EDITORIAL FOCUS

Revisiting protein acetylation and myocardial fatty acid oxidation

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Submitted 2 June 2017; accepted in final form 22 June 2017

In the 20th century, our knowledge of posttranslational modifications (PTMs) and their impact on protein function/enzyme activity was largely confined to that of protein phosphorylation and their regulation via kinases and phosphatases. However, as our scientific tools have become more sophisticated, and as we have advanced our knowledge of cellular/molecular biology, which has further been augmented by the sequencing of the human and mouse genomes, we are now aware that protein function can be influenced by a variety of PTMs. This includes glycosylation, sumoylation, sulfation, and, of relevance to this particular Editorial Focus, acetylation, to name a few. Illustrating the predominance of protein phosphorylation regarding our overall knowledge of protein PTMs, there are nearly nine times as many published entries dealing with protein phosphorylation than there are entries dealing with protein acetylation on the Swiss-Prot database (8), which curates sequence information on the proteome from the published literature (2). Of interest, extensive evidence supports protein acetylation as a critical regulator of energy metabolism (4, 11). Indeed, the substrate for protein acetylation, acetyl CoA, is the common metabolic intermediate linking oxidative metabolism of all exogenous and endogenous fuel sources within our bodies (15), and the vast majority of cellular acetyl CoA is localized within mitochondria, the organelle that drives energy production.

Because the heart is the most metabolically demanding organ on a per gram basis (15), while containing the highest cellular levels of CoA alongside the liver, of which the vast majority is present as acetyl CoA (13), protein acetylation is now widely recognized as a major regulator of myocardial energy metabolism. In a recent article in the American Journal of Physiology-Heart and Circulatory Physiology, Thapa et al. (14) demonstrated that acetylation of the fatty acid oxidation enzymes short-chain acyl CoA dehydrogenase (SCAD), long-chain acyl CoA dehydrogenase (LCAD), and β-hydroxyacyl CoA dehydrogenase (βHAD) is increased in hearts of obese mice. Furthermore, Thapa et al. attributed these increases in SCAD, LCAD, and βHAD acetylation to increased expression/activity of amino acid synthesis 5-like 1 (Gcn5l1). Of particular interest, these increases in SCAD, LCAD, and βHAD acetylation were associated with increased enzyme activity. Finally, to confirm a key mechanistic role of Gcn5l1 in explaining their observations, the authors also generated various stable Gcn5l1 knockout cell lines in H9c2 myoblasts, which decreased SCAD/LCAD acetylation and subsequent SCAD/LCAD activity, ultimately reducing fatty acid oxidation rates in vitro.

The authors’ findings are particularly intriguing considering that current dogma often presumes that protein acetylation reduces enzyme activity/function (4, 11). In the context of fatty acid oxidation, a number of studies have demonstrated that sirtuin 3 (SIRT3), a nicotinamide adenine dinucleotide-dependent deacetylase, increases fatty acid oxidation by deacetylating LCAD. Seminal findings from Hirschey and colleagues (5) have shown that mice with a whole body deficiency for SIRT3 (SIRT3−/−) have reduced fatty acid oxidation rates in the heart, skeletal muscle, liver, and brown adipose tissue (BAT). Reduced BAT fatty acid oxidation likely accounts for the impaired cold tolerance in SIRT3−/− mice, which cannot maintain core body temperature to the same extent as their wild-type littermates (5). Moreover, the reduced peripheral tissue fatty acid oxidation rates in SIRT3−/− mice are associated with an exacerbation of high-fat diet-induced obesity, hepatic steatosis, and insulin resistance (6).

Reasons for the discrepancies between these previous studies and the work of Thapa and colleagues (14) remains enigmatic but could be explained by tissue-specific differences or enzymatic differences controlling acetylation/deacetylation of LCAD and other fatty acid oxidation enzymes. In the work of Hirschey and colleagues (5, 6), much of the phenotype was attributed to changes in hepatic and BAT fatty acid oxidation, whereas the study by Thapa and colleagues (14) focused on myocardial fatty acid oxidation. In addition, the increased acetylation of LCAD, SCAD, and βHAD in the hearts of obese mice was attributed to increased expression/activity of Gcn5l1 (14) versus SIRT3 deficiency accounting for the increased acetylation of LCAD in peripheral tissues of SIRT3−/− mice (5, 6). As the aforementioned studies simply measured overall acetylation of LCAD and not specific lysine residues within LCAD, it is possible that the acetylated lysine residues regulated by Gcn5l1 and SIRT3 within LCAD are different, thereby producing opposing actions on LCAD activity. Moreover, not all lysine residues within LCAD that are acetylated would be expected to impact LCAD activity in the same manner, while certain acetylated lysine residues within LCAD may not be important for controlling fatty acid oxidation. Thus, if Gcn5l1 and SIRT3 do indeed acetylate or deacetylate different lysine residues within LCAD, respectively, they may not have the same end result on fatty acid oxidation. As such, the relative activities of Gcn5l1 versus SIRT3 in a given tissue may dictate whether acetylation increases or decreases fatty acid oxidation in that tissue. With regard to tissue-specific reg-
ulation of LCAD, as the liver is primarily a lipogenic organ and the heart an oxidative organ, it is possible that LCAD acetylation affects fatty acid metabolism in a manner reflecting the physiological function of the organ. During fasting, hepatic SIRT3 expression is increased and fatty acid oxidation rates are elevated. Myocardial fatty acid oxidation rates are also elevated during fasting (15), although the study by Thapa and colleagues (14) did not assess SIRT3 or Gcn5l1 expression in the fasted heart. It is imperative that future studies address this issue, as such findings will most definitely shed light on a potential acetylation-mediated regulation of fatty acid oxidation in a tissue-specific manner.

