded flow to estimate degree of occlusion, and correlate this with myocardial function and metabolism as well as calculated tissue oxygenation.

2. Materials & methods

2.1. Materials

^{3}H-[9,10]-oleic acid and [U-^{14}C] glucose were purchased from Amersham Biosciences (Chalfont, UK); polystyrene microspheres (15 μm mean diameter) from Molecular Probes (Eugene, Oregon, USA); fatty acid-free bovine albumin and all buffer salts from Sigma (Poole, UK); fluorescein-labelled lectin was purchased from Vector laboratories (UK), Cy3-labelled anti-α-smooth muscle actin antibody from Sigma (Poole, UK). All solvents were ANALAR grade and purchased from Fisher Scientific (Loughborough, UK). Ventricular balloons were constructed 'in house' using Saran Wrap polythene film.

2.2. Methods

2.2.1. Animal maintenance

All experiments were carried out in accordance with the UK Home Office, Animal Scientific Procedures Act (1986) and were approved by the University of Birmingham local ethics committee. Male Wistar rats (265 ± 11 g) were maintained and housed at 22 °C 12 h light/12 hr dark with *ad libitum* access to food (Lillico RM3, rat chow) and water throughout the experiment. A total of 35 untreated rats were used.

2.2.2. Tissue isolation and heart perfusion

Hearts were prepared from fed rats as outlined previously [22]. Briefly, anaesthesia was induced with halothane (4% in oxygen) and following thoracotomy hearts were excised and the aorta cannulated (16G cannula), then perfused in retrograde fashion [23]. A small flexible non-elastic balloon was inserted into the left atrium through the mitral valve and into the left ventricle. This fluid-filled balloon was attached to a fine plastic catheter and connected to a pressure transducer and a graduated syringe (0–1000 μl: Hamilton, Nevada, USA). Hearts were maintained at 37 °C and perfused at a constant pressure (100 cm H_2O) with a Krebs–Henseleit crystalloid medium (KH) supplemented with glucose (10 mM) and $CaCl_2$ (1.3 mM) gassed with oxygen/CO_2 (95:5). Developed pressure (peak—end diastolic pressure) was measured following isovolumic contraction and recorded to computer using a digital interface (AD Instruments, Chalgrove, Oxford, UK). The initial balloon volume was adjusted until measured end-diastolic pressure was 0 mm Hg and the systolic pressure was recorded. Balloon volume was increased in incremental steps (50 μl) until the peak systolic pressure developed exceeded 200 mm Hg and developed pressure was recorded in real time. Coronary flow was estimated from timed collections of a known volume of perfusate and expressed as volume/time/unit mass of cardiac tissue. Preliminary experiments were undertaken to establish a single concentration of microspheres needed to produce a significant decrease in cardiac work. For selected experiments, 1×10^5–1×10^7 microspheres (mean diameter 15 ± 0.2 μm, appropriate to block terminal arterioles) were suspended in KH-bovine albumin solution (4% *w*/*v*) and were infused directly into the aortic perfusion line. Given the retrograde nature of this perfusion technique microspheres were infused directly into the coronary circulation. A period of stabilisation (10 min) followed prior to repetition of the performance estimation (Supplemental Fig. 1A). Ventricular performance was calculated off-line as detailed previously [23].

For separate perfusions quantifying 'steady-state' functional parameters of the heart, hearts were perfused as detailed above with intraventricular balloon incorporated in the LV [23]. The LV balloon volume was adjusted to give an end-diastolic pressure (EDP) = 10 mm Hg until EDP was stable (typically 5 min). Estimates of systolic pressure, developed pressure and heart rate were calculated and performance was calculated as above [23].

2.2.3. Perfused working heart

Working hearts were perfused as previously described [22]. Atrial filling pressure was fixed at 10 cm H_2O with an afterload fixed at 100 cm H_2O. Hearts were perfused with KH, together with glucose (10 mM supplemented with U-^{14}C-labelled glucose 0.185 MBq/perfusion) and palmitic acid (0.4 mM pre-bound to bovine albumin + ^{3}H palmitic acid 5.55 MBq/perfusion). All hearts were unpaced. Metabolism was estimated from timed collection of perfusate and effluent gases (see below) for 45 min to quantify utilisation of glucose and palmitate (Supplemental Fig. 1B). The heart was then returned to perfusion in Langendorff mode and microspheres in KH infused directly into the aortic line and the heart retrograde perfused for a further 3 min to ensure transfer of microspheres into the coronary circulation. Hearts were then returned to 'working mode' and perfused through the left atrium at fixed pre- and afterload, as above. Timed collections of both perfusate and effluent gases were then continued for a further 45 min