JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023

EDITORIAL COMMENT

Ketone Bodies Preserve Mitochondria Through Epigenetics*

Risa Mukai, PhD, Junichi Sadoshima, MD, PhD

Ketone bodies have drawn the attention of many investigators in heart failure research because they not only serve as an alternative fuel source during diminished fatty acid utilization but also act as signaling molecules independently of their oxidation, thereby protecting the heart.[1] Ketone bodies, including β-hydroxybutyrate (BHB), acetoacetate, and acetone, are produced by the hepatic ketogenesis pathway and are then oxidized in extrahepatic organs; they are subjected to ketolysis to produce acetyl coenzyme A in mitochondria, which then enters the tricarboxylic acid cycle and produces adenosine triphosphate via oxidative phosphorylation. Utilization of ketone bodies is increased during hypertrophy and heart failure in both mice and humans, thereby playing salutary roles in the failing heart. However, our understanding of the molecular actions of ketone bodies, particularly those acting independently of oxidation, remains limited.

Increasing lines of evidence suggest that BHB affects epigenetics through multiple mechanisms.[2] In the current issue of the *JACC: Basic to Translational Science*, Gambardella et al[3] conducted unbiased mass spectrometry analyses and observed that simultaneous histone 3 methylation at 2 lysine residues, namely dimethylation of histone 3 at lysine 27 and monomethylation of histone 3 at lysine 36 (H3_K27me2K36me1), is induced in postischemic heart failure in mice and humans. They discovered that H3_K27me2K36me1 is negatively regulated by BHB. Furthermore, PPARG coactivator 1α (PGC-1α), a master regulator of mitochondrial function and biogenesis, is negatively regulated by H3_K27me2K36me1. Down-regulation of PGC-1α during heart failure was alleviated in the presence of BHB, accompanied by the reversal of mitochondrial dysfunction and heart failure (**Figure 1**).

The report by Gambardella et al[3] provides novel insights into the signaling actions of BHB. Post-translational modifications (PTMs) of histones are key mechanisms of epigenetic regulation, potentially connecting metabolism to gene expression.[4] BHB promotes acetylation of histones K9 and K14 through inhibition of histone deacetylase 1 (HDAC1), most likely because of the structural similarity of BHB to butyrate, a short-chain fatty acid that directly affects the enzyme activity of HDAC1. This acetylation enhances forkhead box O3-A (FOXO3A)-mediated gene transcription, resulting in protection against stress in mouse kidneys. BHB also promotes the activity of the EP300 family of lysine acetyltransferases, thereby protecting neurons against oxidative stress. In addition, BHB promotes H3K4me3 by inhibiting KDM6a, a histone demethylase, and induces histone lysine β-hydroxybutylation, a PTM of histone non-enzymatically promoted. PTMs of histone by BHB generally confer stress resistance in various organs. Gambardella et al[3] have shown that BHB inhibits H3_K27me2K36me1, thereby most likely alleviating heterochromatin and reactivating a gene expression program for improved mitochondrial function. H3_K27me2K36me1 was increased in the heart in the presence of stress and heart failure and appeared to correlate with the suppression of mitochondrial function. The study by Gambardella et al[3] not only shed light on the role of H3_K27me2K36me1, a novel form of H3 PTM, in the pathogenesis of heart failure, but also suggested how it may be regulated by BHB.