Another key factor that needs to be considered regarding discrepancies of LCAD acetylation and fatty acid oxidation in past studies is the method used for measuring fatty acid oxidation. The original characterization of fatty acid oxidation rates in SIRT3−/− mice simply measured CO₂ production from radiolabeled palmitate in tissue homogenates (5). Although this is an accepted method to measure actual flux through fatty acid oxidation, during the lysis of membranes (e.g., plasma, mitochondrial, etc.) via homogenization, the influence of regulatory factors such as malonyl CoA, a potent endogenous inhibitor of mitochondrial fatty acid oxidation, is removed (10). Assessing fatty acid oxidation rates in tissue homogenates is also limited in that oxidative rates in tissue homogenates are substantially lower than those observed in fully intact tissue in situ. This is a very important limitation when measuring fatty acid oxidation in tissues that perform actual contractile work, such as the skeletal muscle and heart, where ex vivo/in vivo fatty acid oxidation rates are magnitudes higher than that observed in tissue homogenates (10). Unfortunately, this same limitation impedes the conclusions of Thapa and colleagues (14), as their measurements of fatty acid oxidation were performed in H9c2 cells with a stable knockdown of Gcn5l1, and in vitro fatty acid oxidation rates in cultured cells not performing external work are also magnitudes lower than those observed in the working heart ex vivo/in vivo (10). In the Thapa et al. study, H9c2 cells with a stable Gcn5l1 knockdown of ~80% at the protein level resulted in an ~50% reduction in fatty acid oxidation rates, which was associated with a reduction in both SCAD/LCAD acetylation and activity. Despite the limitations in measuring fatty acid oxidation rates in vitro, other studies using a similar in vivo model as that of Thapa and colleagues (14) have observed similar findings. As such, fatty acid oxidation rates are increased in isolated working ex vivo hearts from mice subjected to high-fat diet-induced obesity (1). Furthermore, LCAD acetylation and activity are increased in hearts from these obese mice, such that LCAD acetylation was strongly correlated with myocardial fatty acid oxidation rates (1). Interestingly, this same study demonstrated increased myocardial protein acetylation and subsequent fatty acid oxidation rates in isolated hearts from SIRT3−/− mice, suggesting that discrepancies between LCAD acetylation in the Thapa et al. study versus previous observations likely reflect tissue-specific regulation, since hepatic fatty acid oxidation rates are elevated in SIRT3−/− mice (5, 6).

Adding further confusion to acetylation-mediated regulation of fatty acid oxidation, recent isolated heart studies in SIRT3−/− mice using either working mode or Langendorff perfusions have demonstrated decreased fatty acid oxidation rates (3, 9). These discrepancies between three different studies quantifying myocardial fatty acid oxidation rates in similarly aged SIRT3−/− mice all originating from the Jackson Laboratories is currently unclear. Krebs-Henseleit solution components (e.g., palmitate concentration, bovine serum albumin concentration, etc.) and the inclusion of insulin in the perfusate were not consistent among all three studies and may explain potential differences, but further study is needed to address this issue.

Despite the current debate regarding protein acetylation and its influence on enzyme activity/function, there is clear evidence in the literature that both obesity and/or diabetes lead to marked increases in myocardial fatty acid oxidation rates and subsequent reductions in cardiac efficiency (10, 12, 17). The observations of Thapa and colleagues (14) are in agreement with previous studies and advance our knowledge base in this specific area by demonstrating that increased Gcn5l1 may be a key factor promoting increased myocardial fatty acid oxidation rates during obesity/diabetes by increasing LCAD acetylation. Conversely, they also observed a reduction in SIRT3 expression in hearts from obese mice, consistent with a previous study (1), and it will be important for future investigations to determine the individual contributions of both increased Gcn5l1 and decreased SIRT3 to myocardial protein acetylation profiles during obesity. Whether this is a compensatory mechanism to account for increased fatty acid delivery and subsequent uptake by the heart remains to be elucidated, and studies in whole body or cardiac-specific Gcn5l1-deficient mice will be necessary to further understand the potential role that Gcn5l1 plays in the regulation of myocardial fatty acid oxidation. Because the optimization of cardiac energetics, in particular reducing excessive fatty acid oxidation rates, has been postulated as a novel approach to mitigate obesity/diabetes-related cardiac dysfunction (16, 17), modifying Gcn5l1 activity.

Fig. 1. Divergent actions of long-chain acyl CoA dehydrogenase (LCAD) acetylation of hepatic and myocardial fatty acid oxidation. Shown is an illustration of the divergent actions of lysine (K) acetylation of LCAD on fatty acid oxidation rates observed in the liver versus those observed in the heart.
may represent a novel strategy to achieve this. Taken together, despite these findings contrasting previous literature suggesting that LCAD acetylation decreases LCAD activity and fatty acid oxidation rates in the liver, the outcome should encourage the field to continue exploring why acetylation may reduce fatty acid oxidation in the liver yet enhance it in the myocardium (Fig. 1).

ACKNOWLEDGMENTS

We are grateful for Kim L. Ho’s assistance with the preparation of Fig. 1.

J. R. Ussher is a Scholar of the Canadian Diabetes Association and a New Investigator of the Heart and Stroke Foundation of Alberta, Northwest Territories, and Nunavut. R. Al Batran is a Postdoctoral Fellow of the Canadian Institutes of Health Research and the Canadian Diabetes Association.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.A.B. and J.R.U. drafted manuscript; R.A.B. and J.R.U. edited and revised manuscript; R.A.B. and J.R.U. approved final version of manuscript.

REFERENCES


