The Gift of Light

Using Multiplexed Optical Imaging to Probe Cardiac Metabolism in Health and Disease

Mirwais Wardak, PhD; Patricia K. Nguyen, MD

Downloaded from http://circimaging.ahajournals.org/ by guest on April 25, 2018

Heart failure (HF) resulting from impaired left ventricular (LV) function is associated with significant morbidity and mortality in the United States and worldwide.[1] In the failing heart, there are changes in energy substrate metabolism whose causes and effects are poorly understood.[2] These changes may contribute to deterioration in cardiac contractility and to increasing LV remodeling that are the hallmarks of the failing heart. In HF following cardiac hypertrophy, there is a major metabolic switch in myocardial substrate metabolism from fatty acid to glucose oxidation.[2–5] A key marker of this switch is the coordinated downregulation of fatty acid oxidation enzymes (eg, medium-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase-I β) and mRNA levels in the human LV with a concomitant increase in glucose uptake and oxidation.[2,6] This switch is thought to represent a metabolic reprogramming toward substrate metabolism that is more commonly seen in the fetal heart.[7] The fetus in a womb uses glucose from the mother as the major substrate for energy production; in fact, ≈80% of fetal energy comes from glucose oxidation.

See Article by Panagia et al

Moreover, recent studies have suggested that brown adipose tissue (BAT) may play an important role in obesity, type 2 diabetes mellitus, and HF.[8–13] BAT regulates basal and inducible energy expenditure in humans and is composed of cells that contain numerous lipid droplets, dense packing of mitochondria, and the expression of the mitochondria-associated UCP1 (uncoupling protein 1).[11] UCP1 mediates the biochemical uncoupling of electron transport by allowing proton leakage across the inner mitochondrial membrane into the mitochondrial matrix, thus short-circuiting the usual linkage of electron transport to adenosine triphosphate production that is catalyzed by ATP synthase.[11] Instead, the chemical energy generated by cellular respiration in brown adipocytes is dissipated in the form of heat, which allows thermogenesis by mammals in cold environments and newborn humans. Traditionally, BAT was considered to be no longer present and possessed no significant physiological functions in adult humans; however, positron emission tomography/computed tomography (PET/CT) imaging investigations have clearly demonstrated that discreet depots of BAT are still present in human adults.[14] These studies have relied on the use of clinical PET/CT to correlate focal 2-deoxy-2-[F-18]fluoro-D-glucose (2-FDG) supraclavicular uptake to tissue fat density rather than solely muscle and termed the finding as uptake in supraclavicular area fat or "USA fat."

In this issue of *Circulation: Cardiovascular Imaging*, Panagia et al[15] aimed to develop a multiplexed optical imaging platform to simultaneously assess the uptake of glucose and fatty acid analogs in multiple organs in a murine model of LV hypertrophy (LVH). They hoped that this approach would leverage the strength of optical imaging to examine multiple processes in various tissues in a simple and high-throughput fashion. In their study, the detection of glucose uptake was measured with 2-FDG via Cerenkov luminescence imaging, a technique that was first used to image tumors in mice but also enabled visualization of the heart.[16] In addition, the uptake of free fatty acids (FFA) was quantified using a synthetic, long-chain fluorescent FFA (BODIPY FL C_{16}). Both of these imaging agents were injected at the same time intraperitoneally. Simultaneous uptake of these agents was measured in the myocardium, intrascapular BAT, epididymal white adipose tissue, and skeletal muscle of the quadriceps in mice with and without thoracic aortic constriction/banding (TAC). Although imaging was performed ex vivo after the animals had been euthanized and the tissues harvested, the authors do show that Cerenkov images of 2-FDG uptake in the mouse heart could be acquired in vivo as well.

