Metabolic Control of Hypoxia Sensing in the Diabetic Heart

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Project outline

Background: During a myocardial infarction (MI) the heart becomes hypoxic and responds by activating the Hypoxia-Inducible Factor (HIF)1α, an essential signalling pathway that allows the cells to survive. We have shown that HIF1α activation and adaptation to hypoxia is blunted in type 2 diabetes, accelerating the progression into heart failure (Figure). We have shown that abnormal metabolism is implicated in this blunted HIF response in diabetes, however, we don't fully understand the mechanisms responsible. Unravelling the disease biology that links metabolism to HIF may lead to the development of new therapeutic targets for the treatment of heart failure in type 2 diabetes.

Hypothesis: Dysfunctional metabolism within the diabetic myocardium drives a blunted HIF1α response and adaptation to hypoxia, making the heart less able to recovery post-MI

Aims and description of work to be undertaken:

Aim 1: Identify the signalling pathways activated in response to hypoxia, and which are perturbed in diabetes. Using human cardiomyocytes derived from iPS cells we will use dual transcriptomics and metabolomics to identify the signalling pathways activated as part of the hypoxic response, how these pathways differ in the presence of insulin resistance and the accompanying metabolic signatures. Over-representation analysis will be used to establish enriched pathways found in both significantly changing genes and metabolites in response to diabetes. The Pathway tool will be used to integrate and visualise the transcript and metabolite levels of these pathways to confirm their importance. Key findings will be validated at the protein level.

Aim 2: Identify which aspect of the diabetic environment are driving the blunted hypoxic response. We have evidence that the high fat environment of the diabetic heart is capable of blunting HIF1α activation (Figure), but we do not fully understand how this effect is being exerted. We will use novel lipid-based photoaffinity probes that carry a diazirine photoreactive crosslinking group, which can covalently capture lipid-protein interactions. Biorthogonal conjugation to an affinity tag, followed by proteomics, will allow us to identify which proteins are physically interacting with intracellular fatty acids, and how these change in hypoxia and diabetes. We can adapt these probes to investigate different fatty acids (saturated, unsaturated, chain lengths) and fatty acid derivates (acyl carnitines, acyl CoAs), to understand the specificity and selectively of lipid-induced suppression of hypoxia

signalling pathways. We will also investigate whether other aspects of the diabetic environment can modulate HIF signalling by exposing human cardiomyocytes to increased concentrations of glucose, insulin, ketone bodies and branched chain amino acids in the presence and absence of hypoxia, followed by PCR and western blotting to measure downstream target genes and proteins.

Aim 3: Identify the mechanisms that link abnormal metabolism to an abnormal response to hypoxia. Using siRNA and pharmacological approaches we will inhibit key steps in identified pathways, to confirm the mechanism that link our metabolite of interest to hypoxic signalling pathways. This will be followed up by looking for genetic associations in human GWAS studies, to confirm the association between target genes and disease pathology. Furthermore, using radiotracers and ^{13}C-labelled metabolites we will track the metabolic pathways and how they differ in response to hypoxia, to elucidate which downstream intermediates have accumulated/depleted in response to hypoxia.

Aim 4: Understand the physiological relevance of this metabolic regulation of hypoxia signalling. Metabolites, particularly lipids, show a circadian rhythm through the day, and recently HIF1α was shown to be regulated by the circadian clock, therefore, we will investigate how the rhythm of metabolism and HIF influences the ability to respond to hypoxia at different times of the day. In animal models we will investigate whether the circadian rhythm of circulating lipids and cardiac HIF1α coincide, to advance our understanding of why an MI is more likely to occur in the early hours of the morning.

Outcome: Taken together this work will identify the mechanisms and drivers behind the abnormal response to hypoxia that occurs following MI in diabetes, which may lead to the identification of new targets to slow the progression of heart failure in type 2 diabetes.
The Fellow will gain excellent interdisciplinary training and experience - spanning human cell and disease biology, pharmacology, omics, bioinformatics, and animal models - at the cutting-edge interface of academic and drug discovery research in cardiometabolic disease.