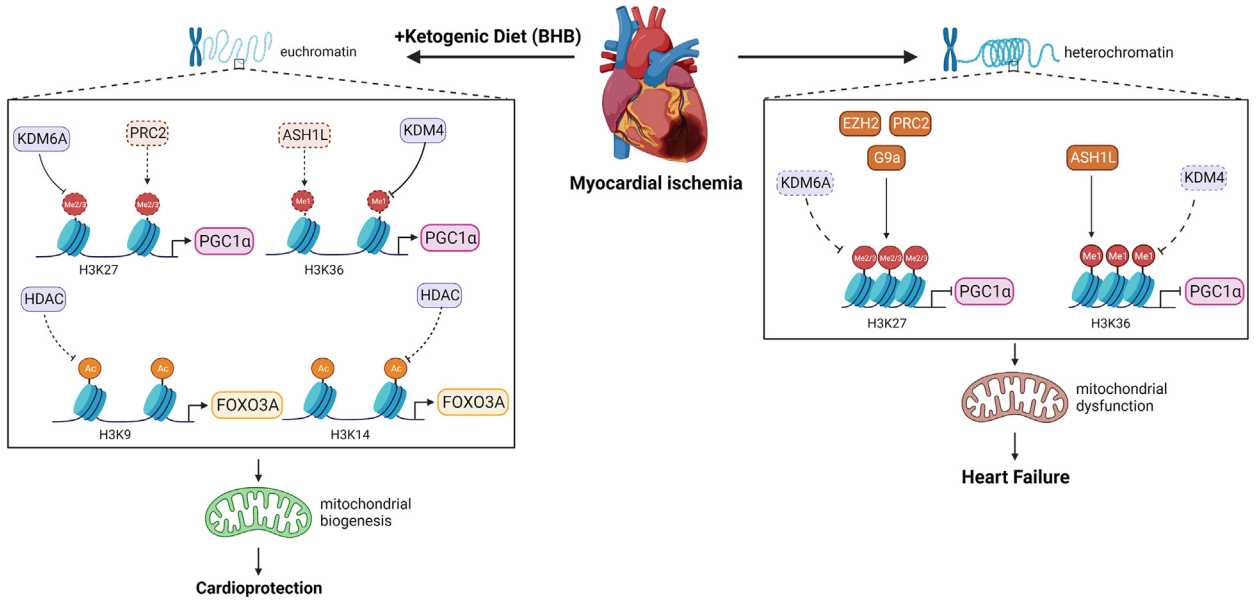
*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Rutgers New Jersey Medical School, Department of Cell Biology and Molecular Medicine, Newark, New Jersey, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.05.013

FIGURE 1 Potential Molecular Mechanisms Through Which Ketogenic Diet Supplemented With BHB Mediates Protective Effects in the Mouse Heart During Myocardial Ischemia



Ischemia and heart failure increase dimethylation of histone 3 at lysine 27 (H3K27me2) and monomethylation of histone 3at lysine 36 (H3K36me1), thereby inducing heterochromatin, which in turn suppresses PPARG coactivator 1α (PGC-1α) transcription. A ketogenic diet supplemented with β-hydroxybutyrate (BHB) inhibits H3K27me2 and H3K36me1, possibly by inhibiting methyltransferases such as polycomb repressive complex 2 (PRC2) and absent, small, or homeotic discs 1-like (ASH1L), and restores euchromatin and PGC-1α transcription. Restoration of PGC-1α expression maintains mitochondrial function and preserves cardiac function. BHB may also up-regulate cell protective mechanisms through acetylation of H3K9 and H3K14 by inhibiting histone deacetylase 1 (HDAC1). EZH2 = enhancer of zeste homolog 2; FOXO3A = forkhead box O3-A; G9a = methyltransferase that targets H3K27; KDM = histone demethylase.

Gambardella et al[3] propose that the effect of H3_K27me2K36me1 is mediated primarily through the suppression of PGC-1α. Although PGC-1α is not uniformly down-regulated in patients with heart failure and animal models, persistent down-regulation of PGC-1α could drive the progression of heart failure. Gambardella et al[3] have demonstrated an increased association of H3_K27me2K36me1 with the PGC-1α promoter during stress and alleviation of PGC-1α down-regulation by BIX01294, an inhibitor of G9a, a methyltransferase that targets H3K27. Thus, H3_K27me2K36me1 appears to promote mitochondrial dysfunction through down-regulation of PGC-1α. The functional significance of H3K27me2/3 in the postnatal heart under stress has just begun to be elucidated recently. Di- and trimethylation of H3K27 catalyzed by enhancer of zeste homolog 2, another methyltransferase and a component of polycomb repressive complex 2, is increased in ischemic cardiomyopathy and may contribute to gene reprogramming. The functional role of H3K27 methylation could be context-dependent: whereas it may protect the heart against pressure overload, it could also act detrimentally by down-regulating ion channels. Furthermore, the function of H3_K27me2K36me1 may be distinct from that of H3K27me2 alone. Thus, further investigation is needed to clarify how increased H3_K27me2K36me1 during cardiac stress affects the chromatin structure, the accessibility of genomic DNA to transcription factors, and the expression of downstream gene sets. For example, assay for transposase-accessible chromatin sequencing may allow the identification of additional factors besides PGC-1α whose expression is regulated by BHB through its effects on epigenetics.

Methylation of H3K36me is associated with euchromatin and enriched along actively transcribed genes. However, a previous study in fungi has shown that H3K36me1/H3K36me2 catalyzed by absent, small or homeotic discs 1-like methyltransferase drives gene repression and increases H3K27me2/3 marks.[5] Although BIX01294 can inhibit H3K36me in addition to its authentic effects on G9a-induced methylation of H3K27, the molecular mechanism through which cardiac stress induces H3K36me1 remains to be elucidated. Furthermore, how H3K36me1 coordinates

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023
SEPTEMBER 2023:1138–1140

with H3K27me to achieve heterochromatin in the heart also remains unknown.