The authors report that within 5 weeks post-TAC, mice developed LVH and BAT activation with upregulation of β_3 adrenergic receptors and increased natriuretic peptide receptor ratio. Recent studies have suggested that communication between the heart and BAT is possibly mediated through sympathetic stimulation and cardiac hormones such as natriuretic peptides.[8,12,13] In addition, there may be reciprocal communication between BAT and the heart.[8] The heart was shown to be significantly increased in weight at both the 5-week and 15-week time points post-TAC when compared with the controls, whereas there was a significant reduction in BAT weight (40%) at the 15-week time point only. It was noted that the pattern of BAT activation was highly influenced by the

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Department of Radiology (M.W.), Molecular Imaging Program at Stanford (MIPS) (M.W.), Division of Cardiovascular Medicine, Department of Medicine (P.K.N.), and Stanford Cardiovascular Institute (M.W., P.K.N.), Stanford University School of Medicine, CA; and Cardiology Section, Veterans Affairs Palo Alto Health Care Administration, CA (P.K.N.).

Correspondence to Patricia K. Nguyen, MD, Cardiology Section, Veterans Affairs Palo Alto Health Care Administration, Bldg 100, 3801 Miranda Ave, Palo Alto, CA 94304. E-mail pknguyen@stanford.edu

(*Circ Cardiovasc Imaging*. 2018;11:e007597. DOI: 10.1161/CIRCIMAGING.118.007597.)

© 2018 American Heart Association, Inc.

***Circ Cardiovasc Imaging* is available at http://circimaging.ahajournals.org**
DOI: 10.1161/CIRCIMAGING.118.007597

duration of hypertrophy and the transition from a preserved (at 5 weeks post-TAC) to a reduced ejection fraction (at 15 weeks post-TAC).

Imaging of BAT 15 weeks post-TAC surgery revealed an increase in glucose ($P<0.01$) and FFA ($P<0.001$) uptake versus controls and an increase in UCP1 ($P<0.01$). Similar but less robust changes were seen in skeletal muscle, whereas glucose and FFA uptake in white adipose tissue remained unchanged. Glucose uptake via the glucose transporters (eg, GLUTs) in the myocardium was significantly increased at 15 weeks post-TAC (>70%), but FFA uptake seemed to decrease and was not statistically significant. Although these findings add to our understanding of the relationship between BAT and LVH, it remains unclear whether BAT activation in LVH represents an adaptive or maladaptive event.

Despite the novel platform presented by the authors, several limitations must be acknowledged. Optical imaging techniques like the one described in this study will generally be confined for use in preclinical studies because of its limited penetration depth. The poor penetration will hold back its applications in humans, although the development of near-infrared fluorescence imaging may allow this approach to delve deeper into the body. The other drawback of optical imaging is its resolution, which is good near the surface, but poorer for deeper structures such as the heart. In human subjects, optical imaging is likely to remain restricted to relatively superficial targets because of the absorbing and scattering properties of tissue in the visible and near-infrared portion of the electromagnetic spectrum.

PET imaging, on the other hand, is relatively depth independent because of its highly penetrating pair of back-to-back 180° gamma rays that are emitted from the body. PET is an imaging technology that is directed at the biochemical processes that underlie all body functions and their alterations in disease.[17] Efforts are currently underway to build a new type of PET scanner that will allow for multiplex imaging so that two or more PET tracers can be injected at the same time. This would reduce the time and imaging costs to simultaneously acquire multiple biochemical and physiological information about an organ or body of interest. The current use of a mouse hotel, which can house at least 4 mice for preclinical PET studies, demonstrates the high-throughput nature of microPET imaging.[18] PET/CT and PET/magnetic resonance imaging (PET/MRI) will, thus, remain vital centerpieces for preclinical and clinical translation of molecular imaging agents in the study of human health and disease, and this is no more important than in the heart.