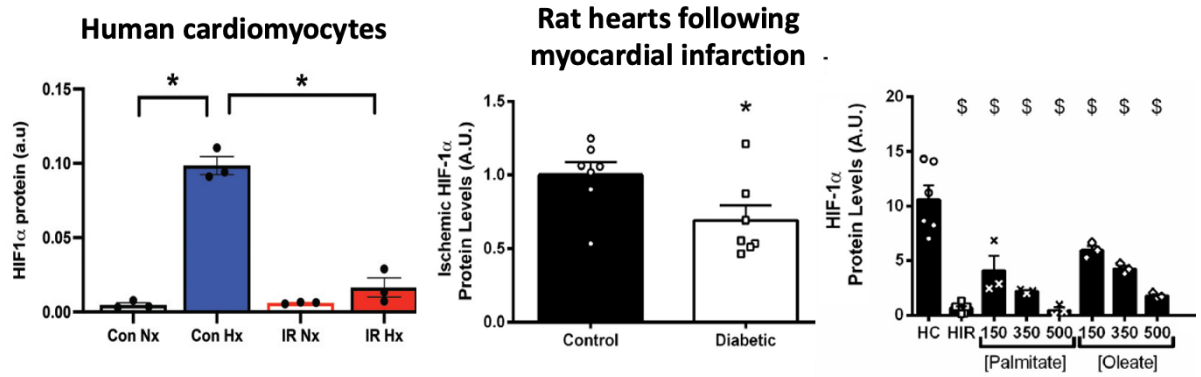

Figure. Abnormal response to hypoxia and myocardial infarction in insulin resistance (IR) and type 2 diabetes. Fatty acids, palmitate and oleate, can suppress HIF1α activation in response to hypoxia.

Contributions of Oxford and NNRCO supervisors:

L Heather will contribute techniques for studying hypoxia and metabolic expertise to investigate the role of metabolism. A Russell will contribute new lipid probes to study the interactions between

lipids and proteins within the cell. T Durrant will contribute expertise in transcriptomics, siRNA and utilisation of human genetics to confirm potential target genes.

Supervisor's recent relevant publications (5 max per supervisor):

1. Sousa Fialho MdaL, Purnama U, Dennis K, Montes Aparicio C, Castro-Guarda M, Massourides E, Tyler DJ, Carr CA and **Heather LC**. Activation of HIF1α Rescues the Hypoxic Response and Reverses Metabolic Dysfunction in the Diabetic Heart. Diabetes, 2021, doi.org/10.2337/db21-0398.
2. Kerr M, Dennis KMJH, Carr CA, Fuller W, Berridge G, Rohling S, Aitken CL, Lopez C, Fischer R, Miller JJ, Clarke K, Tyler DJ, and **Heather LC.** Diabetic mitochondria are resistant to palmitoyl CoA inhibition of respiration, which is detrimental during ischemia. FASEB J, 2021, doi: 10.1096/fj.202100394R
3. Kerr M, Miller JJ, Thapa D, Stiewe S, Timm KN, Aparicio CNM, Scott I, Tyler DJ, **Heather LC**. Rescue of myocardial energetic dysfunction in diabetes through the correction of mitochondrial hyperacetylation by honokiol. JCI Insight, 2020, Sep 3;5 e140326.
4. Dodd MS, Sousa Fialho Md L, Montes Aparicio CN, *et al,* and **Heather LC**. Fatty acids prevent Hypoxia-inducible Factor 1α signalling through decreased Succinate in Diabetes. J Am Coll Cardiol: Basic Transl Sci, 2018.
5. Mansor LM, Sousa Fialho M da L, Yea G, *et al,* **and Heather LC.** Inhibition of sarcolemmal FAT/CD36 by sulfo-N-succinimidyl oleate rapidly corrects metabolism and restores function in the diabetic heart following hypoxia/reoxygenation. Cardiovascular Research, 2017.