Gambardella et al[3] demonstrated that BHB increases the level of S-adenosyl homocysteine, an inhibitor of histone methyltransferase. S-adenosyl homocysteine binds to the catalytic regions of most S-adenosylmethionine-dependent methyltransferases with high affinity, thereby acting as a potent product inhibitor. Thus, BHB may broadly inhibit the activity of histone methyltransferases. Although the observation strengthens the hypothesis that BHB may act as an independent signaling molecule, additional mechanisms through which BHB selectively targets G9a or an absent, small, or homeotic disc 1 homolog need to be clarified. Importantly, because BHB protects the heart through multiple mechanisms, the possibility that the suppressive effect of BHB on H3_K27me2K36me1 could be secondary to the improvement of cardiac function in vivo cannot be entirely excluded.

Gambardella et al[3] indicate that BHB treatment preserves PGC-1α expression by inhibiting its downregulation during ischemia and heart failure. PGC-1α is highly expressed in cardiomyocytes and maintains mitochondrial respiratory function and fatty acid oxidation rates. Thus, understanding the mechanism by which the function of PGC-1α is preserved during heart failure is essential. Expression of PGC-1α is regulated by multiple transcription factors and epigenetic mechanisms, the latter of which include H3K4me3, H3K9me3, and H3K9 acetylation. Thus, the relative importance of H3_K27me2K36me1 over other mechanisms in regulating PGC-1α expression remains to be elucidated. One caveat is that the function of PGC-1α is also regulated by PTMs of itself and epigenetics. Thus, further investigation is needed to clarify how the overall activity of PGC-1α is regulated during heart failure in the presence of BHB.

In summary, the study by Gambardella et al[3] extends our knowledge of the salutary actions of ketone bodies during heart failure. In particular, the study suggests the possibility that, besides its well-established function as an HDAC1 inhibitor, BHB affects H3 methylation through its effect as a signaling molecule. The study shows that BHB has the ability to preserve mitochondrial function, a fundamental feature needed for heart failure treatment. Because the effect on epigenetics could broadly affect multiple gene sites in a sustained manner, it would be interesting to clarify the salutary effect of BHB on chromatin modification in the heart in an extensive and unbiased fashion. Furthermore, it will also be important to investigate the epigenetic effect of ketogenic diets in human hearts.

ACKNOWLEDGMENT The authors thank Daniela Zablocki for critical reading of the manuscript.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported in part by U.S. Public Health Service grants HL67724, HL91469, HL102738, HL112330, HL138720, HL144626, HL150881, and AG23039 (to Dr Sadoshima); American Heart Association 18POST34060247 (to Dr Mukai) and 20 Merit 35120374 (to Dr Sadoshima); and the Fondation Leducq Transatlantic Network of Excellence 15CVD04 (to Dr Sadoshima).

ADDRESS FOR CORRESPONDENCE: Dr Junichi Sadoshima, Rutgers New Jersey Medical School, Department of Cell Biology and Molecular Medicine, 185 South Orange Avenue G609, Newark, New Jersey 07103, USA. E-mail: sadoshju@njms.rutgers.edu. @Sadoshimalab.

REFERENCES

1. Matsuura TR, Puchalska P, Crawford PA, Kelly DP. Ketones and the heart: metabolic principles and therapeutic implications. *Circ Res*. 2023;132(7):882–898.

2. Ruan HB, Crawford PA. Ketone bodies as epigenetic modifiers. *Curr Opin Clin Nutr Metab Care*. 2018;21(4):260–266.

3. Gambardella J, Jankauskas SS, Kansakar U, et al. Ketone bodies rescue mitochondrial dysfunction via epigenetic remodeling. *J Am Coll Cardiol Basic Trans Science*. 2023;8(9):1123–1137.

4. Papait R, Serio S, Condorelli G. Role of the epigenome in heart failure. *Physiol Rev*. 2020;100(4):1753–1777.

5. Bicocca VT, Ormsby T, Adhvaryu KK, Honda S, Selker EU. ASH1-catalyzed H3K36 methylation drives gene repression and marks H3K27me2/3-competent chromatin. *eLife*. 2018;7:e41497.

KEY WORDS epigenetics, heart failure, histone methylation, ketone bodies, PGC-1α