Another limitation of the current study is that the authors restricted themselves to using only 2-FDG for studying glucose uptake and metabolism. There are 2 major classes of glucose transporters in the body, the passive facilitative glucose transporters (GLUTs) and the active sodium-coupled glucose transporters (SGLTs).[19] 2-FDG is only transported through the GLUTs, but not through the SGLTs. New F-18 labeled PET tracers, like methyl-4-FDG, have been shown to be excellent substrates specifically for the SGLTs.[19] Uptake of methyl-4-FDG into the heart may be useful for diagnostic purposes in HF as SGLT genes are expressed in cardiomyocytes. Imaging with both 2-FDG and methyl-4-FDG will therefore give a more complete picture of how glucose is transported and metabolized in healthy and diseased tissue. It is exciting to highlight that although the current study was limited to the imaging of glucose and FFAs, the authors expect that 4 to 5 spectrally resolvable substrates could be imaged simultaneously with their approach. The authors should be commended for their efforts to synergistically merge the fields of optical imaging and energy metabolism to elucidate novel mechanisms that underlie cardiac pathophysiology. Finally, it would have been interesting to include both male and female animals in this study to examine sex differences in cardiac metabolism in LVH and HF. Heart complications often take different forms in men and women.[20] Women are known to adapt differently than men to cardiac pressure overload. Female heart walls tend to stiffen and get thicker, decreasing the ability of blood to enter the LV. Male heart walls get thinner, weakening pumping power. It would, thus, be interesting to compare the bioenergetics and metabolic changes in the failing hearts of males and females and see the role of metabolic imaging in guiding and evaluating therapeutic interventions.

In conclusion, the authors present a novel optical approach that can permit the imaging of multiple metabolic and energetic pathways in the heart and peripheral tissues in a preclinical mouse model of LVH. This method could provide important insights into HF pathogenesis and help resolve its outstanding metabolic mysteries. Moreover, the effects of metabolic therapies in LVH and HF (eg, drugs that can modulate myocardial glucose and fatty acid metabolism) could be investigated in future studies. For human translation, however, PET/CT and PET/MRI will remain the essential imaging modalities for studying the underlying biochemical processes in cardiac disease.

Sources of Funding

We thank the National Institutes of Health grants R01 HL134830 (Dr Nguyen) and the Multidisciplinary Training Program in Cardiovascular Imaging at Stanford University 5T32 EB009035 (Dr Wardak).

Disclosures

None.

References

1. Benjamin EJ, Virani SS, Callaway CW, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, Deo R, de Ferranti SD, Ferguson JF, Fornage M, Gillespie C, Isasi CR, Jimenez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Lutsey PL, Matchar DB, Matsushita K, Mussolino ME, Nasir K, O'Flaherty M, Palaniappan LP, Pandey DK, Reeves MJ, Ritchey MD, Rodriguez CJ, Roth GA, Rosamond WD, Sampson UKA, Satou GM, Shah SH, Spartano NL, Tirschwell DL, Tsao CW, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P. Heart disease and stroke statistics-2018 update: a report from the American Heart Association [published online ahead of print Janurary 31, 2018]. *Circulation*. doi: 10.1161/CIR.0000000000000558.
2. Marín-García J. (2010). Bioenergetics and Metabolic Changes in the Failing Heart. In *Heart Failure: Bench to Bedside* (45-69). London, UK: Springer.
3. Nickel A, Löffler J, Maack C. Myocardial energetics in heart failure. *Basic Res Cardiol*. 2013;108:358. doi: 10.1007/s00395-013-0358-9.
4. Ventura-Clapier R, Garnier A, Veksler V, Joubert F. Bioenergetics of the failing heart. *Biochim Biophys Acta*. 2011;1813:1360–1372. doi: 10.1016/j.bbamcr.2010.09.006.
5. Neubauer S. The failing heart–an engine out of fuel. *N Engl J Med*. 2007;356:1140–1151. doi: 10.1056/NEJMra063052.

Downloaded from http://circimaging.ahajournals.org/ by guest on April 25, 2018

6. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. *Circulation*. 1996;94:2837–2842.
7. Rees ML, Subramaniam J, Li Y, Hamilton DJ, Frazier OH, Taegtmeyer H. A PKM2 signature in the failing heart. *Biochem Biophys Res Commun*. 2015;459:430–436. doi: 10.1016/j.bbrc.2015.02.122.
8. Panagia M, Chen YC, Chen HH, Ernande L, Chen C, Chao W, Kwong K, Scherrer-Crosbie M, Sosnovik DE. Functional and anatomical characterization of brown adipose tissue in heart failure with blood oxygen level dependent magnetic resonance. *NMR Biomed*. 2016;29:978–984. doi: 10.1002/nbm.3557.
9. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One*. 2011;6:e17247. doi: 10.1371/journal.pone.0017247.
10. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest*. 2013;123:215–223. doi: 10.1172/JCI62308.
11. Sampath SC, Sampath SC, Bredella MA, Cypess AM, Torriani M. Imaging of brown adipose tissue: state of the art. *Radiology*. 2016;280:4–19. doi: 10.1148/radiol.2016150390.
12. Collins S. A heart-adipose tissue connection in the regulation of energy metabolism. *Nat Rev Endocrinol*. 2014;10:157–163.
13. Turer AT, Hill JA, Elmquist JK, Scherer PE. Adipose tissue biology and cardiomyopathy: translational implications. *Circ Res*. 2012;111:1565–1577. doi: 10.1161/CIRCRESAHA.111.262493.
14. Cohade C, Osman M, Pannu HK, Wahl RL. Uptake in supraclavicular area fat ("USA-Fat"): description on ^{18}F-FDG PET/CT. *J Nucl Med*. 2003;44:170–176.
15. Panagia M, Chen HH, Croteau D, Iris Chen YC, Ran C, Luptak I, Josephson L, Colucci WS, Sosnovik DE. Multiplexed optical imaging of energy substrates reveals that left ventricular hypertrophy is associated with brown adipose tissue activation. *Circ Cardiovas Imaging*. 2018;11:e007007. doi: 10.1161/CIRCIMAGING.117.007007.
16. Robertson R, Germanos MS, Li C, Mitchell GS, Cherry SR, Silva MD. Optical imaging of Cerenkov light generation from positron-emitting radiotracers. *Phys Med Biol*. 2009;54:N355–N365. doi: 10.1088/0031-9155/54/16/N01.
17. Phelps ME. PET: the merging of biology and imaging into molecular imaging. *J Nucl Med*. 2000;41:661–681.
18. Habte F, Ren G, Doyle TC, Liu H, Cheng Z, Paik DS. Impact of a multiple mice holder on quantitation of high-throughput MicroPET imaging with and without CT attenuation correction. *Mol Imaging Biol*. 2013;15:569–575. doi: 10.1007/s11307-012-0602-y.
19. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. *Physiol Rev*. 2011;91:733–794. doi: 10.1152/physrev.00055.2009.
20. Stefanick ML. Not just for men. *Sci Am*. 2017;317:52–57. doi: 10.1038/scientificamerican0917-52.

KEY WORDS: Editorials ■ adipose tissue ■ cardiac imaging techniques ■ heart failure ■ hypertrophy ■ metabolism ■ mice

Downloaded from http://circimaging.ahajournals.org/ by guest on April 25, 2018

Circulation
Cardiovascular Imaging

American Heart Association®

The Gift of Light: Using Multiplexed Optical Imaging to Probe Cardiac Metabolism in Health and Disease

Mirwais Wardak and Patricia K. Nguyen

Circ Cardiovasc Imaging. 2018;11:
doi: 10.1161/CIRCIMAGING.118.007597
Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2018 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/11/3/e007597

Downloaded from http://circimaging.ahajournals.org/ by guest on April 25, 2018

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Cardiovascular Imaging* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation: Cardiovascular Imaging* is online at:
http://circimaging.ahajournals.org//subscriptions/